EVALUATION OF HEAVY METALS ACCUMULATION ALONG BOTTLENOSE (*TURSIOPS TRUNCATUS*) TROPHIC CHAIN IN NORTHERN ADRIATIC SEA

OCENA KOPIČENJA TEŽKIH KOVIN V PREHRANJEVALNI VERIGI VELIKE PLISKAVKE *TURSIOPS TRUNCATUS*

Dino SCARAVELLI, Nicola DI FRANCESCO, Marina SILVI,
Annalisa ZACCARONI

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Ključne besede: težke kovine, velika pliskavka, severni Jadran

ABSTRACT

Heavy metals are among the most important pollutants in marine environment. Top predator species are particularly at risk of adverse effects due to heavy metal exposure, thus knowing the contribution of diet to pollutants body burden is extremely important. The present work reports on the evaluation of heavy metals presence in some components of bottlenose dolphin diet, trying to define which could be the contribution that each of them give to the body burden of predators. The obtained data confirm the reduced contamination by lead and mercury of the Adriatic Sea, while cadmium is generally present at concentrations within the range of toxic subchronic threshold defined for marine species. Thus it is possible to consider that a diet containing such levels can potentially induce some alteration also in higher organisms, dolphin included. For arsenic As, mean concentrations are in the range of background levels, but the amounts detected are in the range of tissue concentration corresponding to adverse effects in aquatic organisms, so a potential toxic effect can be considered for the species studied and also for dolphins.

IZVLEČEK

Težke kovine sodijo med najhujše onesnaževalce morskega okolja. Zaradi izpostavljenosti pogubnim učinkom teh kovin so še posebno ogroženi vrhunski morski plenilci, zatorej je nadvse pomembno, da vemo, v kolikšni meri k obremenitvi organizmov teh morskih živali prispeva njihova prehrana. Avtorji pričujočega članka so ugotavljali koncentracije težkih kovin v nekaterih sestavinah prehrane velike pliskavke, s čimer so poskušali ugotoviti, v kolikšmi meri prispevajo k obremenjenosti organizmov velikih plenilcev. Zbrani podatki potrjujejo, da je Jadransko morje manj onesnaženo s svincem in živim srebrom, medtem ko se kadmij na splošno pojavlja v koncentracijah znotraj meja toksičnega praga, določenega za morske živalske vrste. Tako je mogoče reči, da prehrana, ki vsebuje takšne ravni kovin, lahko povzroči določene spremembe tudi v višjih organizmih, vključno z delfini. Srednje koncentracije arzenika so sicer na ravni naravnega ozadja, vendar pa se zaznane količine gibljejo v razponu tkivne koncentracije s škodljivimi posledicami za morske organizme. Obstaja torej možnost toksičnega učinkovanja na preučevane morske vrste in tudi na delfine.

1. INTRODUCTION

Studies on the feeding habits of cetaceans can be used to improve knowledge on both predator and prey biology and on the exposure of these species to pollutants. Hence, dietary studies can also be used to monitor the exposure to different contaminants that can affect the health of dolphins and their preys.

Bottlenose dolphins (*Tursiops truncatus* Montagu, 1821) are widely distributed in inshore and offshore waters in the temperate and tropical zones of all oceans and peripheral seas (e.g. Pacific, Atlantic, Indian Oceans, Mediterranean, Black and Red Seas), sometimes entering rivers and estuaries as well as is frequently seen in coastal waters (Wells & Scott, 1999).

Diets of bottlenose dolphins have been studied in various parts of the world, including the northern Atlantic, the Gulf of Mexico, South Africa, Peru, eastern Australia and the Mediterranean (Gunter, 1942; Ross, 1977; Leatherwood et al., 1978; Desportes, 1985; Barros & Odell, 1990; Cockcroft & Ross, 1990; Corkeron et al., 1990; Mead & Potter, 1990; van Waerebeek et al., 1990; Voliani & Volpi, 1990; Relini et al., 1994; Miokovic et al., 1997; Blanco et al., 2001; Santos et al., 2001), but little or no work have been done to evaluate how each component of the diet contributes to dolphins' heavy metals body burden.

The present work describes a heavy metal exposure risk assessment for bottlenose dolphins in Northern Adriatic Sea waters based on the analysis of an artificial diet composed referring to data concerning diet composition available in the literature.

2. METHODS

Sampling was performed directly at fishing boat to fix fish and cephalopods collection location in the study area (Fig. 1). Species were identified and each subject of the sampling group (n= 30 for each group) was then weighed and measured and subsequently stored at -20°C until analysis, which was performed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) for heavy metals analysis (As, Pb, Cd, Hg). Briefly, amounts up to 700 mg of fresh tissue were collected from homogenised animals and microwave digested. The samples were then transferred to the ICP-AES and analysed. Data are reported on a fresh weight basis as mg/kg. When crabs are of concern, they were separated on the basis of gender and the eggs were separated from females and analysed to evaluate the importance of metal excretion with ovoposition.

After analysis, data were treated as follows to obtain an estimation of the amount of metals that could have been ingested with that particular food item:

Starting from literature (Blanco et al., 2001; Santos et al., 2001; Gannon & Waples, 2004; Santos et al., 2007), the mean composition of dolphin diet was defined. Species were then grouped on the basis of the systematic classes (fish, molluscs, crustaceans, other- including all other species) and the percent of contribution to the diet was calculated (Tab. 1).

Assuming a mean weight for stomach content of 10 kg (Santos et al., 2001), the weight of each group in an "ideal" stomach was calculated and the amount of each metal provided

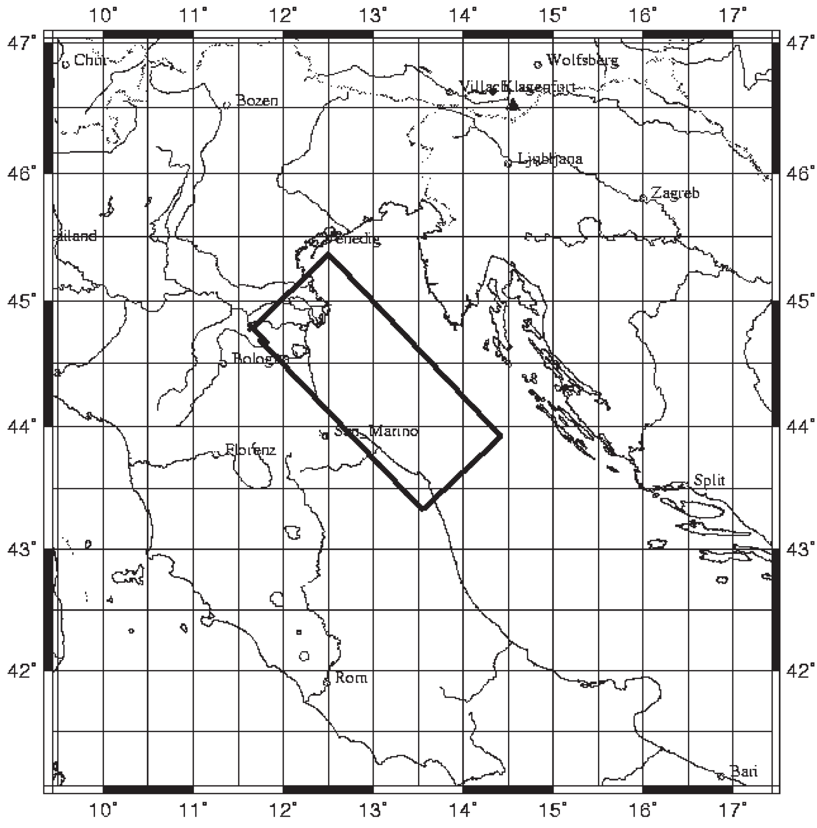

Fig. 1: Sampling area
Slika 1: Območje vzorčenja

with that quantity was calculated in three different scenarios: 1) mean concentration: the concentration/kg was calculated including all species and the mean weight of the stomach content; 2) worst scenario: the mean was calculated considering as the class was composed only by the species presenting higher mean concentration and the highest stomach content found in the literature (15.3 kg); 3) best scenario: the species considered is the one presenting lower concentrations and the lowest stomach content found in the literature (7.6 kg). This calculation allowed to estimate the amount of each toxic metal/year, if the dolphin was feeding on that diet.

Table 1: Percent diet composition as obtained from available literature. The equivalent amount of food in a 10 kg stomach content is also reported.
Tabela 1: Odstotkovna sestava prehrane, pridobljena iz literature. Zabeležena je tudi ekvivalentna količina hrane v desetkilogramski vsebnosti želodca.

Class	**% of the diet**	**Amount (g) estimated in a 10 kg stomach**
Cephalopods	2.15	215
Fish	97.65	9765
Crabs	1.05	105

3. RESULTS

Mean concentrations observed in each species considered are reported in Tab. 2 as mean ± standard error and minimum and maximum value on a wet weight basis.

Highest concentrations were found in all species considered for As, while Pb, Hg and Cd were always detected at low concentrations.

Arsenic seems to be equally distributed in all species considered, as none of them presents extremely high concentrations in any of the samples analysed.

The same is true of all other metals, too, with the only exception of Cd, which reaches mean concentrations in *Sepia officinalis* 4 to 10 folds higher than in other species.

Very low levels of Pb and Hg were observed in all species, with mercury presenting extremely low mean concentration, never higher than 0.02 mg/kg.

Table 2: Mean ± standard error and minimum and maximum value (mg/kg) on a wet weight basis.
Tabela 2: Srednja ± standardna napaka ter najmanjša in največja vrednost (mg/kg) na osnovi mokre teže.

Species/systematic group	**Mean ± s.e. Minimum-maximum**			
	As	***Pb***	***Hg***	***Cd***
Engraulis encrasicolus	3.061 ± 0.168 0.204-5.204	0.023 ± 0.0098 0.001-0.178	0.0047 ± 0.0016 0.001-0.014	0.044 ± 0.0015 0.024-0.068
Sprattus sprattus sprattus	3.183 ± 0.838 1.508-4.067	0.031 ± 0.012 0.008-0.046	0.003 ± 0.0007 0.002-0.0032	0.035 ± 0.0013 0.033-0.037
Solea lutea	6.23 ± 0.804 3.58-11.72	0.066 ± 0.009 0.035-0.130	0.016 ± 0.002 0.008-0.024	0.037 ± 0.007 0.018-0.102
Sardina pilchardus	3.478 ± 0.161 1.279-4.789	0.0709 ± 0.016 0.002-0.279	0.017 ± 0.0028 0.005-0.038	0.035 ± 0.0025 0.023-0.071
Scomber scombrus	2.077 ± 0.046 1.676-3.063	0.0503 ± 0.0209 0.008-0.197	0.008 ± 0.0015 0.002-0.015	0.040 ± 0.0022 0.029-0.080
Solea solea	4.502 ± 0.499 3.573-5.555	0.172 ± 0.039 0.103-0.284	0.010 ± 0.085 0.001-0.41	0.075 ± 0.004 0.066-0.085
Sepia officinalis	5.408 ± 1.117 1.033-9.995	0.048 ± 0.015 0.004-0.187	0.014 ± 0.0037 0.001-0.035	0.119 ± 0.012 0.063-0.239
Carcinus mediterraneus females	6.111 ± 0.533 3.254-9.44	0.092 ± 0.006 0.054-0.122	0.0042 ± 0.001 0.001-0.007	0.045 ± 0.004 0.021-0.069
Carcinus mediterraneus males	1.595 ± 0.170 1.009-2.771	0.025 ± 0.085 0.010-0.102	0.006 ± 0.002 < LOD-0.017	0.0093 ± 0.002 0.002-0.027
Carcinus mediterraneus eggs	3.194 ± 0.612 1.371-5.647	0.068 ± 0.016 0.015-0.103	0.012 ± 0.003 0.001-0.022	0.011 ± 0.002 0.003-0.023

Table 3: Calculation concerning risk assessment and heavy metal load contribution depending on classes and on the scenario considered.

Tabela 3: Ocena tveganja in obremenjenosti s težkimi kovinami glede na posamezne razrede in upoštevani najslabši možni scenarij.

Species/systematic group	**Amount of food (g)**	**Amount of metal with the diet (mg)**			
		As	***Pb***	***Hg***	***Cd***
		"Mean" scenario			
Cephalopods	215	1.1628	0.00873	00234	0.0256
Fish	9765	30.7714	0.2922	0.0474	0.3853
Crabs	105	0.412	0.3096	0.0008	0.00267
		Worst scenario			
Cephalopods	328.95	1,779	0.0133	0.0035	0.0392
Fish	14940.45	47.080	0.4471	0.0726	0.5895
Crabs	160.65	0.6306	0.0132	0.0013	0.004
		Best scenario			
Cephalopods	163.4	0.883	0.0017	0.0195	0.0066
Fish	7421.4	11.087	0.0296	0.0222	0.089
Crabs	79.8	0.127	0.0019	0.00033	0.0007

Starting from the obtained calculation and from the known percent of absorption, a calculation of the annual amount of each metal, which could potentially be absorbed by a dolphin eating 7.6-15.3 kg/day of each item or group of items, was performed, as reported in Fig. 2. The figure reports also a total estimated amount of metal, considering a combination of the 3 food items considered and of total amount. For mean scenario the mean value of known absorption percent, in worst scenario the highest absorption and in the best scenario the lowest absorption were considered.

4. DISCUSSION

The obtained data confirm the reduced contamination by lead and mercury of the Adriatic Sea. Cadmium is generally present at medium-low concentrations in species considered, but despite this, amounts detected are within the range of subchronic threshold defined for marine species (0.5-10 μg/kg exposure level), responsible of decreases in growth, respiratory disruption, moult inhibition, shortened life span of F1 generation crustaceans, altered enzyme levels, and abnormal muscular contractions in crustaceans (Eisler, 1985). Thus it is possible to consider that a diet containing such levels can potentially induce some alteration also in higher organisms, dolphin included. As already shown in other studies from different parts of the world, cephalopods represent the main source for Cd, thus being the highest risk for dolphins, as they are always over the threshold reported (Tab. 3) despite the scenario considered, while fish and crabs are a smaller risk for the species.

Even if mean concentrations are in the range of background levels (Eisler, 1988), the amount of As detected are in the range of tissue concentration corresponding to adverse effects in aquatic organisms, so a potential toxic effect can be considered for the species studied and also for dolphins. Indeed, the percent of absorption of arsenic ranges between 80 and 90%, so it is probable that almost all the arsenic present in preys can be absorbed by bottlenose dolphins. Anyway, it should be remembered that almost all arsenic in marine organisms is represented by organic compounds, like arsenobetaine, which have proved to be little or non-toxic to organisms. Unfortunately, no speciation of As could be performed in present study, so it is impossible to define which were the percent of organic and inorganic arsenic species, to better understand the real risk for As intoxication. Anyway, given that no sign of As intoxication was ever observed in dolphins, and starting from the fact that also in blood of loggerhead high amounts of the metalloid can be found, it is possible to consider that the organic arsenic represents the highest amount of total metalloid; some adaptation mechanism should be also considered for marine organisms, as the high amounts observed are comparable with those producing overt toxicity in terrestrial organisms, but no toxicity was observed (Eisler, 1988).

5. SUMMARY

Starting from UNEP 1999report, Italy is the first producer for heavy metals (Pb, Cd, Cu, Zn) marine pollution, being responsible of 30% of total release of these compounds in the Mediterranean Sea. Being it a closed sea, with a reduced hydric exchange, the Mediterranean is particularly exposed to risks derived from chemical pollution. Knowing pollution degree of this sea by estimating contaminants concentrations in marine species placed at the top of food chains, including marine mammals and cetacean, is thus mandatory. Despite the high number of studies focusing on heavy metals in tissues of different cetacean species, little information is available concerning contaminants transfer along their trophic chains, thus defining which could be the main diet components contributing to toxicant body burden for each of the metals considered.

The present work focuses on the evaluation of heavy metals presence in tissues of bottlenose from the Northern Adriatic Sea and in some components of its diet, trying to define which could be the contribution that each of them give to the body burden of predators.

POVZETEK

Glede na poročilo UNEP (Okoljski program Združenih narodov) za leto 1999 je Italija glavni krivec za onesnaževanje morja s težkimi kovinami (Pb, Cd, Cu, Zn), odgovorna za 30 % vseh izpustov teh snovi v Sredozemsko morje. Ker gre za zaprto morje z omejeno hidrično izmenjavo, je še posebej izpostavljeno tveganjem, ki izvirajo iz kemijskega onesnaževanja. Zatorej je nujno, da ugotovimo stopnjo onesnaženosti morja, in sicer z ocenjevanjem koncentracij onesnaževalcev v morskih živalih na vrhu prehranjevalne verige, vključno z morskimi sesalci. Kljub velikemu številu študij, ki se osredotočajo na težke kovine v tkivih različnih vrst kitov,

je na voljo zelo malo literature o prenosu onesnaževalcev v prehranjevalni verigi, na podlagi katerih bi lahko ugotovili, katere so glavne prehranske komponente, ki prispevajo k toksični obremenitvi organizmov glede vseh upoštevanih kovin.

Avtorji pričujočega članka se osredotočajo na ugotavljanje koncentracij težkih kovin v tkivih velike pliskavke v severnem Jadranu in v nekaterih sestavinah njene prehrane, pri tem pa poskušajo ugotoviti, v kolikšni meri prispevajo k obremenjenosti organizmov velikih plenilcev.

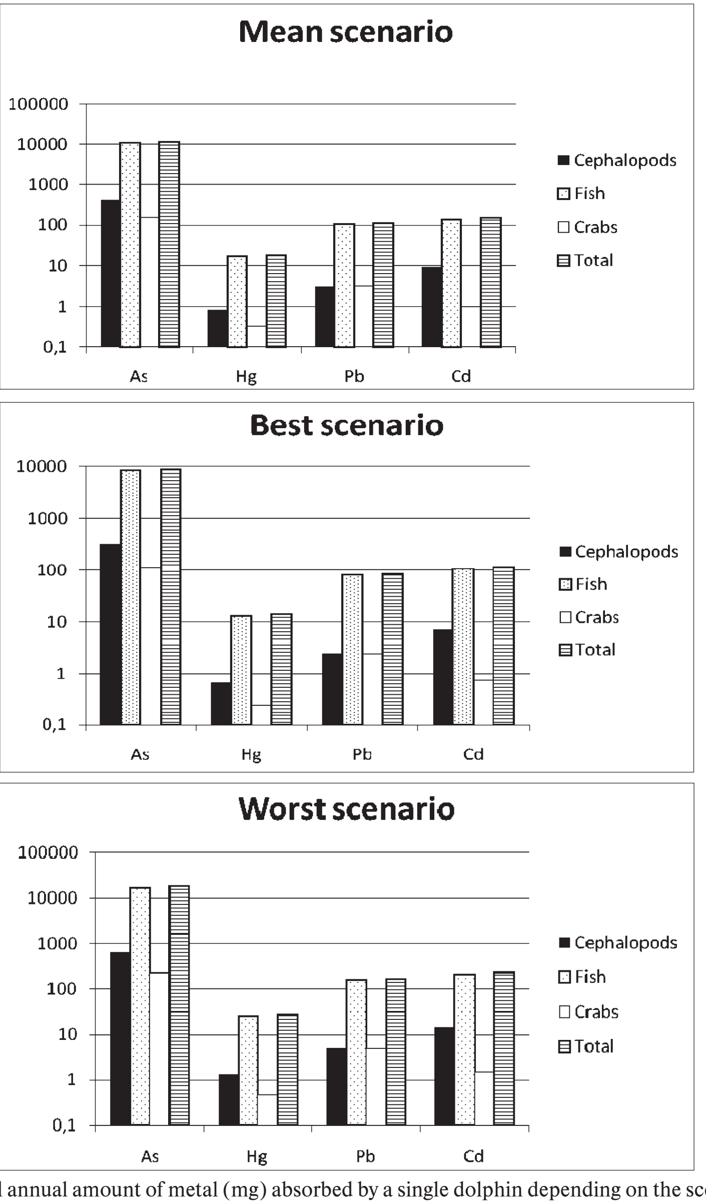

Fig. 2: Hypothetical annual amount of metal (mg) absorbed by a single dolphin depending on the scenario considered (semi-logarithmic scale).

Slika 2: Hipotetična letna količina kovine, ki jo vsrka en sam delfin glede na najslabši možni scenarij (pol-logaritemska lestvica).

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Dino SCARAVELLI
Centre of Aquaculture and Fish pathology
Research Group on Large Pelagic Vertebrates
Veterinary Faculty
University of Bologna
Cesenatico (FC)
dinosc@tin.it