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THESIS

VISUAL SURVEYS FOR ASSESING MEGABENTHIC SPECIES IN THE MARINE PROTECTED AREA OF MESSOLONGHI LAGOON

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TABLE OF CONTENTS

SUMMARY

The study area is part of Messolonghi lagoon, a relatively open lagoon in Patraikos Gulf (Greek Ionian Sea). It is a National Park as well as a Natura 2000 site with great ecological value.

The aim of the present study was the qualitative and quantitative recording of the megabenthic flora and fauna within and in the outer zone of Messolonghi lagoon.

The study was largely conducted by non-destructive visual surveys, through counts of megabenthic species along 100-m transects, by SCUBA diving. Specifically, for the qualitative composition of the study area 24 stations were examined. 15 of these stations were surveyed by two independent observers, recording presence/absence and abundance of megabenthic species. Environmental features like habitat type, temperature and depth were also recorded in order to be correlated with the species distributions. Furthermore, the flora coverage in five 52x52cm quadrats was examined in order to estimate the ecological quality of the area with the Ecological Quality Index (EEI).

Conserning the qualitative synthesis, a total of 148 taxa were identified adding a great value to the existing information of the flora and fauna of the Marine Protected Area. One colonial ascidian was recorded for the first time in the Eastern Mediterranean Sea and another one for the first time in Greece. 17 of the recorded species are protected by various conventions, 8 are endemic in the Mediterranean Sea and 6 are characterized as alien species. During the sampling period, we also recorded a spawning aggregation of the alien nudibranch *Melibe viridis* in the inner part of Messolonghi lagoon, with the densest published population and the largest published sizes.

The differentiation of megabenthic communities was associated with the location of the sites in relation to the effect of the sea (external or internal stations) and the type of habitat, while the above two factors are interrelated.

Regarding the quantitative analysis, occupancy was estimated for 13 important species, which provided information of their distribution and the proportion of the study area that they occupy. The highest occupancies were estimated for the protected seagrass *Posidonia oceanica* covering all of the outer part of Messolonghi lagoon, the endemic fish *Symphodus melanocercus* and the highly protected bivalve *Pinna nobilis*.

Simple abundance estimations were done for 7 abundant megabenthic invertebrates, providing new quantitative evidence for their population and their distribution. A better abundance and density estimation was made for the highly protected bivalve *Pinna nobilis* with the Distance Sampling methodology, revealing a high abundance of the species (381.132 individuals) in the study area.

The Ecological Status of five sampling stations was found through the Ecological Evaluation Index EEI, giving a High Ecological Status of the study area. More stations must be examined to provide a good estimation of the Ecological Status of the whole Messolonghi lagoon.

Conclusively, megabenthic distribution and abundance data were collected by using 3 different survey methodologies. The survey revealed several protected and endemic species and habitats in the Marine Protected Area of Messolonghi lagoon, some of them with noticeable abundances. The survey proposes that a regular monitoring program should be established in the area and protection with actual measures should be applied especially for the highly protected population of *Pinna nobilis*.

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INTRODUCTION

The aim of the present study was the qualitative and quantitative recording of the composition of megabenthic flora and fauna within and in the outer zone of Messolonghi lagoon.

The Marine Protected Area

The Marine Protected Area of Messolonghi lagoon is located in the western Greece (Figure 1a), occupies an area of **441.6km²** and is one of the largest and most significant wetlands in Greece with high ecological value. The whole Natura 2000 protected area of Messolonghi lagoon, with official name: Delta Acheloou, Limnothalassa Messolonghiou – Aitolikou kai ekvoles Evinou, Nisoi Echinades, Nisos Petalas, Dytikos Arakynthos kai Stena Kleisouras" and site code GR2310015 is presented in Figure 1a.
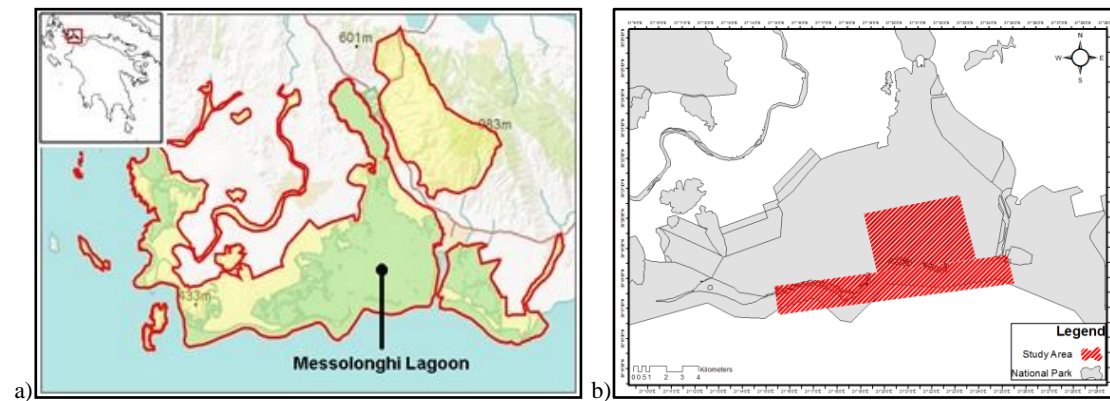
Figure 1: a) Borders of the Protected Area of Natura 2000 Network (http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=GR2310015). b) Borders of the study area which is part of the Marine Protected Area of Messolonghi lagoon

Protection Status

The wider area of the lagoonal system is protected by law as a National Park, as site of the Natura 2000 network of protected areas and under the Ramsar convention. The **management body** of Messolonghi Lagoon was created in 2003, and is responsible to:

- propose and implement conservation projects and promotion of the area
- monitor quality factors of the natural environment
- provides advise on activities and projects in the area
- develop information campaigns - public awareness and attract visitors
- publishe printed and electronic information
- organize events and workshops

The study area

The study area includes part of the inner part and the outer part of Messolonghi lagoon (Figure 1b). The total study area is **48.62** km^2. The position of the study area is important because it includes the transitional zone from the inner part of Lagoon to the outer zone.

Place, Geomorphology

Messolonghi Lagoon, located in the western part of Greece, is the largest lagoon of the country and one of the largest in the Mediterranean Sea. It's part of a wider complex ecosystem, which is one of the most significant wetlands in Greece. Extending 112 km^2, Messolonghi Lagoon predominates in the central part of the wetland (Figure 1a). It lies behind a fragmented sandy coastal ridge (located between the mouth of the river Evinos to the east and the Koutsilaris hill to the west) and is connected with the Ionian Sea and Patraikos Gulf by a large opening (Figure 1a). There are also a number of other smaller lagoons to the north (Aitoliko lagoon), east (Kleisova), and the west (Gourounopoules, Paliopotamos).

A part of the delta of the river Evinos is included in the eastern part of the Messolonghi plain. In the extreme west of the area a part of delta of the river Acheloos is found. Most of the plain is formed from the sediments deposited by these two rivers. Evinos has deposited sediments that cover a part of the area from Messolonghi eastwards, while sediments from the river Acheloos cover the area from Messolonghi to the west. In the western part of the site, the sediments of the river Acheloos surround rocks and hills, of which the Koutsilaris (433 m) is the highest. In the same part several former beds of the river Acheloos can be distinguished. The Messolonghi lagoon system was formed in the Holocene. The basin of the lagoon is divided into the Aitoliko and Messolonghi lagoons, which are connected to each other by a narrow channel. The coastal ridge consists of sand. The lagoon's substrate varies, ranging from sand to clay and locally muddy, consisting of clay silt and fine sand. A feature of the substrate is the existence of many shells breccia.

The lagoons studied are shallow. In the literature, the maximum **depth** is about 2 m but large areas have a depth of about 1m and only the Aitoliko lagoon has a maximum depth of 28 m. As mentioned above, the lagoons of Messolonghi are connected with the sea by means of **several wide openings in the coastal ridge**.

Confinement

Concerning confinement, as defined by Guelorget & Perthuisot (1992), and according to previous publications, (Klaoudatos et al. 1984; Nicolaidou et al. 1988) the whole lagoonal area of Messolonghi is highly confined with zones IV–V dominating and zone VI being well developed (Figure 2). This is due to the large freshwater input in Etolikon and Messolonghi proper and to a barrier at its southern edge. Zones II and III (which are the most interesting for aquaculture) are of a limited extent within the lagoonal system, and are restricted to the central southern part of Messolonghi lagoon and to the Messolonghi channel.
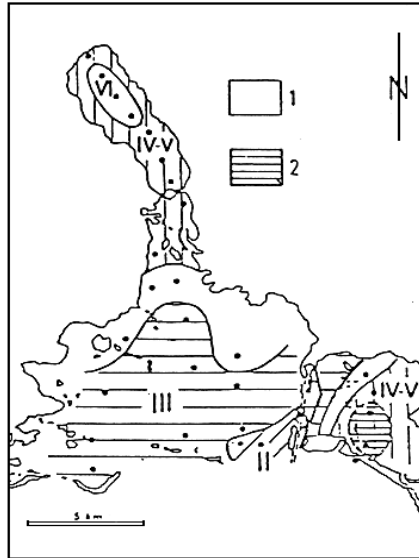
Figure 2: Map of confinement zones in Messolonghi lagoonal area. II: disappearance of marine stenobiotic species, III: dominance of intermediate species, IV-V: dominance of paralic species, VI: paucity of species. 1: transition zone between zones III and IV, 2: organic enrichment (Nicolaidou et al 1988)

The reasons for this are as follows: in Etolikon and Messolonghi lagoons, large freshwater inputs exist, with plus the presence of a net barrier at the southern edge of Messolonghi lagoon. Zones II and III (which are the most interesting for aquaculture) are of a feeble extension within the lagoonal system and restricted to the central southern part of Messolonghi lagoon and to the Messolonghi channel.

Environmental features

The air **temperature** shows seasonal fluctuations. Smaller values (7 ° C) occur during the months of January and February while larger (29 ° C) in August and September.

Mesollonghi lagoon present the highest values of **Chlorophyll-a** of the wider surrounding area. In principle, from December to March, the concentrations of Chlorophyll-a are higher than the other months indicating a spring peak in March. During summer, Chlorophyll-a has a significant decline (Vassilopoulou et al. 2012).

Some parts of the lagoons are rather isolated. This results in large fluctuations of **salinity** during the course of the year. The high salinity in summer, caused by evaporation, is tempered by fresh water flowing into the lagoons from the surrounding fields and some small brooks. During the winter large amounts of fresh water drain into the lagoons. In winter water from the Evinos and Acheloos rivers probably also flows into the lagoons on occasions. Due to the wide communication of Messolonghi Lagoon with Patraikos gulf, the water masses in the frontal area have typical marine salinity (38‰). But, close to Aitoliko the salinity varies, usually being strongly reduced (down to18‰) because of freshwater inflows from pumping stations and drainage work,.

Under prevailing northeastern and secondarily northwestern **winds**, the sandbars act as natural protective embankment to the lagoon, providing protection against the erosive action of the waves.

Pressures

The principal human activity in the lagoons is **fishing**. In the lagoon there are 8 fish farms (dibaria) that are rented by fishermen after an auction for three years. Fishing at the lagoon is still done traditionally, by periodical closure of the fish farms. The extensive fisheries produce mainly mullets, eels, spars, seabream and seabass, plus occasional species, such as Sardinella. There are several **pumping stations** and water inflows (**Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.**) in Messolonghi lagoon. One of the pumping stations in the central and west side of Messolonghi lagoon was in operation during our sampling period, creating large quantities of foam that covered the western coastal zone of the Lagoon. There are also recorded cold water influences from the **river outflows** (Vassilopoulou et al. 2012). In the past, the algal growth, rainfall and winds led to **nutrient fluctuations** over space and time in Messolonghi Lagoon (Friligos 1989).

Messolonghi lagoons were formerly surrounded mostly by extensive salt marshes, large parts of which were drained for agricultural purposes. However, they still remain infertile and serve as a wildlife habitat. Despite land reclamation, **salt marshes**, **sand-banks** and **mudflats** still occur around the lagoons.
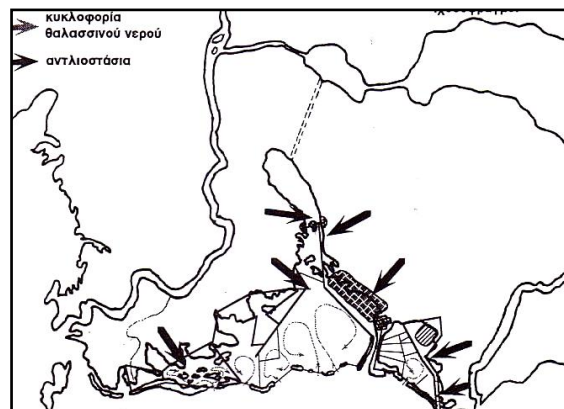
Figure 3: Pumping stations (thick arrows) and water circulation (thin arrows) inside Messolonghi lagoon (Οικονομίδης. Π. 1996)

Previous research

Although the macrobenthic fauna of Messolonghi lagoon is quite well known, there are no studies concerning megabenthic communities. Moreover, this is the first time that non destructive visual surveys are applied in the wider area.

Reports of the benthic flora are recorded by Bogdanos & Diapoulis (1984). Other studies focus on the composition and abundance of benthic fauna like Biological Quality Elements of the Water Framework Directive 2000/60/EK (Bourgoutzani & Zenetos 1983; Nicolaidou et al. 1988). Later Nicolaidou et al. (2005) summarised the available information on the main biological components – phytoplankton, zooplankton, phytobenthos, zoobenthos and fish of Messolonghi and other lagoons and concluded that the most important variable influencing species distribution and diversity in Greek lagoons is the degree of communication with the sea and the nutrient load introduced through fresh water inputs. As stated before, Messolonghi lagoon is highly confined according to the definition of Guelorget & Perthuisot (1983). Therefore its confinement is attributed mostly to the time of renewal of the elements of

marine origin at any given point, and not to features like salinity or sedimentology (Nicolaidou et al. 1988, Nicolaidou et al. 2005). Προβιδάκης 2013 studied the macrobenthic diversity of Messolonghi lagoon.

Chryssanthakopoulou & Kaspiris 2005 have studied the reproductive cycle of the bivalve *Ruditapes decussatus* in Evinos estuaries.

Cabana et al. 2014 have studied the contribution of the habitat complexity in structuring the distribution of the biological traits on macroinvertebrates communities in Messolonghi lagoon. Their results showed a positive correlation of the habitat complexity with the distribution of feeding mode, mobility and habitat location.

Habitat and Species

Generally the area is characterized by a variety of **habitats**. There are extensive areas of shallow water with a rich submerged vegetation of *Ruppia, Enteromorpha* and *Zostera*. A very important habitat type in the area are the *Posidonia oceanica* meadows. The alluvial muddy substrate hosts a large population of *Ruppia maritima spp.spirallis* and *Zostera marina* (maybe misidentification of the species according to Gerakaris et al. 2014) in parts more affected by the sea water. Apart from the seagrasses there are individual plants or assemblages of brown algae (*Cystoseira barbata*), and a few green algae (Cladophora sp.). Algae show a strong growth in the rocky mounds and wooden poles of the pens of the lagoon. The periphyton is particularly rich in species with important participation of Diatoms, green algae and red algae . (ΕΠΕ 1999) The coasts of Echinades islands provide a number of submarine and semi-submarine sea caves wich are important seal habitats.

The **species** *Tursiops truncatus* and *Delphinus delphis* which are recorded in this area, are included in Directive 92/43/EEC, the Bern and Bonn Conventions, CITES and Corine-Biotopes. They are also recorded by the Presidential Decree 67/1981, and *Delphinus delphis* is recorded in the Greek Red Data Book as "Vulnerable". The area of Messolonghi lagoon is characterized by few species of aquatic flora (Bogdanos & Diapoulis 1984) that shape quite developed but scattered meadows.

The species already included in the Natura 2000 Marine Protected Area of Messolonghi lagoon are shown in Table 1 . (http://natura2000.eea.europa.eu/)

Table 1: Certain marine species already recorded in the Natura 2000 Marine Protected Area of Messolonghi lagoon. (http://natura2000.eea.europa.eu/)

FISH	SEAGRASSES	ALGAE
Acipenser sturio	*Posidonia oceanica*	*Cystoseira barbata*
Knipowitschia panizzae	*Zostera noltii*	*Enteromorpha linza*
Aphanius fasciatus	*Zostera marina*	*Enteromorpha intestinalis*
Phoxinellus spp.	*Cymodocea nodosa*	*Caulerpa prolifera*
Alosa fallax	*Ruppia maritima spp.spirallis*	*Acetabularia acetabulum*
Barbus capito		Ulva sp.
Syngnathus abaster		Cladophora sp
Liza ramada		*Valonia aegagropila*
Liza saliens		*Hypnea valentiae,*
CETACEANS		*Hypnea musciformis*
Tursiops truncatus		*Laurensia obtusa*
Delphinus delphis		*Chondria tenuissima*
REPTILES		*Herposiphonia tenella*
Caretta caretta		*Ceramium codii*
Chelonia mydas		Ceramium sp.
		Rytiphloea tinctoria
		Palisada sp.

Visual census

Visual surveys as research methods in Marine Protected Areas are highly recommended for conservation purposes. State variables of interest for the monitoring of marine populations and communities include abundance, population density, biomass, population structure, biodiversity, and occupancy (Katsanevakis et al. 2012). Often abundance or population density estimations of protected species are needed inside Marine Protected Areas and in this case visual surveys are ideal. Direct visual surveys are non-destructive, which should be taken into consideration, especially when dealing with rare or protected species or habitats. Visual surveys have been used extensively for assessing fish community structure and the relative abundances of various megabenthic groups. Generally, different survey methods should be considered according to the biology and behavior of the species of interest (Willis et al. 2000). For example, at shallow depths, direct visual surveys with SCUBA diving are advantageous compared to destructive fishery surveys, as the records are not dependent on catch efficiency (Katsanevakis & Verriopoulos 2004; Katsanevakis 2005).

Different methods of visual surveys for abundance estimations include: Point transects, Strip transects, Line transects, Quadrats, Video, Photography etc. The choice of the best sampling methodology depends mostly on the size and the distribution of the target species as well as on the scope of the study.

Plot sampling

Plot sampling is by far the most commonly applied method for biological monitoring in the marine environment. A wide variety of visual sampling techniques and devices is used for plot sampling like: underwater **quadrat** sampling, **strip transects**, devices for **imaging** (cameras or video recorders and underwater vehicles such as ROVs [remotely operated vehicles] or AUVs [autonomous underwater vehicles]. The choice of the appropriate technique and the most suitable device depends primarily on the surveyed habitat type and the characteristics of the target population or community (e.g. size, distribution of species). (Katsanevakis et al. 2012).

Underwater visual surveys with plot sampling, and especially strip transects, have been used extensively to estimate the density and/or abundance of benthic species. In **strip transects**, the plots are long, narrow strips, and the observer travels along the centre line searching for the animal of interest and counting all individuals within the strip. In plot sampling, the critical assumption is that all individuals present in the surveyed areas are detected. However, this assumption cannot be tested using the survey data, and to ensure that it holds to a good approximation in all habitats and environmental conditions, it may be necessary to use narrow strips, which is problematic for scarce species (Burnham & Anderson 1984, Buckland et al. 2001) and increases the variance of density estimators (Kulbicki & Sarramégna 1999, Buckland et al. 2001). If the assumption that all individuals present in the surveyed areas are detected is not met, there is underestimation of abundance, which is not uncommon in underwater surveys. Failure to properly account for detection probability (i.e. probability of detecting the presence of an individual within a plot) leads to negatively biased estimates of population density or abundance by a factor equal to the probability that an animal in the covered region is detected.

Methods dealing with imperfect detectability

One of the most active areas of biometric and wildlife research is the development of methods and models to properly account for detection probability (e.g. Thompson et al. 1998, Buckland et al. 2001, 2004, Williams et al. 2002, Borchers et al. 2004, Thompson 2004). **Distance sampling**, **mark-recapture** methods, repeated **presence-absence** surveys for occupancy estimation, and **removal** methods estimate detection probabilities and provide unbiased estimates of state variables unlike plot sampling. However, the application of some of these methods in the marine environment seems to lag behind terrestrial and freshwater systems (Katsanevakis 2009a).

- **Distance sampling**

Distance sampling (Buckland et al. 2001) is a widely used group of methods for estimating abundance and/or density of biological populations. Distance sampling has been used extensively in terrestrial ecology (mostly for birds and terrestrial mammals) and for marine mammals. Although it is the standard method for abundance estimations of many species, it is only rarely used for underwater surveys of benthic fauna (e.g. Katsanevakis 2005, 2006). The main distance sampling methods are line transects and point transects. A standardized survey is conducted along a series of **lines** (in line transects) or **points** (in point transects) searching for the animals of interest. For each animal detected, the distance from the line or point is recorded. A detection function is fitted from the set of recorded distances, which is used to estimate the proportion of animals missed by the survey and hence estimate abundance. **When the detection of individuals is difficult, a distance sampling method is typically more efficient than simple strip transect sampling**. This is because densities are corrected with the use of the detection function and the sample size is larger for the same amount of effort as all detected individuals may be recorded regardless of how far they are from the line. (Katsanevakis 2007a)
A more detailed description of Distance Sampling is given in the Methodology section.

- **Occupancy estimation**

Estimation of density or abundance is often costly and requires substantial effort or may be unfeasible for various reasons (e.g. in the case of rare or very cryptic species). Alternatively, species occupancy— defined as the proportion of area, patches, or sampling units occupied (or as the probability of presence in a sampling unit)—may be seen as a low-cost surrogate of abundance (MacKenzie et al. 2006). Moreover, there are cases when occupancy is the most appropriate state variable and would be chosen in the first place, as in studies of distribution and range (Scott et al. 2002), alien invasions (Issaris et al. 2010, Katsanevakis et al. 2011), metapopulation studies (Moilanen 2002), community studies (Martinez- Solano et al. 2003), and large-scale monitoring (Manley et al. 2004). Occupancy estimation is based on **presence absence** data while taking into account the imperfect detection of the target species (MacKenzie et al. 2006). (Katsanevakis et al. 2012)
A more detailed description of Occupancy estimation is given in the Methodology section.

The Ecological Evaluation Index EEI

EEI is an index for estimating the habitat- based ecological status of rocky coastal and sedimentary transitional waters using shallow benthic macrophyte communities (seaweeds, seagrasses) as bioindicators (Orfanidis et al. 2011).

According to Orfanidis et al. 2001, marine benthic macrophytic species can be used to indicate shifts in the aquatic ecosystem from the pristine state with late-successional species (Ecological State Group I) to the degraded state with opportunistic (ESG II) species. Thus, marine benthic macrophytes are used as bioindicators of ecosystem shifts due to anthropogenic stress, from the pristine state with late-successional species (high ecological status class (ESC)) to the degraded state with opportunistic species (bad ESC) (Orfanidis et al. 2003).The first group comprises species with a thick or calcareous thallus, low growth rates and long life cycles (perennials), whereas the second group includes sheet-like and filamentous species with high growth rates and short life cycles (annuals). Seagrasses are included in the first group, whereas Cyanophyceae and species with a coarsely branched thallus are included in the second group. The two main clusters (ESG I, late-successional; ESG II, opportunistic) are futher divided into three and two sub-clusters, respectively: ESG I comprised thick perennial (IA), thick plastic (IB) and shade-adapted plastic (IC) coastal water species, and angiosperm plastic (IA), thick plastic (IB) and shade-adapted plastic (IC) transitional water species. ESG II comprised fleshy opportunistic (IIB) and filamentous sheet-like opportunistic (IIA) species both in coastal and transitional waters (Orfanidis et al. 2011).

The evaluation of ecological status into five categories, from high to bad includes a cross comparison in a matrix of the ESGs and a numerical scoring system (Ecological Evaluation Index) (Figure 4).

The sampling for the estimation of the Ecological Evaluation Index EEI is conducted with **quadrat visual samples** as will be described in the methodology section
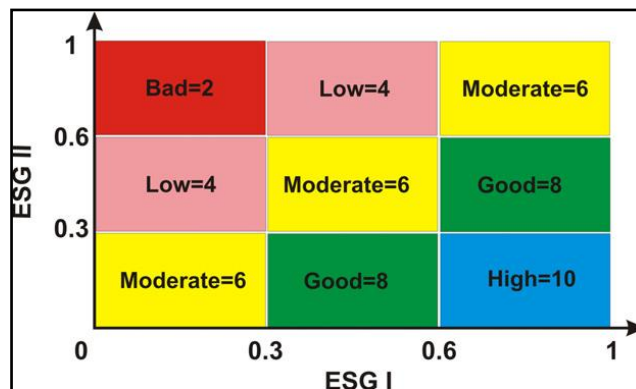
Figure 4: Estimation of EEI and the equivalent ESCs from a matrix based on the mean abundance (%) of ESGs (Orfanidis et al. 2011).

MATERIALS & METHODS

Species composition

Initially, a preliminary survey took place in the study area in October of 2014. During the preliminary survey, we decided the sampling methodology, some target species of greatest interest and our study area. Subsequently, the field work was conducted in November 2014, in two sampling expeditions, mostly by visual non destructive methods. In Figure 5 you can see all the 24 examined sampling stations in the wider area of Messolonghi lagoon for the 3 different scopes of this thesis. With the 24 qualitative stations we wanted to get an insight in the biodiversity of the study area, examining the presence of species of interest.
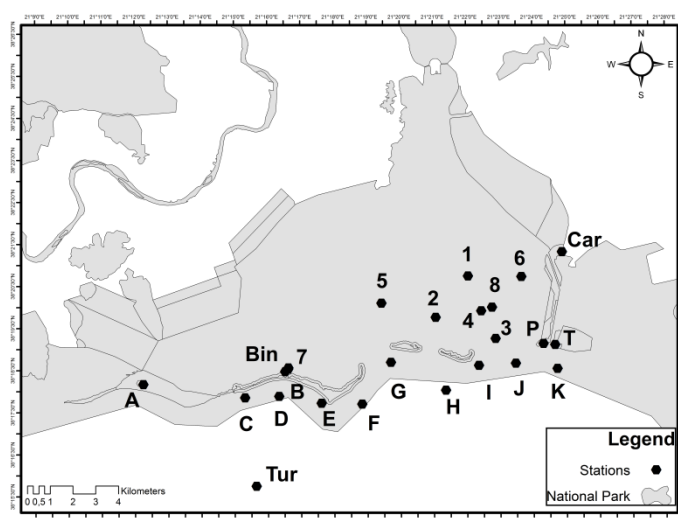
Figure 5: 24 qualitative stations examined. National Park with gray. The sandbars were regarded to be the border between the Inner and Outer Part of Messolonghi Lagoon.

Presence and Abundance of species (Transects)

In Figure 6 you can see the 15 quantitative sampling stations, which were studied through 100m transects. From the 15 quantitative stations, we collected data for the abundance of megabenthic species and of presence of species in 100m transects. External stations of the Lagoon (C, D, E, F, G, H, I, J, K) were selected by using a grid so as to be equally spaced from each other and cover the range of depths 4-12m. The existing bathymetry of the area was used from webapp.navionics.com. The selection of the rest quantitative stations was done randomly, with the objective to cover the majority of habitats of the study area that were accessible by boat. The depth of the inner part of the Lagoon extends from a few centimeters to 3.5 meters, while the P stations and 3 are considered to be on the border of the inner and the outer part of the. The communication of the inner part with the sea, is extended.
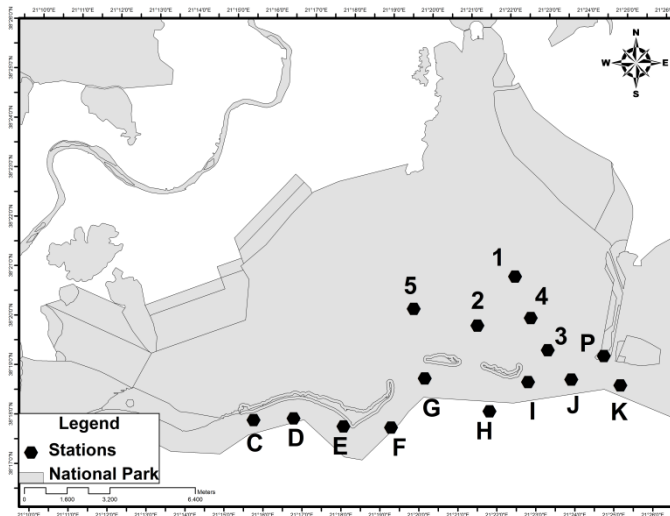
Figure 6: 15 quantitative sampling stations, examined through 100m transects

Table 2: Coordinates of 15 quantitative sampling stations and date on which they were surveyed.

Station	Latitude	Longitude	Date
C	38.300300°	21.261567°	4/11/2014
D	38.301267°	21.278667°	4/11/2014
E	38.299050°	21.300100°	4/11/2014
F	38.299150°	21.320500°	5/11/2014
G	38.316033°	21.334300°	5/11/2014
H	38.305633°	21.362317°	5/11/2014
I	38.315867°	21.378550°	26/11/2014
J	38.317117°	21.397033°	6/11/2014
K	38.315533°	21.418050°	6/11/2014
P	38.325267°	21.410633°	26/11/2014
1	38.351183°	21.371683°	28/11/2014
2	38.334400°	21.356117°	28/11/2014
3	38.326717°	21.386500°	24/11/2014
4	38.337467°	21.378850°	25/11/2014
5	38.339350°	21.328650°	28/11/2014

Occupancy

Most stations were examined by scuba diving, but the stations 1,5 and P were examined from the boat with a similar technique. All the stations depths were from 1 to 12 meters. Every sampling station was approached by boat and GPS. The qualitative and quantitative composition of benthic species was studied with the following method: From the starting point of each station, a dive line of 100 meters was placed on the seabed with direction south by using an underwater compass. Subsequently 2 independent observers were scanning and recording:

1) The presence of megabenthic flora and fauna (including fish)

2) The abundance of megabenthic species

3) Every 5 meters the habitat, the temperature and the depth

4) Length of important species was measured underwater

In cases that a species couldn't be identified underwater, photographs were taken and some samples were collected for further lab identification. All photos used in the text are taken by Christos Kotselis and the writer. Underwater we focused on megabenthic species (>5cm) but especially from the group of algae also smaller samples were identified.

Underwater slates (Figure 7a) were used for recording of data and personal dive computers were used for measuring the depth and the temperature. Length-measurements of certain species were done with a meter with millimeter accuracy. Every diver-observer was wearing diving equipment with backups in case of an emergency.

In the group of algae there is no meaning of individuals, so only data of presence/absence of algae along the transects were used for the analysis and no abundance data. For the group of fish only data of presence/absence were obtained, since for an abundance estimation of fish the observers need to focus in an optical field (water column) which is different from the one for benthic species (substrate).

Samples were preserved in plastic bottles with formalin solution 4 %. The collected samples were identified in the zoobenthos lab of the University of Athens. For each taxonomic group specific keys were used and samples morphological characteristics were examined with the use of stereoscope and microscope.

a)   b)
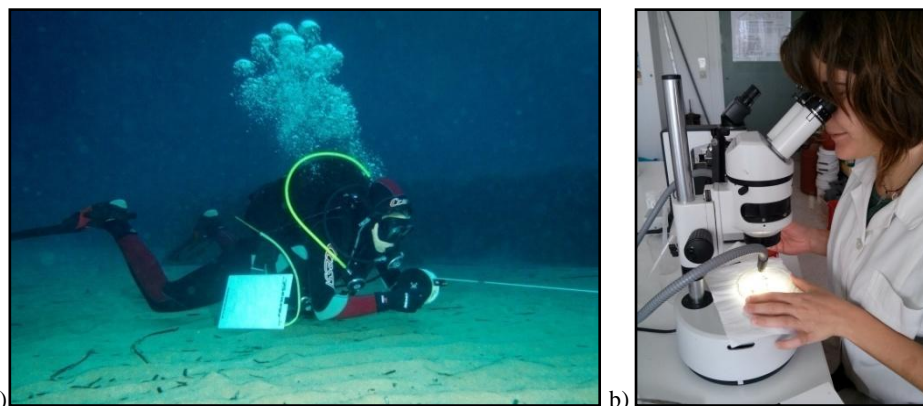
Figure 7: a) Underwater retraction of the diving line. All photos used in the text are taken by Christos Kotselis and the writer. b) Lab identification of species in stereoscope.

Distance Sampling

Distance Sampling methodology (see data analysis) was also applied in the 15 quantitave transects, for a better estimation of the population of the highly protected bivalve *Pinna nobilis*. In practice, the perpendicular distance from the dive line to every individual of *Pinna nobilis* was measured. In the same time the segment of the measured dive line was recorded, so as we can know the exact position of every individual. Moreover, the width of 15 individuals was measured (accurate to the milimeter) with the intention to understand the size distribution of the individuals.

For all the underwater survey methods, the observers were trained before by multiple educational dives and by understanding the assumptions of each method.

The Ecological Evaluation Index EEI (Quadrats)

For the estimation of the Ecological Evaluation Index, five sampling stations were examined, three of which were placed inside the Messolonghi lagoon (stations2,4,7), one in the limit (3) and the last one outside of the lagoon (I) (Figure 8). Macrophyte species were identified in situ into 52x52cm quadrats. The percentage of each species in the whole quadrat was also estimated into the field for every sample. The list provided into Orfanidis et al. 2011, was used for the classification of every identified species into the five functional groups (IA, IB, IC, IIA, IIB). Afterwards, the percentage of each functional group was calculated by adding the percentages of all species that belong to the same group. The numerical scoring system provided in Orfanidis et al. 2011 was used in order to estimate the value of the Ecological Status Class of every sample. The average of the EEIs of the five samples provides information about the Ecological Status of the area examined.
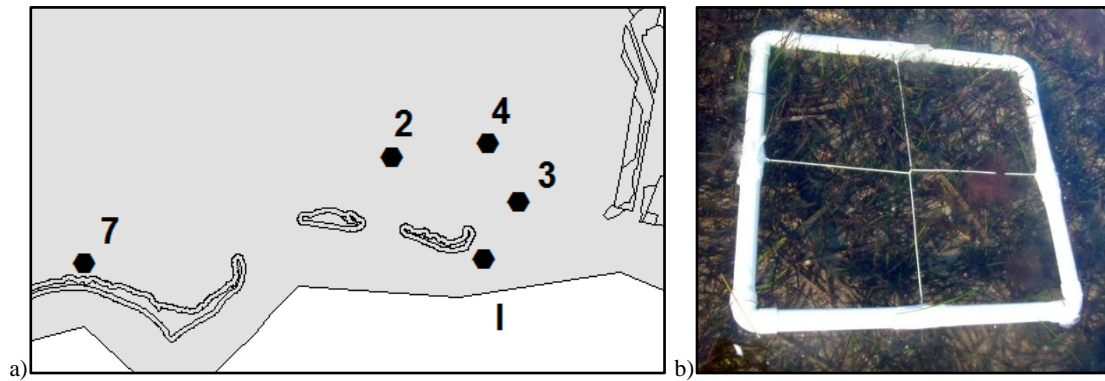
Figure 8: a) the 5 sampling stations for the estimation of the Ecological Evaluation Index b) quadrat 52x52cm placed underwater for the estimation of EEI

Data analysis

Primer

For the grouping of stations, based on the qualitative and quantitative composition of species, cluster analysis and multivariate analysis (MDS) were done with the software PRIMER-E v6 (Clarke and Gorley, 2006). The abundance values were transformed to square root. Similarity between the stations, was calculated by the Bray-Curtis index (Bray and Curtis, 1957).

Occupancy

The program presence 10.0 was used for the occupancy estimation. This program is developed to enable estimation of the proportion of area occupied (PAO), or similarly the probability a site is occupied, by a species of interest according to the model presented by MacKenzie et al. 2002. Typically, species are not guaranteed to be detected even when present at a site, hence the naïve estimate of PAO given by:

$$PAO = \frac{\text{\# sites where species detected}}{\text{total \# sites surveyed}}$$

will underestimate the true PAO. MacKenzie et al. 2002 propose that by repeated surveying of the sites, the probability of detecting the species can be estimated which then enables unbiased estimation of PAO.

For this study, we used the single season model proposed by MacKenzie et al. 2002, for estimating the site occupancy probability (or PAO) for target species, in situations where the species is not guaranteed to be detected even when present at a site. In our case, the transects where scanned by 2 observers.

Single season model description

The single-season model has two fundamental processes, occupancy and detection. Sample units within the region of interest are either occupied by the target species or not (i.e., species is present or absent at each unit) and **the probability of the species being present** at the ith unit is denoted as ψ_i (spelt 'psi'). Given the unit is occupied, the **probability of detecting** the species in the jth survey of that unit is p_{ij}. If the unit is unoccupied then, using the standard single-season models, the species cannot be detected. In order to reliably separate out occupancy from detection (i.e., where the species is vs where the species is found) repeat surveys within the season are required. In our case we had two independent observers scanning all the transects. During the season it is assumed that units are closed to systematic changes in occupancy, the outcome of each survey of a unit is independent and there is no misidentification of species (i.e., no false detections). Because the species is detected imperfectly there is the potential for false absences in the data (i.e., units where the species was never detected, but it was actually present) which will lead to occupancy being underestimated if unaccounted for.

The intent of this modeling is to explicitly **correct for detection** issues leading to improved inferences about occupancy and the factors that may be influencing it. The repeated surveys of a unit will yield a detection history denoting the sequence of detections and nondetections of the species at that unit. From the detection history, a verbal description of the data can be developed and then translated into a probability statement, which is an expression for determining the probability of observing that particular detection history given the model. In an example with four observers, consider the detection history (h_i):

$$h_i = 0101$$

A verbal description would be: the unit was occupied, the species was not detected in the first survey, detected in the second, not detected in the third and detected in the fourth survey. Translating to a probability statement is simply achieved by replacing the relevant phrases associated with certain events in the verbal description by the probability of the event occurring. For example, the unit is occupied with probability ψ_i and the species is not detected in the jth survey with probability $1 - p_{ij}$. Therefore, the probability statement for this detection history is:

$$\Pr(h_i = 0101) = \psi_i(1 - p_{i1})p_{i2}(1 - p_{i3})p_{i4}$$

Probability statements for units where the species was detected at least once are constructed in a similar manner. For units where the species was never detected, e.g., $h_i = 0000$, the same approach is used while recognizing that the species may go undetected at a unit for two reasons; due to either a true or a false absence, and that both possibilities must be accounted for in the verbal description and probability statement. The verbal description for the history 0000 is: the unit was occupied and the species was not detected in all four surveys (i.e., a false absence) OR the unit was unoccupied (i.e., a true absence). To account for the multiple options in the verbal description (that cannot be differentiated between from the available data) within the probability statement, the probability of each option is determined and then added together. That is:

$$\Pr(h_i = 0000) = \psi_i(1 - p_{i},1)(1 - p_{i},2)(1 - p_{i},3)(1 - p_{i},4) + (1 - \psi_i)$$

Given the set of detection histories from the s units that were surveyed, the model likelihood is defined as,

$$L = \prod_{i=1}^{s} \Pr(h_i)$$

Once derived, the likelihood equation is used by substituting in numeric values for the ψ and p parameters and finding what combination of values maximizes the value of the likelihood expression. In practice, **the maximum of the likelihood function, gives the better estimations fot ψ and p**. The parameter values that maximize the likelihood are known as maximum likelihood estimates (MLE's). Further modeling of the ψ's and p's (e.g., to investigate what factors are important covariates for occupancy and detection) is facilitated by using the logit-link function, which is a non-linear transformation used to rescale probabilities from the 0-1 scale to the $\pm\infty$ scale. With the logit-link, occupancy and detection probabilities can be expressed as a function of site-specific and sampling-occasion **covariates**, e.g.,:

$$Logit(\psi) = a_1 + a_2 \ (Cov_1) + a_3 \ (Cov_2) + a_4 \ (Cov_3) + \ldots\ldots + a_U \ (Cov_{U-1})$$

$$Logit(\psi) = \ln(odds(\psi)) = \ln \frac{\psi}{1-\psi} \quad \Rightarrow \quad \psi = \frac{e^{logit(\psi)}}{1 + e^{logit(\psi)}}$$

where **ln** is the natural logarithm, **Cov** are the covariates, **ψ** is the presence probability and **α** are the regression coefficients to be estimated. These resulting equations are essentially logistic regression equations. The terms of the form:

$$ln\left(\frac{\theta_i}{1-\theta_i}\right)$$

are the actual transformations applied to the probabilities (denoted with θ here for generality), and is just the log of the ratio of the probability of success to the probability of failure. This ratio is also known as the odds hence the logit-link is also referred to as the log-odds link. More detail on the use of the logit-link and its interpretation is given in the examples in this chapter and in MacKenzie et al. 2006. In practice, once a number of logistic regression equations for the different parameters of interest is set up, the regression coefficients for each parameter type are estimated simultaneously through the framework of the probability statements which involve a combination of the ψ's and p's, hence are automatically corrected for the effect of the other parameters.

In our case, just the covariate of **depth** was used in our case, assumed to affect occupancy probability of the species. Before entered in the Presence software, the depth was normalized with the following formula: $x - x_m / SD$ where x each transect's depth, x_m the average of depths and SD the Standard Deviation of depths.

Assumptions

A key assumption of the single season model is that all parameters are constant across sites. Failure of this creates heterogeneity. Unmodeled heterogeneity in detection probabilities will cause occupancy to be underestimated. If there is unmodelled heterogeneity in occupancy probabilities, then it is believed that the estimates will represent an average level of occupancy, provided detection probabilities are not directly related to the probability of occupancy. Another major assumption of the MacKenzie et al. 2002 model is that the occupancy state of the sites does not change for the duration of the surveying. Situations where this may be violated, for instance, would be for species with large home ranges, where the species may temporarily be absent from the site during the surveying. If this process of temporary absence from the site may be viewed as a random process, (e.g., the species tosses a coin to decide whether it will be present at the site today), then this assumption may be relaxed. However, this will alter the interpretation of the model parameters ("occupancy" should be interpreted as "use" and "detection" as "in the site and detected"). More systematic mechanisms for temporary absences may be more problematic and create unknown biases. Although, users are reminded that the model assumes closure of the sites at the species level, not at the individual level, so there may be some movement of individuals to/from sites without overly affecting the model.

Models

In our case, four models were fit to the data for each species with the following hypothesis:

Model	Hypothesis
psi(D),p(obs)	The presence probability of the species is **D**epth depended and the 2 **obs**ervers have different detection probabilities (**p₁, p₂**)of the species
psi(.),p(obs)	The presence probability of the species is constant (**.**) in the study area and the 2 **obs**ervers have different detection probabilities (**p₁, p₂**)of the species
psi(D),p(.)	The presence probability of the species is **D**epth depended and the detection probability of the 2 **obs**ervers is constant (**.**)
psi(.),p(.)	The presence probability of the species is constant (**.**) in the study area and the detection probability of the 2 **obs**ervers is constant (**.**)

Model selection

An information theory approach may be followed in order to infer occupancy patterns. According to this approach, data analysis is assumed to be the integrated process of

1) an *a priori* specification of a set of candidate models (based on the science of the problem, as the ones described before)

2) model selection based on the principle of parsimony according to the Akaike information criterion (AIC; Akaike 1973) and

3) the estimation of parameters and their precision (Burnham & Anderson 2002).

The principle of parsimony implies the selection of a model with the smallest possible number of parameters for adequate representation of the data, a bias versus variance trade-off. Furthermore, rather than estimating parameters using only the 'best' model, parameters (i.e. occupancy and detection probabilities) can be estimated using several or even all of the models considered. This procedure is termed multi-model inference and has several theoretical and practical advantages (Burnham & Anderson 2002, Katsanevakis 2006).

In more detail:

The small-sample, bias-corrected form AICc (Hurvich and Tsai 1989) of the Akaike Information Criterion (Akaike 1973; Burnham and Anderson 2002), was used for model selection among the set of candidate models. Specifically,

$$\text{AIC}_c = \text{AIC} + \frac{2k(k+1)}{n-k-1} \text{ and } \text{AIC} = -2\ln\left(\mathcal{L}(\hat{\theta}|\text{data})\right) + 2K$$

where $\ln\left(\mathcal{L}(\hat{\theta}|\text{data})\right)$ is the numerical value of the loglikelihood (natural logarithm) at its maximum point and $\hat{\theta}$ is the vector of the model's estimated parameters. The model with the smallest AIC$_c$ value (AIC$_{c,min}$) was selected as the 'best' among the models tested. The AICc differences, $D_i = \text{AIC}_{c,i} - \text{AIC}_{c,min}$; were computed over all candidate models. Goodness-of-fit

of the most complex model was assessed with x^2 test and the weighted (to give higher weight to distances near zero) Crame´r-von Mises test, as described by Burnham et al. (2004). The models have a good fit to the data, if the x^2 test of the most complex model gives a Pvalue which is higher than 0.05. If Pvalue is lower than 0.05 we should check the index C-hat. If C-hat is close to 1 the model describes adequately our data.

For the estimation of the model averaged psi (of the 4 models that we run), the weighted mean occupancy is given from the formula with w the weight of each model with x occupancy:

$$\bar{x} = \frac{\sum_{i=1}^{n} w_i x_i}{\sum_{i=1}^{n} w_i},$$

Distance Sampling

Distance sampling is a widely used methodology for estimating animal **density** and/or **abundance** of biological populations. Its name derives from the fact that the information used for inference are the recorded distances to objects of interest obtained by surveying lines (as in the present study) or points.

In the case of **lines**, one or more observers perform a standardized survey along a randomly located set of lines (=transects), searching for objects of interest (individuals of *Pinna nobilis*). For each object detected, the observer (1 in our case) record the distances from the line to the object. Not all the objects will be detected, but a fundamental assumption is that all objects that are actually on the line are detected. Intuitively, one would expect that objects become harder to detect with increasing distance from the line, resulting in fewer detections with increasing distance since the probability of detecting an animal decreases as its distance from the observer increases. The key to distance sampling analyses is to fit a **detection function** to the observed distances, and use this fitted function to estimate the proportion of objects missed during the survey. In practice, detection functions model the probability of detecting an animal, given its distance from the transect. From here, we can readily obtain point and interval estimates for the density and abundance of objects in the survey area.

Distance sampling extends quadrat-based methods by relaxing the assumption that all objects within the strip are counted. By measuring distances to the objects that are observed, the probability of observing an object within the strip can be estimated.

In line-transect sampling, a series of (systematic) randomly located straight lines is traversed by one (as in our study) or more observers.

Estimation

Perpendicular distances x are determined from the line to each detected object of interest. Let's suppose k lines of lengths (in our case we examined 15 lines=transects of 100-m length)

$$l_1,...,l_k \text{ (with } \Sigma l_j = L)$$

are positioned according to some randomized scheme, and that animals further than some distance w from the line (the truncation distance) are not recorded. Then, the surveyed area is $a = 2wL$, within which n animals are detected at perpendicular distances $x_1,...,x_n$. Let P_a be the

probability that a randomly chosen animal within the surveyed area is detected, and suppose an estimate $\hat{P}_a$ is available. Animal density D is then estimated by

$$\hat{D} = \frac{n}{2wL\hat{P}_a}$$

To provide a framework for estimating Pa, we define the detection function g(x) to be the probability that an object at distance x from the line is detected, $0 \le x \le w$, and assume that g(0) = 1 (i.e., that we are certain to detect an animal on the transect line). If we plot the recorded perpendicular distances in a histogram, then conceptually the problem is to specify a suitable model for g(x) and to fit it to the perpendicular distance data. As shown in Figure 9, if we define $\mu = \int_0^w g(x)dx$, then $P_a = \mu/w$.
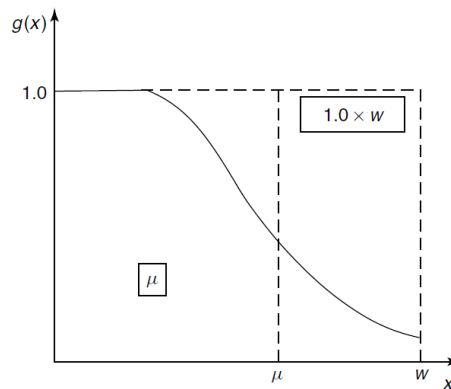
Figure 9: The area μ under the detection function g(x), when expressed as a proportion of the area w of the rectangle, is the probability that an object within the surveyed area is detected; μ is also the effective strip width, and takes a value between 0 and w. (Buckland et al. 1998)

The parameter μ is called the effective strip (half-) width; it is the distance from the line for which as many objects are detected beyond μ as are missed within μ (Figure 9). Thus,

$$\hat{D} = \frac{n}{a\hat{P}_a} = \frac{n}{2wL\hat{\mu}/w} = \frac{n}{2\hat{\mu}L}$$

We now need to estimate μ. We can turn this into a more familiar estimation problem by noting that the probability density function (pdf) of perpendicular distances to detected objects, denoted by f (x), is the detection function g(x), rescaled so that it integrates to unity. That is, f (x) = g(x)/μ. In particular, because we assume g(0) = 1, it follows that f (0) = 1/μ (Figure 10). Hence,

$$\hat{D} = \frac{n}{2\hat{\mu}L} = \frac{n\hat{f}(0)}{2L}$$

The problem is reduced to modeling the pdf of perpendicular distances, and evaluating the fitted function at x = 0. The Distance software uses the methods of Buckland et al. 2001, in which a parametric "key" function is selected and, if it fails to provide an adequate fit, polynomial or cosine series adjustments are added until the fit is judged to be satisfactory by one or more criteria.
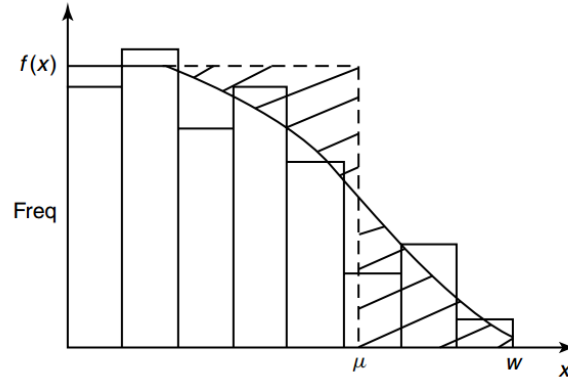
Figure 10: The pdf of perpendicular distances, f (x), plotted on a histogram of perpendicular distance frequencies (scaled so that the total area of histogram bars is unity). The area below the curve is unity by definition. Because the two shaded areas are equal in size, the area of the rectangle, μf (0), is also unity. Hence μ = 1/f (0) (Buckland et al. 1998)

Often (not in our case), the perpendicular distances are recorded by distance category, so that each exact distance need not be measured, or data are grouped into distance categories before analysis. Standard likelihood methods for multinomial data are used to fit such "grouped"(or "interval") data (Buckland et al. 2001).

Variance and Interval Estimation

The variance of $\hat{D}$ is well approximated (Buckland et al. 2001) using the delta method:

$$\widehat{var}(\hat{D}) = \hat{D}^2 \left[\frac{\widehat{var}(n)}{n^2} + \frac{\widehat{var}[\hat{f}(0)]}{[\hat{f}(0)]^2} \right]$$

The variance of n generally is estimated from the sample variance in encounter rates, n_j / l_j. Examination of a range of possible estimators of this variance (Fewster et al. 2009) showed that one based on weighting by line lengths squared performed best when lines are placed at random within the study area. This is the default in the Distance software that we used (Distance v6.2). The authors also proposed and evaluated estimators for the case of systematic random parallel line placement (a recommended design strategy); an updated estimator with even better performance has since been developed (Fewster 2011). Given that f (0) is estimated by maximum likelihood, its variance can be estimated from the information matrix. If we assume that $\hat{D}$ is lognormally distributed, approximately 95% confidence limits are given by $(\hat{D}/C, \hat{D}C)$ where

$$C = \exp\{1.96[\widehat{var}(\ln \hat{D})]^{0.5}\}$$

$$\widehat{var}(\ln \hat{D}) = \ln \left[1 + \frac{\widehat{var}(\hat{D})}{\hat{D}^2} \right]$$

Bootstrap resampling may also be used for variance and interval estimation. In this case, resamples are usually generated by sampling with replacement from the lines, so that independence between the lines is assumed, but independence between detections on the same line is not. If the model selection procedure for the detection function is applied independently to each resample, the bootstrap variance includes a component because of model selection uncertainty.

23

AIC and AIC differences, $\Delta AIC_i = AIC_i - AIC_{min}$, were computed over all candidate models. To quantify the plausibility of each model, given the data and set of 6 models, the Akaike weight w of each model was calculated, where

$$w_i = \exp(-0.5\Delta_i)\Big/\sum_j \exp(-0.5\Delta_j)$$

The Akaike weight is considered as the weight of evidence in favour of model i being the actual best model for the available set of models (Akaike 1983, Burnham & Anderson 2002). To assess absolute goodness of fit, a Pearson's chi-squared statistic was used based on the parametric bootstrap procedure described by MacKenzie & Bailey 2004. Estimates of the relative importance of predictor variables j were made by adding up the Akaike weights across all the models in the set where each variable occurred; the larger the sum of Akaike weights the more important that variable was, relative to the other variables (Burnham & Anderson 2002). So, model-averaged estimates of D (density) and P (detection probability) were calculated by the formula

$$\hat{\bar{\theta}} = \sum_i w_i \hat{\theta}_i$$

where $\hat{\bar{\theta}}$ denotes a model averaged estimate of parameter θ (Burnham & Anderson 2002).

Assumptions

The physical setting for line-transect sampling is idealized as follows:

1. N objects are distributed through an area of size A according to some stochastic process with average rate parameter D = N/A.
2. Lines, placed according to some randomized design, are surveyed and a sample of n objects is detected.

It is not necessary that the objects be randomly (i.e., Poisson) distributed. Rather, it is critical that the line be placed randomly with respect to the local distribution of objects. This ensures that objects in the surveyed strip are uniformly distributed with respect to distance from the line. Thus, if the strip has half-width w, object-to-line distances available for detection are uniformly distributed between zero and w. Random line placement also allows valid designbased extrapolation from the sample to the study area.

There are three key assumptions of the basic method.

1. Objects directly on the line are always detected, i.e., g(0) = 1. Missing objects on the line causes a corresponding underestimation of D.
2. Objects do not move. Conceptually, distance sampling is a "snapshot" method: we would like to freeze animals in position while we conduct the survey. In practice, nonresponsive movement is not problematic provided it is slow relative to the speed of the observer. Responsive movement before detection is, however, problematic (Buckland 1992, Fewster et al. 2008).
3. Distances are measured accurately. Provided distance measurements are approximately unbiased, bias tends to be small in the presence of measurement errors (Buckland et al. 2001, Marques 2007, Borchers et al. 2009 and 2010). Biased measurements, if uncorrected, are problematic.

A fourth assumption is made in many derivations of estimators and variances: whether an object is detected is independent of whether any other object is detected. Point estimates of D are robust to violations of the assumption of independence, and robust variance estimates are

obtained by taking the line to be the sampling unit, either by bootstrapping on lines, or by calculating a weighted sample variance of encounter rates by line. It is also important that the detection function has a "shoulder"; that is, the probability of detection remains at or close to one initially as distance from the line increases from zero (Figure 10). This is not an assumption, but a property that allows more reliable estimation of object density. Given the above, and assuming a suitably flexible method for estimating g(x), the point and interval estimates of D are extremely robust to variation in g(x) due to other factors such as observer, habitat, weather, and so on. This very useful property of standard distance sampling methods is known as pooling robustness (Buckland et al. 2001 and 2004), and is not shared by capture– recapture methods.

Extensions to Standard Methods

More recent research (Buckland et al. 2004) has focused on methods for increasing precision and relaxing the assumptions of the standard methods, and on advanced design issues.

Multiple Covariate Distance Sampling

Generally, probability of detection is a function of many factors other than distance of the object from the line. Other sources of heterogeneity contribute little to bias, provided g(0) = 1. Nevertheless, higher precision might be anticipated if additional covariates are recorded and their effects on g(x) modeled. One approach, is to allow covariates to affect the scale of the detection function but not its shape. In other words, we are assuming the covariates affect the rate at which detectability decreases with distance, but not the overall shape of the detection curve. Building on this work, extensions to the detection function estimation methods allow the scale parameter of the key function to be a function of covariates (Buckland et al. 2004, Marques & Buckland 2003). In the present study, depth of every station was tested as a covariate.

For the understanding and the description of Distance Sampling methodology we used the papers already mentioned, the book of Buckland et al. 1993, the User's Guide Distance 6.2 Release 1, the review of Piegorsch 2013 and the papers of Katsanevakis 2006, 2007a, 2007b 2009a, 2009b who had applied Distance Sampling in *Pinna nobilis* estimations.

RESULTS & DISCUSSION

Presence of Species
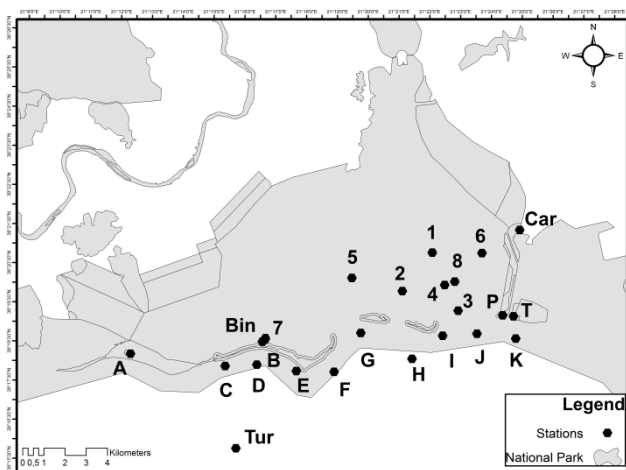
Figure 11: 24 qualitative stations examined

In the 24 stations where qualitative sampling was carried out (Figure 11) a total of 148 taxa were identified with visual, photographic and/or lab identification (Detailed list is found in ANNEX I), Of the 148 taxa, 102 were identified up to species level while 30 were identified up to genus level. The various taxonomic groups and the number of taxa that correspond to each group are shown in the graphs of Figure 12.
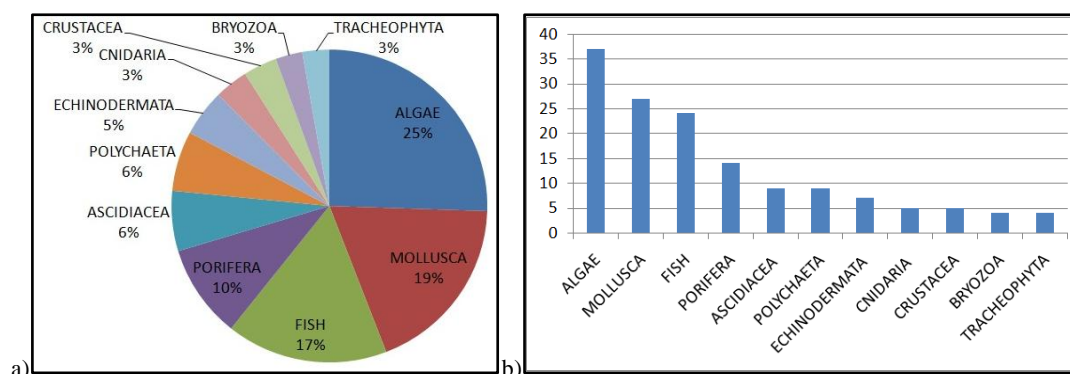
Figure 12: a) Percentage of each taxonomic group b) Number of taxa identified, per taxonomic group

Algae, with 37 taxa was the most numerous group, which is not surprising because algae form the basis of the food chain and it is an abundant polyphyletic group with too many representative species. Most of the algae are also attached to the substrate and therefore easy to be detected with the methodologies of the present study. The group of Molluscs with 27 taxa, include 15 Gastropods, 11 Bivalves and 1 Cephalopod. Numerous species of fish were also detected especially in the outer part of Messolonghi Lagoon. In the inner part of the Lagoon only 2 fish species were detected, probably because of the partial isolation of the inner with the outer part due to aquaculture purposes. Moreover there were also random detections of the dolphins *Tursiops truncatus* (Montagu, 1821) and of the marine turtle *Caretta caretta* (Linnaeus, 1758). In ANNEX I you can see the presence of each taxon in the 24 qualitative sampling stations.

Interesting records

In total, **85 detected species** of ANNEX I are recorded **for the first time in the National Park** of Messolonghi Lagoon and the Natura 2000 Marine Protected Area.

Ascidians is a very interesting taxonomic group because they are an evolutionary link between invertebrates and vertebrates. They have a primitive backbone at some stage of their life cycle, but in other aspects they resemble invertebrates. Generally, in Greece and in other Mediterranean countries, the group of colonial Ascidians is poorly studied. Among the species detected, the colonial ascidian *Aplidium coeruleum* Lahille, 1890 (Figure 13) is recorded for the first time in Greece and the Eastern Mediterranean (species confirmed by Frédéric André pers.com.)· existing records of the species come from the North-East Atlantic, the English Channel (La Manche), the Catalan coast (Banylus) and Tunisia (first record 2010 from Chebbi et al. 2010) (Bisby et al. 2005, Kott P. 1969, Monniot 1978, Peres 1958, Harant 1927, Lahille 1890, Brément 1912). The blue siphons of the species with 6 lobes are characteristic of the species (Figure 13b). Only one colony of *A. coeruleum* was recorded, in the outer part of Messolonghi Lagoon in depth of 4,2m, on the base of *Posidonia oceanica* leaves.
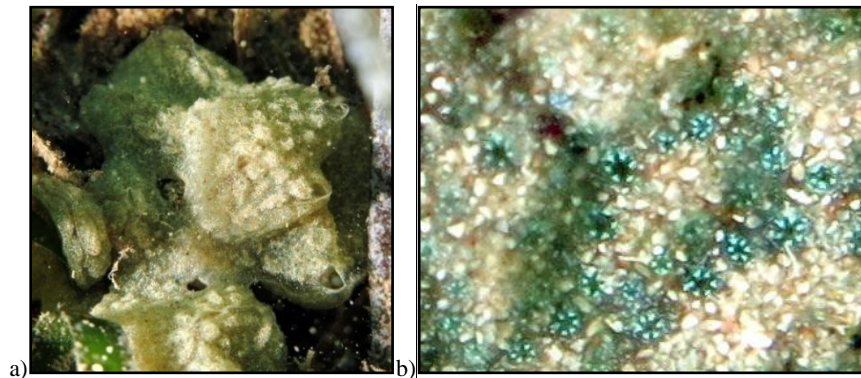
Figure 13: The colonial ascidian *Aplidium coeruleum*, first recorded in the Eastern Mediterranean a) found on the base of *Posidonia oceanica* leaves b) Zoom of the same photo, where the blue siphones, each with 6 lobes, are distinguished.

In addition, the colonial ascidian *Didemnum protectum* (Daumézon, 1908) is recorded for the first time in Greece (species confirmed by Frédéric Andre pers.com.) (Tursi 1980, Hayward & Ryland 1995). *D. protectum* was recorded only in the inner part of Messolonghi Lagoon, at 1.6m depth, surrounding leaves of *Cymodocea nodosa* (Figure 14). Its colonies had a maximum length of 15cm.

Figure 14: The colonial ascidian *Didemnum protectum* first recorded in Greece a) surrounding leaves of *Cymodocea nodosa* and b) detail of the same individual.

The colonial ascidian *Trididemnum cereum* (Giard, 1872) (Figure 15), was extremely abundant in the inner part of the Lagoon. Although the identification of colonial ascidians is quite difficult, due to the good status of the sample, the main taxonomic characteristics were obvious and the species was identified with certainty. A field photograph of *T. cereum* is shown in Figure 15a, taken in the inner part of the Lagoon. The ascidian was grown on a mat of the algae *R. tinctoria* and on leaves of the seagrass *C. nodosa*. Figure 15b,c show parts of dissected ascidians where the main morphological characteristics of *T. cereum* that led to its identification . (According to Tursi 1980, and Hayward & Ryland 1995) can be seen.

T. cereum was reported in 2 sampling stations of 100m transects, in the inner part of Messolonghi Lagoon. The number of separate colonies we counted in each station of 100m transect was for S2:158ind and S4:1ind. The observers were counting individuals in a 4m distance, 2 m on the right and 2 m on the left of the dive line, examining a total area of 400m^2 for every station. An indicative abundance estimation of the species was done in station 2, where we recorded 158 individuals along the 100m transect. So the abundance estimation for station 2 was **0.40** individuals / m^2.
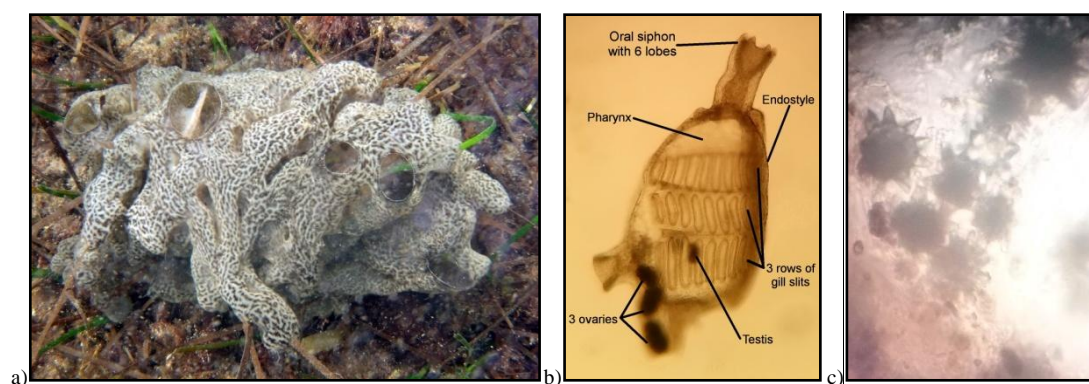
Figure 15: a) Colonial ascidian *Trididemnum cereum*. a) macroscopic view in the field b) microscopic view (10x) of a zooid with three rows of gill slits, oral siphon with 6 lobes and c) microscopic starlike granules (40x)

Another interesting taxon found is the angiosperm Ruppia sp. (Figure 16) which was recorded in three inner stations: S1, S7, SB$_{in}$. Ruppia sp. can be easily mistaken for *Cymodocea nodosa* on the field, but samples allowed us to identify the genus with certainty. Flowering parts were not present, so the species could not be identified. *Ruppia* is a cosmopolitan genus, characteristic of many coastal brackish waters and inland salt-water habitats; Two different species of *Ruppia* are recorded in the Greek lagoons: *R. cirrhosa* and R. *maritima*· They form dense submerged meadows in many lagoons, especially in Macedonia and Thrace (Nicolaidou et al. 2005).

Figure 16: The angiosperm Ruppia sp. in the inner part of Messolonghi Lagoon

The Mediterranean Sea is a marine biodiversity hot spot and hosts high concentrations of endangered, threatened, or vulnerable species (Coll et al. 2010). Among the 148 taxa detected in the wider area of Messolonghi Lagoon, 17 species are protected by various conventions, 8 are endemic in the Mediterranean Sea and 6 are characterized as alien species.

Protected species

A total of **17** protected species were recorded and are listed in

Table 3 with the conventions by which they are protected.

PROTECTED SPECIES	Habitat Directive (92/43/EEC)	Presidential Decree 67/1981	Presidential Decree 109/2002	Presidential Decree 227/2003	Bern Convention	Barcelona Convention	Greek Red Book	CITES
Callista chione (Linnaeus, 1758)								
Cladocora caespitosa (Linnaeus, 1767)			+		+	+		+
Caretta caretta (Linnaeus, 1758)	+				+	+		+
Cymodocea nodosa (Ucria) Ascherson					+	+		+
Cystoseira barbata (Stackhouse) C.Agardh					+	+		
Cystoseira foeniculacea (Linnaeus) Greville					+	+		
Hippocampus hippocampus (Linnaeus, 1758)					+	+		+
Holothuria sp.			+		+	+		
Maja squinado (Herbst, 1788)			+		+	+		+
Palaemon serratus (Pennant, 1777)			+		+	+	+	
Paracentrotus lividus (Lamarck, 1816)	+	+			+	+	+	
Pinna nobilis Linnaeus, 1758 (Πίνα)	+	+			+	+	+	
Posidonia oceanica (Linnaeus) Delile					+	+	+	
Sarcotragus foetidus Schmidt, 1862					+	+		
Scyllaridae	+				+	+		+
Tursiops truncatus (Montagu, 1821)	+			+	+	+		+
Venus verrucosa Linnaeus, 1758			+					

Table 3: Protected species, recorded in the Marine Protected Area of Messolonghi Lagoon and the national or/and international conventions that establish their protection.

Some of the protected species showed extended coverage. For example, meadows of *Posidonia oceanica* covered all the outer part of Messolonghi Lagoon (also around stations C-J) while *Cymodocea nodosa* had a wide expansion mostly in the inner part of the Lagoon. The algae *Cystoseira barbata* and *Cystoseira foeniculacea* were present in all the inner stations, with more than 10 independent thallus, counted along the 100m of station 4. Usually they were attached to the substrate, among the mat of algae and *Cymodocea nodosa* roots (Figure 17a). The form of *Cystoseira barbata* that lives floating, without being attached to the substrate was also recorded. The protected Holothuria sp. was quite abundant (50 individuals/100m transect) in station 3 which is in the border between the inner and the outer part. Finally, a quite dense population of the highly protected bivalve *Pinna nobilis* was recorded and will be discussed further in the following section.
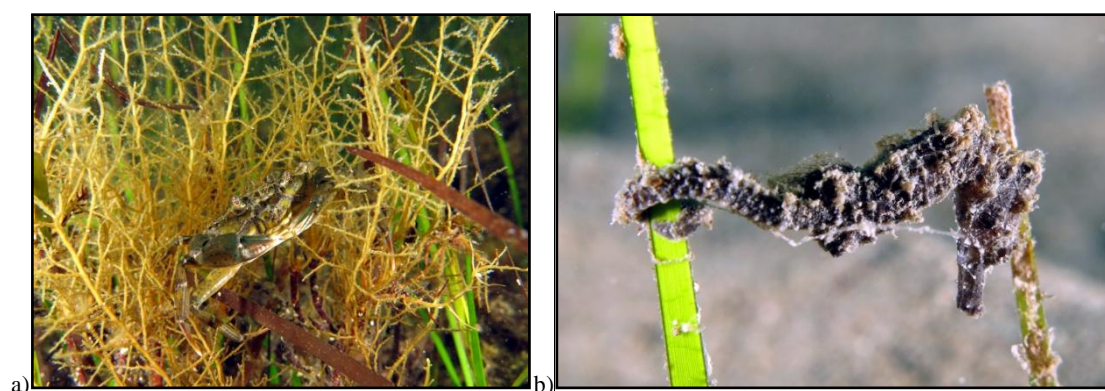
Figure 17: a) The protected algae *Cystoseira barbata* around the crab *Carcinus aestuarii*, found in the inner part of the Lagoon b) The protected fish *Hippocampus hippocampus* recorded in the shallow station E, in the outer part of the Lagoon wrapping its tail around a *Cymodocea nodosa* leaf.

Endemic species of the Mediterranean Sea

Mediterranean Sea, due to its geological history, hosts a high number of endemic species, 79 of which are fish (Coll et al. 2010). In our study we recorded the presence of 8 endemic species of the Mediterranean Sea (endemism checked from fishbase.org, marinespecies.org) that are listed in Table 4. In detail, we recorded 5 endemic invertebrates, 1 seagrass and 2 fish of the genus Symphodus. The fish were detected in the outer part of Messolonghi Lagoon and the other invertebrates mostly in the inner part. *Pinna nobilis* was present everywhere but with higher densities in the border between the inner and the outer part. of *P. oceanica*, as noted before, expands in the outer part of the Lagoon.

Table 4: Endemic species of the Mediterranean Sea recorded

ENDEMIC SPECIES OF MED.	
Astropecten cf *spinulosus* (Philippi, 1837)	*Posidonia oceanica* (Linnaeus) Delile
Carcinus aestuarii Nardo, 1847	*Reteporella mediterranea* Hass, 1948
Paranemonia cinerea (Contarini, 1844)	*Symphodus melanocercus* (Risso, 1810)
Pinna nobilis Linnaeus, 1758	*Symphodus doderleini* Jordan, 1890

Alien Species

In the wider study area 6 alien species listed in Table 5 were recorded (origin checked from elnais.hcmr.gr, marinespecies.org), A quite wide coverage of the invasive alien alga *Caulerpa cylindracea* was recorded in shallow artificial rocks at 20 cm depth (Figure 18a), in station T in Tourlida. The seagrass *Halophila stipulacea* was present in the outer part of Messolonghi Lagoon, in the station K in front of Tourlida. This established and invasive seagrass has become widespread in Greece. The colonial ascidian *Botryllus schlosseri*, which was present in the inner part of the Lagoon, is still in question whether it should be considered as an alien species or not since it is not included in the official Ellenic Network on Aquatic Invasive Species (Elnais). The invasive, and widespread in Greece, lessepsian fish *Siganus luridus*, was present in two stations of the Outer part of the Lagoon. *S. luridus* can provoke serious grazing problems in rocky infralittoral ecosystems (Sala et al. 2011). The alien bryozoan *Amathia verticillata* (ex *Zoobotryon verticillatum*), which is a fouling organism was present in the Inner part of the Lagoon.

Table 5: Alien species recorded

ALIEN SPECIES	
Caulerpa cylindracea Sonder	*Botryllus schlosseri* (Pallas, 1766) * not in ELNAIS
Halophila stipulacea (Forsskål) Ascherson	*Siganus luridus* (Rüppell, 1829)
Melibe viridis (Kelaart, 1858)	*Amathia verticillata* (delle Chiaje, 1822)
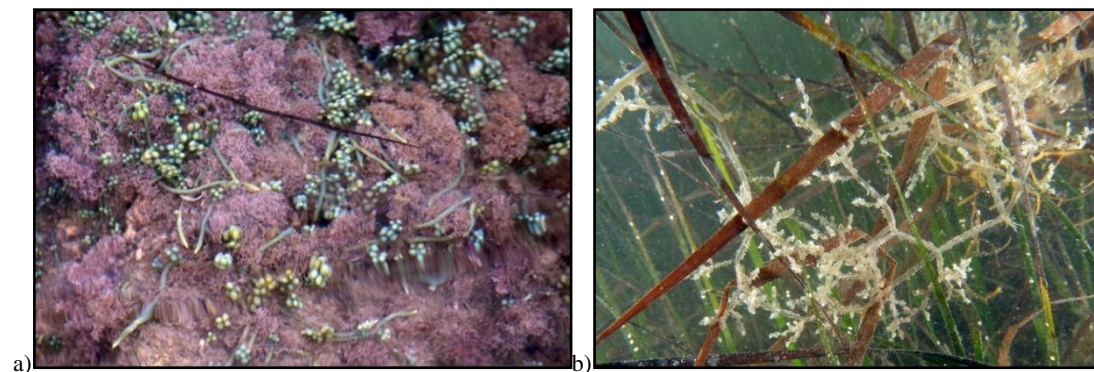
Figure 18: a) Dense thallus of the alien algae *Caulerpa cylindracea* in shallow artificial substrate in Tourlida and b) the alien bryozan *Amathia verticillata* found in the inner part of messolonghi Lagoon

Another interesting record is that of the alien nudibranch *Melibe viridis* (ex. *Melibe fimbriata*) (Figure 19). In the Inner part of Messolonghi Lagoon an extremely high abundance of the species, with a maximum of 171 counted individuals per 100m of transect was recorded. *M. viridis* was present in all the inner stations of the Lagoon with the mentioned higher abundance in station 2. Also, 5 individuals were collected for dissection purposes in the laboratory and stomachic content analysis. A probable newborn individual of the species (Figure 21b) was found in the outer station H, which is very close to the inner station 2 with the densest population of *M. viridis*. However, it is not certain whether this individual was alive or transferred there by currents. The morphological characteristics of the species can be seen in Figure 19a-h.
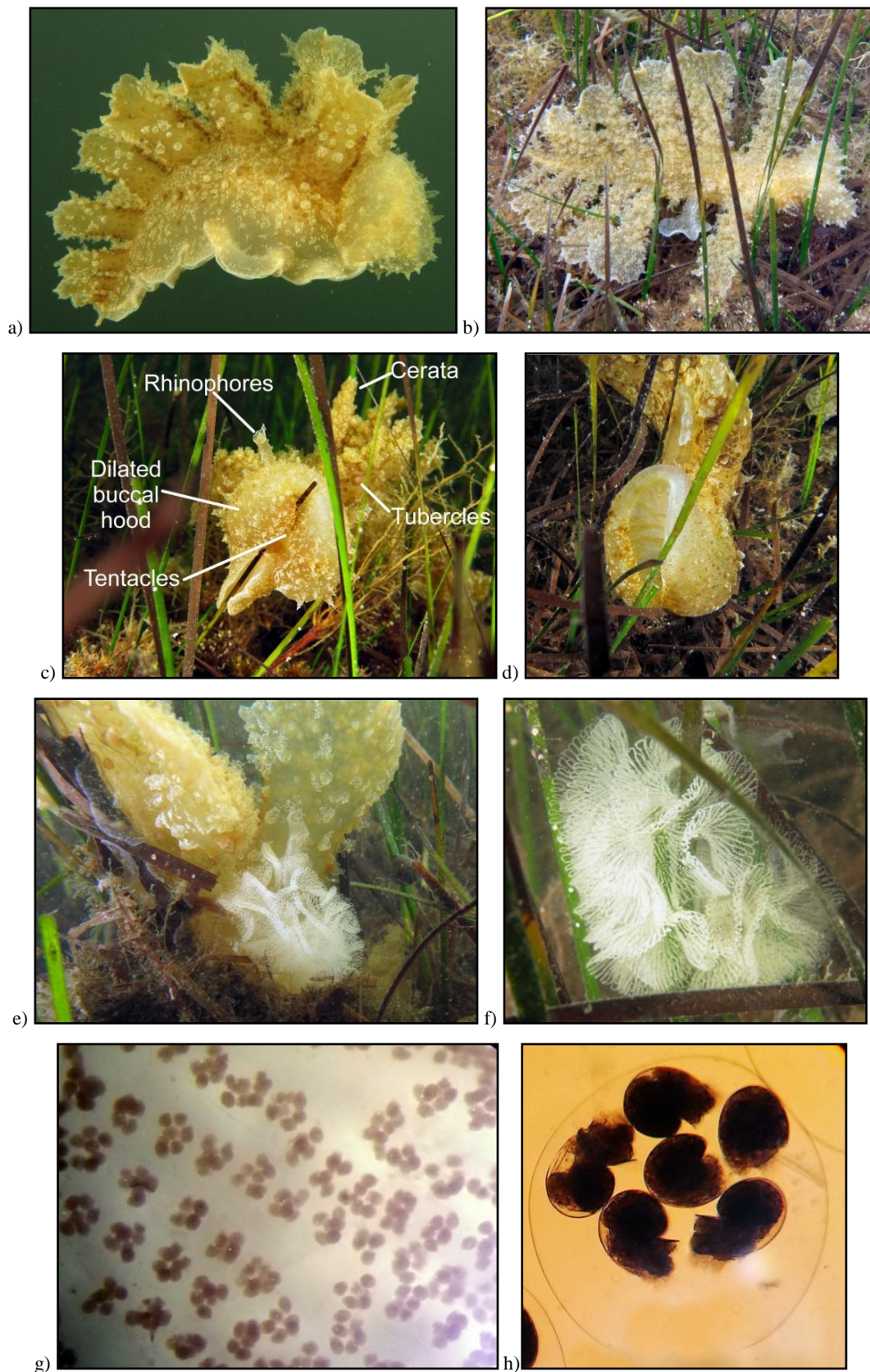
Figure 19: the alien nudibranch *Melibe viridis*, extremely abundant in the inner part of Messolonghi Lagoon a) while swimming with body contraction, b) regeneration of a ceras c) eating macroinvertebrates from a *Cymodocea nodosa* leaf with its main morphological characteristics according to Thompson & Crampton, 1984 d) its elongated trunk and the buccal hood, e) Spawning of an individual f) Jelly-ribbon spawn g) Microscopic view of the spiralling strings of egg capsules h) Microscopic view of one egg capsule with 6 embryos

Body characteristics: (Figure 19a-c) The maximum body size that was measured in the field was 29cm. The body is flattened and consists of maximum 10 pairs of flattened cerata (foot-like formations). Dilated and rounded oral hood around its mouth, fringed with retentive tentacles. Rhinophores were emerging from tall sheaths, into which they can be retracted. Each ceras was oar-like shaped and could easily be autotomized and regenerated. The surface of the body and cerata had tubercles and ramified appendages (gills). Yellow-brown colour. When brought out of the water, individuals gave off a charateristic smell like watermelon.

Substrate: Individuals of *M. viridis* were usually found on sandy/muddy bottoms among *Cymodocea nodosa* leaves on a mat of dead leaves and live algae *Rytiphlaea tinctoria* or *Cladophora* sp (Figure 19b-e).

Feeding behavior: Many individuals were seen feeding on macroinvertebrates from *Cymodocea nodosa* leaves (Figure 19c). Precisely, they extended their buccal hood, surrounding the leaves and detaching small invertebrates like small gastropods that were also found in the stomachic content of the 5 individuals that were sampled.

Behavior: Autotomy of some cerata was observed when some individuals were collected for laboratory analysis. In different individuals, many of the cerata showed signs of regeneration (Figure 19b). Individuals that were seen swimming in the whole water column, had their buccal hood retracted and were in an upside-down position (Figure 19a). With side contractions of their body, the individuals were able to swim with a characteristic movement. Members of the family Tethyidae, like *M. viridis* can undertake swimming excursions, either to elude enemies (like us taking pictures) or to bring about aggregation for feeding or mating or oviposition (Thompson & Crampton, 1984). Due to the high abundance of the individuals, the spawn masses detected and a number of individuals that were photographed during spawning (Figure 19e), it is believed that this was a **spawning aggregation of *M. viridis***.

Egg morphology: The spawn formed a delicate colorless jelly-ribbon (Figure 19e,f) and contained spiralling strings of egg capsules (Figure 19g). Each egg capsule contained usually 6 embryos (Figure 19h) unlike the individuals that Thompson & Crampton (1984) described that contained up to 3 embryos.

Distribution: Worldwide: Indo-Pacific; recorded from South Africa, East Africa, Australia, Philippines and Japan. Mediterranean: recorded first in 1970 in Cephalonia Island, Greece (Mooseleitner, 1986); successive records from Korinthiakos Gulf, Greece (Moosleitner, 1986); Strait of Messina (Mojetta, 1998); Croatia, middle Adriatic (Despalatovic et al., 2002); Montenegro (Jančić 2004); south Italy (Mastrototaro et al. 2004); Tunisia (Antit et al.2011) and Cyprus (Tsiakkiros & Zenetos 2011).

Melibe viridis originates from the Indian and Pacific oceans. It should be noted that neither in its origin countries nor in the other Mediterranean countries that has invaded, such high abundance and large body sizes are recorded (Table 6).

Figure 20: Distribution of M. viridis in Greece (http://elnais.hcmr.gr/)

M. viridis was present in all the 4 sampling stations of 100m transects, in the inner part of Messolonghi Lagoon. The number of individuals counted in each station of 100m transect was S1:7ind, S2:171 ind, S4:62ind, S5:24ind. The transects 2 and 4 were studied with the same methodology (strip transects while diving) while stations 1 and P were examined with boat-transects. Since the detectability of the observers in the two methods may differ only the first two stations are used for the average abundance estimation of *M. viridis*. Along the diving transects of 100m the observers were counting individuals in a 4m vertical distance, 2 m on the right and 2 on the left of the dive line (total area of 400m^2 for every station). The average abundance of *M. viridis* from stations 2 and 4 was **0,29 individuals/m^2**. In Table 6 there is a comparison between some previous studies of *M. viridis* and the present study. According to the records of Table 6, *M. viridis* must be a eurythermal (13.6-25°C) species. It can be found in a range of depths from 0.9m up to 30m. The spawning of the alien species in the Mediterranean occurs from September to December. In the present study the densest population, the largest individuals, the lowest temperature and the smallest depth of occurance of the species were recorded. The species is shown to prefer shallow sandy, sandy-muddy or muddy bottoms with seagrasses meadows like *Cymodocea nodosa, Posidonia oceanica, Zostera* (Thompson & Crampton 1983, Koutsoubas & Cinelli 1997, Despalatovic et al. 2002, Present study).

Table 6: Comparison of studies of *M. viridis*. In the present study we recorded the densest population and the largest individuals in the lowest temperature.

Place	Date of record	Temperature (°C)	Depth	Length (cm)	ind/m^2	Area examined (m^2)	Spawning	Egg ribbons/m^2	Reference
Greece, Astakos	September 1982	25	-	7-14	-	-	+	-	Thompson & Crampton 1983
Croatia	October 2001	18	2-15	10-15	0,01	-	+	-	Despalatovic et al. 2002
Italy, Porto Cesareo	December 1999	14.5	30	-	-	-	+	-	Mastrototaro et al. 2004
Italy, Taranto basin	October 2003	23.3	10	6-14,3	0,03	200	+	-	Mastrototaro et al. 2004
Italy, Taranto, Mar Piccolo	June - July 2004	-	4-13	-	0,13	520	-	-	Carriglio et al. 2004
India, Gulf of Kachchh	March 2010	-	-	8-12	0,16	300	+	0,07	Parasharya & Patel 2014
Cyprus, Limassol	August 2002	27	6	-	-	-	-	-	Tsiakkiros & Zenetos 2011
Greece, Messolonghi Lagoon	November 2014	13.6-15	0.9-1.6	1,5-29	0,29	800	+	0,06	Present study

Figure 21: a) Swimming individual of *M. viridis* of about 20cm length. In the background an indicative view of the abundance of the individuals in station 2 b) A probable newborn individual of *M. viridis*, of 1.5cm length found in the outer station H.

Species inreractions

Individuals of the crab *Carcinus aestuarii* were recorded in the inner part of the Lagoon, having attached the algae Ulva sp. probably for camouflage (Figure 22).
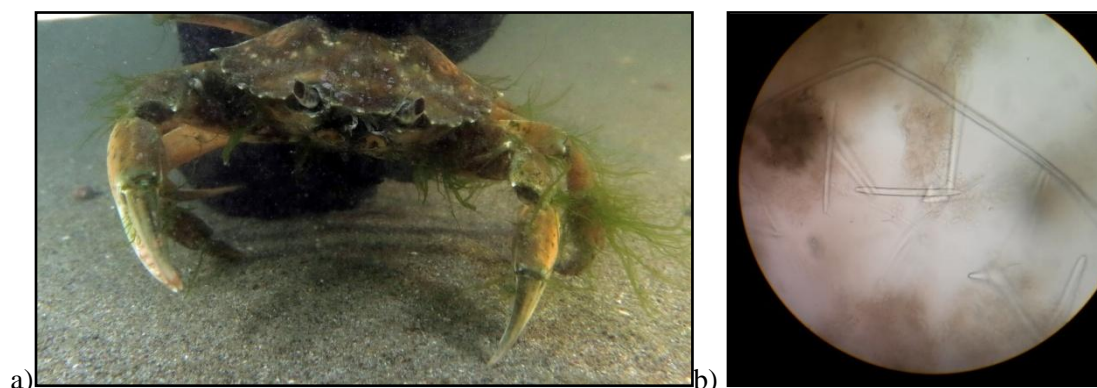
Figure 22: a) The crab *Carcinus aestuarii* with attached Ulva sp. algae on its body b) Microscopic view of the spicules of the sponge *Haliclona (Reniera) mediterranea* that facilitated the species identification.

The pink sponge *Haliclona (Reniera) mediterranea* (Figure 22b) was recorded in the inner stations 2 and 4, hosting abundant polychaetes of the species *Haplosyllis spongicola* (Figure 23). Many individuals of the polychaete were found among the skeleton of the sponge. *H. spongicola* is commonly found associated with different sponge species, but according to (Martin & Britayev 1998, Lattig & Martin 2009) it is more likely a specialized predator than a true sponge symbiont.
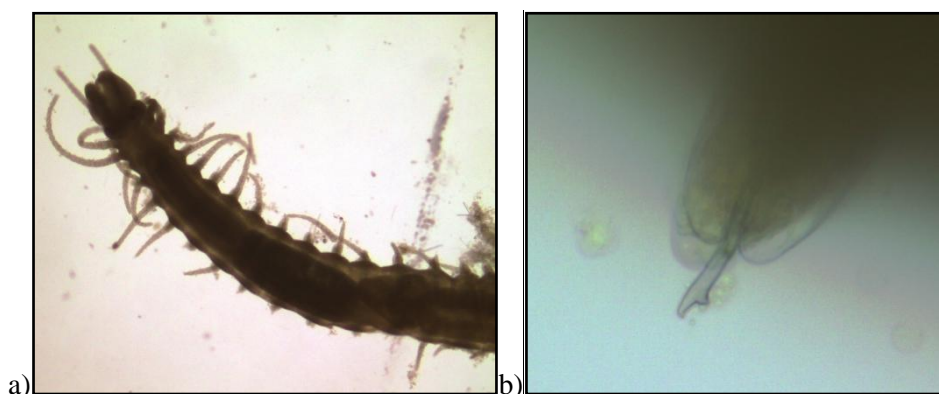
Figure 23: a) Microscopic view of the polychaete *Haplosyllis spongicola* and b) detail of its setae which led to the identification of the species.

Generally, many species observed in the field, were in close relationship with other species. Another example is the protected bivalve *Pinna nobilis* which hosted a great variety of sponges, ascidians, crustaceans and algae that used its surface as a hard substrate. Other characteristic examples are many species that lived among rhizomes of the seagrass *Posidonia oceanica*: many sponges, bryozoans, ascidians, encrusting red algae.

The fish *Symphodus melanocercus* was seen cleaning individuals of the fish *Coris julis* and *Serranus scriba*. In the Mediterranean Sea, *S. melanocercus* is considered the most conspicuous cleaner fish, with the females being more specialized than males (Arnal & Morand 2001).

Quantitative data in transects of 100m
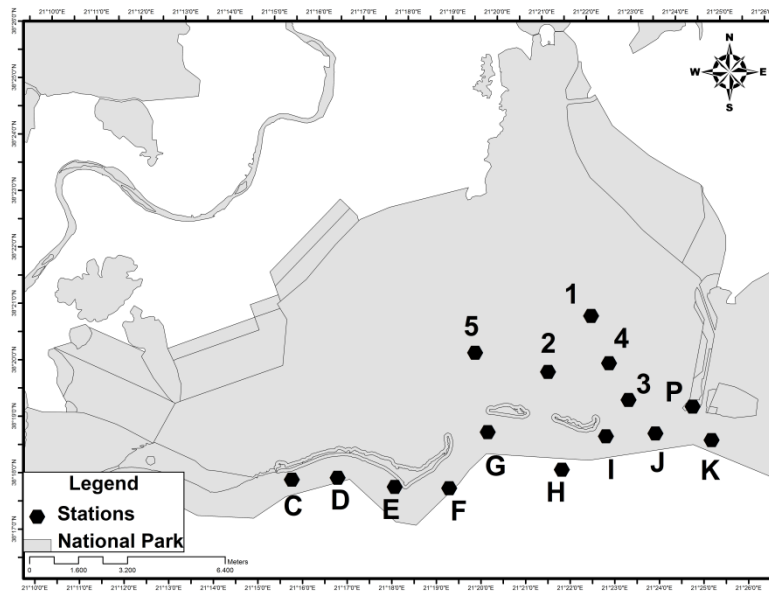
Figure 24: 15 quantitative sampling stations, examined through 100m transects. The stations 3 and P are located on the border of the Inner and the Outer part of the Lagoon.

Table 7: Charateristics of the 15 quantitative sampling stations. The habitats represent P: *Posidonia oceanica*, **S: Sand, M: Mud, CN:** *Cymodocea nodosa*, **CP:** *Caulerpa prolifera*, **RT:** *Rytiphlaea tinctoria*, **CL:** *Cladophora* **sp.**

Station	Habitat	Mean T(°C)	Mean Depth (m)	Distance from entrance (m)	NOTES
C	P	20,2	6,7	11116	
D	P	20,2	11,9	9669	
E	S, P	20,7	4,2	7935	
F	P	19,6	8,6	6244	
G	P	19,3	7,1	4564	bad visibility
H	P	20,8	12,4	2796	
I	P	17,3	6,2	1303	
J	P	20,6	9,2	1301	
K	CN,M	19,7	10,4	3041	
P	P,S,CN,CP		1,6	2228	from boat
1	CN, RT		0,7	3189	from boat
2	CN,RT	13,6	1,6	2746	
3	P,CN	16	1,4	323	
4	CN,CL	15	0,9	1555	
5	CN,RT		1,4	5167	from boat
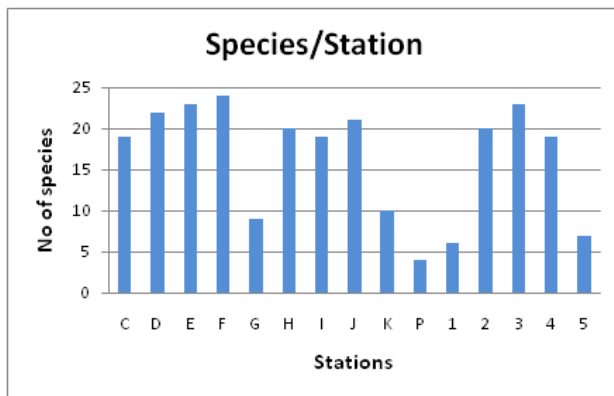
Figure 25: Number of species detected in every sampling station (100m transect)

On the whole, the **presence/absence data of 91 taxa**, was obtained from the 15 sampling stations. As shown in Figure 25, the number of species detected in every sampling station, does not seem to be affected by the position of the station in the inner or the outer part of Messolonghi Lagoon. Stations 1,2,4,5 are considered to be in the inner part of the Lagoon (Figure 24). In the sampling stations P, 1 and 5 a lower number of species were detected, because of the sampling methodology (visual survey from the boat), which does not allow the detection of very small or cryptic species. Additionally, during the survey of the outer station G the visibility was pretty bad, more than in every other station. Also, the outer station K was not similar with the other stations. This station is located in a spot, where underwater excavations took place in the past and its fauna differs from the other outer stations.

According to Guelorget & Perthuisot (1992), in a Lagoonal system the number of species decreases from the outer to the inner part. This does not seem to be happening in Messolonghi Lagoon due to the extensive communication of the Lagoon with the sea. Also, the study area of this study didn't include the most inner part of Messolonghi Lagoon.

Population density of species
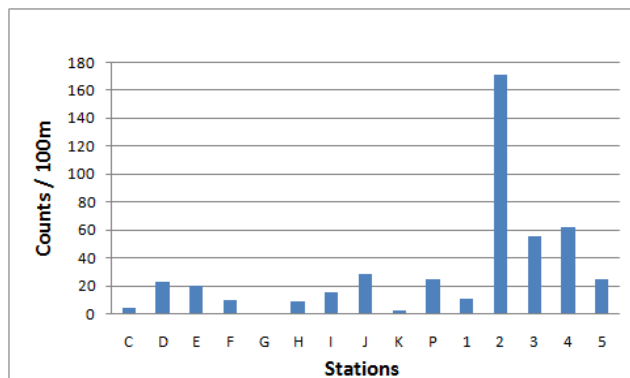
Figure 26: Counts of the most abundant species, in each sampling station of 100m

Although the number of species didn't seem different among the inner and outer stations of Messolonghi Lagoon (Figure 25), there's a difference in the maximum number of the most abundant species. In Figure 26 you can see the number of individuals of the most abundant species of each sampling station. The inner stations 2, 3, 4 seem to host species with higher number of individuals, probably due to their position in the inner part of the Lagoon. As stated before the data obtained from stations 1,5, P cannot be compared because they were collected by a different sampling technique (from the boat).

The counts/100m of megabenthic species appears to follow the same pattern with macrobentic species's abundance from previous research. As described by Προβιδάκης 2013, macrobenthic species were extremely abundant in the inner part of Messolonghi lagoon.
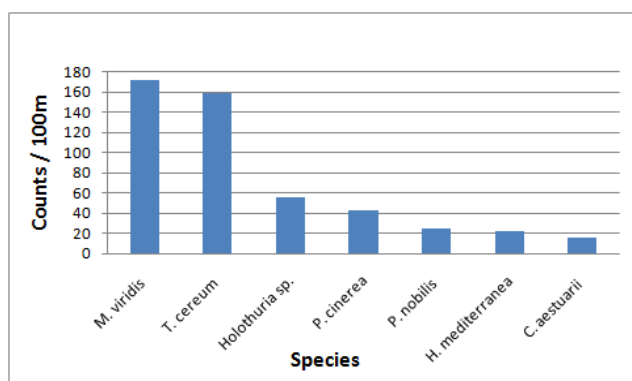
Figure 27: Counts per 100m of the 7 most abundant species

In Figure 27 you can see the 7 most abundant species we recorded, and the maximum number of individuals we counted in 100m. It must be noted that all of the 7 most abundant species were recorded in stations of the inner part of Messolonghi Lagoon, apart from *Pinna nobilis* in station P which is in the border between the inner and the outer part. The most abundant species recorded was the alien nudibranch *Melibe viridis* from which we counted 171 individuals in the 100m transect of station 2. Also in station 2 we counted a great number of colonies of the colonial ascidian *Trididemnum cereum* with 158 counted colonies and the anemone *Paranemonia cinerea* with 43 counted individuals.

In Table 8 you can see the estimated density of 7 abundant species. The density was estimated in relation to the surface examined in each transect. For example, the anemone *P. cinerea* was reported in all the 4 sampling stations of 100m transects, in the inner part of Messolonghi Lagoon. The number of individuals we counted in each station of 100m transect was S1:10ind, S2:43ind, S4:17ind, S5:5ind. The observers were counting individuals in a 2m distance (for this species **width=2**), 1 m on the right and 1 m on the left of the dive line ($200m^2$ for each transect). The transects 2 and 4 were done with the same methodology (strip transects while diving) so only these 2 station are used for the average density estimation of the species. Conclusively, the average density of *P.cinera* in the two inner stations was **0,15 individuals/m^2**. Other density estimations of *P.cinera* come from Kune lagoon in Albania, reporting five individuals 0.04 individuals/m^2 (Boncagni et al. 2009), whereas the species' average density has been estimated from nine Adriatic lagoons and three Tyrrhenian ones as 0.03 and 0.72 respectively (Munari & Mistri 2008). Conclusively the density of the species is within the already recorded Mediterranean density range.

Table 8: Density estimations of 7 abundant species in relation to the characteristics of the area surveyed for their detection in, out or in the border zone of Messolonghi lagoon.

Species	Density estimation individuals/m^2	Area surveyed (m^2)	No of transects	Width of transects	Place of station
Melibe viridis	0.291	800	2	4	In
Trididemnum cereum	0.395	400	1	4	In
Paranemonia cinerea	0,150	400	2	2	In
Holothuria sp	0.138	400	2	2	Border
Pinna nobilis	0.004	12000	15	8	In/Out/ Border
Carcinus aestuarii	0.065	400	2	2	In
Calpensia nobilis	0.018	2400	12	2	Out

Grouping of stations
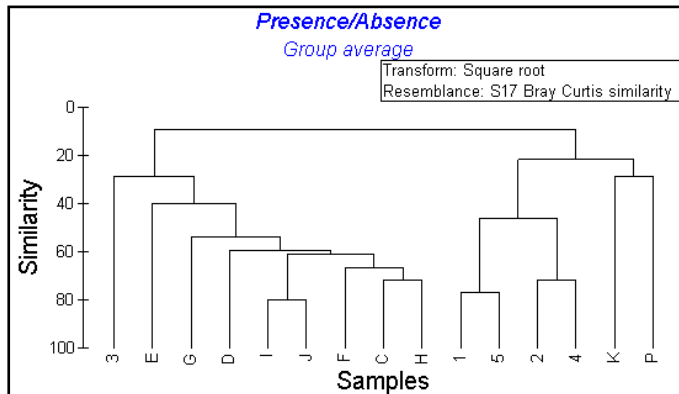
Figure 28: Cluster analysis of the 15 quantitative sampling stations based on the Bray – Curtis similarity

The program Primer PRIMER-E v6 (Clarke and Gorley, 2006) was used in order to group the stations, based on the data of presence/ absence and the abundance of species in every sampling station. The resemblance of the stations was estimated with the Bray – Curtis index (Bray and Curtis, 1957), after a transformation of abundance data with square root (Figure 28). You can see in detail the habitat and the characteristics of each station in Table 7.

According to the MDS analysis (Figure 29), the outer Stations show high percentage of similarity, have *Posidonia oceanica* as a dominant habitat and hosted many common benthic species like fishes, red algae, sponges and Bryozoans. The Inner stations of the Lagoon show also some similarity, have *Cymodocea nodosa* as a dominant habitat and are generally more shallow stations with abundant colonial ascidians, nudibranchs and anemones. Stations 3 and P do not have a high percentage of similarity with the other stations. They are in the border of the Inner and the Outer part of the Lagoon and have *Posidonia oceanica* and *Cymodocea nodosa* in successive segments. Station P had the higher abundance of the bivalves *Pinna nobilis* and station 3 many *Pinna nobilis* individuals and high abundance of Holothurians. The outer station K, have sparse *C. nodosa* and wasn't similar with the other stations. This station is located in a spot, where underwater excavations took place in the past and its fauna differs from the other outer stations. Conclusively, the presence and absence of taxa in every station, seems to be influenced by the position of every station in the Inner or in the Outer part of Messolonghi Lagoon.
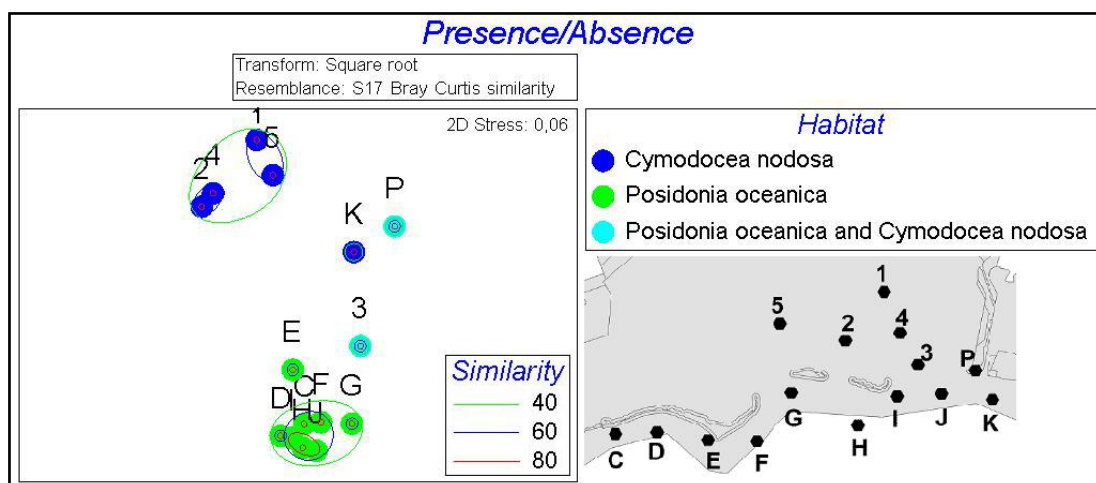
Figure 29: MDS analysis which clusters the stations based on the presence and absence data of 91 taxa in every quantitative station. The countours show the similarity between stations and different colors show the dominant habitat of every station.

As shown in the graphs of Figure 30, the outer stations are deeper than the inner ones. The Distance of every station from the main entrance point does not seem to affect the grouping of the stations. That's because Messolonghi Lagoon is a quite open Lagoon and have many communication points with the sea. The main communication area was considered the one close to the station 3.
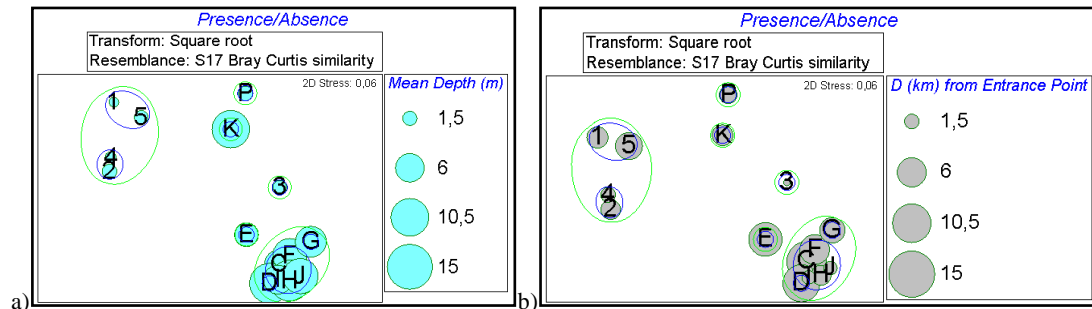
Figure 30: a) Depths of every sampling station and b) Distance from the communication point of Messolonghi Lagoon with the sea

Totally, the abundance data of **55** taxa were used for the abundance analysis. In station G, examined with the worst conditions of visibility, no abundance of taxa had been found, so it was excluded from the abundance analysis.

A comparison between an analysis with presence/absence data and another one with abundance data was done (Figure 31). The collection of presence/ absence data was easier and we collected data from 91 taxa, while we managed to collect abundance data for 55 taxa. Higher percentages of similarity are shown among stations, when using the presence/absence data. Generally presence/absence data can be collected easier and more accurately with this methodology and are ideal for studies of presence and expansion of megabenthic species.
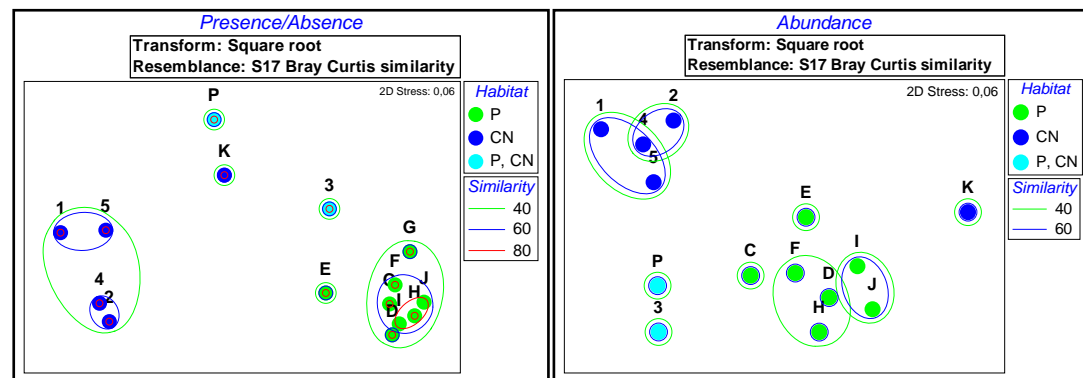
Figure 31: Comparison between grouping of stations with a) presence/absence data of 91 taxa and b) abundance data of 55 taxa

Occupancy Estimation

In the following section, the **occupancy** estimations of 13 important species in the study area are presented. The software PRESENCE v10.0 that was used (MacKenzie et al. 2002, 2006) enables the estimation of the proportion of area occupied, or similarly the probability a site is occupied, by a species of interest. The data for these species came from the 15 sampling stations of 100m transects.

the sampling size was too low to allow the inclusion in the models of many covariates (Table 7); that's why only depth was finally used.

It should be also recalled that the depth of each stations is somehow a reflection of its position in the study area, since the inner stations and the 2 in the border of the Messolonghi Lagoon are really shallow, while the outer stations are deeper.

Four models were fit to the data for each species with the following hypothesis:

Model	Hypothesis
psi(D),p(obs)	The presence probability of the species is **D**epth depended and the 2 **obs**ervers have different detection probabilities (p_1, p_2)of the species
psi(.),p(obs)	The presence probability of the species is constant (.) in the study area and the 2 **obs**ervers have different detection probabilities (p_1, p_2)of the species
psi(D),p(.)	The presence probability of the species is **D**epth depended and the detection probability of the 2 **obs**ervers is constant (.)
psi(.),p(.)	The presence probability of the species is constant (.) in the study area and the detection probability of the 2 **obs**ervers is constant (.)

The best model, with the higher AICwgt is listed first, next is listed the second and so on. Every model estimates a mean presence probability of the species (mean psi) and the detection probability of the observers (p). In the last column of the following tables, the model averaged value of occupancy is provided, estimated as a weighted average of the estimates of the 4 models, using Akaike weights . The models have a good fit, if the x^2 test of the most complex model gives a P-value which is higher than 0.05. If Pvalue is lower than 0.05 we should check the index C-hat. If C-hat is close to 1 the model describes adequately our data.

Pinna nobilis

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(obs)	31.49	0.00	0.8539	1.0000	4	23.49	0.6108	
psi(.),p(obs)	35.73	4.24	0.1025	0.1200	3	29.73	0.5333	0.6059
psi(D),p(.)	37.65	6.16	0.0392	0.0460	3	31.65	0.6651	
psi(.),p(.)	42.04	10.55	0.0044	0.0051	2	38.04	0.8333	

Best &most complex model	Detection probability		χ2 test, P-value	C-hat
	p_1	p_2		
psi(D),p(obs)	0.8732	0.2183	0.2673	0.8168

According to the best model psi(D),p(obs) with the maximum AIC wgt, the presence probability psi of the bivalve *Pinna nobilis* is depth – dependent. Precisely, **as depth increases, psi decreases**. The shallowest inner station 4 of 0.9m has psi estimate 1.0000 and the deepest outer station H of 12,4m has psi estimate 0.0034. According to the best model, the detection probability of the first observer (0.87) is higher than the second one (0.22) because the first observer was also doing the distance sampling methodology and she was more focused in *Pinna nobilis* than the second observer. Furthermore in the outer part of the Lagoon, individuals were inside *Posidonia oceanica* meadows, and difficult to be detected. The models have a good fit to the data according to the x^2 test. The model averaged psi shows that *P. nobilis* has presence probability in 100-m transects of 0.61 which means that the species estimated occupancy is 61%.

Melibe viridis

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike	Mean psi	model-averaged psi
psi(D),p(.)	11.92	0.00	0.7247	1.0000	3	5.92	0.2667	
psi(D),p(obs)	13.92	2.00	0.2666	0.3679	4	5.92	0.2667	
psi(.),p(.)	21.40	9.48	0.0063	0.0087	2	17.40	0.2667	0.2667
psi(.),p(obs)	23.40	11.48	0.0023	0.0032	3	17.40	0.2667	

Best model	Detection probability p
psi(D),p(.)	1.0000

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.5941	1.0282

According to the best model psi(D),p(.) with the maximum AIC wgt, the presence probability psi of the megabenthic nudibranch *Melibe viridis* is depth – dependent. Precisely, **as depth increases, psi decreases**. *M. viridis* was only recorded in the Inner shallow stations, with an extremely high abundance. It seems that the alien invasive species have found an ideal niche to occupy in the inner part of the Lagoon. According to the best model the detection probability of the species was the same for the 2 observers and equal to 1, since the species was really abundant and easy to detect. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.27. Therefore, more studies must be conducted to monitor a possible expansion of the alien, abundant species in the outer part of Messolonghi Lagoon.

Posidonia oceanica

Model	AIC	Deltaic	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	11.92	0.00	0.7247	1.0000	3	5.92	0.7333	
psi(D),p(obs)	13.92	2.00	0.2666	0.3679	4	5.92	0.7333	0.7333
psi(.),p(.)	21.40	9.48	0.0063	0.0087	2	17.40	0.7333	
psi(.),p(obs)	23.40	11.48	0.0023	0.0032	3	17.40	0.7333	

Best model	Detection probability p
psi(D),p(.)	1.0000

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.6733	0.9983

According to the best model psi(D),p(.) with the maximum AIC wgt, the presence probability psi of the seagrass *Posidonia oceanica* is depth – dependent. Precisely, **as depth increases, psi increases**. According to the best model the detection probability of the species was the same for the 2 observers and equal to 1, since the species was really easy to detect and constitutes a habitat-type. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is high and equal to 0.73, since it was present in all the outer stations of Messolonghi Lagoon and in the border zone. *P. oceanica* meadows is a typical habitat type of the Outer part of Mediterranean Lagoons (Guelorget & Perthuisot 1992). Since it is also a protected habitat type, a more detailed habitat mapping must be done for the wider area in order to facilitate the species management and protection.

Cymodocea nodosa

Model	AIC	DeltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	16.56	0.00	0.7190	1.0000	3	10.56	0.5333	
psi(D),p(obs)	18.56	2.00	0.2645	0.3679	4	10.56	0.5333	0.5333
psi(.),p(.)	24.73	8.17	0.0121	0.0168	2	20.73	0.5333	
psi(.),p(obs)	26.73	10.17	0.0044	0.0062	3	20.73	0.5333	

Best model	Detection probability p
psi(D),p(.)	1.0000

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.3861	1.0178

According to the best model psi(D),p(.) the presence probability psi of the seagrass *Cymodocea nodosa* is depth – dependent. Precisely, as depth increases psi decreases. According to the best model the detection probability of the species was the same for the 2 observers and equal to 1, since the species was easy to detect and constitutes a habitat-type. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.53 and it was present in all

the inner stations of Messolonghi Lagoon and in the border zone. *C. nodosa* meadows is a typical habitat type of the inner part of Mediterranean Lagoons (Guelorget & Perthuisot 1992). Since it is also a protected habitat type, a more detailed habitat mapping must be done for the inner area in order to facilitate the species management and protection.

Carcinus aestuarii, Paranemonia cinerea

We had exactly the same presence/absence data and the same outcome for the anemone *Paranemonia cinerea*, which was also present exclusively in the inner part of Messolonghi Lagoon.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	17.87	0.00	0.5633	10.000	3	11.87	0.2696	
psi(D),p(obs)	18.42	0.55	0.4279	0.7596	4	10.42	0.2667	0.2689
psi(.),p(.)	27.28	9.41	0.0051	0.0090	2	23.28	0.2722	
psi(.),p(obs)	27.90	10.03	0.0037	0.0066	3	21.90	0.2667	

Best model	Detection probability p
psi(D),p(.)	0.8654

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.6238	0.9769

According to the best model psi(D),p(.) the presence probability psi of the crab *Carcinus aestuarii* and the anemone *Paranemonia cinerea* is depth – dependent. Precisely, as depth increases psi decreases. According to the best model, the detection probability of the 2 species was constant in the study area and equal to 0.87, which is high since the 2 species were quite abundant in most stations that they were present. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of both species in 100-m transects is 0.27, since they were present only in all the Inner stations of Messolonghi Lagoon. *C. aestuarii* is a typical species of Mediteranean lagoons and estuaries (Ragionieri & Schubart 2013), though it is not well studied in Greek lagoons. *Paranemonia cinerea* is a typical brackish water anemone that tolerates well large temperature and salinity variations (Antoniadou et al. 2015). It lives attached on marine plant species and in all our detections, it was attached on *Cymodocea nodosa* leaves.

Rytiphlaea tinctoria

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	28.06	0.00	0.3860	10.000	3	22.06	0.3370	
psi(D),p(obs)	28.66	0.60	0.2860	0.7408	4	20.66	0.3333	0.3355
psi(.),p(.)	29.49	1.43	0.1888	0.4892	2	25.49	0.3375	
psi(.),p(obs)	30.10	2.04	0.1392	0.3606	3	24.10	0.3333	

Best model	Detection probability p
psi(D),p(.)	0.8903

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.6238	0.1084

According to the best model psi(D),p(.) the presence probability psi of the red algae *Rytiphlaea tinctoria* is depth – dependent. Precisely, as depth increases psi decreases. According to the best model, the detection probability of the species was constant in the study area and equal to 0.89, which is high since *R. tinctoria* was quite extended in most stations that was present and created a mat on the substrate. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.34, since it was present only in all the Inner stations of Messolonghi Lagoon and the outer station K. Along with the species extended substrate coverage in our Inner transects, we consider *R. tinctoria* as a wide spread and typical species of the Inner part of Messolonghi Lagoon. The species was recorded again in the area and was characterized as locally abundant in Messolonghi Lagoon by Providakis et al. 2013.

In the following section, are presented the occupancy estimations of 6 important species in the study area. The data for these species came from 12 sampling stations of 100m transects because they couldn't be detected from the "boat-transects:" that were done in stations P,1,5.

Symphodus melanocercus

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	27.93	0.00	0.6536	10.000	3	21.93	0.6667	
psi(D),p(obs)	29.68	1.75	0.2725	0.4169	4	21.68	0.6667	0.6913
psi(.),p(.)	32.97	5.04	0.0526	0.0805	2	28.97	1.0000	
psi(.),p(obs)	34.77	6.84	0.0214	0.0327	3	28.77	1.0000	

Best model	Detection probability p
psi(D),p(.)	0.4375

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.5446	0.5051

According to the best model psi(D),p(.) the presence probability psi of the fish *Symphodus melanocercus* is depth – dependent. Precisely, as depth increases psi increases. According to the best model, the detection probability of the species was constant in the study area and equal to 0.44, which is quite low since *S. melanocercus* is a mobile fish and it is more difficult to be detected than the megabenthic invertebrates. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.69, since it was wide spread in the outer part of Messolonghi Lagoon. During the analysis of this species models numerical convergence may not have been reached

Trididemnum cereum, Haliclona (Reniera) mediterranea, Palaemon serratus

The colonial ascidian *Trididemnum cereum*, the sponge *Haliclona (Reniera) mediterranea* and the shrimp *Palaemon serratus* have the same occupancy estimations, since their presence/ absence data were exactly the same.

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	9.65	0.00	0.6796	10.000	3	3.65	0.1667	
psi(D),p(obs)	11.65	2.00	0.2500	0.3679	4	3.65	0.1667	
psi(.),p(.)	14.81	5.16	0.0515	0.0758	2	10.81	0.1667	0.1667
psi(.),p(obs)	16.81	7.16	0.0189	0.0279	3	10.81	0.1667	

Best model	Detection probability p
psi(D),p(.)	1.0000

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.4257	1.0141

According to the best model psi(D),p(.) the presence probability psi of the 3 species is depth – dependent. Precisely, as depth increases psi decreases. According to the best model, the detection probability of each species was constant in the study area and equal to 1. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of each species in 100-m transects is 0.17, since they were only recorded in the inner part of Messolonghi Lagoon.

Calpensia nobilis

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	30.65	0.00	0.3946	10.000	3	24.65	0.5011	
psi(.),p(.)	31.19	0.54	0.3012	0.7634	2	27.19	0.5104	0.5021

| psi(D),p(obs) | 32.30 | 1.65 | 0.1729 | 0.4382 | 4 | 24.30 | 0.4917 | |
| psi(.),p(obs) | 32.85 | 2.20 | 0.1313 | 0.3329 | 3 | 26.85 | 0.5000 | |

Best model	Detection probability p
psi(D),p(.)	0.5820

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.4950	0.0118
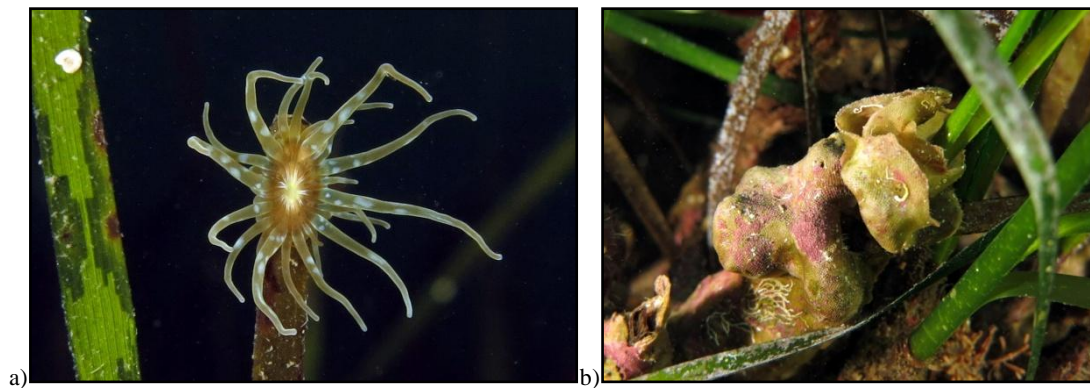
Figure 32: a) the anemone *Paranemonia cinerea* attached to a leaf of *Cymodocea nodosa* and b) the bryozoan *Calpensia nobilis* overgrowing *Posidonia oceanica* shoots

According to the best model psi(D),p(.) the presence probability psi of the bryozoan *Calpensia nobilis* is depth – dependent. Precisely, as depth increases psi increases. According to the best model, the detection probability of the species was constant in the study area and equal to 0.58, which is not very high probably due to the cryptic behavior of the bryozoan, which overgrows the basis of *Posidonia oceanica* shoots. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.50, since it was common only in outer stations of Messolonghi Lagoon. As mentioned before, *P. oceanica* which was also present in the outer stations had a higher presence probability of 0.73. It is believed that the overgrowth of *C. nobilis* over *P. oceanica* shoots, harms the development and the seagrass (Cigliano et al. 2007, Colmenero & Sánchez Lizaso 1999), so further studies can be done in the outer part of Messolonghi Lagoon where both species are abundant.

Echinaster (Echinaster) sepositus

Model	AIC	Delta AIC	AIC wgt	Model Likelihood	no.Par.	-2*Log Like	Mean psi	model-averaged psi
psi(D),p(.)	19.46	0.00	0.6342	10.000	3	13.46	0.4167	
psi(D),p(obs)	21.66	2.20	0.2111	0.3329	4	13.66	0.2500	0.3609
psi(.),p(obs)	23.32	3.86	0.0921	0.1451	3	17.32	0.2500	
psi(.),p(.)	24.09	4.63	0.0626	0.0988	2	20.09	0.3333	

Best model	Detection probability p
psi(D),p(.)	0.4000

Most complex model	χ^2 test, P-value	C-hat
psi(D),p(obs)	0.5248	0.8223

According to the best model psi(D),p(.) the presence probability psi of the common red starfish *Echinaster (Echinaster) sepositus* is depth – dependent. Precisely, as depth increases psi increases. According to the best model, the detection probability of the species was constant in the study area and equal to 0.4 which is not very high probably due to the cryptic behavior of the starfish which was found among *Posidonia oceanica* shoots. The models have a good fit to the data according to the x^2 test. According to the averaged psi, the probability of presence of the species in 100-m transects is 0.36, since it was present only in some outer stations.

Conclusively, for all the 13 species tested, except *Pinna nobilis*, the best model psi(D),p(.) assumes that the presence probability (psi) of the species is depth – dependent and the detection probability (p) is constant in the study area. For *Pinna nobilis* the detection probability differed among the two observers. Given that the depth of the inner part of Messolonghi lagoon is shallower than the outer part, the models reflect mostly the differential occupancy (presence probabilities) of species between the inner and the outer part of the Lagoon. In Table 9 you can see the best model for each species and occupancy estimation for each species. The species with the highest presence probability in the study area were *Posidonia oceanica*, *Symphodus melanocercus* and *Pinna nobilis*.

Table 9: Summary results of the occupancy estimations of 13 species in the study area with the use of the software Presence 10.0

Species	Best model	Occupancy
Posidonia oceanica	psi(D),p(.)	73
Symphodus melanocercus	psi(D),p(.)	69
Pinna nobilis	psi(D),p(obs)	61
Cymodocea nodosa	psi(D),p(.)	53
Calpensia nobilis	psi(D),p(.)	50
Echinaster (Echinaster) sepositus	psi(D),p(.)	36
Rytiphlaea tinctoria	psi(D),p(.)	34
Melibe viridis	psi(D),p(.)	27
Carcinus aestuarii	psi(D),p(.)	27
Paranemonia cinerea	psi(D),p(.)	27
Trididemnum cereum	psi(D),p(.)	17
Haliclona (Reniera) mediterranea	psi(D),p(.)	17
Palaemon serratus	psi(D),p(.)	17

Distance Sampling

For the estimation of *Pinna nobilis* abundance in the study area, models were run in the Distance v6.2 software. The functions that we used for simulating the detection function were the uniform (un), half normal (hn) and hazard rate (hr) combined with the three series expansions of cosine (cos), simple polynomial (sp), hermite polynomial (hp). Of all the combinations, the list of the selected models from best to worst, according to the Akaike criterion, is provided in Table 10. The data have been truncated till 4m perpendicular distance.

Table 10: Results of the models run in Distance v6.2 software. uniform (un)**, half normal** (hn)**, hazard rate** (hr) **cosine** (cos)**, simple polynomial** (sp)**, hermite polynomial** (hp)**. In the columns are provided, the number of** parameters **used by each model, the** DeltaAIC **of each model with the best model, the** AIC score, the D**ensity estimation, the Density's Lower Limit of Confidence Level** (D LCL)**, the Density's Upper Limit of Confidence Level** (D UCL) **and the estimation of detection probability of the observer** (P)**.**

MODEL	# params	Delta AIC	AIC	D	D LCL	D UCL	D CV	P
hr	2	0	109,5708	8458,246	2524,822	28335,44	0,645914	0,42365
hn cos	2	0,457802	110,0286	7775,708	2587,629	23365,65	0,559281	0,460837
un cos	2	1,276001	110,8468	6763,773	2259,002	20251,7	0,55613	0,529783
hn	1	2,802597	112,3734	5708,091	1933,454	16851,87	0,543478	0,627764
un sp	2	3,322403	112,8932	5794,365	1952,937	17191,88	0,548016	0,618417

In Figure 33 you can see the graph of the best model's (hazard rate) detection probability of the Distance Sampling analysis in relation to the perpendicular distance of the individuals. The detection function is monotonically decreasing from a value of 1 at zero distance.
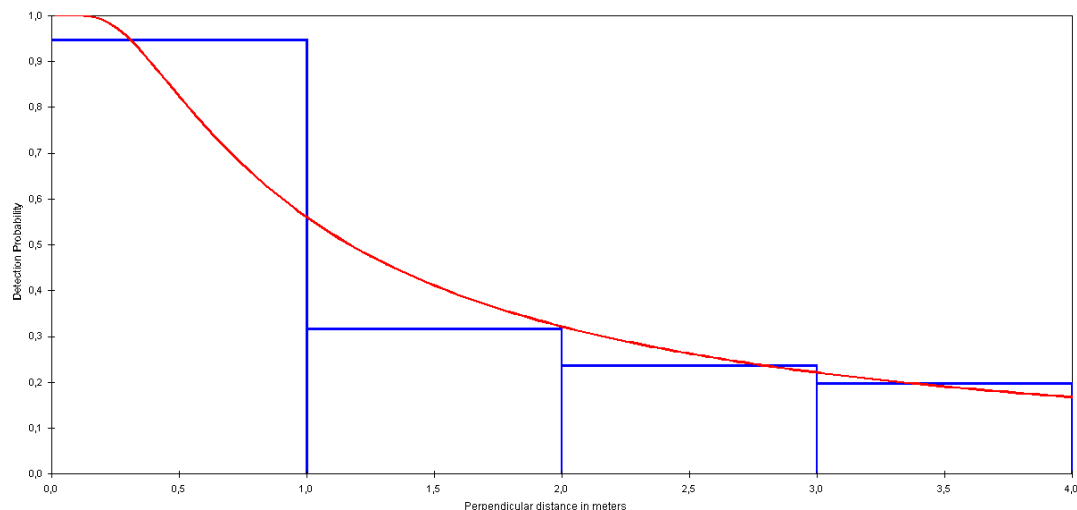
Figure 33: Best model's graph of detection probability (x axis) and perpendicular distance in meters (y axis). The detection probability is maximum close to the dive line (0m) and decreases as perpendicular distance from the line increases.

From the 3 first models (Table 10) that have significant support from the data (with DeltaAIC<2), the **weighted mean of Density** was estimated: D_w**=78ind/ha** (1ha=0.01km^2) and the 95% confidence interval: **[13, 199]**. The confidence interval results from Bootstrap with free choice of the best model in each try, by the Akaike criterion. However for an ideal bootstrap procedure we need at least 20 transects. By multiplying the Density with the surface area of the study area (48,62km^2=4862ha) we get the abundance of *Pinna nobilis* individuals in the study area: **Abundance n= 381.132 [60.775, 968.121]** individuals in the study area.

In the same way, we estimated the weighted mean of the detection probability using the 3 first models. The estimation of the weighted detection probability was: P_w**=0.46 [0.30, 0.72]**. The seemingly low detection probability may be attributed to the complexity of the habitat e.g. in the *Posidonia oceanica* meadows and in the big transect width of 4m, after the truncation.

With the traditional way of plot sampling with simple transects the estimation of *Pinna nobilis* abundance would be n=174 individuals in the whole study area and the density would be D=0.036ind/ha. (15 transects, 100m long, 8m width, 43 individuals found)
The results are summed up in the following table:

	Abundance (ind.) in the study area	Density ind/ha	Detection Prob.
D.S. Estimation	381.132 [60.775, 968.121]	78 [13, 199]	0.46 [0.30, 0.72]
Plot sampling =simple transects	174.221 [7.293, 355.533]	38 [2,73]	-

From the table above it is obvious that there's a serious **underestimation of abundance values when applying simple data analysis for plot sampling**, as discussed in the introduction section. The 95% confidence interval for the simple transects were calculated from the *Pinna noblis* counts from the 15 transect (100m x 8m), by the simple formula: $\overline{X} \pm 1.96\,\sigma/\sqrt{n}$ where X is the average of the 15 values of *Pinna nobilis* in each transect, σ is the Standard Deviation and n the sample size. Subsequently, a reduction in the whole study area and to one hectare was done as shown in the table above.

The results of the abundance estimation: 381.132 [60.775, 968.121] individuals reveals a quite high population of *Pinna nobilis*, living in the inner and the outer part of Messolonghi lagoon.

Abundance estimates for *Pinna nobilis* with Distance Sampling analysis have been already done in Greece in Lake Vouliagmeni estimating 8.501 [4.106, 12.896] individuals during the summer of 2004 (Katsanevakis 2006) and 6.770 [5.460 , 8.393] individuals during the summer of 2006 with a density surface modeling (Katsanevakis 2007a) presuming a decline of the species in 2 years. In Souda Bay the abundance estimation was 130.900 [100.600 , 170.400] individuals (Katsanevakis & Thessalou-Legaki 2009). Abundance estimates and, more importantly, trends in such estimates are a prerequisite for assessing the status of the species and proposing prompt measures to ensure the viability of local populations (Katsanevakis & Thessalou-Legaki 2009). The numbers clarify that in the inner and the outer part of Messolonghi lagoon breaths a high population of *Pinna nobilis* and the place should be protected with actual measures.

Size distribution of *Pinna nobilis*

In our study area, shell widths of *Pinna nobilis* individuals (N = 19) ranged from 6 to 22 cm with a total mean width of 14.4cm. Smaller (and younger) individuals of *Pinna nobilis* were more abundant in shallow waters of the inner part of Messolonghi Lagoon (mean width 12.3cm) and larger (and older) individuals were more abundant in deeper waters of the outer part (mean width 18.8cm). The same size structure of the species, with smaller individuals in more shallow waters has been also noticed by Katsanevakis 2006 in the marine Lake Vouliagmeni (Korinthiakos Gulf, Greece).

Ecological Evaluation Index EEI
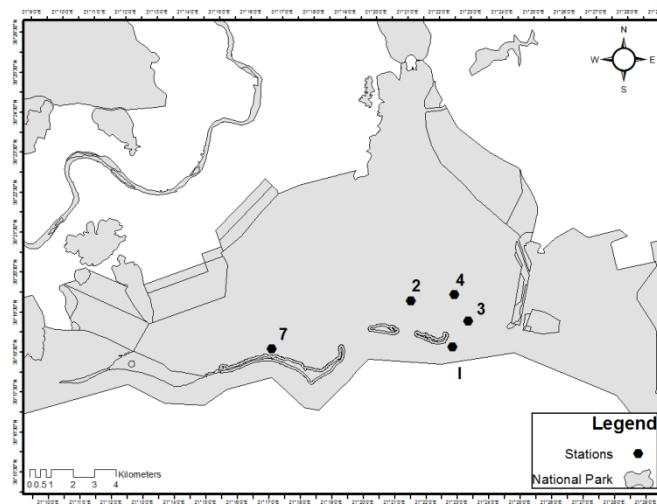
Figure 34: a) the 5 sampled quadrats (52x52cm) for the estimation of the Ecological Evaluation Index

In Table 11 you can see the results of the estimation of the Ecological Evaluation Index EEI. You can see the list of the species identified in every quadrat, the functional group to which they belong and their percentage of coverage in the quadrat. The percentage of each functional group in each quadrat was used to estimate the EEI value and the Ecological Status Class, of every station as described by Orfanidis et al. 2011. Stations 2,3,7 and I (Figure 34), appear to have a High Ecological Status, while the inner station 4 appear to have a Low Ecological Status, because of the high coverage of Cladophora sp. which belongs to the Ecological Status Group IIB of opportunistic species. The average of the EEIs show a High Ecological Status of the frontal area of the Lagoon (Table 11). The current pilot application of the Ecological Evaluation Index, cannot estimate the Ecological Status of the whole Messolonghi Lagoon, due to the limitation of the sampling stations in the border of the Lagoon. Furthermore, the index must be calibrated for lagoons where hard substrate is absent.

Table 11: results of the estimation of the Ecological Evaluation Index EEI.

Species	Functional group	Percentage of coverage (%)				
		2	**3**	**4**	**7**	**I**
Posidonia oceanica (Linnaeus) Delile	IA		65			85
Chondria capillaris (Hudson) M.J.Wynne	IIA				3	
Cladophora sp.	IIB			75	3	
Cymodocea nodosa (Ucria) Ascherson	IB	30		20	65	
Cystoseira barbata (Stackhouse) C.Agardh	IB			5		
Dictyota dichotoma var. *intricata* (C.Agardh) Greville	IIA		1			
Flabellia petiolata (Turra) Nizamuddin	IC		14			
Peyssonnelia sp.	IC					1
Polysiphonia sp.	IIB				12.5	
Rytiphlaea tinctoria (Clemente) C.Agardh	IB	60			2	
Valonia utricularis (Roth) C.Agardh	IIB				0.5	
Total percentage of IA		0	65	0	0	85
Total percentage of IB		90	0	25	67	0
Total percentage of IC		0	14	0	0	1
Total percentage of IIA		0	1	0	3	0
Total percentage of IIB		0	0	75	16	0
Value of EEI		11,221	11,217	2,873	8,636	11,922
Ecological Status		High	High	Low	High	High
Average of EEIs		9,1738 (High)				

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CONCLUSIONS

- A total of 148 taxa were recorded in the wider area of Messolonghi Lagoon. One species is recorded for the first time in the Eastern Mediterranean Sea, one for the first time in Greece, 85 species recorded for the first time in the Marine Protected Area of Messolonghi Lagoon.

- 17 of the recorded species are protected by various conventions, 8 are endemic in the Mediterranean Sea and 6 are characterized as alien species.

- The differentiation of megabenthic communities is associated with the location of the sites in relation to the effect of the sea (external or internal stations) and the type of habitat, while the above two factors seem interrelated.

- Record of spawning aggregation of the alien nudibranch *Melibe viridis* in the inner part of Messolonghi lagoon, with the densest published population and the largest published sizes.

- Occupancy was estimated for 13 important species, providing data of their distribution. The species with the higher presence probability in the study area (higher occupancy) were the protected seagrass *Posidonia oceanica* covering all of the outer part of Messolonghi lagoon, the endemic fish *Symphodus melanocercus* and the highly protected bivalve *Pinna nobilis*.

- Simple abundance estimations were done for 7 abundant megabenthic invertebrates, providing new quantitative evidence for their population and their distribution. A better abundance and density estimation was made for the highly protected bivalve *Pinna nobilis* with the Distance Sampling methodology, revealing a high population of the species (381.132 individuals) in the study area, which should be protected with actual measures.

- The Ecological Status of five sampling stations was found using the Ecological Evaluation Index EEI, giving a High Ecological Status of the study area. More stations must be examined to provide a good estimation of the Ecological Status of the whole Messolonghi lagoon.

- Conclusively, megabenthic distribution and abundance data were collected by using 3 different survey methodologies. The survey revealed the presence of several protected and endemic species and habitats in the Marine Protected Area of Messolonghi lagoon, some of them with noticeable abundances. The survey proposes that a regular monitoring program should be established in the area and protection with actual measures should be applied especially for the highly protected population of *Pinna nobilis*.

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ANNEX I: List of Species in the 24 qualitative sampling stations. * : optical identification on field, **: optical & photographic identification, ***: optical, photographic & sample identification in lab, (dead): species found only dead. Greek common names, if valid, are written in parenthesis.

SPECIES / STATION	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5	6	7	8	B In	B	A	T	Tur.	Car.
FISH																								
Boops boops (Linnaeus, 1758) (Γόπα)*						+	+	+																
Chromis chromis (Linnaeus, 1758) (Καλογριά)**	+	+		+	+	+	+	+																
Coris julis (Linnaeus, 1758) (Γύλος)**	+	+	+	+	+	+	+	+																
Dentex dentex (Linnaeus, 1758)(Συναγρίδα)*						+																		
Diplodus annularis (Linnaeus, 1758) (Σπάρος)*	+	+	+	+	+	+	+	+					+											
Diplodus sargus sargus (Linnaeus, 1758) (Σαργός)*		+																						
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (Καμπανάς)*		+	+																					
Hippocampus hippocampus (Linnaeus, 1758) (Ιππόκαμπος)**			+																					
Oblada melanura (Linnaeus, 1758) (Μελανούρι)*	+	+		+	+	+	+	+																
Serranus hepatus (Linnaeus, 1758) (Καψομούλα)*									+															
Serranus scriba (Linnaeus, 1758) (Πέρκα)*	+	+		+		+		+																
Siganus luridus (Rüppell, 1829) (Γερμανός)*						+		+																
Sparus aurata Linnaeus, 1758 (Τσιπούρα)*															+									
Spicara sp. (Τσέρουλα ή Μένουλα)*	+	+				+	+	+																
Spondyliosoma cantharus (Linnaeus, 1758) (Σκαθάρι)*		+																						
Symphodus cinereus (Bonnaterre, 1788) (Φαγανέλι)*						+	+																	
Symphodus doderleini Jordan, 1890 (Ταινιολαπίνα)**				+																				
Symphodus melanocercus (Risso, 1810) (Μαυροουρολαπίνα)**	+	+		+		+	+	+																
Symphodus rostratus (Bloch, 1791) (Μυτολαπίνα)**			+	+																				
Sympodus sp.(Λαπίνα)*		+	+																					
Syngnathus acus Linnaeus, 1758 (Σακοράφα)**													+											
Syngnathus typhle Linnaeus, 1758 (Κατουρλίδα, Φυκόψαρο)**																						+		
Thalassoma pavo (Linnaeus, 1758) (Γαϊτανούρι)**			+																					
Zosterisessor ophiocephalus (Pallas, 1814)(Πρασινογωβιός)**													+											
ALGAE																								
Alsidium corallinum C.Agardh***													+	+		+	+							
Amphiroa rigida J.V.Lamouroux***													+											
Amphiroa sp.**			+										+											
Anadyomene stellata (Wulfen) C.Agardh***													+											
Caulerpa cylindracea Sonder**																							+	
Caulerpa prolifera (Forsskål) J.V.Lamouroux*									+															
Chaetomorpha sp.***													+		+									

STATION / SPECIES	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5	6	7	8	B In	B	A	T	Tur.	Car.
Chondria capillaris (Hudson) M.J.Wynne***																+	+		+					
Chondria sp.***												+												
Cladophora sp.***												+	+	+		+	+	+						
Codium bursa (Olivi) C.Agardh**												+												
Corallinales**	+	+	+	+	+	+	+	+				+												
Cystoseira barbata (Stackhouse) C.Agardh***												+		+			+							
Cystoseira cf. *compressa* (Esper) Gerloff & Nizamuddin**												+												
Cystoseira foeniculacea (Linnaeus) Greville***												+	+	+			+							
Cystoseira sp.*															+									
Dictyota dichotoma var. *intricata* (C.Agardh) Greville***												+												
Dictyota sp. **				+									+											
Flabellia petiolata (Turra) Nizamuddin***	+	+	+	+	+	+	+	+	+			+												
Halopteris sp.***												+												
Jania adhaerens J.V.Lamouroux***												+			+									
Jania sp.***												+												
Jania virgata (Zanardini) Montagne***												+												
Laurencia sp.***											+		+		+									
Lophosiphonia sp.***																	+							
Padina sp.***			+									+									+			
Palisada patentiramea (Montagne) Cassano, Sentíes, Gil-Rodríguez & M.T.Fujii***															+									
Palisada sp.***									+															
Peyssonnelia rubra (Greville) J.Agardh***						+						+												
Peyssonnelia sp.**	+	+	+	+	+	+	+	+				+												
Phyllophora sp.***				+																				
Polysiphonia sp.***												+		+		+	+							
Rytiphlaea tinctoria (Clemente) C.Agardh***									+			+	+		+	+	+	+	+					
Ulva sp.**																	+							
Ulva rigida C.Agardh***												+												
Valonia utricularis (Roth) C.Agardh***											+	+	+	+			+							
Valonia ventricosa J.Agardh**		+																						
TRACHEOPHYTA																								
Cymodocea nodosa (Ucria) Ascherson***			+									+	+	+	+	+	+	+	+	+	+	+		
Halophila stipulacea (Forsskål) Ascherson***									+															
Posidonia oceanica (Linnaeus) Delile**	+	+	+	+	+	+	+	+	+	+		+												
Ruppia sp.***											+						+		+					
PORIFERA																								
1 Unidentified yellow sponge**	+	+		+		+																		
2 Unidentified orange sponge***	+	+		+		+	+	+																
3 Unidentified white sponge**			+																					

SPECIES \ STATION	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5	6	7	8	B In	B	A	T	Tur.	Car.
4 Unidentified whitish sponge**												+												
5 Unidentified demospongiae**								+																
Chondrilla nucula Schmidt, 1862***												+												
Chondrosia reniformis Nardo, 1847**				+								+												
Clathrina clathrus (Schmidt, 1864)**											+		+											
Dictyoceratida**							+	+																
Fasciospongia cavernosa (Schmidt, 1862)***	+						+																	
Haliclona (Reniera) mediterranea Griessinger, 1971***											+		+											
Ircinia cf *variabilis* (Schmidt, 1862) ***		+										+												
Sarcotragus foetidus Schmidt, 1862*	+		+																					
Terpios gelatinosa (Bowerbank, 1866)**		+		+																				
BRYOZOA																								
Amathia verticillata (delle Chiaje, 1822)**													+											
Calpensia nobilis (Esper, 1796)**	+	+	+	+		+																		
Margaretta cereoides (Ellis & Solander, 1786)**			+																					
Reteporella mediterranea Hass, 1948**	+																							
BIVALVIA																								
Acanthocardia echinata (Linnaeus, 1758)**(dead)									+															
Acanthocardia tuberculata (Linnaeus, 1758)***(dead)			+																+	+	+			
Barbatia sp.***(dead)																			+					
Callista chione (Linnaeus, 1758)***(dead)			+																+		+			
Cerastoderma sp. (Linnaeus, 1758)**(dead)																			+	+				
Laevicardium oblongum (Gmelin, 1791)***(dead)																			+					
Mactra sp.**(dead)									+										+	+				
*Pecten jacobaeus***(dead)									+															
Pinna nobilis Linnaeus 1758 (Πίνα)**	+		+	+					+		+	+	+	+										
Solecurtus strigilatus (Linnaeus, 1758)***(dead)																					+			
Venus verrucosa Linnaeus, 1758 (Κυδώνι)***(dead)												+												
GASTROPODA																								
Bulla cf *striata* Bruguière, 1792**(dead)									+															
Bosellia mimetica Trinchese, 1891**												+												
Cerithium vulgatum Bruguière, 1792***									+					+			+							
Dendrodoris limbata (Cuvier, 1804)**											+													
Gibbula sp.**											+		+											
Hexaplex trunculus (Linnaeus, 1758)***												+												
Melarhaphe neritoides (Linnaeus, 1758)**																						+		
Melibe viridis (Kelaart, 1858)***						+	+		+	+														

STATION / SPECIES	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5	6	7	8	B In	B	A	T	Tur.	Car.
Phorcus sp. **																				+				
Potamides conicus (Blainville, 1829)*** (dead)																			+					
Rissoa sp.**			+											+										
Smaragdia viridis (Linnaeus, 1758)**			+																					
Tricolia sp.**														+										
Tylodina perversa (Gmelin, 1791)**		+																						
Umbraculum umbraculum (Lightfoot, 1786)**						+																		
CEPHALOPODA																								
Sepia officinalis Linnaeus, 1758**																				+				
ASCIDIACEA																								
Aplidium coeruleum Lahille, 1890**			+																					
Ascidiacea sp1.**				+																				
Ascidiacea sp3.**							+																	
Botryllus schlosseri (Pallas, 1766)**												+		+										
Didemnidae**		+	+			+																		
Didemnum protectum (Daumézon, 1908)**												+												
Halocynthia papillosa (Linnaeus, 1767)**		+	+			+	+																	
Pyura dura (Heller, 1877)***													+											
Trididemnum cereum (Giard, 1872)***												+		+			+							
POLYCHAETA																								
Haplosyllis spongicola (Grube, 1855)***												+		+										
Harmothoe sp.***													+											
Hermodice carunculata (Pallas, 1766)**			+																					
Nereis sp.***													+											
Phyllodocidae***													+											
Polychaete sp1.*									+															
Sabella cf *discifera* Grube, 1874***						+																		
Sabella pavonina Savigny, 1822**			+			+	+	+													+			
Sabella sp.**						+																		
ECHINODERMATA																								
Arbacia lixula (Linnaeus, 1758)*													+											
Asterina gibbosa (Pennant, 1777)***												+			+									
Astropecten cf *spinulosus* (Philippi, 1837)**			+																					
Echinaster (Echinaster) sepositus (Retzius, 1783)*			+		+		+																	
Holothuria sp.**													+											
Ophiuroidea**	+		+											+		+	+							
Paracentrotus lividus (Lamarck, 1816)***													+											
CNIDARIA																								
Calliactis parasitica (Couch, 1842)**																						+		

57

STATION / SPECIES	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5	6	7	8	B In	B	A	T	Tur.	Car.
Cerianthus membranaceus (Spallanzani, 1784)*							+																	
cf Paractinia striata (Risso, 1826)**								+				+												
Cladocora caespitosa (Linnaeus, 1767)**												+												
Paranemonia cinerea (Contarini, 1844)**									+	+			+	+										
CRUSTACEA																								
Amphipoda***														+										
Carcinus aestuarii Nardo, 1847***									+	+			+	+		+								
Maja squinado (Herbst, 1788) (Καβουρομάνα)***(dead)												+												
Palaemon serratus (Pennant, 1777)***												+		+			+							
Scyllaridae*						+																		
MAMMAL																								
Tursiops truncatus (Montagu, 1821) (Ρινοδέλφινο)**																						+		
MARINE TURTLE																								
Caretta caretta (Linnaeus, 1758)**																								+
OTHER																								
Cyanobacteriaceae colony**								+																
Eggs of Melibe viridis***										+		+												
Eggs of gastropod or polychaete**								+																

ANNEX II: Abundance data of 55 taxa in the 15 quantitative sampling stations

SPECIES \ STATION	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5
FISH															
Hippocampus hippocampus (Linnaeus, 1758)			1												
Syngnathus acus Linnaeus, 1758												1			
Zosterisessor ophiocephalus (Pallas, 1814)												1			
PORIFERA															
1 Unidentified yellow sponge	1	1		8		7									
2 Unidentified orange sponge	1	22		7		8	15	28							
3 Unidentified white sponge				1											
4 Unidentified whitish sponge													1		
5 Unidentified demospongiae								1							
Chondrilla nucula Schmidt, 1862													7		
Chondrosia reniformis Nardo, 1847				1									1		
Clathrina clathrus (Schmidt, 1864)												6		2	
Dictyoceratida**(Ircinia sp. or Dysidea sp.)							1	1							
Fasciospongia cavernosa (Schmidt, 1862)	1						2								
Haliclona (Reniera) mediterranea Griessinger, 1971												2		22	
Ircinia cf *variabilis* (Schmidt, 1862)		1											15		
Sarcotragus foetidus Schmidt, 1862	1		1												
Terpios gelatinosa (Bowerbank, 1866)		1		1											
BRYOZOA															
Calpensia nobilis (Esper, 1796)	1	1	2	9		2									
Margaretta cereoides (Ellis & Solander, 1786)			1												
Reteporella mediterranea Hass, 1948	1														
Amathia verticillata (Delle Chiaje, 1822)														1	
BIVALVIA															
Pinna nobilis Linnaeus, 1758	4		1	1						24		2	1	1	2
GASTROPODA															
Bulla cf *striata* Bruguière, 1792							1								
Cerithium vulgatum Bruguière, 1792							1								
Dendrodoris limbata (Cuvier, 1804)												4			
Gibbula sp.												6		2	
Hexaplex trunculus (Linnaeus, 1758)													2		
Melibe viridis (Kelaart, 1858)											7	171		62	24
Smaragdia viridis (Linnaeus, 1758)			1												
Tylodina perversa (Gmelin, 1791)		1													
Umbraculum umbraculum (Lightfoot, 1786)						1									
ASCIDIACEA															
Aplidium coeruleum Lahille, 1890			1												
Botryllus schlosseri (Pallas, 1766)												1		1	
Didemnidae		1	3				1								

STATION / SPECIES	C	D	E	F	G	H	I	J	K	P	1	2	3	4	5
Halocynthia papillosa (Linnaeus, 1767)			1	4			1	3							
Pyura dura (Heller, 1877)													5		
Trididemnum cereum (Giard, 1872)												158		1	
POLYCHAETA															
Hermodice carunculata (Pallas, 1766)			2												
Sabella sp.			1				2	1	1						
ECHINODERMATA															
Arbacia lixula (Linnaeus, 1758)													1		
Asterina gibbosa (Pennant, 1777)												1			
Astropecten cf *spinulosus* (Philippi, 1837)				1											
Echinaster (Echinaster) sepositus (Retzius, 1783)				2		1		1							
Holothuria sp.												55			
Ophiuroidea	1														
Paracentrotus lividus (Lamarck, 1816)													2		
CNIDARIA															
Aiptasia diaphana (Rapp, 1829)							1						2		
Cerianthus membranaceus (Spallanzani, 1784)						1									
Cladocora caespitosa (Linnaeus, 1767)													1		
Paranemonia cinerea (Contarini, 1844)											1	43		17	5
CRUSTACEA															
Carcinus aestuarii Nardo, 1847											1	16		1	3
Palaemon serratus (Pennant, 1777)												4		1	
Scyllaridae						1									
OTHER															
Cyanobacteria colony									2						

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