Ecotoxicology (2012) 21:496–511
DOI 10.1007/s10646-011-0811-1

DNA damage in cichlids from an oil production facility in Guatemala

Christopher W. Theodorakis · John W. Bickham · Kirby C. Donnelly · Thomas J. McDonald · Philip W. Willink

Accepted: 23 October 2011 / Published online: 12 November 2011

Abstract This study focused on several wetlands in Laguna del Tigre National Park (Guatemala) as part of Conservation International's Rapid Assessment Program. Sediment and water samples were collected from a laguna near Xan field, Guatemala's largest oil facility, and three other sites for determination of levels of polycyclic aromatic hydrocarbons (PAHs). Cichlid fish (*Thorichthys meeki* and *Vieja synspila*) were collected for determination of DNA strand breakage (by gel electrophoresis), chromosomal breakage (flow cytometry), and fin erosion. For *T. meeki* from Xan field, chromosomal breakage and strand breakage was greater than in at least two of the three reference sites. For *V. synspila*, chromosomal breakage and strand breakage were greater in Xan than one of the two reference sites. Fin erosion was observed only at the Xan laguna. Genetic biomarker effects and fin erosion, along with patterns of aqueous PAH concentrations, indicate that fish are affected by anthropogenic contaminants. PAHs were elevated at some reference sites, but environmental forensic analysis suggested a pyrogenic or diagenic origin. It is possible that oil field brines injected into the ground water caused fin erosion and genotoxicity in fish at Xan field, and it is also possible that pyrogenic PAHs influence levels of DNA damage in reference sites. These analyses represent one of the first efforts to examine genotoxicity in native Mesoamerican cichlids.

Keywords DNA strand breakage · Flow cytometry · Cichlid · Guatemala · Genotoxicity · Fin erosion · Crude oil · Environmental forensics

Kirby C. Donnelly—Deceased.

C. W. Theodorakis
Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University Edwardsville, Edwardsville, IL, USA

J. W. Bickham (✉)
Center for the Environment and Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA
e-mail: bickham@purdue.edu

K. C. Donnelly
Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX, USA

T. J. McDonald
School of Rural Public Health, Texas A&M University, College Station, TX, USA

P. W. Willink
Fish Division, Department of Zoology, Field Museum of Natural History, Chicago, IL, USA

Introduction

Contamination of aquatic and marine ecosystems from crude oil is well documented. Considerable information is available on the acute, chronic, and sublethal effects of oil spills such as the Exxon Valdez on marine organisms (Loughlin 1994; Cronin and Bickham 1998; Harvey et al. 1999; Page et al. 2002). Data also has been collected on the effects of oil wells and oil production facilities on marine ecosystems (Barron et al. 2003; Barron et al. 2005; Carls et al. 2005; Chen et al. 2004; Couillard et al. 2005; Doong and Lin 2004; Liu et al. 2006; Alonso-Alvarez et al. 2007; Di Toro et al. 2007; McIntosh et al. 2010). However, there is less information on the possible effects of oil production facilities on freshwater aquatic organisms, especially in the tropics.

Aquatic organisms that are exposed to crude oil and its byproducts may be affected by various chemicals such as

aliphatic, aromatic, and polycyclic aromatic hydrocarbons (PAHs). Of these, PAHs are probably the most persistent in the environment because of their lower volatility. PAHs and other petroleum contaminants may biodegrade under aerobic conditions, but might persist in aquatic ecosystems under anaerobic conditions for far longer than in terrestrial systems (Ashok and Saxena 1995). As a consequence, PAH compounds are characteristic components of petroleum contamination that accumulate in the sediment (Mille et al. 1998).

However, determination of the accumulation of PAHs into fish tissues is problematic, due to the fact that endogenous metabolic processes rapidly break down these compounds (Levin et al. 1982). Therefore, biomarkers may be particularly useful for documenting PAH exposure. Such biomarkers are also useful for a number of other reasons. First, the relationship between environmental concentrations, tissue levels in wildlife, and the potential to produce adverse effects is not well established (Shugart et al. 1992). The availability of certain chemicals for uptake into fish tissues ("bioavailability") is often determined by certain environmental variables (e.g., the amount of naturally occurring hydrocarbons in the water and sediment). Also, environmental contaminants are usually present as complex mixtures and the toxicity of these mixtures may not be accurately predicted from measurements of concentrations of single chemicals (Shugart et al. 1992). Therefore, measurements of the physiological responses of fishes living in these environments are needed to rigorously evaluate the impacts of hydrocarbon contamination.

The particular biomarker used for this study was DNA damage, which has proven useful for monitoring xenobiotic exposure and effects in a number of aquatic species (Bickham et al. 1998b; Costa et al. 2002; Jha 2004; Neuparth et al. 2006; Theodorakis 2008; Shugart et al. 2010). DNA damage has been found to be characteristic of exposure to crude oil (Bickham et al. 1998a; Custer et al. 2000; Pérez-Cadahía et al. 2004; Baršienė et al. 2006a, b; Bolognesi et al. 2006; Laffon et al. 2006; Goanvec et al. 2008; Deasi et al. 2010). Specifically, we used DNA strand breakage and chromosomal breakage (Shugart et al. 1992; Jha 2004). If the breaks are on only one of the DNA strands (single-strand breaks), the chromosome remains intact but the integrity is compromised. If strand breaks occur on two adjacent strands, the chromosome may become fragmented which can be measured as an increase in cell-to-cell variation in DNA content (Bickham 1990). Exposure to PAH and petroleum contamination is reflected in increases in the amount of single-strand breakage and cell-to-cell variation in DNA content (Bickham 1990).

Most studies documenting the occurrences of environmental contaminants such as PAHs, or their effects such as genotoxicity, have been conducted in temperate latitudes, whereas similar studies in tropical ecosystems are relatively scarce. Some tropical areas have high levels of biodiversity, which is being lost at an alarming rate due to habitat destruction and other human impacts (Myers et al. 2000). Thus, environmental pollution may act synergistically with habitat destruction to further reduce biodiversity in affected areas (Bickham et al. 2000). In fact, environmental contamination is considered one of the primary threats to aquatic biodiversity in Neotropical areas (Agostinho et al. 2005). This is compounded by a lack of knowledge of the effects of contaminant exposure on tropical species. Thus, tropical ecosystems that are potentially vulnerable to pollution are of special concern.

One location in which there is the potential for such contamination is Laguna del Tigre National Park (LTNP) in Northern Guatemala. Within the boundaries of this park is the Xan oil production facility. In this study we test the hypothesis that fish living in proximity of the oil production facility show evidence of stress relative to animals from reference sites. To test this hypothesis we examined sediment and water concentrations of PAHs, frequency of fin erosion caused by bacterial infections, and DNA damage in two fish species, the firemouth cichlid (*Thorichthys meeki*) and the redhead cichlid (*Vieja synspila*; formerly *Cichlasoma synspilum*) near the Xan facility and in other sites around LTNP. Cichlids are a diverse and ecologically important group of fish in Central America, and are potentially valuable sentinels for the ecology and biodiversity of Mesoamerican wetlands (Keenleyside 1991).

Materials and methods

Study sites and sample collection

This endeavor was part of Conservation International's Rapid Assessment Program to determine possible impacts of Xan field, Guatemala's largest oil production facility, on LTNP (Alonso et al. 2000). LTNP is part of the Mayan biosphere preserve located in the Petén province in Northern Guatemala (Fig. 1). The major aquatic ecosystems in the park are rivers and small lakes or "Lagunas". During the dry season these lagunas are separate entities, but during the rainy season much of the intervening land may be inundated such that the lagunas are interconnected and/or there may be flow of surface or ground water between lagunas.

Environmental samples (sediment and water) and/or fish samples were collected from one river and four lagunas within LTNP (Fig. 1). The laguna sampled on Xan field (Xan three laguna, a.k.a. Consejo Nacional de Areas Protegidas [CONAP] Laguna) was located approximately

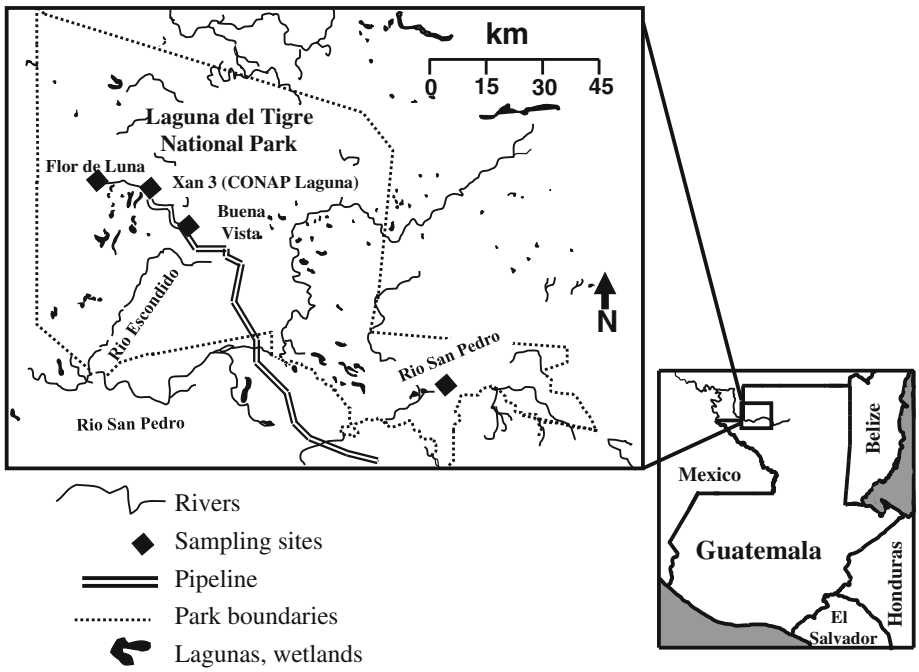

Fig. 1 Map of LTNP and sampling sites within

100–200 m from an injection well that injects oil field brines and other wastewater into the ground water. Sediment samples were collected by manually scooping from the upper 5 cm of sediment with a chemically clean glass jar. Water samples were collected by opening a chemically clean glass jar a few centimeter below the surface of the water. Adult cichlids were captured by cast net from each of the sites. Fish and water samples were collected from 1–31 May, 1999, which was during the dry season in Guatemala.

After capture, fish were then anesthetized in tricane methanosulfonate (MS 222; 0.5 g/l). Standard length (mm) was measured for each individual, and sex was determined from examination of the gonads. Blood was collected by caudal vein puncture or severing the dorsal aorta, diluted by at least 50% with freezing medium (10 mM Tris, 1 mM EDTA, 50 mM NaCl, 10% glycerol, 1% DMSO) and snap-frozen in liquid nitrogen.

DNA damage analysis

Flow cytometry

For flow cytometric analysis, nuclei were isolated from blood cells and stained with propidium iodide according to a modification of the methods of Vindeløv et al. (1983) and Vindeløv and Christensen (1990)as described in Theodorakis et al. (2000, 2001). Cells were suspended in citrate buffer and lysed with Nonidet NP40. Cytoplasm was then digested with trypsin and RNase A, and nuclei were stained with propidium iodine. An increase in the amount of chromosomal damage is reflected by an increase in cell-to-cell variation in DNA content (Bickham 1990). This variation was measured using an Epics Profile II Flow Cytometer (Coulter Corp., Hialeah, FL), which calculates the half-peak coefficient of variation (CV) in DNA content among cells in the G_1/G_0 phase of the cell cycle. Alignment, focus, and instrumental gain were set prior to analysis using 0.097 mm fluorescent microspheres (Coulter Corp.). For each experiment, all samples were run in 1 day in order to minimize the day-to-day variation in machine running conditions. Cells were gated using 90° side scatter and the identity of samples was unknown to the machine operator.

DNA strand breakage

For DNA extractions, 20 µl blood/freezing medium mixture (50/50) was suspended in 500 µl TEN (50 mM Tris, 10 mM EDTA, 100 mM NaCl, pH 8.0). DNA was then extracted and purified according to Theodorakis et al. (1994), and dissolved in TE (10 mM Tris, 1 mM EDTA, pH 8.0). Procedures for minimizing and controlling for shear of DNA during extraction from tissues are described in Theodorakis et al. (1999). DNA was quantified spectrophotometrically at 260 nm (1 AU = 50 µg DNA/mL).

The DNA was subjected to electrophoresis with alkaline or neutral running buffer (Theodorakis et al. 1992, 1994, 1997, 1999; Costa et al. 2002; Neuparth et al. 2009). Alkaline electrophoresis was performed with 30 mM NaOH and 2 mM EDTA (pH 12.5) as the running buffer. A total of 0.5 µg of DNA was loaded into each well of a 0.8%

agarose gel and subjected to electrophoresis at 5 V/cm for 5 h. The buffer was constantly recirculated and cooled in an ice bath. For neutral gel electrophoresis, TBE (45 mM Tris, 45 mM borate, 0.5 mM EDTA, pH 8.0) was used as the running buffer. A total of 0.05 μg DNA was loaded into a 0.3% agarose gel and subjected to electrophoresis at 0.75 V/cm for 18 h. Because such a low percentage gel is very fragile, the gels were cast on a basement of 3% agarose. After electrophoresis, gels were stained in ethidium bromide and photographed under UV light. The average molecular length (Ln) of the DNA in each sample was calculated as in Theodorakis et al. (1994, 1997, 1999).

The Ln calculated under alkaline conditions is affected by both single- and double-strand breaks, while the Ln of neutral gels is affected by double-strand breaks only. Because DNA is a long and fragile molecule, double-strand breaks can be caused not only by genotoxicant exposure, but also by physical shearing which may take place during extraction, purification, and analysis. In order to take this into account, the number of single-strand breaks (per 10^5 bases) was determined by subtracting the double-strand Ln from the single-strand Ln according to the following formula, as modified from Freeman and Thompson (1990):

$$\#\mathrm{SSB} = \left(\frac{1}{\mathrm{Ln\ (single)}} - \frac{1}{\mathrm{Ln\ (double)}}\right) \times 100$$

Because the electrophoresis was run at alkaline conditions, the number of single-strand breaks includes those that were present before alkaline treatment and those that were induced by alkaline-induced cleavage of alkaline-labile sites (Theodorakis et al. 1999).

Weight of evidence analysis

Differences between each site were summarized as a "+" (statistically significantly different from Xan) or a "−" (not statistically insignificantly different from Xan) for both species for both DNA damage variates. Any + was taken as evidence of a difference from Xan. If the number of +'s were greater than the number of −'s, this was taken as strong indication of a difference of the site in question from the Xan three oil facility. If the number of +'s was equal to the number of −'s, this was taken as a probable difference between the site and Xan. If the number of +'s was less than the number of −'s, this was taken as weak evidence of a possible difference from Xan. An absence of +'s was taken as an absence of evidence for a difference between Xan and the reference sites (the null hypothesis).

Statistical analysis

Because data were characterized by heteroscedacity and non-normality that could not be remedied using transformations, data were analysed using non-parametric analysis. The Kruskall–Wallis test with multiple comparisons was used for testing differences among sites. Multivariate MANOVA and multivariate t-tests were used to test for differences among groups using both variates combined, and discriminant analysis and cluster analysis (using Bray–Curtis similarity matrices and neighbor-joining clustering) were used to visualize similarity among sites. For multivariate analyses, data were rank-transformed, and the analyses were performed on the ranks. Fish included were those for which both flow cytometry and strand break analyses were performed. Correlation was performed to test the dependency of CV or SSB on (a) distance from the Xan injection well, (b) concentration of total PAHs in the sediment, and (c) concentration of total PAHs in the water. A significance level of 0.05 was assumed throughout.

Chemical residue analysis

Field samples were extracted in batches of no more that 18 samples. All laboratory batches included a procedural blank, matrix spike/matrix spike duplicate pair (or blank spike/blank spike duplicate pair if insufficient material is available), laboratory duplicate, and a standard reference material (sediment only, as one is not available for water). Surrogate compounds were added prior to extraction to account for extraction efficiencies. All extracts (field and laboratory QA) underwent a column cleanup. The column included silica gel/alumina/copper/sodium sulfate.

Sediment extractions

An automated extraction apparatus (Dionex ASE200 Accelerated Solvent Extractor) was used to extract various organics from 2 to 15 g of pre-dried samples (EPA Method 3545). The extractions were performed using methylene chloride solvent inside stainless-steel extraction cells held at elevated temperature and solvent pressure. The extracts dissolved in the solvent were then transferred from the heated extraction cells to glass collection vials containing activated copper granules to minimize matrix interference during quantitative determinations. Extracts were then concentrated to a final volume of 1-mL using an evaporative solvent reduction apparatus (Zymark TurboVap II). Final extracts were submitted for determination of aromatic hydrocarbons.

Water extractions

Water samples were serially extracted with methylene chloride in separatory funnels and the extracts were concentrated to a final volume of 1 mL (EPA Method 3510). A cleanup step was provided, for use before instrument

analysis, to remove possible matrix interference. The protocol was designed for 1-l water samples, but water samples of other sizes may be collected and extracted by appropriately adjusting the volume of methylene chloride used during the extraction process. The extracts were analysed by capillary gas chromatography/mass spectrometry (GC/MS) for quantification of PAHs.

GC/MS analysis

Quantitation of PAHs and their alkylated homologues in extracts of water, sediment, and biological tissue was performed by GC/MS in selected ion monitoring mode (SIM) (Modified EPA Method 8270C). The gas chromatograph was temperature-programmed and operated in splitless mode. The capillary column was a J&W Scientific DB-5MS® (60 m long by 0.25 mm ID and 0.25 μm film thickness) or equivalent. Carrier flow was controlled by electronic pressure control. The autosampler was capable of making 1 to 5 μl injections. The mass spectrometer was capable of scanning from 35 to 500 AMU every second or less, utilizing 70 eV energy in electron impact ionization mode. The data acquisition system allows continuous acquisition and storage of all data during analysis and capable of displaying ion abundance versus time or scan number.

Calibration solutions were prepared at five concentrations ranging from 0.02 to 1 μg/mL by diluting a commercially available solution containing the analytes of interest (typically NIST SRM 1491). For each analyte of interest, a relative response factor (RRF) was determined for each calibration level. All five response factors were then averaged to produce a mean RRF for each analyte.

All calibration and matrix spike standards were purchased directly from the National Institute of Standards and Technology (NIST). The deuterated internal and surrogate standards were purchased from Cambridge Isotope (Cambridge MA).

Environmental forensic analysis

Several metrics were used to draw inferences about the origin (pyrogenic, petrogenic, diagenic) of the PAHs in the sediment. There are a number of indices that have been developed to distinguish petrogenic from pyrogenic PAHs. These ratios were calculated from the PAH concentrations measured in the sediments at each of the sites. A conclusion was drawn from each index (petrogenic vs. pyrogenic), and a weight of evidence approach was used (considering all indices) to infer the relative contribution of petrogenic and pyrogenic PAHs at each site. Finally, perylenes can be formed from both anthropogenic and diagenic (originating from natural processes in the sediment) sources (Colombo et al. 2006, and refs. therein). Thus, if the ratio of the [perylenes]/[total non-alkylated PAHs] > 10%, this is generally used to indicate a diagenic origin for perylenes.

In addition, there are certain other indicators that were used to infer if the PAHs originated from pyrogenic or petrogenic sources. Wang and Brown (2008) report that PAHs from pyrogenic sources tend to have more high molecular weight (>4 ring) constituents than petrogenic sources, so a higher concentration of these compounds was taken to indicate a greater contribution of pyrogenic sources to the overall PAH pool. Also, Wang et al. (1999) have developed a "pyrogenic index" as $\sum$(all other 3–6 ring PAHs)/$\sum$(alkylated [napthalenes, phenanthrenes, dibenzothiophenes, fluorenes, and chrysenes]). A higher value indicates a greater contribution of pyrogenic relative to petrogenic PAHs (Wang et al. 1999). Garrigues et al. (1995) have suggested that examination of the relative amount of phenanthrene and chrysene was an effective indicator of pyrogenic versus petrogenic PAH sources. Therefore, the ratio of chrysene to phenanthrene was measured. A higher value was used to infer a greater contribution of PAHs from pyrogenic sources to the total PAH pool. Cluster analysis was also performed using all three indices [(1) concentration of PAHs with four or more rings, (2) pyrogenic index (3) chrysene/phenanthrene] as an overall indication of the similarly among sites with regards to relative contribution of pyrogenic and petrogenic sources of PAHs.

Results

The standard lengths and numbers of males and females for *T. meeki* and *V. synspila* collected at each site are reported in Table 1. None of the other fish collected from any other site showed similar indications of fin erosion. For both measures of DNA damage, there was no correlation between length and DNA damage ($p > 0.05$) for either species. There was also no difference between males and females ($p > 0.05$, Mann–Whitney *U*-test) for either DNA damage metric for both species, so values from males and females were combined.

DNA damage

Flow cytometry

For *T. meeki*, at all three sites the CV in DNA content among cells (a reflection of the amount of chromosomal damage) was less than at the Xan facility (Fig. 2a; $p < 0.05$, Kruskall–Wallis test, comparing each site vs. Xan). For *V. synspila*, the CV was lower in Rio San Pedro

Table 1 GPS coordinates for each site and mean (±SD) length (mm) and number of males and females for two cichlid species collected from LTNP, Guatemala

Sampling site	Coordinates	Distance (km) from Xan injection well	*Thorichthys meeki*		*Vieja synspila*	
			Length	Number of males/ number of females	Length	Number of males/ number of females
Laguna Flor de Luna	17° 36.00′ N 90° 53.80′ W	15.9	54.7 (±9.2)	7/7	NA	NA
Rio San Pedro	17° 14.76′ N 90° 15.49′ W	72.7	67.5 (±20.8)	6/5	56.6 (±7.5)	4/8
Buena Vista (a.k.a. Xan 2 Laguna)	17°30.78′ N 90° 45.09′ W	21.5	67.7 (±9)	5/7	87.6 (±3.6)	4/2
Xan 3 (CONAP) Laguna	17° 26.74′ N 90° 39.98′ W	0.2	91.7 (±22.3)	4/6	120 (±14.7)	2/3

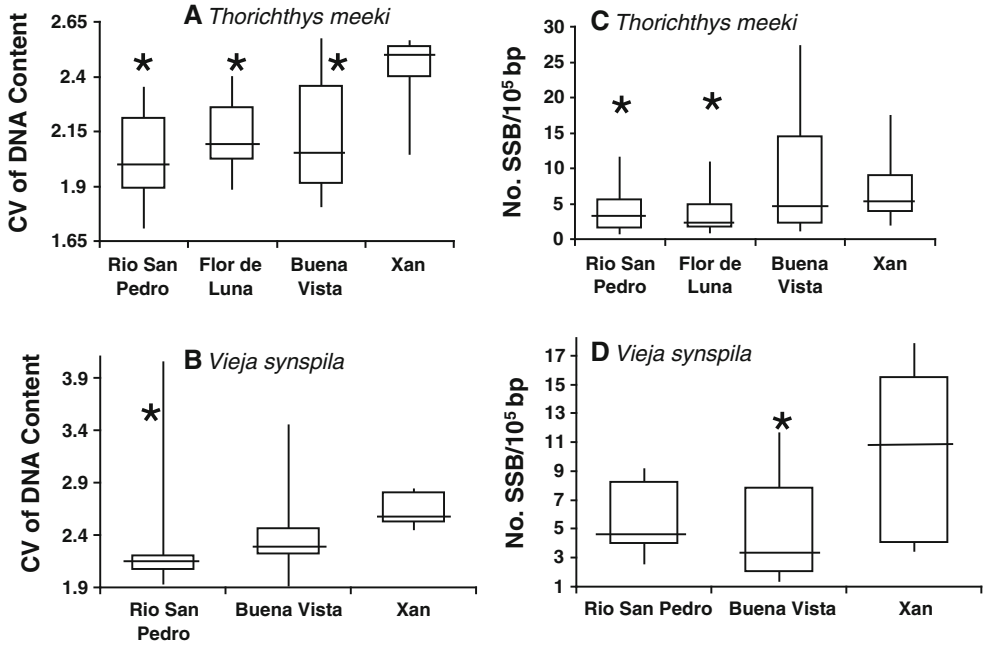

Fig. 2 Amount of chromosomal damage as reflected by flow cytometric determination of cell-to-cell variation in DNA content (**a**, **b**) and amount of single-strand breaks (**c**, **d**) in fish from LTNP For *T. meeki* (**a**, **c**), sample sizes are as follows: Laguna Flor de Luna—20, Laguna Buena Vista—14; San Pedro—18; Xan—12. For *V. synspila* (**b**, **d**), sample sizes are as follows: Laguna Buena Vista—8; San Pedro—14; Xan—6. *Lines* passing though the rectangles are medians, the upper and lower bounds of the rectangles are third and first quartiles, respectively, and *error bars* represent the range (highest and lowest values). *Site statistically different from Xan ($P \leq 0.05$, Kruskall–Wallis test)

than in fish from the Xan facility (Fig. 2b; $p < 0.05$, Kruskall–Wallis test, comparing each site vs. Xan). The amount of chromosomal damage in fish from Laguna Buena Vista was not statistically different from Xan.

DNA strand breakage

For *T. meeki*, at Rio San Pedro and Laguna Flor de Luna there were fewer single-strand breaks than at the Xan facility (Fig. 2c; $p < 0.05$, Kruskall–Wallis test, comparing each site vs. Xan). The samples from Laguna Buena Vista were highly variable and this variation could be due either to analytical problems or the result of real biological effects. No reasons for analytical problems were observed and indeed the samples used were the same as for flow cytometery which did not show unusually high variability for this population. We thus conclude the variation in these samples is a true characteristic of the population. For *V. synspila*, the number of DNA single-strand breaks in fish from the Xan facility was greater than that in fish from

Table 2 Correlation coefficient (R) and *p*-values[a] for correlation of DNA damage versus distance[b] or total PAHs[c] for two species of cichlid fish

Species	Matrix	Statistic	DNA damage metric	
			CV[d]	SSB[e]
Distance vs. DNA damage				
T. meeki	–	R	−0.339	−0.114
	–	*p*-Value	*0.022*	*0.445*
V. synspila	–	R	−0.516	−0.294
	–	*p*-Value	*0.006*	*0.174*
Total PAHs vs. DNA damage				
T. meeki	Sediment	R	0.601	0.294
		p-Value	*0.001*	*0.045*
	Water	R	0.459	0.106
		p-Value	*0.021*	*0.571*
V. synspila	Sediment	R	0.436	−0.399
		p-Value	*0.015*	*0.059*
	Water	R	0.289	0.397
		p-Value	*0.180*	*0.061*

[a] *Ho* amount of DNA damage is independent of distance from Xan 3 oil brine injection well

[b] Distance (km) from the site where the fish were collected to the Xan 3 oil brine injection well

[c] Measure in water samples

[d] Correlation between distance/PAHs and half-peak CV of DNA content in fish blood cells. CV is positively correlated to the amount of clastogenic effects

[e] Correlation between distance/PAHs and Single-Strand Breaks (SSB) in DNA from blood. Ln is negatively correlated to the amount of single-strand breaks

Laguna Buena Vista (Fig. 2d; $p < 0.05$, Kruskall–Wallis test, comparing each site vs. Xan). The amount of single-strand breakage in fish from Rio San Pedro was not statistically different from Xan.

Correlations

There were weak correlations between the DNA damage metrics and distance from the Xan injection well, concentrations of total PAHs in the sediment, and/or concentrations of PAHs in the water. For the flow cytometric data, there were statistically significant ($p < 0.05$) correlations between CV and distance for both species of fish, between CV and total PAHs in both water and sediment in *T. meeki*, and between CV and sediment PAHs in *V. synspila* (Table 2). For strand breaks, there was a statistically significant ($p < 0.05$) correlation between SSBs and sediment PAHs for *T. meeki*, and marginally non-significant ($p \leq 0.061$) correlations between SSBs and both sediment and water PAHs for *V. synspila* (Table 2).

Multivariate analysis

For *T. meeki*, Rio San Pedro clustered with Laguna Flor de Luna, while Laguna Buena Vista clustered with Xan (Fig. 3a). For *V. synspila*, Rio San Pedro clustered with Laguna Buena Vista (Fig. 3d). The discriminant analysis indicated that, for *T. meeki*, Rio San Pedro was least similar to Xan, and Laguna Flor de Luna and Laguna Buena Vista were equally similar to Xan (Fig. 3b, c). For *V. synspila*, Rio San Pedro and Laguna Buena Vista were equally similar to Xan (Fig. 3e, f). The MANOVA analysis indicated that (a) all sites were significantly statistically different from Xan 3 for both species (b) Laguna Buena Vista was different from Laguna Flor de Luna and Rio San Pedro for *T. m*eeki, and (c) all sites were different for *V. synspila* ($p < 0.05$; Fig. 3b, e). The results from the discriminant function analysis (Table 3) indicated that the Wilk's lambda was lower for discriminant function 1 than discriminant function 2 for both species. Because a lower lambda indicates more discriminating power, these data indicate that the data were discriminated better by function 1. This is reflected by the fact that discriminant function 1 explains more of the variance than function 2 (Table 3). The results also indicate that the flow cytometry results (CV in DNA content) has a higher standardized discriminant function score (a higher contribution) than the strand breakage for discriminant function 1 (Table 3). Taken together, these results indicate that the CV in DNA content was better able to detect differences among sites than the strand breakage.

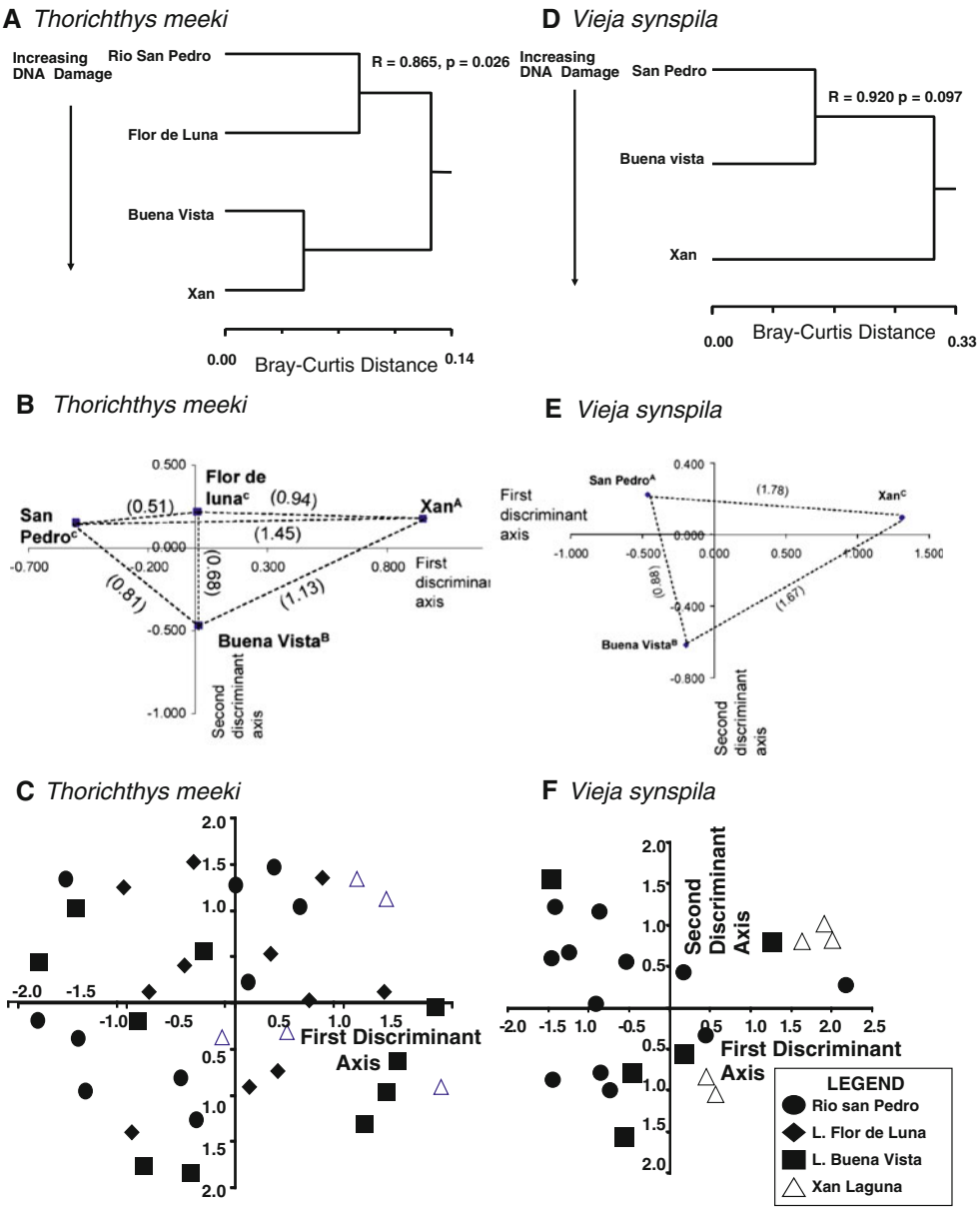

Fig. 3 Multivariate analyses for DNA damage (CV of DNA content and single-strand breaks [SSB]) in cichlid fish collected from four sites in LTNP, Guatemala. Data from neighbor-joining dendrogram (**a**, **d**) are produced by multivariate clustering analysis (using a Bray–Curtis similarity matrix) using DNA damage and chromosomal damage as the variates in **a** *T. meeki* and **d** *V. synspila*. *R* correlation coefficient and *p* significance level for cophenetic correlation (a test of goodness of fit of the data to the dendrogram). Data in **b**, **c**, **e**, and **f**) are from discriminant analysis for **b** and **c** *T. meeki* and **e** and **f** are from *V. synspila*. **b**, **e** Represent group centroids, **c** and **f** are data points for individual fish. Sites labeled with the same superscripts in **b** and **e** are not statistically significantly different ($p < 0.05$, MANOVA)

Weight of evidence

The weight of evidence analysis is presented in Table 4. The Xan 3 Laguna differed by weight of evidence from both Laguna Flor de Luna (two positive results) and Rio San Pedro (three positive and one negative result). The evidence for a difference between Laguna Buena Vista and Xan is inconclusive with two positive results and two negative results (Table 4).

Fin erosion

All individuals of both species collected from the Xan 3 laguna showed symptoms of fin erosion (Fig. 4). No individuals of either species showed indications of fin erosion from any other site.

Analytical chemistry

PAH concentrations

The total PAH sediment concentrations for Rio San Pedro, Laguna Flor de Luna, Laguna Buena Vista and Xan oil facility were, respectively 591.6, 200.3, 1176.9, and 220 ng PAH/g dry sediment. The total water PAH concentrations for Rio San Pedro, Laguna Buena Vista and Xan were 7.5, 20.3, and 38.4 ng/l, respectively. The concentrations of individual PAH isomers in sediments and

Table 3 Results of discriminant function analysis using two measures of DNA damage (CV of DNA content and number of single-strand breaks) in the cichlids *Thorichthes meeki* and *V. synspila* collected from four lagunas in and around LTNP, Guatemala

Discriminant analysis statistic	DNA damage metric	*Thorichthys meeki*		*Vieja synspila*	
		Discriminant function 1	Discriminant function 2	Discriminant function 1	Discriminant function 2
Eigenvalue	–	0.219	0.096	0.617	0.077
% of variation	–	69.453	30.547	88.844	11.156
Wilks' lambda	–	0.748	0.912	0.574	0.928
Standardized discriminant	CV of DNA content	0.965	0.263	0.929	−0.370
Function coefficient	Strand breaks	−0.286	0.959	0.350	0.937

Table 4 Weight of evidence (WoE) analysis for determining if the reference sites are different from the Xan oil production facility in terms of overall genotoxicity responses for two species of cichlid fish

Reference site	Different from Xan?[a]				
	Thorichthes meeki		*Vieja synspilm*		
	CV	SSB	CV	SSB	WoE[b]
Rio San Pedro	+	+	+	–	Yes
Laguna Flor de Luna	+	+	NA	NA	Yes
Laguna Buena Vista	+	–	–	+	Inconclusive

[a] + response variable in Xan greater than in reference site; − response variable not different between Xan and reference site; *NA* not applicable due to lack of data

[b] Overall conclusion as to whether or not the reference site is different from Xan based upon weight of evidence

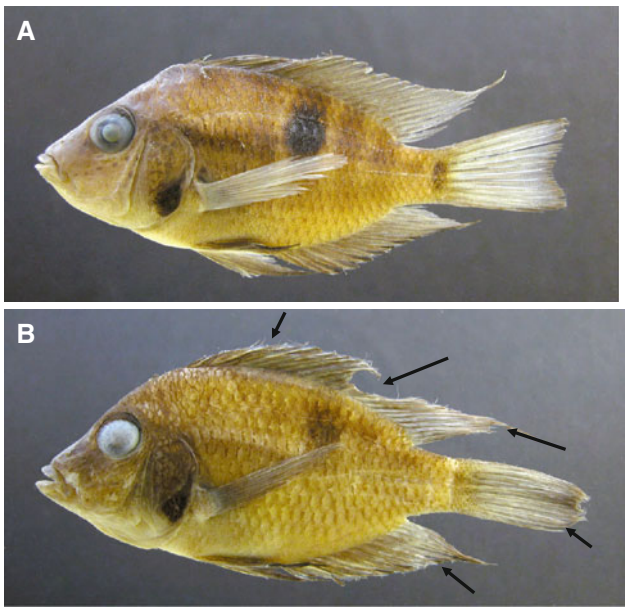

Fig. 4 Photographs illustrating an example of fin erosion in *T. meeki*. **a** An individual from a reference site (Laguna Flor de Luna). **b** An individual from a site near an oil brine injection well (Xan 3 Laguna). *Arrows* indicate representative areas of fin erosion

water are listed in Table 5. Limit of detection for PAHs in sediment ranged from to 0.10 to 0.41 ng/g; minimum detection limits for PAHs in water ranged from to 0.6 to 6.6 ng/l.

Environmental forensic analysis

Overall, the ratio indices reported in Table 6 suggest that all sites have a mixture of petrogenic and pyrogenic PAHs in the sediments. However, the sediment PAHs at Rio San Pedro seem to be primarily of pyrogenic and diagenic origin, and the pyrogenic PAHs seem to be higher in the sediments of Laguna Buena Vista than in Xan or Laguna Flor de Luna (Table 6). The concentrations of PAHs with ≥4 rings (an indication of pyrogenic PAHs) are highest in Rio San Pedro, followed by Laguna Buena Vista (Fig. 5a). The pyrogenic index was higher in Rio San Pedro than in all other sites (Fig. 5b). The ratio of chrysene/phenanthrene (an indication of pyrogenic PAHs) is greatest in Rio San Pedro, followed by Laguna Buena Vista, Laguna Flor de Luna, and finally Xan. Cluster analysis indicated Rio San Pedro clustered with Laguna Buena Vista, Xan clustered with Laguna Flor de Luna. The data

Table 5 Concentrations[a] of selected PAHs in sediment and water samples collected from LTNP and adjacent waterways[b]

	Rio San Pedro		Laguna Flor de Luna		Laguna Buena Vista		Xan 3		ERL[c]	ERM[c]
	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water		
Naphthalene	56.0	10.7	2.8	–	15.4	6.4	6.1	18.7		
C1–C4 Naphthalenes	145.6	19.2	48.2	–	255.1	8.0	54.3	12.8		
Fluorene	17.0	1.0	1.8	–	29.2	0.3	5.5	ND	19	540
C1–C3 Fluorenes	103.8	ND	29.9	–	281.1	ND	51.8	ND		
Phenanthrene	23.7	0.4	12.2	–	69.3	0.5	15.4	0.5	240	1,500
Anthracene	4.6	2.5	0.5	–	5.8	3.0	1.4	2.3	85	1,100
C4-Phenanthrenes/anthracenes	63.4	ND	54.3	–	215.1	ND	41.0	ND		
Dibenzothiophene	5.5	0.7	1.7	–	12.8	0.7	1.1	0.7		
C1–C3 Dibenzothiophenes	15.5	ND	17.5	–	125.9	ND	15.9	ND		
Fluoranthene	10.0	1.2	2.3	–	17.6	0.6	1.8	1.0	600	5,100
Pyrene	8.4	0.8	2.0	–	13.5	0.8	1.5	0.8	670	2,600
C1-Fluoranthenes/pyrenes	6.0	ND	0.2	–	4.2	ND	1.5	ND		
Benz(a)anthracene	3.3	1.2	0.2	–	1.8	ND	0.3	ND	261	1,600
Chrysene	3.1	0.6	0.3	–	2.1	ND	0.3	ND	384	2,800
C1–C4 Chrysenes	48.9	ND	21.8	–	114.1	ND	19.3	ND		
Benzo(b)fluoranthene	2.8	1.0	0.7	–	1.8	ND	0.6	ND		
Benzo(k)fluoranthene	2.7	0.3	0.8	–	2.1	ND	0.2	ND		
Benzo(e)pyrene	1.3	1.1	0.4	–	1.6	ND	0.4	0.3		
Benzo(a)pyrene	1.5	0.5	0.7	–	0.8	ND	0.3	0.2	430	1,600
Perylene	64.2	0.8	0.3	–	4.4	ND	0.3	0.5		
Indeno(1,2,3-c,d)pyrene	1.7	ND	0.7	–	1.5	ND	0.5	0.3		
Dibenzo(a,h)anthracene	0.4	ND	0.5	–	0.6	ND	0.3	ND	63	260
Benzo(g,h,i)perylene	2.2	ND	0.5	–	1.1	ND	0.2	0.3		
Total PAHs	591.6	7.5	200.3	–	1176.9	20.3	220	38.4		

[a] Sediment concentrations are in ng/g, dry weight; water concentrations are ng/l. Values are means ($n = 3$)

[b] Water samples from Laguna Flor de Luna were not available for analysis. Values are means ($n = 3$)

[c] *ERL*, sediment quality guideline of effects range low; *ERM*, sediment quality guideline of effects range median according to Long et al. (1995)

presented in Fig. 5 suggest that (1) pyrogenic PAHs predominate in Rio San Pedro, (2) petrogenic PAHs are higher in the Xan sediments (relative to other sites), and (3) the relative ratio of pyrogenic/petrogenic PAHs decreases in the order of Rio San Pedro > Laguna Buena Vista > Laguna Flor de Luna > Xan 3 Laguna.

Discussion

DNA damage

The data from the DNA strand break and chromosomal damage analyses indicate that these fish most likely are being exposed to one or more DNA-damaging agents. The Xan laguna sampled in this study was located near an injection well that pumped oil field-produced brines into the groundwater. The data from the univariate and multivariate analyses indicate that the amount of DNA damage in *T. meeki* increases in the order of San Pedro < Laguna Flor de Luna < Laguna Buena Vista < Xan (Fig. 3a). For *V. synspila*, the trend was San Pedro < Laguna Buena Vista < Xan (Fig. 3b). The fact that Laguna Buena Vista was most similar to Xan is in accordance with the fact that Laguna Buena Vista was the site that was closest in proximity to Xan. Laguna Buena Vista was also in close proximity to the oil pipeline leading from the Xan facility (Fig. 1). It would be expected that Laguna Flor de Luna shows less of an effect than Laguna Buena Vista because (1) it is upstream from Xan and thus less likely to receive contaminants during the rainy season (when the lagunas are connected), and (2) it is not in close proximity to the oil pipeline. The finding that San Pedro was the least similar to Xan is in concordance with the fact that it is the furthest away from Xan (Fig. 1). In addition, correlations between DNA damage and distance from the Xan injection well and/or water/sediment PAH concentrations provides further evidence of causality that the PAHs contribute to

Table 6 Ratio[a] of selected PAHs diagnostic of pyrogenic, petrogenic, or diagenic sources[b], and the overall conclusion as to the source

Metric	Site				Diagnostic cutoff
	Rio San Pedro	Laguna Flor de Luna	Laguna Buena Vista	Xan 3	
Anthrracene/(anthracene + phenanthrene)	0.16 (pyrogenic)	0.04 (petrogenic)	0.08 (petrogenic)	0.08 (petrogenic)	<0.1 petrogenic, ≥0.1 pyrogenic[c]
(C1–C4 [anthracene & phenanthrene])/ (anthracene + phenanthrene)	2.2 (pyrogenic)	4.3 (pyrogenic)	2.9 (pyrogenic)	2.4 (pyrogenic)	<1 petrogeneic, >2 pyrogenic
Fluoranthene/(fluoranthene + pyrene)	0.54 (pyrogenic)	0.53 (pyrogenic)	0.57 (pyrogenic)	0.55 (pyrogenic)	<0.4 petrogenic, 0.4–0.5 mixture, >0.5 pyrogenic[c]
(Fluoranthene + pyrene)/ (fluoranthene + pyrene + C1–C4 [anthracene & phenanthrene])	0.22 (mixture)	0.07 (petrogenic)	0.13 (mixture)	0.07 (petrogenic)	<0.1 petrogenic, 0.1–0.75 mixture, >0.75 pyrogenic
Chrysene/benzo[a]anthrcene	0.9 (pyrogenic)	1.5 (petrogenic)	1.1 (petrogenic)	1.0 (petrogenic)	<1 pyrogenic, ≥1 petrogenic[d]
Indeno(1,2,3-c,d)pyrene/Benzo(g,h,i)perylene	0. 75 (petrogenic)	1.4 (petrogenic)	1.4 (petrogenic)	2.5 (petrogenic)	<0.5 pyrogenic, >0.5 petrogenic[e]
% perylene of total unsubstituted PAHs[f]	45 (diagenic)	1.1 (anthropogenic)	2.4 (anthropogenic)	0.84 (anthropogenic)	<10% anthropogenic, >10% diagenic[g]
Weight of-evidence conclusion[h]	Primarily pyrogenic and diagenic	Mixture of pyrogenic and petrogenic	Mixture, pyrogenic elevated[i]	Mixture of pyrogenic and petrogenic	

[a] Values are the ratio followed by diagnosis as to whether the PAHs are of petrogenic or pyrogenic origin based upon the diagnostic cutoff. For example "antrhacene/(anthracene + phenanthrene)" is the concentration of anthtracene divided by the concentration of both anthracene and phenanthrene and "(petrogenic)" means that the value of this ratio is indicative of a petrogenic source of PAHs

[b] *Diagenic* originating from naturally occurring processes in the sediment

[c] According to Pies et al. (2008), and refs. therein. E.g., "<0.1 petrogenic, ≥ 0.1 pyrogenic[c]" means that a ratio less than 0.1 is indicative of PAHs that are petrogenic in origin while a ratio greater than or equal to 0.1 is indicative of PAHs that are pyrogenic in origin

[d] According to Scolo et al. (2000), and refs. therein

[e] According to Qiao et al. (2006) and refs. therein

[f] (Amount of perylene/Σ[all unsubstituted PAHs]) × 100. "*anthro.*" anthropogenic source (vs. naturally-derived or diagenic sources). A percentage of less than 10% is indicative of an anthropogenic source of perylene while a percentage of greater than 10% is indicative of a diagenic origin of perylene

[g] According to Colombo et al. (2006), and refs. therein

[h] Conclusion as to the source of the PAHs in the sediment based upon all available evidence

[i] Relative to Xan and Laguna Flor de Luna

genotoxicity in these species. Finally, the finding of evidence for DNA damage in Xan is in accordance with previous studies showing oil field waters to exhibit genotoxic potential (Odeigah et al. 1997; Li et al. 2008, 2010; Xu et al. 2010), and that exposure to PAHs can lead to DNA strand breakage and chromosomal aberrations (Taban et al. 2004; Bihari and Fafanel 2004; Baršienė et al. 2006b; Neuparth et al. 2009).

Both SSBs and CV are well-used biomarkers of genotoxicity (Shugart et al. 1992). One of the advantages of the use of biomarker expression in biomonitoring programs is that they can be used to screen for the effects of multiple contaminants (Shugart et al. 1992). Using biomarkers as a preliminary screen may result in cost reduction of environmental monitoring because performing multiple chemical analyses on a large number of sites with unknown and potentially complex mixtures of contaminants is costly. An alternative would be to screen for possible effects using a small number of biomarkers that respond to a wide array of contaminants in order to prioritize efforts for more intensive chemical analyses. Such biomarkers are also useful because they are able to integrate effects of exposure to complex mixtures of chemicals. In this study, the types of possible contaminants at the Xan facility were unknown, but it was suspected that PAHs may be a problem. Subsequent chemical analysis indicated that PAH levels were higher in Xan water compared to the reference sites, but not in the sediment. Nonetheless, total PAH levels were very low overall in both water and sediment and it is unlikely that PAHs alone caused the observed biomarker effects. However, the fish also may have been exposed to other genotoxins. Had the environmental monitoring simply focused on PAH analysis, evidence of environmental contamination may have been overlooked because of the low levels of PAH contamination.

Although there is evidence for increased DNA damage in both fish species from the Xan laguna, there are differences between them that could stem from a number of causes. First, there could be differential exposure to different genotoxic compounds, as a result of differences in habitat or food preferences. Unfortunately, there is very little information of this type for Neotropical cichlids. Also, there could be differences in the molecular physiology between the two species. Again, such information is scant. It is clear that more research is needed on natural history and physiology before definitive conclusions can be drawn as to differences between these two species. Additionally, the dissimilarity in genotoxic responses between species could be mediated by differences between the flow cytometry and DNA damage assays. This could be related to differences in sensitivities of the assays or the type of damage that they measure. The electrophoretic assay measures single-strand DNA breaks, which could be due to direct nicking of the sugar-phosphate backbone or to conversion of certain DNA base modifications (also indicative of contaminant exposure) to single-strand breaks in vitro at alkaline pH (so-called "alkaline-labile sites"). The flow cytometric assay, on the other hand, measures chromosomal damage, a result of double-strand breaks. Chromosomal breaks are not repaired (Ward 1988), so they may be a less transient effect than single-stand breaks or alkaline-labile sites (i.e., they only disappear when the cell dies). This is reflected by the fact that CV in DNA content contributed more to differences among sites than did strand breakage (Table 3).

Fin erosion

The findings of genotoxic effects in the fish from the Xan laguna is in accordance with the observation that many of these fish—but no fish from any other site—showed signs of fin erosion. Fin erosion was apparent, to a greater or lesser degree, in all fish from the Xan laguna and none of the other sites. Correspondence between two or more endpoints provides stronger evidence of environmental contamination than does one endpoint alone. This is especially true if there is concordance between effects at different levels of biological organization, in this case the effects are at both the molecular (DNA damage) and at the organismal (fin erosion) level. Previous studies have used multiple endpoints at various levels of biological organization to demonstrate that effects on fish are due to anthropogenic, rather than natural variables (Theodorakis et al. 1992).

The primary cause of fin erosion is bacterial infection. It is usually not seen in wild fish populations except under stressful conditions. It has been well documented that exposure to petroleum hydrocarbons and other pollutants can increase the occurrence of bacterial and parasitic infections by suppressing the immune response in fish (Arkoosh et al. 1998; Landsberg et al. 1998).

Analytical chemistry and environmental forensics

An unexpected finding was that the amount of PAHs in the sediment was much higher in Laguna Buena Vista than in any other site. This may explain the elevated amounts of DNA strand breakage and chromosomal damage in the fish from this site. There were also relatively high (mg/kg) levels of PAHs in the sediments of the other sites. However, there may be other sources of PAHs in Laguna del Tigre than petrochemical emissions. The data from Table 6 and Fig. 5 suggest that pyrogenic, and, in Rio San Pedro, diagenic sources of PAHs are also significant. In general, univariate and multivariate analyses indicate that the ratios of pyrogenic/petrogenic PAHs decrease in the order of San

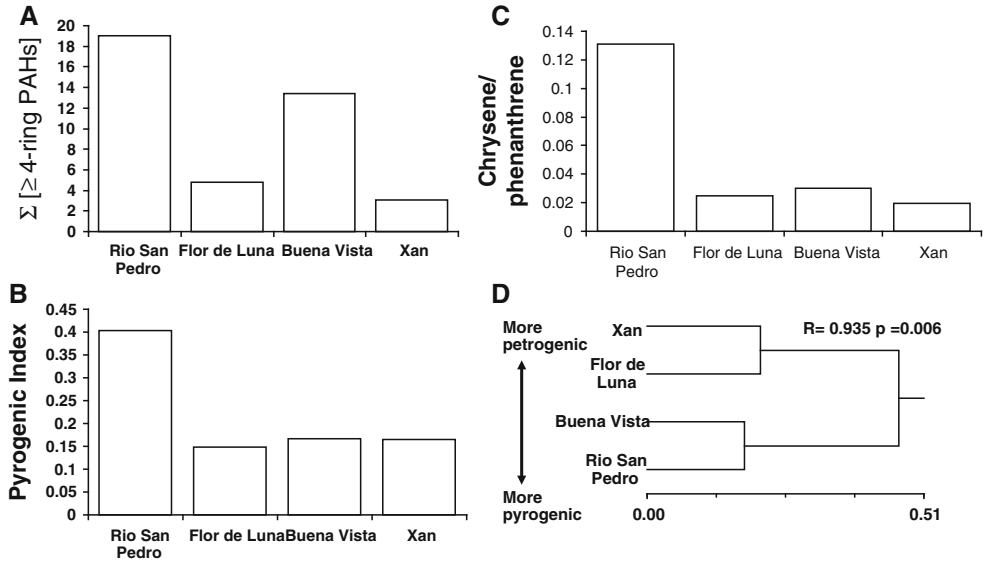

Fig. 5 **a** Concentration (mg/kg) of PAHs with four or more rings, **b** "pyrogenic index" defined by Wang et al. (1999), and **c** ratio of chrysene/phenanthrene in sediments collected from three lagunas and Rio San Pedro in LTNP, Guatemala. **d** Neighbor-joining dendrogram produced by multivariate clustering analysis (using a Bray–Curtis similarity matrix) using the three variates in **a**–**c**. *R* correlation coefficient and *p* significance level for cophenetic correlation (a test of goodness of fit of the data to the dendrogram)

Pedro > Laguna Buena Vista > Laguna Flor de Luna > Xan 3. For example, wildfires are very common in Laguna del Tigre NP, and most originate by arson (NASA 2008). Two of the authors (C.W.T and P.W.W.) personally witnessed burned-over forests and an active wildfire along the shores of Rio San Pedro during the sampling period.

The elevated PAHs seen at Rio San Pedro and Laguna Buena Vista are not reflected in an increased level of DNA damage. However, the environmental forensic analysis indicated that pyrogenic sources may contribute more to the overall PAH load in these sites, especially in Rio San Pedro. Hylland (2006) reported that PAHs from pyrogenic sources are less bioavailable than PAHs from petrogenic sources. Thus, differences among sites in terms of bioavailability cannot be ruled out as a contributing factor in the patterns of DNA damage seen here.

Nonetheless, sediment and water concentrations at Guatemala sites, even with the highest levels of PAHs, do not appear to have much contamination. With the exception of fluorine, sediment concentrations do not exceed the Effects Range Low concentrations (Table 5; Long et al. 1995). In comparison to other published sources, the concentrations of total PAHs in this study are within the range reported at freshwater sites throughout the world (Table 7). In general, the concentrations reported here are at the low end of the distribution in Table 7, and are below most published accounts of areas with "high levels" of contamination (see Table 7 for references). Similar to results in the present study of concentrations of PAHs in the water, previous studies have found that the ranges of PAHs in the water are 1–2 orders of magnitude below sediment concentrations. Again, the concentrations of PAHs reported here are at the low end of concentrations reported at other sites (Table 7). This may partially explain why the levels of DNA damage in LTNP cichlids do not dramatically differ among sites.

Conclusions

The amount of DNA damage in fish from the Xan facility was greater than that in fish from Rio San Pedro, the site farthest from the Xan facility, or in Laguna Flor de Luna, the site upstream from the Xan facility in the rainy season. The amount of DNA damage in fish from Laguna Buena Vista was intermediate between Xan and Rio San Pedro. Total PAH levels in sediment and water were low, but trends in DNA damage in fish among sites were similar to that for PAH concentrations in the water. Although the causative agent(s) of DNA damage and fin rot was not identified in this study, the Oil Production facility at Xan appears to be the most likely source. The concordance of fin erosion and elevated DNA damage in fish from the laguna adjacent to the Xan injection well suggests that these fish are being stressed by anthropogenic chemicals, and it is possible that oil field brines injected into ground water may produce immunosuppressive and genotoxic effects in fish in nearby surface waters.

The results from this study make significant contributions to the field of ecotoxicology for a couple of reasons.

Table 7 Examples of the range of concentrations of total PAHs in rivers and lakes from various countries

Location	Range	References
Sediment[a]		
Dalio River Watershed, China	62–841	Guo et al. (2007)
Gao-Ping River, China	8–356	Doong and Lin (2004)
Aojiang River, China	404–2,605	Li et al. (2010)
Zhejiang Province, China	91–1,835	Zhu et al. (2008)
Taihu Lake, China	290–1,500	Qiao et al. (2006)
Lake Clark, USA	1,291–6,271	Ko et al. (2007)
Lake Aldred, USA	7–3,747	Ko et al. (2007)
Upper Conowingo Reservior, USA	3,743–18,073	Ko et al. (2007)
La Plata River, Argentina	3–2,100	Colombo et al. (2006)
Biobio River, Chile	15–276	Barra et al. (2009)
Laja Lake, Chile	226–620	Quiroz et al. (2005)
Jialu River, China	466–2,605	Fu et al. (2011)
Laguna del Tigre National Park, Guatemala	200–1,177	This study
Surface water[b]		
Dalio River Watershed, China	95–13,448	Guo et al. (2007)
Gao-Ping River, China	23–45,100	Doong and Lin (2004)
Aojiang River, China	910–1,520	Li et al. (2010)
Zhejiang Province, China	70–1,844	Zhu et al. (2008)
Mississippi River, USA	63–145	Zhang et al. (2007)
York River, USA	2–123	Countway et al.(2003)
Surface Waters in Hangzhou, China	989–9,663	Chen et al. (2004)
Laguna del Tigre National Park, Guatemala	8–38	This study

[a] Concentration in ng/g

[b] Concentration in ng/l

First, the data emphasize the importance of coupling biomarker measurements with environmental monitoring. The results of biomarker monitoring indicate an acute toxic effect (fin erosion) and DNA damage in Xan fish. Second, there are relatively few toxicological studies on natural populations of fish in Neotropical waters. Research examining DNA damage or other biomarker responses in Neotropical fish is lacking, and to our knowledge this is the only such study on Central American fish.

Acknowledgments This article is dedicated to the memory of K.C. Donnelly, an exceptional friend, excellent colleague, and outstanding scientist. The authors would like to thank Barry Chernoff (Department of Biology, Wesleyan University, Middletown, CT, USA), Herman Kinh (Unidad de Investigación, Vida Silvestre, Guatemala City, Guatemala) and Christian Barrientos (CI-ProPetén, Ciudad Flores, Guatemala) for their assistance in field collections; Greg Love and Leeanne Alonso (Conservation International, Washington, DC, USA) for technical and logistical assistance; and the Las Guacamayas Biological Field Research Station, Petén, Guatemala, for use of facilities. This research was conducted in collaboration with ProPetén, Flores, Guatemala, and was funded in part by Conservation International, Washington, DC, and by NIEHS grant number ES04917. This study was presented in part at the 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry, 12–16 November 2000, Nashville, TN, and is publication No. 102 of the Center for Biosystematics and Biodiversity, Texas A&M University. The intent of this article is purely for dissemination of scientific knowledge, and is neither endorsement nor condemnation of the activities of any corporation, their employees or subsidiaries, nor to imply liability on their part.

References

Agostinho AA, Thomaz SM, Gomes LC (2005) Conservation of the biodiversity of Brazil's inland waters. Conserv Biol 19:646–652

Alonso LE, Bailey AC, Barrientos C, Bestelmeyer BT, Callisto M, Castañeda Moya F, Castillo Villeda ML, Chernoff B, García EJ, Herrera K, Kihn HA, Lara O, León B, Mancilla M, Morales J, Ordoñez J, Ponciano I, Pérez SG, Queral Regil A, Rodríguez OC, Selvin Pérez E, Theodorakis CW, Willink PW, Zarza H (2000) Biological assessment of Laguna del Tigre National Park, Petén Guatemala. RAP Bulletin of Biological Assessment = Boletín RAP de Evaluación Biológica. 16. Conservation International Center for Applied Biodiversity Science, Washington DC

Alonso-Alvarez C, Munilla I, López-Alonso M, Velando A (2007) Sublethal toxicity of the prestige oil spill on yellow-legged gulls. Environ Int 33:773–781

Arkoosh MR, Casillas E, Clemons E, Kagley AN, Olson R, Reno P, Stein JE (1998) Effect of pollution on fish diseases: Potential impacts on salmonid populations. J Aquat Anim Health 10: 182–190

Ashok BT, Saxena S (1995) Biodegradation of polycyclic aromatic-hydrocarbons—a review. J Sci Ind Res India 54:443–451

Barra R, Quiroz R, Saez K, Araneda A, Urrutia R, Popp P (2009) Sources of polycyclic aromatic hydrocarbons (PAHs) in sediments of the biobio river in south Central Chile. Environ Chem Lett 7:133–139

Barron MG, Carls MG, Short JW, Rice SD (2003) Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. Environ Toxicol Chem 22:650–660

Barron MG, Carls MG, Short JW, Rice SD, Heintz RA, Rau M, DiGiulio RD (2005) Assessment of the phototoxicity of weathered Alaska North Slope crude oil to juvenile pink Salmon. Chemosphere 60:105–110

Baršienė J, Andreikėnaitė L, Rybakovas A (2006a) Cytogenetic damage in perch (*Perca fluviatilis* L.) and duck mussel (*Anodonta anatina* L.) exposed to crude oil. Ekologija 1:25–31

Baršienė J, Dedonyte V, Rybakovas A, Andreikenaite L, Andersen OK (2006b) Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil. Aquat Toxicol 78:S99–S104

Bickham JW (1990) Flow cytometry as a technique to monitor the effects of environmental genotoxins on wildlife populations. In: Sandhu S, Lower WR, DeSerres FJ, Suk WA, Tice RR (eds) In situ evaluation of biological hazard of environmental pollutants. Environmental research series vol. 38. Plenum Press, New York, pp 97–108

Bickham JW, Mazet JA, Blake J, Smolen MJ, Ballachey BE (1998a) Flow cytometric determination of genotoxic effects of exposure to petroleum in Mink and sea Otters. Ecotoxicology 7:191–199

Bickham J, Rowe G, Palatnikov G, Mekhtiev A, Mekhtiev M, Kasimov RY, Hauschultz D, Wickliffe J, Rogers W (1998b) Acute and genotoxic effects of Baku Harbor sediment on Russian sturgeon, *Acipenser guildensteidti*. Bull Environ Contam Toxicol 61:512–518

Bickham JW, Sandhu S, Hebert PDN, Chikhi L, Athwal R (2000) Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutat Res-Rev Mutat Res 463:33–51

Bihari N, Fafanel M (2004) Interspecies differences in DNA single strand breaks caused by benzo(a)pyrene and marine environment. Mutat Res-Fund Mol M 552:209–217

Bolognesi C, Perrone E, Roggieri P, Pampanin DM, Sciutto A (2006) Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquat Toxicol 78:S93–S98

Carls M, Heintz R, Marty G, Rice S (2005) Cytochrome P4501A induction in oil-exposed pink Salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. Mar Ecol Prog Ser 301:253–265

Chen B, Xuan X, Zhu L, Wang J, Gao Y, Yang K, Shen X, Lou B (2004) Distributions of polycyclic aromatic hydrocarbons in surface waters, sediments and soils of Hangzhou City, China. Water Res 38:3558–3568

Colombo JC, Cappelletti N, Lasci J, Migoya MC, Esperanza E, Skorupka CN (2006) Sources, vertical fluxes, and equivalent toxicity of aromatic hydrocarbons in coastal sediments of the Rio de la Plata estuary, Argentina. Environ Sci Technol 40:734–740

Costa FO, Neuparth T, Costa MH, Theodorakis CW, Shugart LR (2002) Detection of DNA strand breakage in a marine amphipod by agarose gel electrophoresis: exposure to X-rays and copper. Biomarkers 7:451–463

Couillard CM, Lee K, Légaré BÎ, King TL (2005) Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. Environ Toxicol Chem 24:1496–1504

Countway RE, Dickhut RM, Canuel EA (2003) Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VA estuary. Org Geochem 34:209–224

Cronin MW, Bickham JW (1998) A population genetic analysis of the potential for a crude oil spill to induce heritable mutations and impact natural populations. Ecotoxicology 7:259–278

Custer T, Custer C, Hines R, Sparks D, Melancon M, Hoffman D, Bickham J, Wickliffe J (2000) Mixed-function oxygenases, oxidative stress, and chromosomal damage measured in lesser scaup wintering on the Indiana Harbor Canal. Arch Environ Contam Toxicol 38:522–529

Deasi S, Verlecar X, Ansari Z, Jagtap T, Sarkar A, Vashistha D, Dalal S (2010) Evaluation of genotoxic responses of *Chaetoceros tenuissimus* and *Skeletonema costatum* to water accommodated fraction of petroleum hydrocarbons as biomarker of exposure. Water Res 44:2235–2244

Di Toro DM, McGrath JA, Stubblefield WA (2007) Predicting the toxicity of neat and weathered crude oil: toxic potential and the toxicity of saturated mixtures. Environ Toxicol Chem 26:24–36

Doong R, Lin YU (2004) Characterization and distribution of polycyclic aromatic hydrocarbon contaminations in surface sediment and water from Gao-Ping River, Taiwan. Water Res 38(7):1733–1744

Freeman SE, Thompson BD (1990) Quantitation of ultraviolet radiation-induced cyclobutyl pyrimidine dimers in DNA by video and photographic densitometry. Anal Biochem 186:222–228

Fu J, Sheng S, Wen T, Zhang Z-M, Wang Q, Hu Q-X, Li Q-S, An S-Q, Zhu H-L (2011) Polycyclic aromatic hydrocarbons in surface sediments of the Jialu River. Ecotoxicology 20:940–950

Garrigues P, Budzinski H, Manitz M, Wise S (1995) Pyrolytic and petrogenic inputs in recent sediments: a definitive signature through phenanthrene and chrysene compound distribution. Polycycl Aromat Comp 7:275–284

Goanvec C, Theron M, Lacoue-Labarthe T, Poirier E, Guyomarch J, Le-Floch S, Laroche J, Nonnotte L, Nonnotte G (2008) Flow cytometry for the evaluation of chromosomal damage in turbot *Psetta maxima* (L.) exposed to the dissolved fraction of heavy fuel oil in sea water: a comparison with classical biomarkers. J Fish Biol 73:395–413

Guo W, He M, Yang Z, Lin C, Quan X, Wang H (2007) Distribution of polycyclic aromatic hydrocarbons in water, suspended particulate matter and sediment from Daliao River watershed, China. Chemosphere 68:93–104

Harvey J, Lyons B, Page T, Stewart C, Parry J (1999) An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species. Mutat Res-Gen Tox En 441:103–114

Hylland K (2006) Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems. J Toxicol Env Heal A 69:109–123

Jha AN (2004) Genotoxicological studies in aquatic organisms. Mutat Res-Fund Mol M 552(1–2):1–17

Keenleyside MHA (1991) Cichlid fishes: behaviour, ecology and evolution. Chapman and Hall, New York

Ko FC, Baker J, Fang MD, Lee CL (2007) Composition and distribution of polycyclic aromatic hydrocarbons in the surface sediments from the Susquehanna River. Chemosphere 66:277–285

Laffon B, Fraga-Iriso R, Pérez-Cadahía B, Méndez J (2006) Genotoxicity associated to exposure to prestige oil during autopsies and cleaning of oil-contaminated birds. Food Chem Toxicol 44:1714–1723

Landsberg J, Blakesley B, Reese R, McRae G, Forstchen P (1998) Parasites of fish as indicators of environmental stress. Environ Monit Assess 51:211–232

Levin W, Wood A, Chang R, Ryan D, Thomas P, Yagi H, Thakker D, Vyas K, Boyd C, Chu SY (1982) Oxidative metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens. Drug Metab Rev 13:555–580

Li J, Ma M, Cui Q, Wang Z (2008) Assessing the potential risk of oilfield produced waters using a battery of bioassays/biomarkers. Bull Environ Contam Toxicol 80:492–496

Li J, Shang X, Zhao Z, Tanguay RL, Dong Q, Huang C (2010) Polycyclic aromatic hydrocarbons in water, sediment, soil, and plants of the Aojiang River waterway in Wenzhou, China. J Hazard Mater 173:75–81

Liu B, Romaire R, Delaune R, Lindau C (2006) Field investigation on the toxicity of Alaska North Slope crude oil (ANSC) and dispersed ANSC crude to gulf killifish, Eastern oyster and white shrimp. Chemosphere 62:520–526

Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuary sediments. Environ Manage 19:81–97

Loughlin TR (1994) Marine mammals and the Exxon Valdez. Academic Press, San Diego

McIntosh S, King T, Wu D, Hodson PV (2010) Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). Environ Toxicol Chem 29:1160–1167

Mille G, Munoz D, Jacquot F, Rivet L, Bertrand JC (1998) The Amoco Cadiz oil spill, evolution of petroleum hydrocarbons in the Iie Grande salt marshes (Brittany) after a 13-year period. Estuar Coast Shelf S 47:547–559

Myers N, Mittermeier RA, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858

[NASA] National Aeronautics and Space Administration (2008). http://earthobservatory.nasa.gov/IOTD/view.php?id=5338. Accessed 25 Aug 2010

Neuparth T, Bickham J, Theodorakis C, Costa F, Costa M (2006) Endosulfan-induced genotoxicity detected in the gilthead seabream, *Sparus aurata* L., by means of flow cytometry and micronuclei assays. Bull Environ Contam Toxicol 76:242–248

Neuparth T, Costa FO, Theodorakis CW, Costa MH, Bickham JW (2009) Assessment of DNA damage in blood cells of *Sparus aurata* L. exposed to benzo[a]pyrene using three distinct genotoxicity assays. Fresenius Environ Bull 18:461–467

Odeigah P, Nurudeen O, Amund O (1997) Genotoxicity of oil field wastewater in Nigeria. Hereditas 126:161–167

Page DS, Boehm PD, Stubblefield WA, Parker KR, Gilfillan ES, Neff JM, Maki AW (2002) Hydrocarbon composition and toxicity of sediments following the Exxon Valdez oil spill in Prince William Sound, Sound, Alaska, USA. Environ Toxicol Chem 21:1438–1450

Pérez-Cadahía B, Laffon B, Pásaro E, Méndez J (2004) Evaluation of PAH bioaccumulation and DNA damage in mussels (*Mytilus galloprovincialis*) exposed to spilled Prestige crude oil. Comp Biochem Phys C 138:453–460

Pies C, Hoffmann B, JPetrowsky J, Yang Y, Ternes TA, Hofmann T (2008) Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. Chemosphere 72:1594–1601

Qiao M, Wang CX, Huang SB, Wang DH, Wang ZJ (2006) Composition, sources, and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. Environ Int 32:28–33

Quiroz R, Popp P, Urrutia R, Bauer C, Araneda A, Treutler HC, Barra R (2005) PAH fluxes in the Laja Lake of south central Chile Andes over the last 50 years: evidence from a dated sediment core. Sci Total Environ 349:150–160

Scolo HH, Garrigues PH, Ewald M (2000) Origin of Polycyclic aromatic hydrocarbons (PAH's) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. Mar Poll Bull 40:387–396

Shugart LR, Bickham J, Jackim G, McMahon G, Ridley W, Stein J, Steinert S (1992) DNA alterations. In: Huggett RJ, Kimerle RA, Mehrle PM Jr, Bergman HL (eds) Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers, Boca Raton, pp 125–154

Shugart LR, Theodorakis C, Bickham JW (2010) Evolutionary toxicology. In: DeWoody JA, Bickham JW, Michler CH, Nichols KM, Rhodes OE, Woeste KE (eds) Molecular approaches in natural resource conservation and management. Cambridge University Press, New York, pp 320–362

Taban IC, Bechmann RK, Torgrimsen S, Baussant T, Sanni S (2004) Detection of DNA damage in mussels and sea urchins exposed to crude oil using comet assay. Mar Environ Res 58:701–705

Theodorakis CW (2008) Mutagenesis. In: Jørgensen SE (ed) Encyclopedia of ecology. Elsevier, Amsterdam, pp 2475–2484

Theodorakis C, D'surney S, Bickham J, Lyne T, Bradley B, Hawkins W, Farkas W, McCarthy J, Shugart L (1992) Sequential expression of biomarkers in bluegill sunfish exposed to contaminated sediment. Ecotoxicology 1:45–73

Theodorakis CW, D'Surney SJ, Shugart LR (1994) Detection of genotoxic insult as DNA strand breaks in fish blood cells by agarose gel electrophoresis. Environ Toxicol Chem 13:1023–1031

Theodorakis CW, Blaylock BG, Shugart LR (1997) Genetic ecotoxicology I: DNA integrity and reproduction in mosquitofish exposed in situ to radionuclides. Ecotoxicology 6:205–218

Theodorakis CW, Elbl T, Shugart LR (1999) Genetic ecotoxicology IV: survival and DNA strand breakage is dependent on genotype in radionuclide-exposed mosquitofish. Aquat Toxicol 45:279–291

Theodorakis CW, Swartz CD, Rogers WJ, Bickham JW, Donnelly K, Adams SM (2000) Relationship between genotoxicity, mutagenicity, and fish community structure in a contaminated stream. J Aquat Ecosys Stress Recovery 7:131–143

Theodorakis CW, Bickham JW, Lamb T, Medica PA, Lyne TB (2001) Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada Test Site, USA. Environ Toxicol Chem 20:317–326

Vindeløv LL, Christensen IJ (1990) A review of techniques and results obtained in one laboratory by an integrated system of methods designed for routine clinical flow cytometric DNA analysis. Cytom Part A 11:753–770

Vindeløv LL, Christensen IJ, Keiding N, Spang-Thomsen M, Nissen NI (1983) Long-term storage of samples for flow cytometric DNA analysis. Cytom Part A 3:317–322

Wang Z, Brown C (2008) Chemical fingerprinting of petroleum hydrocarbons. In: Mudge SM (ed) Methods in environmental forensics. CRC Press, Boca Raton, pp 43–112

Wang Z, Fingas M, Shu Y, Sigouin L, Landriault M, Lambert P, Turpin R, Campagna P, Mullin J (1999) Quantitative characterization of PAHs in burn residue and soot samples and differentiation of pyrogenic PAHs from petrogenic PAHs—the 1994 mobile burn study. Environ Sci Technol 33:3100–3109

Ward JF (1988) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and repairability. Prog Nucl Acids Res Mol Biol 35:95–125

Xu C-J, Zhou X, Zhang G-F, Yuang G-X, Shi D-Y (2010) Research on chromosome aberration of horsebean root tips induced by oil produced waste water. Heilongjiang Agricultural Sciences [online]. http://en.cnki.com.cn/Article_en/CJFDTOTAL-HLJN201001005.htm

Zhang S, Zhang Q, Darisaw S, Ehie O, Wang G (2007) Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA. Chemosphere 66:1057–1069

Zhu L, Chen Y, Zhou R (2008) Distribution of polycyclic aromatic hydrocarbons in water, sediment and soil in drinking water resource of Zhejiang Province, China. J Haz Mat 150:308–316