



Correlations between oxidative DNA damage and formation of hepatic tumours in two flatfish species from contaminated environments

Ciara Baines, Richard Meitern, Randel Kreitsberg, Jérôme Fort, Jörn Peter Scharsack, Pedro Nogueira, Mathieu Giraudeau, Tuul Sepp

► To cite this version:

Ciara Baines, Richard Meitern, Randel Kreitsberg, Jérôme Fort, Jörn Peter Scharsack, et al.. Correlations between oxidative DNA damage and formation of hepatic tumours in two flatfish species from contaminated environments. *Biology Letters*, 2023, 19 (5), 10.1098/rsbl.2022.0583 . hal-04111579

HAL Id: hal-04111579

<https://hal.science/hal-04111579>

Submitted on 27 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Correlations between oxidative DNA damage and formation of hepatic tumours in two flatfish species
from contaminated environments

Ciara Baines^{1,2}, Richard Meitern¹, Randel Kreitsberg¹, Jérôme Fort³, Jörn Peter Scharsack⁴,
Pedro Nogueira⁴, Mathieu Giraudeau³, Tuul Sepp¹

¹Institute of Ecology and Earth Sciences, University of Tartu, Liivi 2, 50409, Tartu, Estonia

²Estonian Marine Institute, University of Tartu, Mäealuse 14, 12618 Tallinn, Harju County,
Estonia

³Littoral, Environnement et Sociétés (LIENSs), UMR7266 CNRS - La Rochelle Université, 2
rue Olympe de Gouges, 17000 La Rochelle, France

⁴Thünen Institute of Fisheries Ecology, Herwigstraße 31, 27572 Bremerhaven, Germany

Abstract

Many species in aquatic environments face increased exposure to oncogenic pollution due to anthropogenic environmental change which can lead to higher cancer prevalence. The mechanistic relationship connecting environmental pollution and cancer is multifactorial and poorly understood, and the specific mechanisms are so far still uncharacterized. One potential mediator between pollutant exposure and cancer is oxidative damage to DNA. We conducted a study in the field with two flatfish species, European flounder (*Platichthys flesus* L.) and common dab (*Limanda limanda* L.) with overlapping distribution and similar ecological niche, to investigate if the link between oncogenic pollutants and cancer described in ecotoxicological literature could be mediated by oxidative DNA damage. This was not the case for flounders as neither PAH bile metabolites nor metallic trace element concentrations were related to oxidative DNA damage measurements. However, dabs with higher PAH metabolite

concentrations did exhibit increased oxidative damage. High oxidative DNA damage also did not predict neoplasm occurrence, rather, healthy individuals tended to have higher oxidative damage measurements compared to fishes with pre-neoplastic tumours. Our analyses showed that flounders had lower concentrations of PAH bile metabolites, suggesting that compared to dab, this species is less exposed or better at eliminating these contaminants.

Keywords: Oxidative stress, hepatic tumours, marine contamination, European flounder, *Limanda limanda*, wildlife cancer

Introduction

Cancer is a disease that arose with multicellularity, potentially affecting all multicellular organisms [1]. Increasing evidence suggests some species have evolved stronger tumour suppressor mechanisms than others [2,3]. Whilst these mechanisms are the result of species'-specific life-history and/or environmental exposure to natural oncogenic substances [4,5], many species face increasing pressure from anthropogenic activities that could affect cancer prevalence in natural populations [6,7].

There is evidence that some species can adapt to increased concentrations of oncogenic pollutants, especially if the selection pressures are high and pollution is present for extended time periods [8,9]. One step towards understanding the mechanisms behind evolved cancer defence mechanisms in natural populations is to focus on specific physiological pathways to explore why cancer prevalence varies between populations and species living in environments contaminated with hazardous chemicals. One potential mediator between pollutant exposure and cancer is oxidative DNA damage, as both organic pollutants [10] and metallic trace elements [11] increase it. This can lead to cancer initiation and progression by increasing DNA mutations or inducing genome instability and cell proliferation [12]. However, relatively little is known about how the amount of oxidative DNA damage impacts cancer risk in pollution-

exposed animal populations, as current studies published mostly focus on changes in antioxidants in response to a specific pollutant in laboratory settings [13-15].

Flatfish, such as European flounders (*Platichthys flesus* L.) and dabs (*Limanda limanda* L.) have been studied for both pollution exposure and hepatic tumours [16, 17]. These benthic species are regularly used in biomonitoring surveys throughout the North Sea and Baltic Sea marine areas, which have been subjected to high concentrations of pollution for over a hundred years [18,19], through atmospheric and riverine inputs, and industrial point-sources (e.g. oil and gas platforms, [20-22]). Histopathological analyses have indicated that dabs have approximately 10 times higher liver neoplasia prevalence than flounders [23-28]. However, the causes behind this species-specific difference are not known.

This field study investigates correlations between the formation of hepatic neoplastic lesions, and (a) concentrations of polycyclic aromatic hydrocarbon (PAHs) bile metabolites, (b) liver concentrations of metallic trace elements (Hg, Cd, As, Pb). We also investigate whether oxidative DNA damage (measured via 8- hydroxy-2'-deoxyguanosine, 8-OhdG) acts as a mediator between contaminant exposure and neoplasm occurrence.

Methods

Field Sampling

Fish were collected using a trawler at ten Baltic and North Sea locations (See Table S1 for locations and numbers sampled) in August 2019. Fishes were weighed and measured before being killed by a percussive blow to the head that was followed by destruction of the brain using a surgical knife. Bile and liver samples were flash frozen in liquid nitrogen and stored at -80°C until analysis. A 3 mm liver section for histopathology analysis was stored in 4% formalin for 48 hours, then in 70% ethanol. Otoliths were collected to measure age. Sites were

allocated as 'reference' or 'polluted' based on OSPAR/HELCOM data on local anthropogenic pressures (Table S1, [29-31]).

Histopathology

Liver samples were processed according to Feist et al [32] for histopathological analysis, prepared onto slides and analysed using a Nikon Eclipse 80i microscope. Foci of cellular alteration (FCA) and neoplastic lesions were recorded [32] (see supplementary Table S1 and figures S1, S2 for further details).

PAH bile metabolites

PAH bile metabolites were measured using fixed fluorescence methods. Bile samples were diluted with 48% ethanol (1:1600) [33] and read using a fluorescence spectrophotometer (BMG Omega Fluostar) measuring fixed wavelength pairs (excitation and emission, respectively). 290/380 nm (2-ring PAH); 256/380 nm (3-ring PAH); 341/383 nm (4-ring PAH); and 380/430 nm (5-ring PAH) [34]. The results were presented as fluorescence units (FU) [35].

Trace metal concentrations

Liver samples were freeze-dried for 48 hours and ground to powder. Total Hg concentrations were measured, in duplicate (relative standard deviation for aliquots <10%), in subsamples of ~1mg of homogenized liver using an advanced Hg analyser spectrophotometer (Altec AMA 254) [36]. Remaining samples were analysed for trace elements arsenic (As), cadmium (Cd), and lead (Pb). Samples were digested with a mixture of 3 mL HNO₃ and 5 mL HCl Suprapur quality, heated in a microwave oven and diluted to 50 ml with deionized water, and analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian Vista-Pro ICP-AES) and Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific).

Oxidative stress markers

Biomarker 8-hydroxy-2'-deoxyguanosine (8-OHdG) was used to measure oxidative lesions formed in nuclear or mitochondrial DNA induced by free radicals. DNA, extracted using the

Purelink Genomic DNA Mini Kit (Invitrogen), was quantified using a Qubit 4 fluorometer. Standards were read on the dsDNA HS setting to calibrate the machine. 1µL of sample was added to 199µL of the working solution in triplicates, analysed, and the means calculated in ng/µL.

Samples were prepared for ELISA kits (Cell Biolabs Inc, cat number STA-320) following the protocol for cell or tissue DNA samples by converting to single strand DNA, digesting DNA using nuclease P1 (M0660S, New England Biolabs), adding alkaline phosphatase (CIP, M0371S New England Biolabs), centrifuging at 6000g for 5 minutes and collecting supernatants. Plates were washed using BioTek ELx50 microplate washer and absorbance (wavelength 450nm) measured using a spectrophotometer (Biotek Synergy 2). Results were normalised against the DNA concentrations, converted from 1 to 4 strand DNA and presented as ng oxidised DNA bases per mg DNA.

Statistical analysis

Statistical analyses were run using R 4.0.5[37]. Principle component analysis (PCA), using packages “stats”[38] and “psych”[39], grouped 2 components (see Figures S3-S4) splitting the variables into metals (As, Cd, Pb, Hg) and hydrocarbons (2, 3, 4, 5 ring). Generalised linear models tested whether oxidative stress or tumours could be predicted by trace metals or PAH metabolite concentrations, age, location (Baltic or North Sea), or whether sites were polluted/reference (Table S3). Chi-squared tested for a difference between the number of fishes with or without neoplasms/FCAs (3-levels: no abnormalities, FCA, neoplasm). Finally, ANOVA and post-hoc Tukey tests tested for differences between oxidative stress and tumours. Other packages used for plotting and data reorganisation were ggplot2[40] tidyverse [41], car[42] and various dependencies within them. Additional analysis with subsets of data (fish aged 3+ as younger fish as less likely to develop cancer and sites where both species were

present to exclude the influence of site characteristics on the results) are provided as supplementary material alongside all code and data (<https://doi.org/10.5281/zenodo.7846187>).

Results

Histopathology analyses indicated that dabs do not have significantly higher tumour prevalence (FCA or neoplasm) than flounders (Figure 1a, Chi-squared =2.81, df=2, p=0.2). This result could, however, be affected by the bias in the sampling, with more younger dabs in the data set compared to flounders. Linear models found that flounders were older and had higher amounts of oxidative DNA damage than dabs, dabs had significantly higher concentrations of PAH metabolites (Table 1). However, these results may be confounded by differences between sites. When we repeated the species comparison, including only the fish from the three sampling sites where both species were sampled, we found that only age was significantly different between dabs and flounders (Table S4). However, all the fishes with the highest contaminant concentrations were dabs (Figure 1b). There was a significantly higher variation (F-test) in metals ($p<0.001$) and hydrocarbons ($p=0.007$) in dabs than flounders, whereas age ($p=0.057$) and log-transformed oxidative DNA damage ($p=0.555$) did not vary significantly between the species.

The generalised linear model suggested that neither age, contaminant concentrations, oxidative stress, or sea (North vs Baltic) influenced the likelihood of a fish developing neoplastic/pre-neoplastic lesions (both species). However, there was significantly higher proportion of tumours in fish living in reference sites compared to more polluted sites (Table 2). Additionally, older flounders had more tumours than younger ones. As age is known to significantly affect cancer prevalence, and we had an over-representation of young dabs in our dataset, additional analysis excluding fishes under 3 years is provided in supplementary materials. Results of these models were the same as for the full dataset (Table S5). In order to

check that site characteristics were not confounding our results, additional analysis was run with site as a predictor (Supplementary materials: Table S8, Figure S5). The model including site resulted in a singular fit, which suggests preferring a simpler model, as such we chose the models with "polluted/reference" and "sea" for the main results.

There was a significantly higher level of oxidative damage in the fishes from the Baltic Sea than the North Sea for both species ($t=-4.511$, $p<0.001$), flounders ($t\text{-value}=-2.305$, $p<0.031$) but age, contaminant concentrations or whether a site was characterised as polluted/reference did not affect oxidative damage (Table S6). In dabs, oxidative damage was significantly higher in the Baltic ($t\text{-value}=-4.198$, $p<0.001$) and in dabs with higher concentrations of PAH metabolites. In the reduced dataset (only fish aged 3+) oxidative damage was significantly higher in the Baltic but the relationship, for dabs, with hydrocarbons (Table S7).

An ANOVA model indicated that oxidative damage was significantly higher in healthy dabs compared to those with FCA ($p=0.027$) but not different between healthy individuals and those with neoplasms or an FCA (Figure 2). There were no significant differences for flounders.

Discussion

This field study with two flatfish species investigates whether the link between oncogenic contaminants and hepatic tumours, described in ecotoxicological literature, could be mediated by oxidative DNA damage. We did not find evidence for this in flounders, as neither PAH nor metallic trace element concentrations were related to their oxidative DNA damage, whereas in dabs, higher concentrations of PAH metabolites were related to increased oxidative damage. It is possible that including other contaminants (e.g., persistent organic pollutants) could provide a different picture. Furthermore, oxidative DNA damage was not related to neoplasm occurrence in flounders whereas healthy dabs had increased DNA damage compared to dabs with FCAs. When comparing the species with each other (previous studies indicated higher

pollution-induced neoplasm prevalence in dabs than flounders [eg.23-28]) we found flounders had more oxidative DNA damage. However, contaminant concentrations varied more in dab compared to flounders suggesting potential differences in either exposure or pollutant removal mechanisms (Figure 1b). Additionally, PAH metabolite concentrations in flounders were lower than in dabs, suggesting more efficient organic pollutant metabolism in flounders. It must be noted, however, that age structure and sample sizes for age groups differed between the species, with more younger dab samples compared to flounders (Table S2). While age was included in the models, this still might confound the results when comparing species. Additionally, it should be noted that there are fewer ‘reference’ sites than ‘polluted’, and it is possible that adding additional sampling locations could change these results.

Contaminants can induce oxidative stress by either enhanced reactive oxygen species generation and/or weakening of antioxidant/damage repair systems [43]. Fish studies have indicated that environmental contaminants can affect antioxidant levels (organic pollutants [44], heavy metals [45,46]). However, limitations in detecting oxidative stress, through measuring oxidants and antioxidants, occur from time-lagged and hormetic upregulation of protective mechanisms [47]. Therefore, we assessed the oxidative DNA damage (8-OHdG) from the liver tissue of fish exposed to contaminated environments. The levels of 8-OHdG in aquatic species have been studied as a biomarker of oxidative stress caused by contamination, showing increased levels in populations exposed to higher contamination burdens [48,49]. In our study, increased PAH metabolite concentrations in dabs, but not flounders, were related to higher oxidative DNA damage levels, suggesting that PAH metabolism is linked to increased DNA damage in dabs. The different results for dabs and flounders could arise here from the differences in sampling sites, as only 3 of the 10 sampling sites included both species. This result for dabs, whilst unexpected, could be an indication of acclimatization or adaptation processes, resulting from long-term exposure to oncogenic contamination to reduce oxidative

199 DNA damage. However, a study on killifish (*Fundulus heteroclitus*) suggested that most of the
200 DNA damage present following PAH exposure was the result of covalent binding of
201 metabolites to DNA rather than from oxidative damage [50]. Whether this is the case for
202 flounders and dabs remains to be studied. Interestingly, tumours were not related to the levels
203 of oxidative DNA damage in the liver, suggesting other processes must be involved that
204 contribute more to tumour development in wild fish than oxidative DNA damage. Rather, in
205 dabs with no abnormalities detected, there was less oxidative damage than in dabs with an FCA
206 (no difference with neoplastic individuals or in flounders).

207 In this study, older fishes had more oxidative DNA damage than younger ones. Increased DNA
208 damage in older fish have been recorded in dabs using comet assay methods [51]. Additionally,
209 a study on three-spined sticklebacks (*Gasterosteus aculeatus*) found that larger fishes have
210 more DNA damage than smaller ones using comet assay and micronucleus tests [52],
211 potentially resulting from either the higher metabolic need of larger fish, leading to more
212 oxidative DNA damage, or DNA damage accumulation with age resulting from the senescence
213 process.

214 Fishing location had the greatest influence on elevated oxidative damage, demonstrated by
215 Baltic Sea fish exhibiting a significantly higher oxidative damage than North Sea fish. Changes
216 in salinity can cause oxidative stress in fish [53] and a study on olive flounder (*Paralichthys*
217 *olivaceus*) found that hyposaline conditions caused oxidative stress [54] so it is possible that
218 the low saline Baltic environment is increasing oxidative damage in flounders and dabs.

219 Additionally, a report by the European Environment Agency suggests a larger proportion of
220 the Baltic (96%) is polluted than the North Sea area (75%) [55] which could cause increased
221 oxidative damage in the Baltic. Actively removing organic pollutants from the organism is
222 considered a risk factor for cancer, as the by-products of active pollutant metabolism by the
223 cytochrome p450 enzymes (CYPs) lead to carcinogenesis via DNA adduct formation [56,57].

In this study, fish caught in ‘reference’ sites had more tumours than those from ‘polluted’ sites. However, it should also be noted that migration of fishes was not accounted for when categorising sites as ‘reference’ or ‘polluted’ and exposure rates could vary between individual’s dependant on migratory distances. It is possible that fish living in heavily polluted environments can adapt/acclimate to cope with contamination [58]. In conclusion, we showed that in an ecosystem where pollution has persisted for a long time, it is not possible to find a clear link between contaminants (PAH and metallic trace elements), oxidative DNA damage, and neoplasms. In the North and Baltic seas, both flounders and dabs have had approximately 50 generations to adapt to high concentrations of environmental contamination. We can suggest that both species, but especially flounders, could have gone through evolutionary processes, where stronger cancer defence mechanisms, more efficient pollutant metabolism, and protection against oxidative DNA damage was selected for.

Acknowledgements

This work was supported by the Estonian Research council grants PSG653 to Tuul Sepp and PRG1137 to Peeter Hõrak. We are grateful to C. Churlaud and to M. Brault-Favrou from the "Plateforme Analyses Élémentaires" of LIENSs for their assistance during trace element analyses and to the crew of RW Walther Herwig III for all-round help during the fieldwork.

References

1. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. 2018 How the evolution of multicellularity set the stage for cancer. *Br. J. Cancer* **118**, 145-152. (doi.org/10.1038/bjc.2017.398)

2. Tollis M, Schiffman JD, Boddy AM. 2017 Evolution of cancer suppression as revealed by mammalian comparative genomics. *Curr. Opin. Genet. Dev.* **42**, 40-47. (doi.org/10.1016/j.gde.2016.12.004)
3. Baines C, Meitern R, Kreitsberg R, Sepp T. 2022 Comparative study of the evolution of cancer gene duplications across fish. *Evol. Appl.* **15**, 1834-1845. (doi.org/10.1111/eva.13481)
4. Ujvari B, Beckmann C, Biro PA, Arnal A, Tasiemski A, Massol F, Salzet M, Mery F, Boidin-Wichlacz C, Misse D, Renaud F. 2016 Cancer and life-history traits: lessons from host–parasite interactions. *Parasitology* **143**, 533-541. (doi.org/10.1017/S0031182016000147)
5. Vittecoq M, Giraudeau M, Sepp T, Marcogliese DJ, Klaassen M, Renaud F, Ujvari B, Thomas F. 2018 Turning natural adaptations to oncogenic factors into an ally in the war against cancer. *Evol. Appl.* **11**, 836-844. (doi.org/10.1111/eva.12608)
6. Giraudeau M, Sepp T, Ujvari B, Ewald PW, Thomas F. 2018 Human activities might influence oncogenic processes in wild animal populations. *Nat. Ecol. Evol.* **2**, 1065-1070. (doi.org/10.1038/s41559-018-0558-7)
7. Baines C, Lerebours A, Thomas F, Fort J, Kreitsberg R, Gentes S, Meitern R, Saks L, Ujvari B, Giraudeau M, Sepp T. 2021 Linking pollution and cancer in aquatic environments: A review. *Environ. Int.* **149**, 106391. (doi.org/10.1016/j.envint.2021.106391)
8. Wirgin I, Roy NK, Loftus M, Chambers RC, Franks DG, Hahn ME. 2011 Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science* **331**, 1322–1325. (DOI: 10.1126/science.1197296)
9. Reid NM, Proestou DA, Clark BW, Warren WC, Colbourne JK, Shaw JR, Karchner SI, Hahn ME, Nacci D, Oleksiak MF, Crawford DL, Whitehead A. 2016 The genomic

landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish.
Science **354**, 1305–1308. (DOI: 10.1126/science.aah4993)

10. Singh R, Kaur B, Kalina I, Popov TA, Georgieva T, Garte S, Binkova B, Sram RJ, Taioli E, Farmer P B. 2007 Effects of environmental air pollution on endogenous oxidative DNA damage in humans. *Mutat. Res.* **620**, 71–82. (doi.org/10.1016/j.mrfmmm.2007.02.024)

11. Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Götte W, Jung D, Mayer-Popken O, Fuchs J, Gebhard S Bienfait HG. 2003 Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis* **24**, 63-73. (doi.org/10.1093/carcin/24.1.63)

12. Valavanidis A, Vlachogianni T, Fiotakis C. 2009 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health C: Toxicol.* **27**, 120-139. (doi.org/10.1080/10590500902885684)

13. Isaksson C. 2010 Pollution and its impact on wild animals: a meta-analysis on oxidative stress. *EcoHealth* **7**, 342–350. (doi.org/10.1007/s10393-010-0345-7)

14. Liu H, Sun P, Liu H, Yang S, Wang L, Wang Z. 2015 Hepatic oxidative stress biomarker responses in freshwater fish *Carassius auratus* exposed to four benzophenone UV filters. *Ecotoxicol. Environ. Saf.* **119**, 116-122. (doi.org/10.1016/j.ecoenv.2015.05.017)

15. Tu TY, Hong CY, Sasado T, Kashiwada S, Chen PJ. 2016 Early life exposure to a rodent carcinogen propiconazole fungicide induces oxidative stress and hepatocarcinogenesis in medaka fish. *Aquat. Toxicol.* **170**, 52-61. (doi.org/10.1016/j.aquatox.2015.11.014)

16. Lerebours A, Stentiford GD, Lyons BP, Bignell JP, Derocles SA, Rotchell JM. 2014 Genetic alterations and cancer formation in a European flatfish at sites of different contaminant burdens. *Environ. Sci. Technol.* **48**, 10448-10455 (doi.org/10.1021/es502591p)
17. Williams TD, Turan N, Diab AM, Wu H, Mackenzie C, Bartie KL, Hrydziusko O, Lyons BP, Stentiford GD, Herbert JM, Abraham JK. 2011 Towards a system level understanding of non-model organisms sampled from the environment: a network biology approach. *PLoS Comput Biol*, **7**, 1002126. (doi.org/10.1371/journal.pcbi.1002126)
18. HELCOM 2018: State of the Baltic Sea – Second HELCOM holistic assessment 2011-2016. Baltic Sea Environment Proceedings 155.
19. Carpenter A. 2019 Oil pollution in the North Sea: the impact of governance measures on oil pollution over several decades. *Hydrobiologia*, **845**, 109-127. (doi.org/10.1007/s10750-018-3559-2)
20. HELCOM. 2021 Inputs of hazardous substances to the Baltic Sea. Baltic Sea Environment Proceedings No.179.
21. Marappan S, Stokke R, Malinovsky MP, Taylor A. 2022. Assessment of impacts of the offshore oil and gas industry on the marine environment. In: OSPAR, 2023: The 2023 Quality Status Report for the North-East Atlantic. OSPAR Commission, London.
22. Ilyin I, Travnikov O, Rozovskaya O, Strizhkina I. 2022. Trends in Deposition of Heavy Metals to the OSPAR Maritime Area in the period 1990-2019. In: OSPAR, 2023: The 2023 Quality Status Report for the North-East Atlantic. OSPAR Commission, London.
23. Cachot J, Cherel Y, Larcher T, Pfohl-Leszkowicz A, Laroche J, Quiniou L, Morin J, Schmitz J, Burgeot T, Pottier D. 2013 Histopathological lesions and DNA adducts in the liver of European flounder (*Platichthys flesus*) collected in the Seine estuary versus

two reference estuarine systems on the French Atlantic coast. *Environ. Sci. Poll. Res.* **20**, 723-737. (doi.org/10.1007/s11356-012-1287-0)

24. Lang T, Wosniok W, Baršienė J, Broeg K, Kopecka J, Parkkonen J. 2006 Liver histopathology in Baltic flounder (*Platichthys flesus*) as indicator of biological effects of contaminants. *Mar. Poll. Bulletin.* **53**, 488-496. (doi.org/10.1016/j.marpolbul.2005.11.008)

25. Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M, Feist SW. 2003 Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Mar. Envir. Res.* **55**, 137-159. (doi.org/10.1016/S0141-1136(02)00212-X)

26. Stentiford GD, Bignell JP, Lyons BP, Feist SW. 2009 Site-specific disease profiles in fish and their use in environmental monitoring. *Mar. Ecol. Progr. Series.* **381**, 1-15. (doi.org/10.3354/meps07947)

27. Lyons BP, Stentiford GD, Bignell J, Goodsir F, Sivyer DB, Devlin MJ, Lowe D, Beesley A, Pascoe CK, Moore MN, Garnacho E. 2006 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.* **62**, 342-346. (doi.org/10.1016/j.marenvres.2006.04.064)

28. CEFAS, Marine Environment Monitoring Group. 2004. UK National Marine Monitoring Programme - Second Report (1999-2001). ISBN 0 907545 20 3.

29. Hylland K, Skei BB, Brunborg G, Lang T, Gubbins MJ, Le Goff J, Burgeot T. 2017 DNA damage in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas. *Marine Environmental Research* 124, 54-60.

30. OSPAR Commission. 2010. OSPAR Quality Status Report 2010. <https://qsr2010.ospar.org/en/index.html>

31. Zaborska A, Siedlewicz G, Szymczycha B, Dzierzbicka-Głowacka L, Pazdro K. 2019 Legacy and emerging pollutants in the Gulf of Gdańsk (southern Baltic Sea) – loads and distribution revisited. *Marine Pollution Bulletin* 139, 238-255
32. Feist SW, Lang T, Stentiford GD, Köhler AICES. 2004 Biological effects of contaminants: use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. *ICES Techniques in Marine Environmental Sciences* 38. (doi.org/10.25607/OBP-226)
33. Aas E, Baussant T, Balk L, Liewenborg B, Andersen OK. 2000 PAH metabolites in bile, cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil exposure: a laboratory experiment with Atlantic cod. *Aquat. Toxicol.* **51**, 241–258. (doi.org/10.1016/S0166-445X(00)00108-9)
34. Lee RF, Anderson JW. 2005 Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. *Mar. Pollut. Bull.* **50**, 705–723. (doi.org/10.1016/j.marpolbul.2005.04.036)
35. Beyer J, Jonsson G, Porte C, Krahn MM, Ariese F. 2010 Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: a review. *Environ. Toxicol. Pharmacol.* **30**, 224–244. (doi.org/10.1016/j.etap.2010.08.004)
36. Bustamante P, Lahaye V, Durnez C, Churlaud C, Caurant F. 2006 Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. *Sci. Total Environ.* **368**, 585-596.
37. R Core Team. 2021 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

38. Revelle W. 2022 psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.2.9, <https://CRAN.R-project.org/package=psych>.
39. Harrell Jr FE. 2022. Rms: Regression Modelling strategies. *R package version 6.0* Dept. Biostatist., Vanderbilt Univ., Nashville, TN, USA.
40. Wickham H, Chang W, Wickham MH. 2016. Create elegant data visualisations using the grammar of graphics. Package ‘ggplot2’ version 3.3.6.
41. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, et al. 2019. Welcome to the Tidyverse. *J Open Source Softw.* 4:1686. (DOI: 10.21105/joss.01686)
42. Fox J, Weisberg S. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
43. Lushchak VI. 2016 Contaminant-induced oxidative stress in fish: a mechanistic approach. *Fish Physiol. Biochem.* **42**, 711–747. (doi.org/10.1007/s10695-015-0171-5)
44. Chinnadurai K, Prema P, Veeramanikandan V, Kumar KR, Nguyen VH, Marraiki N, Zaghloul NS, Balaji P. 2022 Toxicity evaluation and oxidative stress response of fumaronitrile, a persistent organic pollutant (POP) of industrial waste water on tilapia fish (*Oreochromis mossambicus*). *Environ. Res.* **204**, 112030. (doi.org/10.1016/j.envres.2021.112030)
45. Kumari K, Khare A, Dange S. 2014. The applicability of oxidative stress biomarkers in assessing chromium induced toxicity in the fish *Labeo rohita*. *BioMed Res. Int.* **2014**, 782493. (doi.org/10.1155/2014/782493)
46. Das D, Moniruzzaman M, Sarbajna A, Chakraborty SB. 2017. Effect of heavy metals on tissue-specific antioxidant response in Indian major carps. *Environ. Sci. Pollut. Res.* **24**, 18010–18024. (doi.org/10.1007/s11356-017-9415-5)

47. Meitern R, Sild E, Kilk K, Porosk R, Hõrak P. 2013 On the methodological limitations of detecting oxidative stress: effects of paraquat on measures of oxidative status in greenfinches. *J. Exp. Biol.* **216**, 2713-2721. (doi.org/10.1242/jeb.087528)
48. Machella N, Regoli F, Cambria A, Santella RM. 2004 Oxidative damage to DNA: an immunohistochemical approach for detection of 7, 8-dihydro-8-oxodeoxyguanosine in marine organisms. *Mar. Environ. Res.* **58**, 725-729. (doi.org/10.1016/j.marenvres.2004.03.022)
49. El-Agri AM, Emam MA, Gaber HS, Hassan EA, Hamdy SM. 2022 Integrated use of biomarkers to assess the impact of heavy metal pollution on *Solea aegyptiaca* fish in Lake Qarun. *Environ. Sci. Eur.* **34**, 1-24. (doi.org/10.1186/s12302-022-00656-6)
50. Jung D, Cho Y, Collins LB, Swenberg JA, Di Giulio RT. 2009. Effects of benzo [a] pyrene on mitochondrial and nuclear DNA damage in Atlantic killifish (*Fundulus heteroclitus*) from a creosote-contaminated and reference site. *Aquat. Toxicol.* **95**, 44-51.
51. Akcha F, Leday G, Pfohl-Leszkowicz A. 2004 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-Fund. Mol. M.* **552**, 197-207. (doi.org/10.1016/j.mrfmmm.2004.06.020)
52. Wirzinger G, Weltje L, Gercken J, Sordyl H. 2007 Genotoxic damage in field-collected three-spined sticklebacks (*Gasterosteus aculeatus* L.): a suitable biomonitoring tool?. *Mutat. Res.-Genet. Toxicol.* **628**, 19-30. (doi.org/10.1016/j.mrgentox.2006.11.011)
53. Birnie-Gauvin K, Costantini D, Cooke SJ, Willmore WG. 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish Fish (Oxf)*, **18** 928-942.

54. Lee, D.W., Choi, Y.U., Park, H.S., Park, Y.S. and Choi, C.Y., 2022. Effect of low pH and salinity conditions on the antioxidant response and hepatocyte damage in juvenile olive flounder *Paralichthys olivaceus*. *Mar. Environ. Res.* **175**, 105562.
55. European Environment Agency. 2019. Contaminants in Europe's seas. Moving towards a clean, non-toxic marine environment. 61.
56. Stegeman JJ, Lech JJ. 1991 Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environ. Health Perspect.* **90**, 101–109. (doi.org/10.1289/ehp.90-1519513)
57. Willett KL, Ganesan S, Patel M, Metzger C, Quiniou S, Waldbieser G, Scheffler B. 2006. In vivo and in vitro CYP1B mRNA expression in channel catfish. *Mar. Environ. Res.* **62** S332-S336. (doi.org/10.1016/j.marenvres.2006.04.015)
58. Wirgin I, Waldman JR. 2004. Resistance to contaminants in North American fish populations. *Mutat. Res-Fund. Mol. M.* **552**, 73-100.
59. Sepp T, Baines C, Kreitsberg R, Scharsack JP, Nogueira P, Lang T, Fort J, Sild E, Clarke JT, Meitern R. (2022) ENA archive bioproject PRJEB53201 <https://www.ebi.ac.uk/ena/browser/view/PRJEB53201>.

Table 1: Linear model results for a difference between oxidative damage, age and contaminant burdens between species. * represents p-value <0.05, ** represents p<0.01.

	Coefficient	S.E.	Wald Z	Pr(> Z)
Intercept	-18.7452	5.1216	-3.660	0.000252

Oxidative DNA Damage(log)	2.3344	0.8339	2.799	0.005121
				**

Metals	-0.5968	0.4578	-1.304	0.1924
Hydrocarbons	-0.9054	0.3677	-2.462	0.0138*
Age	1.1217	0.2451	4.577	<0.0001*
				**

Table 2: Model results (N=128) of variables influencing the differences in pre-neoplastic/neoplastic tumours between both species and each species individually. * represents p-value <0.05, ** represents p<0.01.

		Estimate	Std. Error	t value	Pr(> t)	
Both	(Intercept)	3.11513	4.5926	0.678	0.49759	
	Metals	-0.20913	0.21477	-0.974	0.33019	
	Hydrocarbons	0.12996	0.22643	0.574	0.56601	
	Age	-0.02367	0.1649	-0.144	0.88584	
	Oxidative Stress	-0.56179	0.75214	-0.747	0.45511	
	Sea (North)	1.18170	0.71741	1.647	0.09927	
	Polluted	-1.4411	0.55388	-2.602	0.00927	**
Dab	(Intercept)	9.92253	5.94056	1.670	0.0949	
	Metals	-0.20196	0.22902	-0.882	0.3778	
	Hydrocarbons	0.24261	0.28230	0.859	0.3901	
	Age	-0.03751	0.34256	-0.110	0.9128	
	Oxidative Stress	-1.73457	0.96487	-1.798	0.0722	
	Sea (North)	1.14057	1.03903	1.714	0.2723	
	Polluted	-1.56631	0.75596	-2.142	0.0383	*
Flounder	(Intercept)	-23.9688	12.3031	-1.948	0.0514	
	Metals	-2.0686	1.4950	-1.384	0.1665	
	Hydrocarbons	-0.5449	0.6646	-1.820	0.4123	
	Age	0.9142	0.4362	2.096	0.0361	*
	Oxidative Stress	3.1787	1.9040	1.669	0.0950	
	Sea (North)	1.6869	1.5987	1.055	0.2913	
	Polluted	-3.1224	1.4183	-2.201	0.0277	*

Figure 1: A: The prevalence of cancer and foci of cellular alterations (FCA) in flounder (n=40) and dab (n=88) livers. Individual numbers on bars. B: PCA loadings for metallic trace elements and polycyclic aromatic hydrocarbons (PAHs).

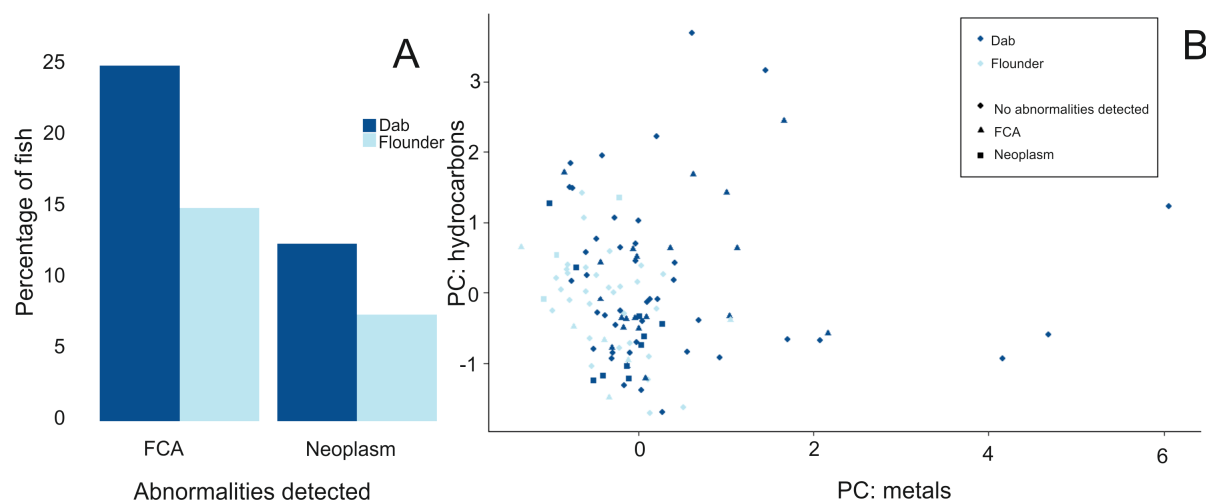


Figure 2: Mean (log-transformed) oxidative DNA damage between fish with neoplasms, foci of cellular alterations (FCA) or neither. Asterix (*) represents $p < 0.05$. Bars represent mean \pm standard deviation. Dots are individual data points.

