DEVELOPMENT OF THYROID DIFFERENCES BETWEEN MUMMICHOGS (*FUNDULUS HETEROCLITUS*) FROM A POLLUTED SITE AND A REFERENCE SITE

Mary Anne Carletta,[1] Peddrick Weis,[2] Judith S. Weis[1]
[1]Dept of Biol Sci, Rutgers University, Newark, NJ, 07102;
[2]Dept of Anatomy, Cell Biol & Injury Sci, UMDNJ - New Jersey Medical School, Newark, NJ 07103

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Abstract

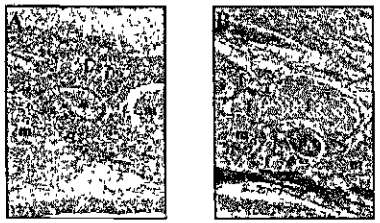
A previous study showed that adult *Fundulus heteroclitus* from a polluted site (Piles Creek or PC) have enlarged follicles and high thyroid (T4) levels when compared to fish from an unpolluted reference site (Tuckerton or TK). This study's purpose was to determine when in development these differences began to appear. Young-of-year fish were captured from both sites, sorted by length, preserved, and processed for light microscopic histological studies. The area of the thyroid follicles was measured. For fish longer than 15 mm (total length), the average follicle area of the PC population was significantly larger than that of the TK population, similar to the pattern found in adult fish. However, for the next smaller size class (12-14.5 mm), the average follicle area of the PC population was significantly smaller. At the smallest size (9-11.5 mm) the two populations were not significantly different. Although not statistically significant, radioimmunoassay measurements of T4 levels in fish were consistent with the follicle findings. In the 20-30 mm size class, the PC fish had a significantly lower T3/T4 ratio than the TK fish. T4 levels in unfertilized eggs were nearly twice as high in PC eggs, consistent with the earlier study on adult fish. The thyroid follicles of normal fish seen in adults began by the time the fish were approximately 15-20 mm long. However, the follicle size reversal for the next smaller size (12-14.5 mm) may also indicate abnormality at this developmental stage for the pollution fish.

Thyroid Follicle Size Differences

At what age do the differences in thyroid follicle size appear?

Differences appear at ~15 mm (TL)
The photos below show the size difference in fish approximately 6 months old (~40 mm)

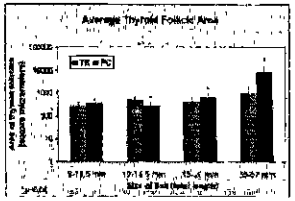
Tuckerton | Piles Creek



Thyroid follicle (f), hematopoietic artery (a), muscle (m)

Methods

- Fish captured in minnow traps, umbrella nets & dip nets in late summer
- Sorted by size
- Preserved in formalin
- Embedded in paraffin, cut sagittally
- Stained with hematoxylin & eosin
- Measured with computer imaging system interfaced with light microscope



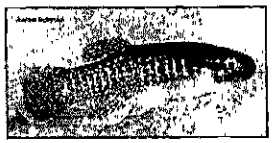
Two Contrasting Sites

Tuckerton	Piles Creek
• Southern New Jersey	• Northern NJ (Lyndhurst)
• Rural, woodland/marsh	• Urban, long industrial use
• Large salt marsh, part of which is wildlife preserve	• Small salt marsh
• Site of Rutgers Marine Experimental Station	• Surrounded by highways and a power plant
• Minimal contamination	• Next to the Passaic River
	• Heavily contaminated sediments

Contaminants Found in Sediments *

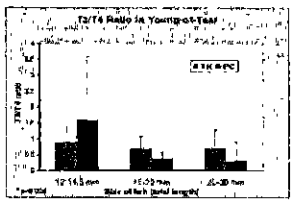
Contaminant	Tuckerton	Piles Creek
Mercury (µg/g)	0.15 ± 0.06	16.41 ± 3.58
Lead (µg/g)	13.68 ± 0.88	55.42 ± 6.03
Copper (µg/g)	7.16 ± 0.04	1895 ± 178
Zinc (µg/g)	32.96 ± 1.18	1697 ± 152
Phenanthrene (µg/g)	13.53 ± 2.04	88.75 ± 6.40
Carbazole (µ/g)	1.90 ± 0.72	7.05 ± 1.45
PCBs (ng/g)	11.9	285
DDTs (ng/g)	1.01	1050
Chlordane (ng/g)	2.48	138

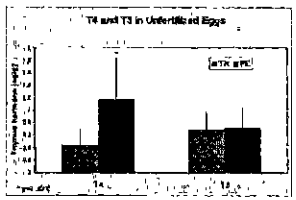
* Source: Bonn et al. 1996. Values are in wet weight. Values are for the surface layer of the sediment. Values were measured in 1993 and 1994. All the PCBs listed were congeners and chromatographically different from the Aroclor mixtures used.



Thyroid Hormone Differences

At what age do differences in thyroid hormones appear?





Methods

Fish:
- Captured in fine mesh umbrella & dip nets
- Sorted by size, frozen at -70°C
- Digestive tract removed for larger 2 size classes
- Whole body used for smallest size class
- Samples pooled as needed for proper sample weight
- Hormones extracted with methanolic ammonia
- Assayed via double antibody radioimmunoassay

Eggs:
- Collected by stripping
- Kept separate by female, frozen at -20°C
- Hormones extracted with methanolic ammonia, then with chloroform
- Assayed via double antibody radioimmunoassay

Size of fish (total length)	# of samples (TK, PC)	Mean T4 (ng/g) TK	PC	Mean T3/T4 TK	PC
12-14.5 mm	14, 4	83.4 ±24.9	161.5 ±65.8	2.1 ±0.8	1.3 ±0.2
	18, 2				
15-19.5 mm (combined)	5, 4	70.8 ±32.9	170.5 ±82.2	12.2 ±5.4	1.3 ±0.5
15.2-19 mm	3, 11	3.7 ±0.6	3.7 ±0.4	11.8 ±0.8	2.3 ±0.5
20-30 mm	T4 16-16	4.0	3.6	2.8	3.9
	T3 15-16	±1.6	±0.7	±0.8	±0.8

Note: Some of the data for the 12-14.5 size class is extremely low and is considered the limit of the standard curve. The last given should be more representative of the sample than the limited sample size which fell within the standard curve.

Conclusions

- The thyroid follicles in PC fish become significantly larger than those of TK fish in their first summer, when both are 15-20 mm long, and continue to be larger into adulthood
- The thyroid follicles in PC fish are significantly smaller than those of TK fish when both are 12-14.5 mm long
- Thyroid hormone levels in PC young-of-year are not significantly different than those of TK, but the numerical trend of the levels is consistent with the follicle size reversal of the 12-14.5 mm size class
- For the 20-30 mm size class, the PC T3/T4 ratio is significantly lower than that of the TK fish
- T4 levels in PC eggs are higher than in TK eggs, consistent with levels found in adult mummichogs in a previous study.

Implications

Earlier research has shown that PC fish behave differently than TK fish: they catch prey less frequently and are less able to avoid predators. Even larval fish from PC behave differently than the same age fish from TK. Other studies have shown that some neurotransmitter levels vary between the two populations. Abnormal thyroid function can affect behavior, which in turn can affect the ability of a population to survive. The differences in thyroid function shown by this study may well contribute to the documented behavioral and developmental differences in the two populations.

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