SUPPORTING INFORMATION

"Developmental toxicity and DNA damage from exposure to parking lot runoff retention pond samples in the Japanese Medaka (*Oryzias latipes*"

This supporting information provides additional results related to: 1) LA-QPCR assay optimization; 2) DNA extraction procedures optimization; and 3) PLRRP sediment PAH concentrations.

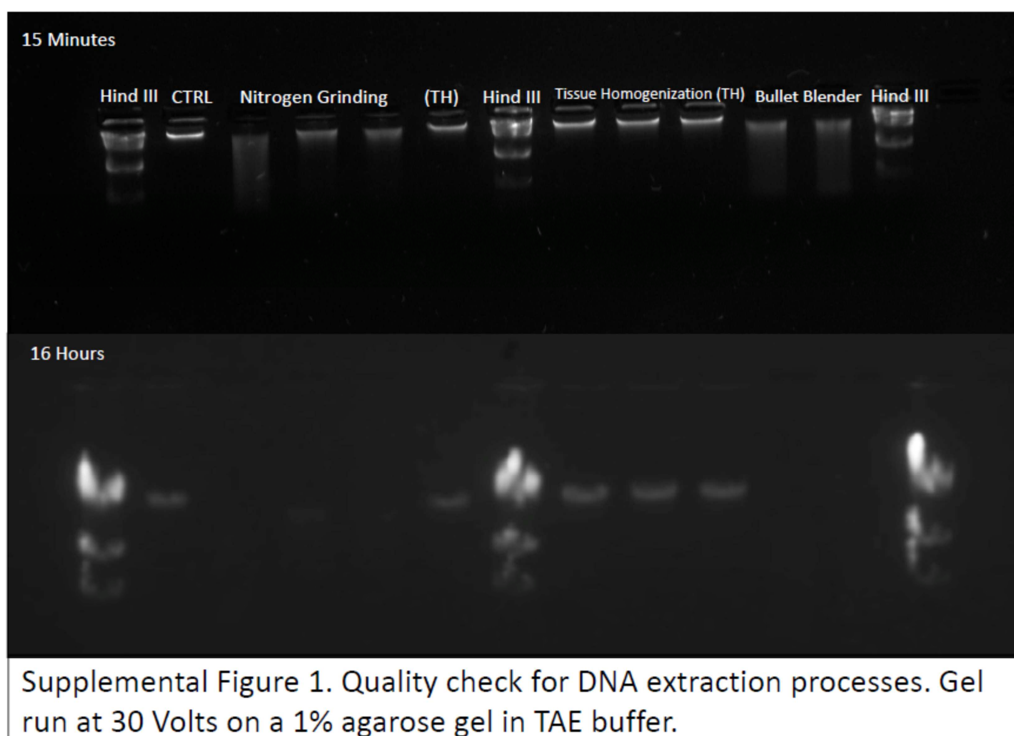
1. DNA EXTRACTION OPTIMIZATION

MATERIALS AND METHODS

Nitrogen grinding, bullet blending, and tissue homogenization were tested as methods of homogenization prior to DNA isolation with Genomic Tips, with DNA integrity assessed by gel electrophoresis as described [Meyer 2010]. The size marker is a HindIII digest of lambda phage DNA, and the control DNA is nematode genomic DNA extracted per our previously-optimized methods [Hunter *et al.*, 2010].

RESULTS

Tissue homogenization generated the highest molecular weight DNA, as seen previously for fish tissues (Supplemental figure 1) [Jung *et al.,* 2009].

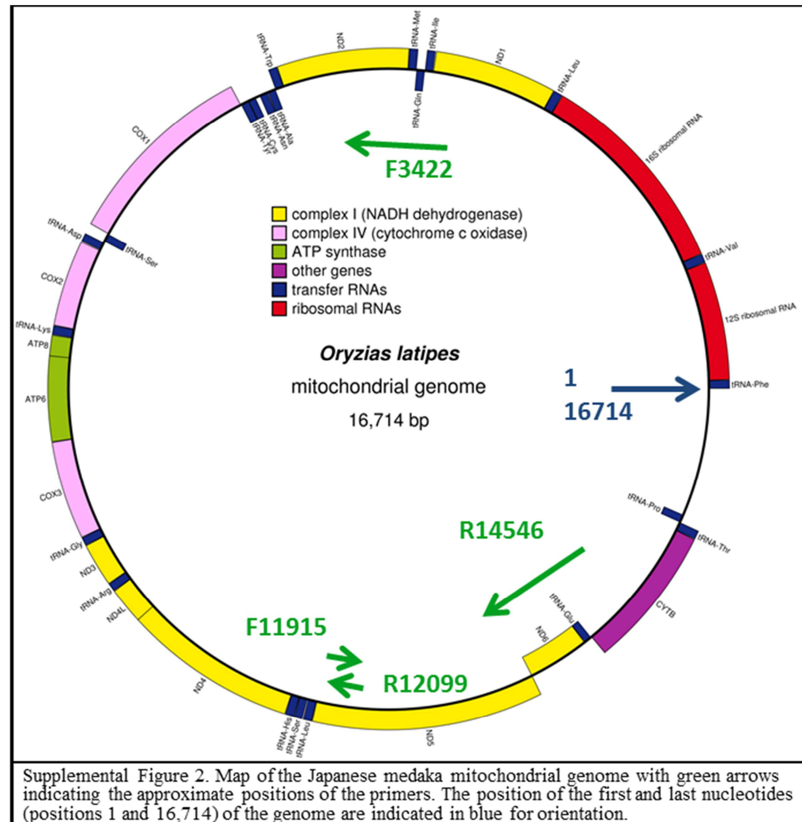


Supplemental Figure 1. Quality check for DNA extraction processes. Gel run at 30 Volts on a 1% agarose gel in TAE buffer.

2. LA-QPCR OPTIMIZATION

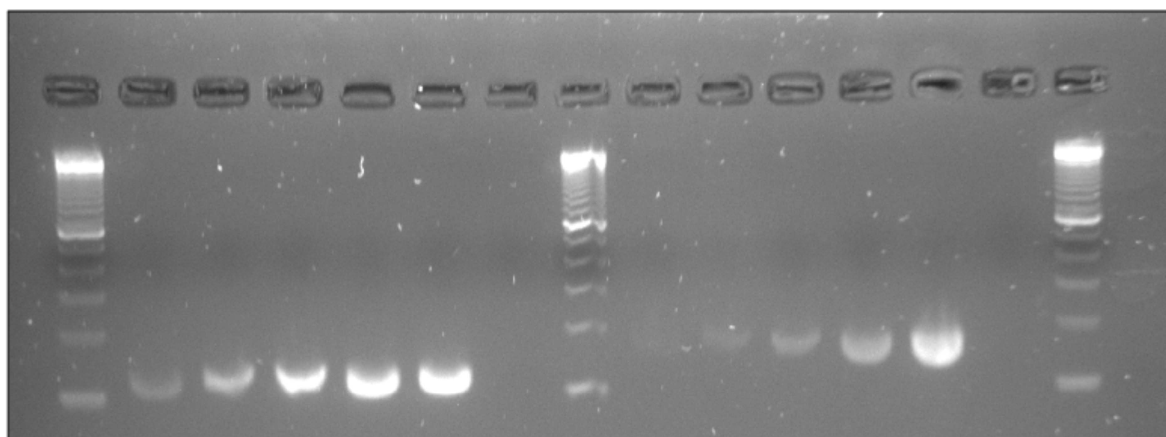
MATERIALS AND METHODS

Several sets of primers were tested for amplification of short and long mitochondrial and nuclear products. The short and long amplicons for the mitochondrial genome are illustrated in Supplemental Figure 2. This map of the medaka mitochondrial genome was created using OGDRAW. Numbers indicate nucleotide positions, based on GenBank accession number NC_004387. After primer choice and temperature optimization (not shown), we carried out cycle tests to identify the optimal cycle number for quantitative reactions. The D loop is the region that lacks any coding sequence between tRNA-Pro and the final base (16714).



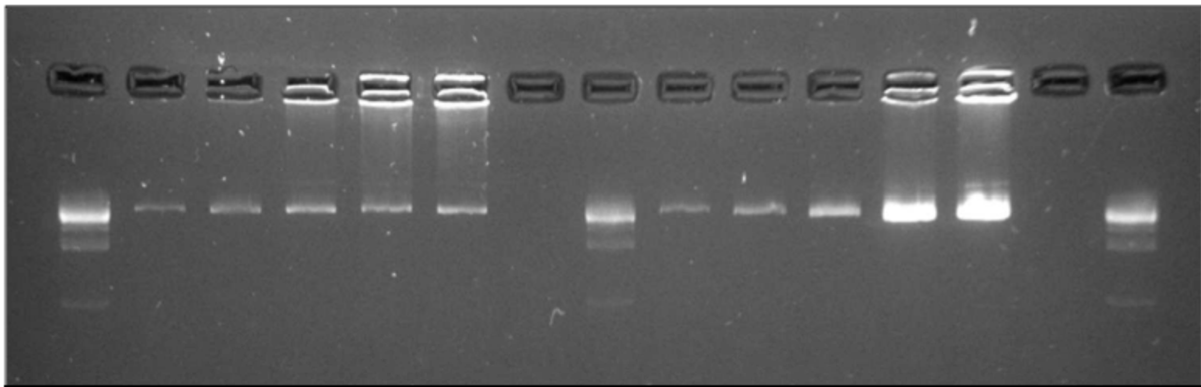
Supplemental Figure 2. Map of the Japanese medaka mitochondrial genome with green arrows indicating the approximate positions of the primers. The position of the first and last nucleotides (positions 1 and 16,714) of the genome are indicated in blue for orientation.

Products were visualized by gel electrophoresis (Supplemental Figures 3 and 4) and quantified by plate reader (Supplemental Figures 5 and 6).



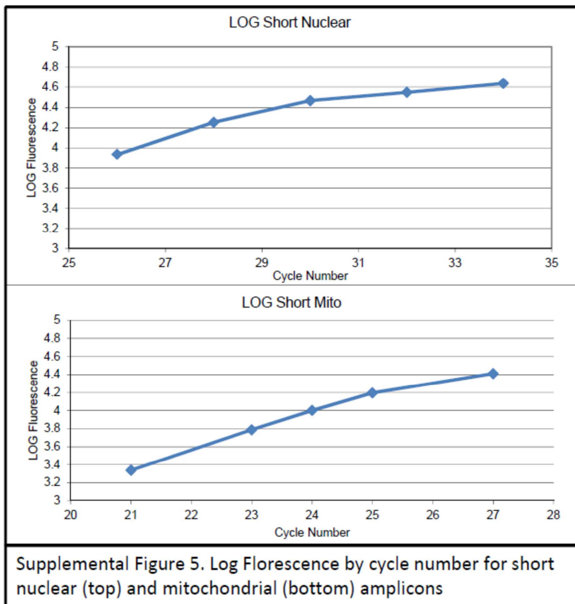
	Short Nuclear							Short Mitochondrial						
Cycle #	-	26	28	30	34	Blank	-	21	23	24	25	27	blank	-

Supplemental Figure 3. Short product cycle optimization. Gels run at 150 volts for 60 minutes on a 2% agarose gel in Sodium Borate buffer.

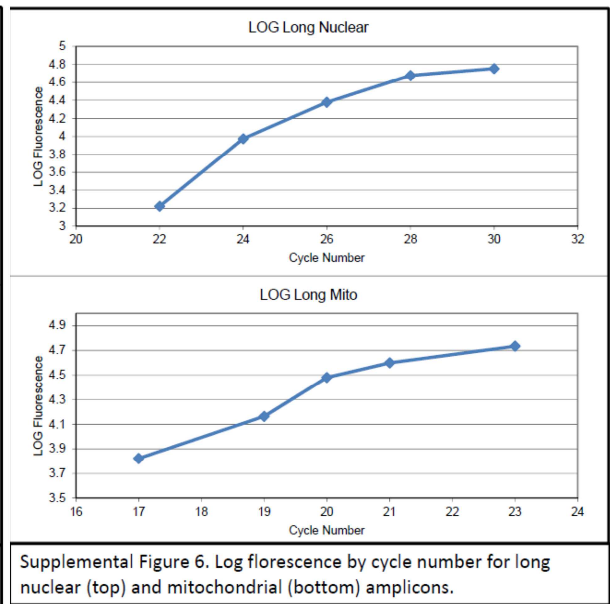


	Long Nuclear							Long Mitochondrial						
Cycle #	-	26	28	30	34	Blank	-	19	20	21	23	25	blank	-

Supplemental Figure 4. Long product cycle optimization. Gels run at 150 volts for 60 minutes on a 2% agarose gel in Sodium Borate buffer.



Supplemental Figure 5. Log Florescence by cycle number for short nuclear (top) and mitochondrial (bottom) amplicons



Supplemental Figure 6. Log florescence by cycle number for long nuclear (top) and mitochondrial (bottom) amplicons.

RESULTS

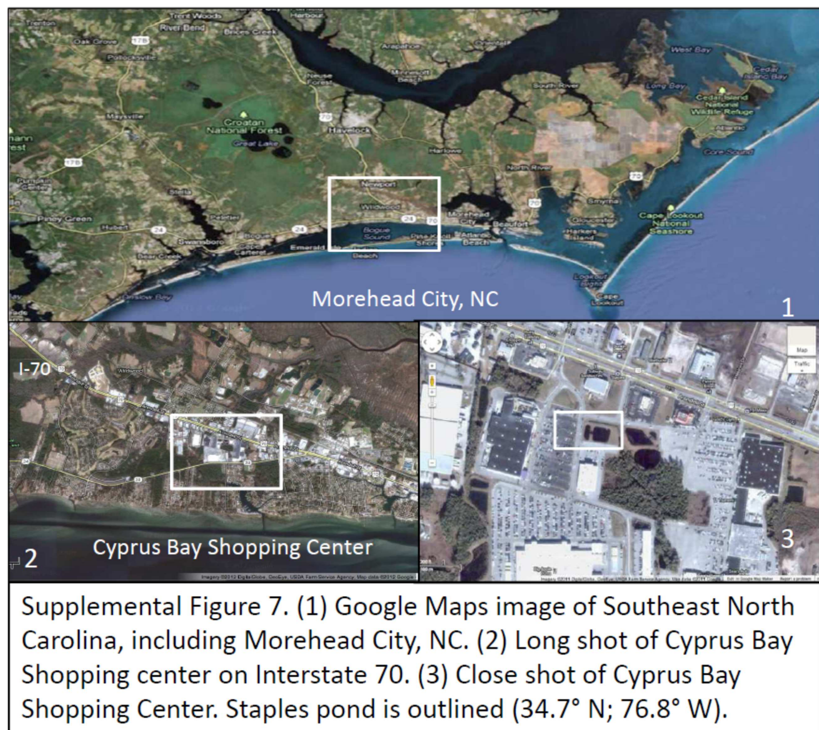
Based on these analyses, typical optimal cycle numbers were determined to be 26, 27, 20, and 22 for short and long nuclear and short and long mitochondrial products, respectively.

3. PLRRP SEDIMENT PAH CONCENTRATIONS

MATERIALS AND METHODS

Sediment Sampling

In May, 2009, modified 60mL syringes were used to collect fifteen sediment core samples per site. The tips of the syringes were cut off, turning them into simple tubes. These tubes were pushed into the sediment at each site, the plungers pulled up to collect sediment, and the tubes then removed from the sand. The sediment was pushed up through the tube with the plunger until only approximately two cubic centimeters of sediment remained in the syringe. This sediment was collected in glass scintillation vials, which were tightly capped and refrigerated at –20°C until homogenization. The sediment sampling site is illustrated in Supplemental Figure 7.



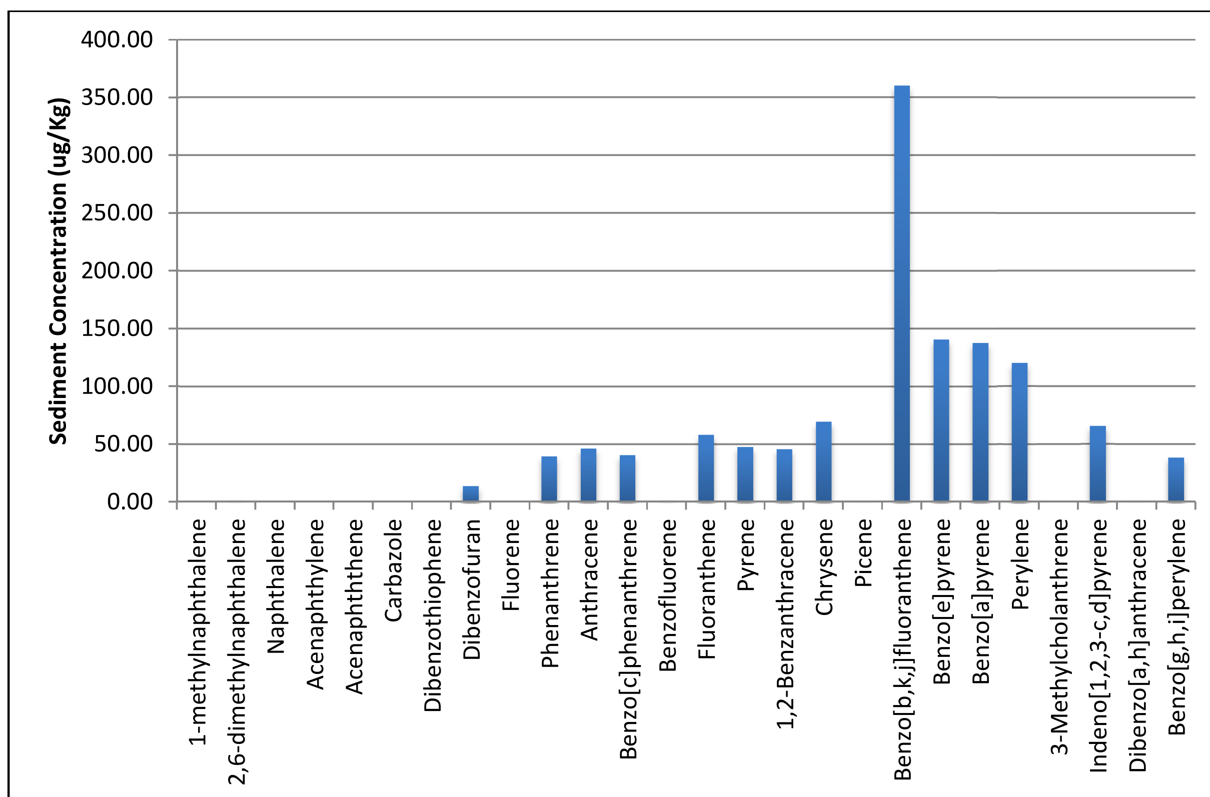
Supplemental Figure 7. (1) Google Maps image of Southeast North Carolina, including Morehead City, NC. (2) Long shot of Cyprus Bay Shopping center on Interstate 70. (3) Close shot of Cyprus Bay Shopping Center. Staples pond is outlined (34.7° N; 76.8° W).

Sediment Homogenization and PAH Analysis

Sediments were first homogenized with a mortar and pestle. Sediment samples were thawed and transferred to a glass graduated cylinder, which was then filled with water to the 15mL mark and shaken. The mixture was then vacuum-filtered on pre-weighed glass fiber filter paper to remove excess water. Then, sediment and filter paper were weighed and homogenized with a stainless steel mortar and pestle. From the ground-up mixture, three aliquots of approximately 0.02g were collected and each placed into a small, labeled aluminum foil sleeve. These sleeves were then placed into an oven at 80°C overnight to achieve a value for dry weight of sediment. Of the

homogenized sediments 2.0g was weighed and placed in 10mL glass test tubes with an equal amount of anhydrous sodium sulfate.

For PAH analysis, wet sediment (approximately 1.5 g) was ground with Na_2SO_4, spiked with a surrogate standard mix (deuterated 2-methylnaphthalene, fluorene, fluoranthene and perylene; Cambridge Isotope Laboratories, Tewksbury, MA, USA), and extracted in 1:1 dichloromethane (DCM) and hexane (v:v) with an accelerated solvent extractor (ASE 200, Dionex, Sunnyvale, CA). Extracts were concentrated with rapid evaporation under N_2 (Turbo Vap, Caliper LifeSciences, Hopkinton, MA, USA) to approximately 0.5 mL, and cleaned with column chromatography using 6% deactivated alumina (4 g) topped Na_2SO_4 with eluting with 50 mL 1:1 DCM/hexane. Purified extracts were concentrated under N_2 with HCl-cleaned copper turnings to remove sulfur, and spiked with an internal standard mix (deuterated naphthalene, phenanthrene, pyrene and benzo[a]pyrene; Cambridge Isotope Laboratories) for quantitation. PAHs were analyzed using a gas chromatograph and mass spectrometer (Agilent GC 6890N, MS 5975, Newark, DE) using electron ionization and selected ion monitoring. Analytes were injected (splitless, 250°C) onto a DB-5 column (30 m, 250 μm nominal diameter, 0.25 μm film thickness; J&W Scientific, Agilent) and separate using a thermal gradient (40°C for 0.6 min, increase to 280°C over 14.6 min, hold at 280°C for 24 min). Recoveries of surrogate standards were 62-101% with the exception of 2-methylnaphthalene, for which recoveries were near 16%. Sediment moisture content was measured gravimetrically by weighing approximately 1.5-2.0 g wet sediment before and after drying at 105°C for 16 hours. Moisture content was calculated as (moist weight – dry weight)/dry weight, and used to correct PAH concentrations to dry weight.



Supplemental Figure 8. PAH concentrations in parking lot runoff retention pond.

RESULTS

Sediment PAH concentrations are presented in Supplemental Figure 8.

REFERENCES

Hunter, S.E., Jung, D., Di Giulio, R.T., Meyer, J.N., 2010. The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. Methods 51, 444-451.

Jung, D., Cho, Y., Meyer, J.N., Di Giulio, R.T., 2009. The long amplicon quantitative PCR for DNA damage assay as a sensitive method of assessing DNA damage in the environmental model, Atlantic killifish (*Fundulus heteroclitus*). Comp. Biochem. Phys. C 149, 182-186.

Meyer, J. N., 2010. QPCR: a tool for analysis of mitochondrial and nuclear DNA damage in ecotoxicology. Ecotoxicology 19, 804-811.