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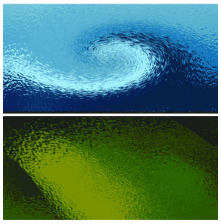

RESEARCH ARTICLE

Genetic vs community diversity patterns of macrobenthic species: preliminary results from the lagoonal ecosystem

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Abstract

1 - The use of molecular data derived from multispecies assemblages in order to test ecological theory has only recently been introduced in the scientific literature.

2 - As a first step, we compared patterns of abiotic environment, polychaeta distribution and their genetic diversity in five lagoon ecosystems in Greece. Our results confirm the hypothesis that higher genetic diversity is expected in the populations of the species occurring in the transitional waters rather than of those occurring in the marine environment.

3 - Patterns derived from the polychaete community level and from the mitochondrial DNA (16S rRNA) obtained from *Nephtys hombergii* and *Hediste diversicolor* showed convergence, indicating the potential use of molecular matrices as surrogates in community analysis.

4 - Finally, the high correlation between the genetic diversity pattern of *H. diversicolor* and the phosphorus concentration in the sediments may imply the broadening of the *hierarchic-response-to-stress* hypothesis towards lower than species level.

Keywords: Biodiversity, *hierarchic-response-to-stress* hypothesis, *Hediste diversicolor*, *Nephtys hombergii*, 16S rRNA, Amvrakikos Gulf, W Greece.

Introduction

There are only a few recent studies aiming at the integration of information patterns deriving from genetic and community levels of the biological organization (*e.g.* Whitehead and Peakall, 2009; Bernatchez *et al.*, 1999). Traditionally, genetic effort is mainly focused on the genetic adaptations of populations and on phylogeographic aspects, while ecology tries to explain the interactions among species and between populations and their environment. Species are frequently considered as homogeneous

units and ecological diversity is thought to exist only between species or between higher levels of biological organization such as assemblages, trophic groups or ecosystems (Wilson and Swenson, 2003). At the species level the variation of the attributes such as composition, abundance or biomass are used as sources of information. However, it has been shown that ecological diversity exists also within species (*e.g.* Hendrickson and Ehrlich, 1971), a fact which primarily derives from the varying responses of the individual to the environmental pressures. Thus, the combination of the disciplines of population genetics with community ecology may well provide an additional insight to the ecosystem analysis (Wilson and Swenson, 2003). A series of mathematical methods have been developed which visualize the community multivariate patterns resulting from the variation in species attributes (*e.g.* Warwick, 1988). Other methods, such as BIOENV, can correlate multivariate patterns derived from communities with those derived from the environmental variables (Clarke and Ainsworth, 1993). The need for the genetic information to be integrated in the ecological thinking and ecological hypothesis testing was obvious already in the fifties. Ford (1964) introduced the term "*ecological genetics*" as "*the adjustments and adaptations of the wild populations to their natural environment*". A modern definition of ecological genetics, according to Lowe *et al.* (2004), is "*the investigation of the origin and maintenance of genetic variation within and between populations, which ultimately leads to adaptation and speciation*". Scientific effort has been spent mostly towards the latter direction, with most of publications referring to plants and bacterial populations (*e.g.* Jackson *et al.*, 2002; Magurran, 2005; Kassen and Rainey, 2004; Davey and O'toole, 2000; Joyce and Rehfeldt, 2013; Wu *et al.*, 2012), while only a small number of published results refer to invertebrates (*e.g.* Lynch, 1989; Jones, 1973; Flight *et al.*, 2012).

Evolvement of genetical approaches such as the development of genetical markers which can describe genetic variation, the expansion of theories (*e.g.* metapopulation theory) and the improvement of procedures and software for analysis, have brought genetics to the forefront of the ecological discipline since it can be used in order to answer many of the scientific questions (Lowe *et al.*, 2004). Theoretical models have been proposed, merging the disciplines of ecology and genetics (*e.g.* Vellend and Geber, 2005), mostly directed towards the ecology-evolution interaction. Alternatively, models developed either for genetic or ecological approaches are applied similarly on both disciplines (*e.g.* Amarasekare, 2000). At the molecular level the genetic variation can be used as a source of information.

In the present study mitochondrial DNA (mtDNA) was used to detect intra-species variation. Animal mtDNA may be variable within and among populations (Parker *et al.*, 1998) and shows high mutation rate compared to the nuclear DNA (Brown *et al.*, 1979; Rand, 1994; Ballard and Kreitman, 1995; Page and Holmes, 1998; Rokas *et al.*, 2003; Ballard and Whitlock, 2004; Bazin *et al.*, 2006; Galtier *et al.*, 2009). The 16S ribosomal RNA (16S rRNA) is a component of the small ribosomal subunit. Parts of the molecule play an important role in the translation of mRNA to peptides, thus they are most probably not underlying evolutionary change (Van Straalen and Roelofs, 2006). One of the most influential theories on benthic ecology was launched by Pearson and Rosenberg (1978). This theory offers a model for the structuring of the benthic communities along an environmental gradient (organic enrichment). Subsequently, the "*taxonomic sufficiency*" concept was developed (Warwick, 1988) which suggests that the species level is not always necessary for the description of the

macrobenthic community patterns, especially when the causes are obvious (*e.g.* in severe pollution gradients and in long-term studies of environmental impacts). Instead, that higher phylogenetic/taxonomic categories reflect pollution gradients more efficiently (*e.g.* Boesch and Rosenberg, 1981; Feraro and Cole, 1990) in these cases. The "*taxonomic sufficiency*" concept has ultimately led to the formulation of the "*hierarchic-response-to-stress*" hypothesis (Olsgard *et al.*, 1998). The hypothesis states that as the environmental stress increases the community patterns best correlated with the environmental ones are deriving from higher categories (*e.g.* families and beyond). However, exploring the opposite direction of the biological organization hierarchy, that is moving from the species down to the molecular level are not, yet, broadly studied.

Constant natural and anthropogenic disturbance have direct effect on the populations and the ecosystems. Genetic analysis can provide information from the population level. Evidence on the influence of the disturbance on individuals can be inferred by inter-and intra-population genetic diversity (*e.g.* detections of bottlenecks).

The focus, therefore, of this preliminary study is to analyze whether the species diversity or the genetic diversity patterns are best correlated with the environmental variables. Consequently, the broadening of the "*hierarchic-response-to-stress*" hypothesis is attempted below the species level for the first time. Mediterranean coastal lagoons are used as a model ecosystem and macrobenthic polychaetes as a model taxon. The data used for the hypothesis testing derive from five Mediterranean coastal lagoons located in the Ionian and the Aegean Sea.

Methods

Study area

The study sites are located in Amvrakikos Gulf (W Greece) and Agiasma lagoon (Nestos River, N Greece). At the northern part of Amvrakikos, rivers Arachthos and Louros are forming a complex of three lagoons: Rodia (39°5′ N; 20°47′ E), Tsoukalio (39°2′ N; 20°47′ E) and Logarou (39°1′ N; 20°52′ E). Rodia is an internal lagoon, connected to Tsoukalio through a narrow opening. Tsoukalio and Logarou are separated from the sea by sand barriers with narrow openings allowing limited water exchange. Muddy substrate sediments dominate the bottom in all lagoons. Tsopeli (39°2′ N; 20°45′ E) is a small lagoon at the mouth of Louros River without any obvious source of pollution (Fig. 1). Agiasma lagoon (40°54′ N; 24°39′ E) is formed by Nestos River Delta. The lagoon is characterized by shallow depth (approximately 1m). Macroalgae were found in Logarou, Tsopeli and on the northern part of Rodia, while angiosperms were present in Tsoukalio and Agiasma lagoons. Ranges of the environmental variables are provided in Table 1. Extensive and semi-intensive fish-farming, fishing, and agriculture are some of the anthropogenic activities carried out in these lagoons. From November to April, there are high water inflows from Arachthos River. For Louros River the period of high water inflows is between December and April, with the highest inflows to be observed on February, while the period from August to October, the inflows are at the lowest levels (Poulos *et al.*, 1993). Tsopeli has been evaluated as lagoon of good ecological quality (Reizopoulou and Nicolaidou, 2007) and thus it is used as reference site.

Sampling

Samples for genetic analysis were collected from 17 stations. Stations arrangement is shown in figure 1. Salinity, temperature and dissolved oxygen were measured in the water overlaying the sediments. Data

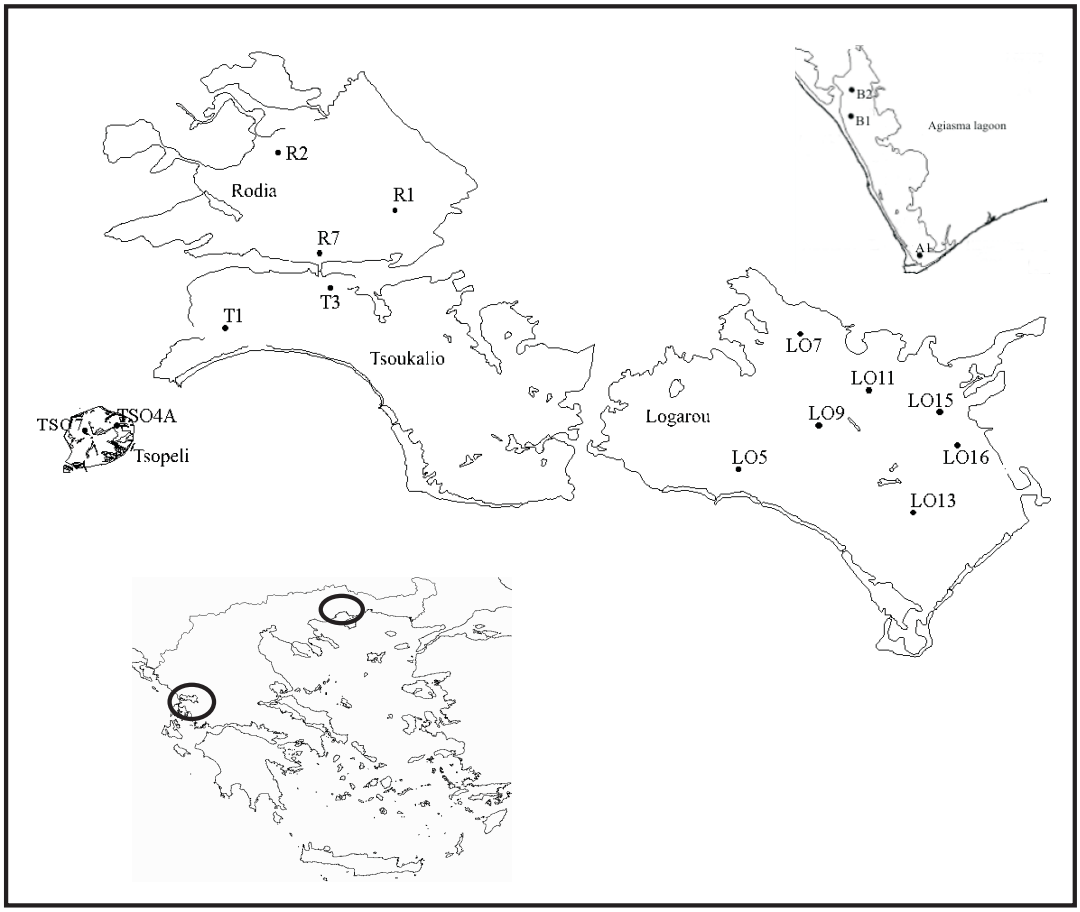

Figure 1. Map of the study areas. Agiasma lagoon is located in northern Greece, while the lagonal complex of Amvrakikos Gulf occurs in western Greece

on particulate organic carbon, nitrate, and phosphate concentrations were estimated from sediment samples as well as data on sediment granulometry by the HCMR Chemistry Lab, based on standard techniques (Strickland and Parsons, 1972; Grasshoff *et al.*, 1983; Parsons *et al.*, 1983).

Samples for faunistic analyses were collected by means of a manually-operated box-corer with a sampling surface of 0.03 square meters. Five replicate units were collected from each station, for macrobenthos analysis. Higher taxa (*e.g.* crustaceans, molluscs, polychaetes) were sorted out from two replicate units from each station designated for genetical process and immediately fixed in alcohol 98%. All polychaetes were identified to family level. Nereididae and Nephthyidae were the most abundant polychaete families found in most stations and thus they were used for genetic analysis. Individuals were identified to species level, morphometric features (wet weight, length, width, number of segments) were measured, the internal parts of each individual were removed and then the remaining tissues were processed genetically. Each individual was given a code and preserved in alcohol 98%.

Molecular analysis

For DNA extraction, approximately 2.5 mg of tissue from each animal were used (smaller

Table 1 - Ranges of the environmental variables measured in the lagoons of Amvrakikos Gulf.

	LOGAROU	TSOUKALIO	TSOPELI	AGIASMA	RODIA
oxygen (mg/l)	7.26-8.00	4.85-5.23	5.29-6.8	5.86-9.85	5.22-5.51
Salinity (psu)	38.21-38.69	27.95-28.52	24.5-26.65	22.10-29.00	26.57-26.74
Temperature (°C)	22.23-23.52	20.57-21.16	21.5-23.05	18.70-20	21.96-22.3
org. C (%) sediment	2.48-3.50	1.80-4.56	1.84-2.05	3.36-1.70	5.13-8.36
tot. N (%) sediment	0.36-0.55	0.21-0.58	0.17-0.27	0.09-0.14	0.58-0.99
P (%) sediment	0.058-0.086	0.046-0.070	0.066	0.002-0.008	0.055-0.84

animal tissue quantities were also attempted but the extraction was not successful in all of the cases). Genomic DNA was extracted from specimens with Nucleospin Tissuekit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The amount of DNA extracted was measured with Nanodrop 1000 Spectrophotometer. Additionally, GenomiPhi DNA Amplification kit (GE Healthcare, Buckinghamshire, England) was used to amplify genomic DNA from specimens from which only insufficient genomic DNA was extracted.

About 660 bp of 16S rRNA gene were amplified with 16SAN-F (TACCTTTTRCATCATGG) and 16SEU-R (ACCTTTGCACGGTCAGGRTACCGC) primers (pers. comm. J. Zanol Silva). Amplification reaction mix for Nephthyidae contained 2 µl 10x buffer, 2.5 µl $MgCl_2$ (25 µM), 0.4 µl dNTPs (100 mM), 2 µl of each primer (10 mM), 0.2 µl Taq DNA Polymerase (5 U/µl) in a total volume of 20 µl per reaction. DNA template concentration was ~150 ng/µl. Reaction mixture for Nereididae and specimens which were elaborated with GenomiPhi DNA Amplification kit, contained 2 µl 10x buffer, 3.5 µl $MgCl_2$ (25 µM), 0.4 µl dNTPs (100 mM), 1 µl of each primer (10 mM), 0.2 µl Genaxxon Taq-Polymerase (5 U/µl) (Genaxxon BioScience, Biberach,Germany) in total volume 20 µl per reaction. For polymerase chain reaction (PCR) of Nephthyidae and Nereididae the following protocols were used: 94 °C for 3 min; 35 cycles with 93 °C for 45 s, 52 °C for 1 min, 72 °C for 1 min; final extension at 72 °C for 7 min. A different PCR temperature file was used for the specimens amplified with GenomiPhi: 96 °C for 4 min; 35 cycles with 93 °C for 45 s, 56 °C for 1 min, 72 °C for 1 min; final extension at 72 °C for 7 min. Amplifications were carried out using DYAD DNAEngine and BIORAD MyCycler. PCR products were purified with Macherey-Nagel Nucleospin Extract II kit.

Sequences of all amplified fragments for both directions were produced by MACROGEN Inc. (Korea), as well as in ABIPrism 3700 DNA Analyzer. Sequencing reactions were performed using the PCR primers. The reaction mixture produced for the alignment contained 2 µl BigDye Enzyme, 0.6 µl of primer, 1 µl of reaction buffer and 2 µl of PCR product. The final volume of each reaction was 10 µl. The sequencing reactions were performed with: an initial step at 96 °C for 3 min, 35 cycles at 96 °C for 10 sec, 50 °C for 15 sec, 60 °C for 4 min. Reactions were purified with EDTA (0,5 M, pH 8), NaAc (3 M, pH 4,3) and 98% EtOH.

Sequences were manually corrected and aligned with BIOEdit software. Different sequences were submitted to GenBank with accession numbers EU221656–EU221670.

Statistical analysis

Phylogenetic networks were derived from the analyses of the genetic data with the Network 4.5.0.1 software (Bandelt *et al.*, 1999).

A Mann-Whitney test (Mann and Whitney 1947) was applied in order to test homogeneity in the distribution of the polychaetes to stations and lagoons. Non-metric multidimensional scaling (nMDS) analysis was performed on the resemblance matrices derived by the application of the Bray-Curtis similarity coefficient on the per station average polychaete abundance values, on the total macrobenthos species abundance values, on morphometric features, and on frequencies of polychaete haplotypes. Number of taxa MDS (ntMDS) analysis was applied on macrobenthic and polychaete data (Arvanitidis *et al.*, 2009). Patterns deriving from the previous analyses were compared by means of the "second stage" MDS (2nd stage MDS) (Somerfield and Clarke, 1995). Following their proposed mathematical approach, a rank correlation, using the harmonic rank correlation coefficient (Clarke and Ainsworth, 1993), was computed between every pair of the resemblance matrices produced by each source of information (polychaetes, macrobenthos, morphometrics, haplotypes). From the above correlations, a final triangular resemblance matrix was constructed, the "second stage" resemblance matrix, containing the resulting values of the harmonic rank correlation coefficient. These correlation values were firstly ranked and subsequently subjected to the "second stage" MDS (Olsgard *et al.*, 1997). The BIOENV analysis was carried out in order to explore correlation between biotic (genetic, community) and abiotic (*e.g.* granulometry, nutrients) patterns (Clarke and Ainsworth, 1993). The PRIMER (v.6) package developed in Plymouth Marine Laboratories (Clarke and Ainsworth, 1993) was used.

Results

Lagoons were found with different macrobenthic assemblages. Assemblages of Logarou were characterized by the large abundances of Iphinoe serrata Norman, 1867 (occupying 30% of the total abundance), *Sphaeroma ghigii* Arcangeli, 1941, *Nephtys hombergii* Savigny in Lamarck, 1818 and *Hydroides dianthus* (Verrill, 1873) (each accounting for the 11% of the macrobenthos abundance). Community structure number was different in Tsoukalio where *Ericthonius difformis* Milne-Edwards, 1830 and *Mytilaster minimus* (Poli, 1795) were dominating, accounting for the 96% of the total abundance. In Rodia lagoon the community was diverse with *Loripes lucinalis* (Lamarck, 1818) accounting for the 39% of the total macrobenthic abundance, *Mytilaster minimus* (13%) and *Abra segmentum* (Recluz, 1843) (10%). The macrobenthic assemblage was very different in Tsopeli and Agiasma lagoons where the oligochaeta were found to be the dominant taxon representing 44% and 50% of the total abundance, respectively.

The most abundant polychaete families in all five lagoons were Nephthyidae and Nereididae. Two species were identified from the family Nephthyidae: *Nephtys hombergii* (64 individuals) and *Nephtys incisa* Malmgren, 1865 (4 individuals) in the replicate units collected for genetic analysis. Another two species were identified from the Nereididae: *Hediste diversicolor* (Müller, 1776) (61 individuals) and *Platynereis dumerilii* (Audouin & Milne Edwards, 1834) (3 individuals). *N. incisa* and *P. dumerilii* were not included in the genetic analysis because of the small number of individuals found. The Mann-Whitney test showed that polychaete abundance distributions are homogeneous both to stations ($U=41.5$; $p>0.05$) and to the lagoons ($U=2$; $p>0.05$) studied.

Ten different haplotypes were obtained among the individuals of *N. hombergii* illustrated by phylogenetic networks (Fig. 2). Individuals used for the genetic analysis represent 62% of the total abundance of the species collected.

Two of the haplotypes (haplotypes 1 and 2) were more common, as they were found in 14 and 15 individuals respectively. The first haplotype, was found in three different lagoons: Logarou (11 individuals), Tsoukalio (2 individuals) and Agiasma (1 individual), and the second haplotype occurred only in Logarou (13 individuals) and Tsoukalio (2 individuals) lagoons. Three more haplotypes were located in Logarou: haplotype 3 was found in five individuals whilst haplotypes 4 and 5 where found in a single individual, each. Analysis showed five more haplotypes in equal individuals from Tsoukalio lagoon.

Similarly, network results showed the existence of eight haplotypes identified from the individuals of *H. diversicolor* (Fig. 3). Genetic data obtained from 37% of the total abundance of the species collected. Sixteen of the individuals shared the same haplotype (haplotype 1), seven of which were found in Tsopeli lagoon and the rest were found in Rodia lagoon. In Tsopeli, three individuals were showed three more haplotypes (haplotype 6, haplotype 7 and haplotype 8), while in Rodia, the results showed the existence of four more haplotypes: haplotype 2 was found in three individuals and haplotypes 3, 4 and 5 in a single individual each.

Nucleotide diversity index (π) and haplotype diversity index (h) were estimated for each one of the polychaetes families. Results of the indices are shown on Table 2.

Indices values are higher for the N. hombergii species than the values resulting from the *H. diversicolor* genetic data. The values of both indices for *N. hombergii* are higher from the 0.5 and 0.5% thresholds respectively, while

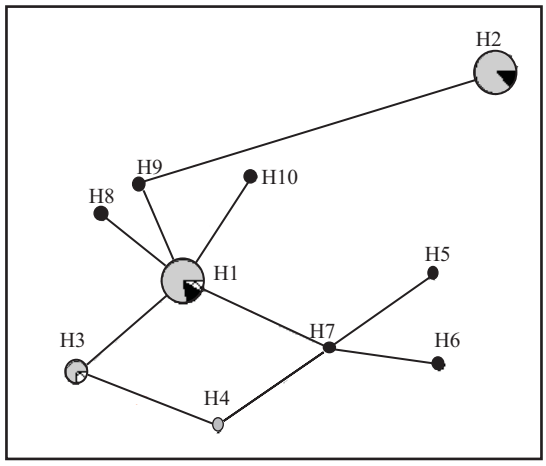

Figure 2. Phylogenetic network for *N. hombergii*. Haplotypes from individuals of Logarou are represented with grey colour, from Tsoukalio lagoon with black colour and from Agiasma lagoon with diagonal crosses. Circle size is proportionate to the frequency of the haplotypes. H: haplotype.

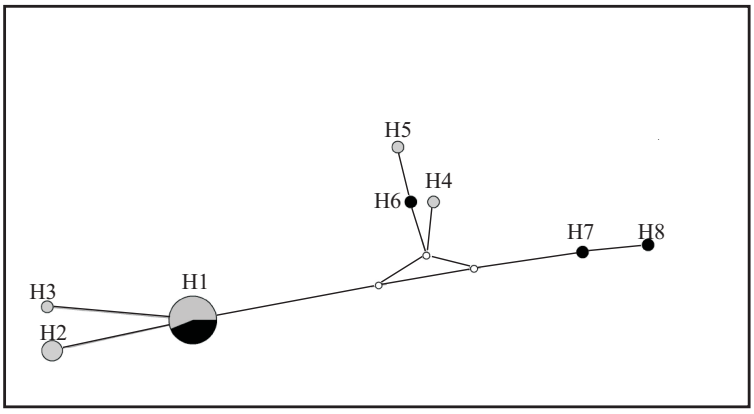

Figure 3. Phylogenetic network for *H. diversicolor*. Haplotypes from individuals of Rodia lagoon are represented with grey colour and from Tsopeli lagoon with black colour. Circle size is proportionate to the frequency of the haplotypes. H: haplotype

Table 2 - Haplotype diversity index and nucleotide diversity index estimated for each species.

	Haplotype diversity index (h)	Nucleotide diversity index (π)
N. hombergii	**0.72**	**0.009**
H. diversicolor	**0.67**	**0.002**

the value of π index for *H. diversicolor* is lower than the 0.5% threshold.

The MDS plot deriving from macrobenthos (Fig. 4), showed a clustering of the stations into three groups. The first group includes all Logarou stations, placed along a gradient from LO15 to LO7. In the second group the stations from Rodia and Tsoukalio were clustered. The stations from Agiasma and Tsopeli formed the third group.

Data derived from the polychaete abundance on the nMDS plot, showed an obvious aggregation of the stations in two major groups (Fig. 5): the first group was represented by Agiasma and Tsopeli stations, arranged from undisturbed towards disturbed areas. Logarou, Tsoukalio and Rodia stations formed the second group, showing the same arrangement. Three groups of stations arose from MDS analysis applied on the polychaete genetics matrix (haplotypes×stations) (Fig. 6): the first group included Logarou and Tsoukalio stations as well as station A1 from Agiasma. In the second group Rodia and Tsopeli stations were included. In the last group only Agiasma stations B1 and B2 were placed. A closer look to the results showed another common characteristic between stations clustered in each group. Levels of salinity in the stations of the first group were measured to be higher than 28. In addition, salinity values measured in the stations of the second group were between 24-27. Finally the last group contained stations with salinity under 24. The plot derived from 2nd stage MDS showed that patterns based on the number of taxa from macrobenthic data and from polychaete data are separated from the remainder patterns (Fig. 7). Patterns deriving from polychaete abundance data are forming a group with the patterns deriving from morphometric data which is also close to the one formed by the patterns deriving from genetic data.

The results of BIO-ENV analysis (Table 3) showed higher harmonic Spearman rank coefficient values between macrobenthos and oxygen, salinity and total nitrate, organic carbon and phosphorus concentration in the sediments (ρw = 0.46). Polychaete

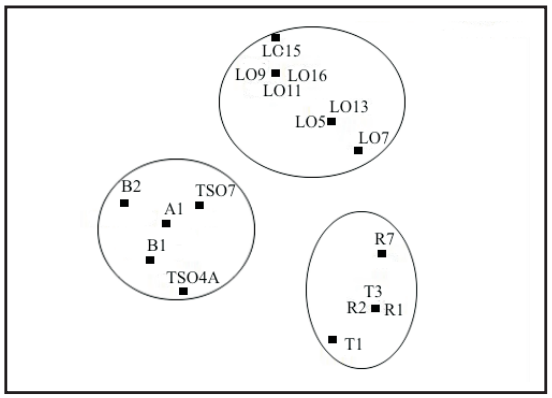

Figure 4. Non-metric MDS plot as derived from macrobenthos abundance data. Station coding: LO, refers to Logarou stations; R, refers to Rodia; T, to Tsoukalio stations; TSO, to Tsopeli; A, B to Agiasma stations. The stress value: 0.14.

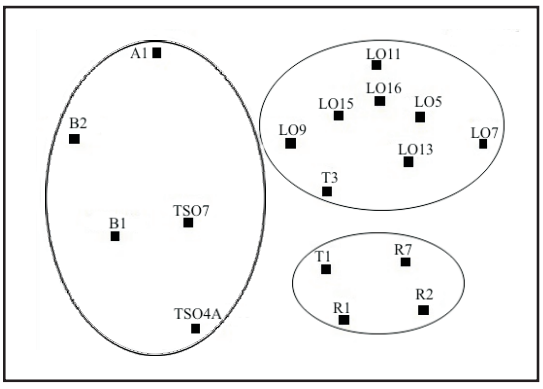

Figure 5. Non-metric MDS plot is derived from polychaete abundance data. Station coding: LO, refers to Logarou stations; R, refers to Rodia; T, to Tsoukalio stations; TSO, to Tsopeli; A, B to Agiasma stations. Stress value: 0.15.

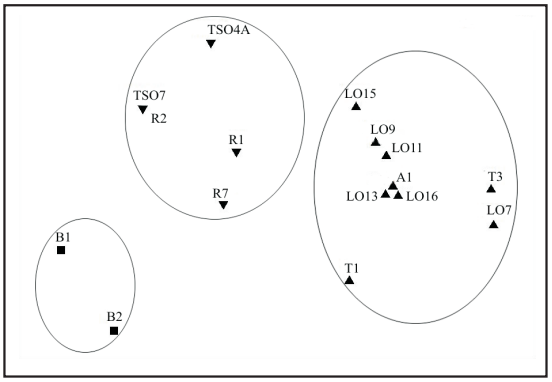

Figure 6. Non-metric MDS plot from molecular data. Triangles represent stations with salinity values over 28, inverted triangles, stations with salinity values ranging from 24 to 27, squares, stations with salinity values under 24. Stress value: 0.02.

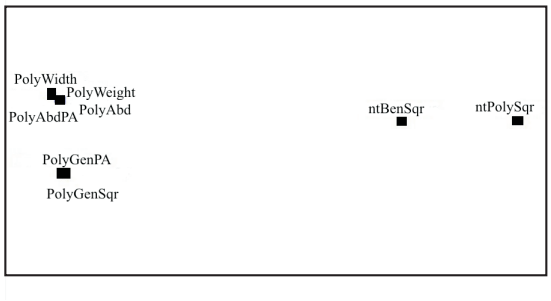

Figure 7. Second-stage MDS analysis. PolyGenPA, PolyGenSqr denote genetic data matrices with presence-absence and square root transformation, respectively; PolyWidth denote matrices with polychaete width data; PolyWeight matrices with polychaete biomass data; PolyAbd, PolyAbdPA, matrices containing polychaete abundance data with fourth-root and presence-absence transformation respectively; ntpolysqr and ntbenthossqr denote patterns deriving from number of taxa MDS (ntMDS) analysis for polychaetes and macrobenthos, respectively. Stress value <<0.05.

pattern appeared to be more correlated with nitrate and phosphorus concentrations with ρw value higher than 0.5. Spearman's coefficient value was even lower (0.19) for polychaete genetic pattern that was found to be correlated with salinity and nitrate and phosphorus concentrations. Spearman correlation coefficient was proportionally low (0.17) when patterns from *N. hombergii* genetic data were correlated to abiotic data. Factors seemed to affect the later pattern were oxygen concentrations and clay percentage in the sediments. Finally, BIOENV revealed that patterns deriving from *H. diversicolor* genetic data were well correlated (0.74) only with the percentage of phosphorus concentration in the sediments.

DISCUSSION

The analysis of macrobenthic data resulted in the differentiation of the Logarou stations from those stations of remaining lagoons. Also, stations from disturbed lagoons were separated from the ones of undisturbed lagoons. Similar results arose from polychaete abundance data. The results differ from the findings of Reizopoulou and Nicolaidou (2004), where stations from Logarou were grouped together with Rodia, Tsoukalio and Mazoma lagoons and the sediment characteristics along with the degree of confinement seemed to be highly associated with their grouping. Results from BIOENV analysis, from the current study, agree with the former, as nutrients from the sediment were correlated to the macrobenthic and polychaete patterns.

Genetic analysis of 16S gene showed the existence of different haplotypes in both polychaete species. There are a number of papers on genetic differences between populations of H. diversicolor (Abbiati and Maltagliati, 1992; Abbiati and Maltagliati, 1996; Breton *et al.*, 2003; Virgilio and Abbiati, 2004a; Virgilio and Abbiati, 2004b; Virgilio *et al.*, 2005; Virgilio and Abbiati, 2006; Virgilio *et al.*, 2006). Although different techniques were used, the individuals of H.

Table 3 - Results from the application of BIOENV analysis showing the degree of correlation between the biotic and abiotic patterns.

	oxygen (mg/l)	Salinity	Temperature (°C)	org. C (%) sediment	tot. N (%) sediment	P (%) sediment	Sand (%)	Silt (%)	Clay (%)	ρw
Macrobenthos	**x**	**x**		**x**	**x**	**x**				**0.46**
Polychaetes					**x**	**x**				**0.53**
Polychaetes genetic data		**x**			**x**	**x**				**0.19**
***N. hombergii* genetic data**	**x**								**x**	**0.17**
***H. diversicolor* genetic data**						**x**				**0.74**

diversicolor were found to be genetically variable in most of the works cited above.

Most of the previously mentioned authors focused on the correlation between various metrics of the genetic variability of *H. diversicolor* populations and a single environmental variable, sometimes severely affected by anthropogenic activities. For example, alloenzymes were used in many studies in order to distinguish populations of *H. diversicolor* and correlate their patterns with tolerance to copper exposure (Virgilio and Abbiati, 2004b) and habitat discontinuity in five estuaries from Adriatic Sea (Virgilio and Abbiati, 2004a). Genetic diversity was correlated with tolerance of the organisms to copper exposure, whereas it did not seem to be affected by habitat discontinuity. An attempt to correlate genetic diversity with heavy metals deposits in the tissue of polychaetes was made by Virgilio and colleagues (2006). In this work, the authors used mtDNA and alloenzymes from individuals of H. diversicolor populations from estuarine communities in the Adriatic Sea. However, correlation between heavy metals and genetic diversity was not proved but, instead, genetic fragmentation within estuaries was detected. In contrast, this study examines the correlation of genetic variability with more than one factors through the BIOENV analysis. Salinity was associated with the multivariate pattern deriving from genetic data although with a relatively low correlation value. Reproductive isolation between populations caused by hydrographic barriers has been reported as an alternative in studies on estuarine populations of *H. diversicolor* (Smith, 1964; Fong and Garthwaite, 1994; Virgilio *et al.*, 2006). It has been proved that salinity levels can be optimal for some larvae and simultaneously lethal for other larvae of the same species preventing their dispersal and gene flow between populations (Smith, 1964, 1977). *H. diversicolor* occurs in fluctuating environments as it is able to tolerate variations of salinity and temperature and live under hypoxic conditions (Scaps, 2002).

N. hombergii is a very common species, tolerant to a wide salinity range and limited oxygen conditions. It is mostly found in clayed or muddy sand (Arndt and Schiedek, 1997). The larvae of *N. hombergii* have a planktonic phase (Noyes, 1980) through which they can disperse widely, a fact that enhances the hypothesis of haplotypes with greater similarity. However, there are two alternative hypotheses on the genetic diversity in transitional ecosystems: according to the first hypothesis, genetic diversity in transitional ecosystems is high (Abbiati and Maltagliati,

1992) as a result of organisms' selection and subsequent dominance ("adaptation"). The second hypothesis suggests that in fluctuating conditions there is one genotype which allows survival and dominates against all others (Abbiati and Maltagliati, 1992). The results of this preliminary analysis showed the existence of more than one haplotypes in the same lagoon making the first hypothesis to be more possible, at least for the lagoons under study.

The genetic variability detected in the studied species could be associated to genetic drift exploited by fragmentation of populations (Scaps, 2002). Population size in coastal lagoons is affected by the temporal and spatial variations of environmental variables. Population instability combined with genetic drift could be influencing genetic patterns as a result of microevolutionary process (Virgilio *et al.*, 2006).

The values of haplotype and nucleotide diversity indices were estimated to be high for *N. hombergii*, while indices values for *H. diversicolor* were found to be relatively low. The results suggest that the populations of *N. hombergii* are large and stable (Lowe *et al.*, 2004). On the contrary, populations of *H. diversicolor* maybe have been through genetic bottleneck that was followed from rapid population growth, as well as increase in mutation frequencies (Lowe *et al.*, 2004). This hypothesis can be underpinned by the presence of anoxia in the Mediterranean lagoon. Anoxic crises are induced by the water enrichment in organic matters, exciting the microbial activity which drives in loss of dissolved oxygen in the water column and the sediment. They are almost always followed by massive mortality of the populations inhabiting the lagoons (Lardicci *et al.*, 2001).

The multivariate pattern obtained from non metric MDS analysis of polychaete abundance data was different from the one resulting from polychaete genetic data analysis. In the polychaete abundance pattern the stations were grouped according to disturbance. Stations from areas with more intense pressure tend to be included in the same group. In the pattern deriving from genetic data stations are clustered according to salinity ranges. The analysis of genetic data provides thus a different pattern in which the likely prevailing factors, which are important for the establishment of the species populations, are highlighted. Thus, the deriving pattern suggests that salinity could be affecting settlement of larvae in the substrates.

Results of 2nd stage MDS analysis depicted small distances between patterns deriving from 16S gene data and patterns from polychaetes abundance and morphometric data, compared to the ones between genetic and ntMDS ones. Convergence between patterns may be considered as an indication that genetic diversity data could be used as a surrogate to access the community diversity. Additionally, high correlation between genetic diversity of 16S gene of H. diversicolor with the percentage of phosphorus concentration in the sediment may well imply the possibility of broadening the "*hierarchic-response-to-stress*" hypothesis.

The hypothesis states that patterns deriving from higher taxonomic categories tend to be best correlated with the environmental variables under increasing stress conditions. The relatively high value of the rank correlation coefficient suggests further investigation in order to further clarify the role of the genes to the description of their association with the environment and expands this hypothesis to lower levels of biological organization than species. The latter would be expected under the assumption that although changes are observed at the species level or higher, the mechanisms imposing these changes may well act at the individual or lower levels. The advantage of the molecular level in environmental monitoring, however, is that all organisms encode information in

their genes by the same way and this may provide the potential for more homogeneous, hence predictable, response patterns to environmental degradation. Nevertheless, the correlation between phosphorus concentration and genetic diversity of populations should be verified with experimental approach, as results from field data can be induced by a number of factors.

CONCLUSION

The results of this preliminary study show interrelation between genetic patterns and environmental factors. Even though 16S gene is believed to have a low mutation rate compared to other genes of mtDNA and it is not widely used to distinguish intra-specific variability, it made it possible to detect differences between individuals. Although the results of the current study provide some evidence to accept the first hypothesis, that of the high genetic diversity in the transitional waters, more studies are needed in order to test whether genetic data could be widely used as surrogates to describe community status and its relation with environmental variables in this highly variable ecosystem. Therefore, studies implementing larger sample size and different genes used in population studies like COI may significantly add more information to integrate to the findings of this first approach and further test the "*hierarchic-response-to-stress*" hypothesis.

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