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HORIZONTAL STRUCTURE OF POTENTIALLY TOXIC ALGAE AND THEIR TOXINS PRODUCTION
STRUKTURA HORINZONTALE E ALGAVE POTENCIALISHT TOKSIKE DHE PRODHIMI I TOKSINAVE TË TYRE

MERJEM BUSHATI[a], ERLINDA KONI[a], MARSELA BREGAJ[a], LEFTER KASHTA[b], ALEKO MIHO[b]
[a]Food Safety and Veterinary Institute, Str. Aleksander Moisiu, No.82, Tirana, Albania.
[b]Faculty of Natural Sciences, University of Tirana, Tirana, Albania.
merjembushati@yahoo.com

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PËRMBLEDHJE
Në lagunën e Butrintit janë marrë 505 mostra dy herë në muaj, gjatë periudhës 2008-2009, në tre stacione. Ky studim ka qëllim, përcaktimin e abondancës të fitoplanktonit potencialisht toksik, e përqëndrimit të toksinave tek *Mytilus galloprovincialis* dhe lidhjen mes tyre. Kryesisht, u numëruan llojet më abondante: *Alexandrium spp., Dinophysis acuminata, D. sacculus, Gonyaulax spinifera, Karenia spp., Pseudonitzschia spp*. Abondanca e këtyre prodhuesve të toksinave dhe përqëndrimi i toksinave përkatëse u analizua statistikisht nga Correl e Regression, Excel dhe për të na dhënë lidhjen midis stacioneve dhe muajve nga ANOVA, Excel. Lloji më dominant ishte *Pseudonitzschia spp.* (abondanca maksimale $1,4x10^6$qel/L, Dhjetor 2008; $4x10^6$qel/L, Janar-Shkurt 2009). Maksimumi i *Pseudonitzschia* në 2008, nuk përkon në kohë me maksimumin e ASP, por në 2009, dy maksimumet përkojnë në kohë. Fitoplanktoni prodhon toksicitet të ndryshëm në varësi të vendndodhjes gjeografike dhe kushteve të ndryshme mjedisore.
Fjalët kyce: Laguna e Butrintit, fitoplanktoni potencialisht toksik, toksina.

SUMMARY
Sampling two times per month was conducted from 2008 - 2009, at three stations, in Butrinti lagoon. This study aimed the determination of the potentially toxic phytoplankton, of the toxins in *Mytilus galloprovincialis* and their relationship. Abundances were focused on the most abundant taxa: *Alexandrium spp., Dinophysis acuminata, D. sacculus, Gonyaulax spinifera, Karenia spp., Pseudonitzschia spp*. The distribution patterns of these toxins producers and the toxins distribution were statistically analyzed by Correl and Regression, Excel and to address relationships between stations and months by ANOVA, Excel. The dominant taxa was *Pseudonitzschia spp.* (maximum abundance $1,4x10^6$cell/L December 2008; $4x10^6$cell/L, January-February 2009). The *Pseudonitzschia* maximum abundance, in 2008 does not coincide in time with the ASP maximum concentration, but in 2009, the two maximums coincide with each-other. The phytoplankton produce variable toxicity depending on the geographical location and environmental conditions of water.
Key words: Butrinti Lagoon, potentially toxic phytoplankton, toxins.

INTRODUCTION
Butrinti lake (Fig. 1) is a typical coastal lagoon, situated in the southern part of Albania. It is connected in the northern part with Bistrica river and in the southwestern part with the Ionian Sea. Monitoring Butrinti lake about chemical, microbiological and biological quality is one of the main aims of Food Safety and Veterinary Institute (ISUV), for secure aquaculture production and trading.

Most phytoplankton are beneficial, forming the base of the food web. However, a small number of phytoplankton species, about 90, also produce potent toxins (13). The rapid growth of these algae to high concentrations results in Harmful Algal Blooms (HABs) (7). These toxic algal cells are filtered out of the water by shellfish during their feeding activity. Without the existing monitoring efforts, these animals could then cause illness or death (Amnesic Shellfish Poisoning-ASP, Paralytic Shellfish Poisoning-PSP and Diarrhetic Shellfish Poisoning-DSP), if consumed by humans.

There is no clear correlation between algal concentration and their harmful effects: *Dinophysis* and *Alexandrium* in very low concentration may produce harmful toxins, whereas *Phaeocystis* is not toxic, but it becomes dangerous in high levels of bloom.

The risk of toxic blooms pushed us to present a survey of the toxins and potentially toxic phytoplankton.

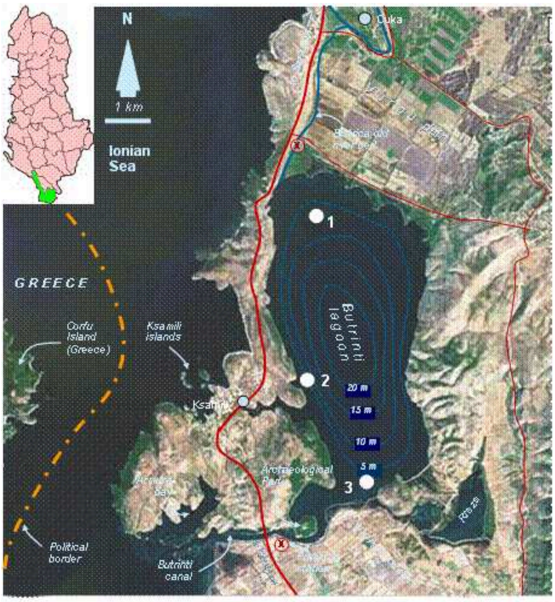

Figure 1. Map of Butrinti lagoon with the three sampling stations (white circles)

MATERIAL AND METHODS

Potentially toxic phytoplankton were analyzed in 138-126 (total 264) samples, respectively during 2008-2009. The presence of toxins in mussels were analysed in 139-102 (total 241) samples during the same period.

Three stations were selected based on the geomorphological conditions of the sea or of the lagoon: **1**-BM1 Nord; **2**-BM1 West; **3**-BM1 South (Fig. 1). The sampling frequency was 2-4 times per month.

Phytoplankton samples were taken on 1m depth, using dark glass bottles. Samples were preserved in alkaline Lugol Solution. The taxonomic list was prepared according to cell counts and photos obtained by the inverted microscope Zeiss Axiovert 40 CFL, equipped with a digital camera. Diatoms cleaning was done using an acid methods (9). Species determinations were done using different keys (7, 9, 18 etc). Subsamples of 25 ml were analyzed after 24 h of sedimentation (17, 4).

For the toxins determination were taken 4 kg mussels. The official method used for the ASP determination is a chemical method (14). Domoic acid-DA content is determined by preparing 50% aqueous methanol extracts of the homogenized tissue, filtering and analyzing the extract by HPLC-UV, wave 242 nm. Chromatographic separation is performed by gradient elution (mobile phase: Acetonitrile 10% with 0.1%TFA). The legal limit for ASP toxins is 20 mg/kg (15).

The PSP determination was done using biological method (1). It is based on a simple aqueous extraction of the saxitoxin and it compounds with HCL (pH=3). One ml of this extract is injected intraperitoneally into albino mice Swiss strain weighing 19 - 21 g. The death od 2 out of 3 injected mice, within one hour, constitutes a positive result (16).

The DSP presence was done using biological method (20) based on the extraction of the lipophilic compounds with acetone followed by partition between diethyl ether or dichloromethane and water. The organic solvent is evaporated and the resultant residue suspended in aqueous solution of 1% Tween 60. One ml of this extract is injected intraperitoneally into albino mice Swiss strain weighing 17 - 22 g. The death of 2 out of 3 injected mice, within a 24 hours, constitutes a positive result (16).

RESULTS AND DISCUSSION

In the present paper, it is mostly focused on the most abundant potentially toxic taxa and their related toxins: *Pseudonitzschia delicatissima, P. seriata* (impact ASP), *Gonyaulax spinifera, Dinophysis sacculus, D. acuminata* (impact DSP) *Alexandrium spp.* (impact PSP), *Karenia spp.* (impact NSP) etc. Also, in this lagoon were present other potentially toxic species, like *Prorocentrum minimum* (VSP), *Scrippsiella spp*. (anoxia in fish, invertebrate), *Cerataulina pelagica* (impact in molluscs, fishes, crustaceans), *Akashiwo sanguinea* (icthyotoxicity), *Lingulodinium polyedrum* (impact DSP), *Ostreopsis spp.* (CFP), *Leptocylindrus minimus* (fish die-off), *Amphidinium carterae* (icthyotoxicity), *Noctiluca scintillans* (fish, invertebrate die-off) etc. (Plate I & II) (7, 13).

2008	J	F	M	A	M	J	J	A	S	O	N	D
Max	6600	2380	880	2060	6700	6360	7120	7920	79120	2.360	26080	1404723
Avg	4491	1355	427	1258	2887	2976	1772	3100	22112	15538	8685	413053
Fr %	98,6											
2009	J	F	M	A	M	J	J	A	S	O	N	D
Max	4353720	4605132	1725126	1080	560	120	1840	7120	26400	105.880	57840	52920
Avg	2631015	3582569	764352	377	218	120	827	6087	8907	45560	20467	25720
Fr %	73,8											

Table 1. Maximal and average values (cells/L) of Pseudonitzschia spp.

The dominance and the great bloom of *Pseudonitzschia* species (frequency 98,6%-2008; 73,8%-2009), (maximum value $1,4x10^6$cell/L: Dec. 2008; $4,6x10^6$cell/L: Feb. 2009) (Tab. 1; Fig. 2), is an indicator of high nutritional conditions and of the eutrophic trend of this environment; known as bloom monospecies in eutrophic enviroment like Kastela Bay, Split (10).

Species like *Gonyaulax spinifera* (max. 160cells/L: Jan-Feb 2008; 160cells/L: Feb-March 2009), *Dinophysis sacculus* (max. 160cells/L: Jan. 2008; 160cells/L: Feb-March 2009), *D. acuminata* (max. 200cells/L: Jan. 2008; 200cells/L: Feb. 2009), *Alexandrium* spp. (max. 320cells/L: June 2008; 200cells/L: July 2009), *Karenia* spp. (max. 1320cells/L: June 2008; 200cells/L: Aug. 2009), *Prorocentrum minimum, Scrippsiella* spp., have not high concentrations in Butrinti lake during 2008-2009 (Tab. 2, Fig. 3). The increase in number of dinoflagellates species by spring-summer (Tab.2, Fig.3) might be a result of the calm habitats where water does not circulate, at high temperatures and there is a lack of nutrients (6), nevertheless their density is low.

In 2009, the ASP maximum concentration (19.85mg/kg-March) was reached after the maximum of *Pseudonitzschia* abundance; but in 2008, the two maximums does not coincide exactly in time (Tab. 1, 3; Fig. 2). It is not clear whether *P.seriata* produces DA under all conditions, since the phytoplankton produce variable toxicity depending on environmental conditions of water (nutrients, bacteria and growth characteristics of organisms). Also, these toxins launched by phytoplankton were filtered time after time from water by mussels and were acumulated in their stomach. Alghthout, we have to emphasize, that the ASP values were within the permitted limits (15).

It seems an evident power correlation (Fig. 4) between *Pseudonitzschia* spp. density and ASP values (determination coefficient=61,5%). Even the linear correlation (Fig. 4) is significantly positive (Regression, Excel). Also using Correl, Excel (correl coeficent=0,62743), we observe the influence of *Pseudonitzschia* in ASP concentrations.

It was calculated by ANOVA, Single Factor that there were no significant differences about *Pseudonitzschia* spp. and ASP values, between the three stations (P-value > significance level 0.05), but there were significant differences (P-value < significance level 0.05) between the months.

Month	*Alexandrium spp*	*Karenia spp*	*Dinophysis sacculus*	*Dinophysis acuminata*	*Gonyaulax spinifera*
2008	Max				
J	120	120	160	200	160
F	120	120	120	120	160
M	200	120	120	160	120
A	120	120	120	120	120
M	120	120	120	120	120
J	320	1320	120	120	140
J	120	120	140	160	120
A	120	120	120	120	120
S	120	480	120	120	120
O	120	200	120	120	120
N	120	360	120	120	120
D	120	120	120	120	120
2009					
J	120	120	120	120	120
F	120	120	160	200	160
M	120	120	160	160	160
A	120	120	120	120	120
M	120	200	120	120	120
J	120	120	140	120	120
J	200	1160	120	160	120
A	120	5680	120	120	140
S	120	120	120	120	120
O	120	120	120	120	120
N	120	120	120	120	120
D	120	120	120	120	120

Table 2. Maximal values (cells/L) of potentially toxic dinoflagellates

2008	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Max	8,21	0,90	0,20	0,90	0,20	0,20	0,50	12,80	4,95	11,85	13,05	7,55
Avg	4,31	0,35	0,20	0,26	0,20	0,20	0,27	5,93	3,40	4,84	9,73	4,42
2009	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Max	17,05	15,85	19,85	0,50	0,30	0,60	0,20	1,30	1,25	2,00	1,00	2,00
Avg	12,64	10,69	18,24	0,33	0,22	0,33	0,20	0,38	0,46	1,10	1,00	1,50

Table 3. Maximal and average ASP values (mg/kg)

Year	J	F	M	A	M	J	J	A	S	O	N	D
2008	66,67	100	0	0	0	0	0	0	0	0	0	8,333
2009	44,44	11,11	33,33	0	0	0	0	0	0	0	0	0

Table 4. DSP percentiles results (%=Positive tests/Total tests x 100)

As we mention above we have not found high densities for *Alexandrium minutum* (PSP-toxin producer) (Tab. 2; Fig. 3), that's why the PSP test-mouse during 2008-2009 resulted always non detected.

Whereas DSP values were not always negative, even though concentrations of *Dinophysis acuminata, D. sacculus, Gonyaulax spinifera* were relatively low. DSP test-mouse resulted positive during Jan-2008: 6 samples positive from 9 samples (= 6/9), Feb-2008: 12/12, Dec-2008: 1/12, Jan-2009: 4/9, Feb-2009: 1/9, March-2009: 3/9 (Tab. 2). But, there is a correlation between total dinoflagellates abundance-DSP producers and DSP percentile tests using Correl, Excel (correl coeficent=0,4503), even though not too strong (determination coefficient=20,2%). We can suspect that the mouse death can be due also to: high concentrations of *Pseudonitzschia* spp. during winter (Tab. 1; Fig. 2); *Dinophysis* in very low concentration may produce harmful toxins; other toxic species that were found in the Butrinti lake: *Lingulodinium polyedrum*, *Dinophysis tripos, D. fortii* etc (DSP).

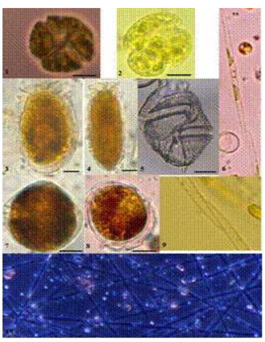

Plate I

Plate II

PLATE I: 1-2: Karenia spp.; 3: Dinophysis acuminata; 4: Dinophysis sacculus; 5: Gonyaulax spinifera; 6: Pseudo-nitzschia spp.; 7: Alexandrium spp. 1; 8: Alexandrium spp. 2; 9: Pseudo-nitzschia cf. delicatissima.

PLATE II: 1: Lingulodinium polyedrum; 2: Ostreopsis spp.; 3: Akashiwo sanguinea; 4: Noctiluca scintillans; 5-6: Prorocentrum minimum; 7: Scrippsiella cf. trochoidea; 8: Protoceratium reticulatum; 9: Cerataulina pelagica
(10 µm).

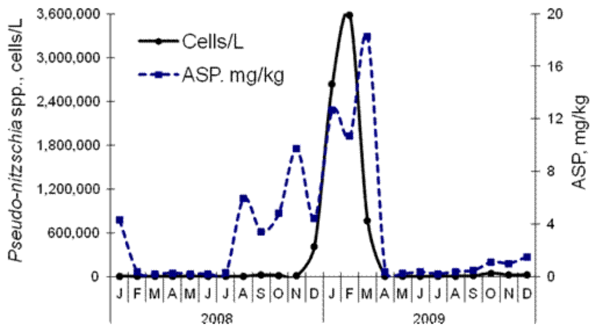

Figure 2. Dynamics of Pseudonitzschia spp. (maximum values) compared with the related dynamics of ASP (maximum values)

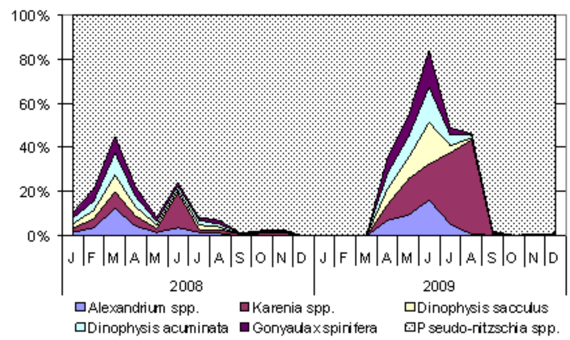

Figure 3. Dynamics of all potentially toxic species (maximum values, %)

In Butrinti lake, the amount of organic matter increases in water as in sediments, even though the living conditions make a favorable habitat for shellfish reproduction, as evidenced by intense growth of *Mytilus galloprovincialis* (6). The optimal depth for mussel growth is about 2-3 m and the most favorable period is during spring-summer beginning (11). Growing mussel sometimes becomes very difficult from HABs, period (winter 2008-2009) during which the growth rate of *Pseudonitzschia* species is high, and from H_2S production due to the anaerobic process at the bottom layers (5, 11). These data should be taken into account, because they represent a potential risk to aquaculture and for the survival of aquatic organisms in general.

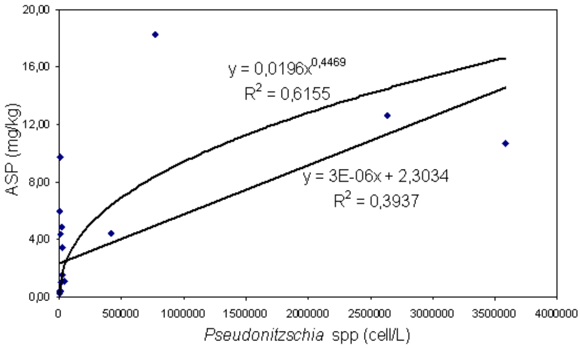

Figure 4. Linear and power correlation between Pseudo-nitzschia spp. and ASP values per months

CONCLUSIONS

Continous knowledge of the growth of toxic phytoplankton species and their related toxins is of enormous importance in ecologic studies, especially in aquaculture activity and human health security. It has also important scientific values, focused on understanding the conditions under which toxic blooms occur and eventually predicting them.

The potentially toxic species observed in Butrinti lake were: *Pseudonitzschia* spp., *Karenia* spp., *Alexandrium* spp., *Dinophysis acuminata, D. sacculus, Gonyaulax spinifera* etc. In January-February 2009 *Pseudo-nitzschia* spp. reached the highest density accompanied with highest values of ASP in March, but within the permit limits. The relative domination and blooming trend of *Pseudonitzschia* species is an indicator of the stressing condition of the wetland system, probably due to the combination of climate changes, nutrients and scarce circulation of water.

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