Monitoring DNA Damage in *Mytilus galloprovincialis* and other Aquatic Animals

III. A Case Study: DNA Damage in Fish from a Florida Marsh

By ARMIN HERBERT

Institut für Molekularbiologie und Biochemie, Freie Universität Berlin

(With 2 figures)

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Introduction

The presence of potentially mutagenic and carcinogenic agents in the aquatic environment has received increasing attention during the past years (ANDELMAN & SUESS 1970, KRAYBILL 1977, GESAMP 1982). To survey the aquatic environment for the occurrence of these pollutants, the study of inducible detoxification systems in fish has successfully been introduced in biological monitoring (PAYNE & PENROSE 1975, BURNS 1976, KURELEC et al. 1977, STEGEMAN 1978). Recently it became possible to estimate directly the genotoxic impact on aquatic animals in their natural environment by biophysical methods (STÜBER & ZAHN 1985, ZAHN et al. 1988, HERBERT & ZAHN 1989a, b). These studies report DNA damage in aquatic animals after an acute oil spill and in the close vicinity of intense human settlement. The case, which is reported here, is an example of the occurrence of DNA damage in fish from a more remote environment, with comparatively less visible human impact. In this study from February and March 1988 DNA characteristics of killifish *(Fundulus heteroclitus)* were investigated, which had been sampled from an estuarine marsh at Marineland/Florida, which is located about 30 kilometers south of St. Augustine and 70 kilometers north of Daytona Beach.

Materials and methods

Animals. Fish *(Fundulus heteroclitus)* were collected by traps and were examined either the following day or after 4 weeks. Control fish were kindly supplied

by Dr. Peter Lin, who had kept these fish for 6 months in laboratory tanks with filtered seawater at ambient water temperatures.

Chemicals. All chemicals were of analytical grade purity. Hydroxyapatite was BIORAD DNA-Grade Bio-Gel HTP.

Formamide was cleaned up prior to use according to SCHLEIF and WENSINK (1981).

Methods. DNA damage was investigated by partial alkaline DNA denaturation and hydroxyapatile elution as described before (HERBERT & ZAHN 1989a, b), using lysis system III at 0 °C with a lysis period of 30 min. Four to six fish from each sample group were examined. EROD (ethoxyresorufin o-deethylase) activities were measured by fluorometric assay of the resorufin metabolite, according to POHL & FOUTS (1980). Protein concentrations were determined by the method of BRADFORD (1976) using the BIORAD protein assay kit. Bovine immunoglobulin G was used as reference for calibration. When DNA characteristics and EROD activities were compared, livers from 6 fish were pooled and homogenized together in 10 ml homogenization buffer (solution A, see HERBERT & ZAHN 1989a). The homogenate was centrifuged for 10 min at 1000 × g. The supernatant was used for the enzyme assay, whereas the nuclear pellet was used for the characterization of the DNA. Since homogenate pools were used in the latter experiment, the given standard deviation is a measure for the variation among parallel determinations of the same sample, but not for the variation of the investigated characteristics within the studied populations.

Results

On January 31, 1988 three oil covered marsh birds were brought to Marineland – one of these birds had been found at Washington Oaks Park, the two others at Anastasia Island. This incident insinuated possible impact of oil pollution onto the marsh ecosystems along the Matanzas River. In order to study possible genotoxic effects, which might be related to oil pollution, fish from the surrounding marsh were collected during the next days and their DNA was examined by partial alkaline DNA denaturation. In figure 1 the DNA characteristics of these fish are compared with two groups of fish from the same population: One group was collected the same day, and then held in the laboratory for four weeks, the other was collected by Dr. Peter Lin in September 1987 and had been held in the laboratory until March 1988. The results are expressed as $-\log F$, as described by HERBERT & ZAHN (1989a), a number, which is typically increased by the presence of DNA lesions. Figure 1 shows that the value of $-\log F$ is higher in fish from the marsh than in laboratory fish. The increased value in marsh fish declines after holding the fish in the laboratory. The relative number of DNA lesions can be calculated by a formula given by KANTER & SCHWARTZ (1979):

$$n = -\log F_x / -\log F_0 - 1,$$

where n is the average number of lesions per DNA strand, and the subscripts x and 0 characterize data from polluted fish and controls. The results show that the fish from the marsh have on average 0.61 lesions per DNA strand.

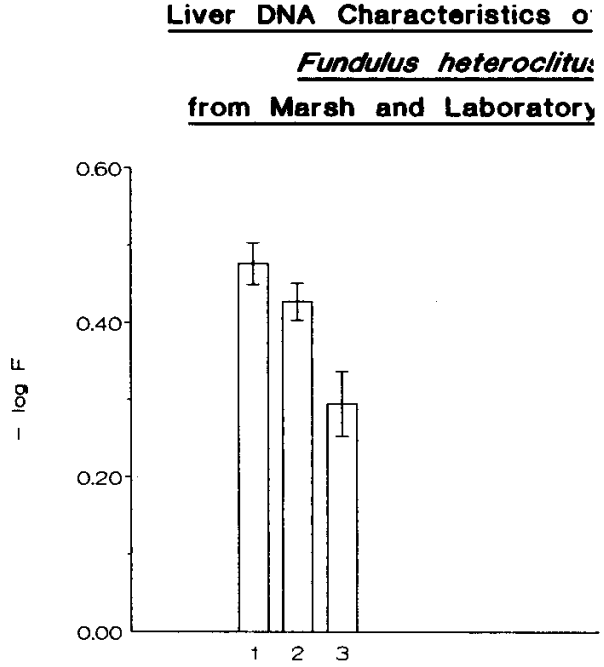

Fig. 1. Comparison of liver DNA from *Fundulus heteroclitus* collected in February 1988 from the Matanzas marshlands (1), collected at the same occasion, but kept for four weeks in laboratory tanks (2), and collected six months before and kept in the laboratory (3).

Although at the time of our investigation there was no opportunity to perform calibration experiments with standard substances, the extent of DNA damage can carefully be compared with results of other authors. The relative number of DNA strand lesions, which were found in the marsh fish, is about the same number as KANTER & SCHWARTZ (1979, page 83) have found in mammalian cells after treatment with 1 Gy X rays. BATEL et al. (1985) kept mosquito fish *(Gambusia affinis)* for two days in water with 100 ppb benzo(a)pyrene and they observed between 0.31 and 0.46 lesions per DNA strand.

Next, the correlation between DNA damage and monooxygenase activity was investigated. Instead of examining livers of individual fish separately, the livers of six freshly caught or laboratory-held fish were pooled and the homogenate was separated by centrifugation into nuclei and cytoplasmic supernatant. The nuclei were used for DNA characterization, whereas the supernatant was used to determine the EROD activity, which is an inducible activity of the monooxygenase system in fish (JAMES & BEND 1980, KLOTZ et al. 1984, SCHELL et al. 1987, JAMES et al. 1988). Inhibition of EROD activity was examined by adding 0.1 mM α-naphtho flavone to the reaction mix. In addition to liver samples, gills were also examined. The data of this comparison are summarized in figures 2 A and 2 B.

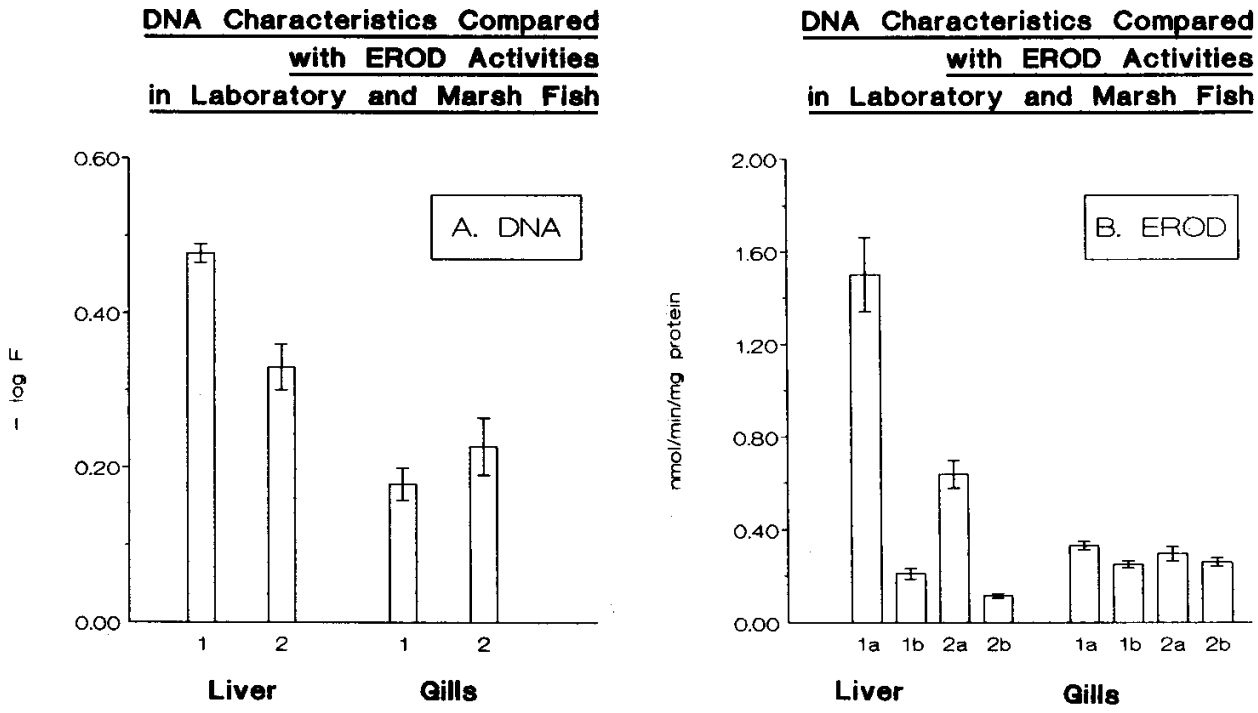

Fig. 2A, 2B. Comparison of DNA and EROD activity from marsh (1) and laboratory (2) fish. Enzyme activities were determined without (a) and with (b) inhibitor present.

The results for the liver DNA were similar to the first study and gave a number of 0.45 lesions per DNA strand. No lesions were discernible in the gills. The slight difference between gills of marsh and laboratory fish, and the general difference between gills and liver might be related to differences in transcriptional activity as previously explained (HERBERT & ZAHN 1989a, b).

Interestingly the pattern, which was found for EROD activities, is almost congruent with the figure of DNA lesions. The activity in the gills is low in laboratory fish and not induced in marsh fish. However, there is a slight activity in the liver of laboratory fish, and this activity is increased by a factor of about 2.4 in the fish from the marsh.

Discussion

Although the marshlands around Marineland in Florida seem to be remote estuaries, some ways of hazardous human impact on the ecosystem can not be excluded. First, there is the possibility of oil pollution brought in from the Atlantic Ocean. Oil and oil derivatives may result from extensive ship traffic in the vicinity of Florida or may enter the Atlantic through currents from the Mexican gulf and from the Caribbean (ROMERO et al. 1981, VAN

VLEET et al. 1983). Second, a major contribution to water pollution may derive from land-based human activities through ground and river water contamination (MILLER et al. 1977). Besides the nearby finding of the three oil covered birds, there was no other sign or a public report of a major oil spill or dumping, and the fish, which were examined, looked externally healthy. Thus, the reason for the high degree of DNA damage in the investigated fish remains somewhat speculative. STEGEMAN (1978) reports induced MFO activities in *Fundulus heteroclitus* years after a local oil spill. On the other hand, chronic pollution, caused e.g. by the recreational use of power boats or cars, entering beaches and shallow river waters, may be the cause for the observed molecular-biological alterations in the fish. Since fish as well as aquatic invertebrates are able to a certain degree to accumulate pollutants like polycyclic aromatic hydrocarbons (LEE et al. 1972a, b, 1976, DUNN & STICH 1976), one might consider human health impacts by seafood consumption as possible. However, this aspect requires further investigation and both detailed chemical and biological studies.

To conclude this report, also the more technical aspects of this investigation should be discussed. Monitoring induction of the MFO system in aquatic animals has been introduced as a sensitive tool in environmental reseach more than a decade ago (PAYNE & PENROSE 1975, BURNS 1976, KURELEC et al. 1977, STEGEMAN 1978), and methods to study successfully DNA damage in aquatic animals have become available recently (ZAHN et al. 1981, STÜBER & ZAHN 1985, HERBERT 1987, ZAHN et al. 1988, KURELEC et al. 1988, SHUGART 1988). A combination of both approaches has been used in this study and the experimental results indicate a correlation between DNA damage and MFO activity. A similar correlation has been reported by STÜBER & ZAHN (1985), and ZAHN et al. (1985) discuss this phenomenon in more detail and suggest that both DNA damage and MFO induction should be studied in environmental monitoring programs. It could be demonstrated here, that it is technically possible to study both parameters in parallel from the same sample by separation of tissue homogenates into nuclear and cytoplasmic fractions. Thus, this procedure may be useful for further studies in environmental research.

Summary

In a case study, possible genotoxic pollution effects were investigated in killifish *(Fundulus heteroclitus)* from a Florida marsh, which appeared to be only under slight impact of human activities. Liver DNA was characterized by partial alkaline unwinding and subsequent hydroxyapatite batch elution. By this method a significant amount of DNA damage was found in the fish from the marsh. In a second investigation a similar result coincided with an increased monooxygenase (ethoxyresorufin o-deethylase) activity in the same fish. Possible sources for these biochemical alterations are discussed.

Zusammenfassung

In einer Fallstudie wurden gentoxische Schadstoff-Wirkungen an Killi-Fischen *(Fundulus heteroclitus)* aus einem Küsten-Feuchtgebiet Floridas untersucht, das nur geringfügig durch menschliche Aktivitäten belastet schien. Leber-DNA wurde durch partielle alkalische Denaturierung mit anschließender Hydroxylapatit-Elution charakterisiert. Anhand dieser Methode wurden DNA-Schäden in signifikantem Ausmaß bei den Fischen des untersuchten Feuchtgebiets gefunden. Dieser Befund konnte in einer zweiten Untersuchung bestätigt werden, hierbei wurde zudem eine Koinzidenz mit einer gesteigerten Monooxygenase (Ethoxyresorufin-o-deethylase)-Aktivität gefunden. Die möglichen Ursachen für diese biochemischen Wirkungen werden diskutiert.

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Author's address:

Dr. ARMIN HERBERT

C. V. Whitney Laboratory for
Experimental Marine Biology and Medicine
University of Florida
9505 A1A South
St. Augustine, FL 32086-9604
USA

Present address:

Institut für Molekularbiologie und Biochemie
Freie Universität Berlin
Arnimallee 22
D-1000 Berlin 33
Federal Republic of Germany