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Seasonal Variations of Nitrosamine Content in Some Black Sea Fish Species

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Abstract

The present study examined the interrelationships of water temperature, phytoplankton concentration and nitrosamine compounds (NA) content in six Black Sea teleoste fish species investigated in 4 seasons. The total level of NA in examined fish ranged from 0.0002 to 0.003 µg per kg that is lower then the legal level in Ukraine (0.003 µg per kg). The significant decrease of NA concentration in all fish species in warmer season was observed. The negative correlations were found between NA level in fish, water temperature and microalgae concentrations in water. The interactions between endogenous and exogenous factors and NA formation in fish are discussed.

Key words: eutrophication, nitrite pollution, phytoplankton, temperature, risk assessment.

Introduction

The anthropogenic modification of nitrogen circulation in biosphere connected with the human activity leads the high distribution and accumulation nitrogen compounds in the environment, including marine ecosystems. Direct sewage into the sea from coastal settlements containing heavy loads of fertilizer and human waste is the major contributing factor to the degradation of the Black Sea environment. There is sufficient historical data to demonstrate that there has been an enormous increase in the nutrient load to the Black Sea in 25 years. In the 1950s, the Danube, the Dniester and Dnieper annually carried 14,000 t of phosphates, 150,000 t of nitrates and 2,350,000 t of organic matter into the North-western Black Sea. By the 1980s, the rivers were carrying an annual average of 55,000 t of phosphate (about 4 times more), 340,000 t of nitrates (about 2 times more) and 10,700,000 t of organic matter (about 4.5 times more) (Michnea, 1998; Rudneva and Petzold-Bradley, 2001). They caused water eutrophication and formation nitrosamines (NA) in water, sediments and in aquatic organisms. The formation of NA is relatively easy from nitrogen containing precursors such as nitrogen oxides, nitrates, nitrites, amines and amides. Exogenous NA synthesis is demonstrated in water, soil, air and endogenous is showed in biota. The NA content in environment depends on the circulation of their nitrogen precursors, their distribution in environment, microorganisms metabolism. The anthropogenic impact on ecosystems including water and air pollution together with solar radiation gives damage to the normal nitrogen balance in biosphere and stimulates the formation of NA compounds.

In recent years, NA have been reported as the most potent groups of known cancerogens. They cause cancer, mutations, embryonic defects and anomalies of development in 40 species of animals including aquatic organisms (Rubenchik, 1990; Winston *et al*., 1992). The precursors of NA compounds (nitrogen oxides, nitrates, nitrites, amines and amides) are highly distributed in the environment. Besides that, NA form from some pesticides and drugs. Reaction between nitrite and certain amine compounds in marine water might be expected to result in the formation of NA.

Bacteria and microorganisms also play an important role in this process. They might reduce nitrate to nitrite (the main precursor of NA) in some cases. In water where excess nitrogenous wasteis received, an imbalance between bacteria nitrification and denitrification can occur, eventually leading to nitrite accumulation. It acts to increase water nitrite that can accumulate to high levels that are toxic to aquatic animals. In this case, NA pose potential hazards to the health of fish and people; because they are transfered via food chains (Deane and Woo, 2007). Thus, seafood should be tested for NA identification for evaluation of safety control and risk.

The aim of the present work was to study the interactions between some seasonal parameters of marine environment (water temperature, nitrogen compounds and phytoplankton concentrations) and NA content in Black Sea fish species.

Materials and Methods

Animal Sampling

Scorpion fish *Scorpaena porcus* L., toad goby *Mesogobius batrachocephalus* Pallas, shore rocking *Gaidropsarus mediterraneus* L., whiting *Merlangus*

merlangus euxinus Nordmann, red mullet *Mullus barbatus ponticus* Essipov and Mediterranean scad *Trachurus mediterraneus* Staidachner were seasonally collected (from winter 2003 to winter 2004) in Sevastopol Bay, Black Sea, Ukraine (Figure 1). Fish were caught, kept on ice, killed by spinal section and brought to the laboratory. Fish meat was prepared and frozen at -20°C for NA determination. NA were extracted by cold ester from 100-150 g of tissue, pooled from 10 - 15 fish.

Procedure

NA detection involved the use of thin-layer chromatographic method on silicagel plates in the chlorophorm : benzol : ethylacetate : acetic acid solution in ratio volume 18:10:8:0.5 in triplicates (Kawabata *et al*., 1974; Acronymous, 1979). NA were identified by UV irradiation light and compared to control and reference samples on the base of R_f. The solutions of dimethylamine, diethylamine, piperidine, pirollidine and dibuthylamine were applied as reference samples.

Summary: NA content was estimated according the following formula:

$$C = 1000\ Kn/Vm$$

C – NA content in the sample, µg per kg;
n – NA content in the silicagel plate, ng;
V –sample volume, µl;
M – examined sample mass, g;
K – extraction coefficient;

Data of water temperature was presented by the Sevastopol Department of Black Sea State Protection Agency. The data of phytoplankton concentration in Sevastopol Bay were cited by the publication of Lopuhina and Manjos (2005).

Statistical Analysis

This was performed using Student t-test for paired values (Lakin, 1990). The values presented are the mean $\pm$ SD of three or more independent measurements per each season. Correlation test was employed to analyze the relationship between water temperature, phytoplankton cell concentration and NA content in examined fish species. In all cases, the significant level adopted was 95% (P= 0.05).

Results

The obtained results demonstrated the common tendency of decreasing NA content in the warmer period in all examined fish species. In winter period, the highest was detected in *S. porcus* and *G. mediterraneus* (higher than those in other fish species) (Figure 2, P<0.05). No significant differences were found between *M. batrachocepalus, M. merlangus euxinus* and *M. batrachocepalus*. The lowest NA concentration was observed in *T. mediterraneus* (P<0.05). In spring, NA content was higher in *S.porcus, M. batrachocepalus* and *M.barbatus ponticus* than in other fish species, while no significant differences were found in NA concentrations in examined fish in summer. In autumn, *S. porcus* and *M. batrachocepalus* showed significantly higher (P<0.05) NA content than the other fish, of which the parameters were not significantly different. Negative correlation between water temperature and NA content in fish was found (Table 1).

The strong correlation (r = -0.63) was showed between water temperature and NA content in *G. mediterraneus*, the medium values were found in *S. porcus* (r = -0.338), *M. barbatus ponticus* (r = -0.328), *M. merlangus euxinus* (r = -0.328), and *T. mediterraneus* (r = -0.292). No correlation was

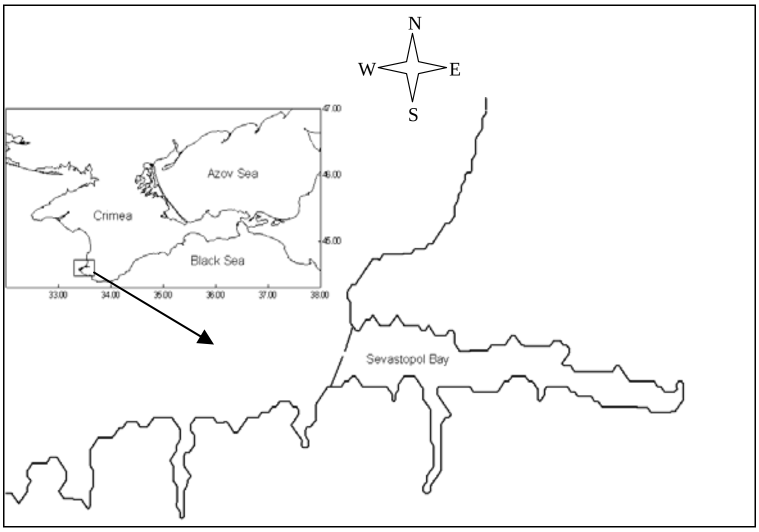

Figure 1. Sampling site of fish specimens in Sevastopol Bay (Black Sea).

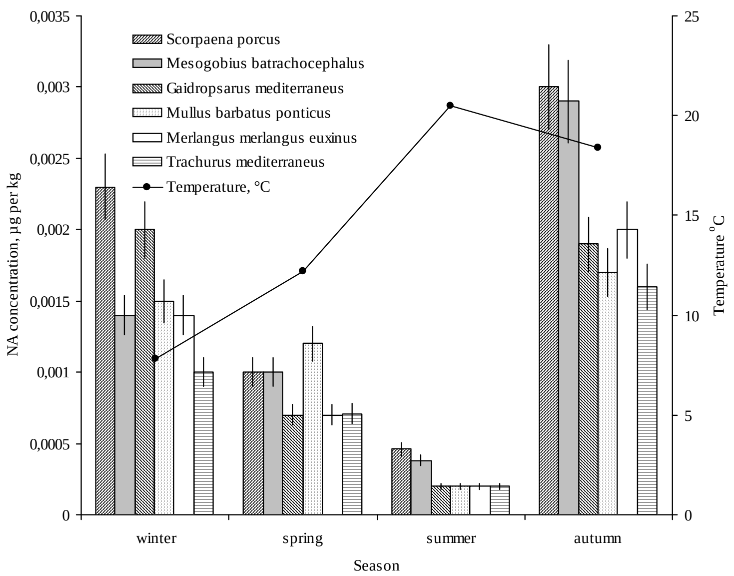

Figure 2. Interactions between NA content in fish and water temperature.

Table 1. Correlation coefficients between water temperature, phytoplankton cells number and NA content in fish

Fish species	Correlation coefficients between water temperature and NA contents in fish	Correlation coefficients between phytoplankton cells number and NA contents in fish
S. porcus	-0.338	-0.741
M. batrachocepalus	-0.096	-0.649
G. mediterraneus	-0.630	-0.806
M. barbatus ponticus	-0.328	-0.766
M. merlangus euxinus	-0.32	-0.766
T. mediterraneus	-0.292	-0.782

observed in *M. batrachocepalus.* Thus, the obtained results showed that water temperature is an important seasonal parameter influences NA formation in fish.

According to the results obtained by Lopuhina and Manjos (2005), the concentrations of microalgae cells in Sevastopol Bay in the examined period were increased in 2 times in summer (Figure 3). Strong negative correlation between NA content in fish tissues and phytoplankton cell concentrations was showed (Table 1). Significantly higher correlation coefficients than in the case of interactions between NA content and water temperature was observed. The highest correlation was detected in *G. mediterraneus* (r = -0.806), the lowest was in *M. batrachocephalus* (r = -0.648), the others were approximately identical. Thus, the correlation between NA content in examined fish species and phytoplankton cells concentration in water was stronger than that between NA content in fish and water temperature.

Discussion

Several field studies with various fish species have shown the presence volatile NA in their tissues (Ijengar *et al*., 1976; Ruiz-Capillas *et al*., 2000). NA at levels ranging from 0.01 to 0.1 ppm have been confirmed in Chinese marine salt fish *Argirops spinifers*, *Nemipterus virgatus*, *Scomberomorus commersoni* and *Pseudosciaena crocea.* In contrast, fresh water fish were shown to contain a lower level of NA and therefore to present a less favourable environment for the formation of NA in general (Fong and Chan, 1976). The levels of N-nitrosodipropylamine were found to be 2.8 µg per kg on an average in raw samples of Alaska pollak *Theragra chalcogramma* (Sung *et al*., 1997). The epidemiological studies of 145 samples of cooked salted fish in China showed that the total volatile NA was 0.028-4.545 mg per kg. The researchers suggested that the consumption of Chinese salted fish is causative factor for nasopharyngel carcinoma (NPC). They demonstrated the positive correlation between the levels of NA and mortality from NPC. Their results support the conclusion that the high NPC risk may be attributed to consumption of salted fish containing high contents of NA (Zou *et al*., 1994).

Our results showed that in examined Black Sea teleoste fish species tissues, NA content ranged from 0.0002 to 0.003 µg per kg. It was lower than the legal level which is 0.003 µg per kg of fish in Ukraine. At the same time, clear seasonal variations were observed. Values were lower in the warmer season

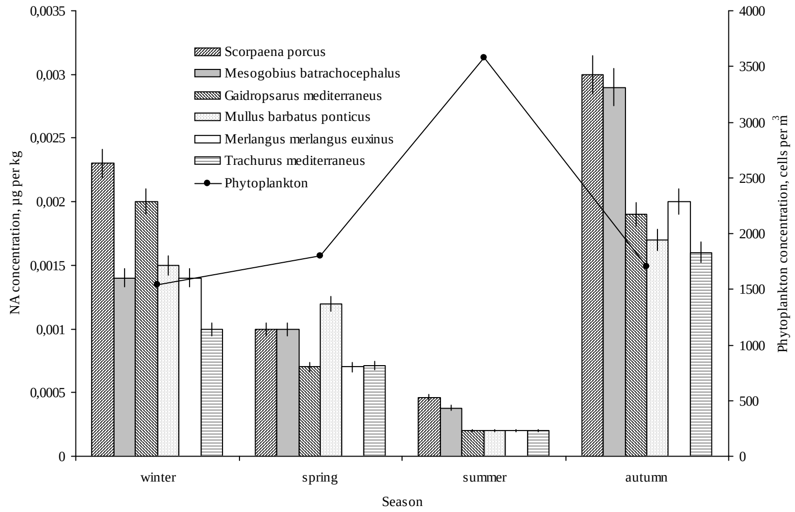

Figure 3. Interactions between NA content in fish and phytoplankton concentration in water.

and increased in autumn. Water temperature and phytoplankton cell concentration also showed significant seasonal variations which negatively correlated with NA content in fish. Medium and low correlations were found between NA content in fish and water temperature, while correlation between NA level and phytoplankton concentration was stronger. So the lower NA values observed in fish collected in spring and summer could be explained by higher concentrations of microalgae cells in warmer period. It is well known that microalgae utilize nitrogen compounds dissolved in water which are needed for their growth and development. Thus, the concentration of NA and especially their precursors, decreases in warmer period; and the risk of NA formation in water and biota also decreases.

An important role of microorganisms in the reactions of NA formation in water was reported by other researchers (Barabasz *et al*., 1995; Kim *et al*., 1996; Sangjindovong, 1997). At the same time, fertilized and hatching eggs are the most sensitive to toxic effects of exogenous NA. In laboratory conditions, it was demonstrated that NA in high concentrations decreased the hatching of fertilized eggs and therefore through their effect on eggs hatchability, may reduce fish populations along with increasing aquatic eutrophication (Bieniarz *et al*., 1996). In autumn time, phytoplankton bloom decreases, microalgae cells finish their vegetation and dump on the bottom, nitrogen compounds dissolve in water and accumulate in bottom sediments. Their increased concentration causes the formation NA precursors in water and in biota.

Besides that, the peculiarities of fish biology and ecology could influence NA content in examined species. In most cases, NA content is higher in bottom fish species (*S. porcus*, *M. batrachocepalus*, *G. mediterraneus*, *M. barbatus ponticus* and *M. merlangus euxinus*) than in pelagic *T. mediterraneus.* These differences are clearer in colder period than in warmer time. It could be suggested that bottom fish species affected by NA precursors are stronger than pelagic ones for three possible reasons. Firstly, they are inhabited at the bottom area and their preferable food is bottom invertebrates containing high levels of xenobiotics including NA and precursors, which transfer through food chains. Secondly, the dead algae cells dump on bottom and thus the lower water layers contain higher concentration of nitrogen compounds than the upper layers. The interaction between them causes the formation of NA and their precursors which involve in fish metabolism. Thirdly, pelagic *T. mediterraneus* migrates and can to leave the polluted and eutrophed waters with unfavourable living conditions, while the bottom fish species cannot migrate in long distances and prefer to stay at the constant locations. Additionally, the differences in metabolism of bottom and pelagic fish species should be also taken into account.

Based on the knowledge obtained from these and other studies, it is our opinion that the seasons and related factors such as temperature, eutrophication and algae bloom play an important role in NA formation in fish. It needs to be assessed in fisheries and seafood production.

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