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Trace elements and nitrosamines concentration in black sea elasmobranch species

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Abstract

The trace elements (Pb, Cu, Zn, Cd, As and Hg) and nitrosamine (NA) concentrations were determined in tissues of three Black Sea elasmobranch species Atlantic spiny dogfish *Squalus acanthias,* buckler skate *Raja clavata and* stringray *Dasyatis pastinaca. The levels of examined chemicals were below the maximum level permitted by Ukraine State Standards. Significant concentration differences among species were detected for Cu, Pb, As, Hg and NAs which were higher in skates than in shark tissues.* The results indicate that the complex of non-organic and organic pollutants in elasmobranchs together with their biological characteristics can be applied to evaluate the ecological status of the marine environment and risk assessment.

INTRODUCTION

Shallow coastal waters of the marine environment are highly impacted to the anthropogenic pollution which leads negative consequences for biota. Direct sewage into the seas and ocean from coastal settlements containing heavy loads of trace elements, biogens, oil hydrocarbons and pesticides is the major contributing factor to the degradation of aquatic ecosystems. Toxic effects of trace elements on marine biota are documented by many researchers [1, 2]. Heavy metals accumulated in seafood (commercial fish and invertebrates) are very harmful to human health [3, 4]. Trace elements impacted negatively on fish population status and biodiversity which we described previously [5].

High concentration of biogens in marine environment causes eutrophication and formation of nitrosamines (NAs) in water, sediments and in aquatic organisms. NAs content in environment depends upon the circulation of their nitrogen precursors, their distribution in environment, and microorganisms metabolism. The anthropogenic impact on ecosystems including water and air pollution together with solar radiation damage the normal nitrogen balance in hydrosphere and stimulate the formation of NA compounds which have been reported as the most potent groups of known cancerogenes [6, 7]. Hence, NAs pose a potential hazards to the health of fish and people because they are transfer via food chains [8, 9].

Sevastopol Bay (Black Sea, Ukraine) is highly polluted marine area. According to the data of State Agency of Black Sea Protection, 60 mln. m^3 of sewage are entered the coastal waters of city Sevastopol annually, including 44 mln. m^3 (73%) waters after mechanical treatment, 8 mln. m^3 (14%) without any treatment and approximately 8 mln. m^3 (13%) after biological treatment [10]. Annually rivers enter into the Sevastopol coastal waters 3187 tons of particular matter, 156 tons of the total nitrogen, 2.1 tons of nitrites, 120 tons of nitrates, 7.6 tones of phosphates, 13.1 tones of total phosphorus, 310 kg of the Cu, 770 kg of Zn, 50 kg Cr and other chemicals [11]. Since the end of 1990s and at present the content of pollutants was decreased caused the economic crisis but not the ecosystem protective actions and special remedies [12, 13].Fish are very sensitive to the changes of biotic and abiotic factors and thus they are specific indicators of different environmental pollutants including heavy metals and biogens. They accumulate chemicals from water and food and sometimes their levels are toxic for themselves and for human consumers [14, 15]. The data of the trace elements and NAs concentration in fish is very important for assessment the ecological status of coastal waters and risk for human health.

Teleost fish species of the Sevastopol coastal waters are studied very carefully during a lot of time and at recent years their biodiversity was decreased caused anthropogenic pollution, overfishing and intruders activity [16]. The information of elasmobranch status in Sevastopol region is very limited. At the same time predators and carnivorous species can be used as indicators for the monitoring of marine environment of trace elements and NAs pollution. In addition, elasmobranches are commercial fish species and their catches are expected to decrease at present and, probably, in the future. In some publications are documented the decrease of elasmobranch populations and one of the reason of this could be associated with the negative environment for these forms caused water and sediment pollution. Both shark and skates have been legally fished for human consumption because their fillets are characterized high food quality, flavor and lack of the bones [17]. They contain great concentrations of fat-soluble vitamins (D, E and A), polyunsaturated fatty acids and antioxidants, especially in the liver. The fat of the elasmobranches is used for pharmacological and food purposes. Hence, the aim of the present study was to document the baseline concentrations of six trace elements (Zn, Cu, Pb, Cd, Hg and As) and NAs in three commercial elasmobranch fish species caught in Sevastopol coastal waters (Ukraine, Black Sea) and to show correlations between trace elements, NA concentration and fish ecological status.

MATERIALS AND METHODS

Sampling sites

Fish were caught at two sites both situated at the Sevastopol coastal waters in 2003-2005 (Figure 1). Marine waters in the

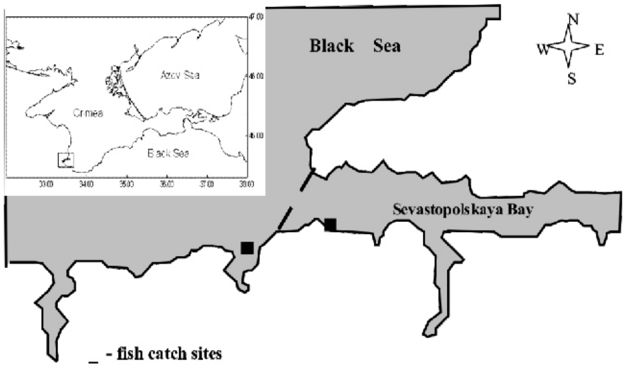


Figure 1: Sampling sites in Sevastopol Bay (44o36'N - 33o32'E, Sevastopol, Black Sea, Ukraine)

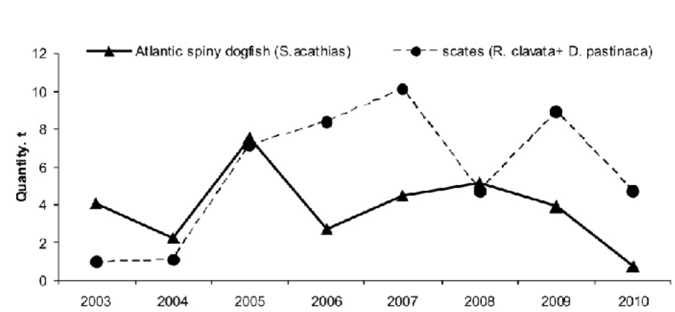


Figure 2: Commercial catches of elasmobranch fish in Sevastopol region (Black Sea, Ukraine) at the period of 2003 2010.

examined region is highly polluted caused of man-made activity including maritime transport, navy, recreation, domestic sewage as we described above.

Sample collection and preparation

Three elasmopbranch species Atlantic spiny dogfish *Squalus acanthias* (L. 1758), buckler skate *Raja clavata* (L. 1758) *and* stringray *Dasyatis pastinaca* (L. 1758) were studied. Scientific species names of the fish were described according Vasil'eva [18] and [19].

Fish were individually measured and weighed and captured. The total (L), disk (Ld) lengths, total weight and soma weight, sex, stages of sexual maturity, weight of gonads and liver were measured. Liver and gonads were weighted for somatic indices calculations (I_H and I_G).

Hepatosomatic indices were calculated according the formula [20]:

$$I_H ‰ = (Wl / Ws) \cdot 1000$$

Gonadosomatic indices were calculated according the formula [21, 22]:

$$I_G \% = (Wg / Ws) \cdot 100$$

where Wg, Wl, Ws gonad weight, liver weight and soma weight in grams of fish.

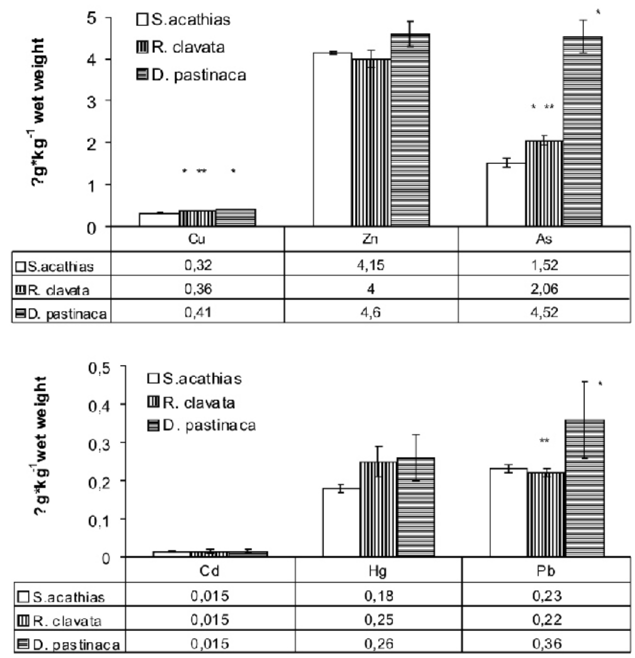
Fillets were frozen and stored at the temperature -20°C and then were used for trace metals determination and biochemical assays.

Chemical analysis

Trace elements determination

Metal concentration levels were determined in single animal according the method described in [23]. Fish tissues were dried for 9 hours at increasing temperature from + 50ºC to +450ºC with the interval of 30 min. Dried samples were digested with concentrated nitric acid. Concentrations of Cd, Cu, Pb and Zn were measured by atomic absorption method used Hitachi (Japan) spectrophotometer. Arsenic concentration was detected by spectrophotometric method using Ag-diethylditiokarbamat reagent in chloroform and calculated on the basis of calibration standards at the wave length of 520 nm.

Mercury concentration was determined on atomic absorption



	Cu	Zn	As
S.acathias	0,32	4,15	1,52
R. clavata	0,36	4	2,06
D. pastinaca	0,41	4,6	4,52

	Cd	Hg	Pb
S.acathias	0,015	0,18	0,23
R. clavata	0,015	0,25	0,22
D. pastinaca	0,015	0,26	0,36

Figure 3: Trace elements content (mean ± S.E., mg kg-1 wet weight) in muscle tissues of elasmobranch fish species, caught in Sevastopol Bay (Black Sea, Ukraine). * - significant differences compared with S. acanthias, ** - significant differences compared with D. pastinaca.

spectrophotometer "Julia-2" (Russia) [24]. 5 g of the tissue was added in 1 ml ethanol, 5 ml distilled water, 5 ml concentrated nitric acid and incubated at the room temperature at 30 min. Then 5 ml concentrated sulphuric acid was added in the mixture and heated during 15-20 min at +70ºC, then added 30 ml of twice distilled water and boiled on water bath during 15-20 min. 1 ml of the sample was added in the cuwett of the spectrophotometer and Hg concentrations was measured.

All determinations were processed in triplicates.

NAs determination

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 [25, 26]. 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 NAs content were estimated according the following formula:

$$C = 1000Kn/Vm$$

C NAs content in the sample, µg per kg;

n NAs content in the silicagel plate, ng;

V sample volume, µl;

M examined sample mass, g;

K extraction coefficient;

Statistical analysis

Chemical analysis was determined individually in 5 organisms of each species, assays run in triplicate. The results were processed to statistical evaluation by ANOVA. All numerical data are given as means ± S.E. [27]. Statistical significant differences were assessed using a Student's t-test, the significance level was $p \leq 0.05$. Correlations were calculated by the least-squares method between trace elements concentration and NAs concentration used the program CURVFIT.

RESULTS

Catches trends and biological characteristics of Elasmobranch fish from Sevastopol Bay in the period of 2003 2010

Three examined elasmobranch species contribute to trawl component of the Sevastopol region fishery (Figure 2). The catches of the elasmobranchs varied during the investigated period and at the recent years they were decreased in Sevastopol coastal waters which could be connected with the poor ecological status of the marine environment and the unfavorable conditions for fish life. Biological characteristics of the examined fish species are differed each from other [18, 28]. Atlantic spiny dogfish *S. acanthias* is gregarious bentic-pelagic fish which inhabits on the depth of 180-200 m and in the upper layers of the water for feeding. It is carnivorous species and its diet includes fish (horse mackerel, pickerel, atherina, red mullet, and etc). The shark is viviparous fish, the maturation period begins at the age of 13-14 years and body length of 1 m. Incubation period of the embryos is 2 years, individual female produces 10-12 embryos and 18 eggs. Usually the length of the adults is estimated as 150-208 cm and the weight 14 kg. Fish life span is approximately 25 years, number of males in the catches are higher than females. According our data the individuals caught in the coastal area of Sevastopol region were juvenile fish. The liver mass of the adult sharks is estimated as 17.9-29.6% of the total body weight [28]. The I_H of fish in our study was significantly less (Table 1).

Buckler skate *R. clavata* is benthic species, it inhabits the bottom area at the depth of 100 m. In Black Sea it buries itself in the bottom sediments or in the soil. Females spawn at the spring time in the shelf. Every female spawns from ten to hundreds eggs on the bottom. The period of the embryogenesis continues 4.5-5.5 months. The length of the male is 70 cm and the female 125 sm. Its diet includes benthic fish species (scomber, pickarel), crustacea and molluscs with the first group predominating [18, 28]. In our study the size of the fish was also significantly smaller as compared of the adults and we suggest that our fish were juvenile.

Stringray *D. pastinaca* inhabits the benthic lays at the depth of 200 m and buries itself in the bottom sediments or in the soil. The size of adults is estimated as 60-70 cm and the mass is 6-10 kg. Maximum length is 1-2.5 m and mass 16 kg. It is viviparous, the development of the embryo continues 4 months. Every female produces 4-12 individuals and 12-32 developmental eggs. The diet includes small fish species, crustacea and mollusks [18, 28]. According to the data presented in Table 1 the examined fish samples were juvenile as in the case of buckler and shark.

Trace elements concentration in Elasmobranch fish in Sevastopol Bay

Content of examined trace elements in elasmobranch fish species is presented in Figure 3. Trace elements concentrations in tissues of tested elasmobranch fish species were below the maximum levels permitted in fish fillets in Ukraine (10 for Cu, 1 for Pb, 0,2 for Cd, 40 for Zn, 5 for As and 0,4 for Hg mg kg^{-1} wet weight respectively). Zn was the highest in tissues of all species examined, followed by As, Cu, Pb, Hg and Cd.

Table 1: Biological characteristics of elasmobranch fish caught in Sevastopol Bay (Black Sea, Ukraine).

Species	L, cm	Ld, cm	Weight, g	I_H, ‰	I_G, %
Atlantic spiny dogfish *S. acanthias* (n=16)	32.2 – 53.4 44.32±1.27	30.1 – 50.8 41.87±1.24	105.9 – 652.96 319.2±33.01	8.39 – 18.08 12.95±0.63	0.01 – 0.263 0.099±0.08
buckler skate *R. clavata* (n=43)	21.0 – 68.5 42.52±1.45	16.4 – 41.6 23.95±0.98	115.01 – 196.62 460.04±62.43	1.67 – 6.41 3.94±0.17	0.048 – 1.02 0.46±0.03
stringray *D. pastinaca* (n=31)	33.70-65.0 43.13±1.24	16.6 – 35.8 22.42±0.90	209.89 – 2399.0 592.59±87.34	4.15 – 14.21 8.47±0.42	0.216 – 1.467 0.81±0.04

Comments: n number of animals; L total length, Ls standard length, IH hepatosomatic indices, IG gonadosomatic indices; above line min-max values, under line - mean ± SEM

The levels of several elements in fish tissues demonstrated interspecies differences. The greatest concentration of Cu was observed in stringray while in buckler skate the value was significantly less ($p<0.01$). At the same time it was higher than in dog fish ($p<0.05$). Concentration of Pb in stringray was significantly higher than in other examined species ($p<0.001$). The level of Pb in dogfish and in buckler skate was the similar. No significant differences were shown in concentration of Cd, Hg and Zn in examined fish species. Content of Hg tended to increase in both skates as compared with shark. The concentration of As was significantly higher in both skates compared to shark ($p<0.001$). Concentration of As in stringray tended to legal level permitted in Ukraine.

Strong liner correlation was observed between Cu and Pb content in fish muscle ($r=0.98$), between Cu and As ($r=0.84$) and between Pb and As ($r=0.89$). Close linkage was found between Cu and Hg level ($r=0.58$), Pb and Zn ($r=0.64$) and Zn and As (0.68). In other cases no relations were shown.

NAs Concentration in Elasmobranch fish caught in Sevastopol Bay

NAs concentration in the tissues of three Black Sea elasmobranchs is presented in Figure 4. The levels were significantly higher ($p<0.01$) in skates than in shark. No differences were observed between skates.

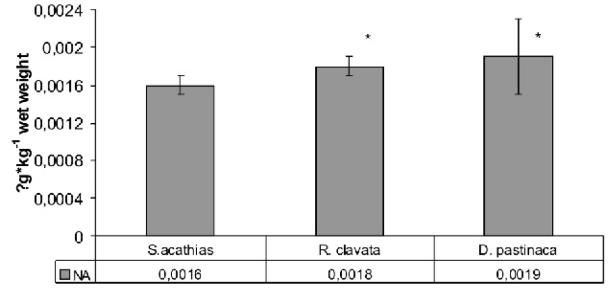


Figure 4: Nitrozamine content (mean ± S.E., μg kg-1 wet weight) in muscle tissues of elasmobranch fish species, caught in Sevastopol Bay (Black Sea, Ukraine). * - significant differences compared with S. acanthias

Discussion

Comparison of mean trace elements and NAs concentration in the tissues of Black Sea Elasmobranchs with mean concentrations reported in Elasmobranch species in other studies

Trace element levels obtained in the tissues of Black Sea elasmobranch species caught in Black Sea (Sevastopol shelf zone) were comparable to those which have been reported in fish worldwide [17, 29]. In the muscle tissues of three examined species concentration of tested elements was Zn > As > Cu > Pb > Hg > Cd. Skates were more contaminated than shark. Cooper concentration in muscle of Black Sea elasmobranchs ranged between 0.32 and 0.41 mg kg^{-1} wet weight. It was generally of the same values as those reported elsewhere in elasmobranch tissues (0.0073 to 5.31 mg kg^{-1} wet weight) [17, 30]. No significant differences were observed for Zn concentration in the tissues of Black Sea elasmobranch species (4.0-4.6 $mgkg^{-1}$ wet weight). They were comparable with the Zn content in the muscle of the elasmobranch fish from different geographical locations (0.2 - 18.3 mg kg^{-1} wet weight) [17, 30, 31]. Lead level was significantly higher ($p<0.01$) in stringray than in both examined species. It was intermediate among the values detected in the elasmobranch from different waters which were varied from 0.01 to 1.88 mg kg^{-1} wet weight [17]. Cadmium concentration was identical in three Black Sea elasmobranchs. It was comparable with the Cd content in the fish from other regions (0.01- 1.08 mg kg^{-1} wet weight) [17]. Mercury concentration was higher in Black Sea skates (0.25-0.26 c wet weight) as compared with shark (0.18 mg kg^{-1} wet weight) but the differences were not significant. The obtained values were less than in the elasmobrachs from Southeastern Australian waters (0.77- 2.99 mg kg^{-1} wet weight) [17, 32] and Japan region (0.019-2.13 mg kg^{-1} wet weight) [30]. Mercury levels in the muscle of examined elasmobranchs caught in Sevastopol Bay were comparable with the values found in the fillets of the fish from Izmir Bay (Eastern Aegan, Turkey), ranging from 0.023 to 0.224 mg kg^{-1} wet weight [33]. Arsenic content was significantly greater in both skates compared to shark. The values were comparable with those measured in sharks worldwide (0.7-29 mg kg^{-1} wet weight) [17].

Hence, the concentrations of trace elements tested in Black Sea elasmobranch tissues are also in the same range at those reported by other investigators [17, 32, 33]. Several field studies have shown the presence of NAs in fish tissues [34]. NAs level ranging from 0.01 to 0.1 ppm has 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 NAs and therefore to present a less favorable environment for the formation of NAs in general [3]. Content of N-nitrosodipropylamine was found to be 2.8 μg per kg on an average in raw samples of Alaska pollak *Theragra chalcogramma* [35]. The epidemiological studies of 145 samples of cooked salted fish in China showed that the total NAs concentration was 0.028-4.545 mg per kg [36]. Our results have been shown that in Black Sea teleost fish species NAs content ranged from 0.0002 to 0.003 μg per kg. It was lower then the legal level in Ukraine which is 0.003 μg per kg of fish [37]. The values of NAs in elasmobranch tissues measured in this study were the same as compared with teleost species caught in Sevastopol Bay.

Trace elements level, fish size and their ecological status

There are several factors including abiotic (physical and chemical parameters of the water and sediments in marine location), biotic (specificity of fish biology, feeding behavior, age, sex, swimming activity and physiological status) and anthropogenic (level of pollution led human activity) that may affect chemicals accumulation in marine organisms. The most important factor is diet [38]. Fish have been recognized as the most important source of mercury in aquatic environments while invertebrates (mollusks and crustacean) are the great source for cadmium [31, 39]. Trace metal accumulation in elasmobranch species from Sevastopol Bay may thus be expected since they are top predators in their corresponding trophic net. Dietary differences among skate species and shark seemed to be the most important causes for differences in their trace elements level in muscle. Content of Cu, As and especially Hg was higher in skates than in shark.

Cu is the essential element and its concentration is regulated in the organism by physiological pathways [40] while As and Hg are non-essential and their accumulation in fish depends on feeding habits. Animal feeding of algae and crustacean appear to retain higher arsenic concentrations than piscivorous forms. Most marine animals have only limited ability to accumulate arsenic from seawater and its concentrations in the fish depends on their food and trophic position in the local food chain. The element assimilated from the food is presented in the tissues in organic form, whereas that accumulated from water remains inorganic and is excreted rapidly or sequestered in non-living tissue compartments [41]. Arsenic in marine organisms is found in the fat or water-soluble forms such as arsenobetaine which is less toxic than inorganic form [17]. It usually represents 50% to more than 95% of the total arsenic in tissues of marine invertebrates and fish, including elasmobranch [41]. Hence, the differences of arsenic concentrations in tested elasmobranches might be related to diet because skates food preferences include benthic crustacean and mollusks while in shark diet fish dominate.

Environmental mercury and its accumulation in the tissues of aquatic animals is often considered with anthropogenic pollution, but in some cases the main sources of Hg are natural connected with geological processes in the earth and water [17]. At 1990s the average concentration of Hg varied from 1 to 7 legal levels (59 ng l^{-1} and 109 ng l^{-1}) in the water of Sevastopol Bay and in general it was estimated as 60% of legal level [42]. Fish retain mercury in their tissues as methylmercury and its concentration increased via trophic chain from inorganic mercury in anaerobic bacteria to organic form associated with fish gills and gut. Methylmercury the most toxic form and it is estimated for more than 95% of organic mercury in fish muscle. It may be biomagnified through all levels of the aquatic food chain as it accumulates in the top tophic level including carnivorous species [33]. The lipophilic nature of methylmercury facilitates its penetration across cell membranes and causes their damage. Hg interacts with SH-groups of the proteins and inactivates them. Long biological harflife causes to its rapid accumulation in fish tissues [39].

Several researchers have reported that trace elements concentrations associated with body size in marine fish [4, 17, 31, 33]. Strong positive correlation between fish length and metal content (mercury, copper and cadmium) was obtained in sharks caught in Argentina region [31]. Data from the literature emphasis the importance of biometric parameters in analysis on the bioaccumulation of mercury [39]. Close relations were documented between Hg concentrations in muscle and the length of teleost and elasmobranch species from Izmir Bay [9], North Queensland [32] and Southeastern Australia waters [17]. The results reflected the increase of trace elements accumulation with age and body size. Strong positive correlation between mercury content and total length of *S. mitsukurii* was found while for other metals (Zn, Cd and Cu) negative correlations were observed. Regression of Hg concentration on age of shark was shown to be linear [30]. As a rule in elasmobranchs, elder fish are larger animals and they require large prey for feeding. Large feeding organisms contain high levels of xenobiotics, including trace elements which are accumulated in the predators body [17]. In contrast, several authors reported that metal contents tended to decrease with fish length which was documented at the case of deep-water sharks caught in North-Eastern Atlantic [40]. The researchers proposed that such changes may reflect a decrease metal exposure at greater lengths due ontogenic changes in diet and bathymetric habit. The increase of mercury content with age and correspondingly with body size and length is connected with slow turnover of Hg in fish [39, 43].

In our studies there were no significant relations between fish length and metal concentrations in muscle because the variations of body size of tested specimen were insignificant and we propose that the fish belonged to the same age classes. Differences of trace elements content in Black Sea elasmobranchs could be associated with the interspecific variations in diet and living conditions. Skates are principally benthic forms whereas the shark is bento-pelagic predator. The higher concentrations of the trace metal in skates may result from increased exposure to them due to the ingestion of toxicant-containing sediments and food [44, 45].

NAs level, fish size and their ecological status

Nutrients level (nitrate and phosphate) is varied in Sevastopol coastal waters and it depends on domestic and river discharges and season fluctuations. In general, the ammonia nitrogen concentration ranged from 5 to 150 mg l^{-1} [10]. In waters that receive excess nitrogenous waste, an imbalance between bacteria nitrification and denitrification can occur, eventually leading to nitrite accumulation. It acts to increase nitrite concentration and the precursors of NAs compounds (nitrogen oxides, nitrates, nitrites, amines and amides) in water that may accumulate to high levels in aquatic animals that are toxic to them and human consumption. They cause of cancer, mutations, embryonic defects and anomalies of development in 40 species of animals including aquatic organisms [6, 7]. The results of few investigators support the conclusion that the high cancerogenesis risk may be attributed to consumption of salted fish containing high contents of NA [36]. Besides that NAs form from some pesticides and drugs. Thus fish especially commercial species should be tested for NAs identification for evaluation of safety control and risk.

High correlation between NAs content and fish size was observed (r=0.69). As we marked above elder fish are larger animals and they require large prey for feeding. Large feeding organisms contain high levels of nitrogen and its compounds including NAs which accumulated in the tissues [17]. Our results demonstrated that skates contain greater concentrations on NAs as compared with shark. There are several reasons which could explain this situation. Firstly, skates are benthic species and their preferable food is bottom invertebrates containing high levels of xenobiotics including NAs and their precursors, which transfer through food chains. Secondary, 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 NAs and their precursors which involve in benthic skats metabolism. Thirdly, benthic- pelagic shark migrates and can to leave the polluted and eutrophed waters with unfavorable living conditions while the benthic skates can't migrate in long distances and prefer to stay at the constant locations. In addition, the differences in metabolism of benthic and pelagic fish species should be also taking into account. The similar results we obtained in teleost fish species caught in Sevastopol Bay: in most cases NAs content was higher in bottom fish species (*S. porcus, M. batrachocepalus, G. mediterraneus, M. barbatus ponticus* and *M. merlangus euxinus)* than that in pelagic *T. mediterraneus* [37].

Relationships between trace elements and NAs content

Strong correlation between Cu, Hg and NAs content was shown (r=0.87 and r=0.91 respectively). The relationship between As concentration and NAs level was less (r=0.48). No relationships between Pb, Cd, Zn levels and NAs content were observed. We could propose that Cu, Hg and As interact with NAs or their precusors and form organic complexes while other trace elements do not react with NAs. In this case the increase of toxic effects of organic forms of Cu, Hg and As are more clearly and it is important to take into account for ecological risk assessment of marine environment and biota.

CONCLUSION

Three tested elasmobranch fish species caught in Sevastopol Bay generally contained lower concentrations of all examined trace elements and NAs in fillets thus there is likely little risk to human health from the consumption of these fishes. The differences between skates and shark are very important from an ecotoxicological view point because skates are benthic forms and could be used as biomonitors in Black Sea ecosystem. In addition in terms of food safety three tested elasmobranchs are commercial species in Black Sea and may be considered suitable for human consumption as metal and nitrosamine concentrations are below than the legal levels permitted in Ukraine.

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