RISK ASSESSMENT OF POLLUTANTS ALONG FOOD CHAIN OF *CARETTA CARETTA*

OCENA TVEGANJA KOPIČENJA ONESNAŽEVALCEV V PREHRANJEVALNI VERIGI GLAVATE KARETE (*CARETTA CARETTA*)

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Key words: heavy metals, loggerhead turtle, Northern Adriatic
Ključne besede: težke kovine, glavata kareta, severni Jadran

ABSTRACT

Several monitoring activities have been performed to assess the exposure and accumulation of different pollutants in the tissues of stranded and living loggerhead turtles, while little or no work has been carried out to define, which could be the contribution of food items to the heavy metal body burden of sea turtles. Understanding the contribution of diet of sea turtles to contaminant body burden is important for their conservation. The monitoring of contaminants in food items and the estimation of the amount of each pollutant that can be provided by each single item in sea turtles is important in the risk assessment of loggerheads' health related to environment pollution.

The present article reports on the evaluation of heavy metals in marine species representing a potential example of loggerhead sea turtle diet and on the assessment of the probable contribution of these species in heavy metal body burden of the turtles.

The obtained data confirm the reduced contamination by lead and mercury in the Adriatic Sea, while cadmium is generally present in 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, sea turtles included. For arsenic As, mean concentrations are within 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, turtles included.

IZVLEČEK

Medtem ko je bilo z namenom, da se oceni kopičenje različnih onesnaževalcev v tkivih nasedlih mrtvih in živih glavatih karet in izpostavljenost teh morskih želv tem nevarnim snovem, opravljena že cela vrsta monitoringov, pa doslej ni bilo narejeno skoraj nič, da bi ugotovili, v kolikšni meri k obremenitvi organizmov glavatih karet s težkimi kovinami prispevajo posamezne vrste njihovega plena. Za varstvo teh živali je zatorej pomembno, da vemo, kakšen je prispevek prehrane glavatih karet k njihovemu zastrupljanju. Monitoring onesnaževalcev v posameznih vrstah plena in ugotavljanje njihove količine v vsakem izmed njih je pomemben za ocenjevanje, v kakšni meri je ogroženo zdravje glavatih karet zaradi onesnaževanje njihovega okolja.

Avtorji pričujočega članka so ugotavljali koncentracije težkih kovin v različnih morskih živalskih vrstah, s katerimi se hrani glavata kareta, in ocenjevali, v kolikšni meri lahko te vrste prispevajo k obremenitvi njenega organizma s težkimi kovinami.

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 morskimi želvami. 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, vključno z želvami.

1. INTRODUCTION

Loggerhead turtle (*Caretta caretta*) is the most common sea turtle species inhabiting the Adriatic Sea. Several monitoring activities have been performed to assess the exposure and accumulation of different pollutants in the tissues of stranded and living loggerhead turtles, while little or no work has been carried out to define, which could be the contribution of food items to the heavy metal body burden of sea turtles.

Understanding the contribution of diet of sea turtles to contaminant body burden is important for their conservation. The monitoring of contaminants in food items and estimation of the amount of each pollutant, which can be provided by each single item to sea turtles, is important in the risk assessment of loggerheads' health related to environment pollution.

Among others, heavy metals represent a great risk for marine organisms, as they can persist in the environment for long period, can accumulate in sediments and living organisms and can experience biomagnifications along the food chain, even if the amplitude of the biomagnification process is not as great as for organic pollutants.

The present work reports on the evaluation of heavy metals in marine species, representing a potential example of loggerhead sea turtle diet, and on the assessment of the probable contribution of these species to the heavy metal body burden of the turtles.

2. METHODS

Sampling was performed with the aid of oceanographic boat "Daphne II" of Agenzia Prevenzione e Ambiente (ARPA) of the Emilia Romagna region. This structure has different aims: the study as well as the research and the control of marine environment and of its interactions with coastal areas. Sampling was performed along two different 1.5 km transects at 10 and 20 km outside Cesenatico coasts (Figure 1). Benthos sampling was performed using a fishing tool called "Rapido", a small trawling net, for a fishing time of 4 minutes. This allowed us to collect animals from an area of 0.001752 km^2.

Benthos was collected and separated by species or, if not possible, by systematic group. Each subject (n= 30 for each group when available) 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 (for molluscs and fish only muscle was

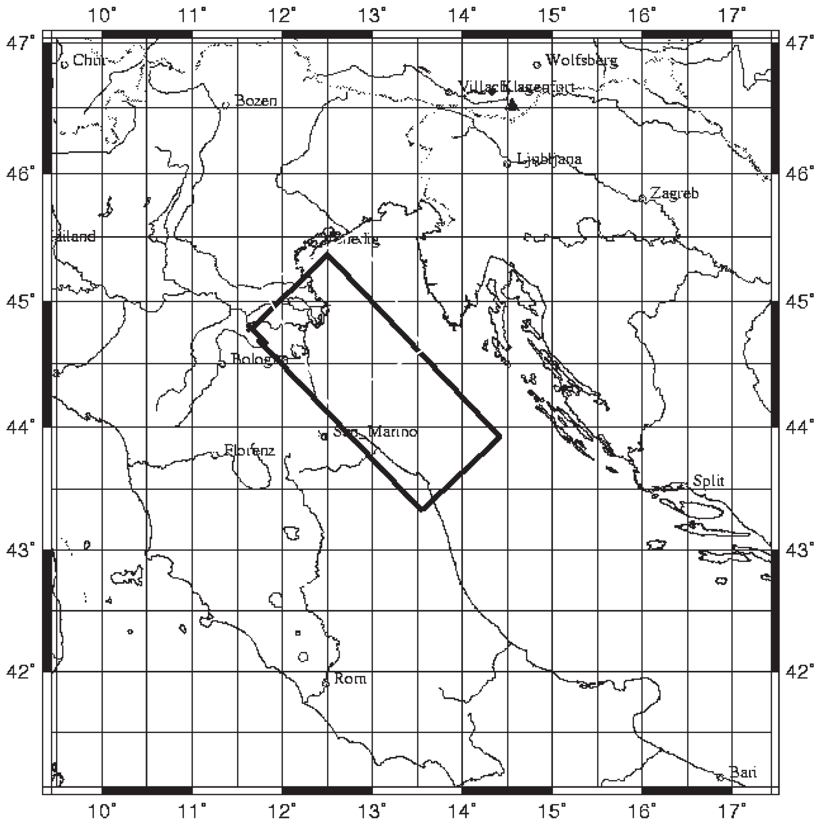

Figure 1: Sampling area
Slika 1: Območje vzorčenja

considered) 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 for other toxicological purposes; for the same reason, eggs were separated from pregnant females and analysed to evaluate the importance of metal excretion with ovodeposition.

After the analysis, the data were arranged to obtain an estimation of the amount of metals, which could have been ingested with that particular food item.

Starting from literature (Bentivegna et al. 2001, Parker et al. 2005, Revelles et al. 2007), the mean composition of loggerhead diet was defined. Species were then grouped on the basis of the systematic or functional classes (fish, molluscs, crustaceans, other - including all other species) and the percent of contribution to the diet was calculated (Table 1).

Assuming a mean volume and weight for stomach content of 370 ml and g respectively, the weight of each group in an "ideal" stomach was calculated and the amount of each metal provided with that quantity was calculated in three different scenarios: 1) mean concentration: the concentration/kg was calculated including all species; 2) worst scenario: the mean was calculated by considering the class was composed only of the species presenting higher mean concentration; 3) best scenario: the species considered is the one presenting lower concentrations. This calculation allowed us to estimate the amount of each toxic metal/year, if the loggerhead was feeding on that diet.

Table 1: Percent diet composition as obtained from available literature. The equivalent amount of food in a 370 g stomach content is also reported.

Tabela 1: Odstotkovna sestava prehrane, pridobljena iz literature. Zabeležena je tudi ekvivalentna količina hrane v 370 g težki vsebnosti želodca.

Class	% of the diet	Amount (g) estimated in one stomach
Molluscs	23.6	87.32
Fish	4.4	16.28
Crabs	26.7	98.79
Other	0.1	0.37

3. RESULTS

Mean concentration observed in each species considered are reported in Table 2 as mean ± standard error and minimum and maximum value on a wet weight basis. It can be clearly seen that *N. millepunctata* is characterised by a very high As load, while lowest levels were found in *S. solea* and in male crabs.

Lead was found at low concentration in all species, never reaching values higher than 0.551 mg/kg. Very low levels of Hg and Cd were found as well, while in *Natica* and in *Phyllonotus* very high levels of Cd were observed.

Table 3 reports the results of calculation concerning risk assessment and heavy metal load contribution depending on classes and on the scenario considered.

Starting from the obtained calculation and from known percent of absorption, a calculation of the annual amount of each metal, which could potentially be absorbed by a sea turtle eating 370 g/day of each item or group of items, was performed, as reported in Figure 2. The figure also gives the total estimated amount of metal, considering a combination of the 4 food items considered. 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.

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*
Tapes philippinarum	3.007 ± 0.085 1.92-4.41	0.177 ± 0.015 0.058-0.551	0.048 ± 0.0035 0.011-0.086	0.073 ± 0.004 0.052-0.178
Natica millepunctata	44.087 ± 6.826 23.75-73.69	0.098 ± 0.016 0.045-0.184	0.036 ± 0.007 0.012-0.066	0.643 ± 0.210 0.149-1.663
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

Nudibrach	2.31 ± 0.375 1.93-2.685	0.189 ± 0.011 0.178-0.200	0.014 ± 0.002 0.012-0.016	0.078 ± 0.016 0.062-0.094
Phyllonotus truculus	7.792 ± 1.509 5.153-10.38	0.182 ± 0.016 0.150-0.199	0.047 ± 0.004 0.039-0.054	0.649 ± 0.049 0.554-0.717
Sea stars	4.502 ± 0.499 3.573-5.555	0.172 ± 0.039 0.103-0.284	0.010 ± 0.085 1.92-4.41	0.075 ± 0.004 0.066-0.085
Aporrhais pespelecani	2.231 ± 0.061 1.685-3.10	0.127 ± 0.011 0.038-0.266	0.021 ± 0.003 < LOD-0.063	0.109 ± 0.007 0.039-0.188
Solea solea	1.494 ± 0.348 1.146-1.842	0.004 ± 0.001 0.003-0.005	0.009 ± 0.085 1.92-4.41	0.012 ± 0.002 0.010-0.014
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 1.92-4.41	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/day with the diet (mg)**			
		As	***Pb***	***Hg***	***Cd***
		"Mean" scenario			
Mollusk	87.32	0.559	0.0133	0.003	0.0129
Fish	16.28	0.0886	0.0009	0.0002	0.0005
Crabs	98.79	0.387	0.008	0.0008	0.0025
Other	0.37	0.001	0.0000657	0.00000468	0.0000282
		Worst scenario			
Mollusk	87.32	4.45	0.0092	0.0035	0.046
Fish	16.28	0.101	0.001	0.0002	0.0006
Crabs	98.79	0.603	0.009	0.0004	0.0044
Other	0.37	0.001	0.00006	0.0000037	0.00002
		Best scenario			
Mollusk	87.32	0.262	0.0155	0.0042	0.0064
Fish	16.28	0.024	0.000065	0.00014	0.00019
Crabs	98.79	0.157	0.0024	0.000649	0.0009
Other	0.37	0.00085	0.000069	0.00000518	0.00002

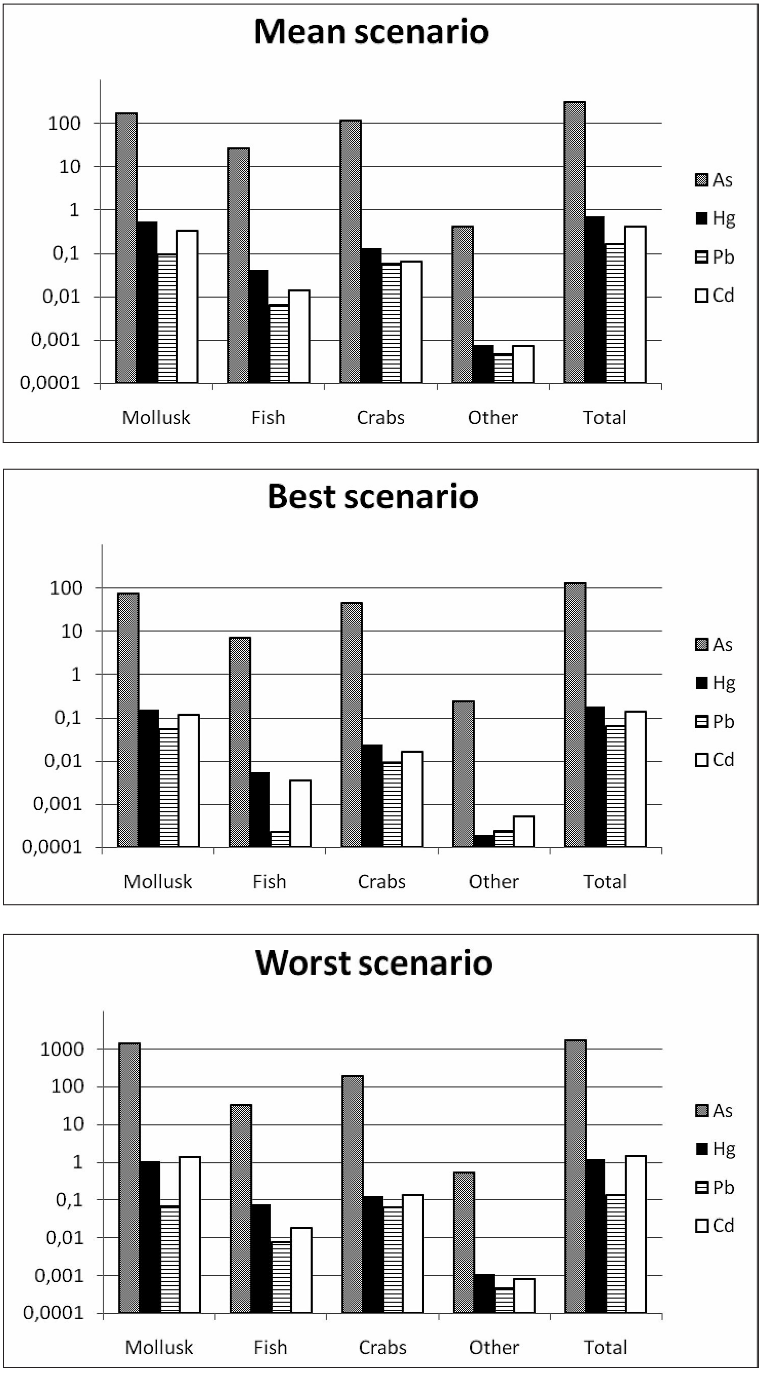

Figure 2: Hypothetical annual amount of metal (mg) absorbed by a single sea turtle depending on the scenario considered (semi-logarithmic scale).

Slika 2: Hipotetična letna količina kovine, ki jo vsrka ena sama glavata kareta glede na najslabši možni scenarij (pol-logaritemska lestvica).

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, even if *N. millepunctata* and *P. truculus* seem to be important accumulating species and can potentially represent a notable source of cadmium for benthos-eating species. Despite the medium-low concentrations, 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, such as sea turtles. It should be noted that molluscs represent highest risk for sea turtles, as they are always over the threshold reported (Table 3) despite the scenario considered, while fish and crabs are a smaller risk for the species.

Most interesting data are those concerning As, which seems to be the most important contaminant in the Adriatic Sea. The obtained data are indeed in agreement with those already observed in loggerhead turtles from the same area, with main contaminant being As (A. Zaccaroni et al., unpublished data).

Even if mean concentrations are within the range of background levels (Eisler 1988), the amount detected are within the range of tissue concentration corresponding to adverse effects in aquatic organisms, so a potential toxic effect can be considered for the species studied, including sea turtles. 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 sea turtles. 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 the 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 sea turtles, and starting from the fact that in blood of loggerhead turtles 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. CONCLUSIONS

The present data seem to be indicative of a reduced contribution of diet to sea turtles heavy metals body burden as far as Pb, Cd and Hg are concerned, while a great contribution should be considered for As. Anyway, it should also be noted that the mean concentrations of Pb, Hg and Cd in sea turtles' tissues and blood are very low, lower than expected starting from the available literature. Thus, it is probable that low levels of metals can come mainly from the diet.

6. SUMMARY

Various studies have been focused on the monitoring of contaminants in tissues of *Caretta caretta*, using stranded dead animals. Scarce are studies reporting not only on pollutants levels, but also on possible contribution of various diet components in contaminants, i.e. heavy metals, to sea turtles body burden.

The present work evaluates accumulation of toxic heavy metals (As, Pb, Cd, Hg) along trophic chain of *Caretta caretta* in the Northern Adriatic Sea, trying to define which could be main diet components contributing to toxicant body burden for each of the metals considered.

The research focuses on the dynamics of heavy metals transfer from the environment to the highly endangered species like sea turtles, as well as on the assessment of possible toxic effects occurring in the studied animals, including non-acute, highly relevant effects, like immunosupression.

POVZETEK

O kopičenju onesnaževalcev v tkivih nasedlih poginulih glavatih karet *Caretta caretta* je bilo opravljenih že veliko različnih raziskav, medtem ko študij o ravni onesnaževalcev in potencialnem prispevku različnih prehranjevalnih sestavin k obremenjenosti organizmov morskih želv s težkimi kovinami skorajda ni zaslediti.

Avtorji predstavljenega članka so ocenjevali koncentracije toksičnih težkih kovin (As, Pb, Cd, Hg) v prehranjevalni verigi glavate karete v severnem Jadranu in poskušali oceniti, katere so glavne prehranjevalne komponente, ki v največji meri prispevajo k toksični obremenitvi njenega organizma.

Pričujoča raziskava se osredotoča na dinamiko prenosa težkih kovin iz okolja na močno ogrožene vrste, kakršne so morske želve, kot tudi na oceno potencialnih toksičnih vplivov, ki jih je zaslediti v preučevanih živalih, vključno z neakutnimi, a zelo pomembnimi vplivi, kakršna je imunosupresija.

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