

**Environmental
variations in a
semi-enclosed
embayment**

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Environmental variations in a semi-enclosed embayment (Amvrakikos Gulf, Greece) – reconstructions based on benthic foraminifera abundance and lipid biomarker pattern

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The evolution of environmental changes during the last decades and the impact on the living biomass in the western part of Amvrakikos Gulf was investigated using abundances of benthic foraminifera and lipid biomarker concentrations. These proxies indicated that the gulf has dramatically changed due to eutrophication. Eutrophication has led to a higher productivity, a higher bacterial biomass, shifts towards opportunistic and tolerant benthic foraminifera species (e.g. *B. elongata*, *N. turgida*, *T. agglutinans*, *A. tepida*) and a lower benthic species density. Close to the Preveza Straits (connection between the gulf and the Ionian Sea), the benthic assemblages appeared to be less productive and more diversified under more oxygenated conditions. Sea grass meadows largely contributed to the organic matter at this sampling site. Isorenieratane, chlorobactane and lycopane together with oxygen monitoring data indicated that anoxic (and partly euxinic) conditions prevailed seasonally throughout the western part of the gulf with more severe hypoxia towards the east. Increased surface water temperatures have led to a higher stratification, which reduced oxygen resupply to bottom waters. These developments are reasons for mass mortality events and ecosystem decline observed in Amvrakikos Gulf.

1 Introduction

Coastal development, pollution and a range of anthropogenic activities including extensive agriculture, aquaculture, urban and industrial wastes are main causes of decline and loss of coastal habitats observed over the last decades (Airoldi and Beck, 2007; Diaz and Rosenberg, 2008).

Amvrakikos Gulf, located in north-western Greece, is a semi-enclosed embayment characterized by a complex lagoonal system, extensive delta (Kapsimalis et al., 2005) and a fjord-like oceanographic regime (Ferentinos et al., 2010). The gulf is protected under the international Ramsar Convention as Wetlands of International Importance. In

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addition it is designated as a Special Protection Area (SPA), according to the European Union Directive 79/409/EU and it is included in the Natura2000 Network. Despite the efforts, which have been made for the protection and conservation of this unique area, the gulf is suffering from seasonal hypoxia. The dysoxic/anoxic conditions appeared during the last 20 to 30 yr and have been caused by the excessive use of fertilizers, the increase in animal stocks, intensive fish farming and domestic effluents (Ferentinos et al., 2010; Kountoura and Zacharias, 2011). Recently, in February 2008 the environmental stress in the gulf reached a peak, as documented by a sudden massive mortality of fish in aquaculture rafts in the north-eastern part of the gulf (Ferentinos et al., 2010).

The purpose of the present paper is to illuminate the evolution of the environmental conditions over historic timescales in the Amvrakikos Gulf over the last 50 yr through the study of proxies in the sediment. Benthic foraminifera have been proven useful in the reconstruction of palaeoenvironmental conditions since changes in abiotic and biotic parameters such as salinity, eutrophication, oxygen concentration, substrate, water depth and pollution do modify benthic assemblages (Scott et al., 1979; Jorissen, 1987; Debenay et al., 2005; Murray, 2006). Due to their short reproductive life cycles their study can detect short-term environmental changes like oxygen conditions, organic matter (OM) supply and lithology at the sea bottom (Murray, 2001).

More specific in environments where oxygen depletion in the bottom water occurs, benthic foraminifera populations and their diversity are usually reduced and the assemblages are dominated by dysoxic or seasonally by anoxic tolerant species (Sen Gupta and Machain-Castillo, 1993). The changes in benthic assemblages' characteristics have been used to evaluate the past evolution of oxygen depletion in a wide spectrum of coastal environments (Platon et al., 2005; Filipsson and Nordberg, 2004).

Lipid biomarkers have been used as tracers for human alteration and eutrophication of water bodies (e.g. Naeher et al., 2012; Smittenberg et al., 2004). Specific indicators of severe oxygen depletion are the pigments isorenieratene, chlorobactene and okenone (or related derivatives), which have been used to trace photic zone euxinic

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conditions (e.g. Brocks and Summons, 2003). Apart from oxygen depletion, water column properties such as stability of stratification and salinity can be traced by tetrahymanol and gammacerane (Sinninghe Damsté et al., 1995; Thiel et al., 1997; Bechtel and Schubert, 2009), whereas alkenones have been useful in reconstructing surface water temperatures (Prahl and Wakeham, 1987; Müller et al., 1998).

In this study the combined approach of benthic foraminifera and lipid biomarker proxies was used to characterise environmental changes and the implications for the living biomass during the recent history in Amvrakikos Gulf.

2 Regional setting

Amvrakikos Gulf was formed during the Mid-Quaternary period (ca. 50–11 ka BP) (Kapsimalis et al., 2005; Anastasakis et al., 2007) and is approximately 35 km long and 6–15 km wide (Fig. 1). It is separated from the open Ionian Sea by a beach-barrier complex and is connected to the open sea through a narrow, elongated channel, the Preveza Straits, which is approximately 6 km long, 0.8 to 2.5 km wide and 20 m deep. The delta of the Arachthos and Louros Rivers and associated lagoons are located at the northern border of the gulf.

The water column is stratified during the year with a brackish surface layer and a saline bottom layer (Ferentinos et al., 2010). The surface layer is well oxygenated with concentrations ranging from 7.5 to 9 mg l⁻¹, but the dissolved oxygen content in the bottom water layer only reaches 0–2 mg l⁻¹ during the summer months in the western part and year round in the eastern part of the Gulf (Ferentinos et al., 2010 and unpublished data). Brackish water flows out through the strait with the surface layer, whereas saline water enters the Gulf through the bottom layer. Summer months temperatures and salinity in the surface water ranged between 28.3 and 29.3 °C and between 32.9 and 33.7 psu, respectively. Temperatures in the bottom water ranged between 15.8 and 16.0 °C, whereas the salinity was around 37.7 psu. Seasonal hypoxia has been

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established at the sampling sites since the last 2–3 decades (Kountoura and Zacharias, 2011).

Since the 1970s the gulf has been altered, mostly due to extensive agriculture, aquaculture and urban development, the construction of two dams, which control the run-off of the Arachthos River, and the establishment of oil stations along the southern border of the gulf. Now also the surface water layer suffers occasionally from oxygen depletion (Ferentinos et al., 2010). In 2008, it seemed that high density water filled the deeper parts of the basin and caused uplifting of the anoxic layer leading to a massive fish mortality event (Ferentinos et al., 2010). Based on the testimonies of the local Fisheries Commission, fish mortality events in aquaculture rafts had also been observed in the past (at 1988, 1992 and 1998), although less intense than in 2008.

The sediment in cores Amvr15 and Amvr13 consisted of grey mud. The colour of the sediments in the top 5 cm in each core appeared darker in relation to the rest of the core and was attributed to an increase in the total organic carbon (TOC, Fig. 2). At the sampling site of core Amvr15, seagrass meadows were present on the sediment surface.

3 Methods

Two short sediment cores were retrieved in October 2010 from Amvrakikos Gulf by a KC Kajak sediment core sampler. Core Amvr15 (42 cm long) was collected from an area close to the entrance of the gulf (38°56'53" N, 20°48'31" E) at a water depth of 32 m (Fig. 1). The 30 cm long core Amvr13 was retrieved from the inner part of the gulf (38°59'15" N, 20°51'48" E) at a water depth of 40 m (Fig. 1).

The actual sediment accumulation rates in the cores Amvr13 and Amvr15 were calculated from the vertical distribution of ^{210}Pb , following the constant rate of supply (CRS) model of Appleby and Oldfield (1978). The downcore ^{210}Pb activity was determined through the activity of its α -emitting granddaughter ^{210}Po , assuming secular equilibrium with ^{210}Pb . The supported ^{210}Pb activities, which correspond to sediment

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layers deposited earlier than the last 100–120 yr, were calculated from the vertical profiles of ^{226}Ra published by Tsabaris et al. (2011) in the same area.

Benthic foraminifera were studied on 29 samples from core Amvr15 and 20 samples from core Amvr13. The mean sampling interval for faunal analyses was 1.4 cm in each core. Samples were washed over a 63 μm sieve and dried in an oven. About 200 specimens of benthic foraminifera were picked and identified from each sample; a microsplitter device was used. Each taxon was expressed as a percentage of the total benthic assemblage. An estimation of the species diversity was performed using the $H(s)$ index following the Shannon-Wiener equation (Shannon, 1948; Buzas and Gibson, 1969). The ratio of the number of benthic foraminifera per weight of dry sediment ($>63\ \mu\text{m}$) was used as an index of benthic foraminifera productivity (Blackwelder et al., 1996). Hierarchical cluster analysis (R-mode) performed on 18 benthic foraminifera species and genera which were sufficiently abundant in both cores. The tree diagram was constructed using the Ward's method based on Euclidian distance on SPSS software.

Bulk parameters were analysed and measured as described previously (Naehler et al., 2012): In short, total carbon (TC), total nitrogen (TN) and total organic carbon (TOC) were determined on untreated and decalcified sediment samples, respectively, with errors of up to $\pm 0.2\ \text{wt}\%$ by means of an elemental analyser (Carlo Erba 2500). The total inorganic carbon (TIC) was calculated from the difference between TC and TOC. The isotopic composition of OM ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was analysed by an Isoprime mass spec connected to an elemental analyser (Carlo Erba 2500) The error was $\pm 0.3\ \text{‰}$ and values are reported against the international standards Vienna Pee Dee Belemnite (VPDB, carbon) and air (nitrogen). The chlorin index (CI) and total chlorin concentrations were determined according to (Schubert et al., 2005). For biomarker analysis, the same extraction and treatment produce was used as described in Naehler et al. (2012). An internal standard was added for quantification (α -Cholestane, C_{19} *n*-fatty acid, C_{19} *n*-alcohol) before extraction with MeOH/DCM. After saponification, neutrals were further separated into apolar and polar fractions over NH_2 columns (Hinrichs et al., 2003).

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The polar fraction was derivatised with BSTFA for 1 h at 80 °C. FA were converted into methyl esters with 14 % BF₃/MeOH. FA double bond positions were determined according to Spitzer (1997). A sample aliquot of the polar fraction was desulfurized with Raney-Nickel catalyst (Sinninghe Damsté et al., 1988), followed by hydrogenation for 2 h with PtO₂ as catalyst in a solution of concentrated acetic acid and ethyl acetate (1 : 1, v : v). Instruments and measurement conditions are described in Naeher et al. (2012). Alkenones were quantified on an Agilent 7890A GC system, equipped with an Agilent column (30 m long × 320 μm inner diameter × 0.32 μm film thickness) and a flame ionization detector (FID). The GC oven temperature program was: 70 °C to 180 °C at 40 °C min⁻¹, then to 320 °C at 2 °C min⁻¹ and held for 10 min.

4 Results

4.1 Age model

According to the CRS model, the estimated average sediment accumulation rates in core Amvr15 were 0.6 cm yr⁻¹ and 0.8 cm yr⁻¹ in core Amvr13. Regarding core Amvr15, the estimated rate was in agreement with the one calculated by Tsabaris et al. (2011) from the same area. From the vertical profiles of ²¹⁰Pb, no significant bioturbation was observed. Based on the age models, the cores comprised sediments deposited since 1967 (Amvr15) and 1972 (Amvr13).

4.2 Benthic foraminifera abundance

Benthic foraminifera were present throughout both cores. A total of 127 foraminiferal species were recognized in samples from core Amvr15. The number of benthic foraminifera specimen per sediment was high, except in the intervals 25–30 cm, 10–15 cm, 6–7 cm and 1–2 cm depth (Fig. 3). The *H*(*s*) diversity index ranged between 2.6 and 3.6 and exhibited lower values from around 18 cm to the top of the core. Within

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the upper 3 cm of the core the $H(s)$ index represented small scale oscillation with low values in the dark colour laminae. Furthermore, at around 1 cm the reduction of the $H(s)$ index was accompanied by a reduction of the benthic foraminifera population. A shift of both indices to higher values occurred in the light-coloured muddy sediments at the core top. The downcore variation of the abundances of selected taxa is shown in Fig. 3. Benthic foraminifera assemblages consisted of highly diversified porcelaneous (*Quinqueloculina seminulum*, *Q. oblonga*, *Q. laevigata*, *Q. stelligera*, *Q. lata*, *Q. subpoezana*, *Miliolinella subrotunda*, *Triloculina* spp.) and hyaline (*Rosalina globularis*, *Discorbis* spp., *Planorbulina mediterraneensis*, *Cibicides* spp.) epifauna (Murray, 2006). Infauna was represented by *Bulimina aculeata*, *Bolivina dilatata*, *B. spathulata*, *Ammonia beccarii* and *A. tepida*, *Nonion depressum*, *Nonionella turgida* and *N. bradii* (Murray, 2006). Epifauna species dominated the benthic assemblages between 20 and 42 cm. The increased participation of diverse *Quinqueloculina* spp., *Rosalina* spp., *Cibicides* spp. together with *P. mediterraneensis* and other epifauna species may be attributed to the presence of seagrass meadows colonized locally on the coring site (Murray, 2001; Mateu-Vicens et al., 2010). Furthermore, the presence of *Cibicides* spp. could also be correlated to the hydrodynamic regime at the coring site since high abundances of this taxon are related to high current velocities (Szarek et al., 2006). Infaunal species showed increased abundances in the upper 20 cm and *B. aculeata* dominated the benthic assemblages with up to 40 % of the total association. This taxon occurs “predominantly surficial” and reacts quickly to labile OM supply (Mojtahid et al., 2010).

A total of 77 foraminiferal species were recognized in samples from core Amvr13 (Fig. 4). The $H(s)$ diversity index ranged between 1.8 and 2.7 and exhibited lower values at around 20 cm and 1 cm depth. The number of benthic foraminifera specimen per sediment was generally higher than in core Amvr15. Lower values were observed at around 20 cm, 17 cm, 7 cm and 3 cm (Fig. 4). Shallow and deep infaunal species dominated the benthic assemblages almost throughout the core, including high abundances of *Bulimina elongata*, *B. aculeata*, *B. dilatata*, *B. spathulata*, *Hopkinsina pacifica*, *A. tepida*, *N. turgida* and *N. bradii*. All these species have been reported as common in

shelf environments, associated with high contents of OM, and being stress-tolerant taxa (Jorissen, 1987; Barmawidjaja et al., 1995; van der Zwaan, 2000; Mendes et al., 2004; Murray, 2006). The agglutinated species included mostly *Textularia conica* and *T. agglutinans* and the porcelaneous included Miliolids. *Quinqueloculina* spp. and *Miliolinella* spp. showed similar fluctuations and higher abundances at around 25 cm, 15 cm and 8 cm.

4.3 Bulk parameters and biomarkers

The TOC profile of core Amvr13 increased slightly towards the core top (1.3–2.7 wt %; Fig. 2). In core Amvr15, the TOC was higher and increased stronger towards the surface (1.4–6.1 wt %; Fig. 2). TN and $\delta^{15}\text{N}$ values were also higher in core Amvr15 and increased in both cores towards the sediment top (Fig. 2). Whereas chlorin concentrations in core Amvr15 were constant throughout the core with 0.5 mg g^{-1} TOC, core Amvr13 showed a strong increase in concentrations from 0.5 mg g^{-1} TOC to a maximum concentration of 2.4 mg g^{-1} TOC over the last 8 yr (Fig. 2). The CI was slightly lower indicating fresher OM material throughout core Amvr15 compared to Amvr13 and decreased towards the core top (Fig. 2). For comparison, CI values from the Swiss lake Rotsee were added, which were similar to CI values in core Amvr15 (Fig. 2).

The atomic C/N ratio was lower in core Amvr13 than in core Amvr15 with values slightly below 10 and 13–16, respectively. In 2–3 cm (2006–2008) and 18–20 cm (1985–1988) of core Amvr13 the total nitrogen concentrations were below the detection limit, which hindered the calculation of the C/N ratios. In core Amvr15 below 20 cm (before 1977) C/N values ranged between 7 and 27 (Fig. 2). While the $\delta^{13}\text{C}_{\text{TOC}}$ remained almost constant throughout both cores with on average about 3–4‰ higher values in core Amvr15.

The profiles of branched alkanes and isoprenoids were very similar to hopanoids in both cores (Fig. 5); all three are bacterial biomarkers (Rohmer et al., 1984; Summons et al., 2007). These lipids were relatively constant throughout core Amvr15, whereas

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in core Amvr13 they were most abundant in 28–29 cm (1974–1975) and in the upper 10 cm (since about 1998) and lowest in 12–14 cm (1993–1995) (Fig. 5).

Short chain *n*-alcohols and phytol increased quite continuously towards the surface sediment (Fig. 5). Short chain *n*-alcohols and phytol mainly originate from phytoplanktonic sources (Meyers and Ishiwatari, 1993; Rontani and Volkman, 2003). Dinosterol (4,23,24-trimethylcholest-22E-en-3 β -ol, 4-Me 30 Δ 22; Fig. 5) which also increased towards the surface sediment is of diatom origin in Amvrakikos Gulf, because C_{22:6}FA, which would hint to dinoflagellates as the source organisms is absent (Volkman, 2003; Withers, 1983). In contrast, β -sitosterol (24-ethylcholest-7-en-3 β -ol, C_{29:1} Δ 7; Fig. 5) with a similar concentration profile as dinosterol could not be assigned to a single source but has its origin in phytoplankton, higher land plants and emerged macrophytes (Volkman, 1986). The P_{aq} proxy is an indicator for macrophytes (Ficken et al., 2000) and ranged between 0.2 and 0.6 in both cores (Fig. 5).

Tetrahymanol is found in bacteriovoric ciliates and was often used as a stratification, stagnation and/or salinity indicator (Sinninghe Damsté et al., 1995; Bechtel and Schubert, 2009; Thiel et al., 1997). While it was relatively constant throughout core Amv15 and in the lower part of Amvr13, it increased in the most recent decade in Amvr13 (Fig. 5).

After hydrogenation, traces of isorenieratane and chlorobactane were found throughout both cores, except the lowermost sample of core Amvr13, in which chlorobactane could not be detected. Both pigments are specifically derived from phototrophic sulphur bacteria (Chlorobiaceae) and have been used as tracers for photic zone euxinia and anoxia (Brocks and Summons, 2003). Lycopane concentrations and the (lycopane + C₃₅*n*-alkane)/C₃₁*n*-alkane ratio remained constant in core Amvr15, whereas both parameters increased with depth in core Amvr13 (Fig. 6). Although the source of lycopane is unknown, it has been used to reconstruct palaeo-redox conditions in the bottom water in marine settings (Sinninghe Damsté et al., 2003; Wakeham et al., 1993).

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Alkenones were determined in both cores. These markers are specifically derived from haptophyte algae (Herbert, 2003). The UK'₃₇ index ($\text{Me C}_{37:2} / [\text{Me C}_{37:2} + \text{Me C}_{37:3}]$; by Prahl and Wakeham, 1987) is an alkenone based proxy, which is highly correlated with mean annual sea surface temperatures (Prahl et al., 1988; Müller et al., 1998). This index showed increasing values towards the top of both cores, between 0.61 and 0.84 in core Amvr15 and 0.63 and 0.78 in core Amvr13.

5 Discussion

5.1 Benthic foraminifera clusters

Cluster analysis (R-mode) revealed four clusters (Fig. 8). Cluster I was composed of *B. elongata*, *N. turgida*, *T. agglutinans* and *A. tepida* (Fig. 8). In many studies *N. turgida* appeared as the most tolerant species to oxygen depletion and its increase in abundance is associated with enhanced OM supply (Sen Gupta and Machain-Castillo, 1993; Blackwelder et al., 1996), usually of terrestrial origin (Mojtahid et al., 2010; Goineau et al., 2011). *A. tepida* is considered as a species, which is tolerant to large environmental variations (Almogi-Labin et al., 1992; Debenay and Guillou, 2002), including hypoxia (Blackwelder et al., 1996) and anthropogenic pollution (Debenay et al., 2005). *B. elongata* is associated with food-enriched sediments related to river plumes (Guadiana River, Iberia; Mendes et al., 2004), closed embayment regimes (Yugoslavia; Murray, 2001) and fish farming products (Croatia; Vidović et al., 2009). *T. agglutinans* is an opportunistic species and exhibits a preference for food-enriched conditions and a tolerance to oxygen deficiency (Barmawidjaja et al., 1995). Furthermore, *N. turgida* (as *N. opima*) and *T. agglutinans* are major species correlated with a high OM content at areas under the influence of Po River in Adriatic Sea (Jorissen, 1987; Murray, 2001).

Cluster II was composed of *H. pacifica*, *B. dilatata* and *B. spathulata* (Fig. 8). All these species are known to participate in benthic assemblages in oxygen poor and organic

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rich environments (Sen Gupta and Machain-Castillo, 1993; Murray, 2006). However their low degree of opportunism (Barmawidjaja et al., 1995) in combination of their presence in areas of a river-influenced outer shelf (*Bolivina* spp.; Goineau et al., 2011) and of well oxygenated substrates (Hyams-Kaphzan et al., 2009) suggests that Cluster II should represent benthic foraminiferal associations in lower stress environments than these of Cluster I.

Cluster III was composed of *Quinqueloculina* spp., *Miliolinella* spp., other porcelaneous, *Textularia conica* and *P. mediterraneensis* (Fig. 8). High abundances of *Quinqueloculina* spp. and in general Miliolids were found in oligotrophic environments and at sufficient bottom water oxygen concentrations (Murray, 2006; Blackwelder et al., 1996; Hyams-Kaphzan et al., 2009). Furthermore, the reduction in the abundance of both porcelaneous and agglutinated groups of benthic foraminifera was used to trace palaeo-hypoxic evolution (Platon et al., 2005).

Cluster IV was composed of *Cassidulina* spp., *Nonion* spp., *Cibicides* spp., *Rosalina* spp., *B. aculeata* and *A. beccarii* (Fig. 8). As mentioned previously, species belonging to the genera of *Cibicides*, *Rosalina*, *Nonion* and *Ammonia* are known as epiphytic in the Mediterranean Sea (Murray, 2001). However, *B. aculeata* and *A. beccarii* have been reported in high abundances on the seaward part of shelves with high OM supply (Goineau et al., 2011; Debenay et al., 2005). A similar trend exhibited species belonging to the genera of *Cibicides* and *Rosalina* which have been reported in high abundances seaward on shelves under the influence of aquaculture products (Croatia; Vidović et al., 2009).

The downcore fluctuations compared to the upper core in the abundances of the benthic species of Cluster I could be used to trace high OM supply and oxygen depletion. Cluster II would represent similar environments, but under lower stress conditions. The abundances of the benthic foraminifera species of Cluster III could be used to trace environments of well oxygenated conditions and that of Cluster IV of similar environments, but probably influenced by higher marine OM input and/or seagrass development.

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5.2 Eutrophication, productivity and OM sources

The TOC increased in both cores with time (Fig. 2), indicating a higher OM supply recently. This can be explained by an increased productivity, which is supported by the increase in TN values in both cores (Fig. 2). This interpretation agrees with the higher abundance of Cluster I species in core Amvr13 and Cluster IV species in core Amvr15, which indicated a larger OM input (Fig. 9) (Sen Gupta and Machain-Castillo, 1993; Blackwelder et al., 1996; Goineau et al., 2011; Debenay et al., 2005). Also other processes like oxygen availability or degree of opportunism can affect the abundance of these species (Sen Gupta and Machain-Castillo, 1993; Murray, 2006; Barmawidjaja et al., 1995), which might explain that Cluster II species did not increase. Many biomarkers also traced an enhanced productivity with time, for instance by the increase in short chain *n*-alcohols, phytol and dinosterol towards the uppermost sediment in both cores (Fig. 2). These markers indicated an increased abundance of primary producers (Meyers and Ishiwatari, 1993; Rontani and Volkman, 2003; Volkman, 2003). The higher productivity was a direct result of progressive eutrophication in the gulf, as suggested by increasing $\delta^{15}\text{N}$ values, which have been used to reconstruct sewage supply and eutrophication in other settings (Cole et al., 2004; Wu et al., 2006). The higher chlorin concentrations in core Amvr13 within the most recent decade (Fig. 2) agrees with this explanation. In contrast, the constant chlorin levels throughout core Amvr15 indicated that productivity hardly changed at this site.

The atomic C/N ratio below 10 (Fig. 2) indicated that the OM in core Amvr13 mainly originated from algal sources, in agreement with observations in other settings (Meyers and Ishiwatari, 1993). In contrast, the higher C/N ratios in core Amvr15 suggested significant supply of plant derived OM sources (Fig. 2). The constant $\delta^{13}\text{C}_{\text{TOC}}$ values throughout both cores indicated that the OM source remained constant during the last decades. The $\delta^{13}\text{C}_{\text{TOC}}$ values in Amvr15 (average: -18.6% ; Fig. 2) are typical for marine organic matter, whereas terrigenous OM sources average at -26% (Sackett, 1964; Jasper and Gagosian, 1990). The lower $\delta^{13}\text{C}_{\text{TOC}}$ values in Amvr13

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(average: -22.6% ; Fig. 2) might indicate mixed marine and terrigenous sources, but this explanation disagrees with the low C/N values (Fig. 2), which are characteristic for a predominantly marine OM origin (Meyers and Ishiwatari, 1993). Therefore, the lower $\delta^{13}\text{C}_{\text{TOC}}$ values in Amvr13 were more likely the result of mixing and uptake of dissolved inorganic carbon derived from OM degradation, which can lead to isotopic shifts towards depleted values in eutrophic systems (van Breugel et al., 2006). This would mean that Amvrakikos Gulf is not in equilibrium with the atmosphere. Therefore, complicating factors that can affect the $\delta^{13}\text{C}_{\text{TOC}}$ like the Suess effect (Meyers, 2006; McCarroll and Loader, 2004) are expected to be of minor importance in Amvrakikos Gulf.

The relatively enriched $\delta^{13}\text{C}_{\text{TOC}}$ values in core Amvr15 most likely resulted from additional OM input by seagrass meadows, which were present at this sampling location. In the western Mediterranean Sea it was shown that the presence of seagrass meadows led to an overprint of the OM in the sediment (Papadimitriou et al., 2005). $\delta^{13}\text{C}_{\text{TOC}}$ values were enriched at sites with seagrass meadows compared to sites with predominant phytoplankton derived OM sources by 4–6‰, which is well within the offset observed between cores Amvr15 and Amvr13. Furthermore, seagrass meadows can lead to 2–3‰ depleted $\delta^{15}\text{N}$ values (Papadimitriou et al., 2005), which would also explain lower $\delta^{15}\text{N}$ values in Amvr15.

β -Sitosterol is the major sterol of emerged macrophytes (Volkman, 1986). Therefore, seagrass might be a main source of this marker at least in Amvr15 (Fig. 5). The high P_{aq} index above 0.2 in both cores (Fig. 5) traced the predominance of mid chain over long chain *n*-alkanes (Ficken et al., 2000), which indicated that macrophytes are predominant lipid sources compared to higher land plant sources.

The disagreement between constant chlorin concentrations and the increase of many lipid biomarkers in Amvr15 (for instance short chain *n*-alcohols, phytol, dinosterol, β -sitosterol; Figs. 2 and 5) indicated that degradation also affected the biomarker profiles. The CI is an estimate of the OM freshness (Schubert et al., 2005). CI values obtained in the gulf sediment indicated a lower degree of degradation in the uppermost

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part of both cores (Fig. 2). The CI profile in core Amvr15 was almost identical with the profile obtained in the Swiss lake Rotsee (Naeher et al., 2012), suggesting similar degradation rates at both settings. The CI values were higher in core Amvr13 (Fig. 2), indicating higher degradation rates at this location. These results suggest that the increase in concentration of many biomarkers (for instance short chain *n*-alcohols, phytol, dinosterol, β -sitosterol; Fig. 5) are also affected by degradation. In agreement with the constant chlorin concentration values in Amvr15 (Fig. 2), the slight biomarker concentration decrease in this core might be due to degradation. But the much higher increase in biomarker concentrations in Amvr13 (for instance short chain *n*-alcohols, phytol, dinosterol, β -sitosterol; Fig. 5) must be mainly due to a higher productivity, which is supported in the increase in chlorin concentrations in this core (Fig. 2).

5.3 Impact of eutrophication on benthic foraminifera and bacteria – hypoxia reconstructions in the gulf

The benthic assemblages increase in population was associated with a decreasing diversity as depicted by the high negative correlation between the $H(s)$ and the benthic productivity indices (Pearson coefficient $r = -0.71$) for the entire dataset of the cores. This is in contrast to the usual trend of microfauna which appeared less diverse and less abundant in stressful, fluctuating environments (Blackwelder et al., 1996). However, the sediments of the gulf are under the influence of fish farming and urban waste. Eutrophication can lead to an increase in benthic foraminifera density (Angel et al., 2000) in conjunction to a decrease of diversity where the opportunistic species are dominant (Debenay et al., 2005). Therefore, the summed abundances of the benthic species of Clusters I and II showed high positive correlation with the benthic productivity index (Pearson coefficient $r = 0.83$) and high negative correlation with the diversity index ($r = -0.87$) for the entire dataset. The opposite trend presents the summed abundances of the benthic species of Clusters III and IV, which showed high positive correlation with the diversity index (Pearson coefficient $r = 0.72$) and negative correlation with the benthic productivity index ($r = -0.74$) for the entire dataset.

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The benthic foraminifera in core Amvr13 represent low diversity, but high abundance assemblages (Fig. 9). The coring site of core Amvr13 is under the influence of large OM supply and stratified water masses promoting the development of low oxygen bottom water. This bottom-water environment is suggested by the dominance of species of Cluster I and II over those of Cluster III and IV. Furthermore, fluctuations in the abundances of the four clusters in conjunction with fluctuations in the benthic abundance and diversity index indicate fluctuating sea bottom environmental changes for the last 35 yr. The development of more unfavourable benthic environments occurred at around 1985–1988, 1994–1997 and after 2000, as shown by decreases of Cluster III and increases of Cluster I (Fig. 9).

Around 1980 and since the end of the 1990s, a higher bacterial biomass was observed in the same core, as indicated by higher concentrations of branched alkanes, isoprenoids and hopanoids (Fig. 5). The profiles of these markers were especially similar to *B. aculeata* (Fig. 3), which is indicative of a higher supply of OM and severe oxygen depletion. These results suggest that the higher supply of OM has led to increased OM mineralisation and oxygen consumption rates. These developments might be related to aquacultures, which started around 1980. They excessively supplied nutrients and OM to the gulf, which was a main reason for eutrophication (Kountoura and Zacharias, 2011; Ferentinos et al., 2010). Furthermore, the testimonies of the fishermen often refer to a large number of fish deaths and reduction of fish populations in aquaculture rafts between 1988 and 1997.

The most stressful conditions started at around 2000, as recorded by a gradual increase in the abundance of Cluster I over Cluster III. These conditions peaked with the almost absence of Cluster III in 2008 at the time of the recently recorded seasonal hypoxic event in the gulf. However, the rapid increase of Cluster III and the $H(s)$ index shortly after the event suggests a fast recovery of the benthic environment (Fig. 9). These conditions starting at around 2000 and peaking in 2008 were already indicated by higher concentrations of bacterial biomarkers since the mid/end 1990s (Fig. 5). These increased concentrations were in good agreement with the intensified

productivity (Sect. 5.2) and the resulting higher OM supply to the sediment, which had the same implications as during the 1980s.

In contrast, the constant abundance of chlorins and lower concentrations of bacterial markers throughout core Amvr15 indicated less severe conditions than at site Amvr13 without significant changes in productivity and OM supply (Figs. 2 and 5). Benthic assemblages at site Amvr15 appeared to be less productive and more diversified than those of core Amvr13. The dominance of Cluster III and IV species (Fig. 9) suggested lower OM supply, hence, higher sea floor oxygenation. This can be attributed to the location of site Amvr15 at the entrance of the gulf characterized by sufficient oxygen supply due to the water replenishment from the Ionian Sea (Ferentinos et al., 2010). Tziavos and Vouloumanos (1994) also reported a reduction of the benthic diversity eastwards in the surface sediments of the gulf. The observation of seagrass meadows at site Amvr15 and their absence at site Amvr13 further supports the lower impact of eutrophication (Green and Short, 2003).

More opportunistic species of Cluster IV replaced Cluster III species at site Amvr15 during times of nutrient enrichment and oxygen depletion (Fig. 9). This was especially the case between 1976 and 1980, the time interval when aquaculture development started. However, the reduction of both Clusters III and IV at 1985–1987 and 1995–1997 (Fig. 9) and a higher bacterial biomass around 1980 (Fig. 5) coincided with relative changes at site Amvr13, which suggested similar control mechanisms and time synchronicity in the bottom water oxygen regime between the two coring sites. Furthermore, similar to site Amvr13, the most severe sea floor conditions in respect to oxygen at site Amvr15 appeared in 2008 as suggested by the almost absence of Cluster III and a higher bacterial biomass around that time (Figs. 5 and 9). This indicates that the effects of that hypoxic event did not only influence the benthic fauna of the inner part of the gulf, but was also spread in areas which are considered as throughout the year being well oxygenated. However the impact of hypoxia on the benthic microfauna at the bottom of the gulf entrance was less intense than that occurred in the inner part

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of the gulf, since species considered being sensitive to hypoxia (Cluster IV) were still highly abundant (Fig. 9) and seagrass meadows were also present at this site.

Furthermore, the more severe oxygen depletion at Amvr13 compared to Amvr15 was also indicated by the increase of the (lycopane + C₃₅n-alkane)/C₃₁n-alkane ratio with depth (Fig. 6). In contrast, the quite constant ratio in core Amvr15 (Fig. 6) suggested more oxygenated conditions.

Isorenieratane and chlorobactane were used as tracers for phototrophic sulfur bacteria (Chlorobiaceae) and phototrophic zone euxinia and anoxia (Brocks and Summons, 2003). The observation of these carotenoids throughout both cores indicated regularly occurring developments of anoxic and euxinic conditions reaching into the photic zone at both sites. The monitoring data showed that oxygen depletion has occurred seasonally during summer and fall in the western part of the gulf (Ferentinos et al., 2010; Kountoura and Zacharias, 2011). Due to the low concentrations of these pigments in the sediment of both cores only the existence of photic zone anoxia but no temporal changes could be reconstructed.

5.4 Impact of climate on stratification and oxygen replenishment

Stratification is another key factor in the oxygen budget of the gulf water column, because oxygen is resupplied by mixing. The relatively constant tetrahymanol concentrations in core Amvr15 (Fig. 5) suggested that stratification did not change since the 1970s. In contrast, the increase of tetrahymanol in the uppermost part of core Amvr13 (Fig. 5) clearly showed a higher stratification and stagnation, probably due to higher water temperatures and/or salinities in the gulf. To prove these relationships, sedimentary proxies were compared with monitoring data.

The UK'₃₇ index ($\text{Me C}_{37:2} / [\text{Me C}_{37:2} + \text{Me C}_{37:3}]$; by Prahl and Wakeham, 1987) was used to estimate surface water temperatures in Amvrakikos Gulf. The calibration of Prahl et al. (1988) yielded values between 16.9 and 23.6°C and between 17.4 and 21.9°C in cores Amvr15 and Amvr13, respectively. For comparison, the temperature calibration by Müller et al. (1998) which is based on sediment core top samples

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worldwide yielded very similar results (16.5–23.4 °C in core Amvr15 and 17.0–21.7 °C in Amvr13; Fig. 7). The difference between estimated surface water temperatures using both calibrations was only up to 0.4 °C.

The temperature estimates from both cores indicated a trend with increased surface water temperatures, which matched well with increasing air temperatures observed at the Preveza (Aktio) weather station during the last decades (Fig. 7). The UK₃₇ derived surface water temperatures in both cores (16.5–23.4 °C, 1974–2010; Fig. 7) were within the range of monitored average annual minimum and maximum air temperatures with values between about 12 and 22 °C (1970–2010), except the uppermost part of core Amvr15 with larger UK₃₇ temperature estimates. The annual average air temperatures ranged between 16.2 and 18.0 °C (1970–2010), and were lower than estimated UK₃₇ temperatures (Fig. 7). Although the timing of blooms of the source organisms is unknown for Amvrakikos Gulf, the best agreement between UK₃₇ derived and monitored temperatures was obtained if the average temperature of March until August was used. The air temperatures ranged from 18.2 to 21.1 °C (1970–2010; Fig. 7). The monitoring data showed that especially the annual minimum temperatures increased during the last decades, whereas the annual maximum temperatures hardly increased. By using only monthly average minimum temperature data, the best fit was obtained with the average minimum temperature data of June and July with a range of 16.8–21.2 °C (Fig. 7). These results indicated that alkenones might have captured the lowermost surface water temperatures during June and July. In contrast, this and other calibrations showed strong correlations either with annual average temperatures or seasonal average temperatures at times of blooms (Herbert, 2003; Müller et al., 1998; Prah1 et al., 1988). Therefore, a shift of the time of blooms from spring towards summer is more likely, which would also explain the large increase in estimated temperatures during the last decades.

Nonetheless, the warming trend of UK₃₇ derived temperatures together with the monitoring data indicated that the surface water temperatures in Amvrakikos Gulf increased during the last decades. Previous studies showed that steep temperature

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gradients separate surface and bottom waters (Ferentinos et al., 2010). Higher temperatures in the surface water may further increase these differences, which can explain the observation of a higher stratification during the last years.

Furthermore, also salinity controls stratification by steep salinity gradients in the water column due to the inflow of high salinity water masses through the Preveza Straits from the Ionian Sea and brackish water outflow (Ferentinos et al., 2010). This circulation pattern is similar to the Black Sea, but the reduced outflow of the Black Sea prevents the reduction of its permanent stratification (Murray et al., 2007; Ozsoy and Unluata, 1997), which is not the case for Amvrakikos Gulf (Ferentinos et al., 2010). However, the similar developments regarding circulation patterns, eutrophication and temperature have led to a strong decrease in oxygen concentrations, which resulted in mass mortality events and ecosystem collapse in both settings (Ferentinos et al., 2010; Kountoura and Zacharias, 2011; Lancelot et al., 2002; Mee et al., 2005).

6 Conclusions

The analysis of benthic foraminifera and lipid biomarkers revealed that Amvrakikos Gulf exhibited dramatic environmental changes due to eutrophication during the last decades. The higher productivity and OM supply to the sediment (higher concentrations of chlorins, TOC, TN and $\delta^{15}\text{N}$ values) led to a higher abundance of tolerant and opportunistic benthic species and bacteria, whereas the benthic species density decreased. Especially the increased abundance of Cluster I (*B. elongata*, *N. turgida*, *T. agglutinans*, *A. tepida*) over Cluster III species (*Quinqueloculina* spp., *Miliolinella* spp., other porcelaneous, *Textularia conica*, *P. mediterraneensis*) indicated more severe OM supply and oxygen depletion in 1976, 1980, 1985–1987, 1995–1997, 2000 and 2008. Cluster III and IV species (Cluster IV: *Cassidulina* spp., *Nonion* spp., *Cibicides* spp., *Rosalina* spp., *B. aculeata*, *A. beccarii*) rapidly recovered after environmental disturbances. In core Amvr15 the benthic assemblages appeared to be less productive and more diversified with a dominance of species of Clusters III and IV under conditions of

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lower OM supply and higher bottom water oxygen concentrations than in core Amvr13. The presence of seagrass at site Amvr15 largely influenced the values of $\delta^{13}\text{C}_{\text{TOC}}$, C/N ratio and mid chain *n*-alkanes. Nonetheless, the presence of isorenieratane and chlorobactane in both cores traced temporarily photic zone euxinic conditions throughout the gulf. The increasing air temperatures have led to stronger stratification and hence oxygen depletion during the last decade.

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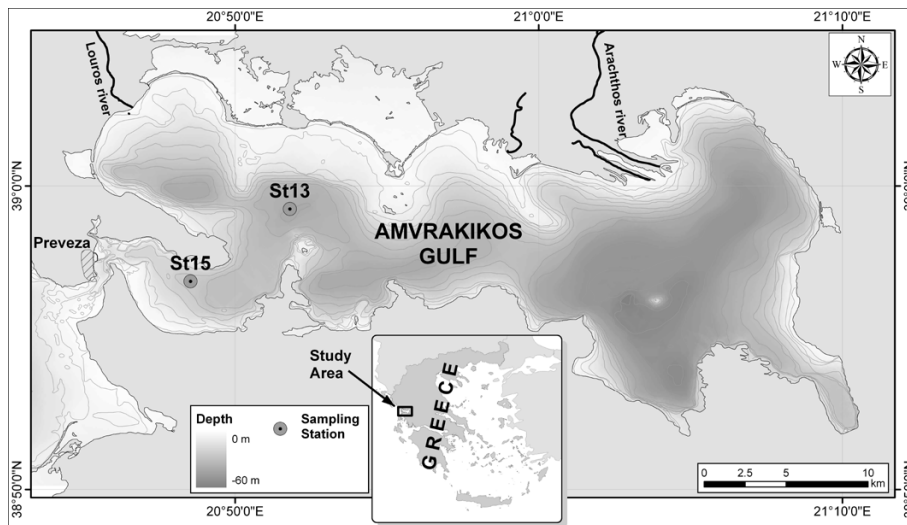


Fig. 1. Map of Amvrakikos Gulf, Greece. The major rivers (Louros and Arachthos Rivers), the Preveza Straits (connection with the Ionian Sea) and the sampling stations of cores Amvr13 and Amvr15 are illustrated.

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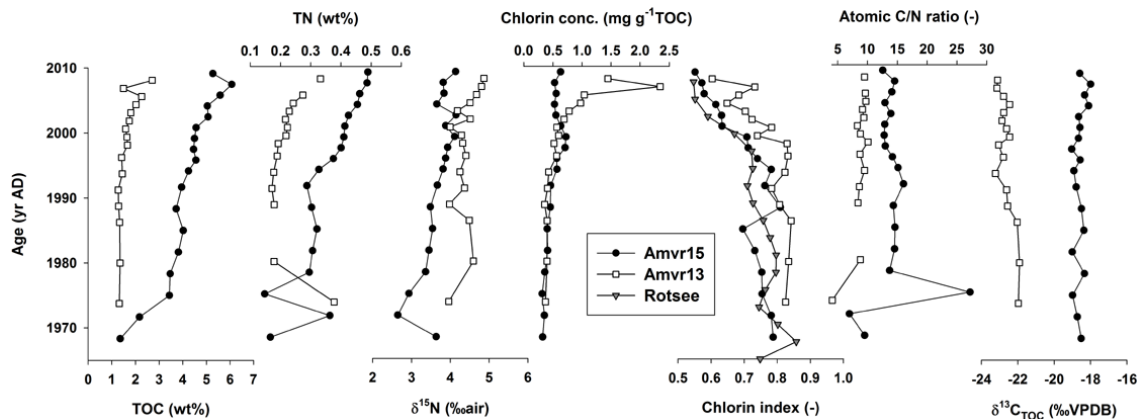


Fig. 2. Bulk parameters of cores Amvr13 and Amvr15 plotted vs. age (yr AD), including the concentrations of total organic carbon (TOC), total nitrogen (TN), nitrogen isotopic composition ($\delta^{15}\text{N}$, ‰air), chlorin concentrations ($\text{mg g}^{-1}\text{TOC}$), chlorin index (CI, including values from the Swiss lake Rotsee, Naeher et al., 2012), the atomic C/N ratio and the TOC isotopic composition ($\delta^{13}\text{C}_{\text{TOC}}$, ‰VPDB).

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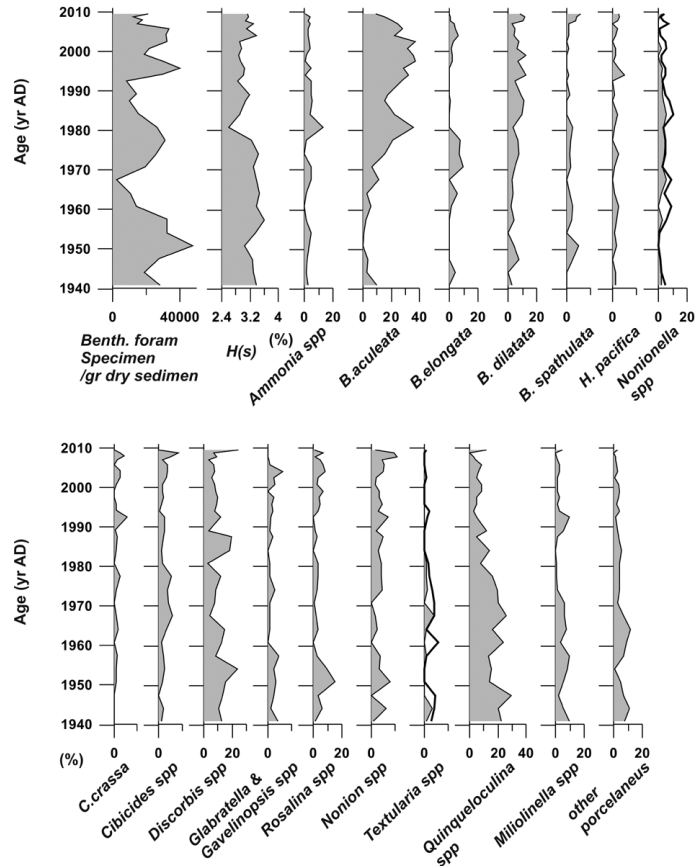


Fig. 3. Downcore abundance variations of selected benthic foraminifera in core Amvr15 versus sediment depth together with the indices of benthic productivity (benthic foraminifera specimen/g of dry sediment) and diversity ($H(s)$). Grey bands in the diagrams of *Textularia* spp. and *Nonionella* spp. indicate the participation of *T. agglutinans* and *N. turgida*, respectively.

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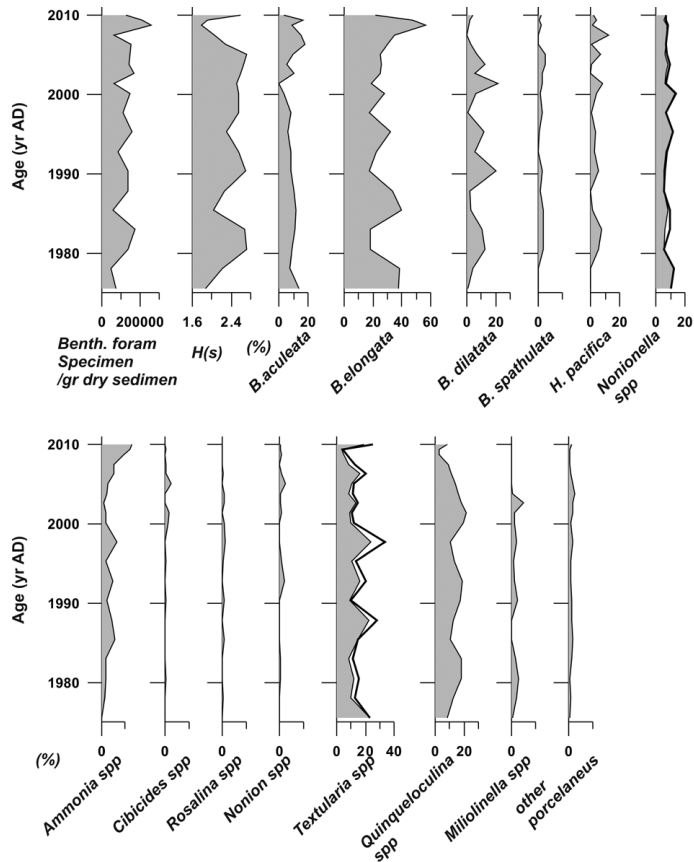


Fig. 4. Downcore abundance variations of selected benthic foraminifera in core Amvr13 versus sediment depth, together with the indices of benthic productivity (benthic foraminifera specimen/g of dry sediment) and diversity ($H(s)$). Grey bands in the diagrams of *Textularia* spp. and *Nonionella* spp. indicate the participation of *T. agglutinans* and *N. turgida*, respectively.

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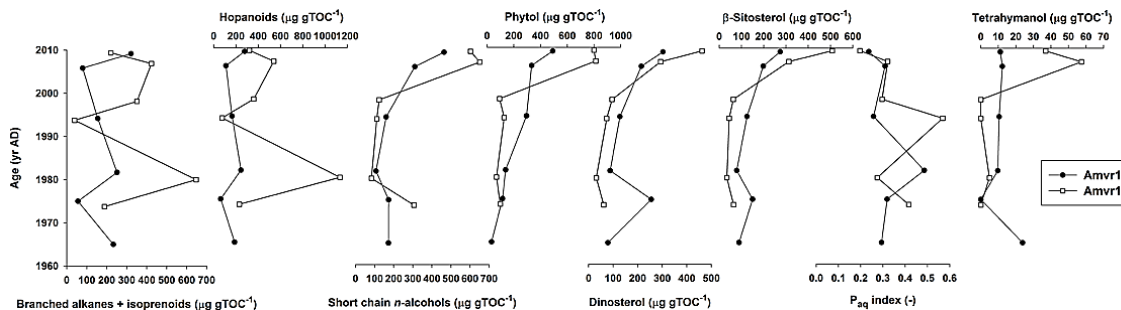


Fig. 5. Concentrations ($\mu\text{g g}^{-1}\text{ TOC}$) of the sums of branched alkanes/isoprenoids, hopanoids, short chain *n*-alcohols (C_{11} – C_{20}), phytol, dinosterol, β -sitosterol, P_{aq} index (by Ficken et al., 2000) and tetrahymanol plotted vs. age (yr AD).

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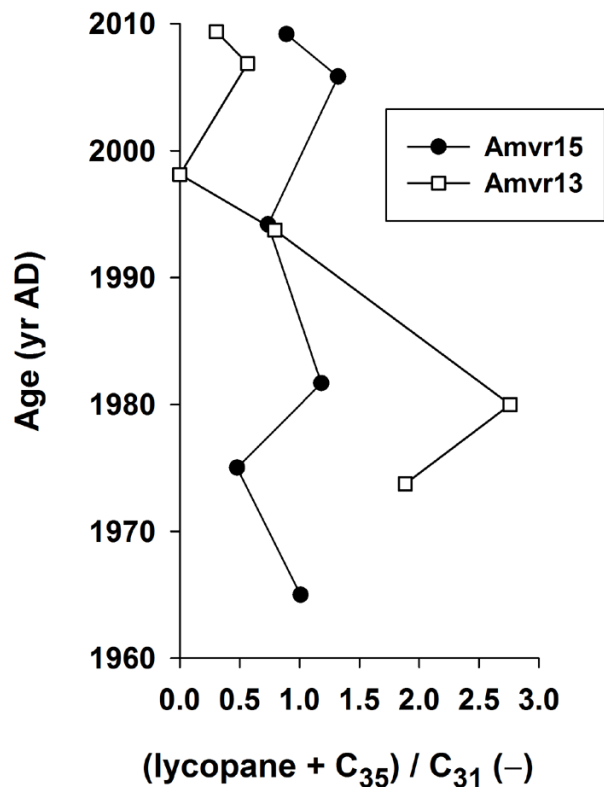


Fig. 6. Profiles of $(\text{lycopane} + C_{35}n\text{-alkane})/C_{31}n\text{-alkane}$ ratio vs. age (yr AD).

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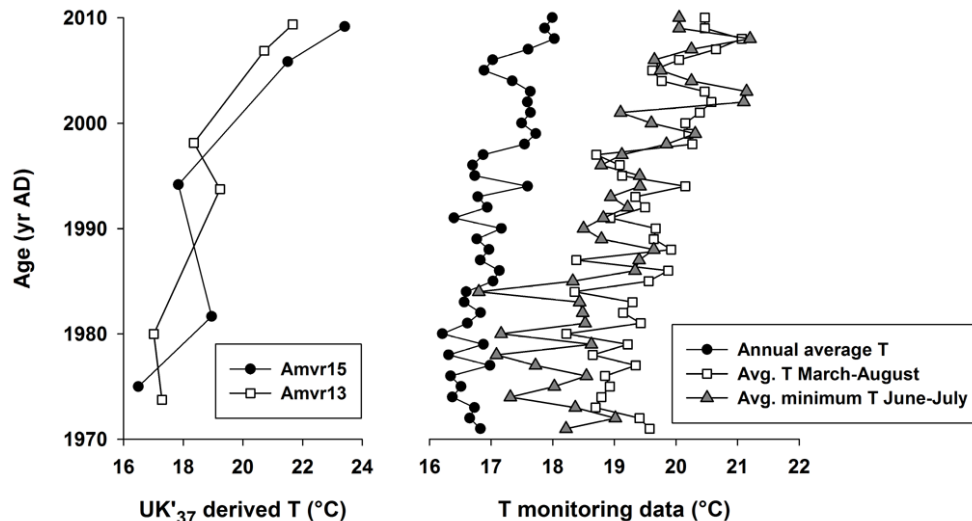


Fig. 7. UK'37 index derived surface water temperatures (left) according to the correlation of Müller et al. (1998), $UK'_{37} = 0.033 T + 0.069$ ($R^2 = 0.981$) with T = mean annual sea surface temperature, plotted vs. age (yr AD). For comparison, temperature data (°C) between 1970 and 2010 from the meteorological station Preveza (Aktio) were added: Annual average temperatures, average temperatures of March–August, average minimum temperatures of June–July.

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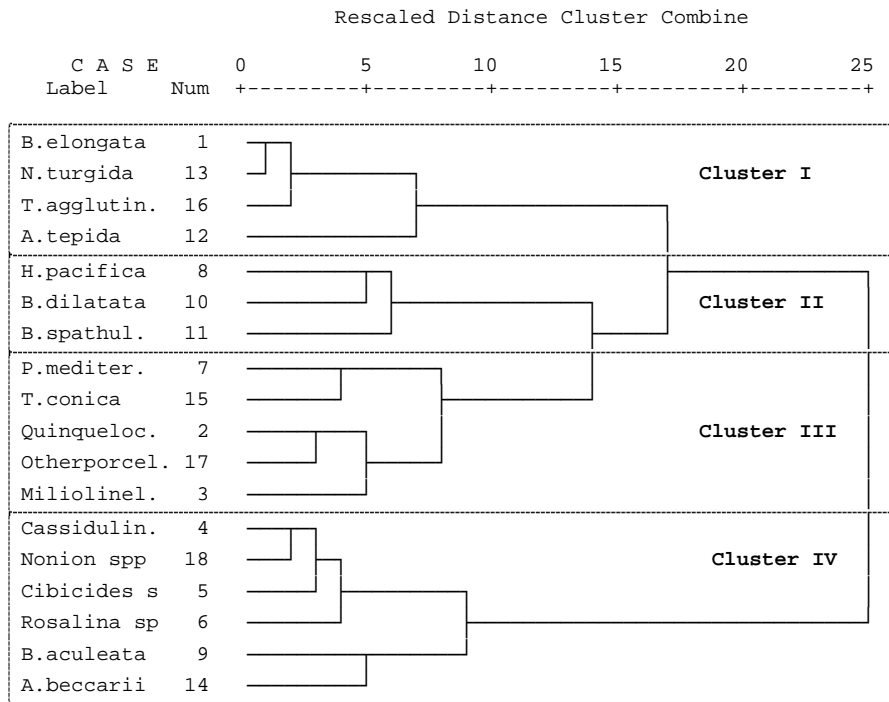


Fig. 8. R-mode cluster analysis for the entire dataset of the two cores.

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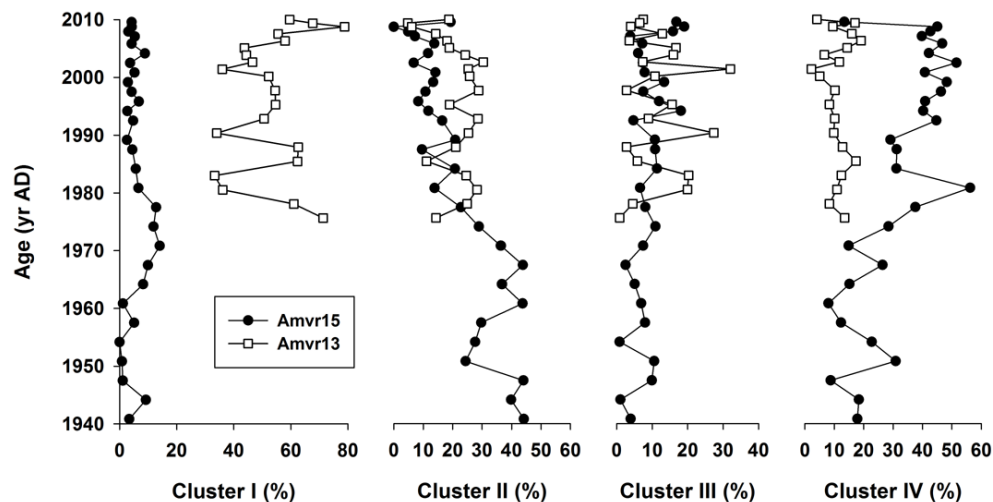


Fig. 9. Downcore variations of the sum abundances of the benthic foraminifera clusters (I–IV) in cores Amvr13 and Amvr15 vs. age (yr AD).

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