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Scientific Paper

# CAGE EXPOSURE OF EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*) FOR *IN SITU* ASSESSMENT OF POLLUTION-RELATED GENOTOXICITY

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Genotoxic effects are often the earliest signs of pollution-related environmental disturbance. In this study, we used the comet assay and micronucleus test to assess DNA damage in the erythrocytes of the European sea bass (*Dicentrarchus labrax*) exposed to environmental pollution *in situ.* Fish were collected from a fish farm in the Trogir Bay and their cages placed at an unpolluted reference site Šolta (Nečujam Bay) and a polluted site Vranjic (Kaštela Bay) for four weeks. A group of fish which remained at the fish farm Trogir Bay were used as the second control group. Fish exposed at the Vranjic site showed a significantly higher erythrocyte DNA damage, measured by the comet assay, than either control group. Micronucleus induction showed a similar gradient of DNA damage, but did not reach statistical significance. Our results show that cage exposure of a marine fish *D. labrax* can be useful in environmental biomonitoring and confirm the comet assay as a suitable tool for detecting pollution-related genotoxicity.

**KEY WORDS:** *Adriatic Sea, comet assay, fish, ecogenotoxicology, marine biomonitoring, micronucleus test*

Anthropogenic environmental pollution presents an increasing challenge to coastal waters, and bays are particularly endangered by contamination input due to limited self-renewal (1). The wider Kaštela Bay is a densely populated area with the heaviest load of genotoxic agents along the eastern Adriatic coast (2). Intensive industrial development and urbanisation have introduced various types and quantities of pollutants into the bay (3-5).

The assessment of DNA damage is of primary concern when evaluating the causal relationships between contaminant exposure and biological effects in aquatic organisms. Therefore the use of sensitive biomarkers in sentinel species has become a major issue in environmental genotoxicity monitoring (6, 7).

Fish are often organisms of choice in environmental biomonitoring because of their role in biotic communities and because of their sensitivity to mutagens and environmental pollutants, even at low concentrations (6, 8, 9). Several studies have investigated the genotoxic effects of polluted aquatic environments on fish species, either through the sampling of native populations (6, 9-13) or by cage exposure *in situ* (14-16). When assessing the impact of pollution in aquatic environments using native fish populations, research may be compromised by migration of fish for feeding and breeding, availability of certain species at a particular site of interest, or difficulty to obtain enough specimens for desired analyses. *In situ* cage exposure of sentinel species offers several advantages such as the knowledge of

the precise location and duration of exposure (17). Furthermore, it reduces inter-individual variability (life history, genetic background, and developmental stages)(18) and obviates the influence of adaptive mechanisms, more likely to develop in native pollution-stressed fish populations.

In comparison with studies that utilised the comet assay on freshwater species, a limited number of studies has been focused on marine fish. These studies have often implemented species that live close to sediments, where most contaminants tend to accumulate (19). Hatchery-reared turbot (*Scophthalmus maximus*) was experimentally exposed to the sediment collected from polycyclic aromatic hydrocarbons (PAH) and heavy metal polluted sites (20), while native populations of gray mullet (*Mugil sp*.), sea catfish (*Netuma sp*.), and marine flatfish dab (*Limanda limanda*) were used in studies investigating the effects of coastal and estuarine water contamination (13, 21-23).

There have not been many studies using transplanted (caged) fish for genotoxicity assessment, and most of them involved freshwater species (14, 15, 24, 25). Studies using caged fish in marine environment include the research work assessing the influence of the Laranjo basin (Aveiro, Portugal) contamination on caged golden grey mullet (*Liza aurata*) by measuring erythrocyte nuclear abnormalities (16) and the study of genotoxic response in caged eel (*Anguilla anguilla*) after exposure to harbour waters by observing DNA integrity and nuclear abnormalities in erythrocytes (26).

European sea bass (*Dicentrarchus labrax*) is suitable for assessing pollutant-induced effects in marine environments, since it is sensitive enough to detect the effects of a wide range of pollutants at low doses and it is naturally widespread in the studied ecosystems (7). This species was implemented in many studies that investigated biotransformation and biochemical and genotoxic responses to benzo(a)pyrene [B(a)P], $\beta$-naphthoflavone, 4-nonylphenol, 17$\beta$-estradiol, and resin acids (27-33), contaminated water samples (34-36), or environmental pollution *in situ* (7, 37). Caged sea bass has already been used to assess marine pollution in the Mediterranean Sea, and the effects were evaluated using biochemical markers (EROD, GST, and AChE) (38). Sea bass is one of the few marine fish species commercially available from aquaculture and therefore easy to obtain for cage exposure.

There are many different assays for detecting DNA damage, among which micronucleus test (MNT) and the comet assay (single cell gel electrophoresis assay) have proven their reliability and sensitivity in detecting pollution-related genotoxic effects in aquatic environments (13, 39, 40).

MNT is a relatively fast, simple, sensitive, and inexpensive procedure. Micronuclei (MN) are small cytoplasmic masses of chromatin resulting either from chromosome breaks during cell division or from chromosomes that are lagging in anaphase (41).

The comet assay is a sensitive technique for detecting DNA damage (single-strand and double-strand breaks, alkali labile sites or DNA-DNA and DNA-protein cross-links) induced by alkylating agents, intercalating agents, or oxidative damage (42). It requires a small number of cells, detects genotoxic damage at the single cell level, and allows for an early response evaluation in a biota (19).

The aim of this study was to assess the applicability of cage exposure of sea bass (*D. labrax*) in biomonitoring marine environments and to evaluate the genotoxic potential of the polluted Kaštela Bay site (Vranjic) and the control Nečujam Bay site (Šolta) using the MNT and the comet assay on sea bass erythrocytes.

## METHODS

### *Study areas*

The Kaštela Bay (Figure 1) is a semi-enclosed bay in the Eastern Adriatic with the average depth of 23 m, 61 km$^2$ surface, and a total volume of 1.4 km$^3$ (43). The area is one of the most densely populated and industrialised coastal areas along the eastern Adriatic. Industrial and urban wastewater outlets, located in the eastern part of the bay, discharge untreated or partly treated effluents into the bay. The bay also receives agricultural and urban runoffs and untreated stormwater. This basin receives 32 million m$^3$ of untreated municipal wastewater and 20 million m$^3$ of partly treated industrial wastewater per year (44).

The Vranjic site in the Kaštela Bay is likely to be exposed to various sources of contamination. It is placed near the mouth of the Jadro River (8 m$^3$ s$^{-1}$) and receives effluents from various industries (brewery, cement plant, etc.), the harbour, Split shipyard, Vranjic shipworks, domestic sewage, and agricultural discharge that enters the bay without any treatment (2, 39).


**Figure 1** *Map showing the study areas and exposure sites: 1 - Fish farm reference site; 2 - Nečujam Bay reference site; 3 - Vranjic site*

The fish farm, situated on the western side of the Trogir Bay, was chosen as the first reference site. The second reference site was the Nečujam Bay (on the island of Šolta, situated outside the Kaštela and Trogir Bays). This area is considered to have no known local sources of contamination.

*Cage exposure*

The cage experiment took place between September and October 2003. European sea bass (*D. labrax*) specimens were collected from the fish farm in the Trogir Bay and transplanted to the Nečujam Bay site and Vranjic, where they were kept in polyethylene cages (1.5 m x 1 m, mesh size 12 mm) for a month. One cage contained about fifty fish.

*Blood sampling*

Peripheral blood was collected from the caudal vein of each fish with heparinised syringes. Blood samples were kept on ice and immediately processed for genotoxicity testing (7 to 16 samples for the comet assay and 6 to 16 samples for MNT).

Unless specified otherwise, chemicals and reagents used were purchased form Sigma Chemical Co., St. Louis, MO, USA.

*The comet assay*

The comet assay was performed according to the basic procedure of Singh et al. (45) with slight modifications. Five microlitres of blood diluted in PBS (1:200) were mixed with 95 μL of 0.5 % low melting point (LMP) agarose and placed on 1 % normal agarose-precoated microscope slides. After solidifying for 2.5 min at 0 °C, a third layer of 0.5 % LMP agarose was added and left to solidify. The cells were lysed in a freshly made lysing solution (2.5 mol $L^{-1}$ NaCl, 100 mmol $L^{-1}$ EDTA, 10 mmol $L^{-1}$ Tris-HCl, 10 % DMSO, 1 % Triton X-100, pH 10), for one hour at 4 °C. After rinsing with redistilled water, the slides were placed on the horizontal gel box, covered with cold alkaline buffer (0.3 mol $L^{-1}$ NaOH, 1 mmol $L^{-1}$ EDTA, pH>13), and left for 20 min. Electrophoresis was run in the same buffer at 25 V (0.83 V $cm^{-1}$) and 300 mA for 20 min at 4 °C. After electrophoresis, the slides were neutralised in a cold neutralisation buffer (0.4 mol $L^{-1}$ Tris-HCl, pH 7.5), 2x5 min, fixed in methanol:acetic acid (3:1) for 5 min, and stored in the dark at room temperature. Prior to examination with a Zeiss Axioplan epifluorescence microscope, the slides were rehydrated and stained with 10 μg $mL^{-1}$ ethidium bromide. At least 50 cells were examined per slide (each slide corresponding to one animal), and the extent of DNA migration was determined as a percentage of the tail DNA, using the Komet 5 image analysis system by Kinetic Ltd. (UK).

*The micronucleus test*

Smears were prepared from 10 μL heparinised blood and left to dry. They were fixed with 1 % glutaraldehyde in PBS for 5 min, stained with bisbenzimide 33258 (Hoechst) at the final concentration of 1 μg $mL^{-1}$ for 5 min, and then washed and mounted in glycerol-McIlvaine buffer (1:1). The slides were kept in the dark at 4 °C before scoring under the Zeiss Axioplan epifluorescence microscope at 1000x magnification. 2000 cells were counted on each slide. MN were identified according to the criteria described by Kirsch-Volders et al. (41, 46) as small round structures in the cytoplasm, smaller than 1/3 of the nucleus diameter. Furthermore, the MN had to be in the same optical plane as the main nucleus, and its boundary had to be distinguishable from the main nucleus. Only intact cells with distinct nuclear and cellular membranes were scored.

*Statistical analysis*

DNA damage in each group is expressed with the mean ± SEM for both the comet assay and MNT. For statistical analysis we used the Mann-Whitney *U*-test. The level of significance was $P \leq 0.01$.

## RESULTS

### *Comet assay data*

The level of DNA damage in fish erythrocytes is presented as the percentage of migrated tail DNA (% tDNA). Fish from the Vranjic site showed a significantly higher DNA damage than fish from either reference site (10.84 % vs. 7.06 % tDNA for the fish farm and 6.25 % tDNA for the Nečujam Bay site on Šolta; Figure 2). The frequency of cells exceeding 50 % tDNA did not vary greatly, but was still the highest in fish from the Vranjic site (Table 1).

**Table 1** *Percentage of sea bass erythrocytes with more than 50 % tDNA*

| Location | Cells exceeding 50 % tDNA / % |
|---|---|
| Fish farm | 0.65 |
| Nečujam Bay | 0.35 |
| Vranjic | 1.06 |

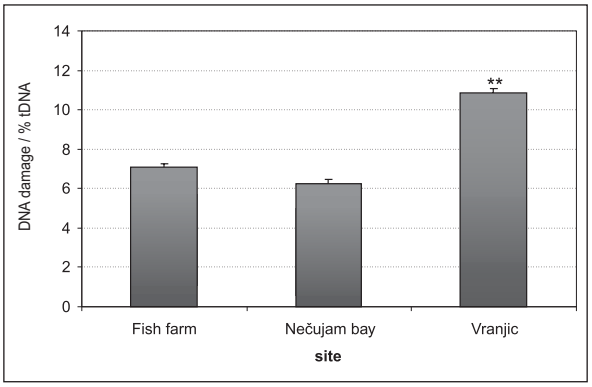


**Figure 2** *The level of DNA damage measured by the comet assay (percentage of tail DNA, mean+SEM) in sea bass erythrocytes after four weeks of in situ cage exposure) ** - significantly increased compared to both reference sites; P≤0.01*

### *Micronucleus test data*

Fish from all three sites showed similar MN frequency (Figure 3). Fish at the Vranjic site had a small increase over other fish, but the difference was not statistically significant.

## DISCUSSION

Our comet assay has confirmed that the Vranjic site, and therefore the Kaštela Bay, is polluted. This site has already been identified as the site with high genotoxic pressure on mussels (*Mytilus galloprovincialis*) measured by the comet assay, MNT (39), and Fast Micromethod® (2).

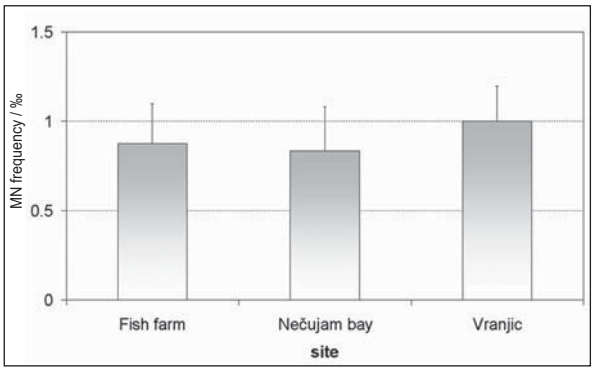


**Figure 3** *MN frequency in sea bass erythrocytes (mean+SEM) after four weeks of in situ cage exposure*

The baseline level of DNA damage in erythrocytes of *D. labrax* at the reference sites, measured with the comet assay, was between 6.25 % and 7.06 % tDNA, while at the polluted Vranjic site it was 10.84 % tDNA, which is a statistically significant increase. It is sometimes difficult to compare the results of the comet assay between different authors due to differences in the applied protocols. For this reason we compared our results with studies using similar protocols. A similar baseline DNA damage was observed in fish species of unpolluted sites studied using similar protocols. Baseline DNA damage in carp (*Cyprinus carpio*) caged at the unpolluted site in the nature park "Kopački rit" was within 5 % to 7 % tDNA (47). In native three-spined sticklebacks (*Gasterosteus aculeatus*) captured in a clean environment, tDNA was 6.33 % (48).

Our comet assay showed 1.7-fold increase in DNA damage between the polluted Vranjic site and the clean Nečujam Bay site. These results are comparable with the findings in three-spined sticklebacks (*G. aculeatus*) from a polluted site receiving effluent of a large sewage treatment plant. Their DNA damage was 1 to 1.5 times higher than in fish from the unpolluted site (48). One to five times higher rate of DNA strand breaks was measured in the erythrocytes of caged Sacramento sucker (*Catostomus occidentalis*) after one week of exposure in waters receiving agricultural chemical runoff (14). Caged chub (*Leuciscus cephalus*) liver cells showed a two fold increase in DNA damage after four weeks of exposure to contaminated river water (15).

Comparable DNA damage between the reference sites indicates an absence of genotoxic effects caused by translocation or cage exposure-induced stress. A comet assay study on feral and caged chubs in rivers with different pollution (*L. cephalus*) reported very similar hepatocyte DNA damage (15).

In our study the MN frequency in sea bass erythrocytes varied between 0.83 ‰ and 1 ‰. Similar MN incidence in this species was observed in a study investigating genotoxic response of juvenile sea bass to resin acids. In a control group it was <0.86 ‰ (29). Two other studies investigated the effects of B(a)P (30) and of a soluble fraction of secondary treated industrial/urban effluent (SF-STIUE) (36) on MN frequency in sea bass erythrocytes. In control groups the MN frequency varied between 1 ‰ and 2.5 ‰.

In organisms with many small chromosomes it is likely that MN formed after a clastogenic event will be very small in size, some of them not even visible under light microscopy (49). This may also be true for *D. labrax* whose karyotype consists of 48 subtelocentric and acrocentric chromosomes (50). Therefore, fish species with fewer but larger chromosomes are recommended for MNT, such as Christy's lyretail (*Aphyosemion christy*) (2*n*=18), cowfish (*Galaxias maculates*) (2*n*=22), killifish (*Nothobranchius rachowi*) (2*n*=16), central mudminnow (*Umbra limi*) (*2n*=22) and eastern mudminnow (*Umbra pygmaea*) (2*n*=22). Several papers have reported a good correlation between MN frequency and pollution or chemical concentrations in fish species with a similar number of chromosomes, such as the three-spined stickleback (*G. aculeatus)* (2*n*=42) and loach (*Misgurnus anguillicaudatus*) (2*n*=50) (48, 51, 52). Sea bass erythrocytic MN frequency seems to be sensitive and suitable enough for assessing the genotoxic potential of pollutant mixtures, and is therefore justified for use in environmental studies (36).

Although both assays applied in our study demonstrated a similar level of DNA damage, the comet assay was more sensitive. Similar was observed in a study of the effects of anthropogenic contamination on caged carp (*C. carpio*) erythrocytes (47). The comet assay showed statistically significant differences between grey mullets (*Mugil* sp.) and sea catfish (*Netuma* sp.) from polluted and clean sites while MN frequency was not significantly different (13). The lower sensitivity of MNT for various feral fish species has also been described elsewhere (53). In contrast, butterfish (*Pholis gunnellus*) from contaminated areas in Firth of Forth (Scotland) showed higher MN frequency but no increase in DNA strand breaks measured by the comet assay (54). Similar was reported for three-spined sticklebacks (*G. aculeatus*) (48). Differences in the sensitivity of these two assays confirm the need for using them together since they complement each other in different aspects of DNA damage. MNT detects more persistent DNA damage (double strand DNA breaks) and aneugenic effects that can not be repaired and last as long as the cell itself (47). On the other hand, the comet assay detects mostly repairable DNA lesions (alkali labile sites and single strand DNA breaks), indicating recent pollution.

A study using native and caged mussels (*M. galloprovincialis*) on several locations in the Kaštela and Trogir Bays at the same time as in this study showed a similar pattern of DNA damage caused by pollution (39). In comparison with the sea bass, measured DNA damage in mussels was 3.7 times higher. This suggest that mussels are more sensitive organisms in assessing genotoxicity of marine pollution. On the other hand, MN frequency in both studies did not show statistically significant difference between polluted and unpolluted areas for either species. This could be attributed to the absence of aneugenic stressors at the polluted Vranjic site.

## CONCLUSION

Our study confirms the presence of genotoxic burden on organisms in the Kaštela Bay. The comet assay on caged sea bass erythrocytes appears to better distinguish the genotoxic effects of polluted environments than MNT. Nevertheless, it is advisable to implement both methods in genotoxicity studies, as they reveal different aspects of DNA damage and therefore complement each other. The results of this study have also confirmed that cage exposure of European sea bass is a suitable method in marine genotoxicity monitoring and encourage the use of fish erythrocytes in environmental pollution assessment.

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## REFERENCES

1. Vlahogianni T, Dassenakis M, Scoullos MJ, Valavanidis A. Integrated use of biomarkers (superoxide dismutase, catalase

and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. Mar Pollut Bull 2007;54:1136-71.

2. Jakšić Ž, Batel R, Bihari N, Mičić M, Zahn RK. Adriatic coast as a microcosm for global genotoxic marine contamination - a long-term field study. Mar Pollut Bull 2005;50:1314-27.
3. Knezić S, Margeta J. Integrated management of coastal sewerage systems: the case of Kaštela Bay, Croatia. Water Resour Manag 2002;16:279-305.
4. Milun V, Barić A, Zvonarić T. Temporal and spatial distribution of chlorinated hydrocarbons in mussels from the Kaštela Bay (Adriatic Sea). Fresenius Environ Bull 2004;13:1237-43.
5. Kljaković-Gašpić Z, Odžak N, Ujević I, Zvonarić T, Horvat M, Barić A. Biomonitoring of mercury in polluted coastal area using transplanted mussels. Sci Total Environ 2006;368:199-209.
6. Russo C, Rocco L, Morescalchi MA, Stingo V. Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. Ecotoxicol Environ Saf 2004;57:168-74.
7. Ahmad I, Maria VL, Oliveira M, Serafim A, Bebianno MJ, Pacheco M, Santos MA. DNA damage and lipid peroxidation vs. protection responses in the gill of *Dicentrarchus labrax* L. from a contaminated coastal lagoon (Ria de Aveiro, Portugal). Sci Total Environ 2008;406:298-397.
8. Gustavino B, Scornajenghi KA, Minissi S, Ciccotti E. Micronuclei induced in erythrocytes of *Cyprinus carpio* (teleostei, pisces) by X-rays and colchicine. Mutat Res 2001;494:151-9.
9. de Lemos CT, Rödel PM, Terra NR, Oliveira NCD'A, Erdtmann B. River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol Environ Saf 2007;66:391-401.
10. Pandrangi R, Petras M, Ralph S, Vrzoc M. Alkaline single cell gell (Comet) assay and genotoxicity monitoring using bullheads and carp. Environ Mol Mutagen 1995;26:345-56.
11. Devaux A, Flammarion P, Bernardon V, Garric J, Monod G. Monitoring of the chemical pollution of the river Rhône through measurement of DNA damage and cytochrome P4501A induction in Chub (*Leuciscus cephalus*). Mar Environ Res 1998;46:257-62.
12. Brown JS, Steinert SA. DNA damage and biliary PAH metabolites in flatfish from Southern California Bays and harbors, and the Channel Islands. Ecol Indic 2004;3:263-74.
13. de Andrade VM, da Silva J, da Silva FR, Heuser VD, Dias JF, Yoneama ML, de Freitas TRO. Fish as bioindicators to assess the effects of pollution in two southern Brazilian rivers using the Comet assay and micronucleus test. Environ Mol Mutagen 2004;44:459-68.
14. Whitehead A, Kuivila KM, Orlando JL, Kotelevtsev S, Anderson SL. Genotoxicity in native fish associated with agricultural runoff events. Environ Toxicol Chem 2004;23:2868-77.
15. Winter M, Day N, Hayes RA, Taylor EW, Butler PJ, Chipman JK. DNA strand breaks and adducts determined in feral and caged chub (*Leuciscus cephalus*) exposed to rivers exhibiting variable water quality around Birmingham, UK. Mutat Res 2004;552:163-75.
16. Guilherme S, Válega M, Pereira ME, Santos MA, Pacheco M. Erythrocytic nuclear abnormalities in wild and caged fish (*Liza aurata*) along an environmental mercury contamination gradient. Ecotoxicol Environ Saf 2008;70:411-21.
17. Oikari A. Caging techniques for field exposures of fish to chemical contaminants. Aquat Toxicol 2006;78:370-81.
18. Pellacani C, Buschini A, Furlini M, Poli P, Rossi C. A battery of *in vivo* and *in vitro* tests useful for genotoxic pollutant detection in surface waters. Aquat Toxicol 2006;77:1-10.
19. Frenzilli G, Nigro M, Lyons BP. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat Res Rev Mutat 2009;681:80-92.
20. Hartl MGJ, Kilemade M, Sheehan D, Mothersill C, O'Halloran J, O'Brien NM, van Pelt FNAM. Hepatic biomarkers of sediment-associated pollution in juvenile turbot, *Scophthalmus maximus* L. Mar Environ Res 2007;64:191-208.
21. Akcha F, Leday G, Pfohl-Leszkowicz A. Potential value of the Comet assay and DNA adduct measurement in dab (*Limanda limanda*) for assessment of *in sit*u exposure to genotoxic compounds. Mutat Res 2003;534:21-32.
22. Akcha F, Vincent Hubert F, Pfohl-Leszkowicz A. Measurement of DNA adducts and strand breaks in dab (*Limanda limanda*) collected in the field: effects of biotic (age, sex) and abiotic (sampling site and period) factors on the extent of DNA damage. Mutat Res 2004;552:197-207.
23. Lyons BP, Stentiford GD, Bignell J, Goodsir F, Sivyer DB, Devlin MJ, Lowe D, Beesley A, Pascoe CK, Moore MN, Garnacho E. A biological effects monitoring survey of Cardigan Bay using flatfish histopathology, cellular biomarkers and sediment bioassays: Findings of the Prince Madog Prize 2003. Mar Environ Res 2006;62(Suppl 1): S342-6.
24. Barbee GC, Barich J, Duncan B, Bickham JW, Matson CW, Hintze CJ, Autenrieth RL, Zhou G-D, McDonald TJ, Cizmas L, Norton D, Donnelly KC. *In situ* biomonitoring of PAH-contaminated sediments using juvenile coho salmon (*Oncorhynchus kisutch*). Ecotoxicol Environ Saf 2008;71:454-64.
25. Bony S, Gillet C, Bouchez A, Margoum C, Devaux A. Genotoxic pressure of vineyard pesticides in fish: Field and mesocosm surveys. Aquat Toxicol 2008;89:197-203.
26. Maria VL, Correia AC, Santos MA. Genotoxic and biochemical responses in caged eel (*Anguilla anguilla* L.) after short-term exposure to harbour waters. Environ Int 2004;29:923-9.
27. Maria VL, Gravato C, Correia AC, Santos MA. Biotransformation and genotoxicity responses to PAHs in two teleost species. Fresenius Environ Bull 2002;11:609-15.
28. Gravato C, Santos MA. β-naphthoflavone liver EROD and erythrocytic nuclear abnormality induction in juvenile *Dicentrarchus labrax* L. Ecotoxicol Environ Saf 2002;52:69-74.
29. Gravato C, Santos MA. Juvenile sea bass liver biotransformation induction and erythrocytic genotoxic responses to resin acids. Ecotoxicol Environ Saf 2002;52:238-47.
30. Gravato C, Santos MA. Genotoxicity biomarkers' association with B(a)P biotransformation in *Dicentrarchus labrax* L. Ecotoxicol Environ Saf 2003;55:352-8.

31. Teles M, Gravato C, Pacheco M, Santos MA. Juvenile sea bass biotransformation, genotoxic and endocrine responses to β-naphthoflavone, 4-nonylphenol and 17β-estradiol individual and combined exposures. Chemosphere 2004;57:147-58.
32. Teles M, Pacheco M, Santos MA. Biotransformation, stress and genotoxic effects of 17β-estradiol in juvenile sea bass (*Dicentrarchus labrax* L.). Environ Int 2006;32:470-7.
33. Maria VL, Ahmad I, Santos MA. Juvenile sea bass (*Dicentrarchus labrax* L.) DNA strand breaks and lipid peroxidation response following 17β-estradiol two mode of exposures. Environ Int 2008;34:23-9.
34. Gravato C, Santos MA, Magalhães I. Juvenile *Dicentrarchus labrax* L. biochemical and genotoxic responses after short-term exposure to β-naphthoflavone and contaminated harbour waters. Fresenius Environ Bull 2000;9:269-74.
35. Gravato C, Santos MA. Juvenile sea bass liver biotransformation and erythrocytic genotoxic responses to pulp mill contaminants. Ecotoxicol Environ Saf 2002;53:104-12.
36. Gravato C, Santos MA. *Dicentrarchus labrax* biotransformation and genotoxicity responses after exposure to a secondary treated industrial/urban effluent. Ecotoxicol Environ Saf 2003;55:300-6.
37. Mohmood I, Maria VL, Oliveira M, Ahmad I, Pacheco M, Santos MA. Seasonal assessment of a contaminated coastal lagoon (Ria de Aveiro, Portugal) using Dicentrarchus labrax L. erythrocytic nuclear abnormalities. Fresenius Environ Bull 2008;17:1924-31.
38. Stien X, Percic P, Gnassia-Berelli M, Roméo M, Lafaurie M. Evaluation of biomarkers in caged fishes and mussels to assess the quality of waters in a Bay of the NW Mediterranean Sea. Environ Pollut 1998;99:339-45.
39. Klobučar GIV, Štambuk A, Hylland K, Pavlica M. Detection of DNA damage in haemocytes of *Mytilus galloprovincialis* in the coastal ecosystems of Kaštela and Trogir Bays, Croatia. Sci Total Environ 2008;405:330-7.
40. Klobučar GIV, Pavlica M, Erben R, Papeš D. Application of the micronucleus and Comet assays to mussel *Dreissena polymorpha* haemocytes for genotoxicity monitoring of freshwater environments. Aquat Toxicol 2003;64:15-23.
41. Kirsch-Volders M, Sofuni T, Aaderma M, Albertini S, Eastmond D, Fenech M, Ishidate M, Lorge E, Norppa H, Surallés J, von der Hude W, Wakata A. Report from the *in vitro* micronucleus assay working group. Environ Mol Mutagen 2000;35:167-72.
42. Cotelle S, Férard JF. Comet assay in genetic ecotoxicology: a review. Environ Mol Mutagen 1999;34:246-55.
43. Odžak N, Zvonarić T, Kljaković-Gašpić Z, Horvat M, Barić A. Biomonitoring of mercury in the Kaštela Bay using transplated mussels. Sci Total Environ 2000;261:61-8.
44. Ujević I, Odžak N, Barić A. Trace metal accumulation in different grain size fractions of the sediments from semi enclosed bay heavily contaminated by urban and industrial wastewaters. Water Res 2000;34:3055-61.
45. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91.
46. Kirsch-Volders M, Sofuni T, Aaderma M, Albertini S, Eastmond D, Fenech M, Ishidate M, Kirchner S, Lorge E, Morita T, Norppa H, Surallés J, Vanhauwaert A, Wakata A. Report from the *in vitro* micronucleus assay working group. Mutat Res 2003;540:153-63.
47. Klobučar GIV, Štambuk A, Pavlica M, Sertić-Peroć M, Kutuzović-Hackenberger B, Hylland K. Genotoxicity monitoring of freshwater environments using caged carp (*Cyprinus carpio*). Ecotoxicology 2010;19:77-84.
48. Wirzinger G, Weltje L, Gercken J, Sordyl H. Genotoxic damage in field-collected three-spined sticklebacks (*Gasterosteus aculeatus* L.): a suitable biomonitoring tool? Mutat Res 2007;628:19-30.
49. Udroiu I. The micronucleus test in piscine erythrocytes. Aquat Toxicol 2006;79:201-4.
50. Sola L, Bressanello S, Rossi AR, Laselli V, Crosetti D, Cataudella S. A karyotype analysis of the genus *Dicentrarchus* by different staining techniques. J Fish Biol 1993;43:329-37.
51. Chu S, Xi Z, Xu X, Zhang Y, Xu Y. Induction of micronuclei in peripheral erythrocytes of *Misgurnus anguillicaudatus* by polychlorinated biphenyls. Bull Environ Contam Toxicol 1996;57:179-82.
52. Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerklet CA, Schluter D, Kingsley DM. The genetic architecture of divergence between threespine stickleback species. Nature 2001;414:901-5.
53. Lee RF, Steinert S. Use of the single cell gel electrophoresis/Comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat Res 2003;544:43-64.
54. Bombail V, Aw D, Gordon E, Batty J. Application of the Comet and micronucleus assays to butterfish (*Pholis gunnellus*) erythrocytes from the Firth of Forth, Scotland. Chemosphere 2001;44:383-92.

***Sažetak***

KAVEZNO IZLAGANJE LUBINA (*DICENTRARCHUS LABRAX*) U PROCJENI GENOTOKSIČNOG UTJECAJA ONEČIŠĆENJA

Genotoksični učinak često je jedan od najranijih pokazatelja štetnog djelovanja onečišćenja okoliša. U ovom radu procijenjeno je oštećenje DNA u eritrocitima lubina (*Dicentrarchus labrax*) izloženima okolišnom onečišćenju s pomoću komet-testa i mikronukleus-testa. Lubini su prikupljeni na ribogojilištu i kavezno izloženi u periodu od četiri tjedna na dvije postaje različitog stupnja onečišćenja na jadranskoj obali: na kontrolnoj postaji Šolta (zaljev Nečujam) i na onečišćenoj postaji Vranjic (Kaštelanski zaljev). Zasebna skupina lubina skupljena na ribogojilištu poslužila je kao druga kontrola. Rezultati komet-testa pokazali su statistički značajan porast oštećenja DNA na postaji Vranjic u usporedbi s obje kontrolne postaje. Rezultati mikronukleus-testa pokazali su sličan gradijent onečišćenja, iako nisu dosegli statističku značajnost. Ovi rezultati upućuju na primjenjivost kaveznog izlaganja lubina *D. labrax* u biomonitoringu vodenog okoliša te potvrđuju korisnost komet-testa kao prikladne metode za detekciju genotoksičnog utjecaja onečišćenja.

**KLJUČNE RIJEČI:** *biomonitoring morskog okoliša, ekogenotoksikologija, Jadransko more, komet-test, mikronukleus-test, ribe*

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