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Estimation of freshwater influx along the eastern Adriatic coast as a possible source of stress for marine organisms

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Seawater salinity changes and thermal stress have been considered to be among the most important determinants of rocky intertidal zone organism distribution. The aim of present study was to determine the freshwater influx and evaluate the mussel Mytilus galloprovincialis *shells as indicators of natural environmental conditions by comparing $\delta^{13}C$ of shell carbonate layers with seawater salinity and temperature values measured over the 1998-2007 period. Our results show that the salinity was relatively constant in the open sea, with typical values of the northern and southern Adriatic being 37.65 ± 0.40 and 38.37 ± 0.19 respectively. It varied in intertidal zones, estuaries, locations close to under-sea freshwater springs and during rainy days in closed lagoons on different locations along eastern Adriatic coast. Salinity and temperature variations at investigated sites showed freshwater influx which was also reflected in lower $\delta^{13}C$ values of mussel* M. galloprovincialis *shell carbonates. Based on the measured parameters, three groups of locations in the investigated area (25 locations) can be defined: locations with strong to medium freshwater influence (9), with minor freshwater influence (6) and marine environment locations (10). Our results confirm that mussel* M. galloprovincialis *responds sensitively to subtle changes in the environmental conditions. Results of the isotopic analyses presented in this study support the hypothesis that the $\delta^{13}C$ in mussel's shell might be used as an indicator of environmental salinity conditions and hypo-osmotic stress. Therefore such investigations might represent an additional tool for waste water management and environmental protection.*

Key words: salinity, temperature, stable carbon isotopes, shell, *Mytilus galloprovincialis*, biomonitoring

INTRODUCTION

Coastal monitoring programs are valuable tools for assessing the current state of coastal environments, for determining spatial and temporal trends in pollution and identifying potential sources of contaminants. Monitoring often involves organisms from different environments, such as estuarine and intertidal zones, where conditions (e.g. salinity, temperature) fluctuate. Biomarkers "report" on important cellular functions under "normal" and "stressful" states in marine organisms in the course of their normal physiological adaptation to changing environmental conditions (VIARENGO *et al*., 1999; FEDER & HOFMANN, 1999; LUEDEKING & KOEHLER, 2004; HAMER *et al*., 2004; IVANKOVIĆ *et al*., 2005).

The main difficulty in using biomarkers in a monitoring programme is the interference of natural environmental factors, together with pollution, in their biological responses (ICES WGBEC, 1999; HYLLAND, 2006; HAMER *et al*., 2008). When external conditions are closely controlled, biological responses can be accurately assessed and related to exposure events (McCARTHY & SHUGART, 1990). Therefore the use of hydrographic data (temperature, salinity, etc.) and some additional indicators of present and past environmental stress are necessary for the correct interpretation of the organism stress response to changes in environmental conditions and possible pollution in an integrated monitoring programme.

Our guiding hypothesis was that interference of environmental factors with biological (stress) responses to contamination exposure is real, and new approaches and methods for appropriate indicator field evaluation are needed (VIGHI *et al*., 2003). In addition, living organisms reflect exogenous and/or endogenous rhythms in the environment. The mineralogy and composition of their calcified structures are affected equally by both physiological and environmental factors, which indicate the potential of biominerals as appropriate biomonitoring agents.

The bivalve *Mytilus galloprovincialis*, just as other molluscs, produces a shell composed largely of biogenic calcium carbonate ($CaCO_3$), in the form of aragonite and calcite polymorphs (KENNEDY *et al*., 1969; WATABE, 1988). The calcite/aragonite ratio in the shell of *M. galloprovincialis* sampled from the east Adriatic coast, with an annual seawater (SW) temperature variation from 4°C to 28°C, resulted in approximately 75 mol% of calcite and 25 mol% of aragonite (MEDAKOVIĆ *et al*., 1991). Previous preliminary results show that hydrographic parameters measured at localities along the east Adriatic coast were quite different though not strong enough to influence changes in the calcite-aragonite ratio in the examined shell samples (MEDAKOVIĆ, 1995). According to the results of Dodd (DODD, 1966) decreasing salinity caused an increase in the aragonite content in the shell of blue mussel *Mytilus edulis*, contrary to the findings by EISMA (1966) who found that such a relationship could not be confirmed and that the observed mineralogical variation would be better explained as a temperature effect. The factors for precipitation of carbonate polymorphs in the shells include a complex interaction between organisms and environmental conditions, inorganic ions, dissolved organic materials, enzymatic activity, macromolecules, etc. (LORENS & BENDER, 1980; FALINI *et al*., 1996; VAN DER PUTTEN *et al*., 2000).

Due to daily accretion of growth bands, the shell contains a continuous record of physical and chemical conditions of SW, including the inclusion of foreign particles or substitution of heteroatoms within the crystal lattice (FRITZ *et al*., 1990; CARRIKER *et al*., 1991; CARRIKER, 1992). It was shown that exposure of mussels to changed environmental conditions (temperature and salinity) can also cause a shift in the isotopic composition of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) in mussel shells, which means their isotopic and geochemical composition reflects conditions in SW primarily during the shell/mussel growth period (BURCHARD & FRITZ, 1980; RICHARDSON, 2001). Molluscs, in general, are believed to express only minimal metabolic effects on their isotopic composition and thus their isotopic values are representative of environmental water conditions (JONES, 1985). The $\delta^{13}C$ of carbonate is determined by the ratio in the water at which the deposition occurs. Most marine carbonates reflect the $\delta^{13}C$ of total dissolved carbon in the water where they form their shell (ANDERSON & ARTHUR, 1983). Therefore, the relationship between environmental parameters and shell stable isotope profiles is of great value in obtaining a better insight into the variation of environmental factors at investigated locations in periods before sampling (MUTVEI *et al*., 1993).

The mussel *M. galloprovincialis* has been widely used as a sentinel organism for the assessment of pollution in coastal areas and for the biomonitoring of marine environments (GOLDBERG, 1975, 1986; VIARENGO & CANESI, 1991; GOSLING, 1992; CAJARAVILLE *et al.*, 2000; JAKŠIĆ & BATEL, 2003). For example, "Project Adriatic" provides a framework for the systematic research of the Adriatic Sea as a basis for the sustainable development of Croatia. The project was launched in 1997 both because of the increasing awareness that maintaining high quality of the marine environment is one of the basic conditions for future development of the Adriatic region and amid clear indications that its quality was decreasing (increased pollution at "hot spots") (BIHARI *et al.*, 2002, 2004).

The aim of this work was to determine freshwater influx and evaluate mussel *M. galloprovincialis* shells as indicators of environmental stress (hypo-osmotic conditions) by comparing the $\delta^{13}C$ of shell carbonate layers with measured seawater salinity and temperature values (1998-2007) and therewith to distinguish between locations of the Croatian National Biomonitoring Programme "Project Adriatic".

MATERIAL AND METHODS

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich, if not stated otherwise.

Test organism and investigated area of the biomonitoring programme

Mediterranean mussels *Mytilus galloprovincialis* Lamarck, 1819 (Mollusca, Bivalvia) were collected (in October 1998) at 20 sampling sites (where present) out of a total of 25 sites used for the "Project Adriatic" biomonitoring along the Croatian Adriatic coast (Fig. 1). Five mussels (at least 1 year old; ranging from 2.5-6.0 cm in length) were collected, measured and weighed at each site. After dissection of soft tissue, the clean shells were stored at 4°C until crystallographic and isotopic composition analysis.

Seawater temperature and salinity

Over the 1998-2007 period, in addition to mussel collection, standard hydrographic parameters were measured four times per year (March, June, August and October) at investigated locations, including salinity, pH, temperature and oxygen saturation, using a portable WTW Multimeter P4. Measurements of these hydrographic parameters in open Adriatic Sea waters were performed at two stations (Fig. 1; RV001 - northern Adriatic and Stončica - southern Adriatic) on a monthly scale. Temperature was determined by reversing thermometer and salinity by salinometer (YEO-KAL 601Mk1V) after sampling with a Niskin sampler.

$\delta^{13}C$ analysis of mussel shell carbonate layers

Remaining parts of the soft tissues were removed using a plastic knife. For isotopic analysis, shell aragonite and calcite layers of whole shells were separated by careful grinding with emery paper and homogenised. Before stable isotope analysis the organic matter was removed by roasting the carbonate powder for 2 hours at 190°C. The carbonate was transformed into CO_2 by reacting with anhydrous H_3PO_4 at 55°C under vacuum (McCREA, 1950). NBS-18 and NBS-19 were used as standards to report all isotopic signatures in per mil (‰) relative to the Vienna Pee Dee Belemnite (VPDB) (COPLEN, 1996). Precision determined by repeated analysis of the working standard was better than ± 0.1‰ for $\delta^{13}C$. $\delta^{13}C$ was determined using a dual inlet Varian Mat 250 isotope ratio mass spectrometer (IRMS). The stable C ratios are reported in the (δ) notations as the per mil (‰) deviation relative to the VPDB international standard.

Statistical analyses

The $\delta^{13}C$ values for mussel shells are given as mean values of five shell samples. The correlation and significance of the mean values of $\delta^{13}C$ for the year 1998 and average values of seawater salinity and temperature of the period 1998-2007 was performed by Pearson correlation test. Cluster analysis and tree diagram of Euclidean distances (complete linkage) of measured parameters at investigated locations were performed by Statistica '99 (StatSoft, Inc.).

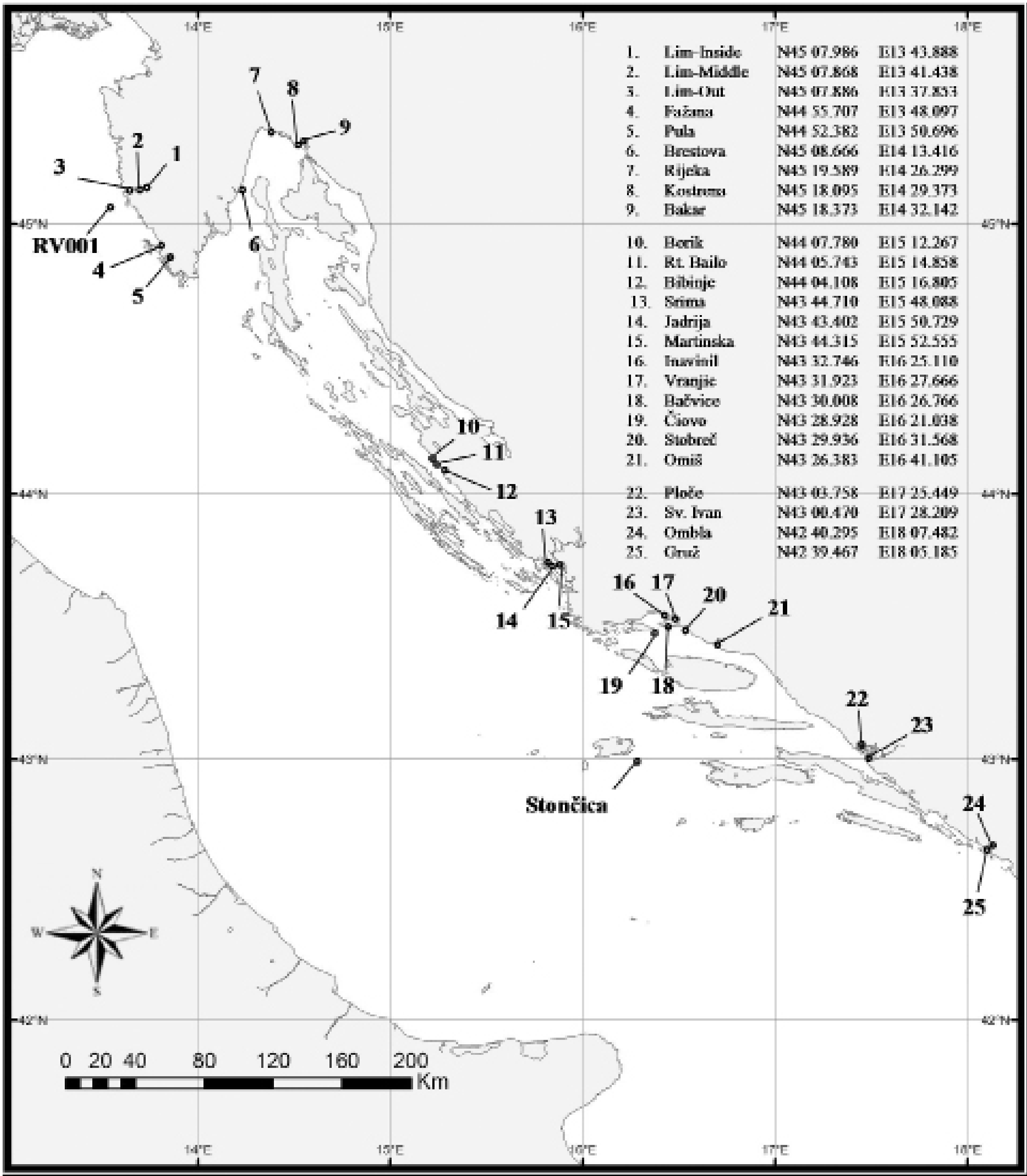

Fig. 1. Positions of sampling locations along the Croatian eastern coastal area of the Adriatic Sea

RESULTS

Environmental conditions in the investigated area

The positions of sampling locations along the Croatian eastern coastal area of the Adriatic Sea are shown in Fig. 1. Fig. 2 shows the comparison between average temperature and salinity values measured at two reference points for the open sea – Rovinj (northern Adriatic) and Stončica (southern Adriatic), in the 1961-1990 and 1998-2007 periods (derived from average monthly values). A small general increase (0.1-1.5^{o}C) of surface seawater temperatures was noted in the 1998-2007 period. Annual average salinity values measured during the 1998-2007

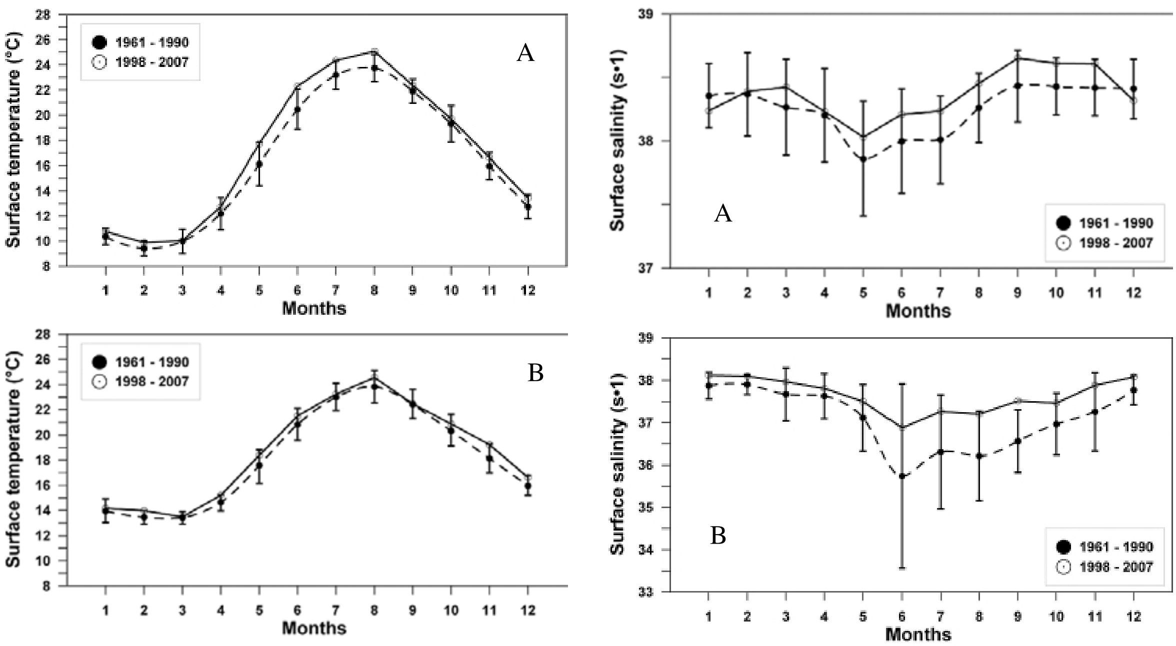

Fig. 2. Average temperature and salinity values of the (●)1961-1990 and (○) 1998-2007 periods for the open Adriatic Sea surface waters: (A) Rovinj RV001 (northern Adriatic) and (B) Stončica (southern Adriatic)

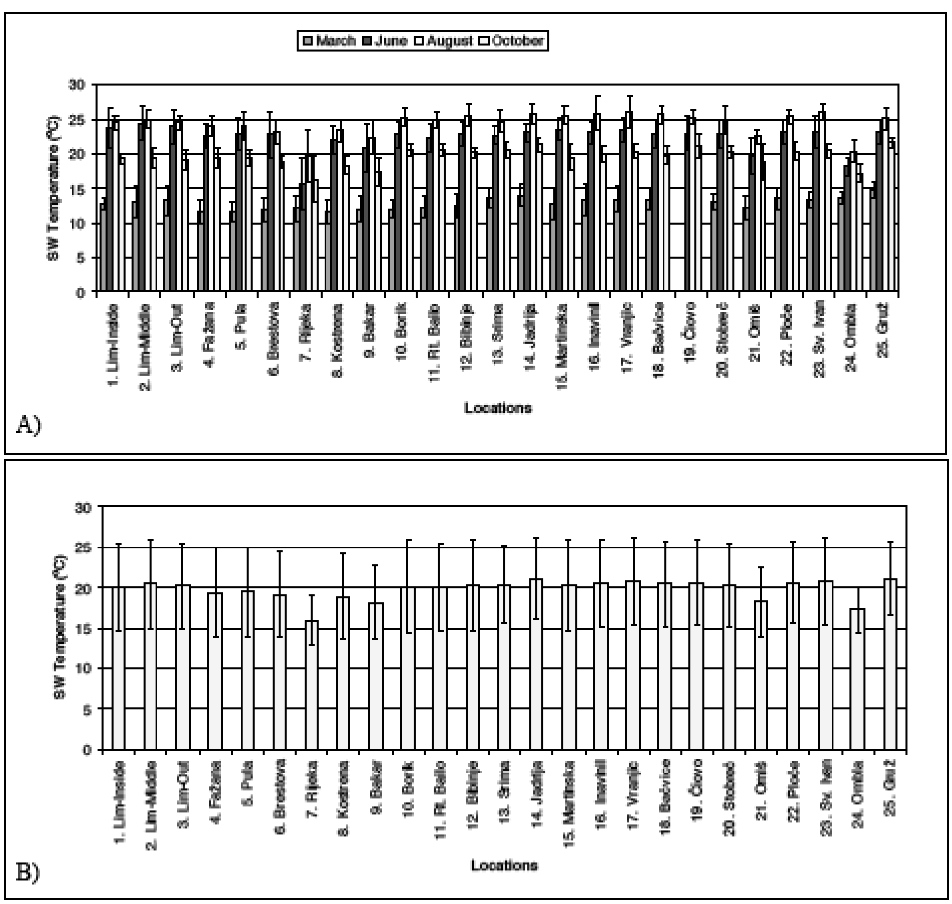

Fig. 3. (A) Seasonally averaged, and (B) total average SW temperatures at investigated locations during the 1998-2007 period

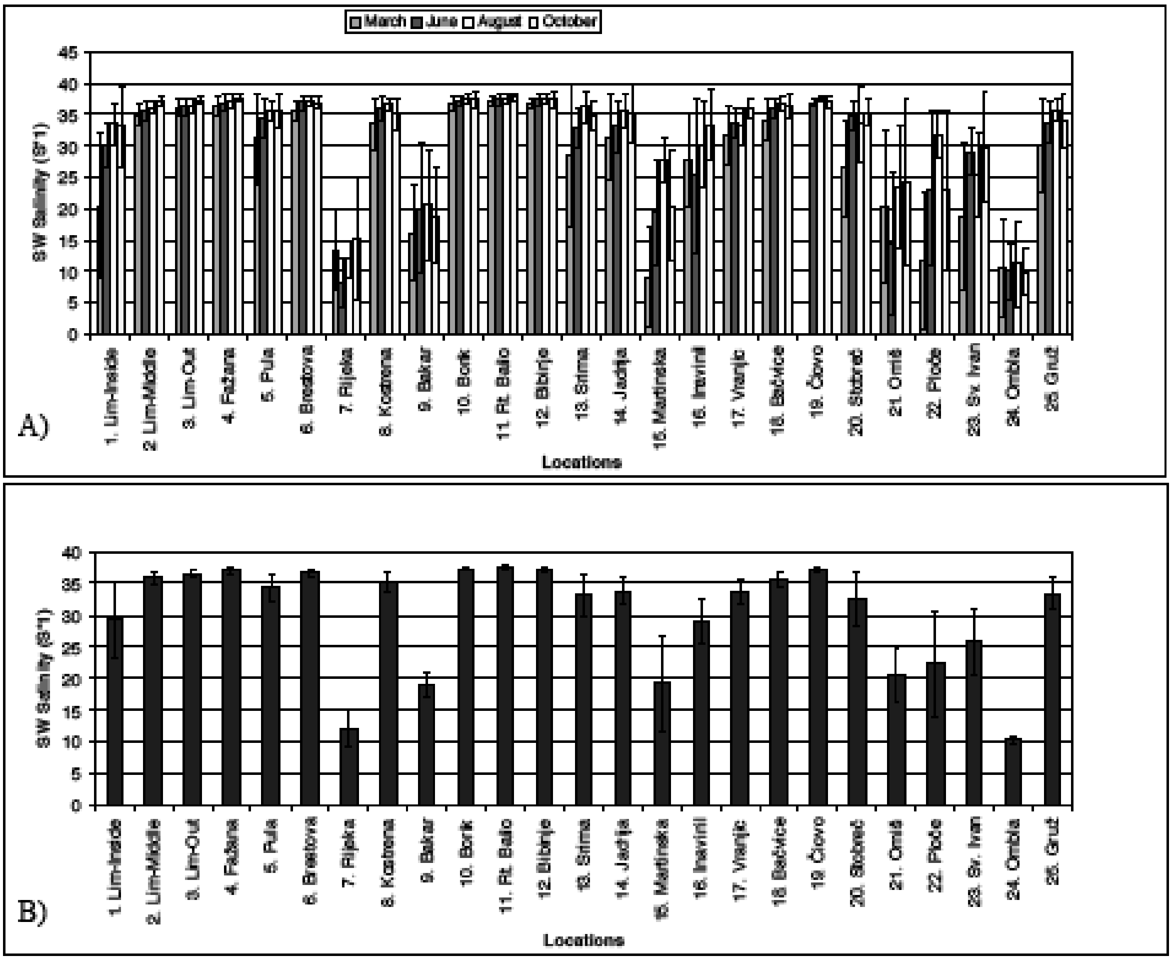

Fig. 4. (A) Seasonally averaged, and (B) total average salinity at investigated locations during the 1998-2007 period

period at Stončica indicated a small variation over the years of 38.37 ± 0.19, similar to the 1961-1990 period (38.25 ± 0.20), and a small increase of the average value (0.12). Salinity data for open waters near Rovinj showed the same trend with 37.08 ± 0.73 and 37.65 ± 0.40 for the two periods respectively) with a small increase of the average values by 0.57.

Mussels *M. galloprovincilis* living in the intertidal zone at the investigated locations of the Adriatic Sea coastal area (Fig. 1) were exposed to a wide range of temperatures that followed a clear annual cycle with seasonally averaged values ranging from approximately 11-14°C in winter (March) to 19-26°C in summer (August) over the 1998-2007 period (Fig. 3A). Total average values show that the Rijeka sampling location (S-7) had the lowest temperature at 16°C while the Gruž sampling location (S-25) showed the highest average temperature at 21.5°C (Fig. 3B).

Cluster analysis of the seasonal average SW temperatures measured at the 25 locations during the 1998-2007 period separated the following four locations: Rijeka (S-7), Bakar (S-9), Omiš (S-21) and Ombla (S-24) that were under strong freshwater influence. Locations S-7 and S-9 were influenced by freshwater springs while stations S-21 and S-24 were estuarine – i.e. sited at the mouth of the rivers Cetina and Ombla, respectively (data not shown).

While the open sea salinity (S) values were relatively constant over the 1998-2007 period (Fig. 2), salinity varied strongly at some of the investigated sites; including those in estuaries, close to under-sea fresh water springs and during rainy days in closed lagoons. Seasonally averaged salinity values ranged from 8-38 over

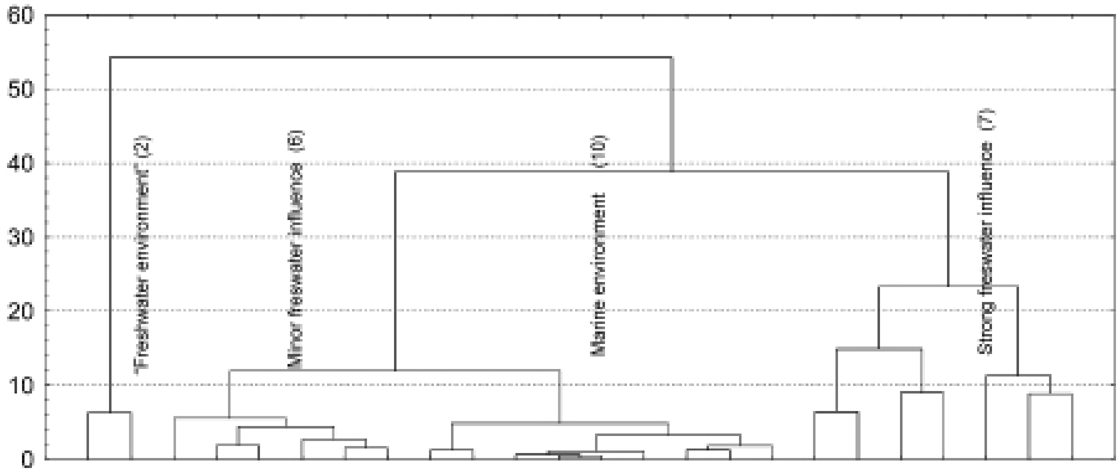

Fig. 5. Tree diagram of linkage distance for 25 locations according to seasonal average seawater salinity over the investigated period (1998-2007)

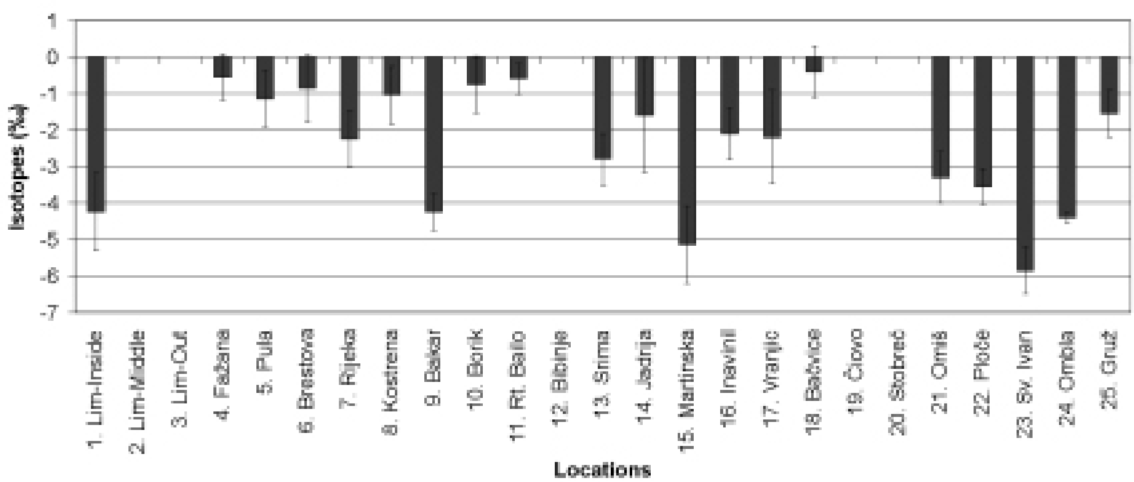

Fig. 6. $\delta^{13}C$ average values in the carbonate shell layers of mussels. Isotope composition was not analysed at S-2, S-3, S-12, S-19 and S-20 due to the lack of mussels at these locations

the investigated area (Fig. 4A). Average annual salinity values were the lowest (12) at Ombla (S-24), and highest at Rt. Bailo (S-11) 37 (Fig. 4B).

A tree diagram of Euclidean distances for 25 locations according to seasonal average SW salinities measured over the investigated area during the 1998-2007 period indicated the following clusters of locations: Ombla (S-24) and Rijeka (S-7) as "freshwater environments"; Lim-Inside (S-1), Sv. Ivan (S-23), Inavinil (S-16), Bakar (S-9), Omiš (S-21), Martinska (S-15) and Ploče (S-22) with strong freshwater influence; Stobreč (S-20), Gruž (S-25), Srima (S-13), Vranjic (S-17), Jadrija (S-14) and Pula (S-5) with minor freshwater influence and locations Bačvice (S-18), Kostrena (S-8), Rt. Bailo (S-11), Bibinje (S-12), Borik (S-10), Fažana (S-4), Brestova (S-6), Lim-Out (S-3) and Lim-Middle (S-2) as no freshwater influence marine environments (Fig. 5).

$\delta^{13}C$ values of *M. galloprovincialis* shells

According to shell composition of 75% of calcite and 25% of aragonite the bulk shell $\delta^{13}C$ is presented in Fig. 6. The $\delta^{13}C$ for aragonite ranges from -5.4 to 0.1‰ and from -6.28 to -0.86‰ for calcite. The aragonite part of the shell was enriched and compared to calcite by 1.1‰ with the heavier carbon isotope at all investigated locations.

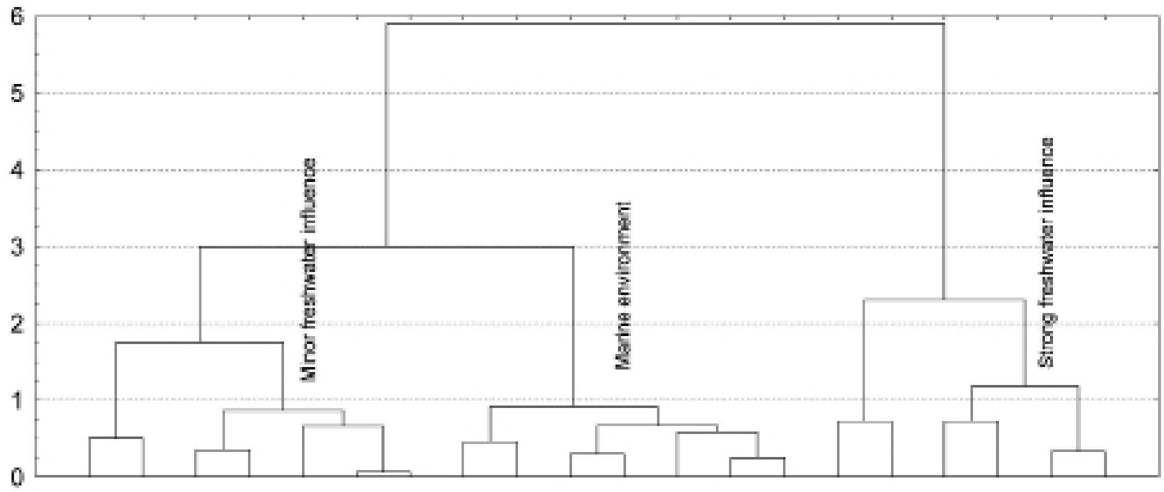

Fig. 7. Cluster analysis of average $\delta^{13}C$ values of the aragonite and calcite layers of the mussel shells

The enrichment factor of isotopic fractionation of carbon isotopes between inorganic precipitated aragonite and bicarbonate was 2.0 ± 0.4‰, for calcite was 1.0 ± 0.2‰ and was not temperature dependent.

The results of $\delta^{13}C$ isotope analysis of aragonite and calcite parts of the *M. galloprovincialis* shell point to the conclusion that the influence of freshwater was the greatest at locations with the lowest $\delta^{13}C$, and minimal at locations with the highest $\delta^{13}C$ values (Fig. 6). According to $\delta^{13}C$ values, the 20 locations could be separated into three groups with respect to varying freshwater influence; 7 locations under strong freshwater influence, 6 under minor freshwater influence and 7 without freshwater influence (pristine marine environment) (Fig. 7).

Table 1. Correlation of average seawater temperature and salinity during the investigation period (1998-2007) with shell average isotopic composition ($\delta^{13}C$ calcite+aragonite) at the investigated locations (October 1998)

	Temperature	Salinity	$\delta^{13}C_{average}$
Temperature	1		
Salinity	0.645159	1	
$\delta^{13}C_{average}$	0.080036	0.720121	1

* We used for correlation 20 sampling sites out of a total 25 sites, where both parameters were measured

The sites were ranked according to $\delta^{13}C$ values and subsequently correlated with measured SW salinity and temperature average values for the entire investigated period (1998-2007) (Table 1).

Ranking of investigated sites according to shell isotopic composition and measured seawater salinity indicates 9 separate locations with strong to medium freshwater influence: Ombla (S-24), Rijeka (S-7), Bakar (S-9), Martinska (S-15), Omiš (S-21), Ploče (S-22), Sv. Ivan (S-23), Lim-Inside (S-1) and Inavinil (S-16).

DISCUSSION

Mussels of the genus *Mytilus* are one of the most common marine molluscs and thus are significant components of coastal ecosystems. As fluctuations in environmental conditions can produce a broad range of cellular responses and organism adaptations (WIDOWS, 2001; SCHROTH *et al*., 2005; HAMER *et al*., 2008), it should be taken into account during the evaluation of data gathered by biotests and in the biomarker analysis.

Changes of physical (temperature, radiation, pressure), geochemical (salinity, tidal regime, pH, oxygen content) or biological conditions (population density, nutrition, parasites, prey) could affect species/population distribution in the shallow intertidal zone. The most interesting question relates to the determination of the quantity of “stressed effects” or toleration level to specific factors or their synergistic combination. In order to primarily elucidate the effect of marine environment salinity changes (e.g. hyposaline stress) on the results of different biomarkers and biotests used for monitoring of environmental pollution, it is necessary to show, apart from the measurement of oceanographic parameters (SW temperature and salinity), the history of temperature and salinity-hypoosmotic stress exposure (BURCHARDT & FRITZ, 1980; RICHARDSON, 2001)

$\delta^{13}C$ in the shell of *M. galloprovincialis* as indicator of freshwater influx

We found that aragonite was, on average, enriched and compared to calcite with the heavier carbon isotope. The enrichment factors of isotopic fractionation of carbon isotopes between inorganic precipitated aragonite and bicarbonate and inorganic precipitated calcite and bicarbonate were not temperature dependent. The results of previous studies indicate that the influx of freshwater into the marine environment causes enrichment with light stable isotopes ^{12}C and ^{16}O, which was also reflected in carbonate shell composition (CRAIG, 1965; KENNEDY *et al*., 1969; LORENS & BENDER, 1980; GROSSMAN & KU, 1986). Lower $\delta^{13}C$ values were found in shells of mussels collected in environments with more freshwater influence, since river water is enriched with ^{12}C and carries more soil CO_2 from terrestrial ecosystems (CARRIKER *et al*., 1980; ANDERSON & ARTHUR, 1983).

M. galloprovincialis forms its shell over the year, and particularly during the intensive growth period (GOSLING, 1992), which means that it reflects isotopic and geochemical composition conditions in SW primarily during the growth period. Correlation of average seawater temperature and salinity during the investigated period (1998-2007) with $\delta^{13}C$ (according to a shell composition of 75% calcite and 25% aragonite) at the investigated sites indicated a high dependence of $\delta^{13}C$ with SW salinity (r=0.720121) and no, or weak, correlation to SW temperature (r=0.080036).

Stable isotope analysis of the growth of biomineralised tissues (e.g. bivalve shells) allows reconstruction of parameters of environmental and ecological importance. In addition, for those tissues that exhibit incremental growth stable isotope analysis can provide time series information relevant to ontogenetic and palaeoceanographic applications (EVANS *et al*., 2002; SCOURSE *et al*., 2004). The establishment of environmental records, using $\delta^{18}O$ and $\delta^{13}C$ as temperature and salinity proxies, respectively, is based on the hypothesis that isotopic equilibrium has been established between seawater and shell carbonate at the time of precipitation. Two species of mussel, *Pecten maximus* and *Pinna nobilis,* with contrasting shell precipitation rates and longevity, were previously used as model organisms to test this hypothesis (RICHARDSON *et al*., 1999; OWEN *et al*., 2002a). They were also used in laboratory calibrations (OWEN *et al*., 2002b) and field validations (KENNEDY *et al*., 2001; OWEN *et al*., 2002c; FREITAS *et al*., 2005). The validation, especially of *Pecten maximus*, has led to its establishment as a high-frequency archive of palaeo-environmental change. The aforementioned stable isotope ratios can also be used to validate the growth of molluscs to construct an age model for molluscan shell records and provide calcification temperature estimates which allows for comparison, on a calendar time scale, of shell elemental data with environmental variables as well as estimation of shell growth rates (FREITAS *et al*., 2005, 2006).

Environmental stress

As noted previously (WIDOWS, 1985; WIDOWS & DONKIN, 1992; HAMER *et al*., 2004, 2008), the combination of pollution and natural stressors such as salinity, seasonal temperature changes, changes in intracellular ionic strength and pH results in severe adverse effects occurring under *in situ* conditions, and their interpretation requires further research. The use of mussels in environmental pollution monitoring programmes is important in understanding the factors which determine the relationships among contaminant levels in the environment (seawater, sediments and suspended particles) and contaminant levels in mussels (including different tissues) and the resulting biological effects. Of course, the (bio) accumulation and pollution effects depend upon both biotic and abiotic factors and are affected by environmental factors such as temperature and salinity. Aqueous solubility is important for metals (but not for organics) and a prerequisite for its accumulation and concentration in mussels. Water solubility and bioconcentration usually are inversely correlated. Since physical factors (salinity, temperature, pH) can influence solubility, they might also be expected to affect bioaccumulation (GOSSLING, 1992). Further uptake of contaminant by mussel is affected by any factor which influences pumping/feeding activity including metabolism, storage and elimination.

This evaluation of $\delta^{13}C$ shell analysis with measured SW temperatures and salinities is a first step in proving and detecting recent and past environmental temperature and salinity conditions at investigated sites during the biomonitoring programme "Project Adriatic". Environmental stress other than chemical pollution (e.g. temperature changes and hypoosmotic conditions) need to be taken into account when interpreting the results of the biomarkers and bioassays used as indicators of water quality and organisms health. However, it is not always clear how to incorporate such information into the biological effect assessment of pollution because hypo-osmotic stress (freshwater influx) and other factors can affect biomarkers results, some in positive and some in negative directions (ICES, 1999; GAITANAKI *et al.,* 2004; IVANKOVIĆ *et al.,* 2005; HAMER *et al.*, 2008). If, for example, strong salinity fluctuation at investigated locations is detected and if there are no chemical data of contamination (water, sediment, biota), the results should not be regarded as completely straightforward. To prevent such possible false positive or negative interpretations of biomarker results, the inclusion of additional parameters/indicators is timely, with further experimental laboratory work and field evaluation being needed.

Results of the isotopic analyses presented in this study support the hypothesis that the $\delta^{13}C$ in mussel shell might be used as an indicator of hypo-osmotic conditions (KANDUČ *et al.,* 2001, 2006). The present research highlights the possibility for detecting/estimating the freshwater influx (hypo-osmotic conditions and possible environmental stress) without continuous measurement of parameters (salinity), which is expensive and time consuming. Continuous measurement of SW temperatures can be easily done using data-loggers at a minute time scale. Those results can then be additionally used for estimation of salinity fluctuation because freshwaters are of different temperatures compared to SW.

For the purposes of the "Project Adriatic" we determined those locations with strong to medium freshwater influx that could possibly interfere with pollution effects in mussels: Ombla (S-24), Rijeka (S-7), Bakar (S-9), Martinska (S-15), Omiš (S-21), Ploče (S-22), Sv. Ivan (S-23), Lim-Inside (S-1) and Inavinil (S-16). Clearly, with this investigation we showed that the composition of biomineralised structures produced by marine organisms and isotopic composition of carbon ($\delta^{13}C$) may act as an archive, and that is a potentially valuable tool for reconstructing environmental conditions as an aid in data interpretation of pollution biomonitoring studies. Furthermore, registering mineralogical composition and phase transitions by measuring stable isotope profiles and tracking inorganic or organic pollutants in the shells and shell layers,may clarify the relationship and interaction between organisms and their environment.

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Procjena dotoka slatke vode uzduž istočne obale Jadrana kao mogućeg izvora stresa za morske organizme

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SAŽETAK

Promjena saliniteta i temperature morske vode u stjenovitom obalnom području ograničava i utječe na raspodjela morskih organizama. U području otvorenog mora salinitet je relativno stalan, a u istraživanom razdoblju (1998.-2007.) se kretao od 37,65 ± 0,40 za sjeverni i do 38,37 ± 0,19 za južni Jadran. Salinitet značajno koleba u zoni plime i oseke, na području ušća rijeka i podmorskim izvorima slatke vode te tijekom kišnih dana u zatvorenim lagunama. Zbog dotoka slatke vode temperatura mora na istraživanim postajama pokazuje unutar sezonske razlike, uz jaka sezonska kolebanja. Kolebanje vrijednosti saliniteta i temperature morske vode na području od Limskog kanala do Dubrovnika (sjeverni - južni Jadran) pod utjecajem dotoka slatke vode izravno su se odrazili i na vrijednosti $\delta^{13}C$ u ljušturama dagnje *Mytilus galloprovincialis*. Vrijednosti $\delta^{13}C$ iz 1998. godine su rangirane i korelirane s prosječnim vrijednostima temperature i saliniteta morske vode tijekom devetogodišnjeg istraživanog razdoblja (1998.-2007.). Na temelju sadržaja $\delta^{13}C$ u ljušturama, temperature i saliniteta morske vode istraživano područje (25 postaja) je grupirano u tri skupine: srednje do jak utjecaj slatke vode (9), manji utjecaj slatke vode (6) i lokacije morskog okoliša (10). Utvrđeno je da su ljušture školjkaša, odnosno vrijednosti $\delta^{13}C$, dobri pokazatelji kolebanja prirodnih čimbenika, naročito saliniteta, posredstvom dotoka slatkih voda. Dagnja *M. galloprovincialis* se može koristiti kao indikator kolebanja ekoloških čimbenika i možebitnih onečišćenja. Stoga navedena istraživanja predstavljaju dodatni koristan alat u biomonitoring programima, primjerice ispitivanju kvalitete okoliša, upravljanju otpadnim vodama i zaštiti okoliša.

Ključne riječi: salinitet, temperatura, stabilni izotopi ugljika, ljuštura, *Mytilus galloprovincialis*, biomonitoring