

## DNA DAMAGE AND RADIOCESIUM IN CHANNEL CATFISH FROM CHERNOBYL

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**Abstract**—The explosion of the Chernobyl Nuclear Power Plant resulted in some of the most radioactively contaminated habitats on earth. Despite evacuation of all human inhabitants from the most contaminated areas, animals and plants continue to thrive in these areas. This study examines the levels of contamination and genetic damage associated with radiocesium in catfish (*Ictalurus punctatus*) from the cooling pond and a control site. In general, catfish from the cooling pond exhibit greater genetic damage, and the amount of damage is related to the concentration of radiocesium in individual fish. Genetic damage is primarily in the form of DNA strand breaks, with few micronuclei being observed in contaminated fish. The possible roles that acclimation and adaptation play in the response to high levels of radiation exposure are discussed.

**Keywords**—Catfish Chernobyl DNA damage Micronuclei Radiocesium

## INTRODUCTION

Reactor 4 of the Chernobyl Nuclear Power Plant (CNPP) exploded on April 26, 1986. Approximately 200 million Ci [1] of radiation was released as a result of the explosion and subsequent fire, which burned for 10 d. Regions surrounding the CNPP were contaminated with particulates from fuel rods and graphite [2], whereas aerosols resulting from the high temperatures associated with the meltdown of core materials were distributed in the upper atmosphere and often carried for hundreds of kilometers before settling to earth [3]. Despite loss of radionuclides with short half-lives, high levels of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  still cover a vast range in Ukraine, Belarus, Russia, Poland, and Scandinavia [4]. Although approx. 135,000 people were evacuated from a zone 30 km in radius around the CNPP, natural fauna and flora continue to thrive in the area. In this article, we report the impact of latent radiation on the integrity of genetic material for one species from the CNPP cooling pond. The population of channel catfish in the pond is vast, and this species thrives only in this lake in northern Ukraine. Freezing of ponds and rivers prevent the channel catfish from surviving in other habitats in the region.

Radiation and other carcinogens are known to cause genetic damage [5–13]. However, few studies have investigated DNA damage in relation to radionuclides in natural settings [14–18]. However, studies that examine genetic damage in natural populations provide useful information about the potential biological hazards associated with such toxic agents and the amelioration or exacerbation of effects via ecological pathways. Until such information is accumulated, our ability to understand and mediate environmental damage resulting from disasters such as the accident at the Chernobyl nuclear reactor [19] will be limited.

The mechanisms whereby radiation causes genetic damage

are generally well known. Ionizing radiation, as it passes through water, produces hydroxyl radicals, hydrated electrons, and hydrogen atoms. In the presence of oxygen, these reactants produce free radicals of oxygen and peroxides [20]. The strong oxidative nature of these free radicals and peroxides break nucleotide–nucleotide bonds in DNA [9,20]. Fortunately, aerobic organisms have evolved enzyme systems, namely superoxide dismutase, catalases, and peroxidases [21,22], as a response to oxygen toxicity [18]. Stimulation of these preexisting enzyme systems may explain why low “adaptive” doses of radiation result in less genetic damage in organisms that are subsequently “challenged” by high doses of radiation [9]. Combining an ability to mediate radical production with good enzyme systems for DNA repair [15,23], it is possible that chronic, low-level exposure to radiation may have little effect on DNA integrity. Thus, the artificially high and acute doses often administered in laboratory experiments may not adequately reflect outcomes in most contaminated sites (e.g., Savannah River Site [15]); however, the CNPP explosion provides an opportunity to examine highly contaminated environments.

Ecotoxicologists are interested in understanding how organisms respond to exposure to environmental toxicants. While laboratory studies of organismal responses to toxicants, such as survivorship analysis [24], are very informative, they often lack the complexity of natural environments. It is the complexity of real ecosystems that make the remediation of impacted systems difficult to realize; only with information from natural settings can we refine our predictive powers for assessment of ecological and health risks. Ultimately, the goal of studies such as this is to provide information that will help mediate the environmental damage resulting from radiation releases like those at Chernobyl.

Assessing the response of whole organisms exposed to toxicants in natural situations is difficult. Survival models would be difficult to construct because of other, nontoxicant, factors

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influencing survivorship. It is often necessary to assess the impact of toxicants by use of a corollary to organismal response. The assessment of genetic damage is of utility in this regard because such damage has an impact on the survival of cells and individuals [11,25]. Several methods have been developed to assess DNA damage. The alkaline unwinding technique is a rapid method of assessing strand breaks. Recent studies have shown that the number of breaks, as determined from this method, can be associated with various toxic agents [11–15,26].

Other methods of assessing genetic damage involve the assessment of chromosomal damage. The micronucleus assay [27–29] is extensively used as an indicator of chromosomal damage because it is a simpler and faster test of clastogenicity than standard metaphase spread preparations. In this assay, micronuclei are observed in polychromatic erythrocytes and are considered to be chromosomal fragments. Such fragments were not incorporated into daughter nuclei at the time of cellular division, have persisted through interphase, and remained in the cell at the time of erythrocyte maturation [27–29]. The micronucleus assay is scored more quickly than the chromosome analysis because it is not dependent on the presence of metaphase spreads. Moreover, larger numbers of cells ( $n = 1,000$  per individual) are analyzed than is typical of chromosome studies ( $n = 50$  to 100). The reason the micronucleus assay was chosen for this study is that preparation and analysis of fish chromosomes is very difficult due to the small size of the chromosomes and the low metabolic rate of the fish. In contrast, very little searching is necessary in the micronucleus assay because interphase erythrocytes, the dominant cell type in peripheral blood, are used. Alkaline unwinding and micronuclei assays give measures of genetic damage at two different levels in a hierarchy, breaks in the DNA and loss of genetic material from the nucleus of a cell.

Obviously, the assessment of genetic damage is only a corollary of organismal response to toxicant exposure. Beyond the obvious differences between genetic damage and realized survival, which goes unmeasured, is the problem that genetic damage among tissues may be heterogeneous [15]. Individual tissues differ in their rates of accumulation of different toxicants, and this is true for radionuclides as well. For example, cesium is a potassium analog and is likely to be incorporated into soft tissues. On the other hand, strontium tends to be found in hard tissues, i.e., bones, where it replaces calcium. Further problems exist in the variable ability of tissues to enzymatically repair genetic damage [15,30]. For these reasons, it is preferable to assess genetic damage in a variety of tissues whenever possible.

The purposes of this study were to quantify the concentrations of radiocesium in catfish from the cooling pond at the Chernobyl reactor; to compare DNA strand breaks and chromosomal damage in catfish from the cooling pond and an uncontaminated population; to compare DNA strand breaks in different tissues of catfish; and to determine what association, if any, there is between radiocesium content and genetic damage in catfish.

## MATERIALS AND METHODS

### *Site characteristics and sampling*

The cooling pond comprises a 22-km<sup>2</sup> impoundment originally constructed for the purpose of cooling the hot water effluent from two nuclear power reactors [3]. Waters for the cooling pond are provided by a diversion of the Pripyat River. The reservoir was expanded in 1983 to facilitate the heavy burden imposed by adding reactors 3 and 4. Constant circulation of

water and continuous warming provided by thermal effluents prevents freezing of this lake in winter.

Channel catfish were stocked in the CNPP reservoir for the first time in 1985, just 1 year prior to the explosion. Originally, catfish from Arkansas and Alabama were transported to a fish hatchery in southern Ukraine in 1972. Fish from this hatchery served as the controls for this study because they would not have been exposed to radiation from Chernobyl. Fish from the cooling pond ( $n = 34$ ) were captured using gill nets (8 to 10-cm mesh) and by angling from the shore. Live fish were transported to a holding facility at the Chernobyl Fisheries Laboratory on the banks of the cooling pond or were transported in large containers to a laboratory in Stracholesse, 30 km south of the CNPP. Control fish ( $n = 29$ ) were transported from the laboratory to a large hatchery operated by Kiev Hydroelectric Energy Ministry. Control fish were held for 30 d in the hatchery facility in tanks measuring approx.  $50 \times 20 \times 5$  m with water from the Dneiper River. Control fish were recaptured by hand nets. Fish were anesthetized with MS222 (3-aminobenzoic acid ethyl ester, A5040; Sigma Chemical Co., St. Louis, MO, USA), and length, weight, and sex were noted. Approximately 1 ml whole blood was taken by syringe from the caudal artery. Anesthetized fish were killed by decapitation, and liver, kidney, and gill tissue was sampled and frozen in liquid nitrogen. Samples of muscle were dried for subsequent assessment of concentrations of radionuclides.

### *DNA strand breaks*

Standard methods for alkaline DNA unwinding [11,12] were used with modifications as indicated in Sugg et al. [15]. These procedures were applied to three tissues (gill, liver, and red blood cells). A brief description of the techniques are provided here. A detailed description of the procedures can be obtained from the authors.

Standard techniques for extraction of DNA were used [31]. Samples of red blood cells (25  $\mu$ l,  $n = 37$ ), liver ( $\cong 0.2$  g,  $n = 55$ ), and gill ( $\cong 0.1$  g,  $n = 60$ ) were placed in separate microcentrifuge tubes containing 500  $\mu$ l of NET buffer [31]. To each of these samples 10  $\mu$ l of proteinase K (20 mg/ml, 25530-015; Gibco BRL, Grand Island, NY, USA) were added, and the tubes were placed on a specimen rotator (13-688-1D; Fisher Scientific, Norcross, GA, USA). After spinning for 3 hr at 36 to 37°C in an incubator, 500  $\mu$ l of Tris-equilibrated phenol [31] was added, and samples were mixed for 10 min on the specimen rotator at room temperature. Next, tubes were centrifuged for 5 min at 6,000 rpm, and the top, aqueous, phase was removed and placed in a clean tube. The phenol extraction was repeated, and using the same procedures, two chloroform extractions were performed to yield purified DNA.

The alkaline unwinding method involved three treatments of the samples, each of which was performed in duplicate for each sample. Prior to treatment, each sample was diluted with distilled, deionized water (DI-H<sub>2</sub>O) to yield a fluorescence approximately equal to that of a calf thymus standard (D-3664; Sigma Chemical Co., St. Louis, MO, USA) diluted to 0.1 mg/ml. The first treatment yielded a double-stranded value for the sample. A sample (50  $\mu$ l) of the diluted DNA was placed in a 6-ml test tube to which 450  $\mu$ l of buffer A (250 mM NaCl, 2.5 mM Tris, pH 7.4, 0.25 mM MgCl<sub>2</sub>, 0.125 mM ethylenediamine tetraacetic acid [EDTA]) was added. Next, 500  $\mu$ l of 0.005 N NaCl was added to the tube, and the solution was mixed by slowly drawing it into a tuberculin syringe (23G needle) and rapidly expelling it into the tube five times. Finally, 2 ml of

phosphate buffer/Hoechst dye 33528 (phosphate: 100 mM  $\text{KH}_2\text{PO}_4$ , 100 mM  $\text{K}_2\text{HPO}_4$ , pH 7.0; Hoechst: 1 mg/ml; mixture: 100  $\mu\text{l}$  dye/300 ml buffer) was added to the tube, and the solution was incubated in the dark for 15 min. After samples had incubated, the fluorescence of each duplicate of the samples, a standard (50  $\mu\text{l}$  of calf thymus DNA), and a blank (50  $\mu\text{l}$  of buffer A) were determined with a TKO-100 fluorometer (Hoefer Scientific, San Francisco, CA, USA).

The other treatments of the samples differ only in that 250 ml of 0.005 N NaOH was added in place of the NaCl and the tubes were incubated for 30 min. The partially unwound treatment was incubated at 38°C, and the completely unwound treatment (single-stranded) was incubated at 85°C. After incubation, 250 ml of 0.05 N HCl was added to each tube, then samples were mixed. After mixing, 2 ml of phosphate buffer/Hoechst dye was added to each tube, and the samples were incubated for 15 min. in darkness. As with the double-stranded treatment, a standard and a blank were used for these determinations as well.

The percent double-stranded DNA in the sample (% DS, an inverse measure of DNA damage) was determined from the following formula:

$$\%DS = \frac{F_{\text{UW}} - F_{\text{SS}}}{F_{\text{DS}} - F_{\text{SS}}}$$

where  $F_{\text{UW}}$ ,  $F_{\text{SS}}$ , and  $F_{\text{DS}}$  were the fluorescence from the partially unwound, single-stranded, and double-stranded determinations, respectively. Because each treatment was done in duplicate, there were eight possible combinatorial values for %DS. Each of these were calculated, and the standard error of the estimate was determined. Any sample that produced a standard error greater than 5% of the mean was rerun. Note that the value for %DS used here was equivalent to the  $F$  value used by Shugart [12], but %DS was used to avoid confusion with the  $F$  ratio obtained from analysis of variance.

#### Micronucleus analysis

Blood smears from 44 catfish were prepared in the field and air dried. Dry slides were sent to Texas A & M University for staining and analysis. Slides were flooded for 1 min with absolute methanol (3041; Mallinckrodt, Paris, KY, USA), tipped to remove the methanol, and allowed to partially dry. The slides were then flooded with approx. 1 ml of Miles stain (Stanbio Laboratory, San Antonio, TX, USA) for 1.5 min. Then the stain was diluted with approx. 1.5 ml of hematological buffer (Stanbio Laboratory, San Antonio, TX, USA) for 3 min. The slide was then rinsed with distilled water, taking care that the metallic sheen on the surface of the stain solution was forced away from the slide. The slides were then set upright to dry.

Dry slides were examined using an Orthoplan microscope (Leitz, Rockleigh, NJ, USA) at 1,000 $\times$  total magnification with an oil immersion objective. Slides were scanned under low power (100 $\times$ ) to find areas of the slide where the blood cells formed a monolayer and were well stained. Well-stained cells had distinct and lightly stained pink cytoplasm and dark blue or purple nuclei. Only slides on which there was minimal background or debris could be accurately analyzed. This meant that only about half of the slides prepared in the field were suitable for analysis. A total of 1,000 cells were scored for each fish. Micronuclei were identified as small, darkly stained bodies of chromatin within the cytoplasm and outside the nucleus. Only structures of similar staining intensity as the nucleus and nonrefractory in appearance were scored as micronuclei.

#### Radiocesium analysis

Radiocesium concentrations were determined only on muscle because no liver and blood tissues remained after other analyses were performed. Radiocesium concentrations are viewed as an index of individual contamination. Samples of skeletal muscle were taken from each individual, placed in 20-ml scintillation vials (3-337-12B; Fisher Scientific, Norcross, GA, USA), weighed, freeze dried, and reweighed prior to analysis for radiocesium content. Radiocesium detection was conducted using a Minaxiγ 5000-series automatic gamma counter (Packard Instruments, Meriden, CT, USA) equipped with a 3-in.  $\times$  3-in. NaI crystal and a 550 to 760-KeV window. Samples were analyzed three times, and the average count per minute was used to calculate the radiocesium content. Standards ( $\text{Cs-137}$ ,  $9.3 \times 10^3$  Bq; Packard Instruments, Meriden, CT, USA) were run before and after each set of samples, and blanks were used to block groups of five samples. Averages of readings for all blanks were used to calculate background radiation. The average of the standards run before and after each set were used to calculate the efficiency. Count times for all samples, blanks, and standards were adjusted to produce counting standard deviations <5% [32]. Lower detection limits were calculated for each block of five samples, and those samples with activities at or below the detection limit were assigned radiocesium activities of zero. Count per minute for samples, background counts, and efficiency were used to calculate the amount of radiocesium in each sample and then divided by the dry weight of the sample to determine the concentration of radiocesium (134 and 137).

#### Statistical analysis

Two types of statistical analysis were used in this study. The first was used to describe heterogeneity among sites for the variables of interest. The second type of analysis was used to determine the association between measures of genetic damage and concentrations of radiocesium. Because the variables of interest (micronuclei, %DS, and radiocesium concentration) were not normally distributed, they were transformed. The %DS was arcsine square-root transformed, and radiocesium concentration was square-root transformed [33]. The number of micronuclei represents count data which is best transformed by adding 0.5 and taking the square root [33]. All statistical analyses were performed using SYSTAT® [34].

Analysis of variance was used to determine if sample localities varied for %DS for each tissue and the average of all tissues, number of micronuclei, radiocesium concentration ( $C$ ), and length ( $L$ ) and weight ( $W$ ). Measures of body size were included to account for possible age-specific uptake rates of radiocesium [15,35]. Because length and weight differed significantly among sites, these variables were used as covariates to test for differences among sites in the remaining variables.

Stepwise regression was used to determine which variables, if any, explained a significant portion of the variation in DNA damage. These analyses were performed using the following model statements:

$$\hat{Y} = \beta_0 + (\beta_1 \cdot C) + (\beta_2 \cdot L) + (\beta_3 \cdot W) \\ + (\beta_4 \cdot C \cdot L) + (\beta_5 \cdot C \cdot W) + (\beta_6 \cdot C \cdot L \cdot W)$$

where the  $\beta$ 's represent the regression coefficients. Procedures for the regression were set so that a variable must have an  $\alpha$  of less than 0.15 and a tolerance of less than 0.1 to be entered and an  $\alpha$  of greater than or equal to 0.15 to be removed from

Table 1. Least-squares means of transformed variables, standard errors (in parentheses), and means of untransformed data (in square brackets) for genetic damage (%DS), number of micronuclei, and radiocesium concentration in catfish from Chernobyl<sup>a</sup>

Location	Average %DS	Liver %DS	Gill %DS	Blood %DS	Number of micronuclei	Cesium concn. (Bq/g)
Control	0.968 (0.024) [0.673]	0.836 (0.033) [0.543]	1.700 (0.021) [0.773]	0.955 (0.033) [0.662]	1.406 (0.121) [1.900]	0.095 (0.081) [0.024]
Cooling pond	0.896 (0.023) [0.605]	0.674 (0.032) [0.392]	1.068 (0.021) [0.751]	0.973 (0.029) [0.678]	0.991 (0.115) [0.591]	6.018 (0.075) [35.485]

<sup>a</sup> Types of transformations for each variable are described in the text. Analysis of covariance indicated significant differences among samples for each variable except %DS in gills and blood.

the model. Separate analyses were performed on average %DS and the %DS for each tissue and the number of micronuclei.

### RESULTS

There was considerable heterogeneity among sites. Percent double-stranded DNA of all fish (untransformed) ranged from 53.6 to 96.5% for gill, from 24.8 to 90.1% for liver, and from 28.1 to 88.1% for blood. The number of micronuclei ranged from 0 to 6 micronuclei per 1,000 cells. The concentration of radiocesium (untransformed) ranged from below the detection limit to 52.97 Bq/g. Analyses of variance showed that %DS for liver ( $p < 0.001$ ), average %DS ( $p = 0.039$ ), number of micronuclei ( $p = 0.006$ ), radiocesium concentration ( $p < 0.001$ ), length ( $p = 0.027$ ), and weight ( $p = 0.035$ ) differed significantly among sites. Percent double-stranded DNA and number of micronuclei were greatest for the control site; radiocesium content, length, and weight were greatest for the cooling pond.

For control catfish, weight ranged from 252 to 820 g, and length ranged from 314 to 449 mm. The weight of cooling pond fish ranged from 236 to 1,696 g, and the length ranged from 319 to 570 mm. Because length and weight differed significantly among sites, and because radiocesium concentration may covary with increasing size, analysis of covariance was used to describe differences in means for the variables of interest (Table 1). In general, means for %DS and number of micronuclei were higher for the control population; mean radiocesium content was much greater for fish from the cooling pond. Significant differences were found only for %DS in liver ( $p = 0.001$ ), average %DS ( $p = 0.034$ ), number of micronuclei ( $p = 0.022$ ), and radiocesium content ( $p < 0.001$ ).

Stepwise regression of %DS against radiocesium content, length, and weight resulted in few significant coefficients (Table 2). For blood, %DS was not significantly associated with any of the independent variables. In gill, a significant negative association was detected with the interaction of length and weight.

Table 2. Stepwise regression coefficients for genetic damage (%DS) in three tissues and the average value for catfish from Chernobyl<sup>a</sup>

Statistical effect	Average %DS	%DS in liver	%DS in gill	%DS in blood
Intercept	0.962	0.838	1.117	0.912
Cesium	-0.010	-0.028	—	—
Length × weight	—	—	-0.001	—

<sup>a</sup> All variables were transformed as described in the text. Coefficients are provided only for variables that explained a significant proportion of the variation.

Percent double-stranded DNA was significantly associated with radiocesium content for liver and the average of all tissues (Fig. 1). These relationships were negative, indicating that increasing radiocesium content is associated with less double-stranded DNA and hence a greater amount of genetic damage.

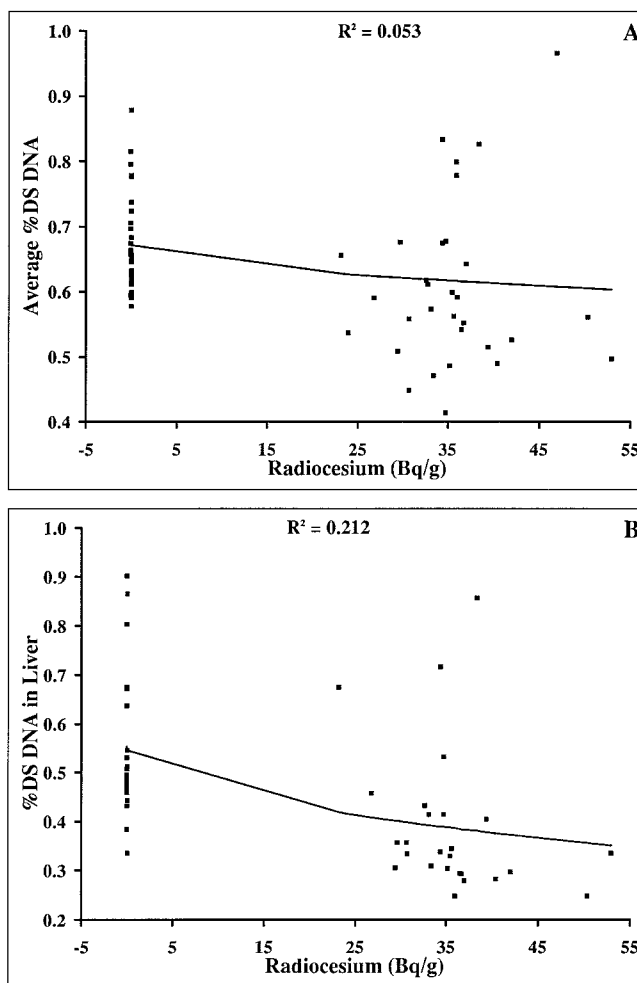


Fig. 1. Association between genetic damage (%DS) and concentration of radiocesium (Bq/g) in catfish from Chernobyl, Ukraine, and a control site. (A) Relationship for genetic damage averaged across all tissues. (B) Association for genetic damage in liver only. Each regression is statistically significant at  $p < 0.05$ , but the models explain little of the variation in genetic damage. Transformations of the variables are explained in the text, and regression coefficients are provided in Table 2.

Table 3. Stepwise regression coefficients for number of micronuclei in catfish from Chernobyl. Three comparisons are used: fish from the control site, fish from the reactor cooling pond, and all fish combined<sup>a</sup>

Statistical effect	Control site	Cooling pond	All fish combined
Intercept	1.430	-0.847	1.301
Cesium	—	0.307	—
Cesium × weight	—	—	-0.001

<sup>a</sup> All variables were transformed as described in the text. Coefficients are provided only for variables that explained a significant proportion of the variation.

Stepwise regression for number of micronuclei resulted in a model that included radiocesium and a negative two-way interaction between weight and radiocesium (Table 3). This result would suggest that as the product of radiocesium and weight increase, the number of micronuclei decreases. A separate regression model for fish from the control site resulted in a model that only included a constant (Table 3). When fish from the cooling pond were examined alone, the concentration of radiocesium was significantly and positively related to the number of micronuclei (Table 3).

## DISCUSSION

In general, catfish from the cooling pond experience a greater number of DNA strand breaks than do control fish; however, they do not exhibit a greater number of micronuclei. Greater genetic damage in aquatic organisms from cooling ponds has been previously related to concentrations of radiocesium and other toxicants [13,15]. The %DS DNA from these studies cannot be compared directly to determine which contaminant results in the greatest genetic insult because the alkalinity of the unwinding solutions were optimized for each species. However, some general patterns can be examined.

Results from a study of genetic damage in largemouth bass on the Savannah River Site [15] suggested that genetic damage was greater in liver and gill tissues for fish from sites with substantial radiocesium contamination, while blood exhibited the least amount of damage in fish from sites with high levels of radiocesium contamination. The differences in genetic damage have been suggested to result from tissue differences in DNA repair and cell turnover rates. The present study found genetic damage to be greatest in liver for both control and cooling pond fish. Gill tissue had the least number of strand breaks for both samples, with genetic damage in red blood cells falling between the other tissue types. Differences between these studies may be due to several factors, including differences in levels of contamination, types of contamination, and the physiology of the fishes.

The relatively high levels of genetic damage reported for liver in the present study and the previous study at the Savannah River Site are interesting. While repair enzyme activities are considered good in this tissue [15,30], genetic damage is considerably greater when compared to other tissues that are likely to have less efficient repair mechanisms. This result may indicate a real response to exposure to contaminants, or it may result from procedural difficulties inherent with the tissue itself. Pancreatic tissues are present within the liver of these fish, and the high nuclease activities associated with this tissue may have resulted in some degradation of the DNA. Because such an outcome is possible, meaningful comparisons of the absolute

amount in genetic damage in liver to other tissues are difficult. However, because liver samples were treated identically during the sampling and analysis phases, the relative amounts of genetic damage are still a valid comparison. The results still demonstrate that the amount of genetic damage in cooling pond catfish is higher than that for control fish.

Levels of radiocesium contamination are great in the cooling pond at CNPP. Catfish from this site have over 50% greater concentrations of radiocesium than bass in the most contaminated cooling pond at the U.S. Department of Energy's Savannah River Site [15]. These extreme differences in contamination for the two studies may explain the greater importance of radiocesium, as it pertains to strand breaks, in the present study. Additionally, the study at the Savannah River Site also examined concentrations of mercury and concluded that it was an important determinant of genetic damage, perhaps because it inhibits the repair of strand breaks. Although 10 samples of fish from the cooling pond at CNPP were found to contain virtually no mercury, it is possible that other contaminants exist in this environment. These potential contaminants include lead and polycyclic aromatic hydrocarbons, both of which could potentially lead to genetic damage [11,12]. Finally, catfish and bass have different physiological attributes and habits. The bass at the Savannah River Site live in warm water and occupy a position in the top trophic level (carnivorous), while catfish typically live in cooler, deeper water and are primarily scavengers. The trophic difference can influence the concentration of radiocesium as it is biomagnified. Physiological and trophic differences may also exist in potassium requirements/uptake (for which cesium is an analog), but it is the physiological differences that may be the most important for genetic damage. The potentially slower metabolic rates of the catfish may lead to slower rates of repair for the DNA.

Several factors determine the amount of genetic damage an organism will exhibit when exposed to toxicants. The balance between the rate at which strand breaks occur and the rate at which they are repaired determines the steady-state number of strand breaks. Repair mechanisms are only one level in a hierarchy of defense systems. Other enzyme systems, such as superoxide dismutase and peroxidase, can serve as a higher-level defense when radiation exposure leads to free-radical production. High activities of both free-radical scavenging and DNA-repair enzymes may effectively mediate the damaging effect of radiocesium in catfish. If this is the case, then the low number of micronuclei in fish from the cooling pond may indicate that either cell lines or individual fish that remain in the cooling pond today have acclimated or adapted to the high levels of contamination, perhaps through selection favoring enzyme systems that are more efficient at scavenging free radicals and repairing strand breaks. Such adaptation may explain the low variance in numbers of micronuclei from this sample [36], which could indicate that most of the strand breaks are repaired before segments of DNA can be lost (Fig. 2). Obviously, further studies into the activities of these enzyme systems will be needed to address this possibility.

The relationship between genotoxicants and permanent, transmissible genetic changes in organisms is unclear [36–39], but most studies have shown that some damage to DNA is associated with radiation [19,36,40–44]. For humans, a definite health risk has been established for exposure to particular doses of radiation [45,46]. Normally, when exposures exceed acceptable bounds, as established by national and international health organizations, then people are excluded from prolonged

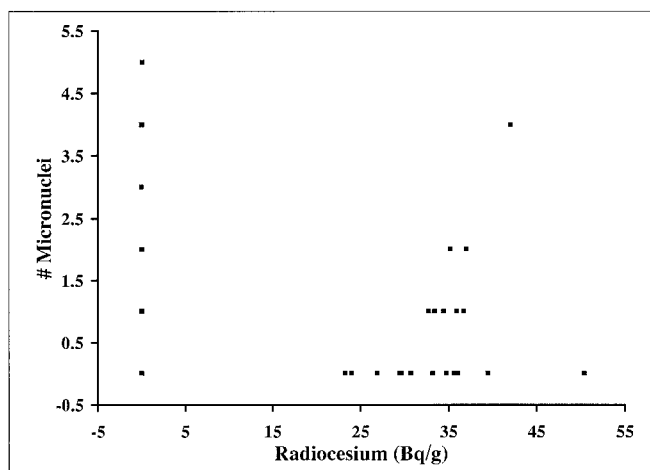


Fig. 2. Association between number of micronuclei per 1,000 cells and concentration of radiocesium (Bq/g) in catfish from Chernobyl, Ukraine, and a control site. Values to the left of the graph represent individuals from the control site, and those to the right came from the reactor's cooling pond. There is no association between these variables for control fish; however, radiocesium concentration explains a significant portion of the variation in number of micronuclei ( $r^2 = 0.875$ ,  $p < 0.001$ ) for fish living in the cooling pond. Transformations of the variables are given in the text, and regression coefficients are provided in Table 3.

occupancy of contaminated regions. Avoidance preempts adaptation (or may arguably be an adaptation itself), and selection for more efficient mechanisms to combat intracellular challenges of ionizing radiation is not possible. Natural populations of organisms do not have such avoidance opportunities and must adapt to pollutants or face local extinction. Clearly, the metabolic costs associated with the continual repair of broken strands of DNA will, at some level, result in reduced efficiencies for growth, maintenance, and reproduction. Thus, it is likely that for the catfish in the Chernobyl cooling reservoir, the process of natural selection is ongoing for enzymes that efficiently remove or neutralize oxygen free radicals and peroxides, prevent oxygen ions bonding to nucleotides, or effectively repair strand breaks when they occur. Thus, assignment of biological risk to organisms inhabiting contaminated regions must be a dynamic process since risk to surviving organisms changes as more vulnerable individuals are removed [47].

Some previous studies have shown similar reductions in variability (somatic or population) resulting from contaminant exposure. In a controlled laboratory dosage study using the clastogenic drug triethylenemelamine, Bickham et al. [36] showed that rats exposed for 5 d exhibited high levels of DNA content variation in somatic cells. However, when exposed rats were allowed to recover for 7 d, DNA content variation was reduced to levels significantly less than observed for control animals. In a field study of black-crowned night herons (*Nycticorax nycticorax*), Custer et al. [48] observed lower levels of DNA content variation in spleen cells from contaminated sites as compared to a reference site.

Determination of ecological risk and the potential impacts of contaminants to natural communities and ecosystems associated with radiation releases is complicated by man's avoidance of these regions. The potential negative impacts to viability and fertility of organisms imparted by radiation is often compensated for by the positive impact brought about by elimination of man's destruction and modification of habitat. Such compensation has been reported for both the Chernobyl exclusion

zones and the Savannah River Site. The Chernobyl cooling reservoir is a tremendously productive ecosystem despite the fact that for a prolonged period of time exposures near the sediments were in excess of 200 to 300 rads/d [3]. Likewise, Pond B and Par Pond at the Savannah River Site [46] have been noted for their importance to fish, plant, waterfowl, and reptilian fauna of the region despite considerable contamination with  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ .

Studies of the relationship of genetic damage and radionuclides at Chernobyl are showing that individuals and populations continue to thrive in regions despite rather alarming levels of radioactive contamination. However, the mechanisms of acclimation and adaptation that are taking place in the exposed organisms are unclear. Knowledge of such processes is vital for accurate assessment of the expected outcomes of unavoidable exposures to radioactive contamination and for development of cogent programs for ecological and individual risk assessment. Bickham and Smolen [49] describe the levels of biological organization, ranging from the molecular level through cells, tissues, organisms, and populations, at which the effects of environmental mutagens can be expressed. They noted that long-term effects can include population genetic and evolutionary changes, and they coined the term "evolutionary toxicology" to describe an emerging field of scientific endeavor that will focus on these issues. Other researchers, including Depledge [50] and Dieter [51], also have noted that contaminant exposure will ultimately have an effect on the Darwinian fitness of organisms. Nonetheless, considering the varied responses of natural populations that have been studied (including the catfish reported herein), it is presently difficult to predict what the outcome of any particular environmental insult on natural populations will be. The environmental disaster at Chernobyl represents an extraordinary opportunity to observe the adaptation of wildlife to highly polluted environments.

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