NOAA FISHERIES

Low level PAH impacts on fish heart shape, what it means, and the search for diagnostic markers

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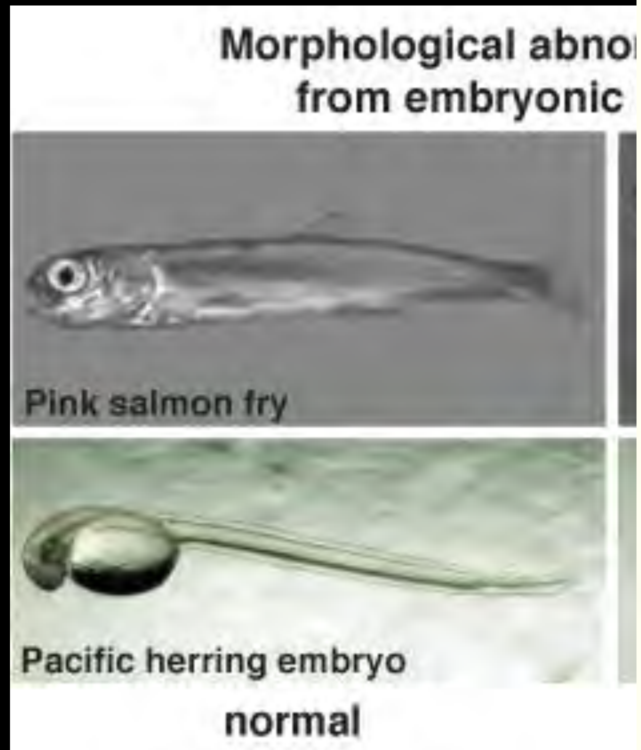
Oiled Wildlife Care Network

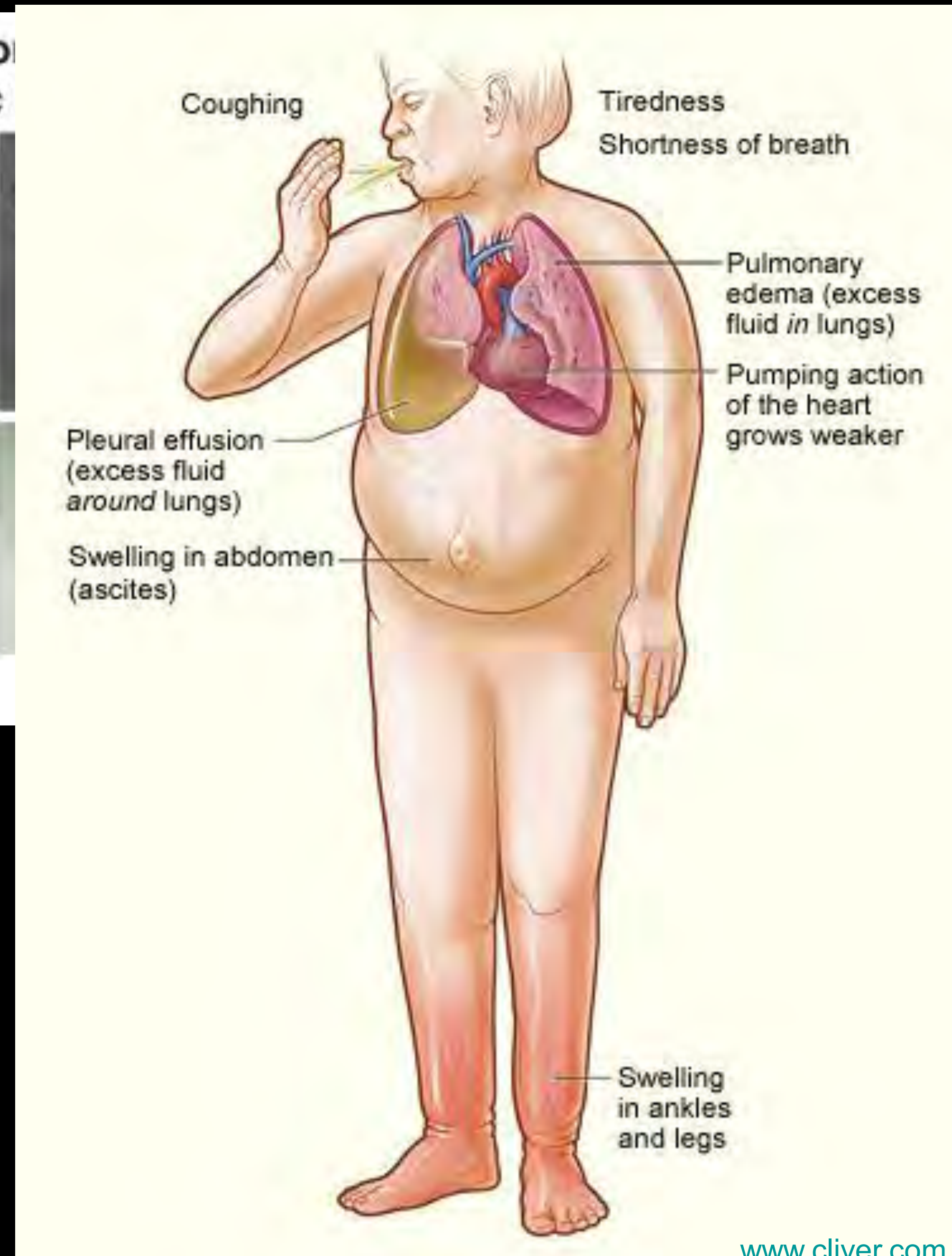
“Literally billions of larvae are produced by most populations of marine fishes annually. In most species, more than 99% of these larvae die in their first year from the combined effects of starvation and predation; the average fish probably dies in less than a week (Miller 1988). Hence very minor shifts in mortality rates can have major implications for later year class strength and for recruitment into older, catchable size classes.”

Helfman, Collette, Facey
The Diversity of Fishes
SFAS Fish 311

What we learned from Exxon Valdez

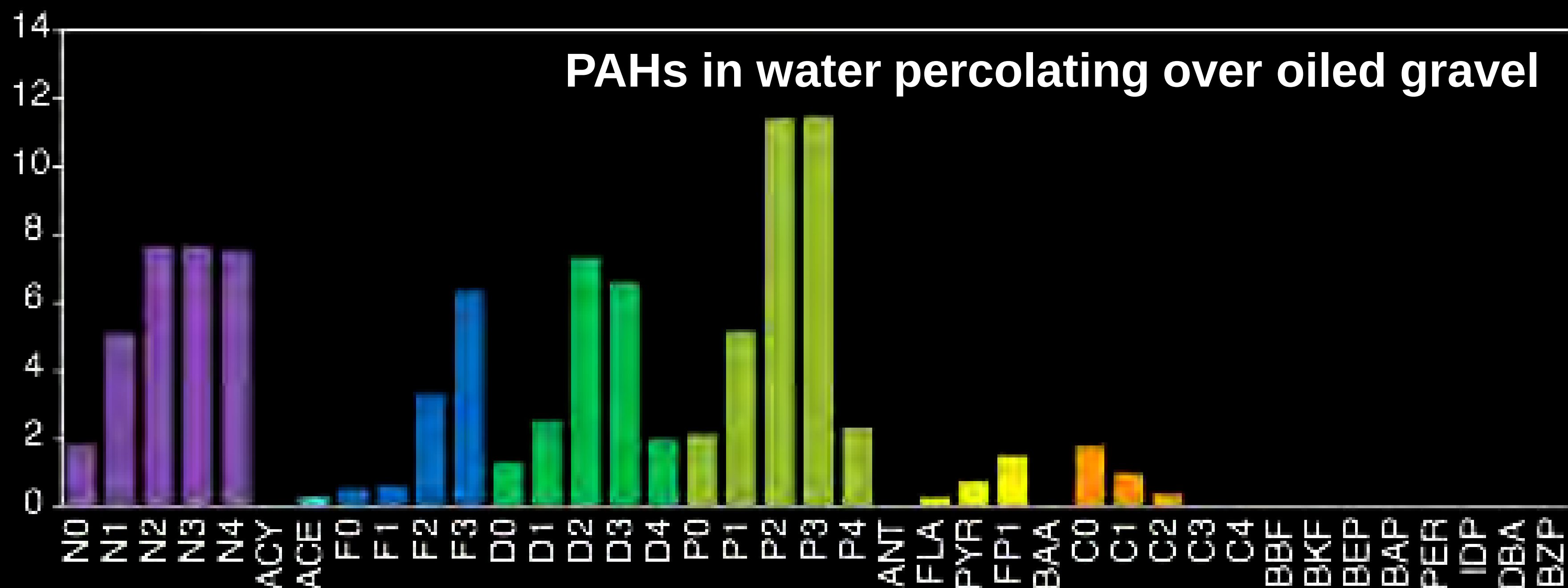
Jeep Rice, Jeff Short, Mark Carls, Ron Heintz, NOAA Auke Bay Labs, Juneau





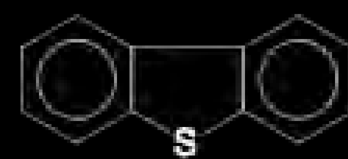
Oil exposure causes heart failure in fish embryos

Polycyclic Aromatic Hydrocarbons (PAHs)
A toxic family of chemicals in petroleum

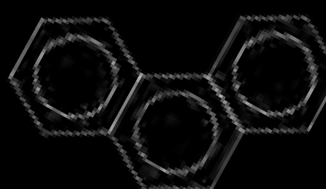


naphthalene

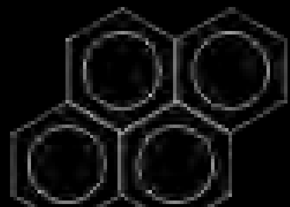
fluorene



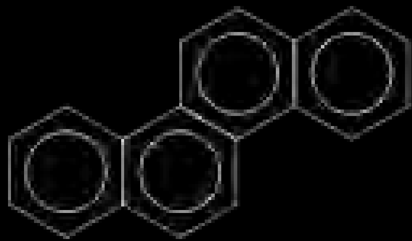
dibenzothiophene



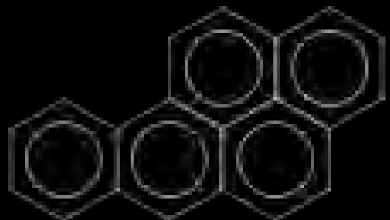
phenanthrene



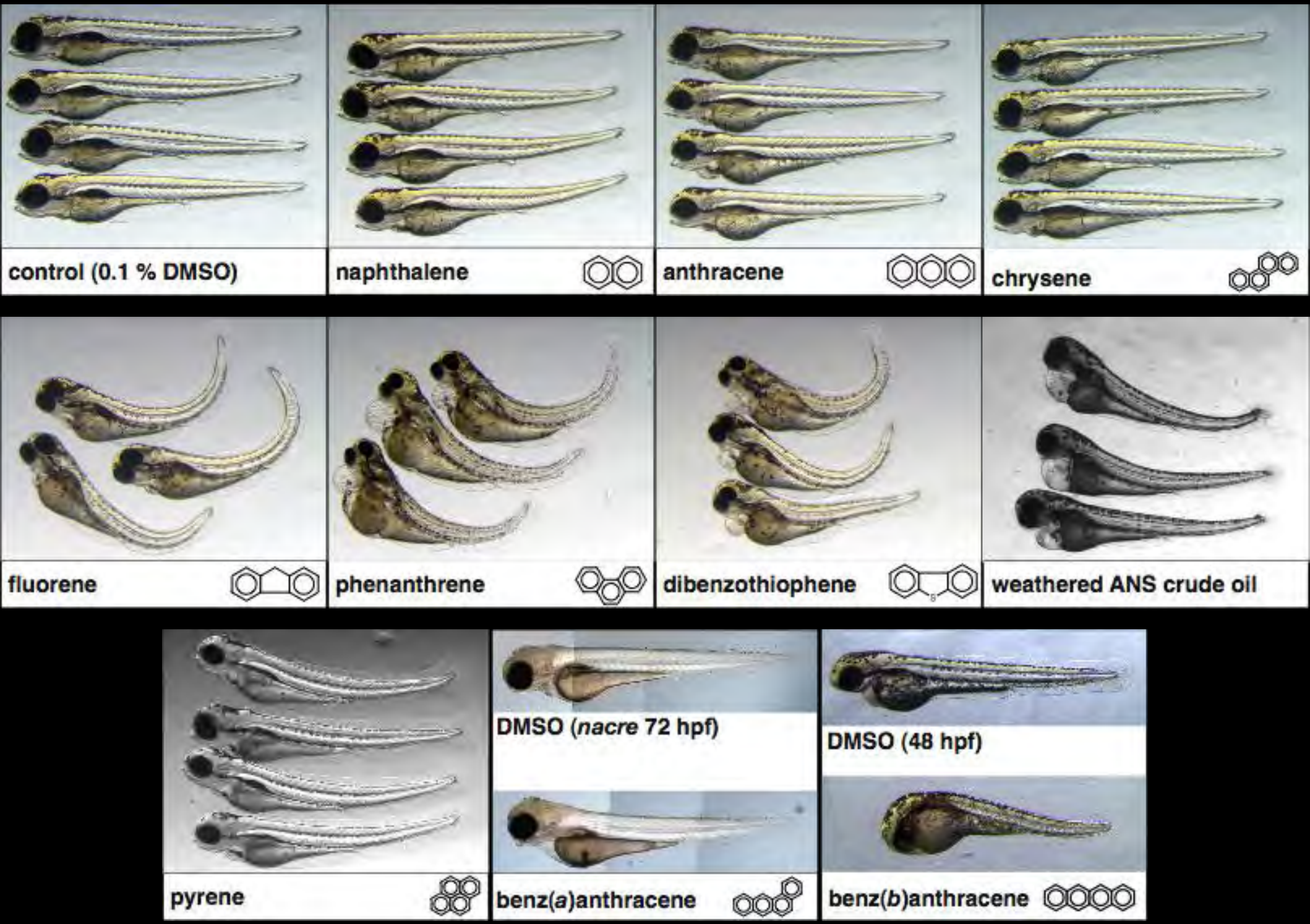
pyrene



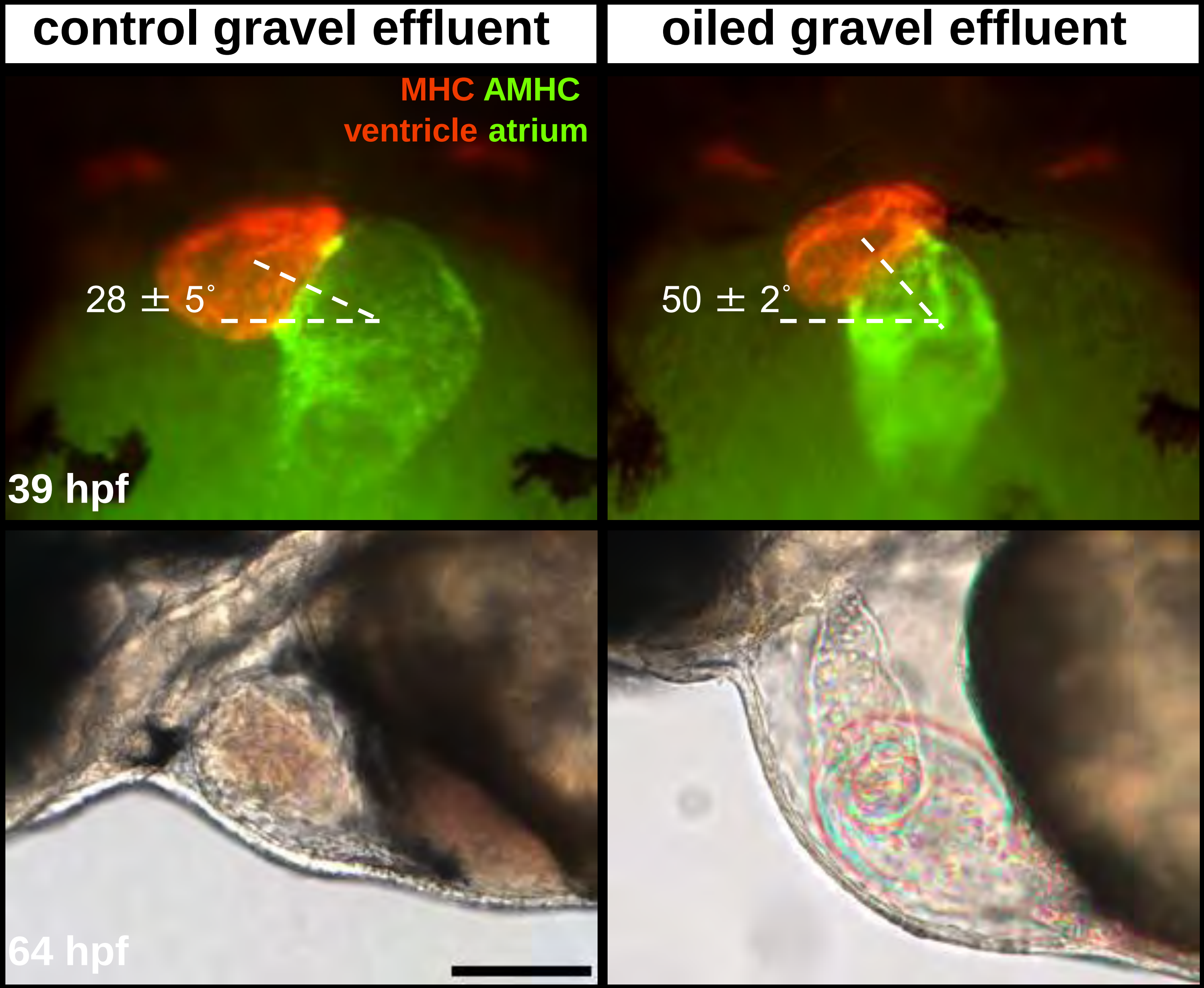
chrysene



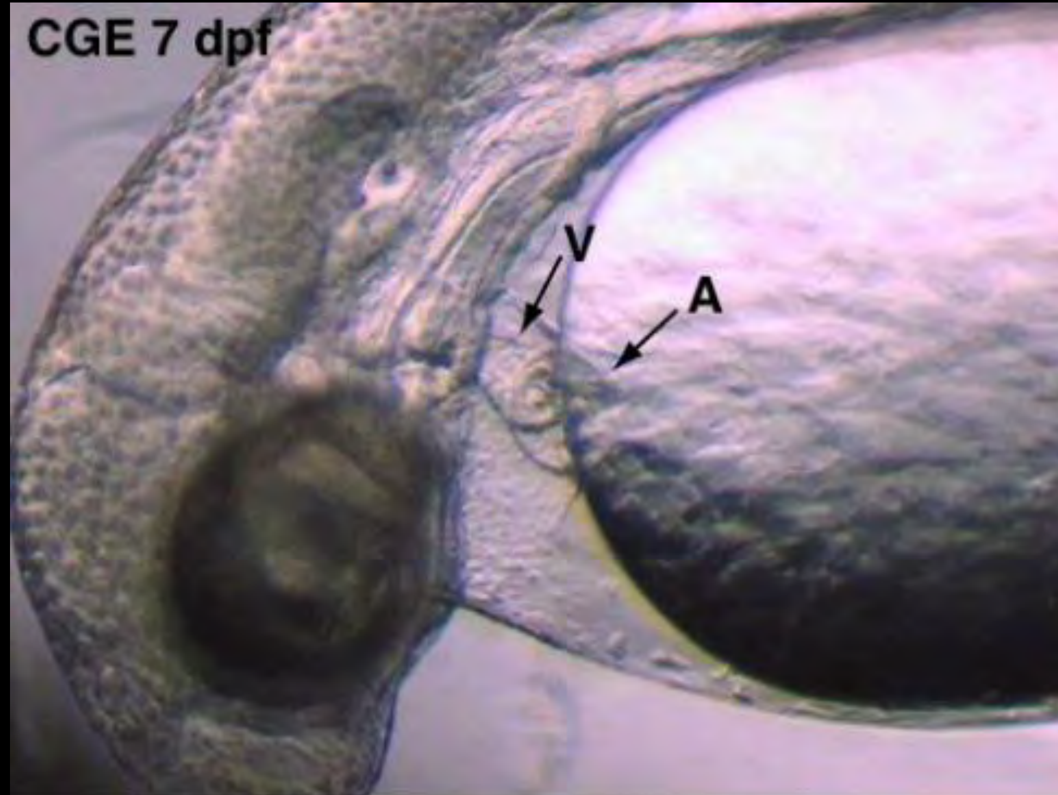
benzo(a)pyrene

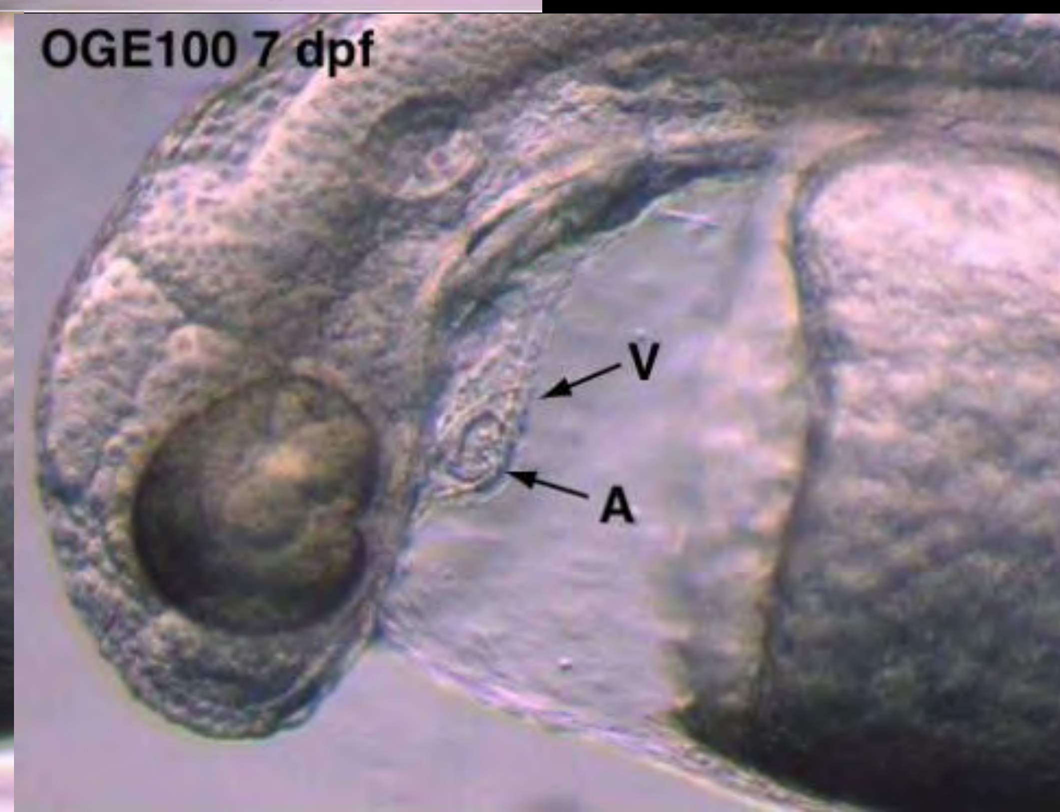

control (0.1 % DMSO)
naphthalene
anthracene
chrysene
fluorene
phenanthrene
dibenzothiophene
weathered ANS crude oil
pyrene
DMSO (nacre 72 hpf)
benz(a)anthracene
DMSO (48 hpf)
benz(b)anthracene

Weathered crude oil causes early cardiac "looping" defects in embryos (zebrafish)

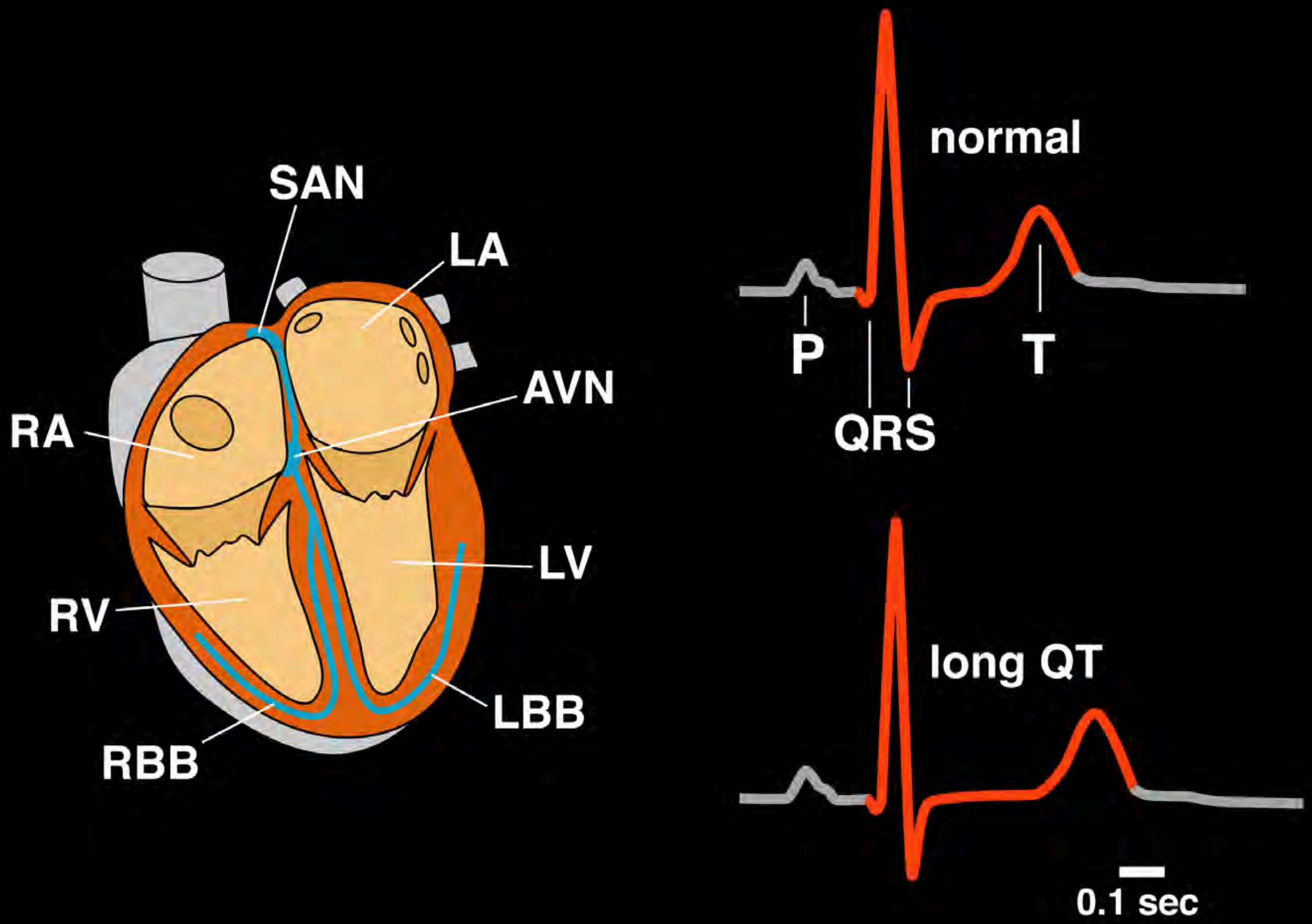


same effects in herring



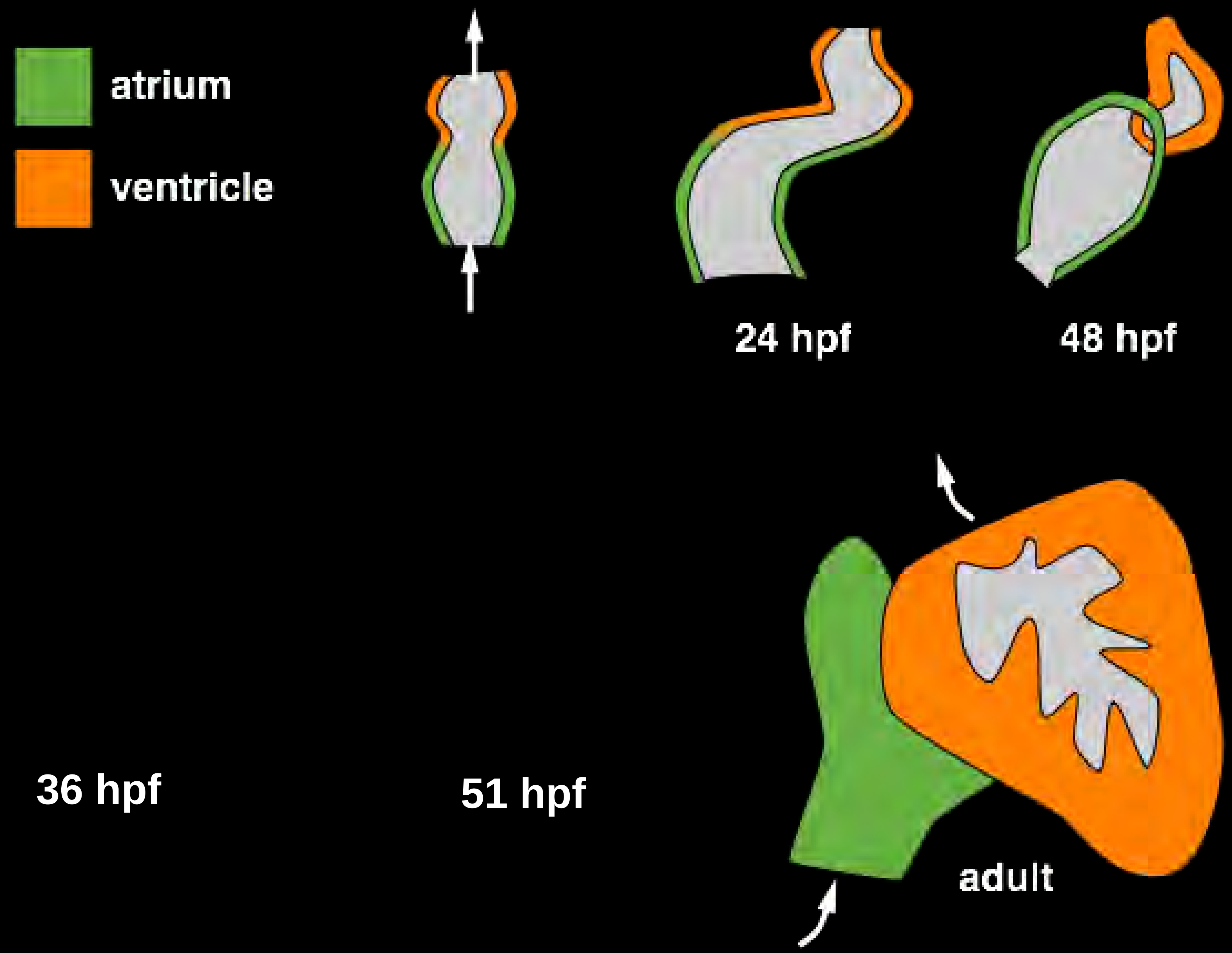


PAHs disrupt the electrical activity of the heart



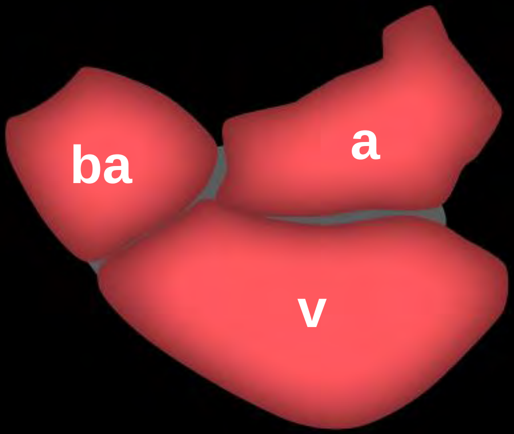
after Keating and Sanguinetti, Cell 104:569

Cardiac function and form are inextricably linked



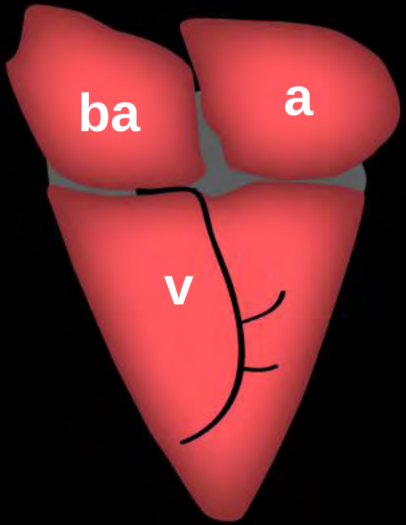
Why heart shape matters for fish

slow fish: burst swimming



saccular ventricle

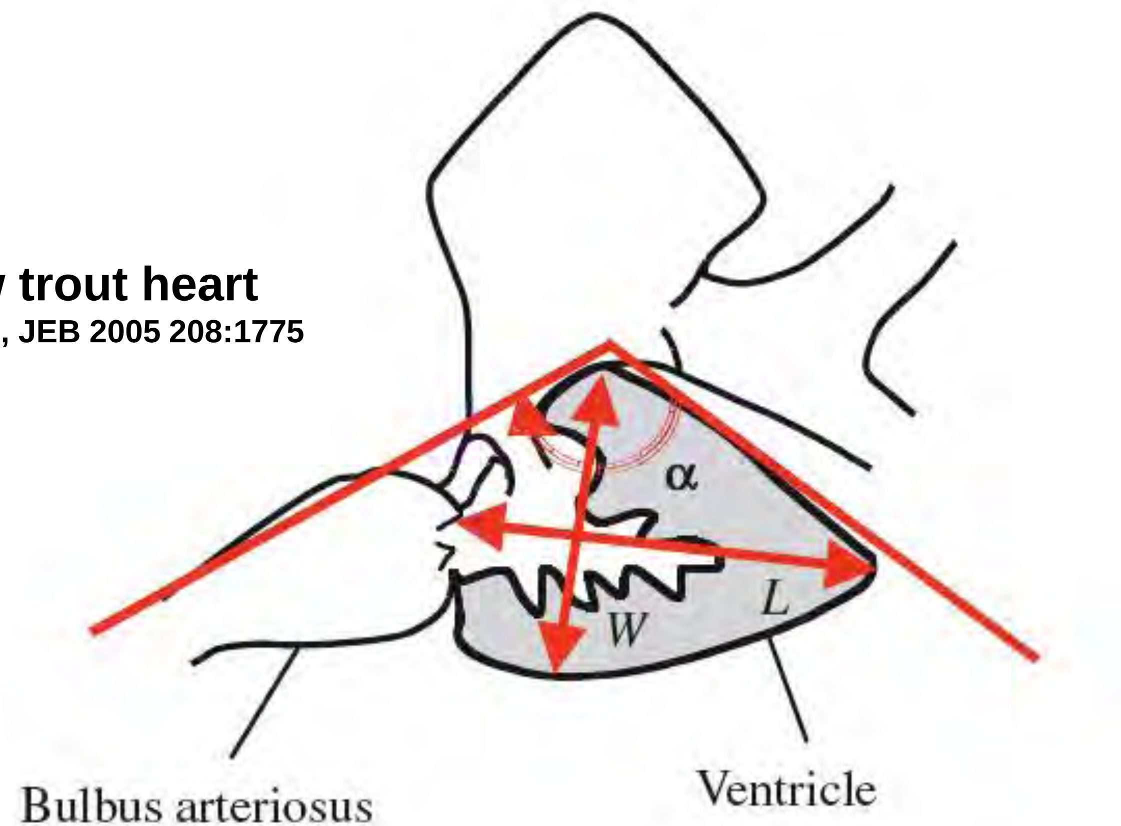
fast fish: sustained swimming



pyramidal ventricle

Heart shape really matters to fast-swimming fish

rainbow trout heart
Claireaux et al., JEB 2005 208:1775



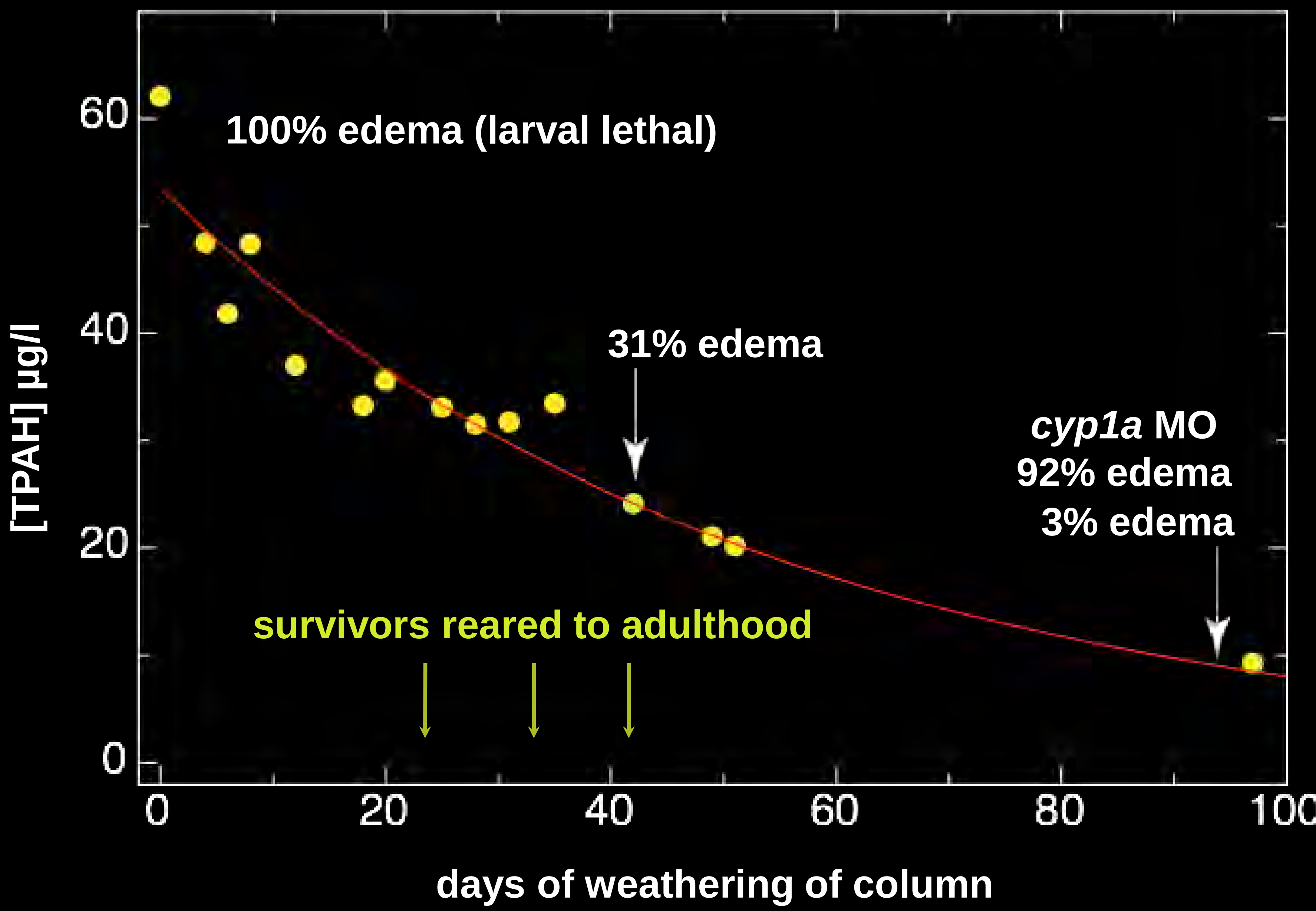
	Good swimmers	Poor swimmers
Angle (deg.)	154±4	153±5
Length (cm)	1.17±0.04	1.06±0.04
Width (cm)	1.16±0.04	1.21±0.05
Length/width ratio	1.01±0.01	0.88±0.04*

Values are mean ± S.E.M., N=9.
*Significant difference between groups (Student's t-test; $P<0.05$).

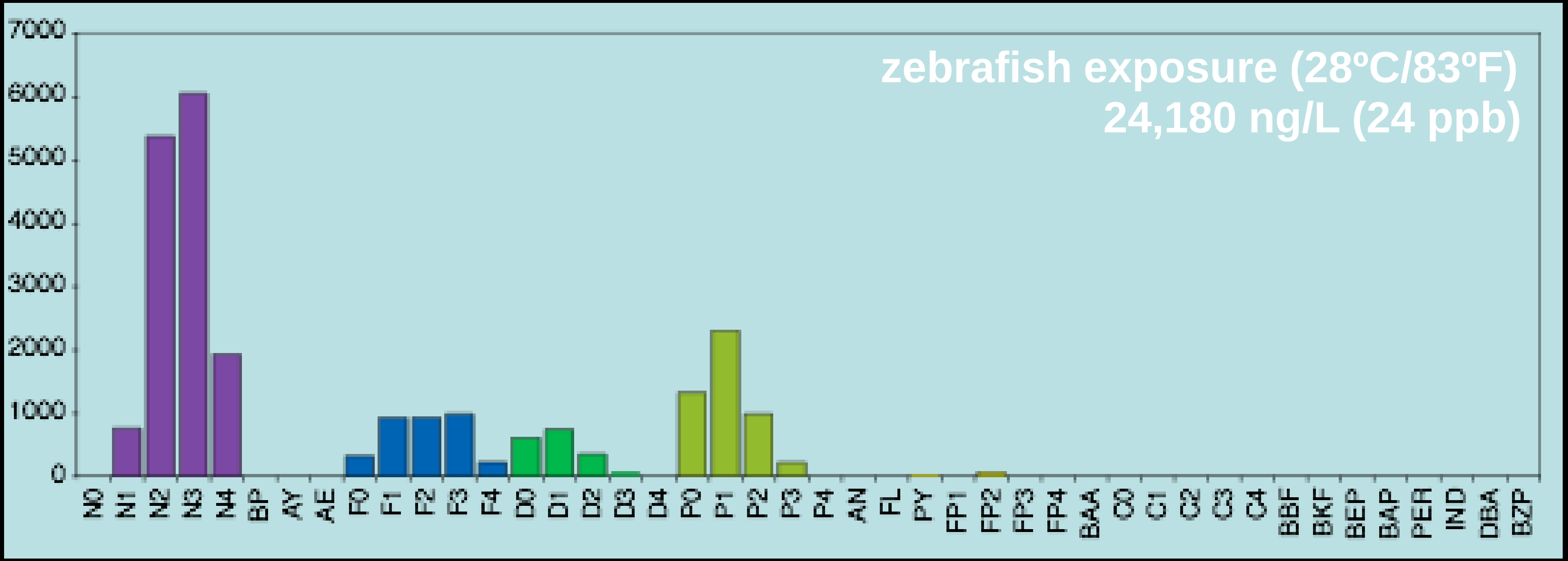
How changes in anatomy are linked to changes in gene expression (and how we get “biomarkers” out of this)

Oil-induced cardiac dysfunction in zebrafish embryos

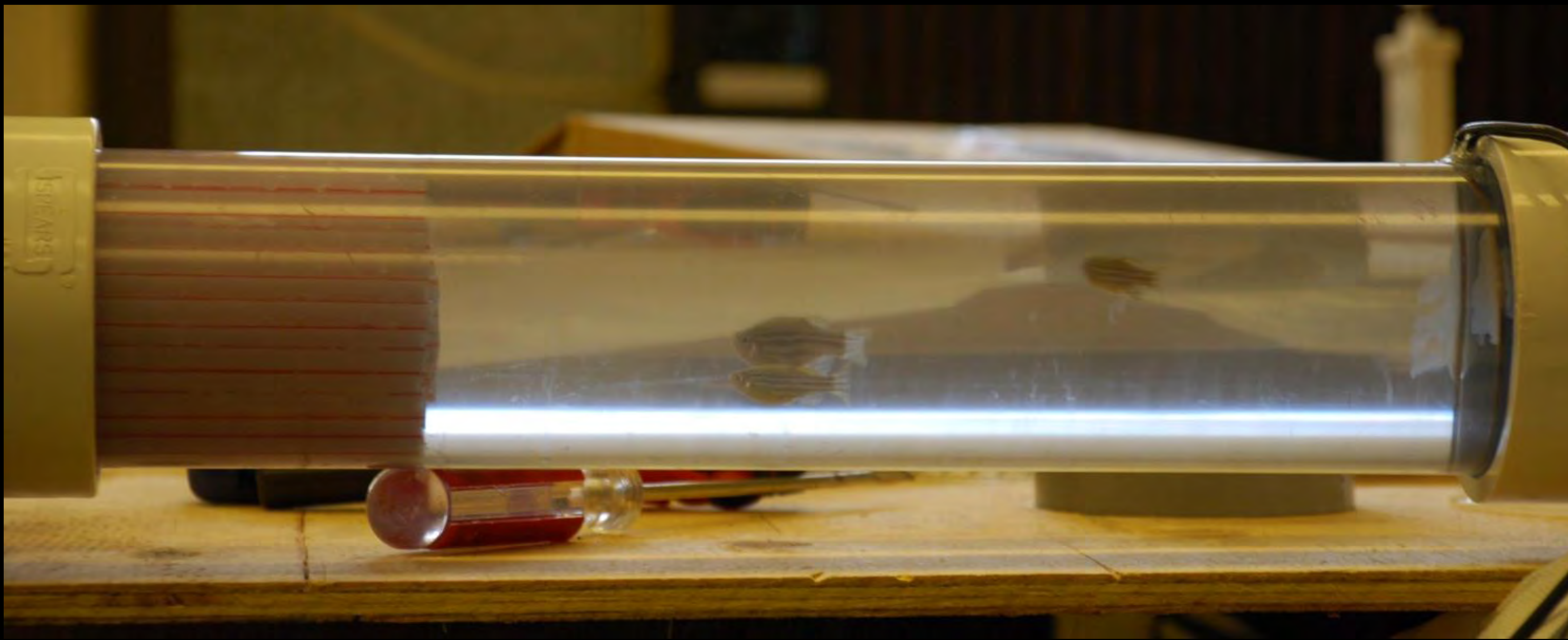
exposed from 4-48 hpf



Zebrafish exposure

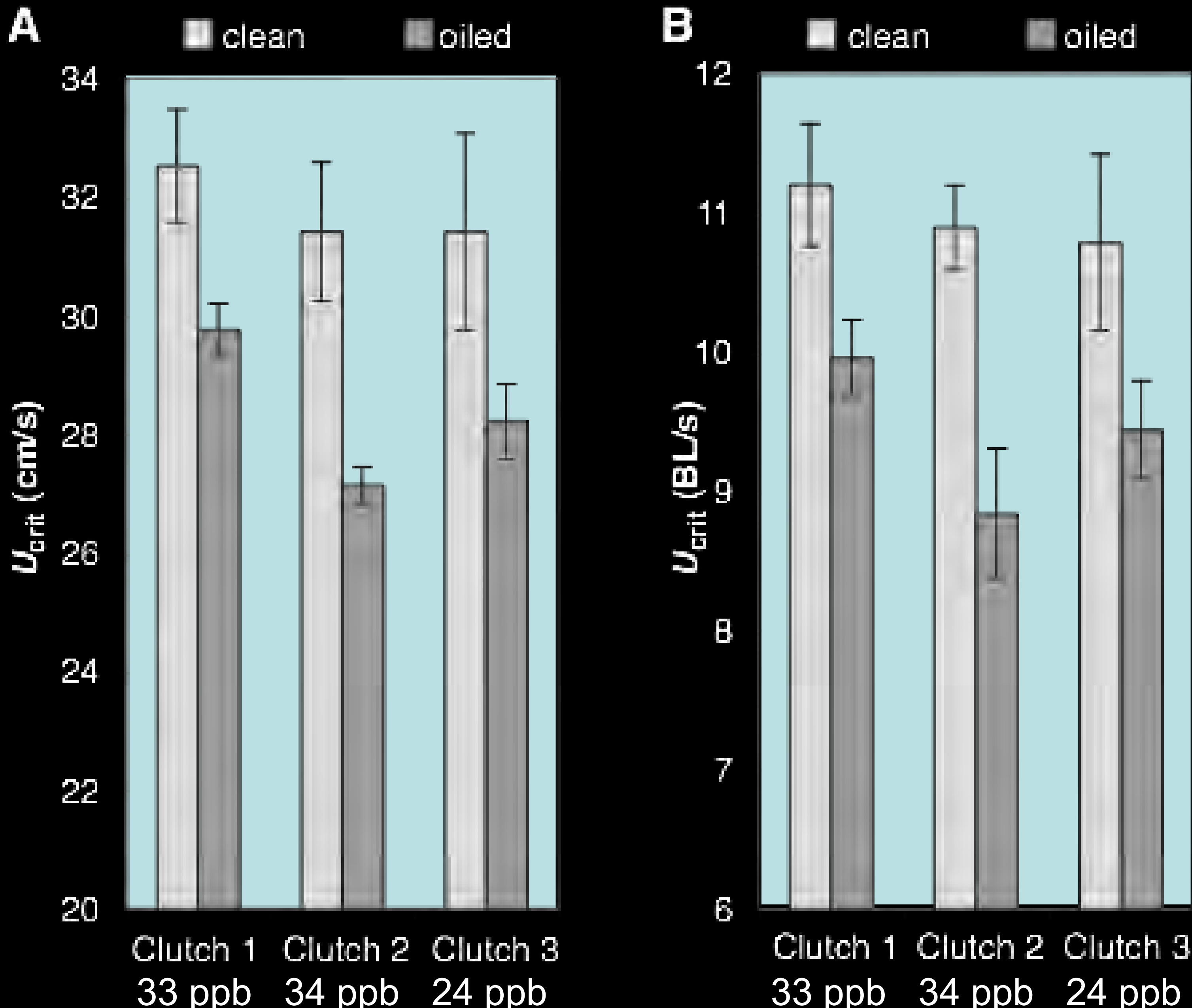


And one year later...
The fish “treadmill”



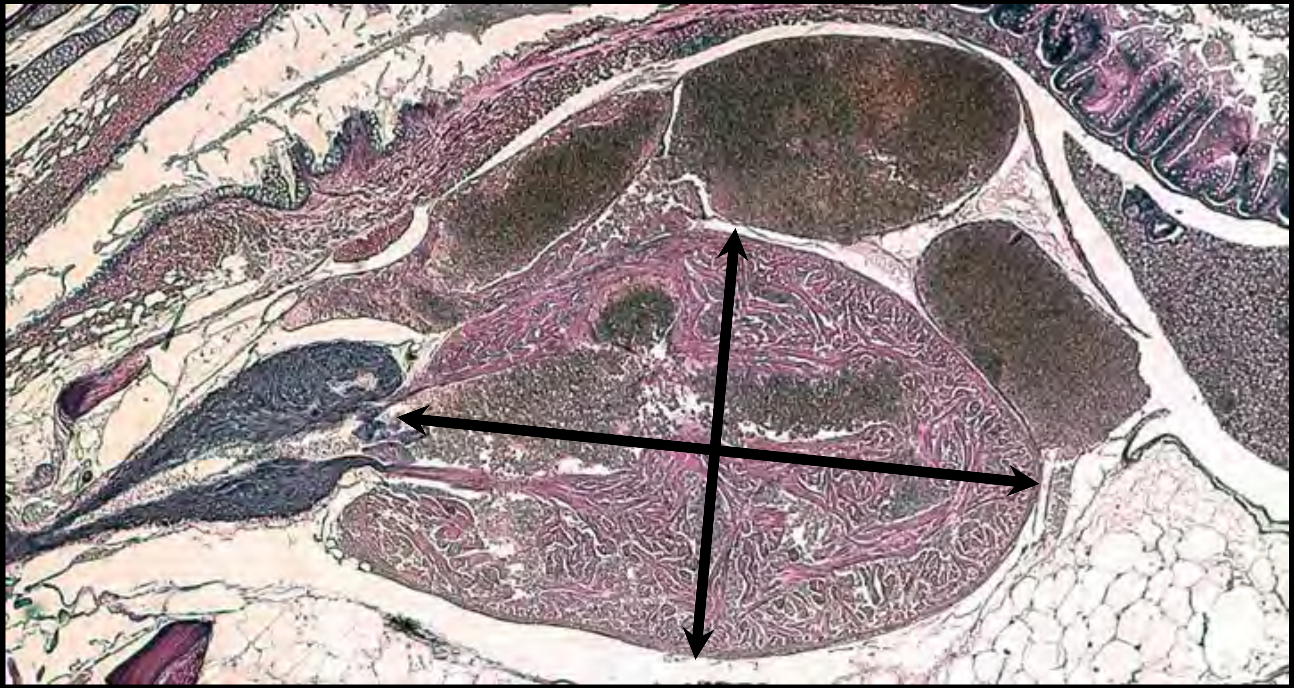
Zebrafish among the fastest fish, capable of sustained swimming up 13 BL/s

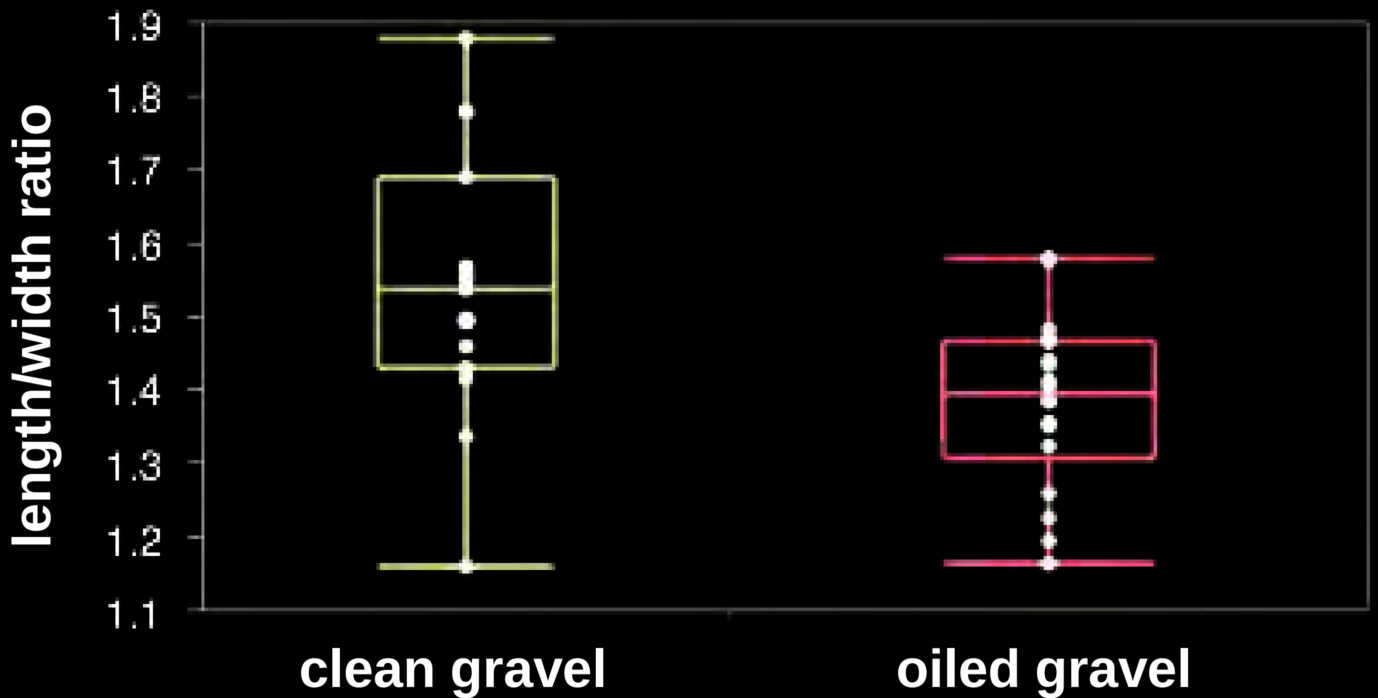
Adult fish exposed to oil as embryos are slower swimmers



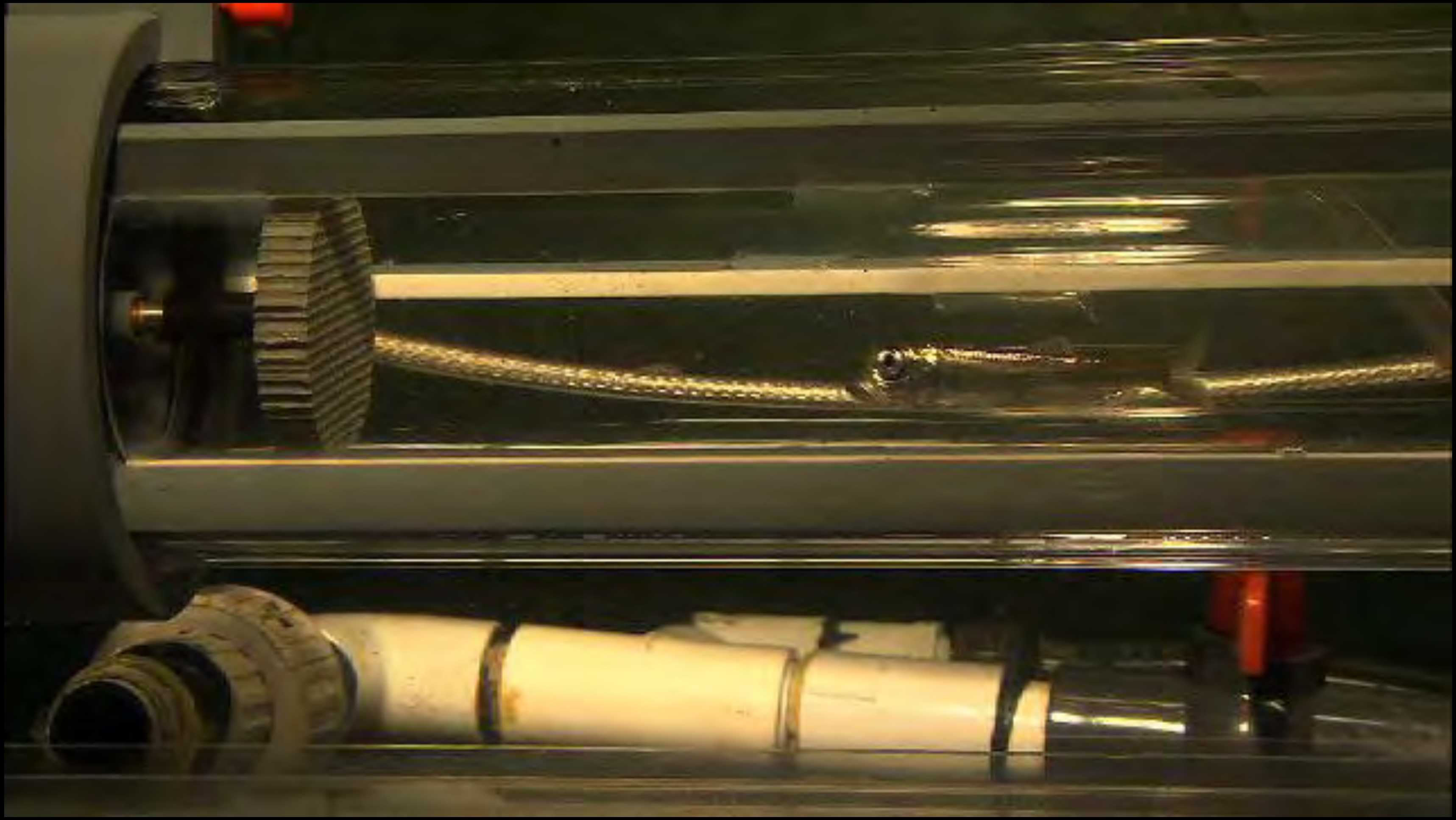
Cori Hicken, UAF-Juneau Center

Adult fish exposed to oil as embryos have rounder hearts

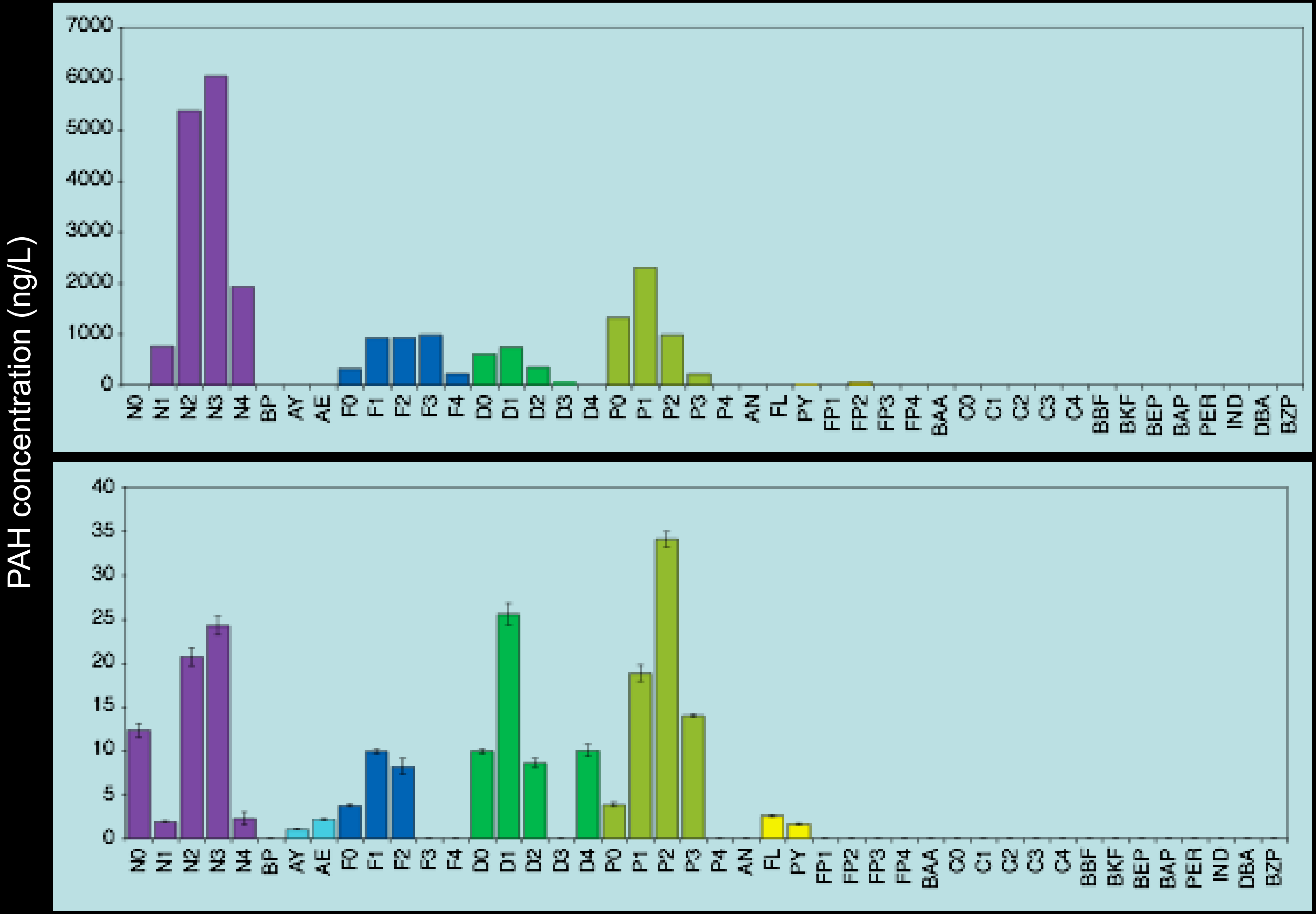




Our much nicer RCAC-funded Scandinavian-built swim tunnel

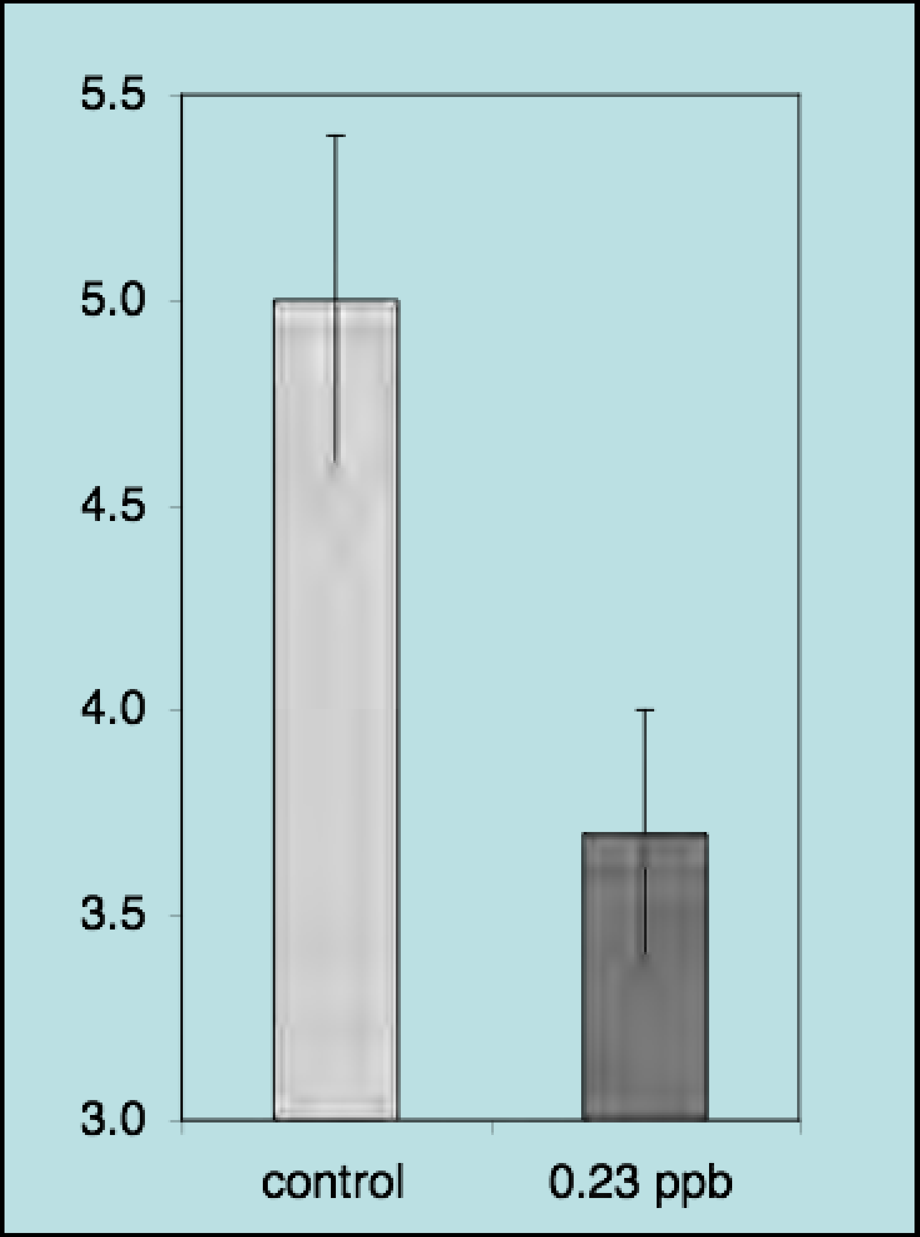


Exposure: zebrafish vs. herring

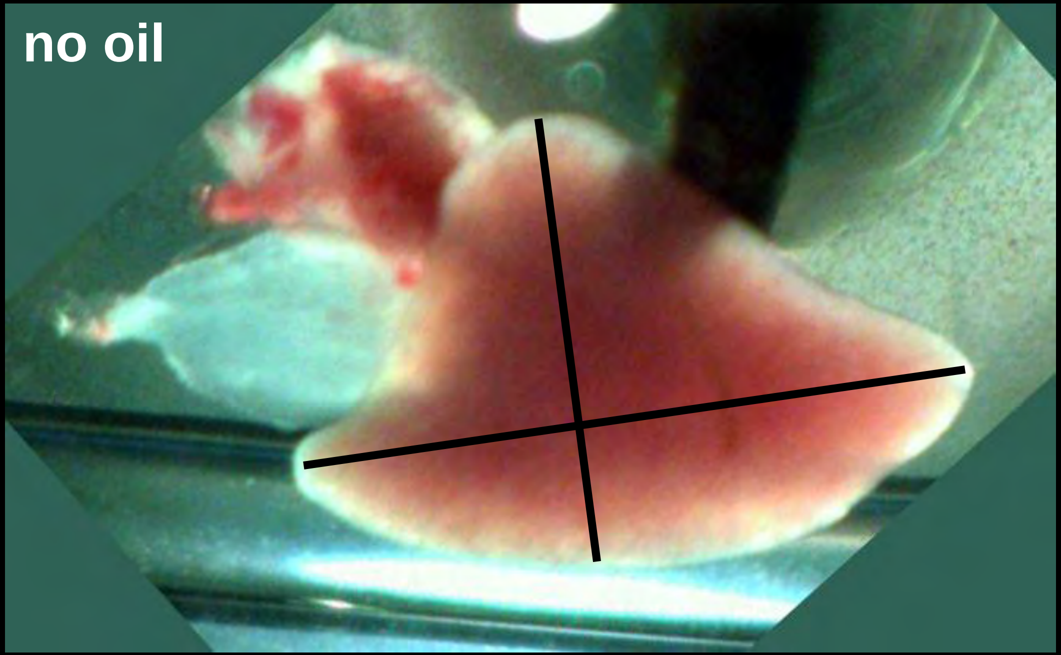


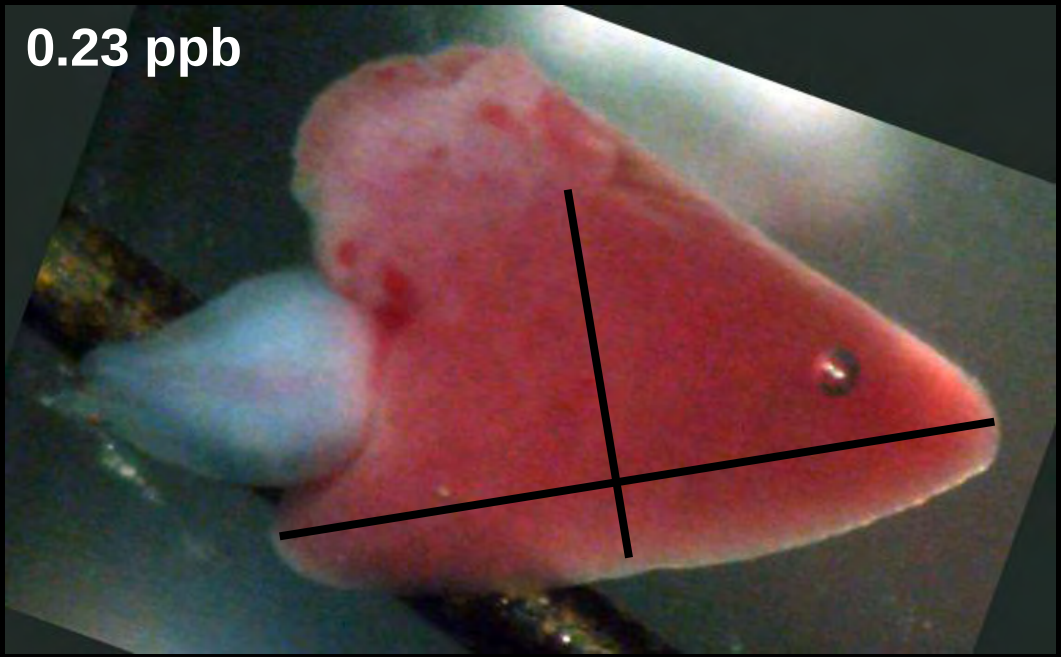
Reduced swimming speed in 7 month old juvenile Pacific herring exposed as embryos

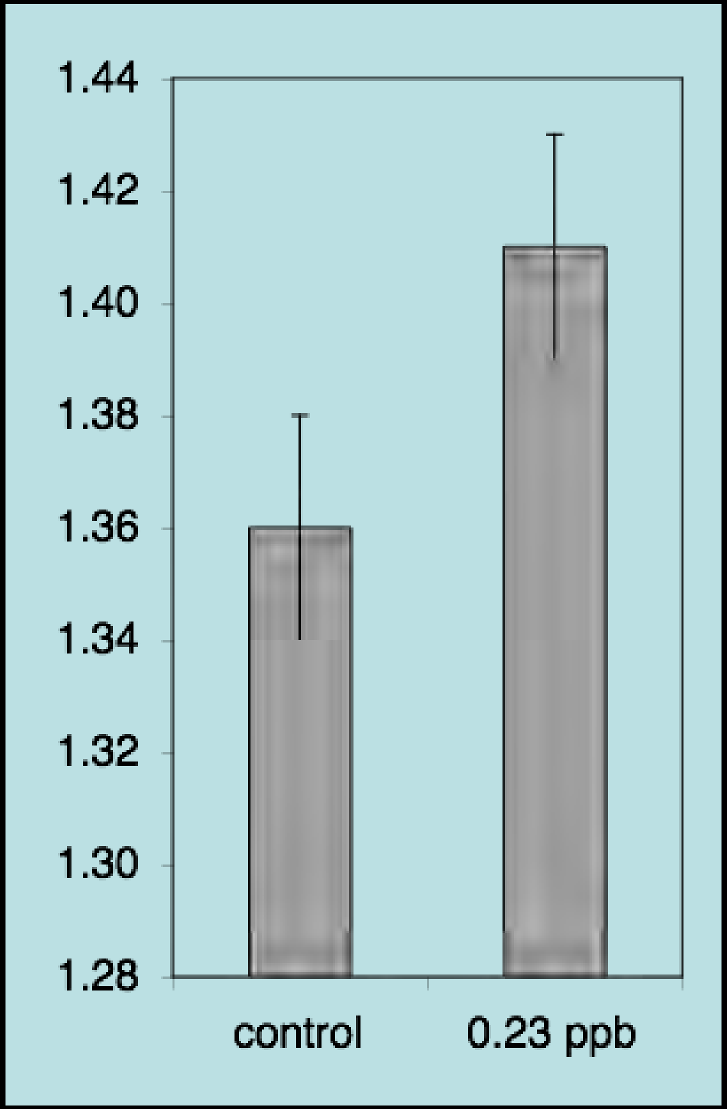
critical swim speed
(body lengths/sec)



Altered heart shape in juvenile Pacific herring exposed as embryos

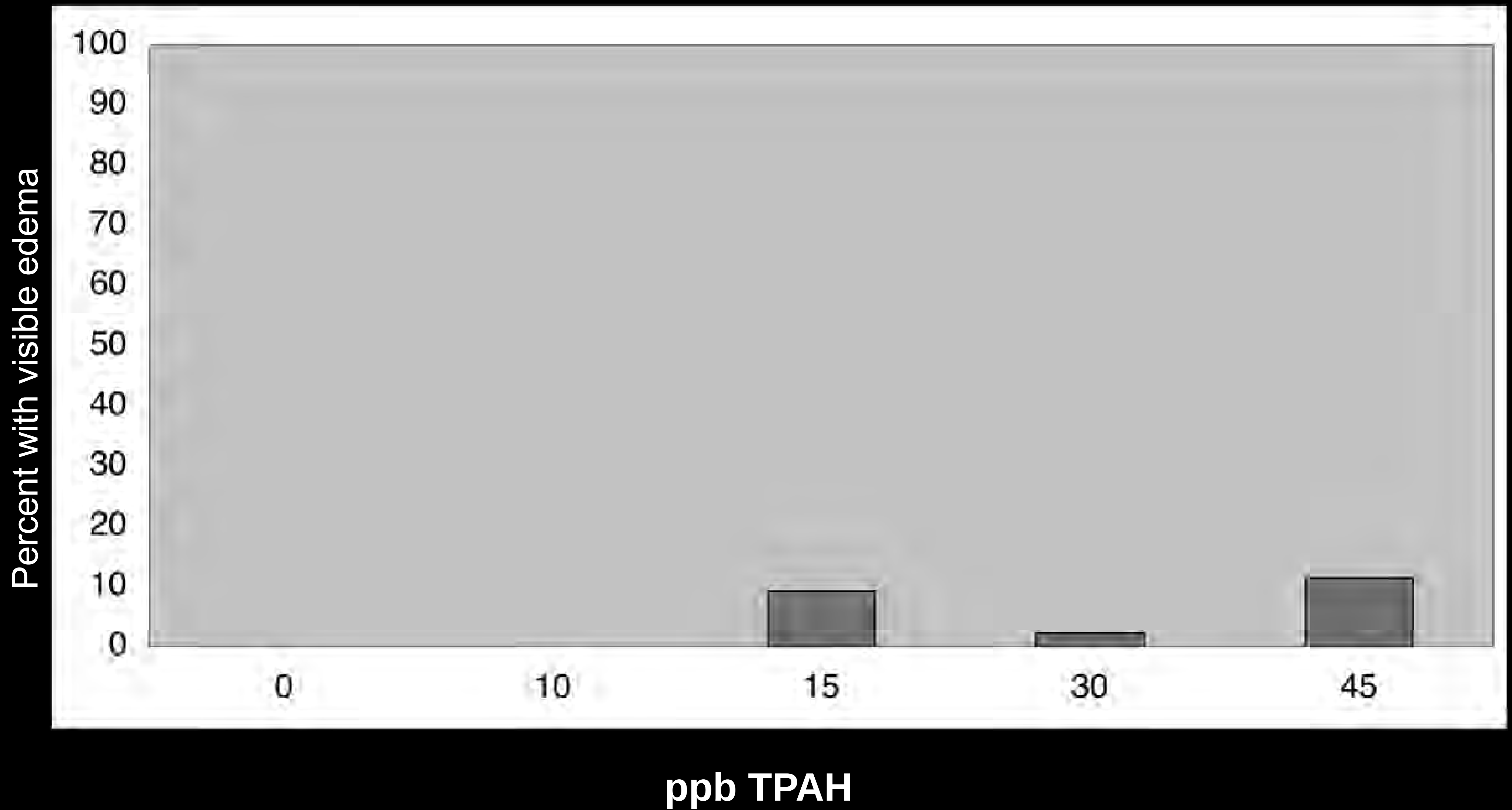






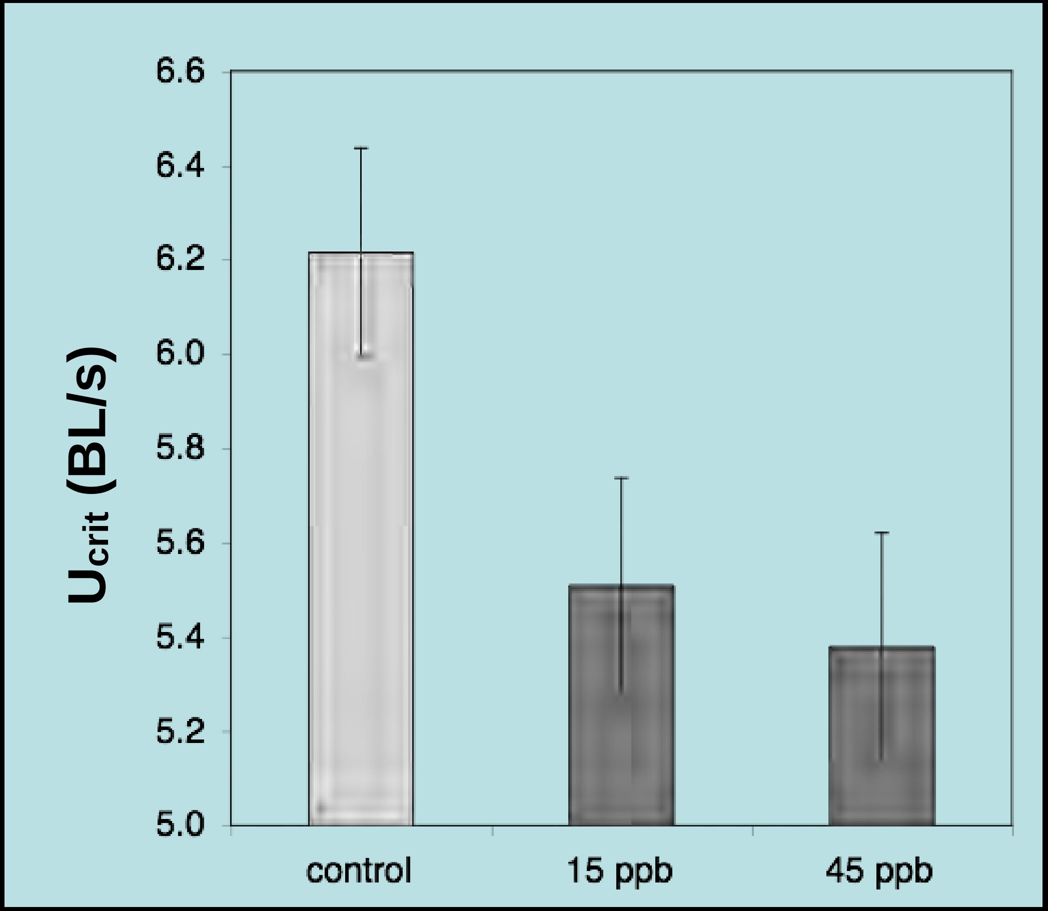
increased length-width ratio

Pink salmon exposure: edema at hatch (Nov 2010)

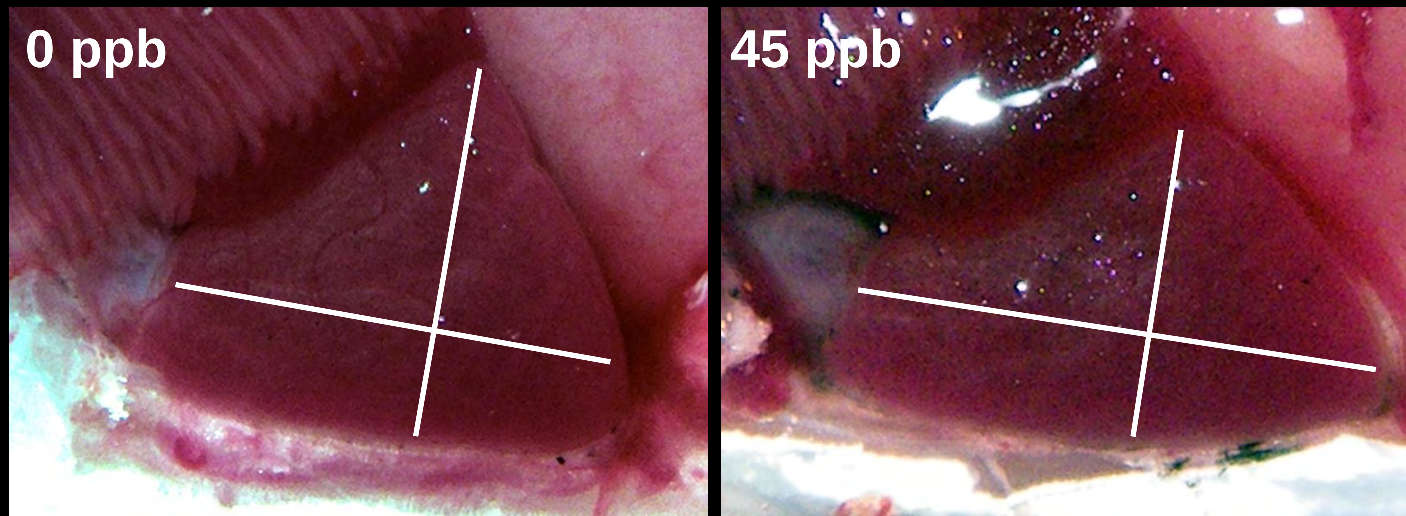


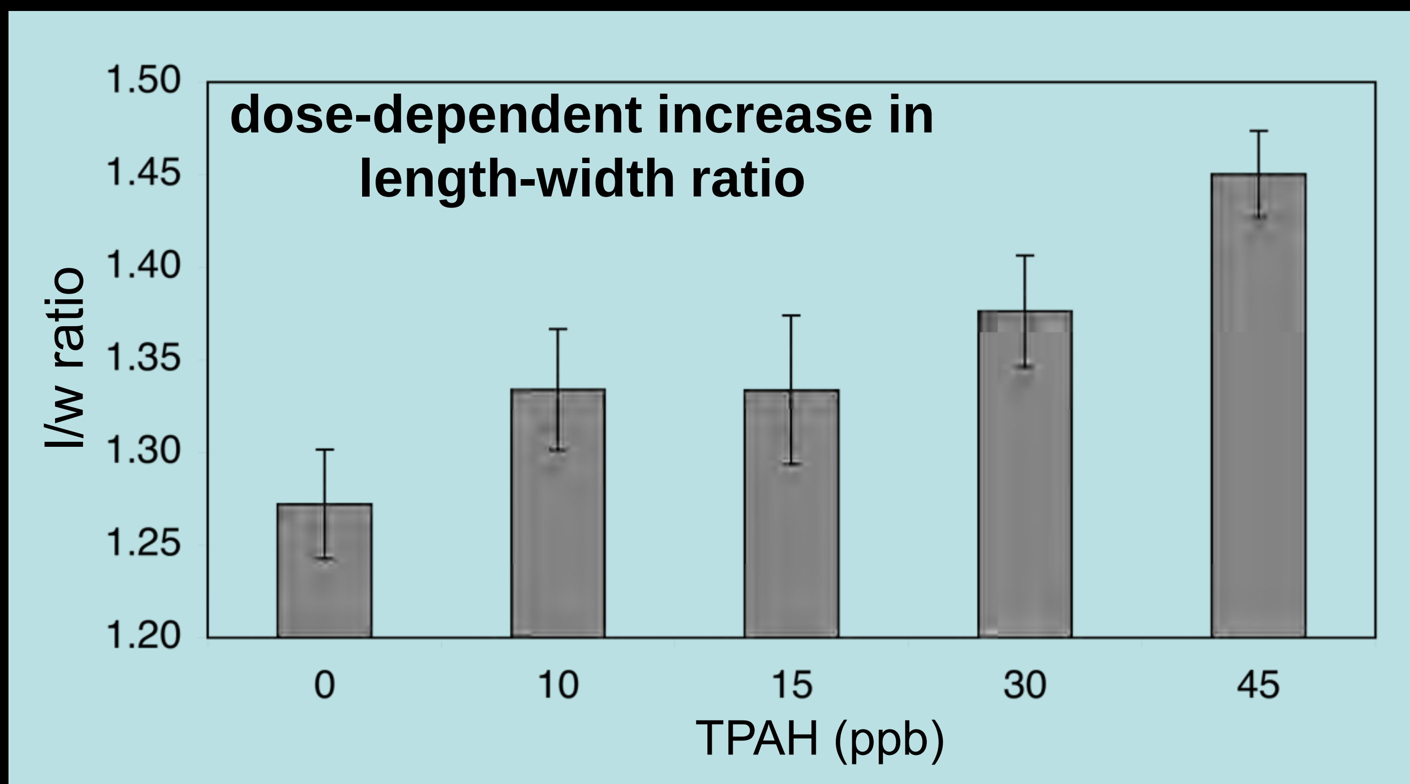
At low doses of PAH, only a small percentage of fish show edema from heart failure. These animals have clearly visible heart defects.

Reduced swimming speed in 9 month old juvenile Pink salmon exposed as embryos for 8 days

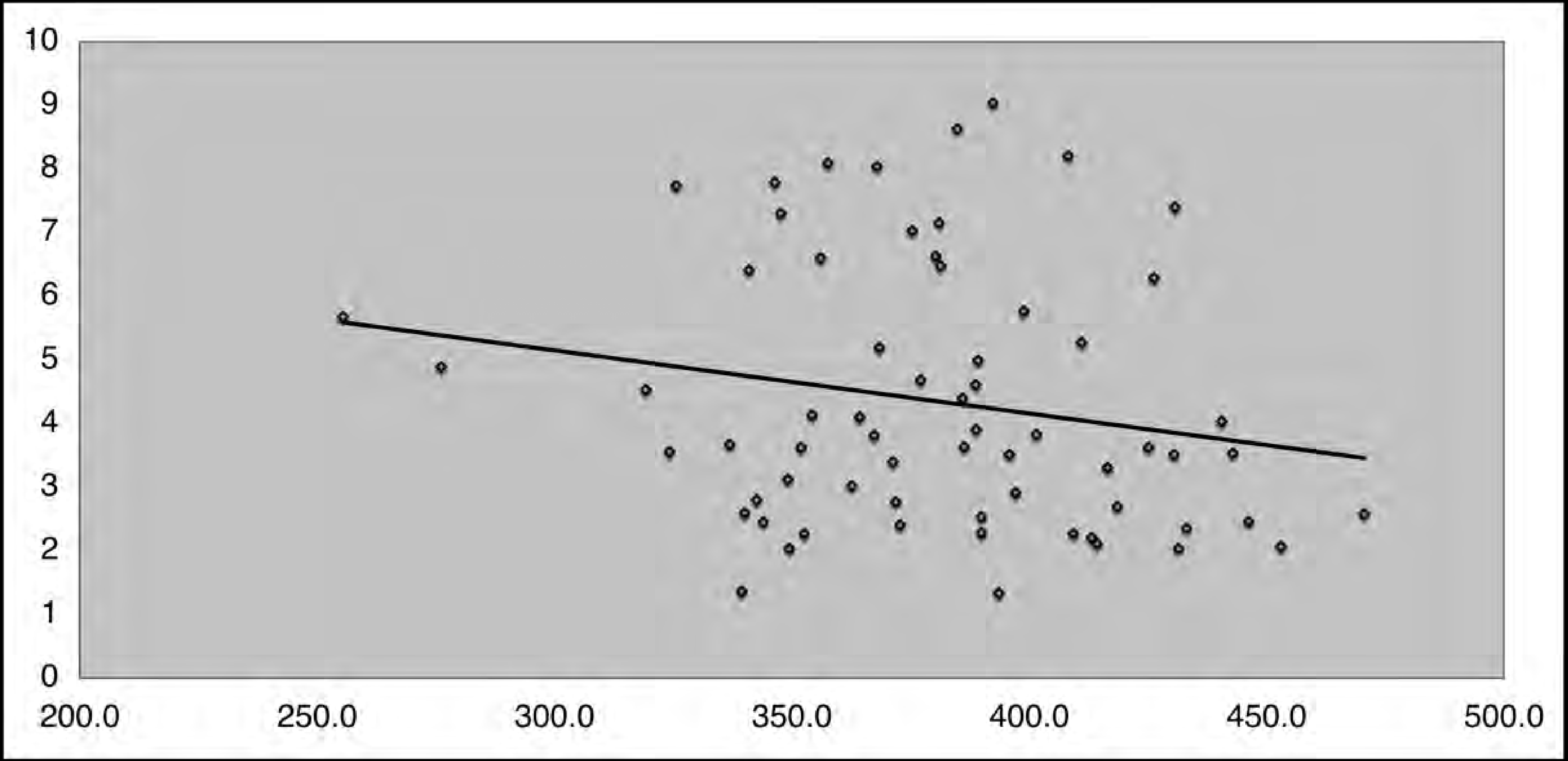


Altered heart shape in juvenile pink salmon and Pacific herring exposed as embryos





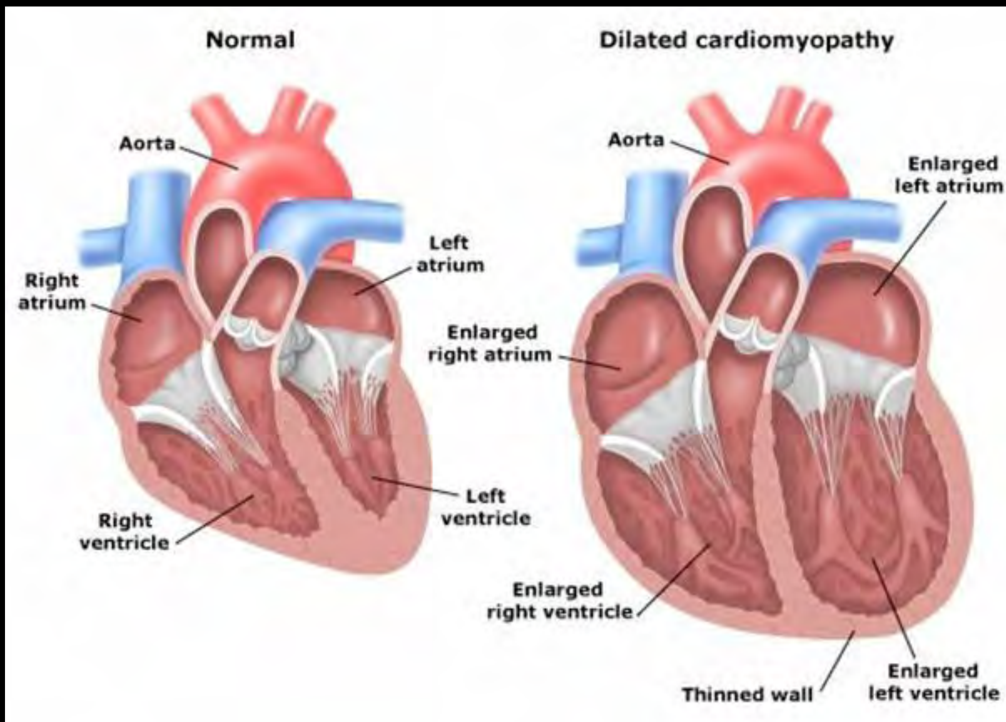
Longer hearts - slower swimming

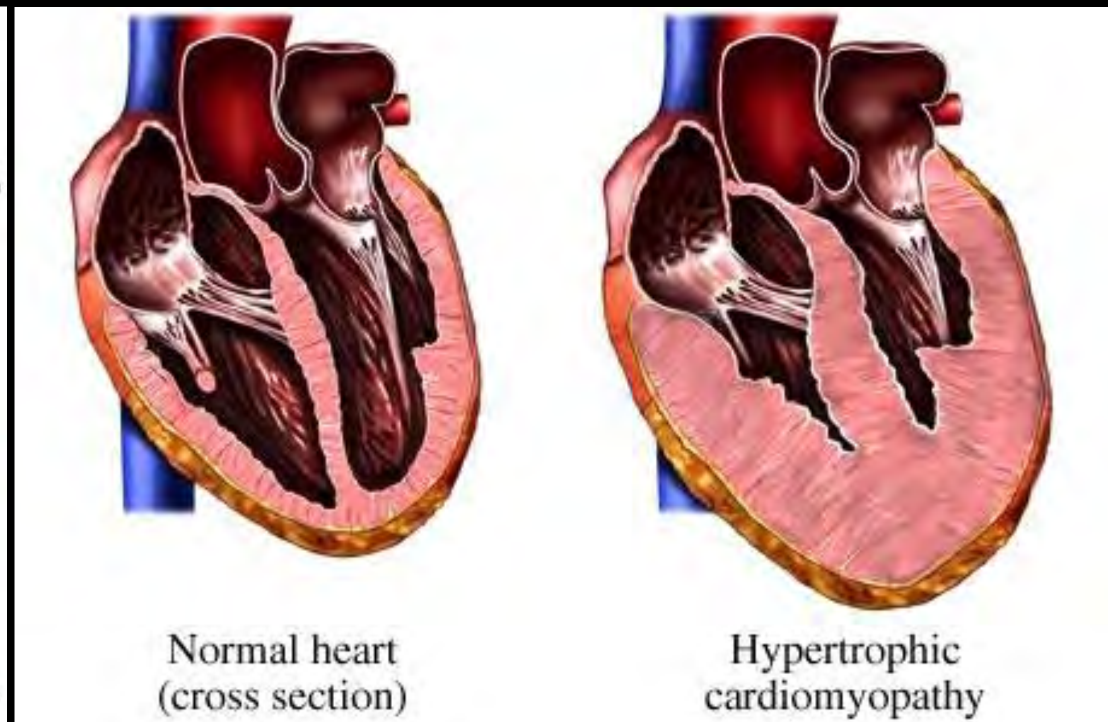


Critical swim speed vs. ventricle length in juvenile herring

Taking a tip from human heart disease

Hearts under stress enlarge by either of two pathways

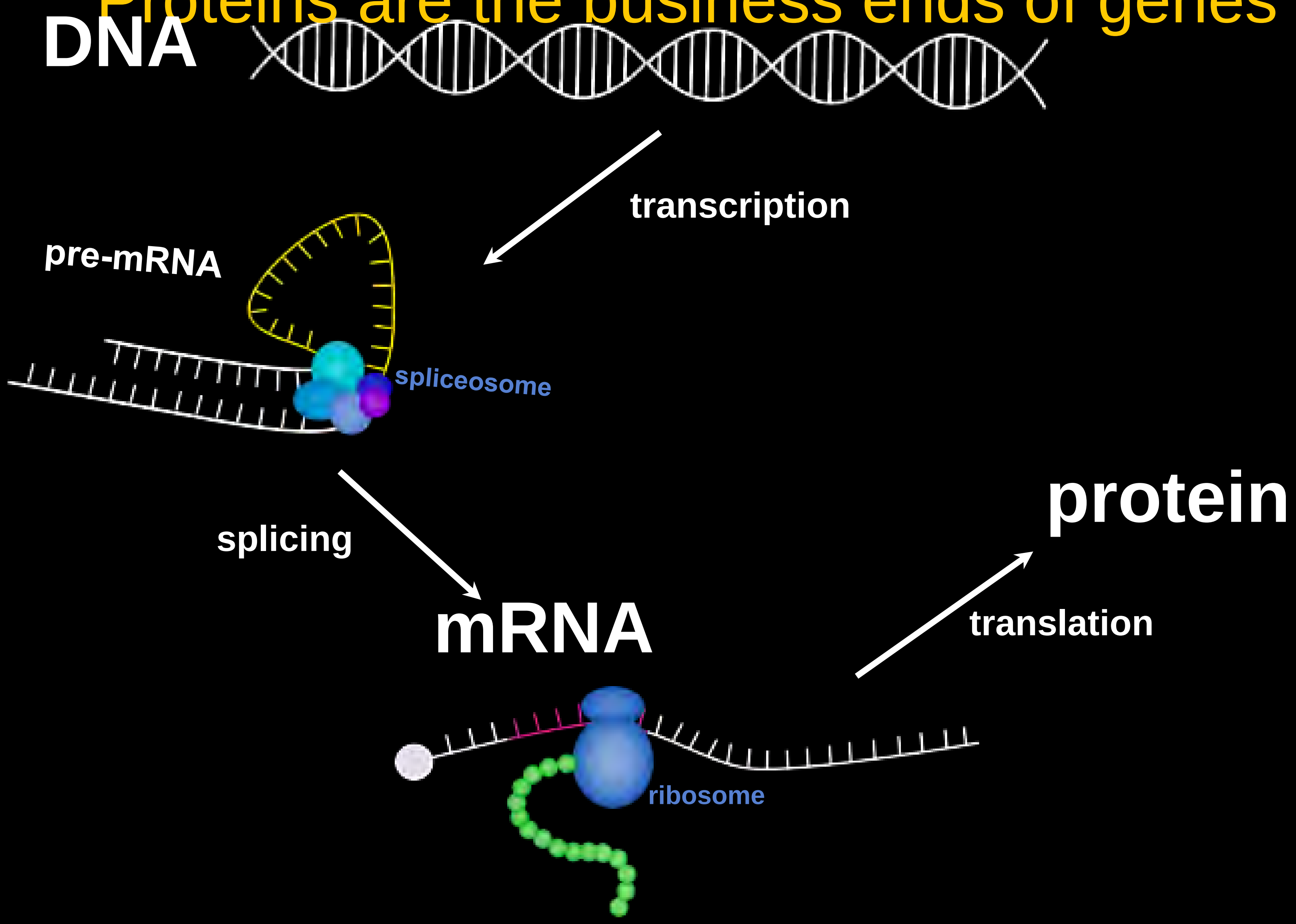




dilated cardiomyopathy **hypertrophic cardiomyopathy**

If elongated juvenile hearts are weaker, they are likely to compensate over time and lead to hypertrophy in adults

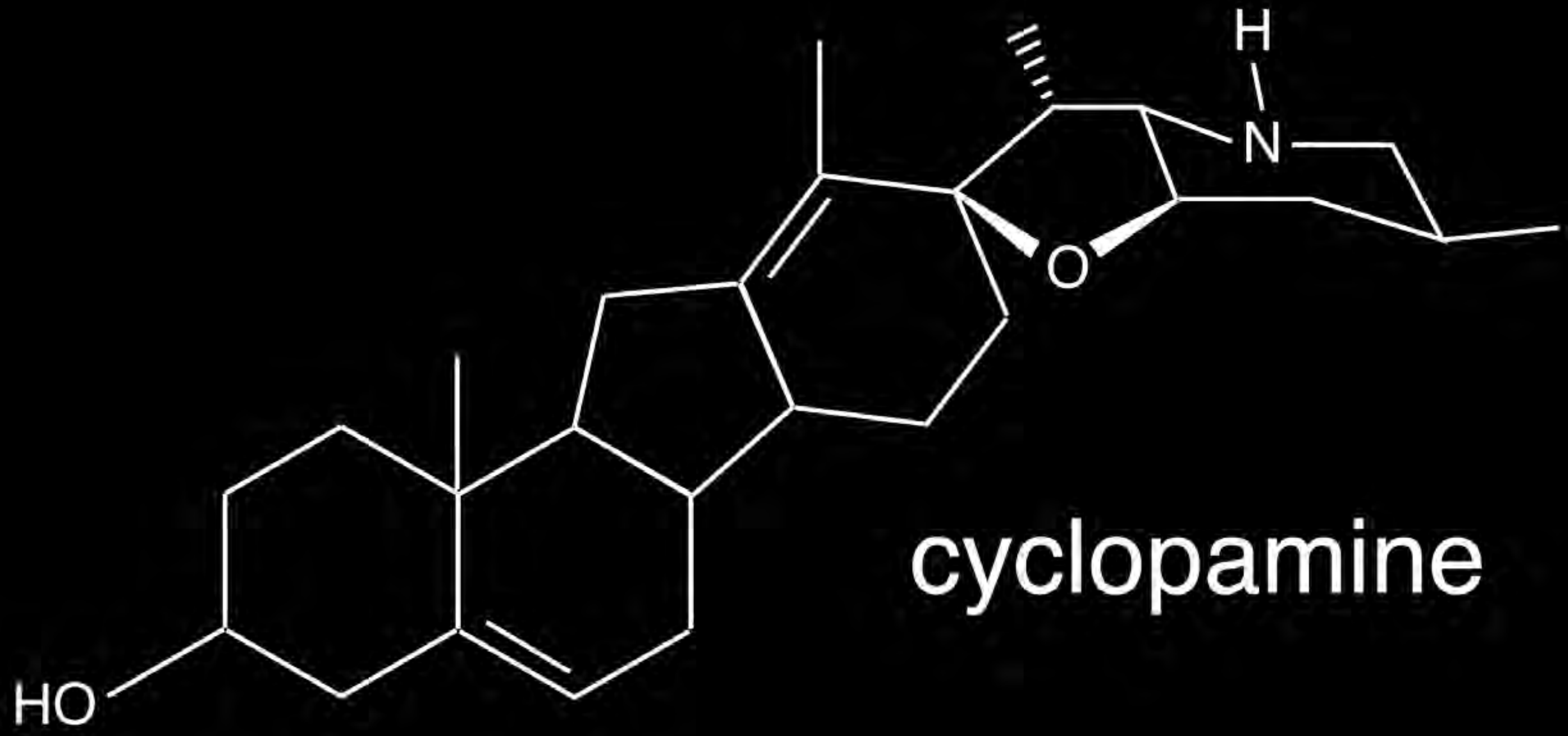
“Gene expression”: Genes make RNA, RNA makes protein
Proteins are the business ends of genes





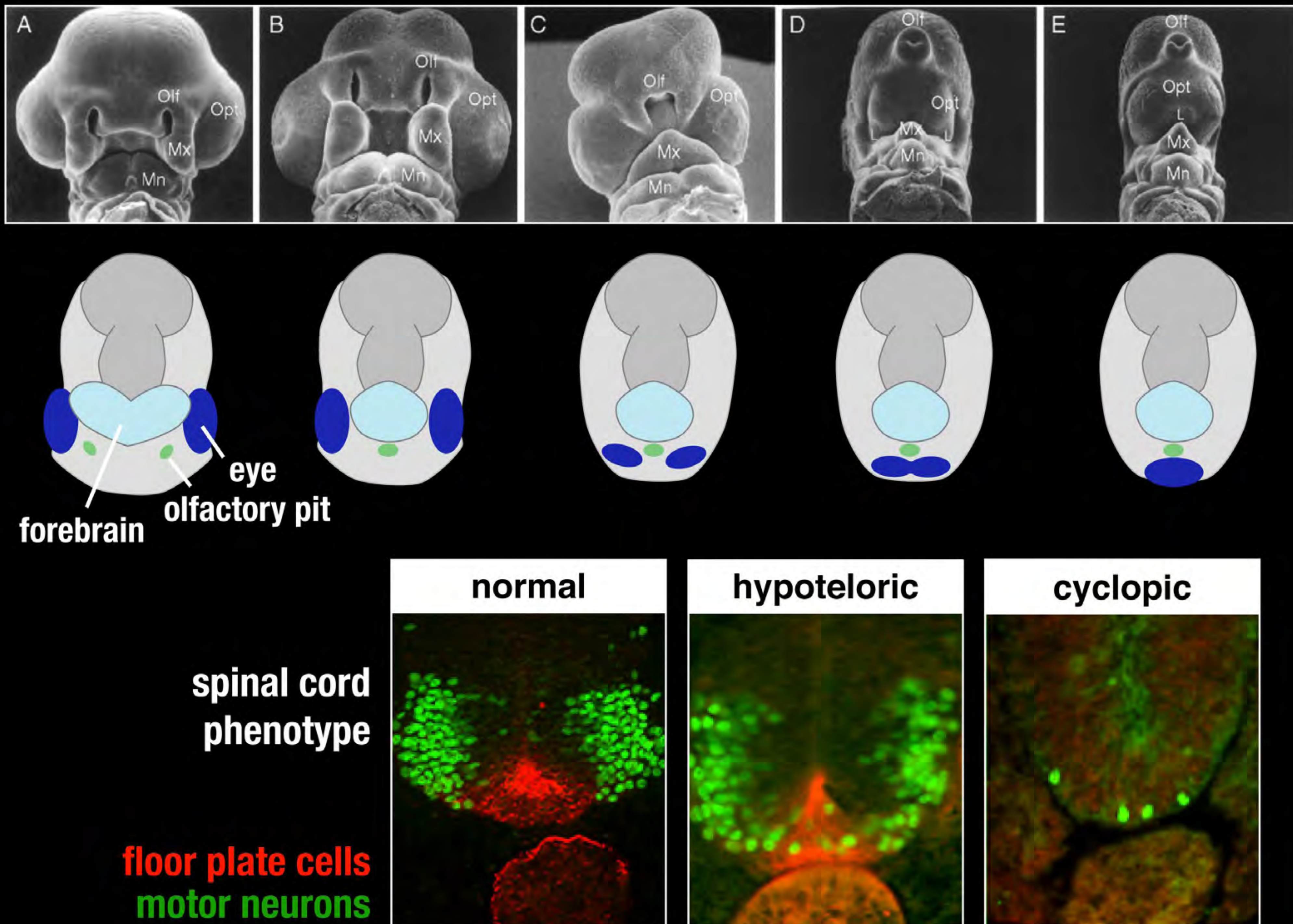
Corn lily, false hellebore

Binns, James, Keeler, et al.
USDA Poisonous Plant Research Lab
Logan Utah



cyclopamine

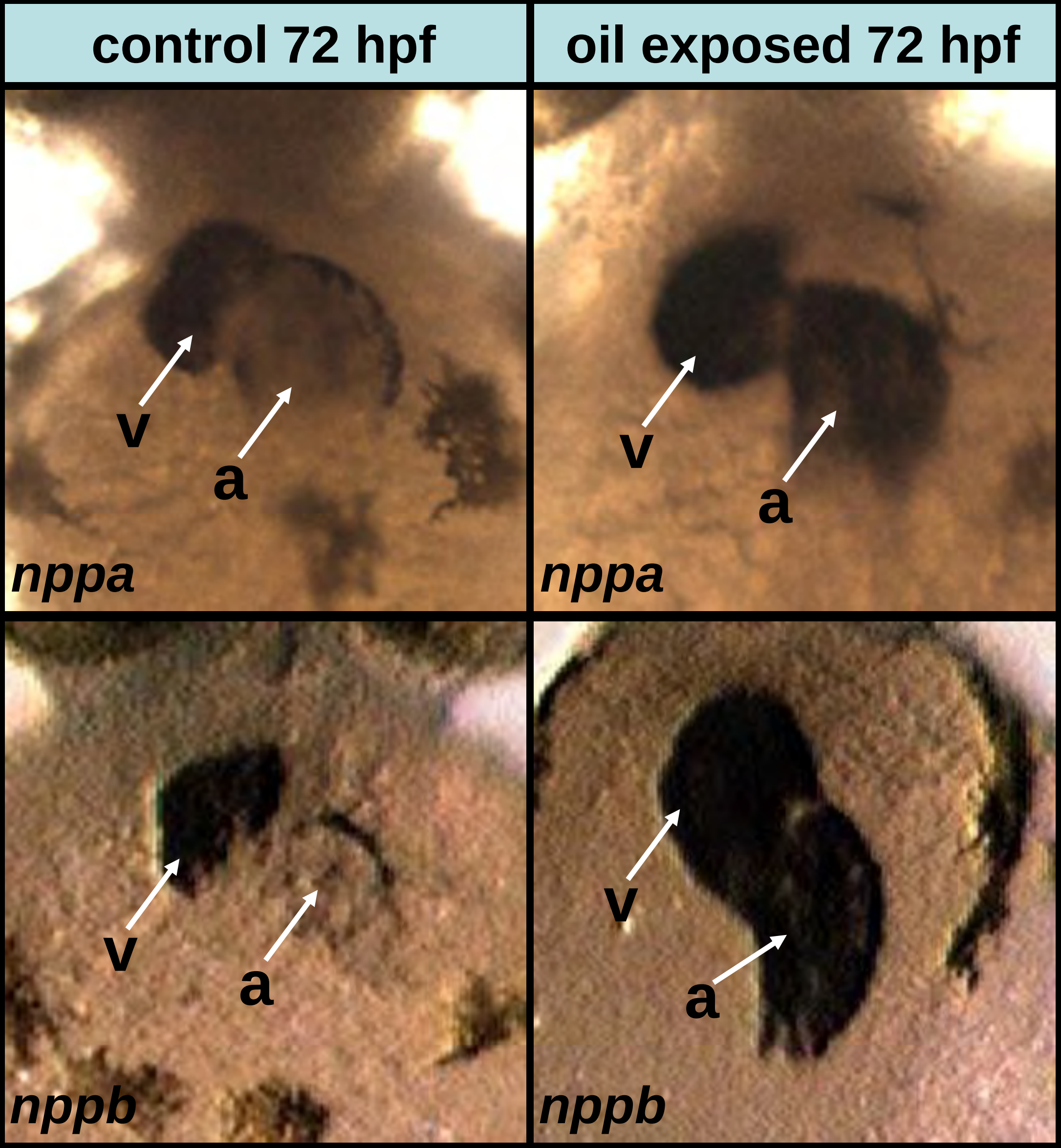
Cyclopamine blocks Sonic Hedgehog signaling during neural tube patterning



Cardiac natriuretic peptides

- **Atrial (ANP), Brain or B-type (BNP), and Ventricular (VNP)**
- **NP genes are turned on during cardiac hypertrophy (excess growth in response to stress)**
- **blood BNP levels diagnostic and prognostic in human heart failure and cardiomyopathies**
- **evolved in fish (younger species and mammals lost VNP)**
- **osmoregulatory (regulate salt water retention) and regulate contractility**
- **may be cardioprotective, particular for wide pressure fluctuations seen by fish atrium**
- **Measure protein levels with antibodies, RNA levels by “quantitative PCR”**

Oil exposure acutely up-regulates natriuretic peptide gene expression

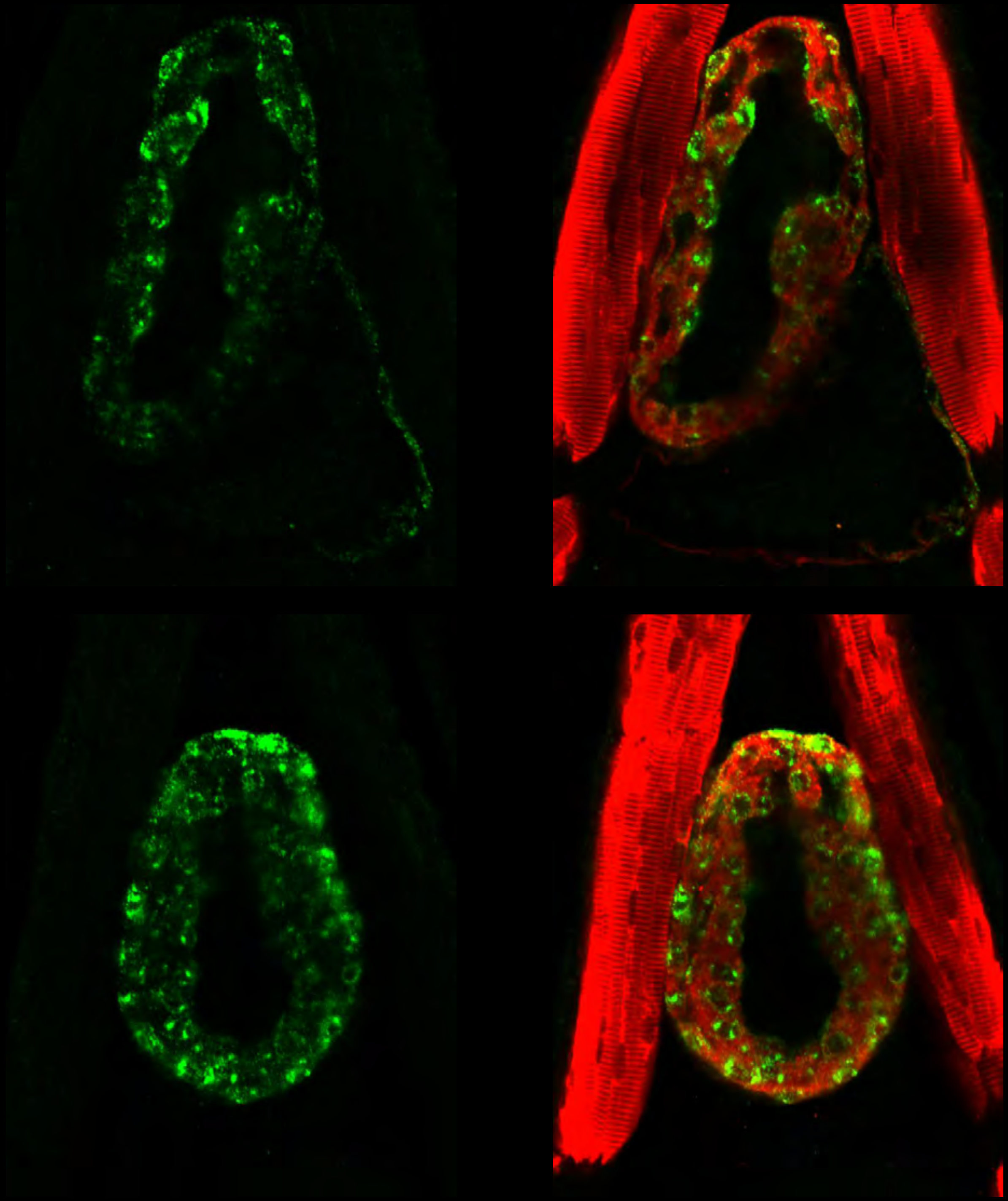


Chronic elevation of mature NPs after oil exposure ends

zebrafish

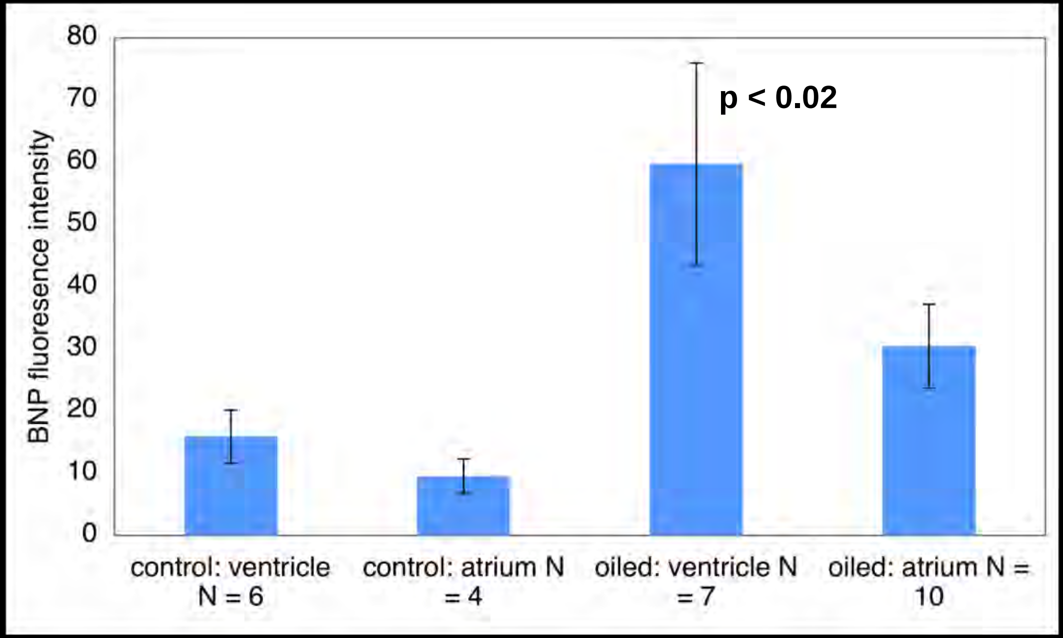
clean gravel 4-48 hpf
BNP/myosin labeled 5 dpf

oiled gravel 4-48 hpf
BNP/myosin labeled 5 dpf

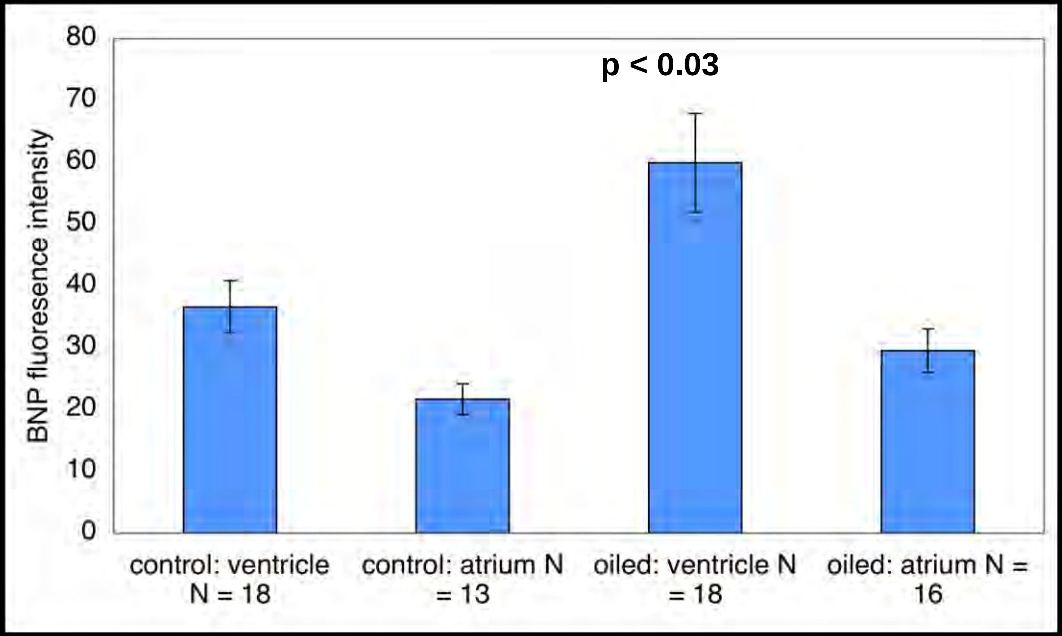


Quantification of BNP protein in embryonic/larval hearts

48 hpf - end of oil exposure



5 dpf - 3 days in clean water



Genes for ANP and BNP in hand for both herring and pink salmon

```
  herring ANP CCCCGAGCAAGACCTTCTCCCCCTGCTTCGGGCCAGACTGGACCGGATCGGAACCTCCA 397
     pink ANP CCAGGAGTAAAGCTGTGTCTGGGTGCTTCGGAGCCAGGATGGACCGGCATCGGGACCTCAA 397
    trout ANP CCAGGAGTAAAGCTGTGTCTGGGTGCTTCGGAGCTAGGATGGACCGGCATCGGGACCTCCA 406
zebrafish ANP CCCGAAGCAAAAGCTTGTCTGGCTGTTTTGGGGAAGGCTGGATCGGCATAGGGTCTTCCA 209
              ** * ** **     * ** *  ** ** ** *  **  **** ** ** **  * ** *

  herring BNP ACGACTCG------AACAGATACTCCGGATCTTTCGGCAGACCGATGGACAGAATCGCAT 382
     pink BNP ATGACTCAAGATCAAAGAGATACTCGGGCTGCTTCGGCGAAGGATGGACCGAATCGGCT 376
    trout BNP ACGACTCAACATCAAACAGATACTCCGGCTGCTTCGGCCGAACGATGGACCGAATCGGCT 376
zebrafish BNP AAGACTCAAAG---AAGAAAAACTCGGGGTGTTTCGGGAGCAAACTGGACAGAATCGGTT 355
              * *****       **** * ******* ** ****** *     ***** ******* *
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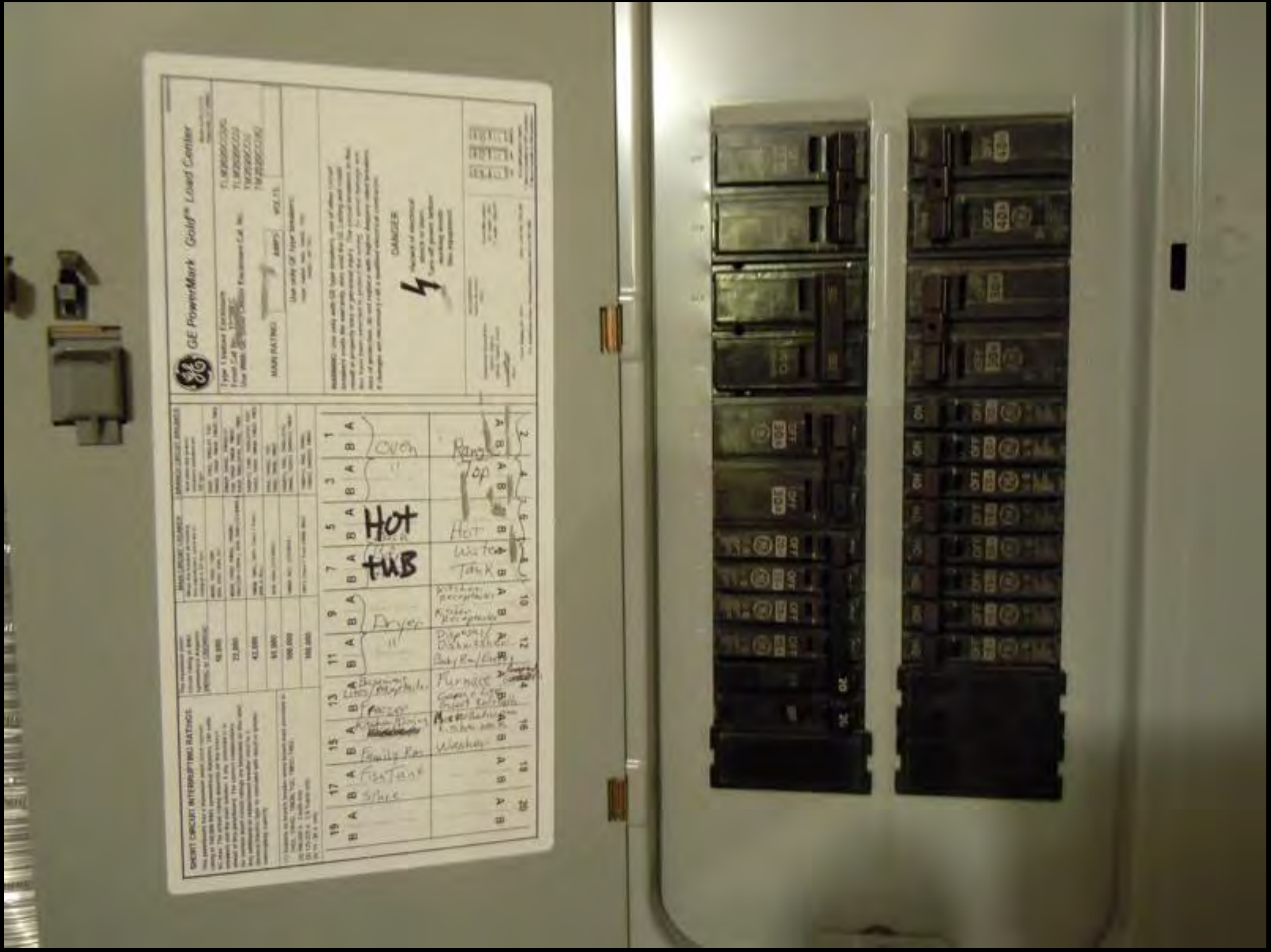
gene sequences matters

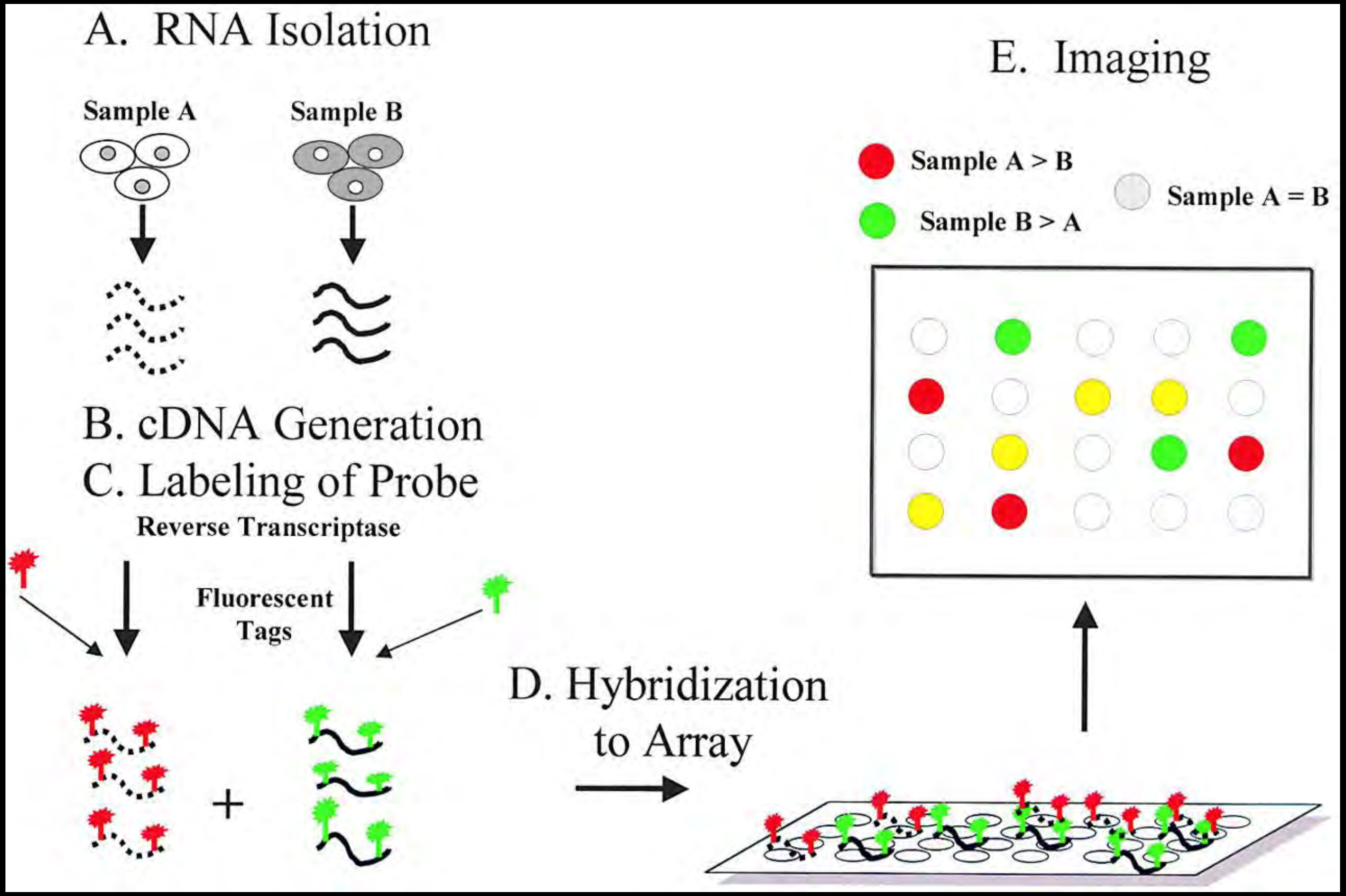
Hypothesis-based science gave us a few candidate biomarkers.

Technologies only a few years old can give us dozens (if not more).

Anatomy is shaped during embryonic development by "master regulater" genes



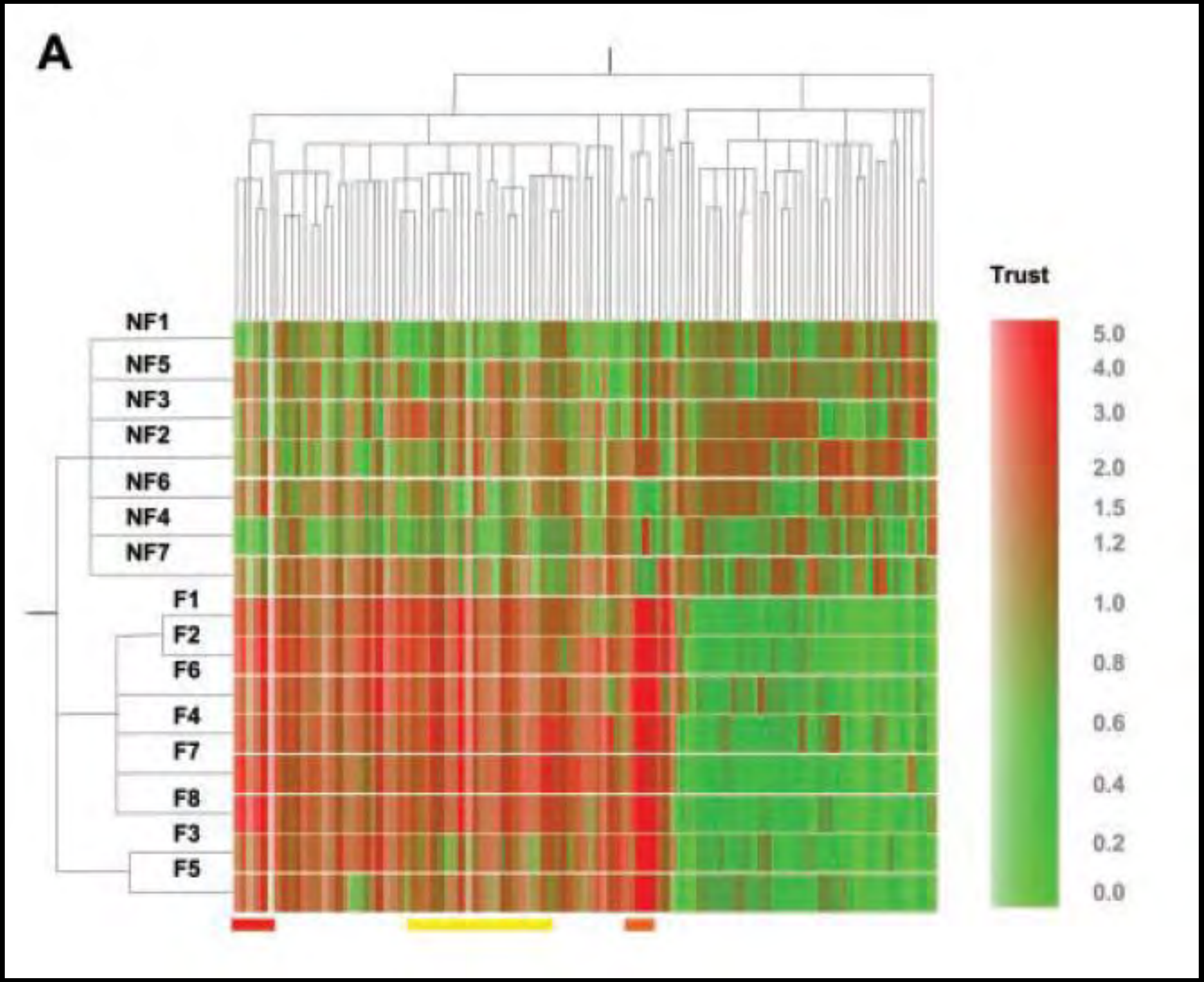
DNA microarray for "transcriptional profiling" or "transcriptomic analysis"



This is already "old school"

Albelda and Sheppard 2000 Am. J. Respir. Cell Mol. Biol., 23:265

103-gene human heart failure fingerprint



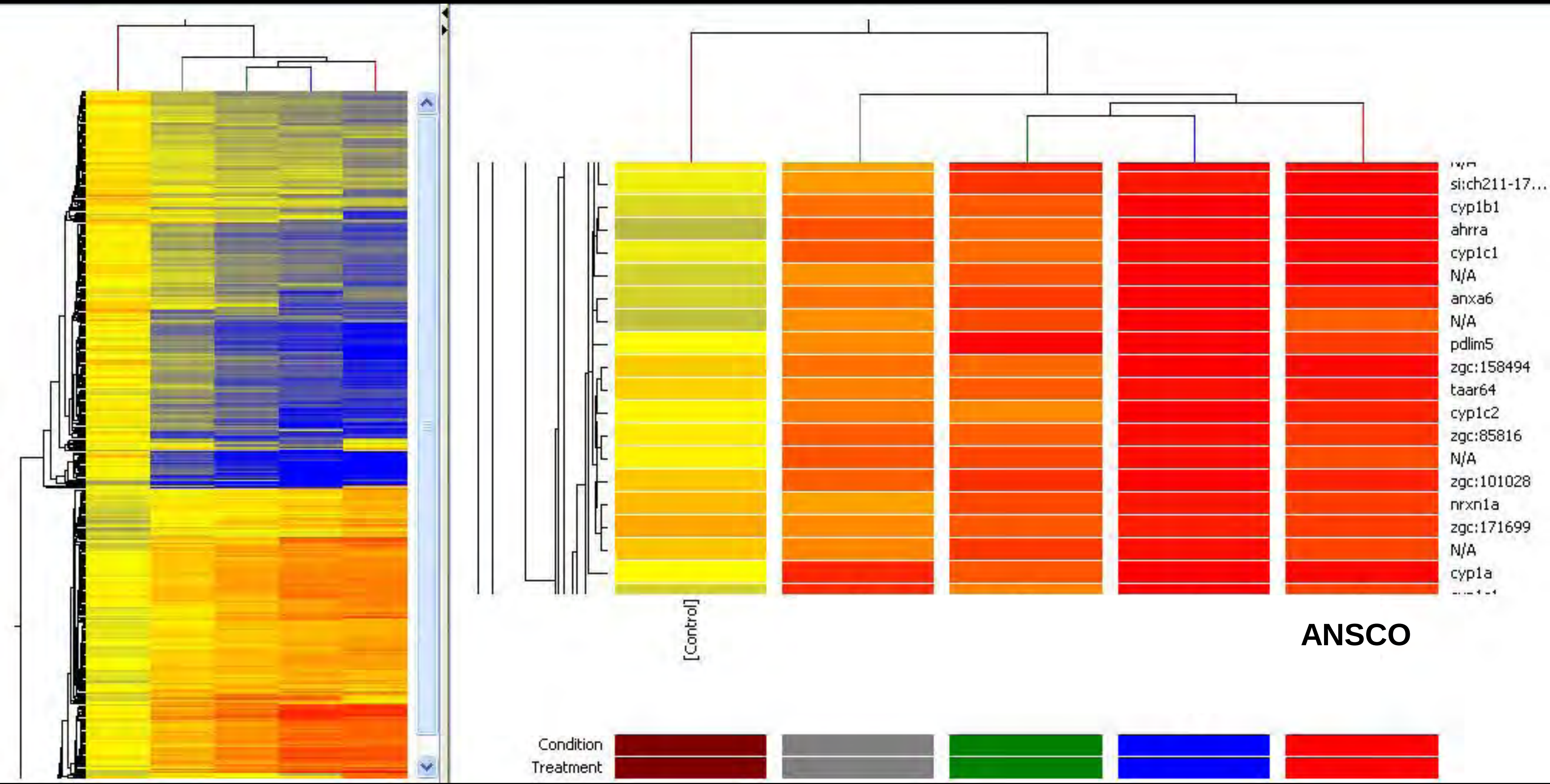
Tan et al., 2002 PNAS 99:11387

Mine this database for other markers of oil-induced cardiac stress in fish

15 best up-regulated in human heart failure

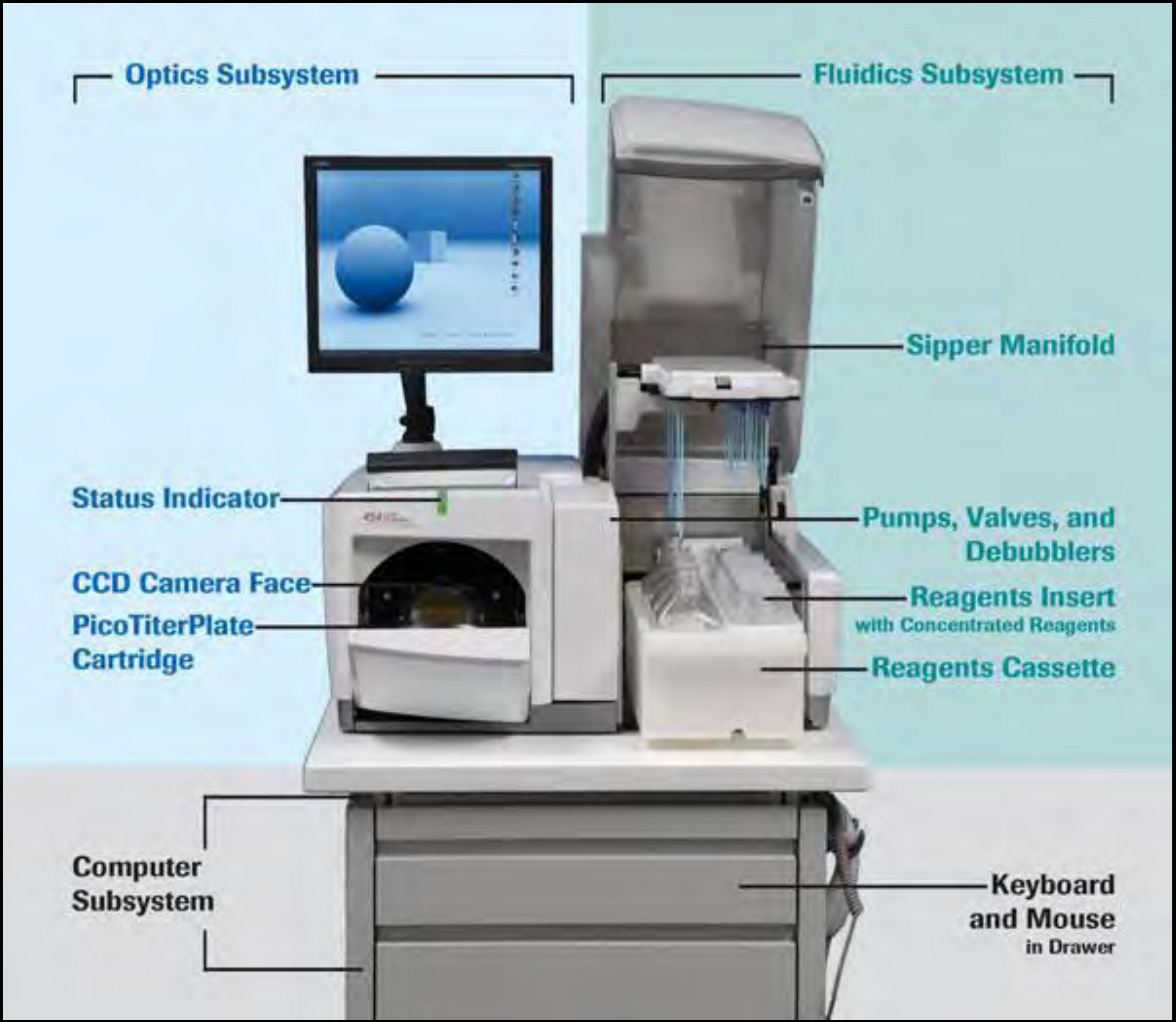
Name	Mean of NF, average difference units ±SD	Mean of F, average difference units ±SD	Fold change
BNP	751 ± 367	5,956 ± 1,908	↑ 7.9
ANF	1,477 ± 1,228	6,249 ± 1,434	↑ 4.2
ANF precursor	2,108 ± 751	6,986 ± 1,137	↑ 3.3
α1 collagen type I	716 ± 319	2,667 ± 1,199	↑ 3.7
Prepro-α_2 collagen type I	98 ± 79	485 ± 312	↑ 4.9
Osteoblast specific factor 2	40 ± 39	474 ± 281	↑ 12
Lumican	462 ± 158	1,753 ± 561	↑ 3.8
Pro-α_1 collagen type III	156 ± 37	510 ± 229	↑ 3.3
Thrombospondin-4	293 ± 101	1,040 ± 425	↑ 3.5
Connective tissue growth factor	246 ± 110	794 ± 435	↑ 3.2
Poly(A) site DNA	196 ± 81	540 ± 114	↑ 2.7
GEM GTPase	131 ± 29	359 ± 114	↑ 2.7
CDC-like kinase 1	126 ± 45	341 ± 84	↑ 2.7
T-plastin	87 ± 54	234 ± 98	↑ 2.7
Mμ-crystallin	1,327 ± 372	3,389 ± 648	↑ 2.6

Zebrafish microarray (40K genes) with embryonic oil exposure



Red genes are up, blue genes are down, yellow no change

“Next Generation” gene sequencing



What we gain from the molecular approach to quantifying injury

- faster
- cheaper (in terms of labor costs)
- more sensitive
- set toxicity thresholds more rigorously
- broader species coverage
- grounded by 20 years of published research on oil toxicity