

Effects of Anacostia River water on growth and development in larval zebrafish (*Danio rerio*)

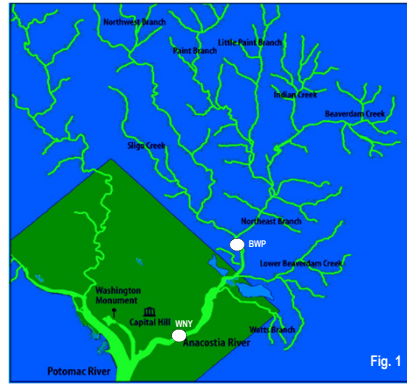
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Introduction

- Poor water quality in the Anacostia River
- Heavily urbanized
- Slow flow
- Persistent presence of toxic chemicals
- Tumors in fish tissue
- Recent clean-up efforts have improved water quality
- A bioassay system could assess overall water quality and River health



Methods

- Collected water samples from 2 different sites (Fig. 1)
 - Washington Navy Yard (WNY), downstream
 - Bladensburg Waterfront Park (BWP), midstream
- Placed fertilized zebrafish (*Danio rerio*) eggs into
 - Filtered BWP water (for 14 days)
 - Filtered WNY water (for 30 days)
 - Control/system water (for 14-30 days)
- Record differences in hatching, survival
- Subsample at 7, 14/15, 20, and 30 days to document
 - Anatomy (length, girth, eye diameter, inter-eye distance)
 - Behavior (general swim behaviors; location in dish, immobility, burst swimming)
- GC-MS identified contaminants in WNY and BWP water samples.

Conclusion: Water quality and biological impact of chemicals differ along the Anacostia.

WNY

- Downstream site
- More contaminants were identified
- Major contaminants
 - (5 β)Pregnane-3,20 α -diol
 - Ethylbenzene
 - Siloxanes
- Early developmental exposure
 - No effect on survival
 - Earlier hatch rates
 - Increased growth/larger larvae (>20dy)
 - Accelerated swim behaviors (>20dy)
 - Thigmotaxis (stress response) – more time at edge of dish (\geq 7dy)
 - Effects developed with time

BWP

- Midstream site
- Fewer contaminants
- Major contaminants
 - Siloxanes (82%)
- Early developmental exposure
 - No effect on survival
 - No effect on hatch rates
 - Shorter, wider larvae (7dy)
 - Increased immobility (7dy)
 - Travel farther with more breaks (14dy)
 - No thigmotaxis – more time/frequency spent in dish center
 - Effects occurred early

Purpose

- Determine the impact of exposure to Anacostia River water on fish early life stages
- Perform water quality analysis to identify potential biologically active contaminants in Anacostia water samples

GC-MS revealed different contaminants along the Anacostia

Multiple contaminants were identified at WNY (>50% certainty).

Chemical Formula	Compound Name	Peak Time (minutes)	Percent Certainty
C ₁₀ H ₁₆ O ₂	Ethyl iso-allochoilate	9.41	66.49
C ₁₀ H ₁₆ O	2-Cyclohexan-1-one,3,5,5-trimethyl-	6.21	61.55
C ₁₇ H ₂₆ O ₅	cyclohexasiloxane, dodecamethyl-	8.76	61.37
C ₁₇ H ₂₆ N ₂ O ₅	Morphinan-4,5-epoxy-3,6-di-ol, 6-(7-nitrobenzofurazan-4-yl)amino-	6.80	58.49
C ₆ H ₁₀	Ethylbenzene	3.23	55.81
C ₆ H ₁₀	Ethylbenzene	3.23	55.81
C ₂₇ H ₄₆ N ₂ O ₄	(5 β)Pregnane-3,20 α -diol, 14 α ,18 α -(4-methyl-3-oxo(1-oxa-4-azabutane-1,4-diy))-, diacetate	5.12	55.12
C ₂₈ H ₄₆ O ₁₁	7 α H-Cyclopenta[a]cyclopropa[1]cycloundecane-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-2,4,7,10,11-pentaacetate	19.25	54.45
C ₈ H ₁₆ O ₂ Si ₂	Octasiloxane	10.98	53.85
C ₁₁ H ₁₈ O	1-Heptasiloxanol	16.97	53.26
C ₁₇ H ₂₆ O ₄	Spirost-8-en-11-one, 3-hydroxy-, (3 α ,5 α ,14 α ,20 α ,22 α ,25R)	8.82	52.11
C ₂₁ H ₂₆ O ₂	Cyclopropa[3,4]benz[1,2-aj]azulene-5-one,9,9a-bis(isoxoy)-1	12.78	50.93

Fewer contaminants were identified at BWP (>60% certainty). Most were siloxanes.

Chemical Formula	Compound Name	Peak Time (minutes)	Percent Certainty
C ₂₇ H ₄₆ O ₂ Si	Bis(di(trimethylsiloxy)phenyl)siloxy	19.40-19.50	81.61
C ₁₈ H ₂₆ O ₂ Si ₂	Decamethylcyclopentasiloxane	6.14-7.23	86.48
C ₁₂ H ₂₀ O ₂ Si ₂	Dodecamethylcyclohexasiloxane	8.68-14.43	88.54
C ₁₄ H ₂₄ O ₂ Si ₂	Eicosamethylcyclododecasiloxane	13.25	62.68
C ₁₆ H ₂₆ Si ₂	Hexadecamethylcyclooctasiloxane	10.71-13.08	87.01
C ₁₈ H ₂₆ O ₂ Si ₂	Octadecamethylcyclononasiloxane	13.63-14.78	89.33
C ₈ H ₁₆ O ₂ Si ₂	Octamethylcyclohexasiloxane	4.24-4.37	69.86
C ₂₁ H ₂₆ BO ₂ PSiW	Pentacarbonyl (4,5-diethyl-2,2,3-trimethyl-1-phenyl-1-phospha-2-sile-5-boracyclohex-2-ene-P1) tungsten	11.12	78.3
C ₁₄ H ₂₄ O ₂ Si ₂	Tetradecamethylcycloheptasiloxane	10.91-11.48	78.84
C ₁₆ H ₂₆ O ₂ Si ₂	1,1,3,3,4,4,5,5,8,8,11,11,13,13,15,15-hexadecamethylcyclooctasiloxane	8.56-11.85	63.73
C ₁₇ H ₂₆ ClN ₂ O ₂	1,2-Dicarboxy-3-(4-chlorophenyl)-2-(3-(1H)-dihydropyrid(1,2-a)benzimidazole	11.68	76.19

Zebrafish larvae reared in filtered WNY water have accelerated growth and altered swim behaviors, consistent with a stress response.

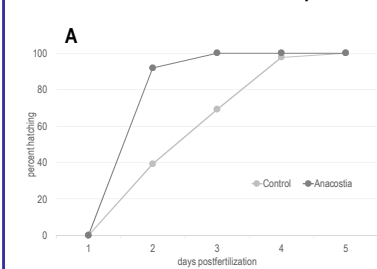


Fig. 2A. Zebrafish larvae hatched earlier in Anacostia (WNY) water

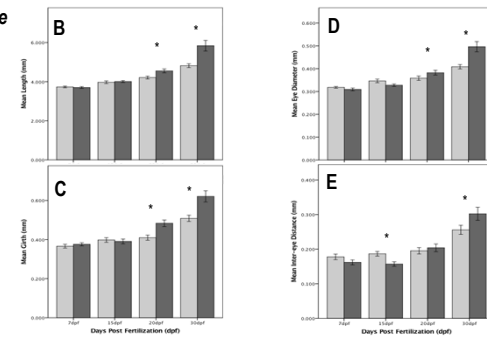


Fig. 2B-E. After 20dy, WNY-treated larvae were significantly larger (*). All parameters were significant at 30dy.

	7dpf	15dpf	20dpf	30dpf
Angular velocity (mm/s)	ns	ns	A > C	ns
Total distance traveled (mm)	ns	ns	A > C	ns
Average velocity (mm/s)	ns	ns	A > C	ns
Duration of overall activity (s)	ns	ns	A > C	A > C
Time immobile (s)	ns	ns	ns	ns
Duration of burst swimming (s)	ns	A > C	A > C	ns
Frequency of burst swimming	ns	A > C	A > C	ns
Time in edge zone	A > C	A > C	A > C	A > C

Fig. 2F. At all ages, WNY-exposed fish (A) spent more time at the edge of the dish (vs. controls, C). Other behaviors were altered after >2wk of exposure.

Larvae reared in filtered BWP water did not display a stress response, but have early anatomical differences.

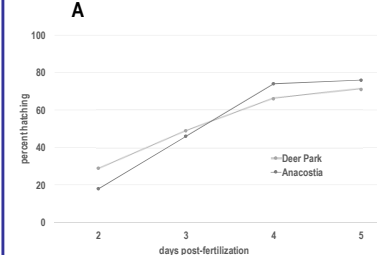


Fig. 3A. No difference in hatching when zebrafish larvae were reared in BWP water.

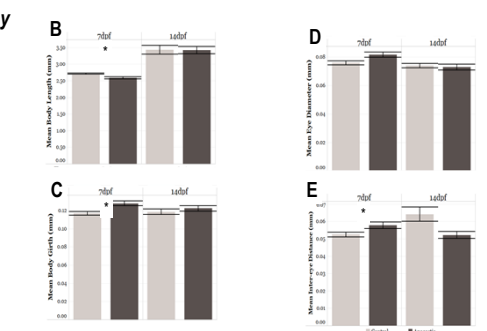


Fig. 3B-E. At 7dy, BWP larvae were smaller and wider than controls (*). No differences were observed at 14dy.

	7dpf	14dpf
Total distance traveled (mm)	ns	A > C
Average Velocity (mm/s)	ns	ns
Turn angle	ns	C > A
Absolute angular velocity (mm/s)	ns	C > A
Time immobile	A > C	ns
Frequency of immobility (breaks)	ns	A > C
Time in center of dish	A > C	ns
Frequency of visits to center	C > A	A > C

Fig. 3F. BWP-larvae (A) were more immobile at 7dy, but spent time in the center of the dish (vs. controls, C). More behavioral differences were observed at 14dy.