NOAA
FISHERIES
SERVICE

Embryonic crude oil exposure causes cardiac hypertrophy & reduced aerobic performance in juvenile pink salmon & Pacific herring

Mark Carls, John Incardona & Nat Scholz

Co-authors: John Incardona, Larry Holland, Tiffany Linbo, David Baldwin, Mark Myers, Karen Peck, Mark Tagal, & Jeep Rice

Fundamental questions

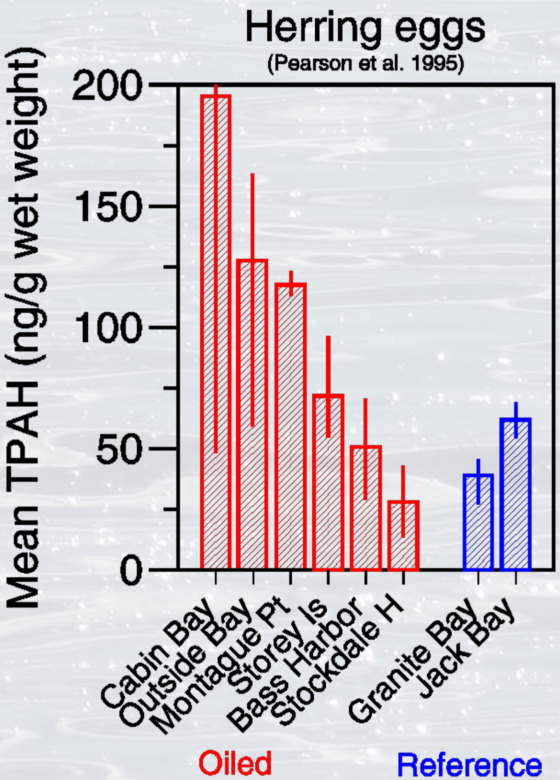
1. Does oil exposure cause long-term damage?
2. What is long-term damage?
3. Does long-term damage affect populations?

Objective

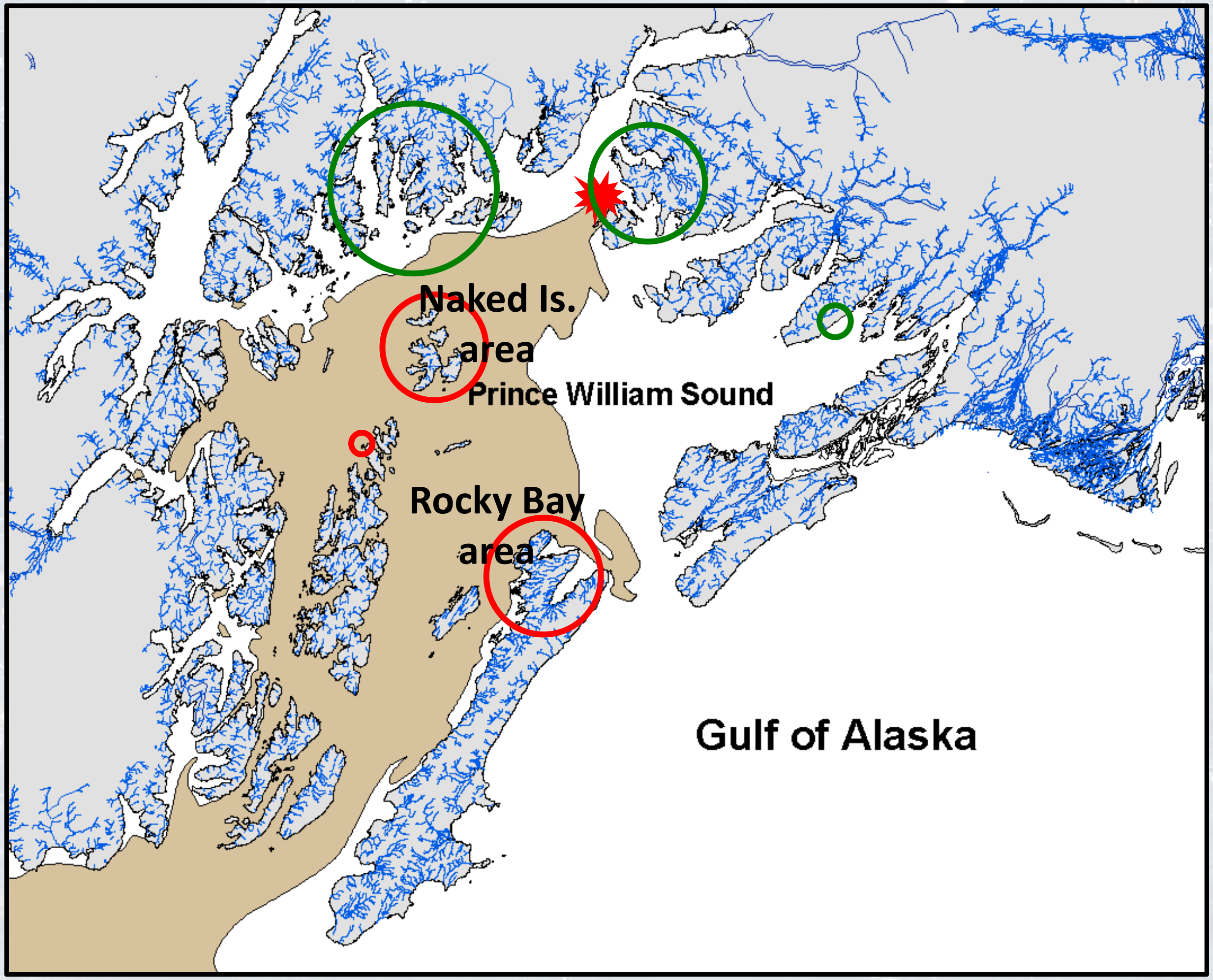
- Assess long–term cardiac damage by oil in
  - Pacific herring
  - Pink salmon

1989: *Exxon Valdez* oil spill

- **Were herring exposed?**



Most herring spawned on Naked & Montague Is. beaches when [PAH] greatest in the water column (McGurk 1992).

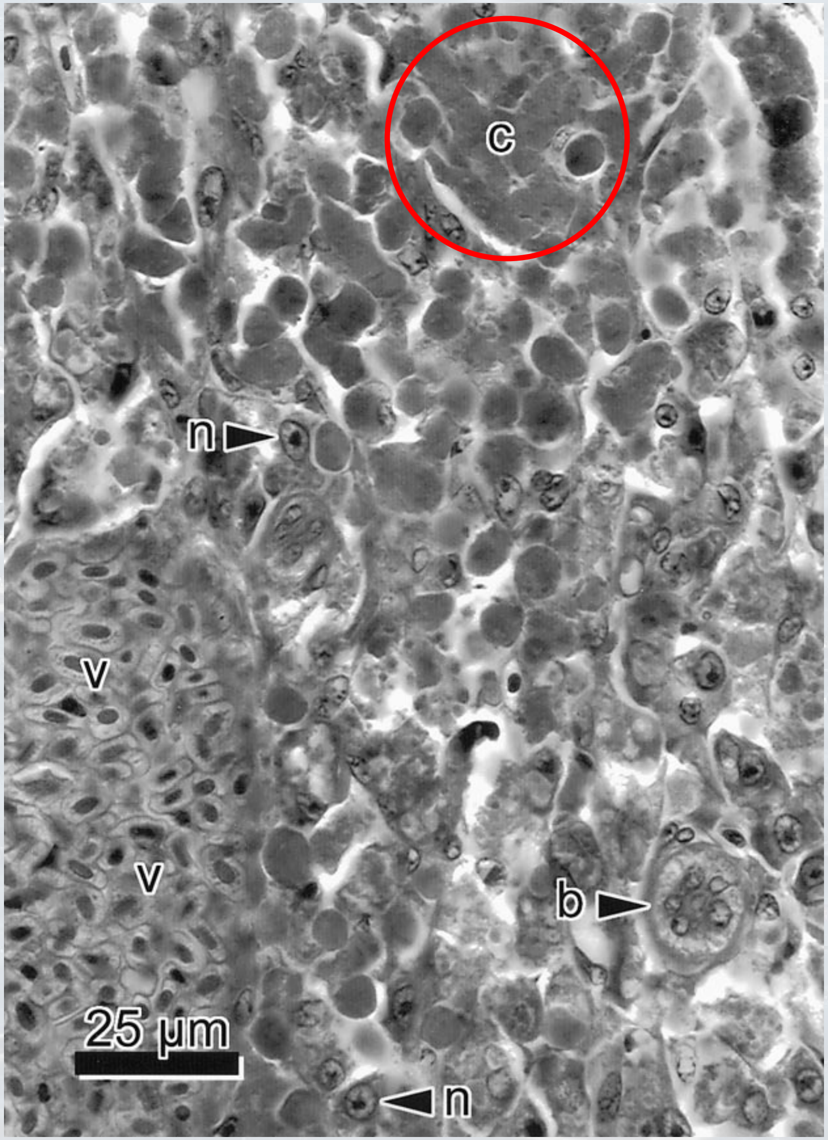


1989: *Exxon Valdez* oil spill

- Were herring exposed?
- **Were fish affected?**

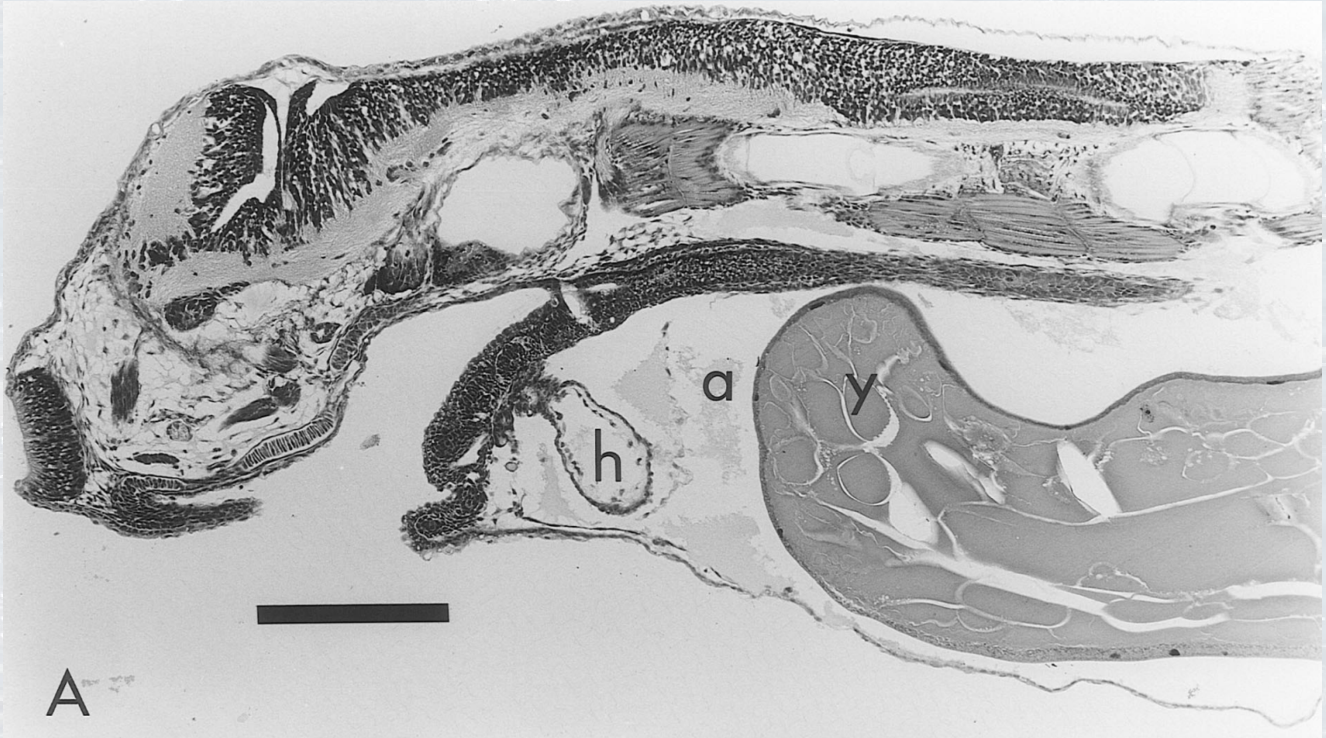
Adult Pacific herring at oiled sites had multifocal hepatic necrosis;

those at reference sites did not (Marty et al. 1999)



1989: *Exxon Valdez* oil spill

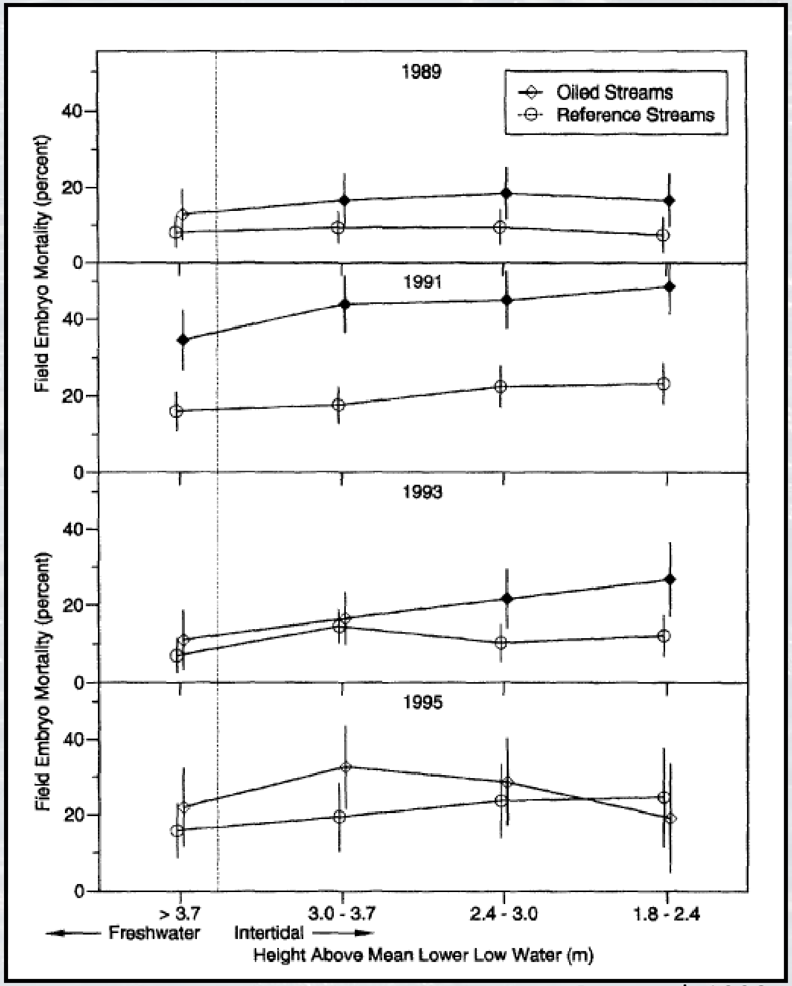
- Were herring exposed?
- **Were embryos affected?**



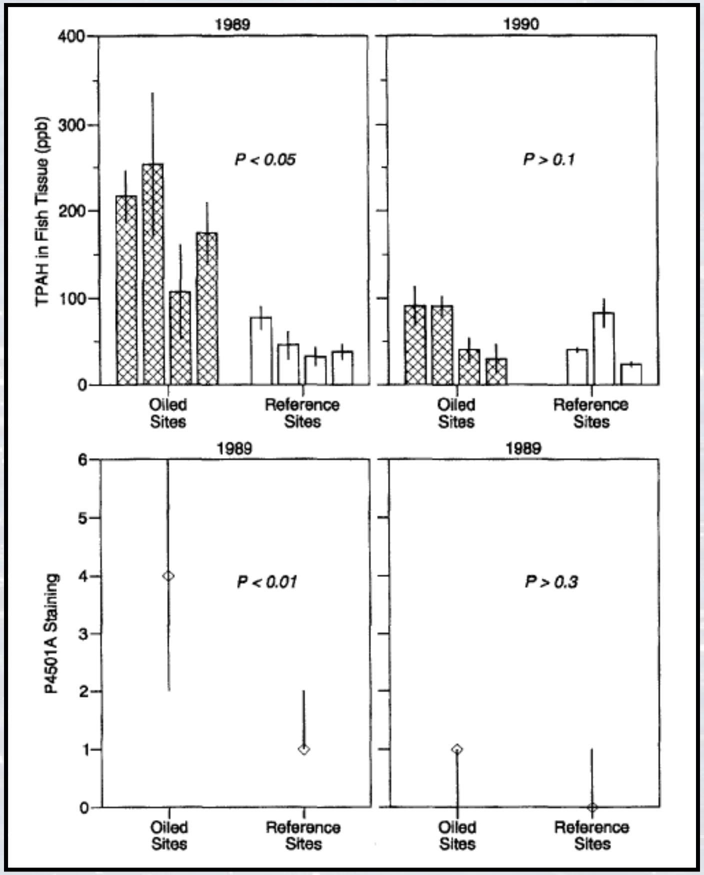
a = severe yolk-sac edema and h = pericardial edema. Oiled herring embryo from Bass Harbor; Marty et al. 1997

1989: *Exxon Valdez* oil spill

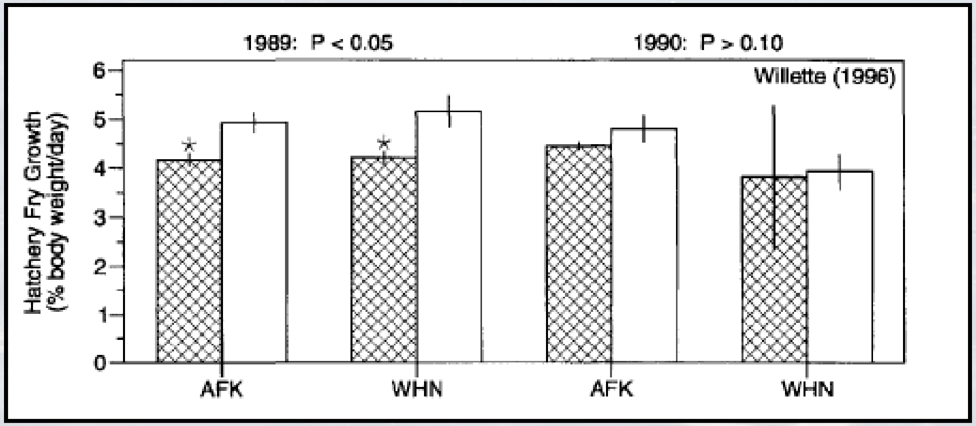
- Were herring exposed?
- Were pink salmon affected?



Bue et al. 1998

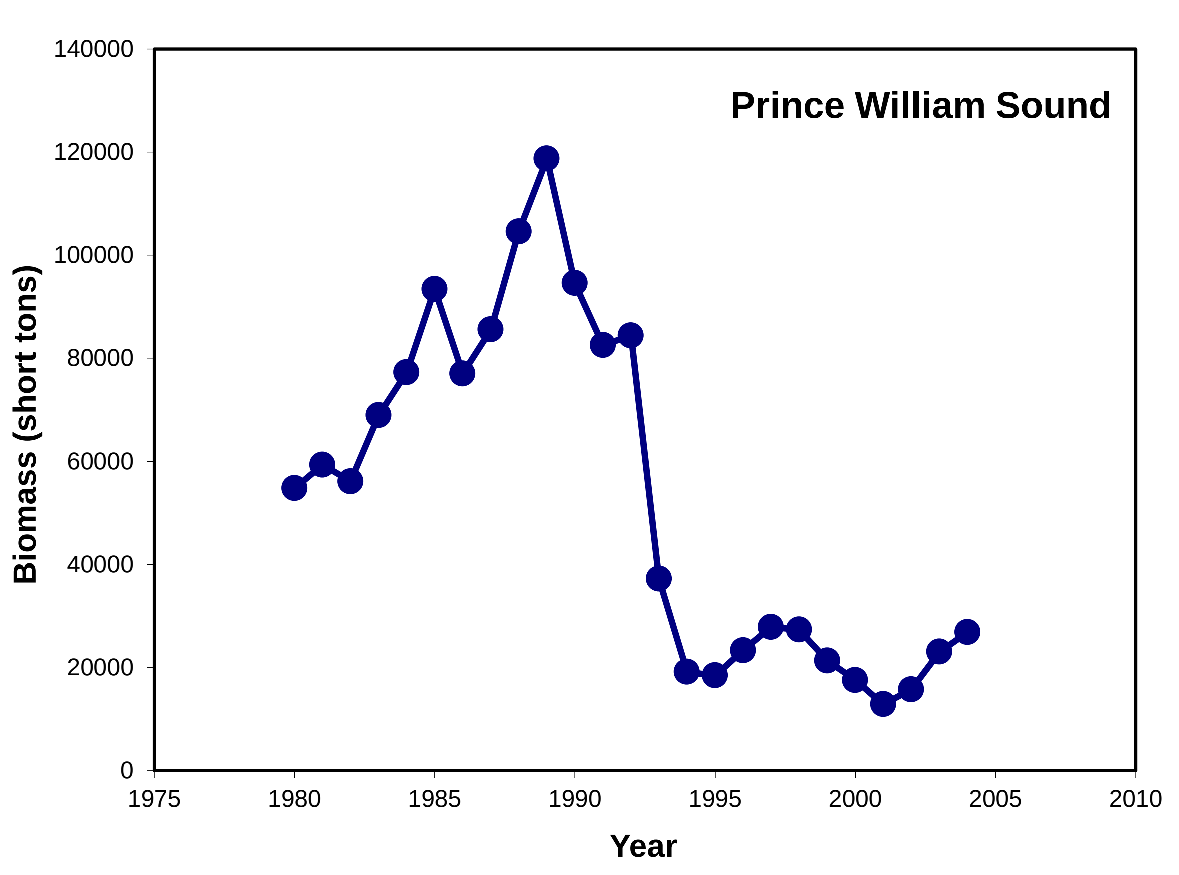


Carls et al. 1996



1989: *Exxon Valdez* oil spill

- Were herring exposed?
- Were embryos affected?
- **Did the oil spill have a role in the population collapse?**



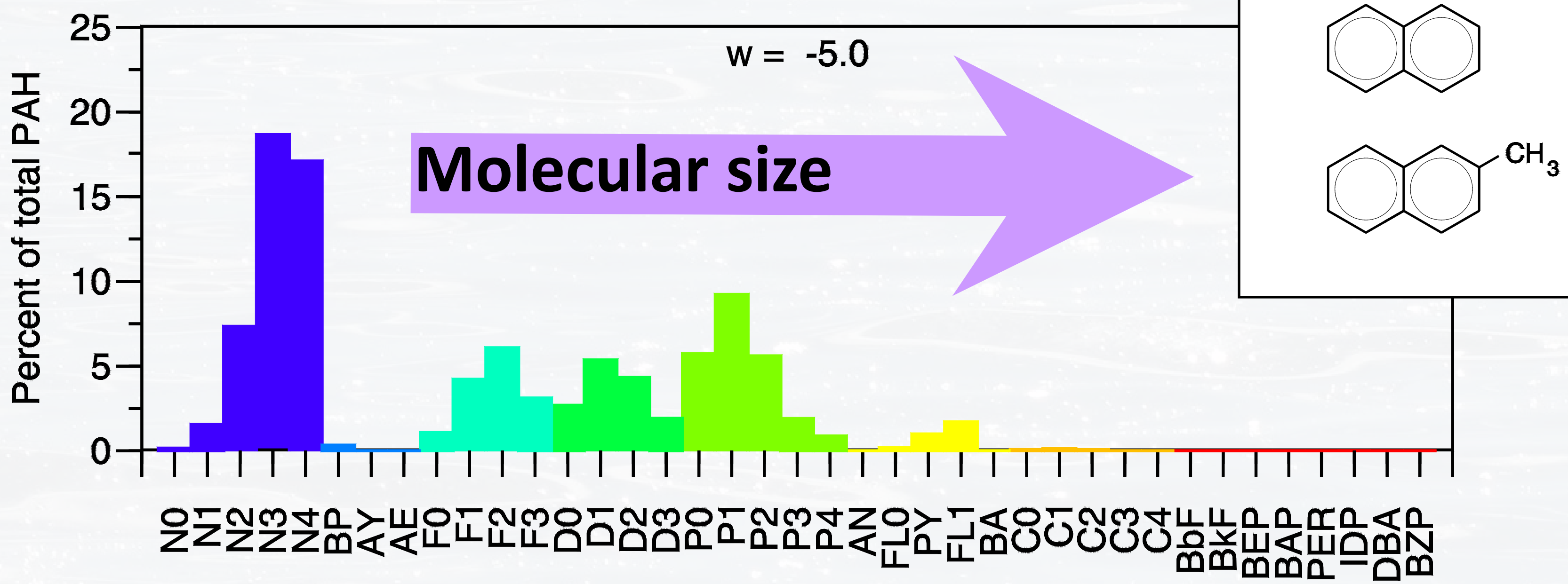
Subsequent laboratory research demonstrated oil-related embryo damage assoc. with PAHs

^ *polynuclear aromatic hydrocarbons*

Pacific Herring

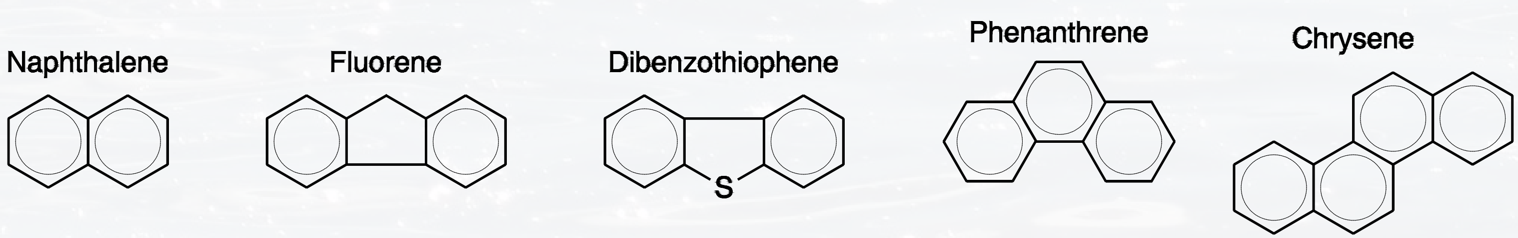
Pink Salmon

PAHs (polynuclear aromatic hydrocarbons)
in *Exxon Valdez* Crude Oil:



substitution:

Naph **Flu** **Dib** **Phen** **Chry**



(Fresh *Exxon Valdez* oil in PWS sediment)

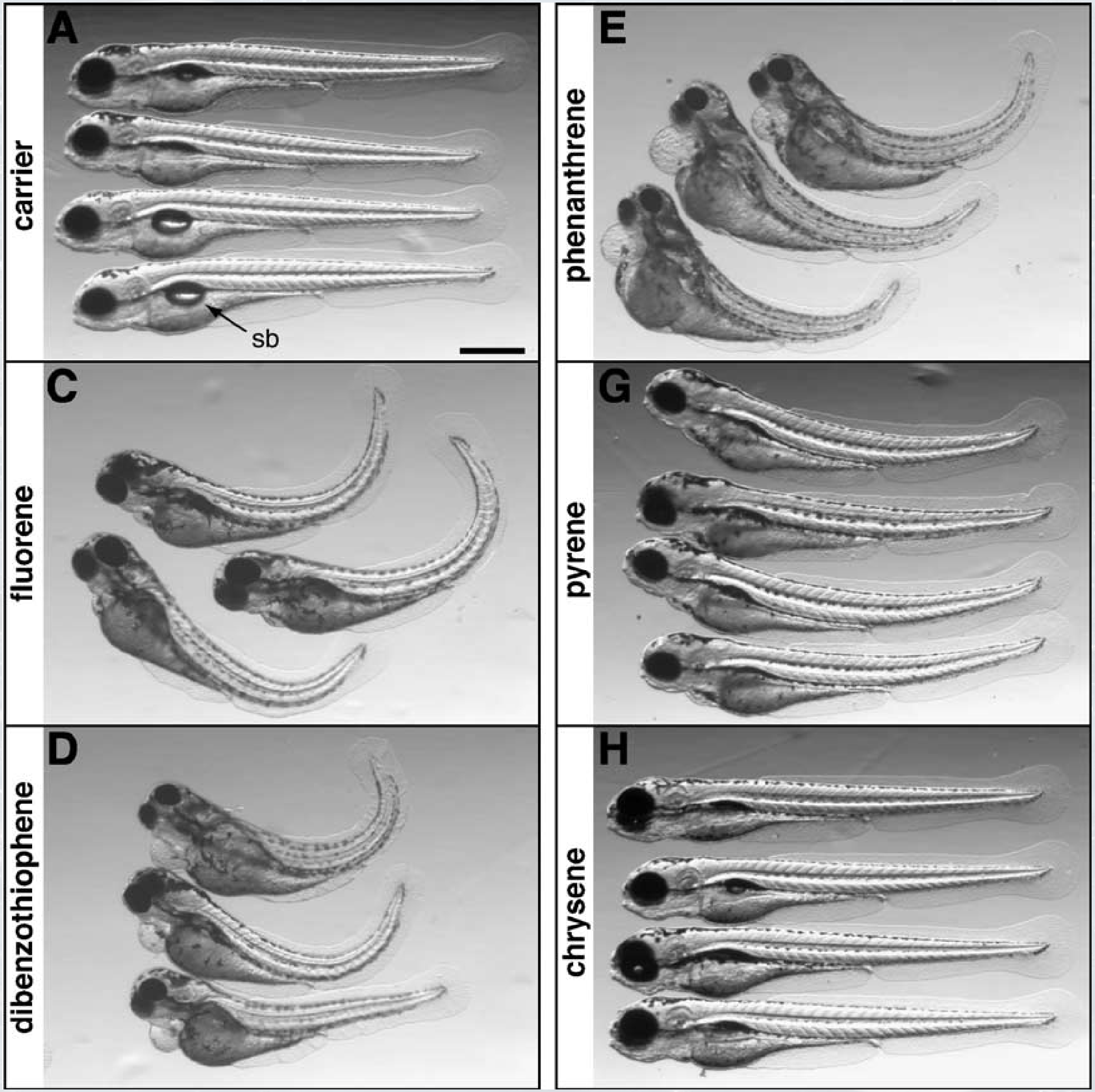
weathering

Embryonic heart failure underlies most defects associated with crude oil

Exposure to individual PAHs can reproduce key aspects of crude oil exposure (complex PAH mixtures)

PAH uptake disrupts the form & function of the developing heart

Disrupted circulation leads to heart malformation, edema, & other defects

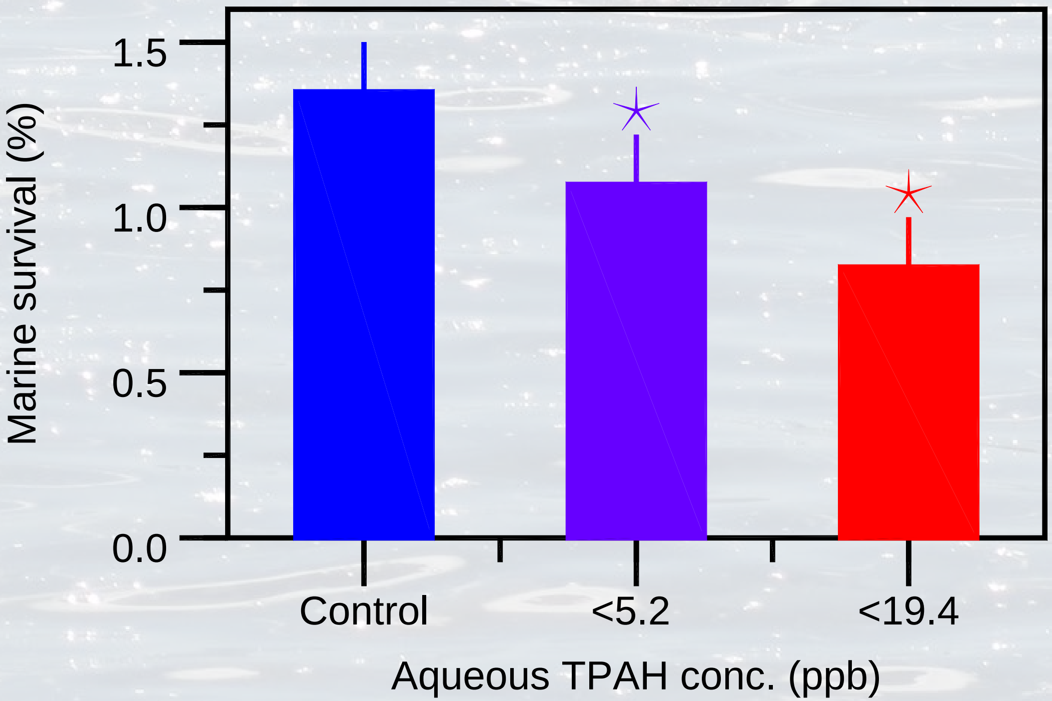


Growth & morphogenesis of the heart into a complex, 3-d structure critically depends on normal pumping and circulation

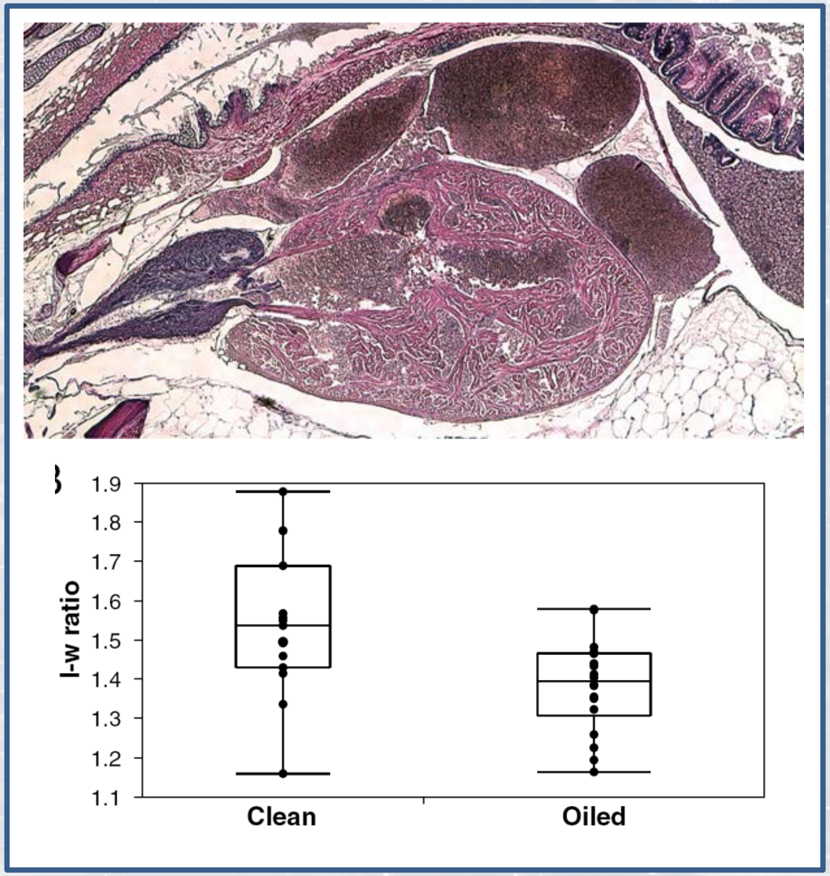
Mild disruption may subsequently alter cardiac shape and performance

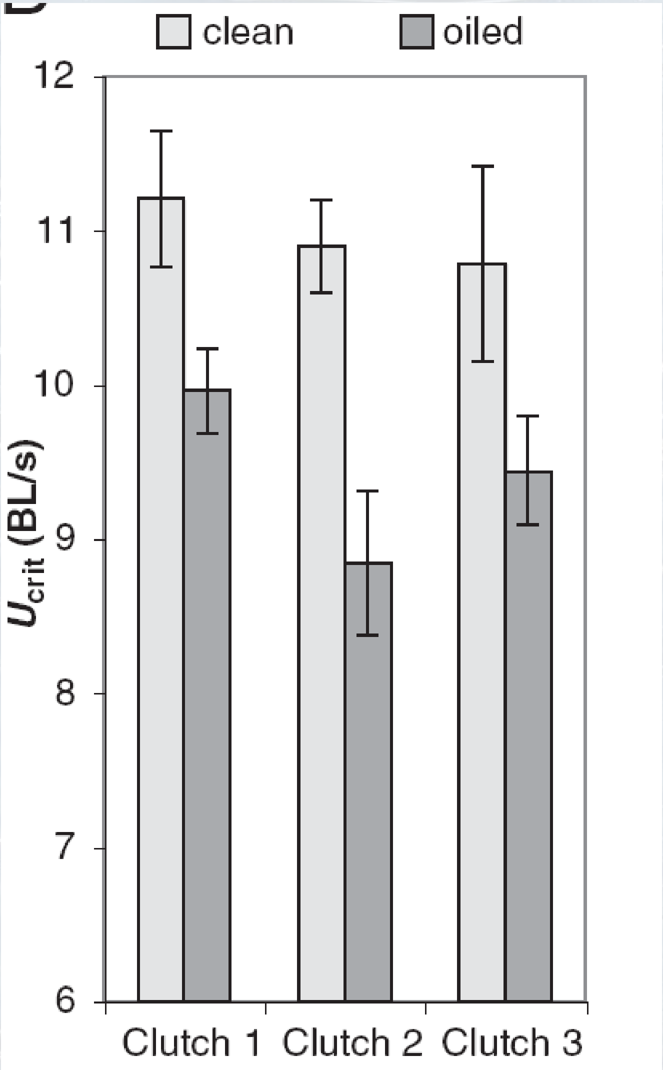
Swimming performance is important for predator avoidance

This may explain reduced marine survival



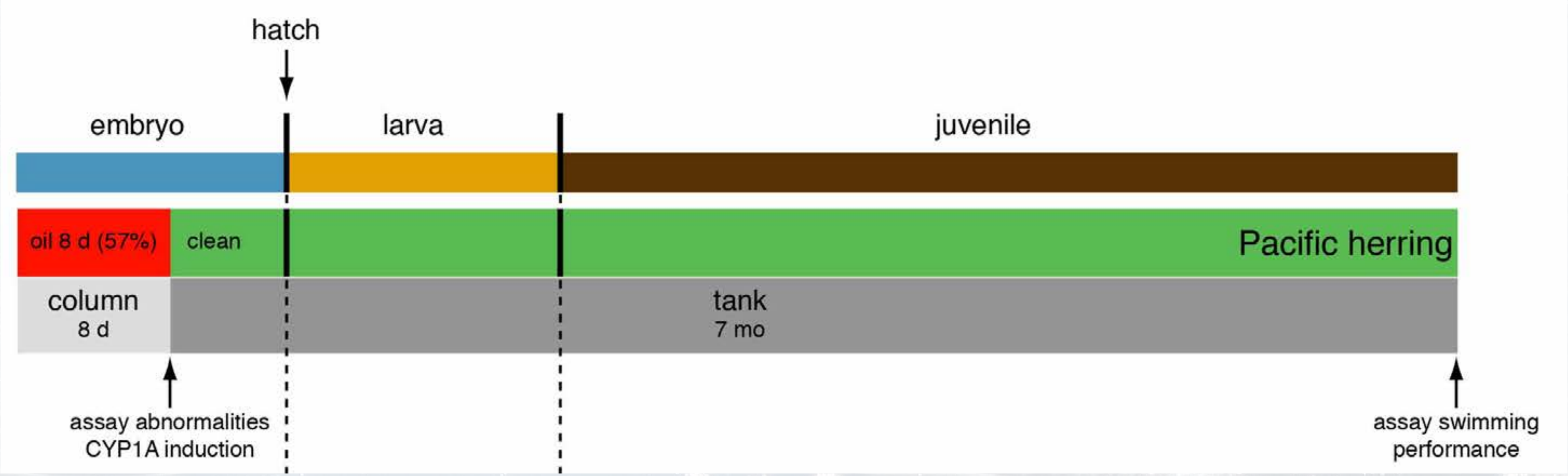
Delayed cardiac effects suggest a mechanism for population loss

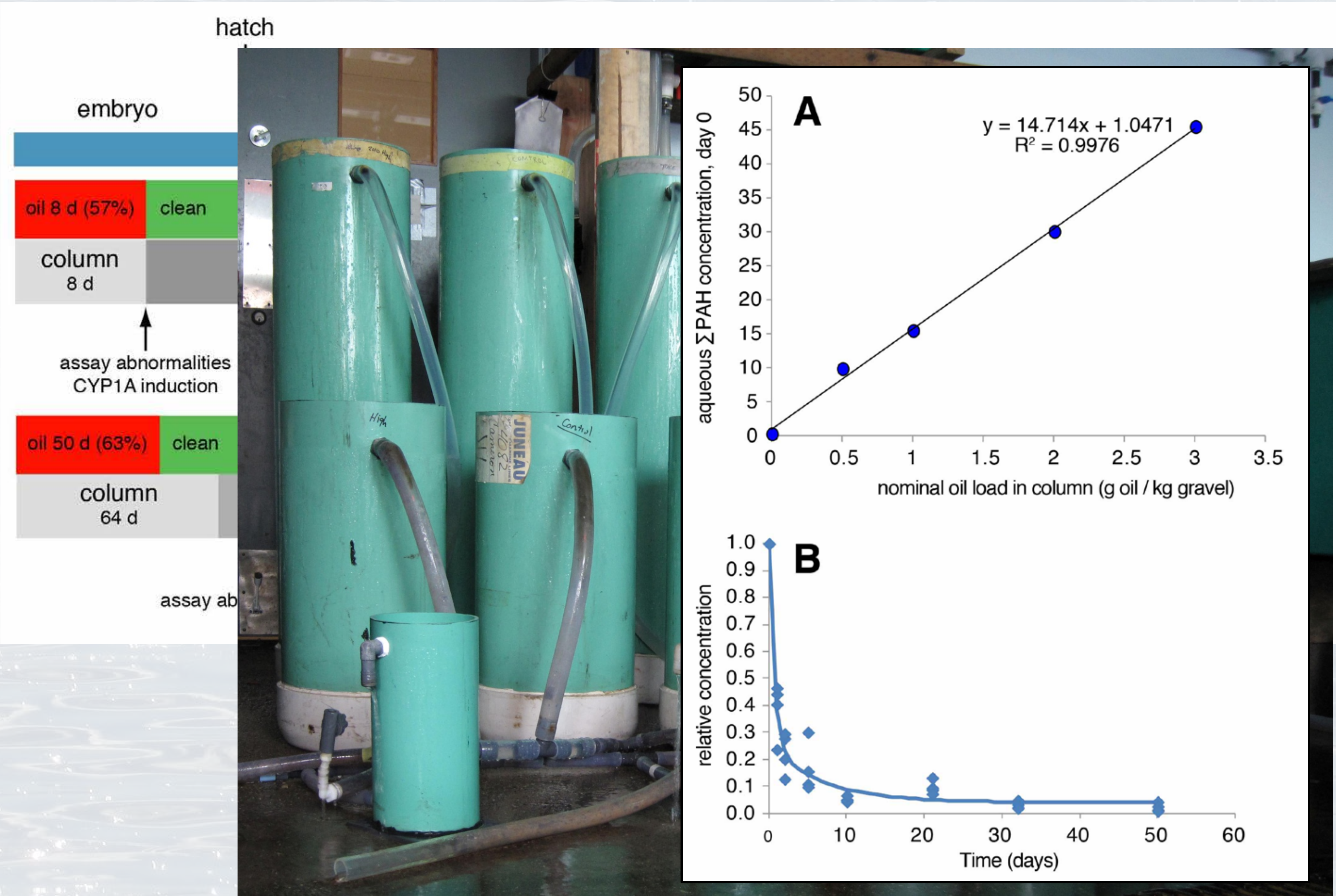




Hicken et al. 2011

Zebrafish


hatch
embryo
larva
juvenile
oil 8 d (57%)
clean
Pacific herring
column
8 d
tank
7 mo
assay abnormalities
CYP1A induction
assay swimming
performance

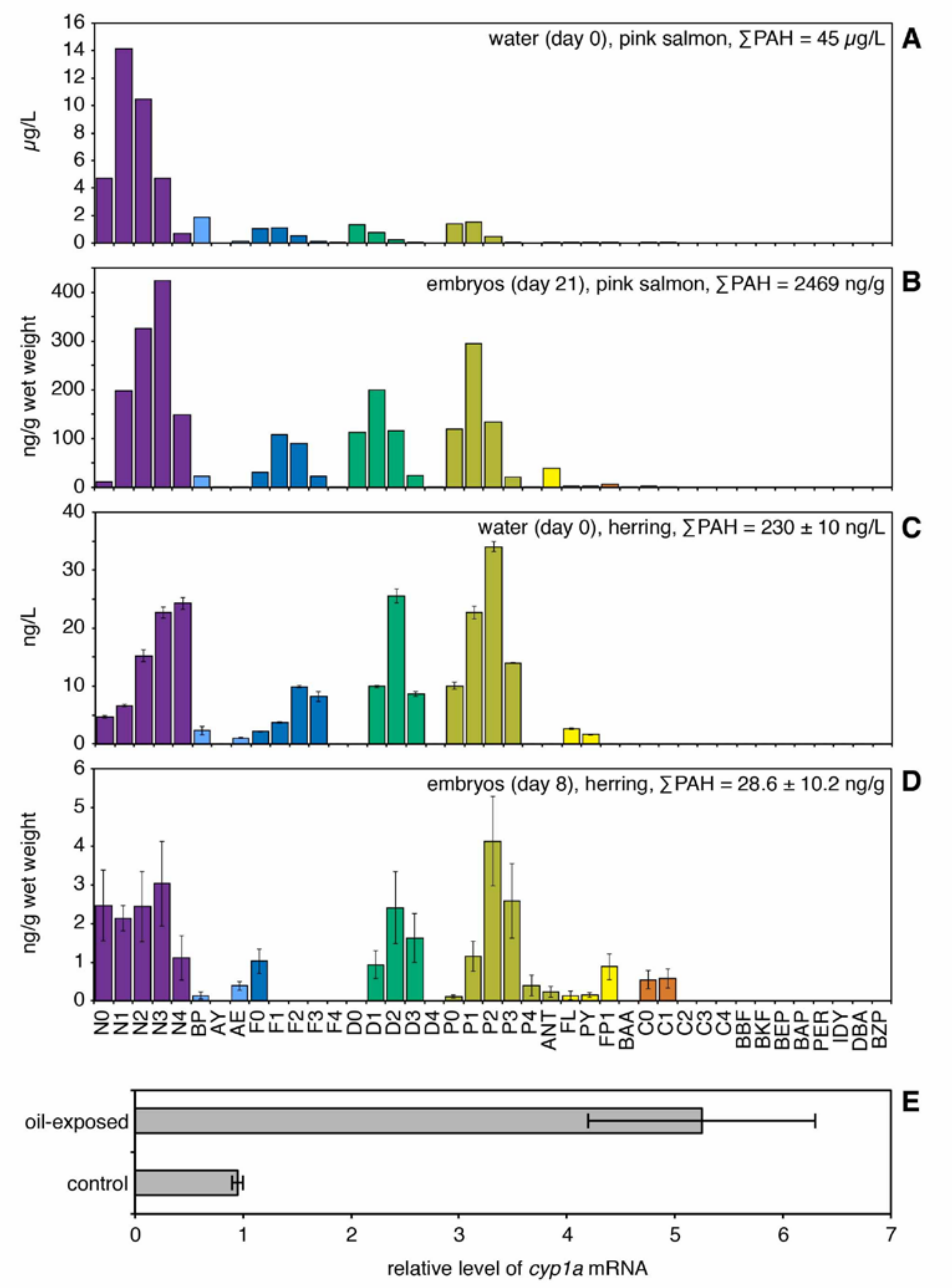

hatch
embryo
oil 8 d (57%)
clean
column
8 d
assay abnormalities
CYP1A induction
oil 50 d (63%)
clean
column
64 d
assay ab
A
y = 14.714x + 1.0471
R² = 0.9976
aqueous ∑PAH concentration, day 0
nominal oil load in column (g oil / kg gravel)
B
relative concentration
Time (days)

PAHs in treatment water

PAHs accumulated in embryos

More weathered oil for herring

Biochemical response to PAH
(herring embryos)



PAH concentrations in tissue were

- **Dose-dependent**

Species	Treatment	∑PAH water (µg/L)	∑PAH in embryos (ng/g wet weight)	∑PAH in embryos (ng/g lipid)
Pink salmon	clean gravel	0.2	26	240
	0.5 g/kg oiled gravel	9.8	222	2,066
	1 g/kg oiled gravel	15.4	634	5,895
	2 g/kg oiled gravel	30.0	1,279	11,900
	3 g/kg oiled gravel	45.4	2,474	23,012
Pacific herring	clean gravel	0.039 ± 0.003	9.3 ± 3.7	582 ± 178
	oiled gravel	0.230 ± 0.010	28.6 ± 10.2	1,787 ± 256

PAH concentrations in tissue were

- **Smaller in herring**

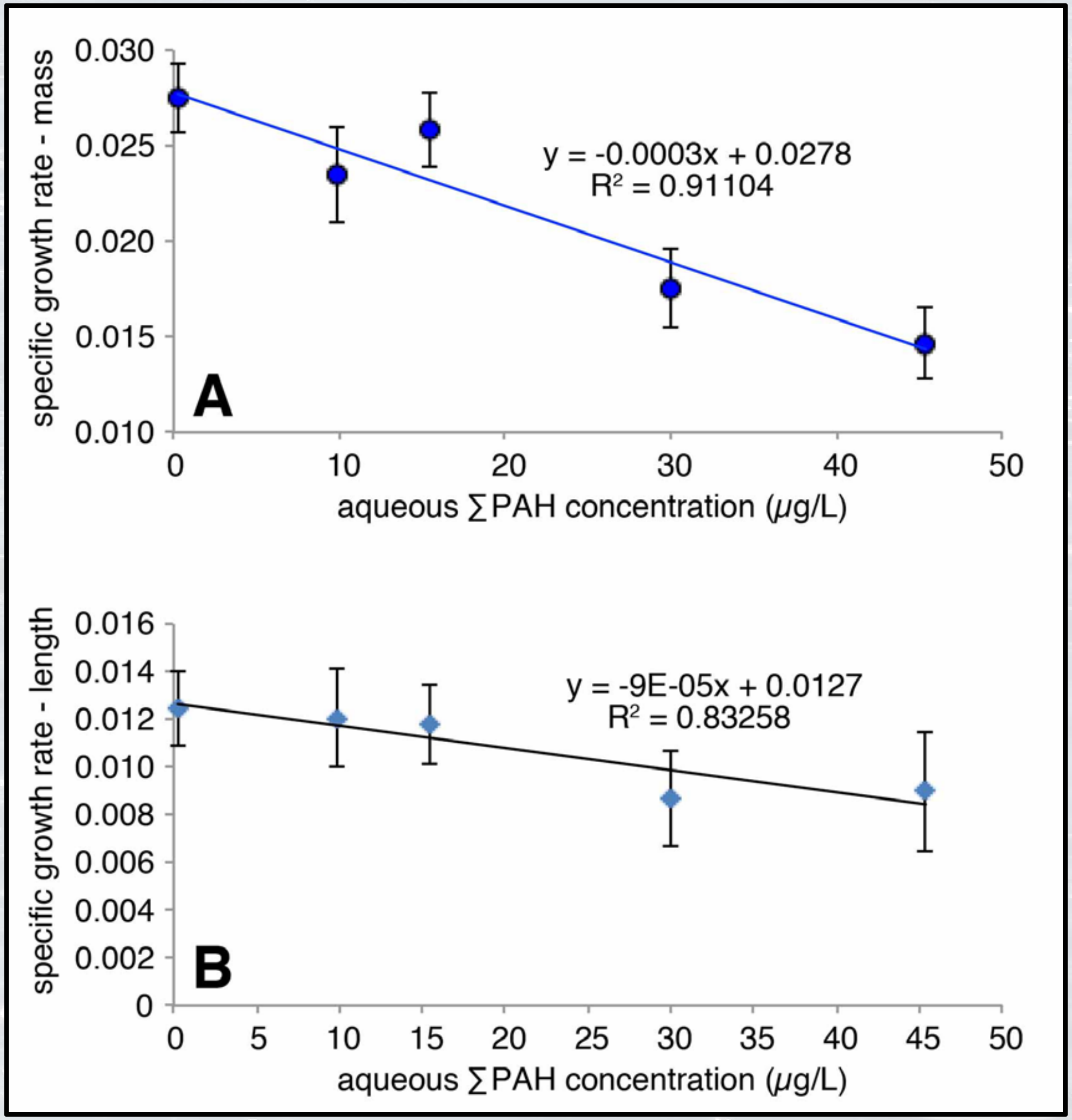
Species	Treatment	∑PAH water (µg/L)	∑PAH in embryos (ng/g wet weight)	∑PAH in embryos (ng/g lipid)
Pink salmon	clean gravel	0.2	26	240
	0.5 g/kg oiled gravel	9.8	222	2,066
	1 g/kg oiled gravel	15.4	634	5,895
	2 g/kg oiled gravel	30.0	1,279	11,900
	3 g/kg oiled gravel	45.4	2,474	23,012
Pacific herring	clean gravel	0.039 ± 0.003	9.3 ± 3.7	582 ± 178
	oiled gravel	0.230 ± 0.010	28.6 ± 10.2	1,787 ± 256

PAHs in tissue were

- **More similar on a lipid basis**
- **Thus overlapping biological effects were expected**

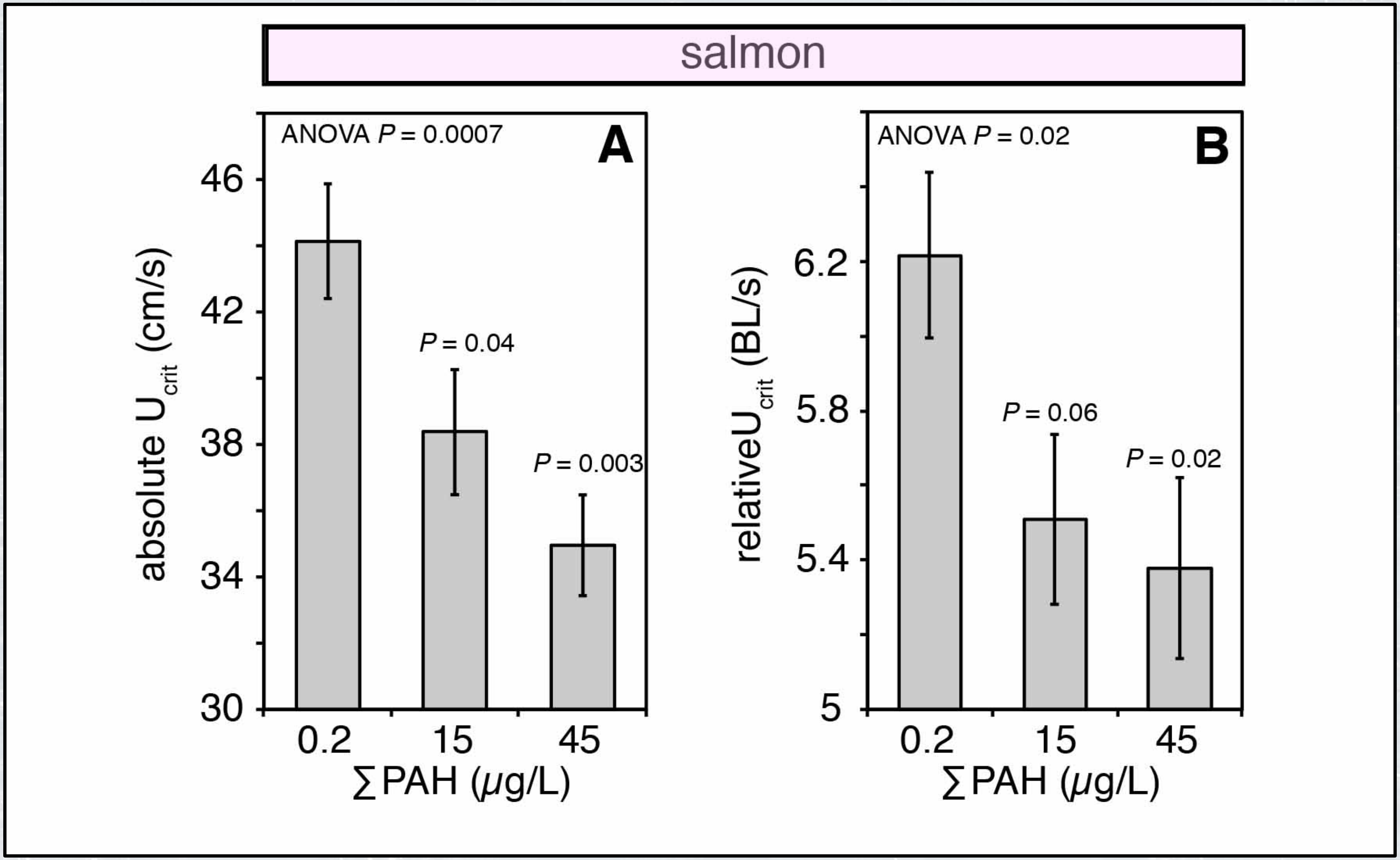
Species	Treatment	∑PAH water (µg/L)	∑PAH in embryos (ng/g wet weight)	∑PAH in embryos (ng/g lipid)
Pink salmon	clean gravel	0.2	26	240
	0.5 g/kg oiled gravel	9.8	222	2,066
	1 g/kg oiled gravel	15.4	634	5,895
	2 g/kg oiled gravel	30.0	1,279	11,900
	3 g/kg oiled gravel	45.4	2,474	23,012
Pacific herring	clean gravel	0.039 ± 0.003	9.3 ± 3.7	582 ± 178
	oiled gravel	0.230 ± 0.010	28.6 ± 10.2	1,787 ± 256

Growth rate was reduced by PAH exposure

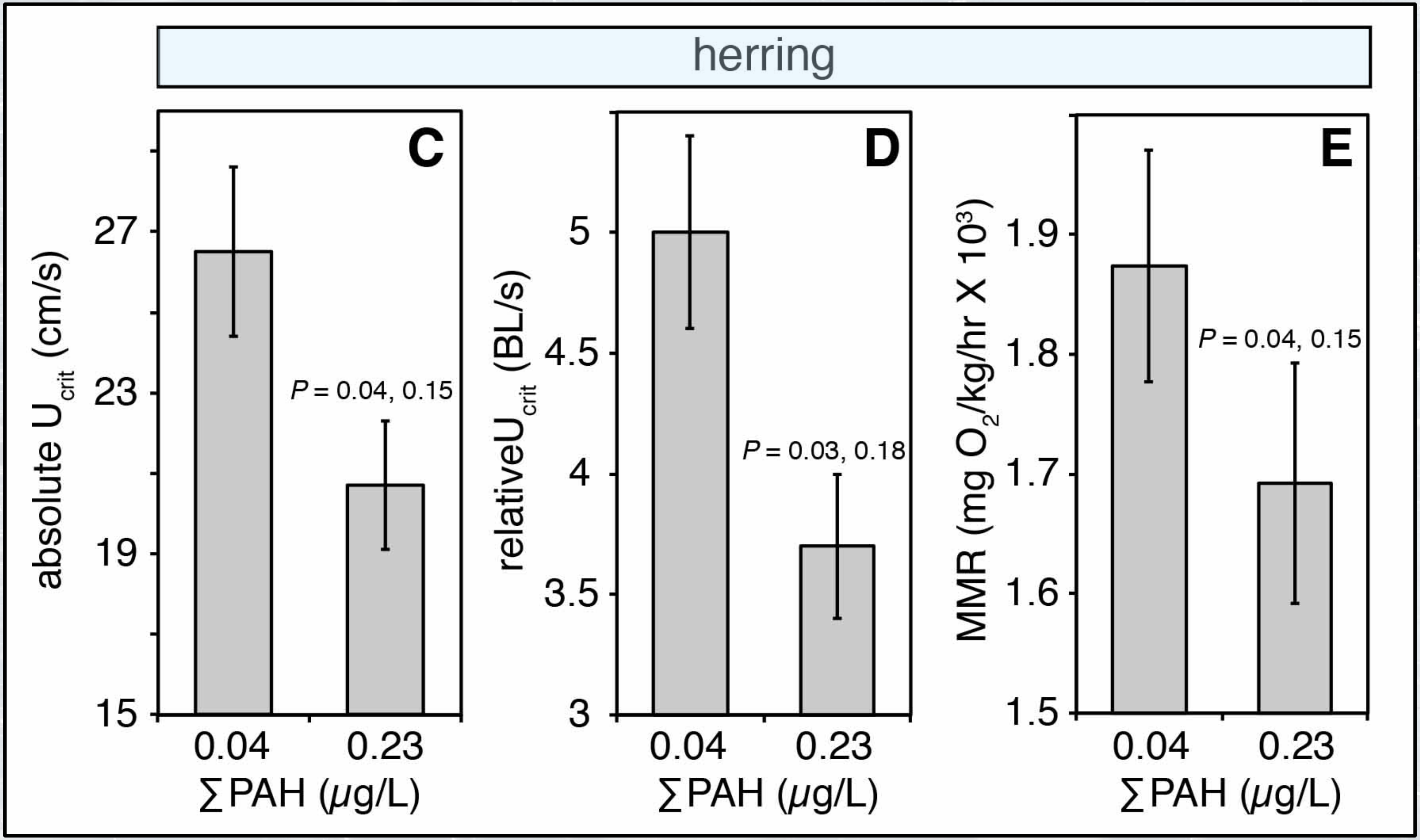


(pink salmon,
post emergence,
exogenous food)

Critical swimming speed was reduced



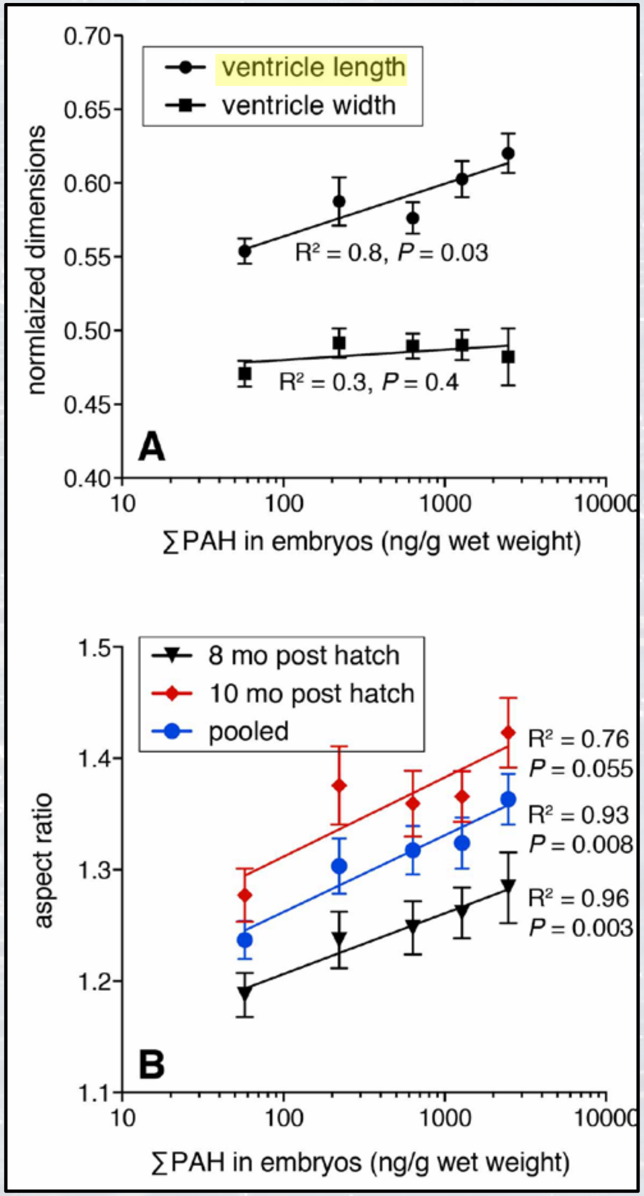
Critical swimming speed was reduced, as was maximum metabolic rate at top speed (indicating reduced delivery of oxygen)

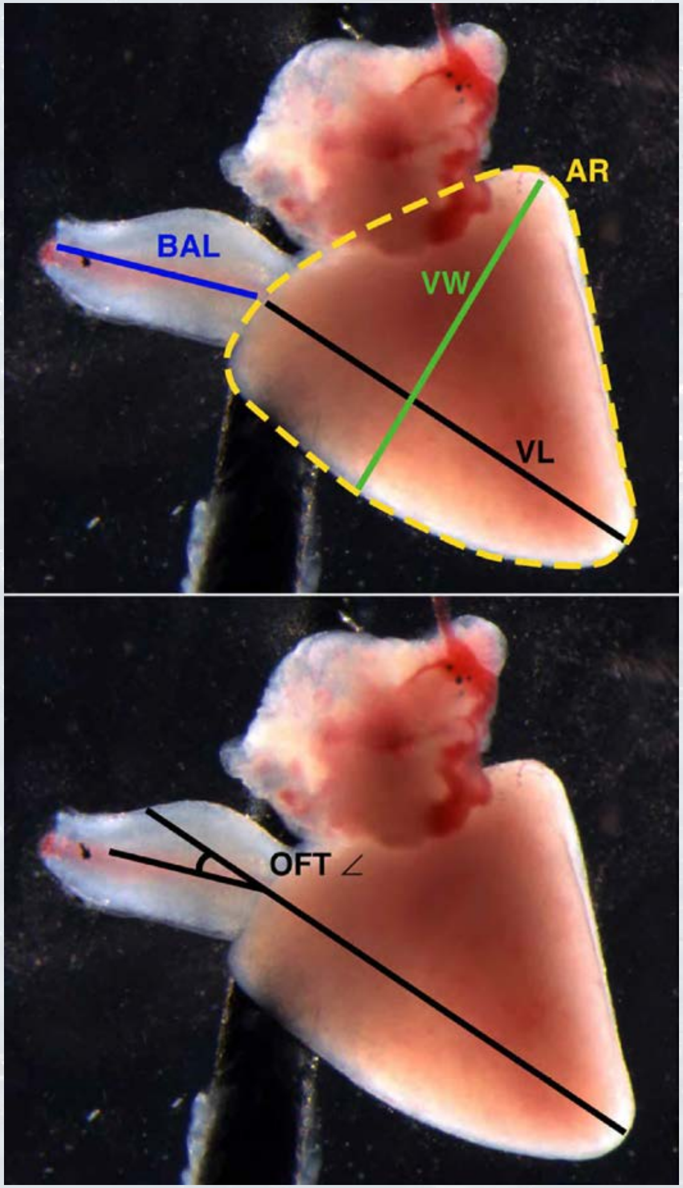


Cardiac morphology changed

Length & width

Aspect ratio

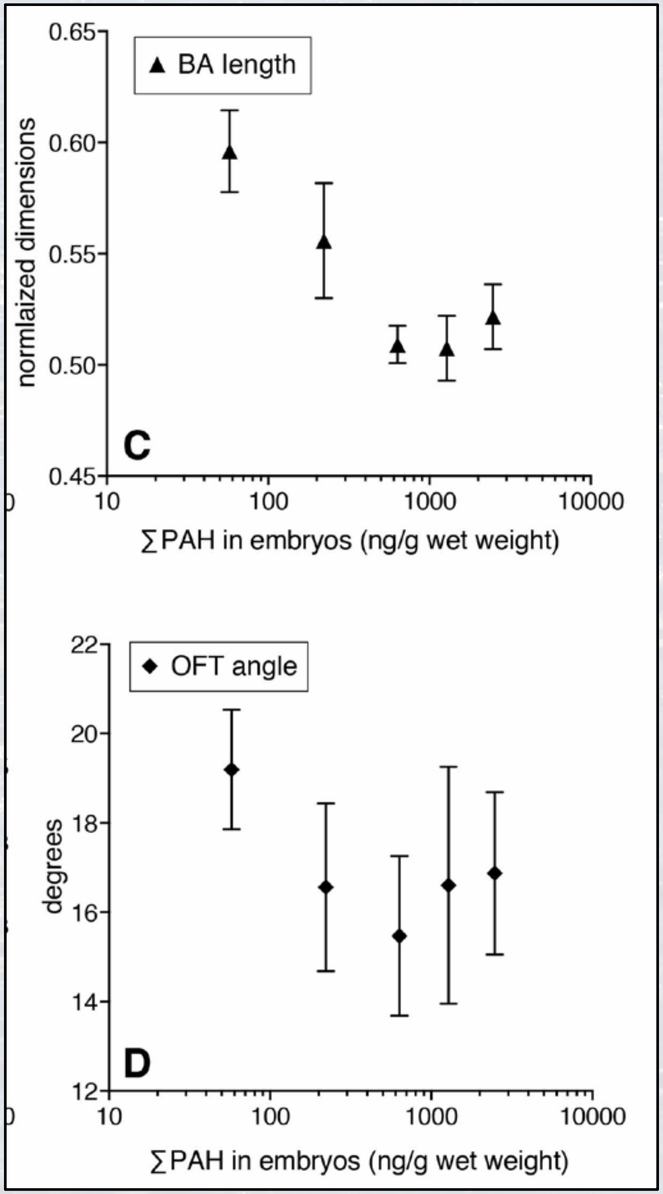


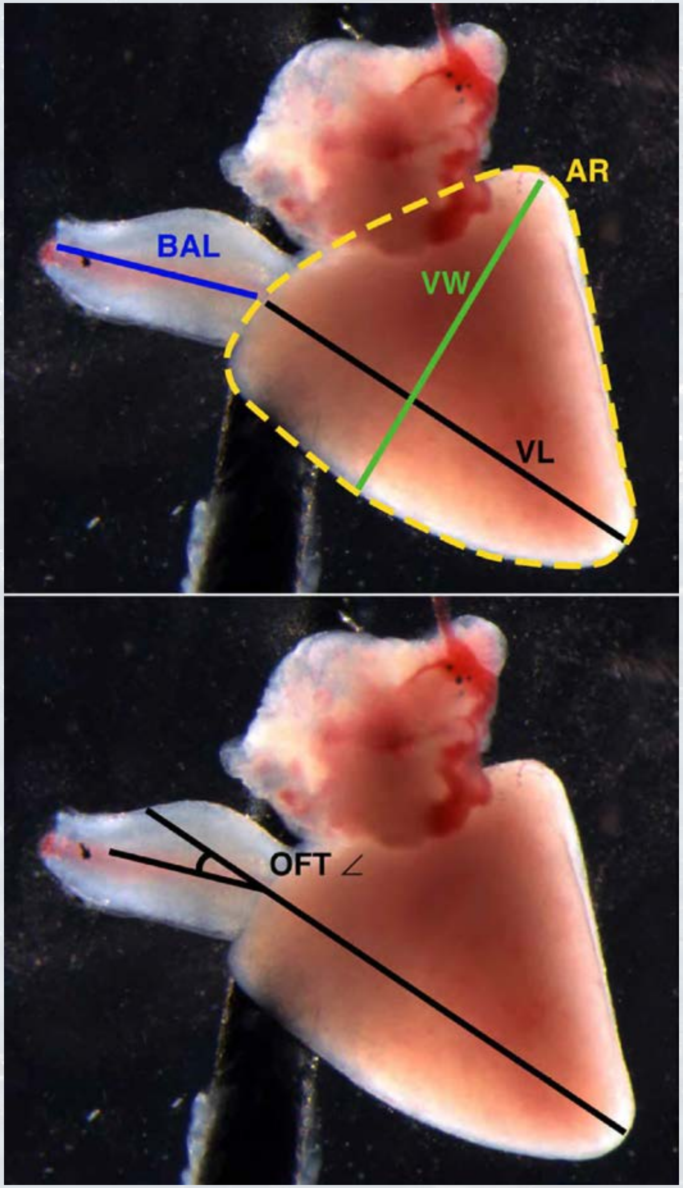


Cardiac morphology changed

Bulbus arteriosus

Outflow tract





Cardiac morphology changed

size changes in herring were opposite those in salmon

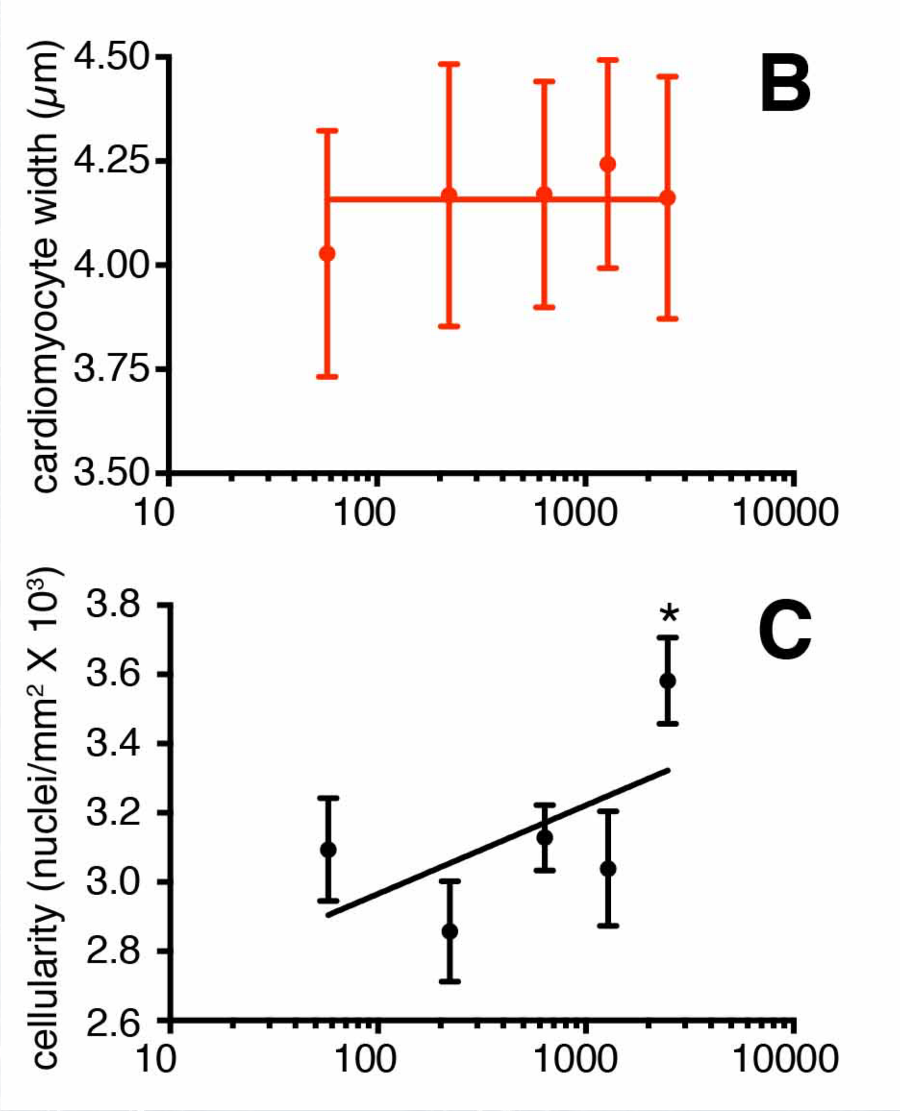
measure	control	oil exposed	*P* oil effect[b]	*P* tank effect[c]
Fork length (cm)	5.3 ± 0.1	5.6 ± 0.1	0.0005	0.0007 (C6)
Mass (g)	1.26 ± 0.07	1.65 ± 0.10	0.0003	0.0004 (O2)
Condition factor	0.82 ± 0.01	0.87 ± 0.01	0.01	0.02 (C11)
Ventricle length - lateral[a]	69.5 ± 1.0	69.8 ± 1.2	0.2	0.001 (O8)
Ventricle width - lateral[a]	51.3 ± 0.9	49.8 ± 0.7	0.7	0.3
Aspect ratio - lateral	1.45 ± 0.02	1.52 ± 0.02	0.04	0.03 (O8)
Ventricle length - ventral[a]	64.0 ± 1.0	63.6 ± 1.5	0.6	0.004 (O8)
Ventricle width - ventral[a]	63.5 ± 0.9	64.3 ± 1.0	0.07	0.01 (O8)
Aspect ratio - ventral	1.14 ± 0.01	1.13 ± 0.01	0.2	0.003 (C11)
Ventricle volume (mm^3)	0.042 ± 0.003	0.050 ± 0.002	0.007	0.002 (C6)
BA length (relative to ventricle)	0.45 ± 0.01	0.47 ± 0.01	0.6	0.5
OFT angle (degrees)	21.9 ± 1.8	15.4 ± 1.4	0.004	0.5

Cardiac morphology changed (herring)

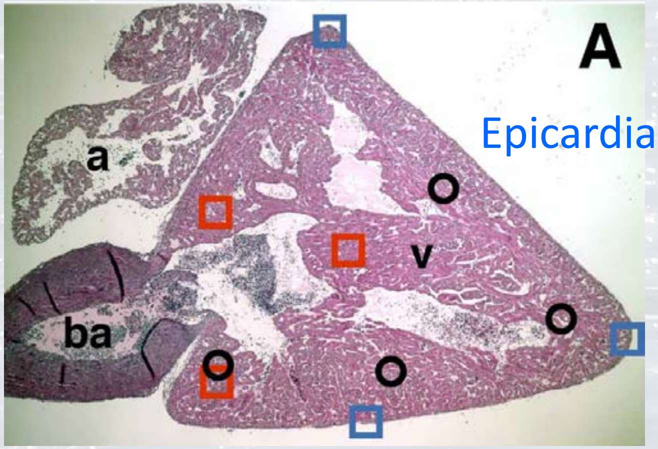
Change in aspect ratio and outflow tract were consistent with salmon

measure	control	oil exposed	*P* oil effect[b]	*P* tank effect[c]
Fork length (cm)	5.3 ± 0.1	5.6 ± 0.1	0.0005	0.0007 (C6)
Mass (g)	1.26 ± 0.07	1.65 ± 0.10	0.0003	0.0004 (O2)
Condition factor	0.82 ± 0.01	0.87 ± 0.01	0.01	0.02 (C11)
Ventricle length - lateral[a]	69.5 ± 1.0	69.8 ± 1.2	0.2	0.001 (O8)
Ventricle width - lateral[a]	51.3 ± 0.9	49.8 ± 0.7	0.7	0.3
Aspect ratio - lateral	1.45 ± 0.02	1.52 ± 0.02	0.04	0.03 (O8)
Ventricle length - ventral[a]	64.0 ± 1.0	63.6 ± 1.5	0.6	0.004 (O8)
Ventricle width - ventral[a]	63.5 ± 0.9	64.3 ± 1.0	0.07	0.01 (O8)
Aspect ratio - ventral	1.14 ± 0.01	1.13 ± 0.01	0.2	0.003 (C11)
Ventricle volume (mm^3)	0.042 ± 0.003	0.050 ± 0.002	0.007	0.002 (C6)
BA length (relative to ventricle)	0.45 ± 0.01	0.47 ± 0.01	0.6	0.5
OFT angle (degrees)	21.9 ± 1.8	15.4 ± 1.4	0.004	0.5

Change in ventricular shape could be cardiac hypertrophy (histological assessment)



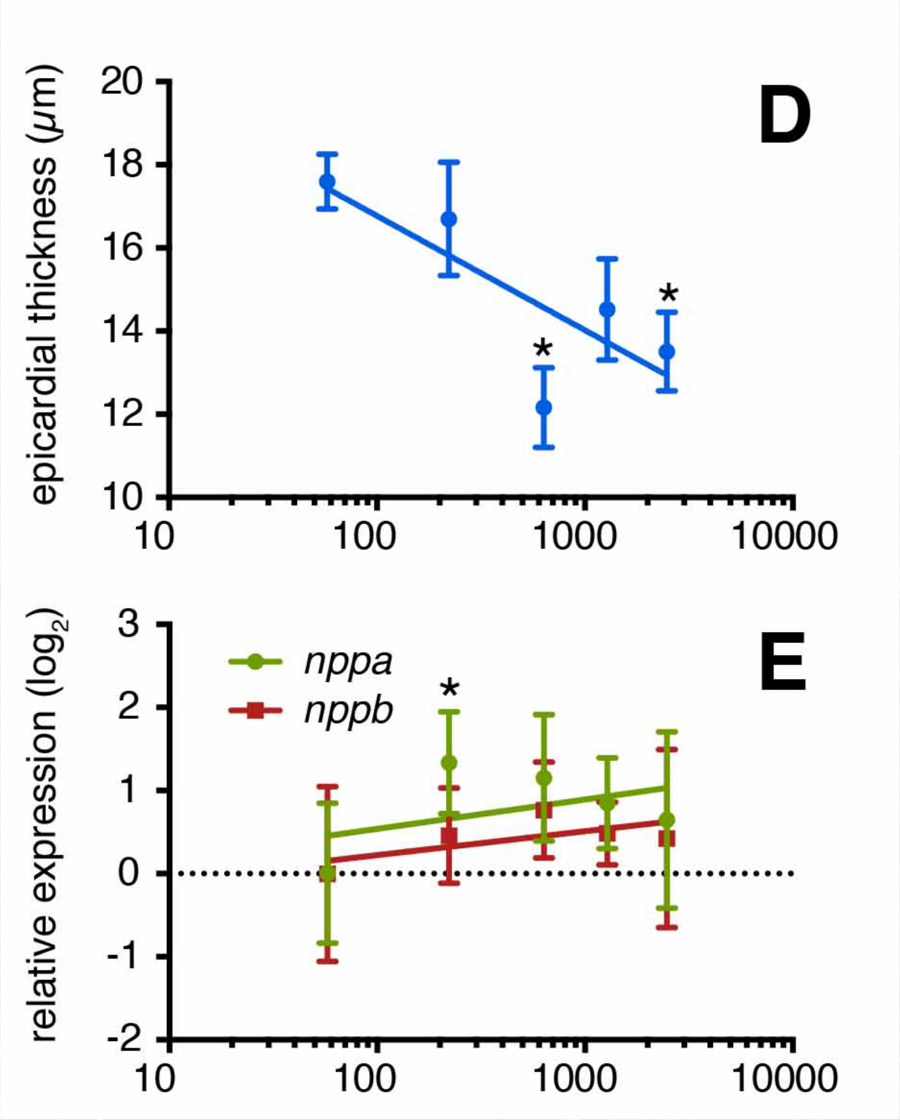
Cardiomyocyte diameter (not sig)



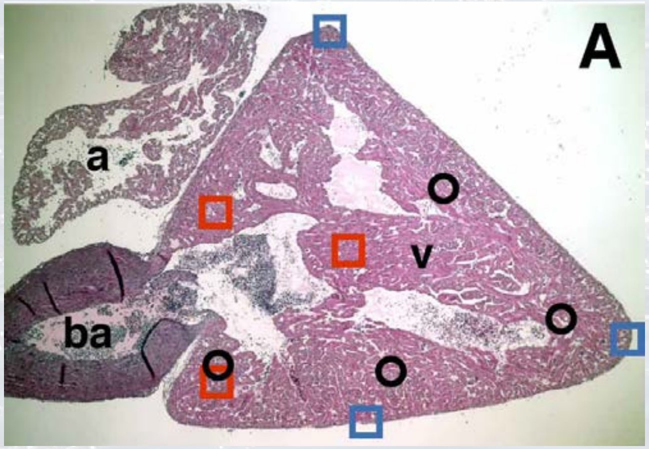
Epicardial thickness

Cardiomyocyte nucleous density (sig)

Change in ventricular shape could be cardiac hypertrophy



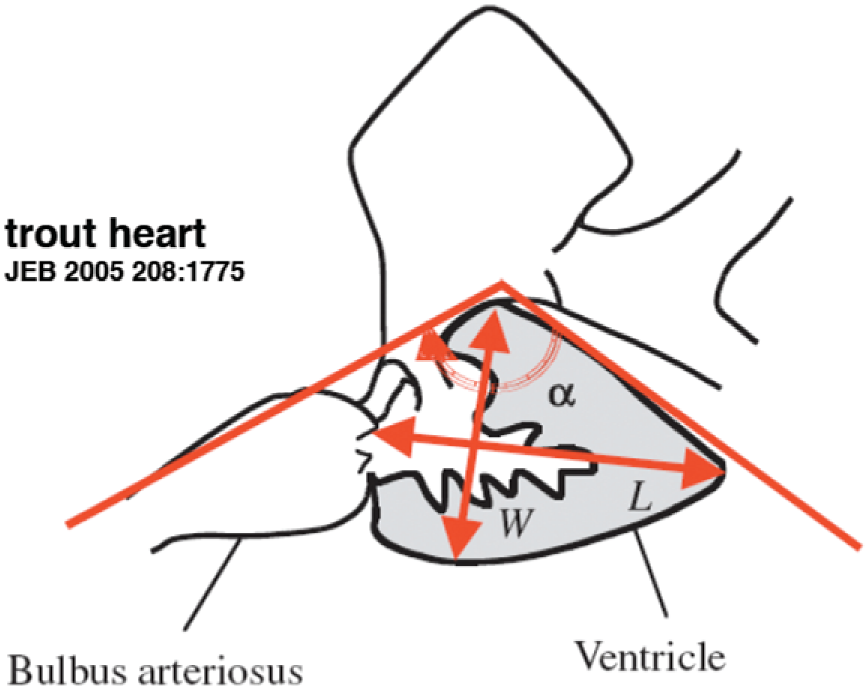
Epicardial thickness (sig)



Natriuretic peptides (trend)

Cardiac output is linked to optimal ventricular shape in numerous fish species

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; $P<0.05$).

Pyramidal ventricles confer high cardiac output for prolonged swimming

Exposed adult rainbow trout with reduced aspect ratios had lower cardiac output *(at left)*

Zebrafish exposed as embryos to ANSCO and assessed 1 y later had reduced aspect ratios and reduced U_{crit} *(Hicken et al. 2011)*

Juvenile zebrafish naturally have more elongated ventricles than adults

Therefore, juvenile salmon hypertrophy may transform into rounded adult morphology

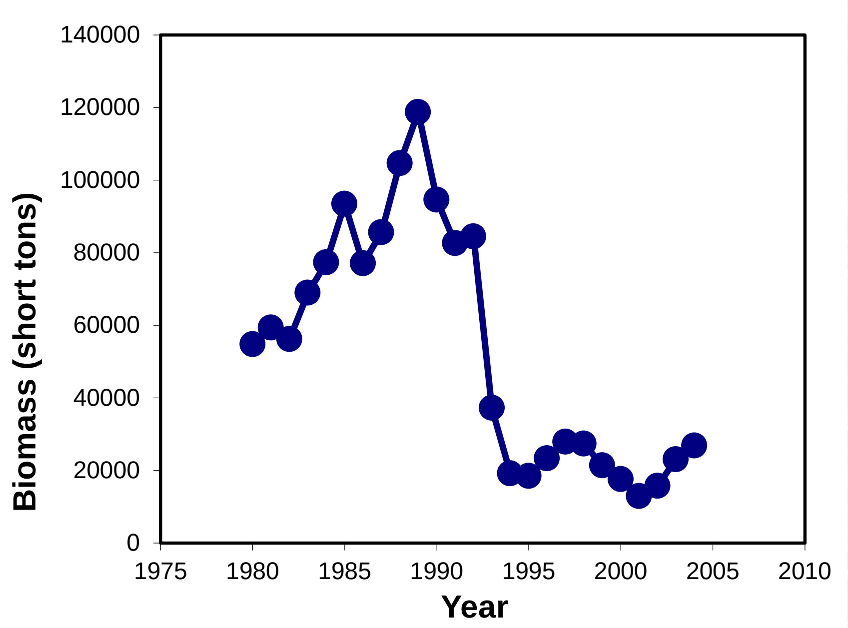
Cardiac output is linked to optimal ventricular shape in numerous fish species

Alternatively, the precise nature of cardiac dysfunction during embryonic exposure likely influences final ventricle shape

e.g., Zebrafish lacking atrial myosin heavy chain have elongated ventricles with a reduced outflow tract angle

Crude oil reduces ventricular contractility in zebrafish without affecting the atrium & causes atrial fibrillation in Pacific herring embryos

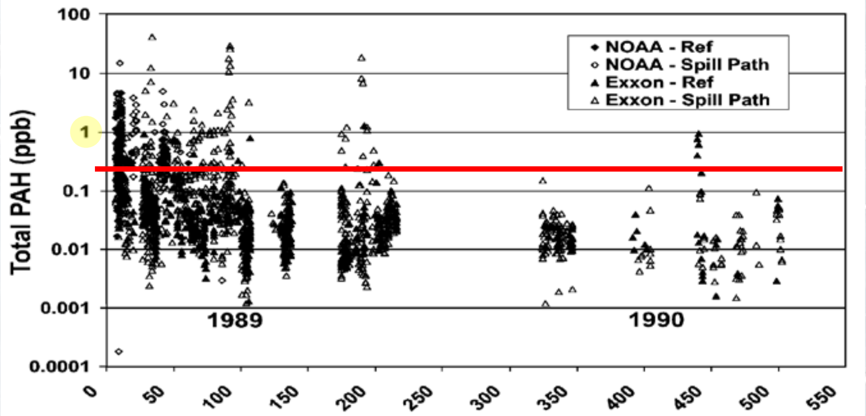
Was the herring population collapse in PWS related to oil exposure?



Cardiac injury occurs in a significant portion of herring that survive transient PAH exposure without outward signs of acute heart failure

Injury threshold: 0.23 μg/L

A previous study suggested 7% of water was injurious *(Boehm et al. 2007)*



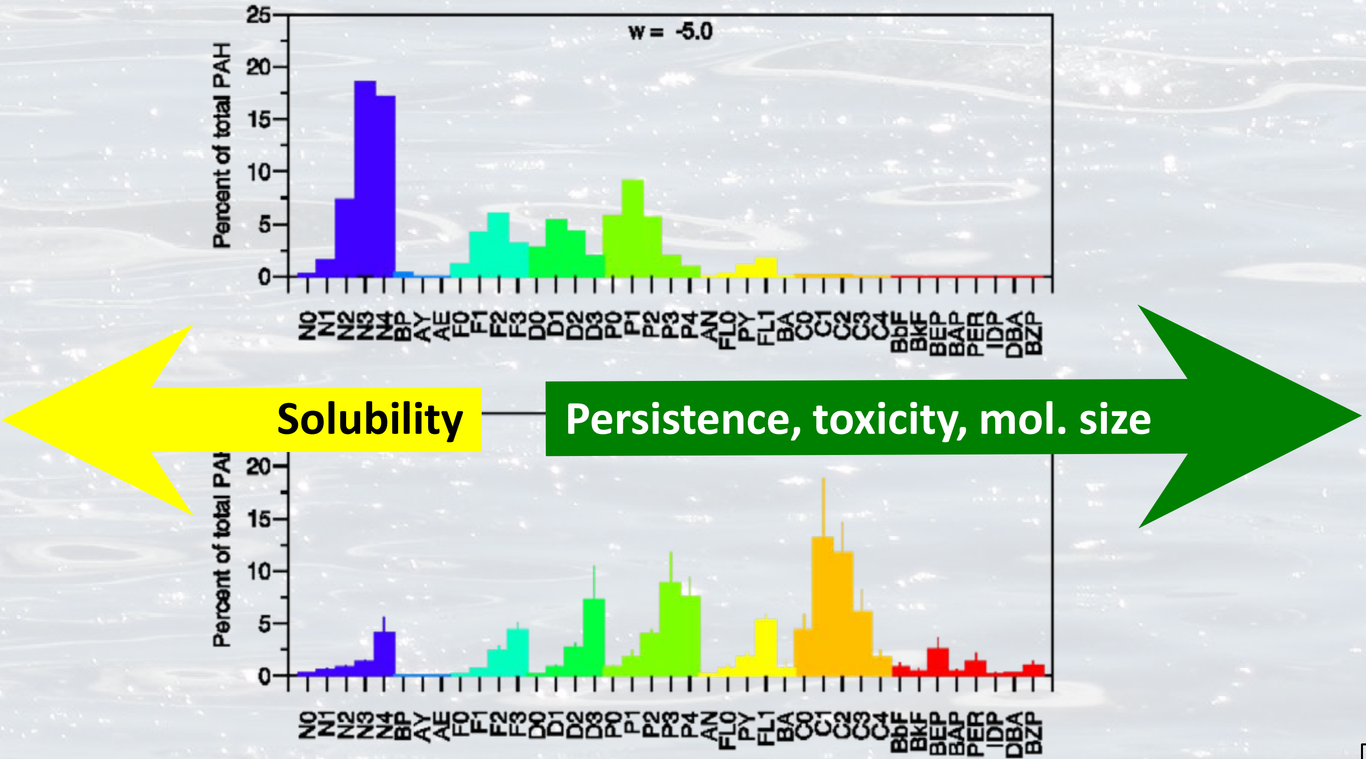
But this study lowers the effects threshold

Therefore, forage fish loss in PWS may have been underestimated

Critical point:

Toxicity is a function of concentration AND composition

Low concentrations of effluent from weathered oil are more toxic than those from unweathered oil because the less toxic, more volatile lower molecular weight compounds are differentially removed by weathering



weathering

Fish hearts are damaged by embryonic exposure to oil

Transient exposure causes permanent changes in heart anatomy

Damaging levels can be very low (0.23 μg/L = parts-per-billion)

Fish with damaged hearts have

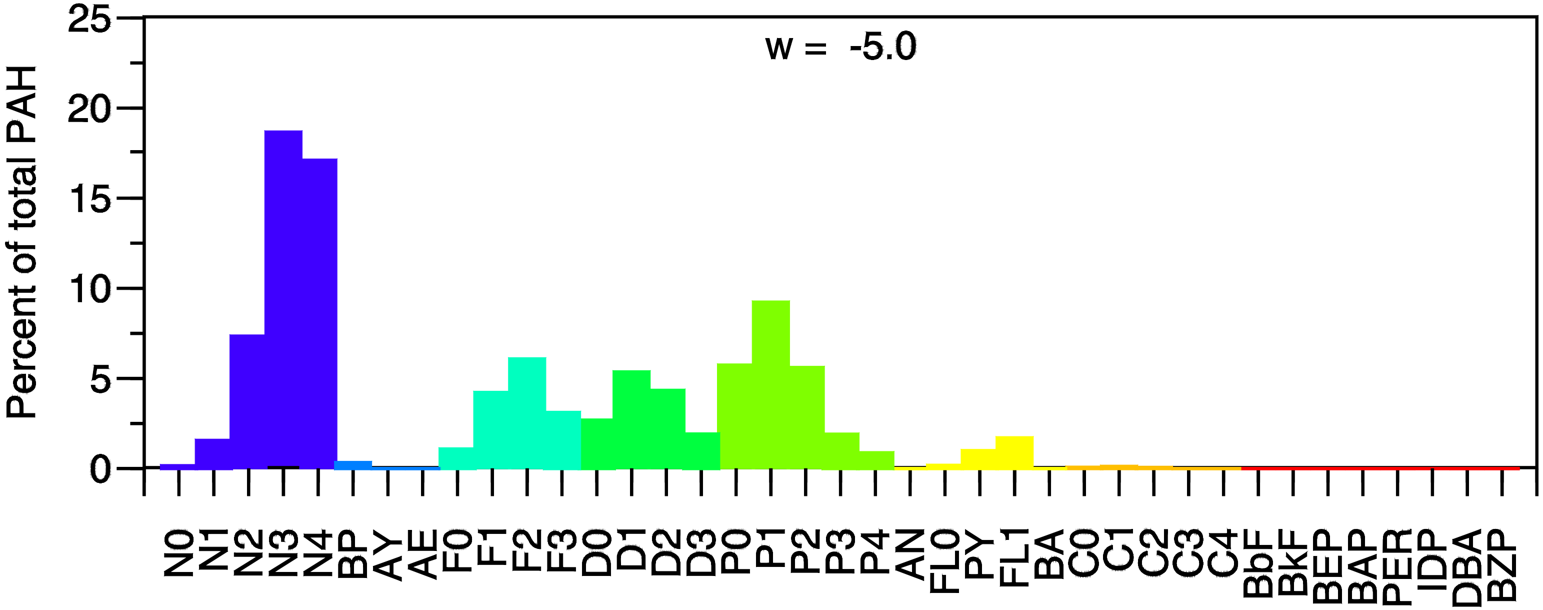
Reduced aerobic capacity

Evidence of ventricular hypertrophy

The Exxon spill in PWS likely impacted pink salmon and Pacific herring more than previously estimated.

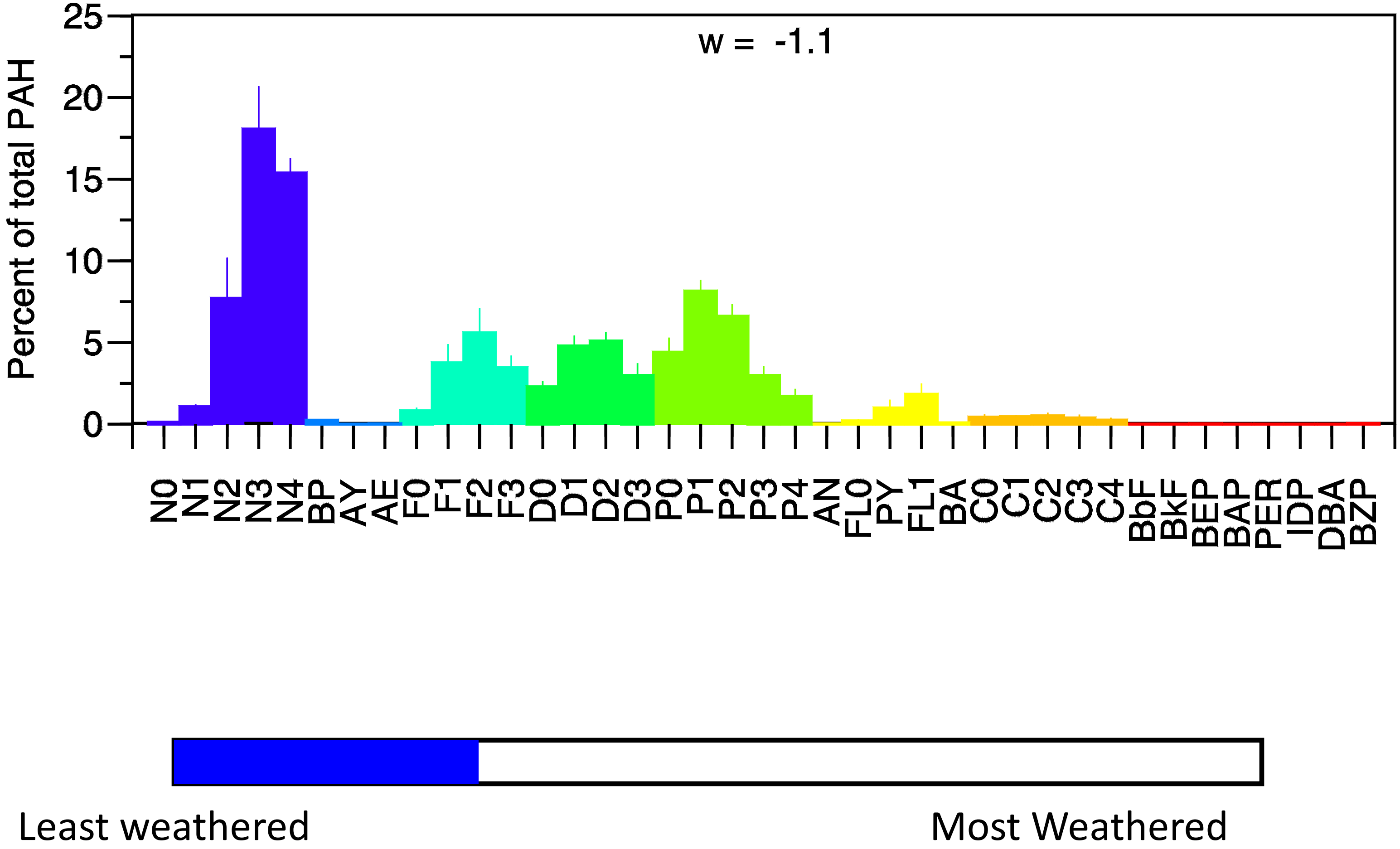
Developmental cardiotoxicity may have contributed to the catastrophic collapse of the PWS herring population

Thank you!

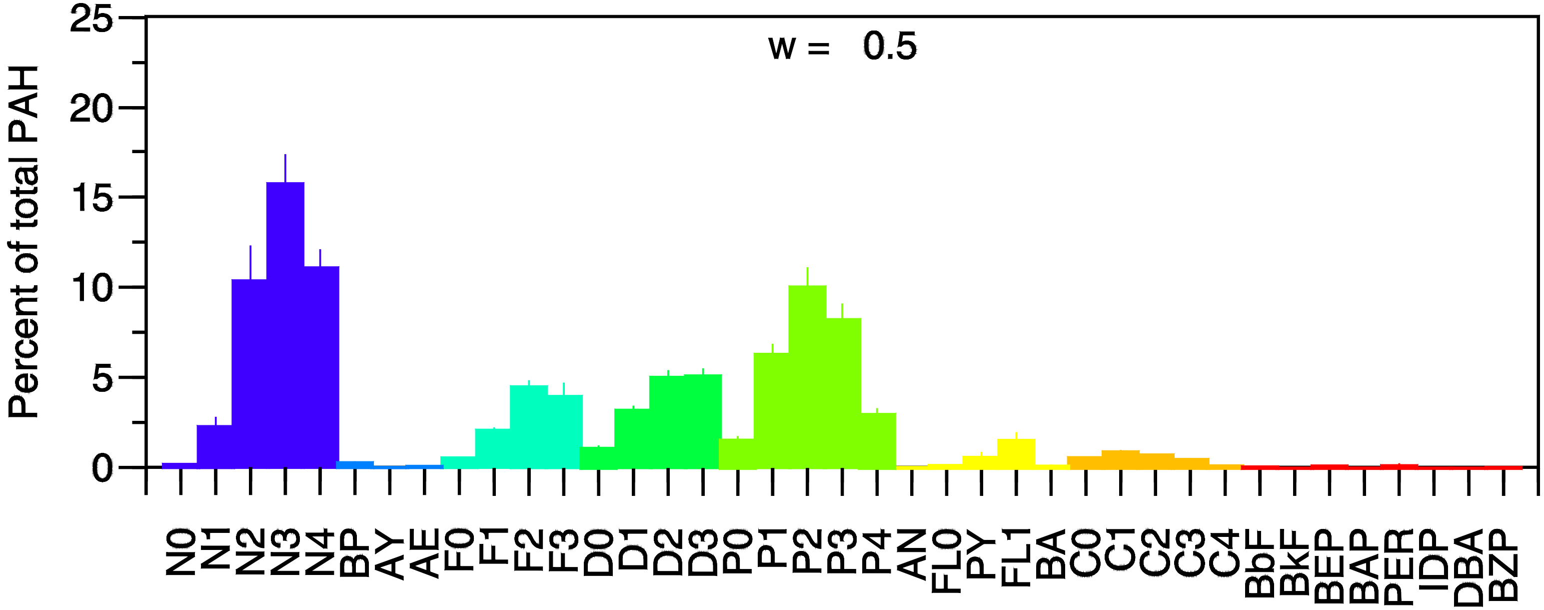


Least weathered

Most Weathered

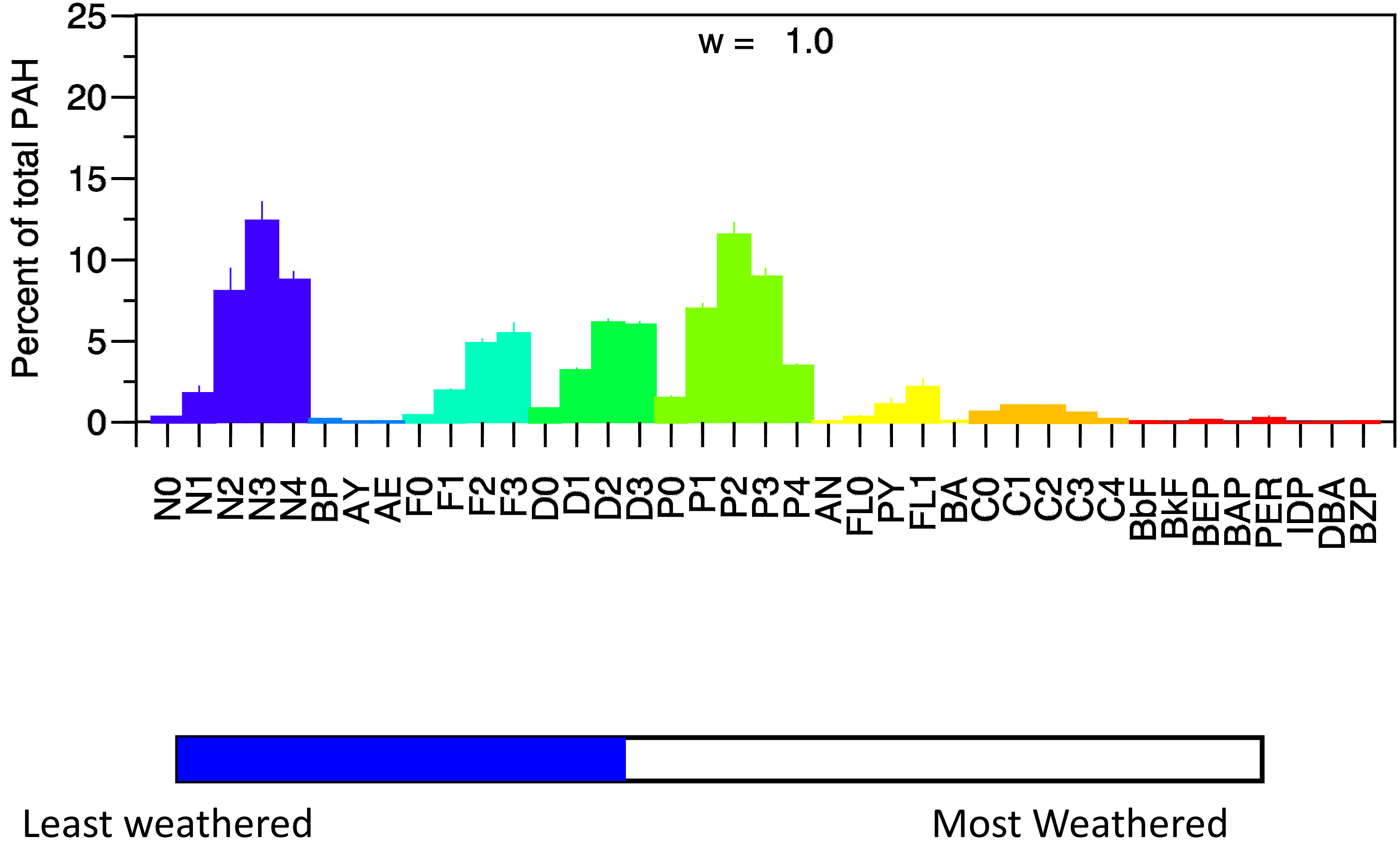


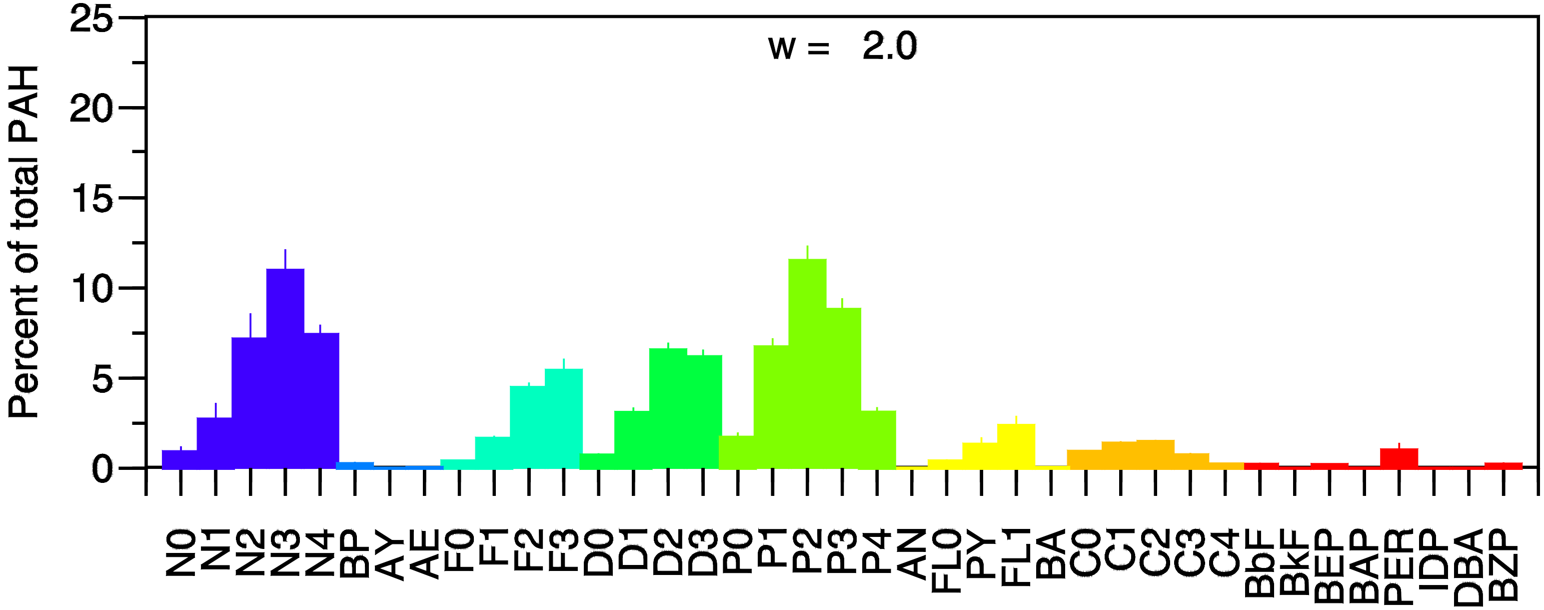
Percent of total PAH
25
20
15
10
5
0
w = -1.1
N0 N1 N2 N3 N4 BP AY AE F0 F1 F2 F3 D0 D1 D2 D3 P0 P1 P2 P3 P4 AN FL0 PY FL1 BA C0 C1 C2 C3 C4 BbF BkF BEP BAP PER IDP DBA BZP
Least weathered
Most Weathered



Least weathered

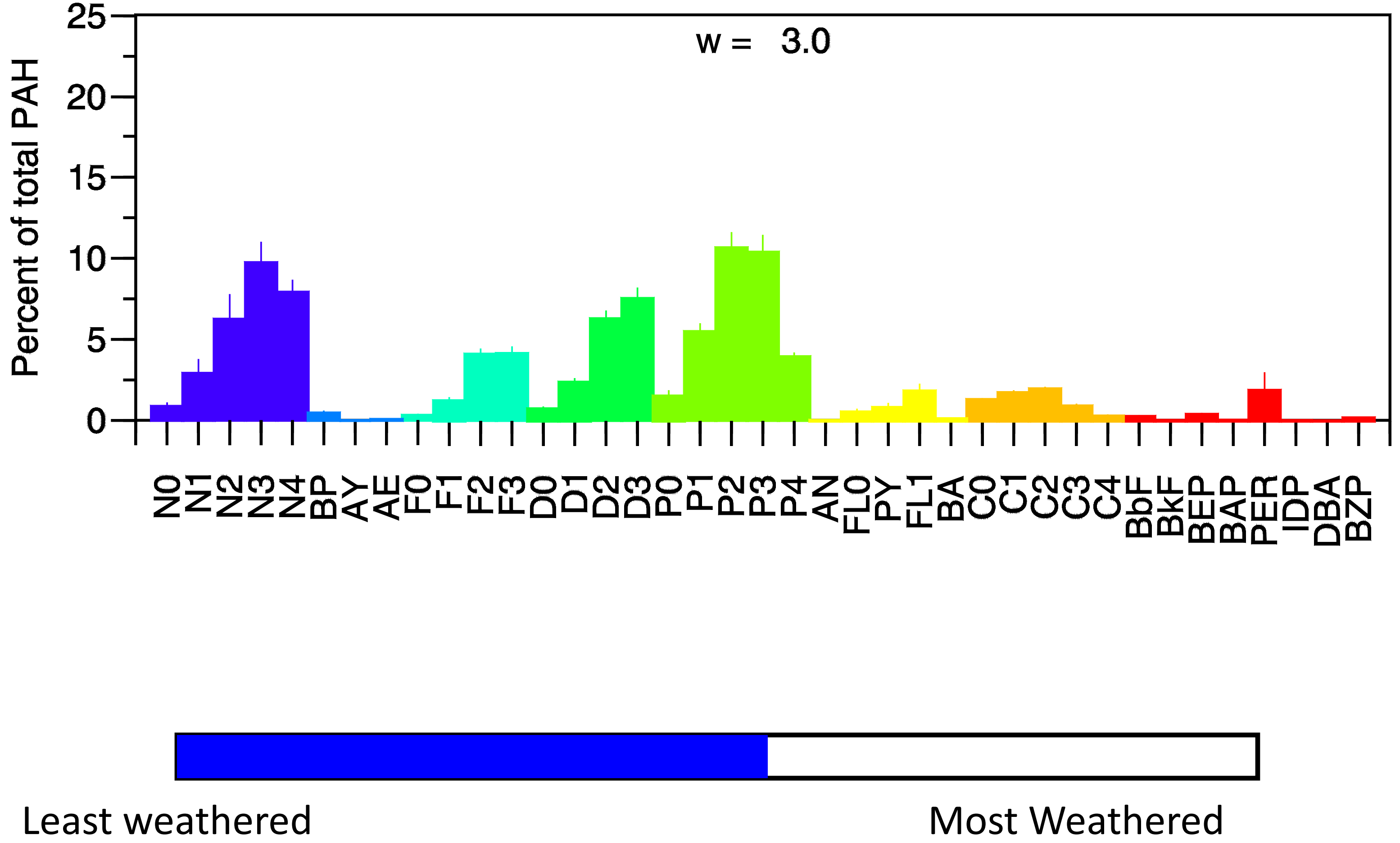
Most Weathered

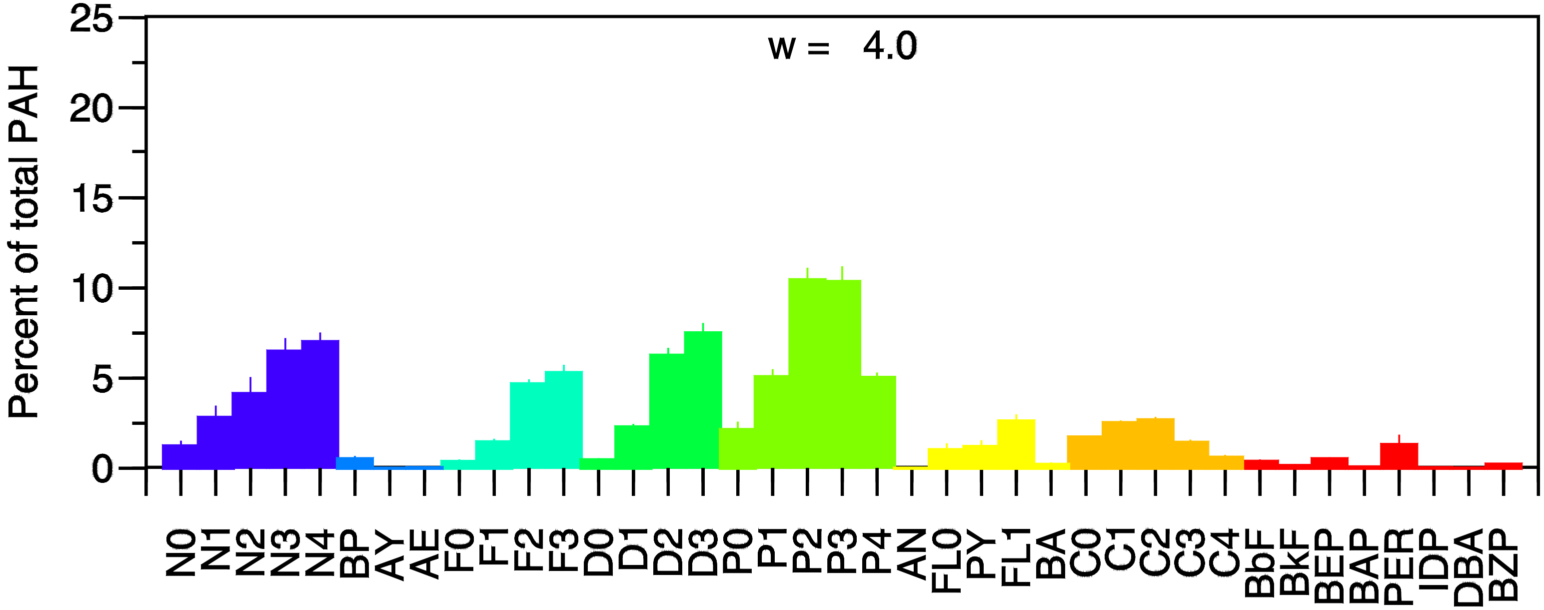

w = 1.0
Percent of total PAH
0
5
10
15
20
25
N0
N1
N2
N3
N4
BP
AY
AE
F0
F1
F2
F3
D0
D1
D2
D3
P0
P1
P2
P3
P4
AN
FL0
PY
FL1
BA
C0
C1
C2
C3
C4
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP
Least weathered
Most Weathered



Least weathered

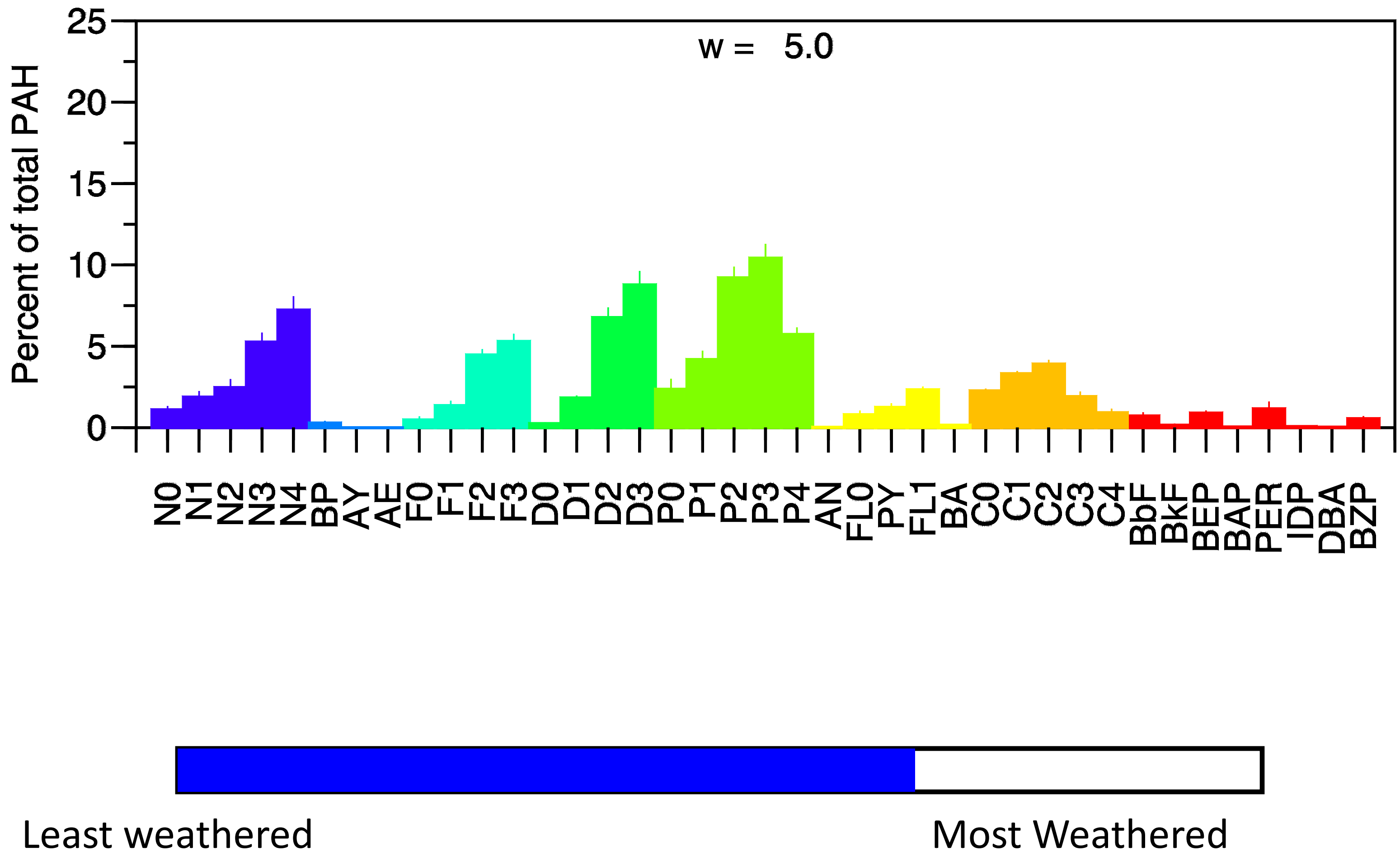
Most Weathered

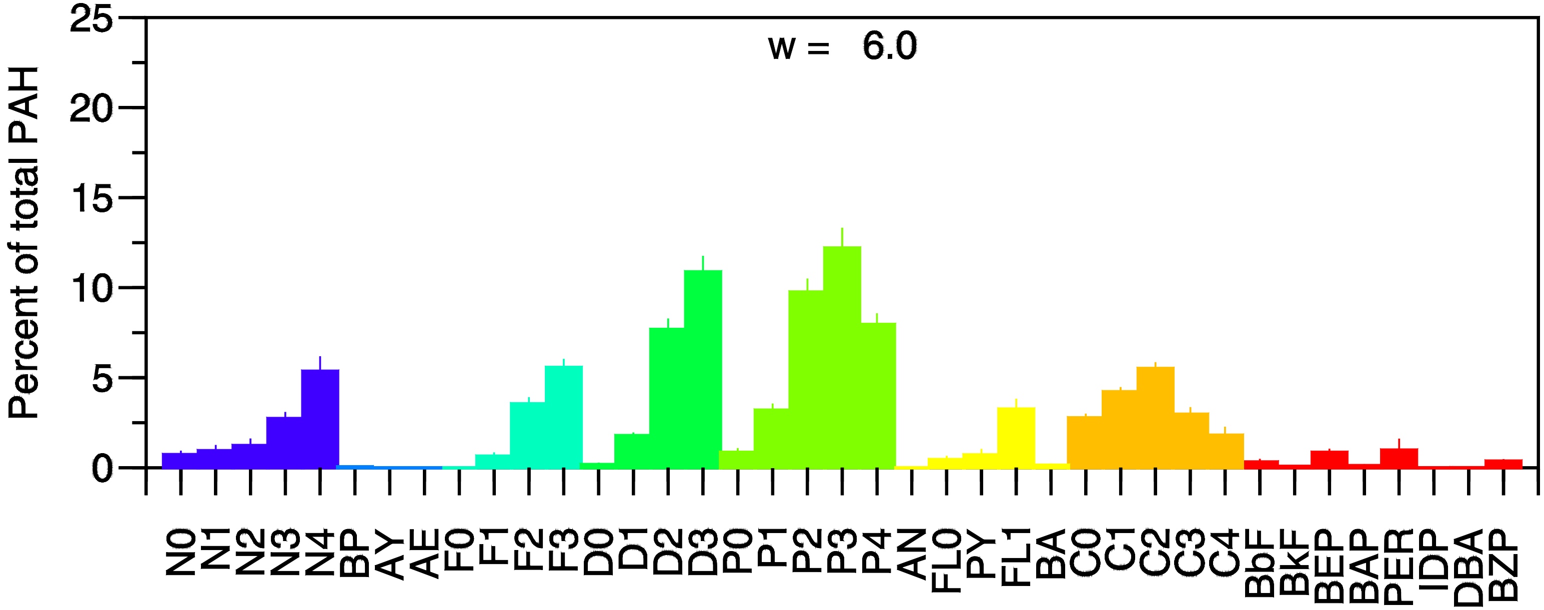

w = 3.0
Percent of total PAH
25
20
15
10
5
0
N0
N1
N2
N3
N4
BP
AY
AE
F0
F1
F2
F3
D0
D1
D2
D3
P0
P1
P2
P3
P4
AN
FL0
PY
FL1
BA
C0
C1
C2
C3
C4
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP
Least weathered
Most Weathered



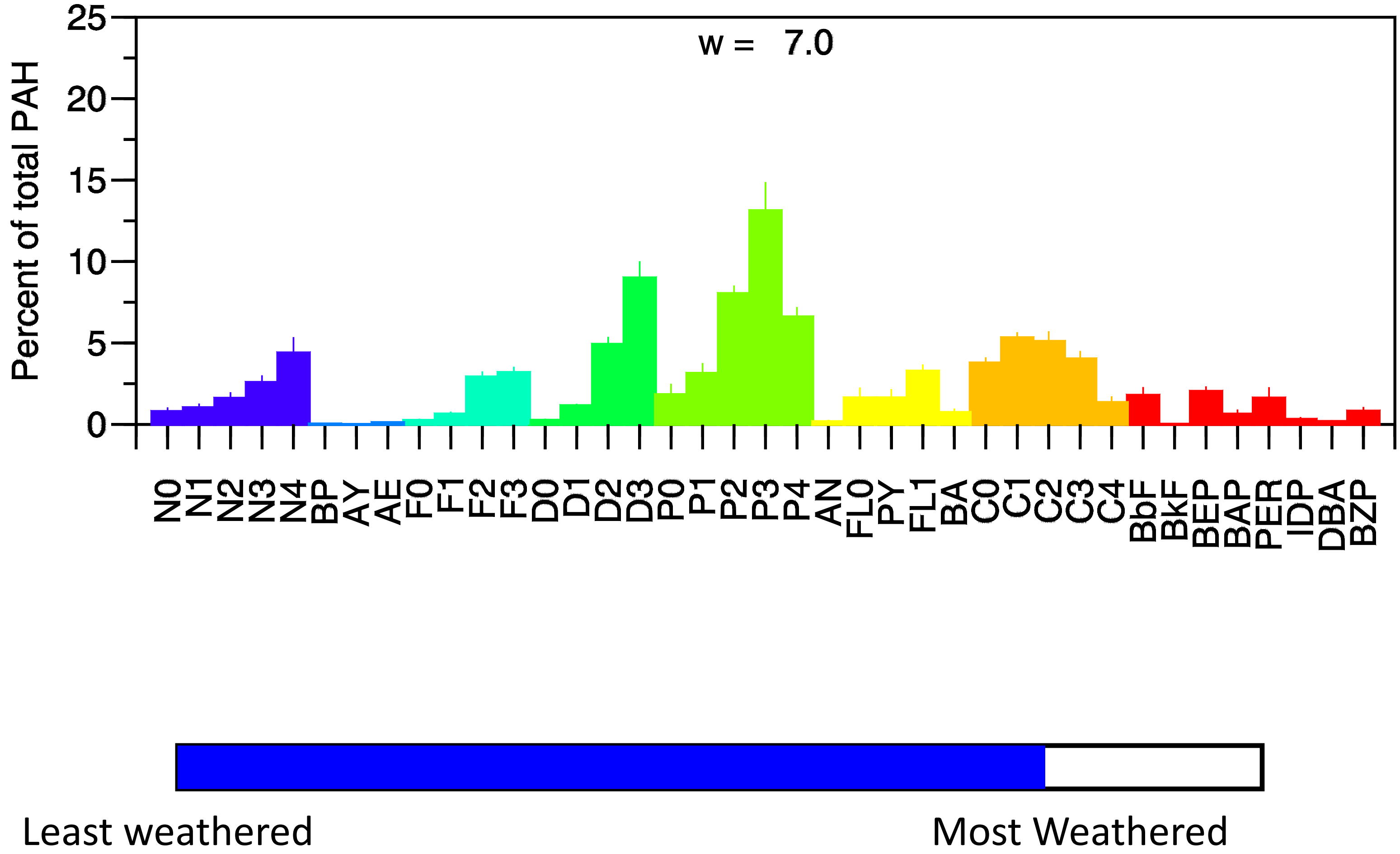
Least weathered

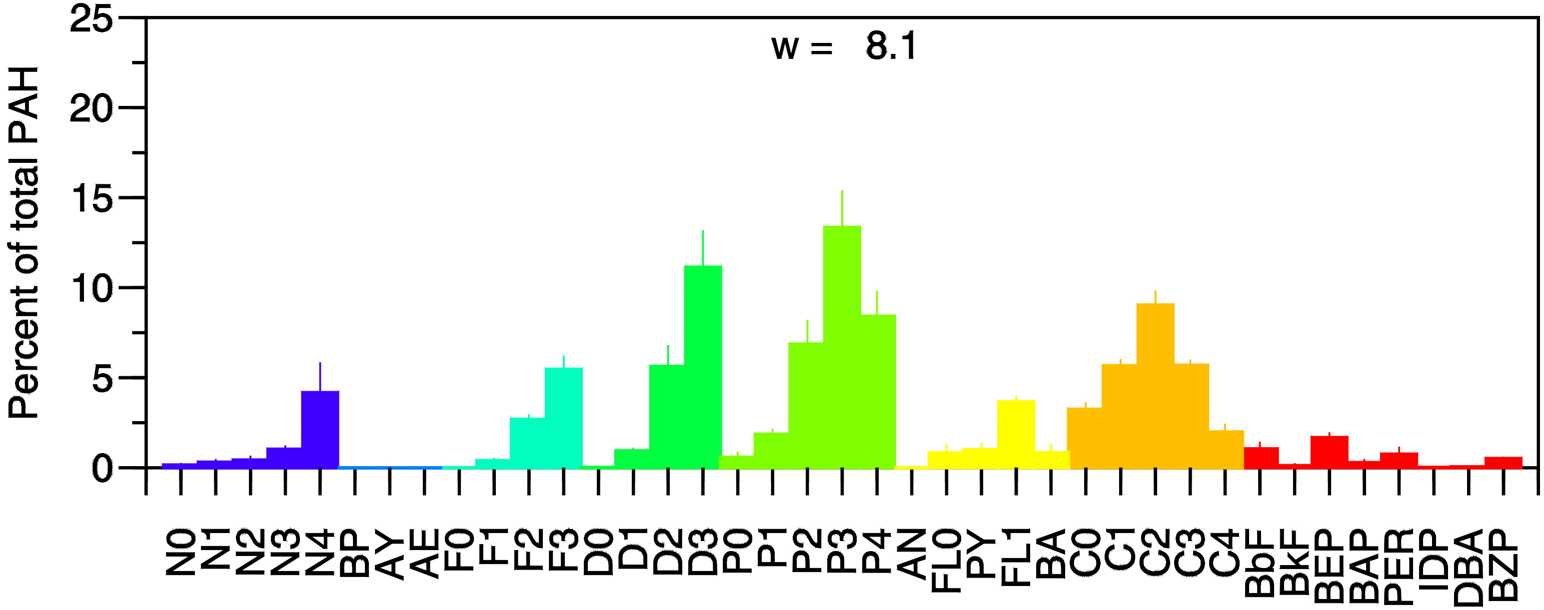
Most Weathered


w = 5.0
Percent of total PAH
0
5
10
15
20
25
N0 N1 N2 N3 N4 BP AY AE F0 F1 F2 F3 D0 D1 D2 D3 P0 P1 P2 P3 P4 AN FL0 PY FL1 BA C0 C1 C2 C3 C4 BbF BkF BEP BAP PER IDP DBA BZP
Least weathered
Most Weathered



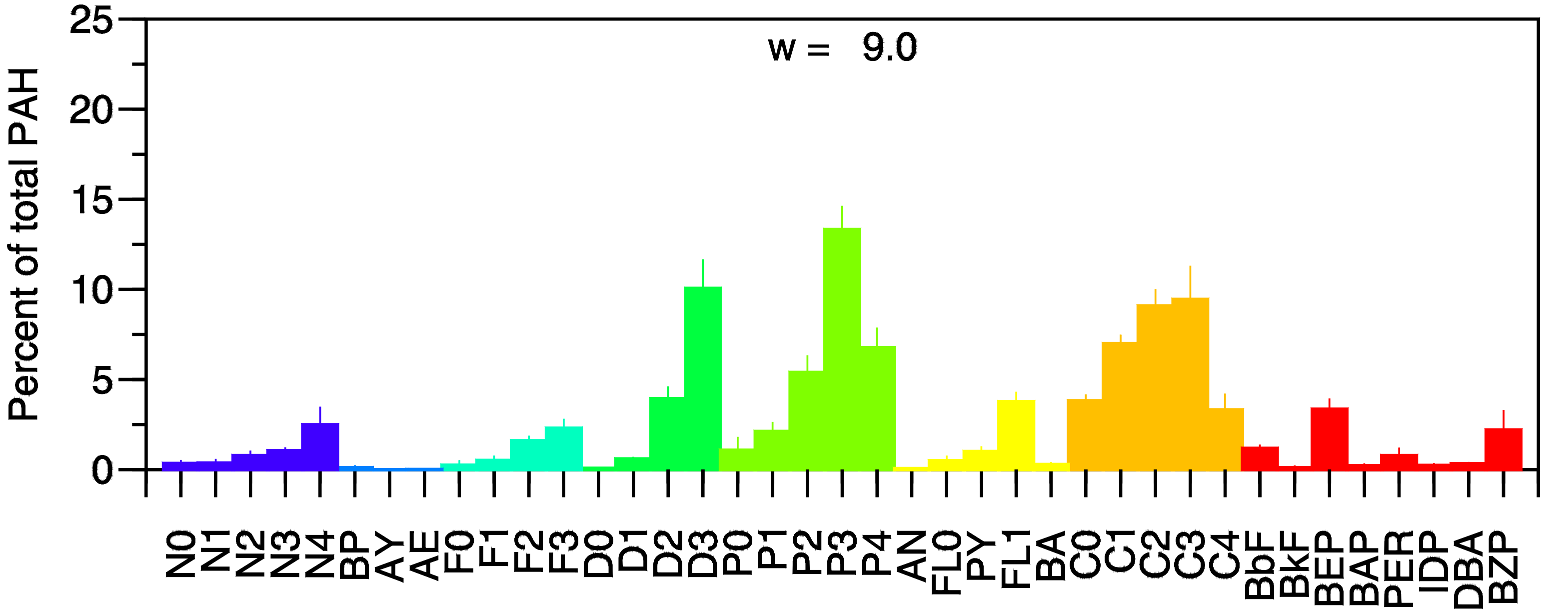
Least weathered Most Weathered


w = 7.0
Percent of total PAH
25
20
15
10
5
0
N0
N1
N2
N3
N4
BP
AY
AE
F0
F1
F2
F3
D0
D1
D2
D3
P0
P1
P2
P3
P4
AN
FL0
PY
FL1
BA
C0
C1
C2
C3
C4
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP
Least weathered
Most Weathered



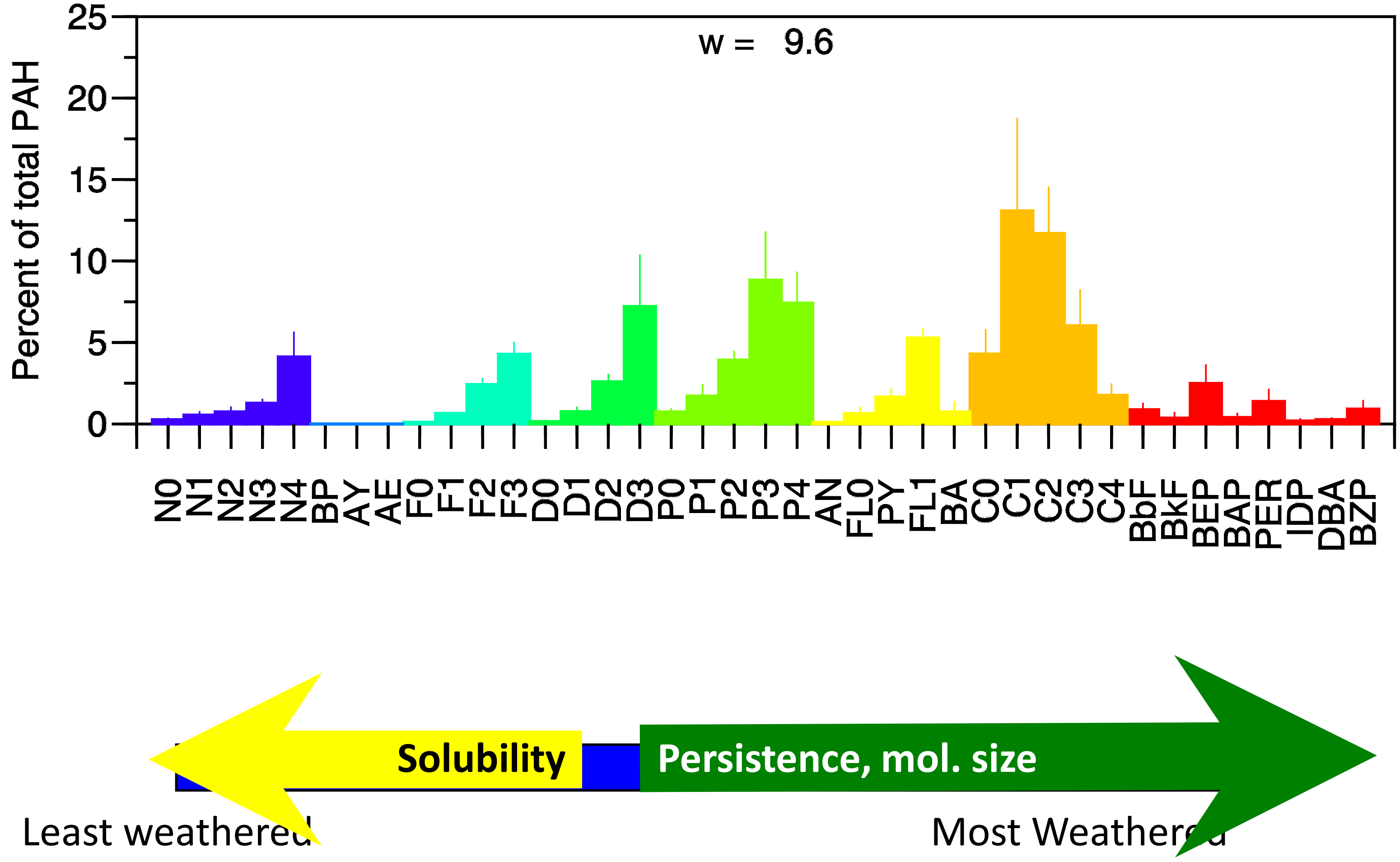
w = 8.1
Percent of total PAH
25
20
15
10
5
0
N0
N1
N2
N3
N4
BP
AY
AE
F0
F1
F2
F3
D0
D1
D2
D3
P0
P1
P2
P3
P4
AN
FL0
PY
FL1
BA
C0
C1
C2
C3
C4
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

Least weathered
Most Weathered



Least weathered

Most Weathered


w = 9.6
Percent of total PAH
25
20
15
10
5
0
N0
N1
N2
N3
N4
BP
AY
AE
F0
F1
F2
F3
D0
D1
D2
D3
P0
P1
P2
P3
P4
AN
FL0
PY
FL1
BA
C0
C1
C2
C3
C4
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP
Solubility
Persistence, mol. size
Least weathered
Most Weathered