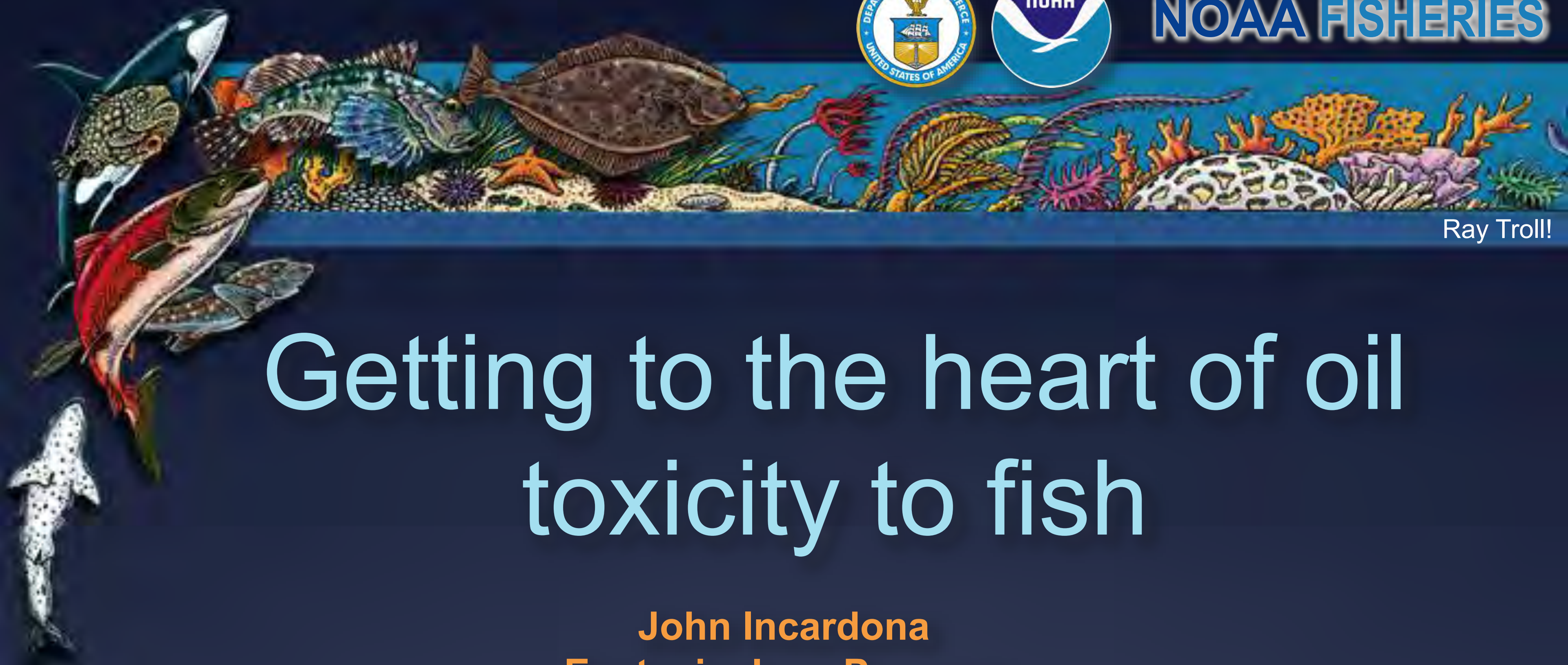
NOAA FISHERIES



Ray Troll!

Getting to the heart of oil toxicity to fish

John Incardona
Ecotoxicology Program
NOAA Northwest Fisheries Science Center, Seattle

Prince William Sound RCAC Regional Citizens' Advisory Council

Hopkins Marine Station Stanford University

Collaborators 2002 to present

Embryology/whole animal studies

- NOAA Fisheries Northwest Fisheries Science Center, Seattle (Incardona, Scholz, Linbo, Tagal, Baldwin, Edmunds, Peck, Swarts, and many others)

- NOAA Fisheries Auke Bay Laboratories, Juneau (Mark Carls and Jeep Rice)

Electrophysiology/gene expression

- Stanford University - Hopkins Marine Station (Barbara Block, Luke Gardner, Fabien Brette, Ben Machado)

Gene expression

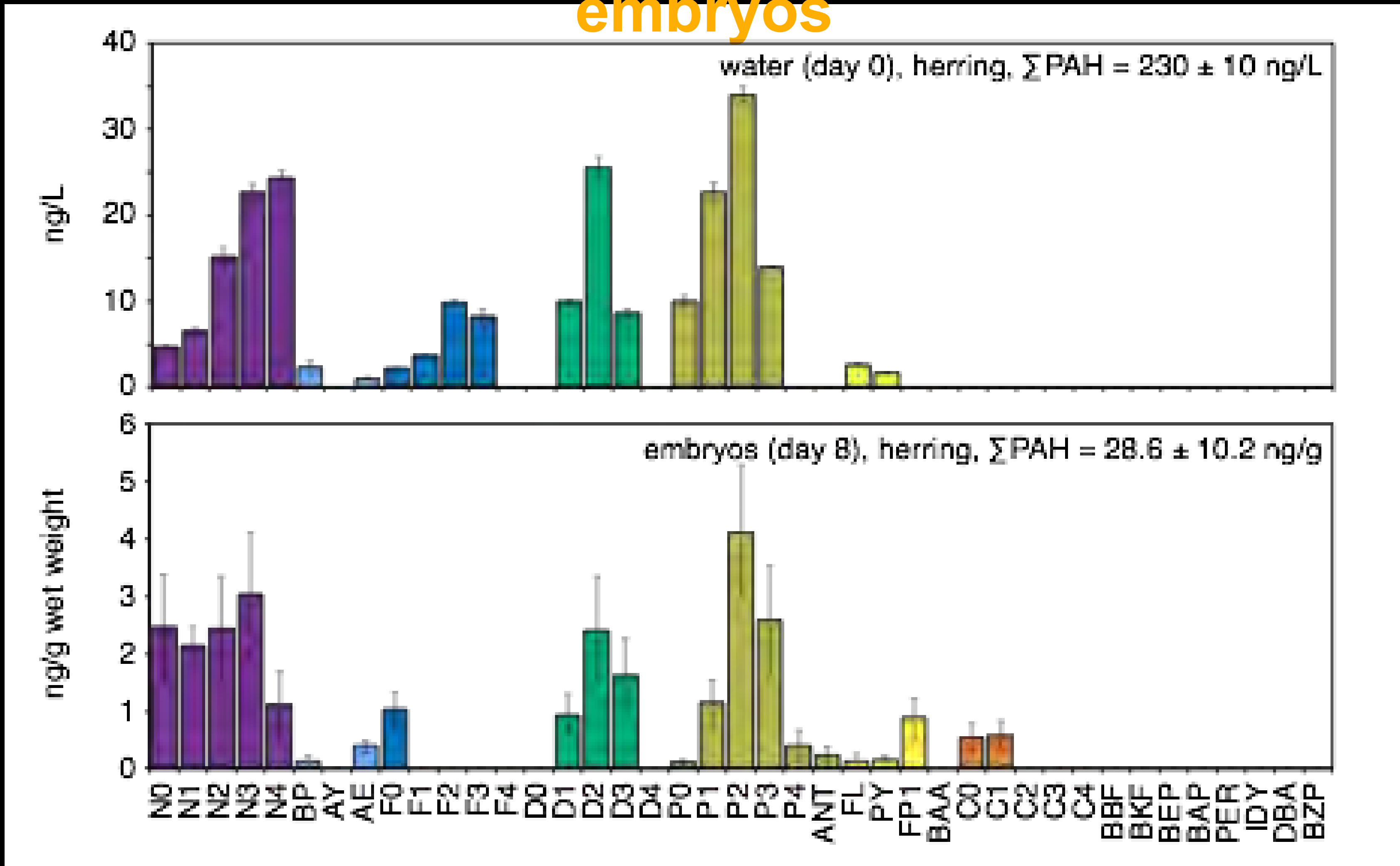
- Institute of Marine Research, Norway; SINTEF, University of Oslo (Sonnich Meier, Rolf Edmundsen, Elin Sørhus)

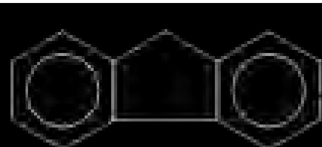
1990s: What NOAA learned from the Exxon Valdez

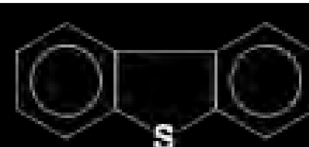
~100K compounds in crude oil
~1% is water soluble

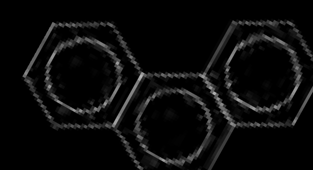
numerous papers
e.g., Marty et al., 1997 Can J Zool 75:989
Carls et al. 1999 ET&C 18:481
Heintz et al., 1999 ET&C 18:494

PAHs are "fat-seeking" and accumulate in fish embryos



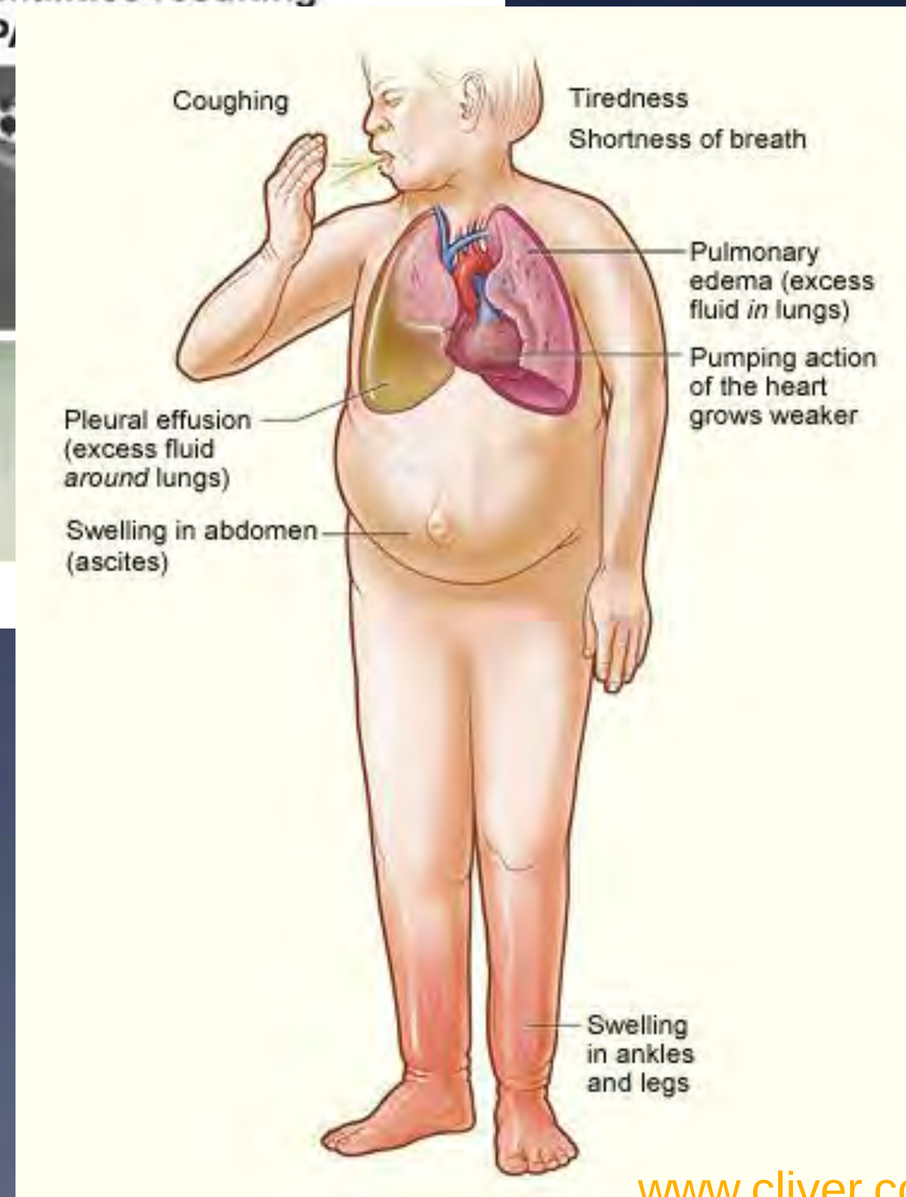






naphthalene fluorene dibenzothiophene phenanthrene

2000s: NOAA learned more from Exxon Valdez

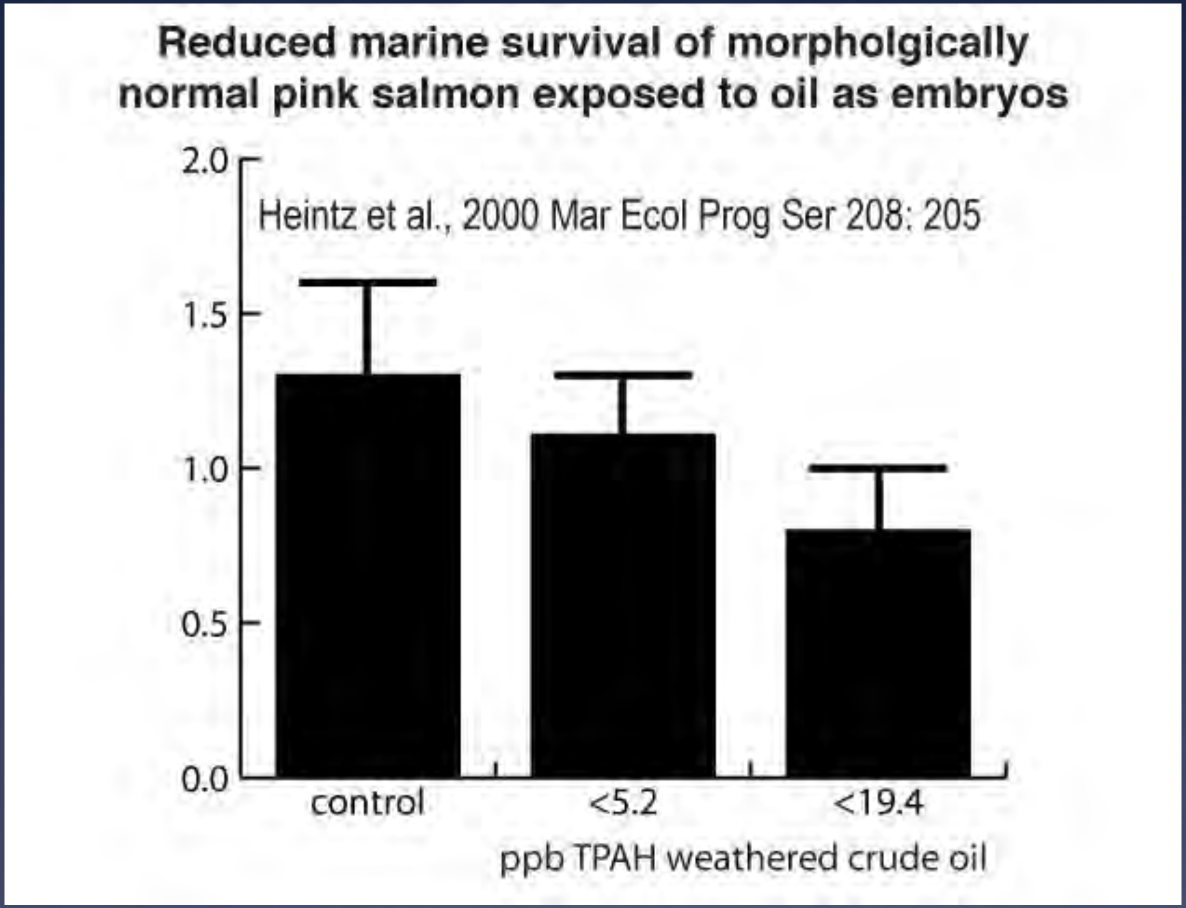


Oil exposure causes heart failure in fish embryos

Incardona et al. 2004 Toxicol Appl Pharm 196:191
2005 Environ Health Persp 113:1755
2009 Environ Sci Technol 43:201

What else NOAA learned from Exxon Valdez

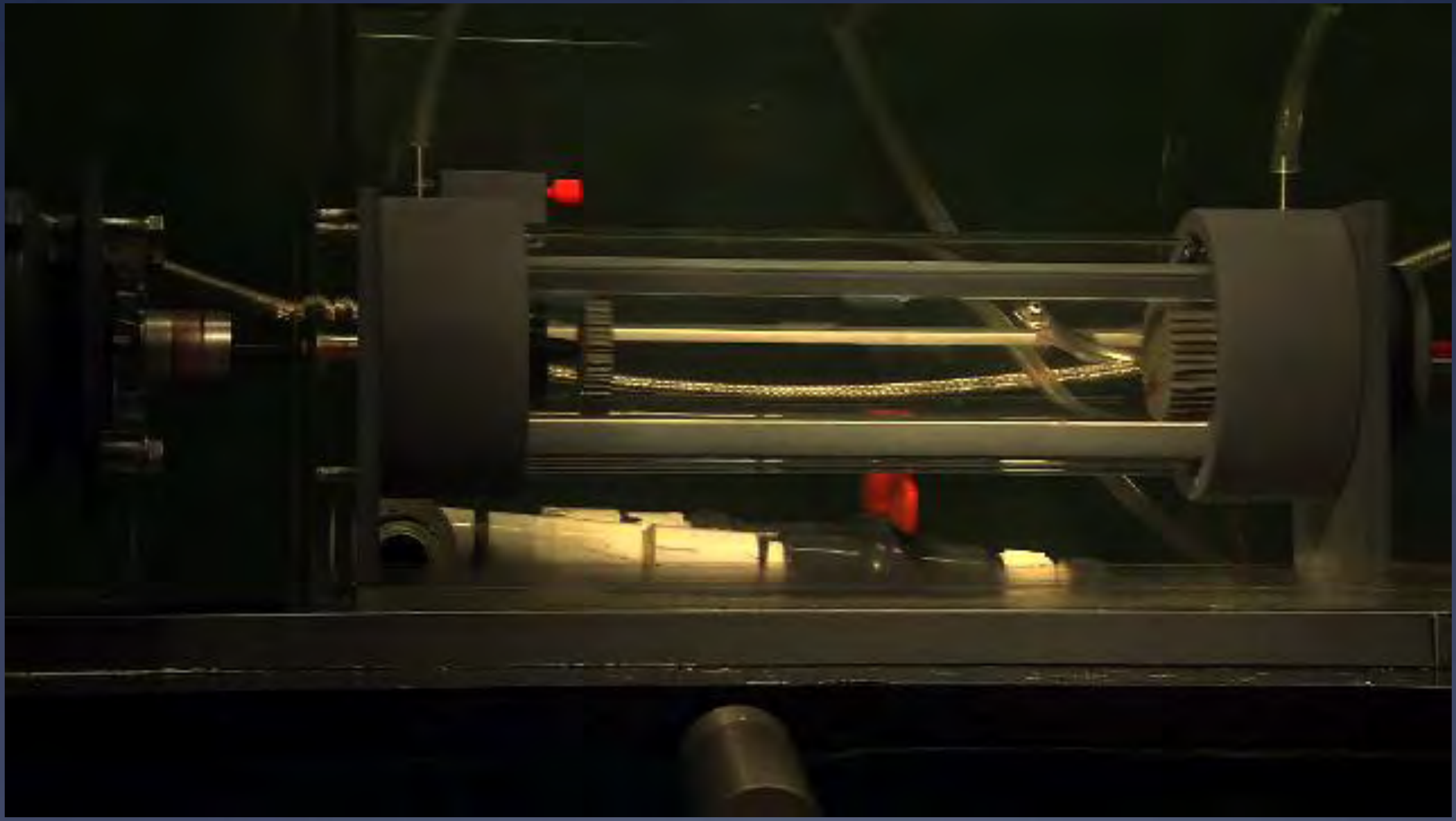
Jeep Rice, Mark Carls, Ron Heintz, NOAA Auke Bay Lab, Juneau



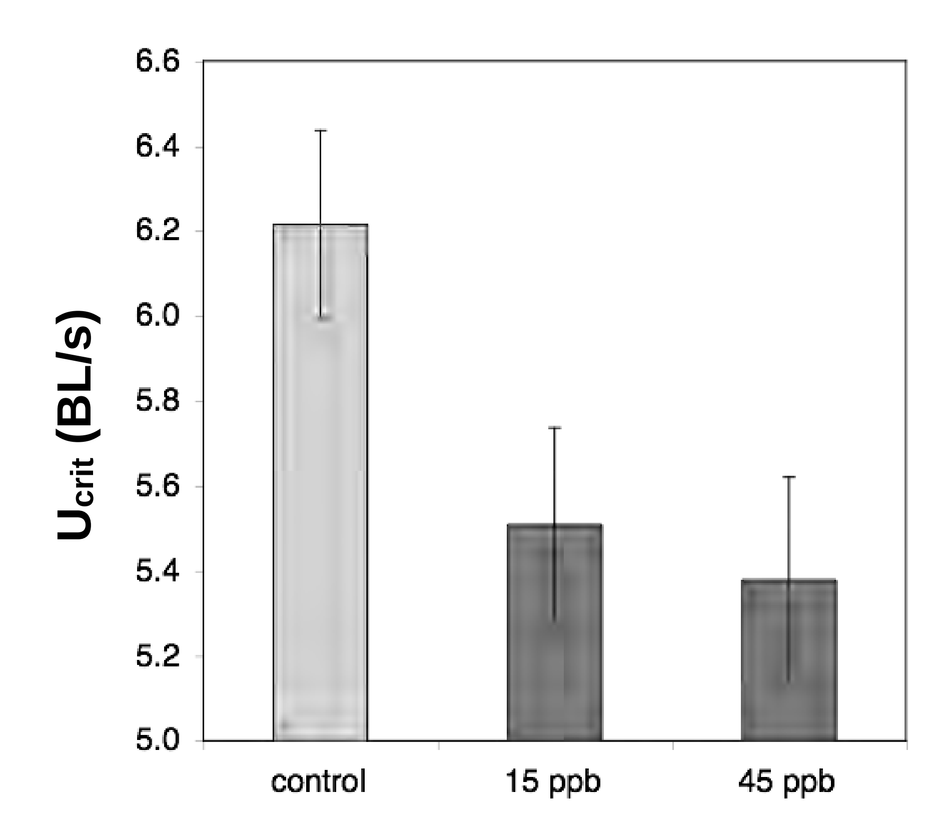
Externally normal salmon fry that survived embryonic oil exposure have reduced survival to adulthood after release to the ocean

Testing swimming ability and heart function in young fish

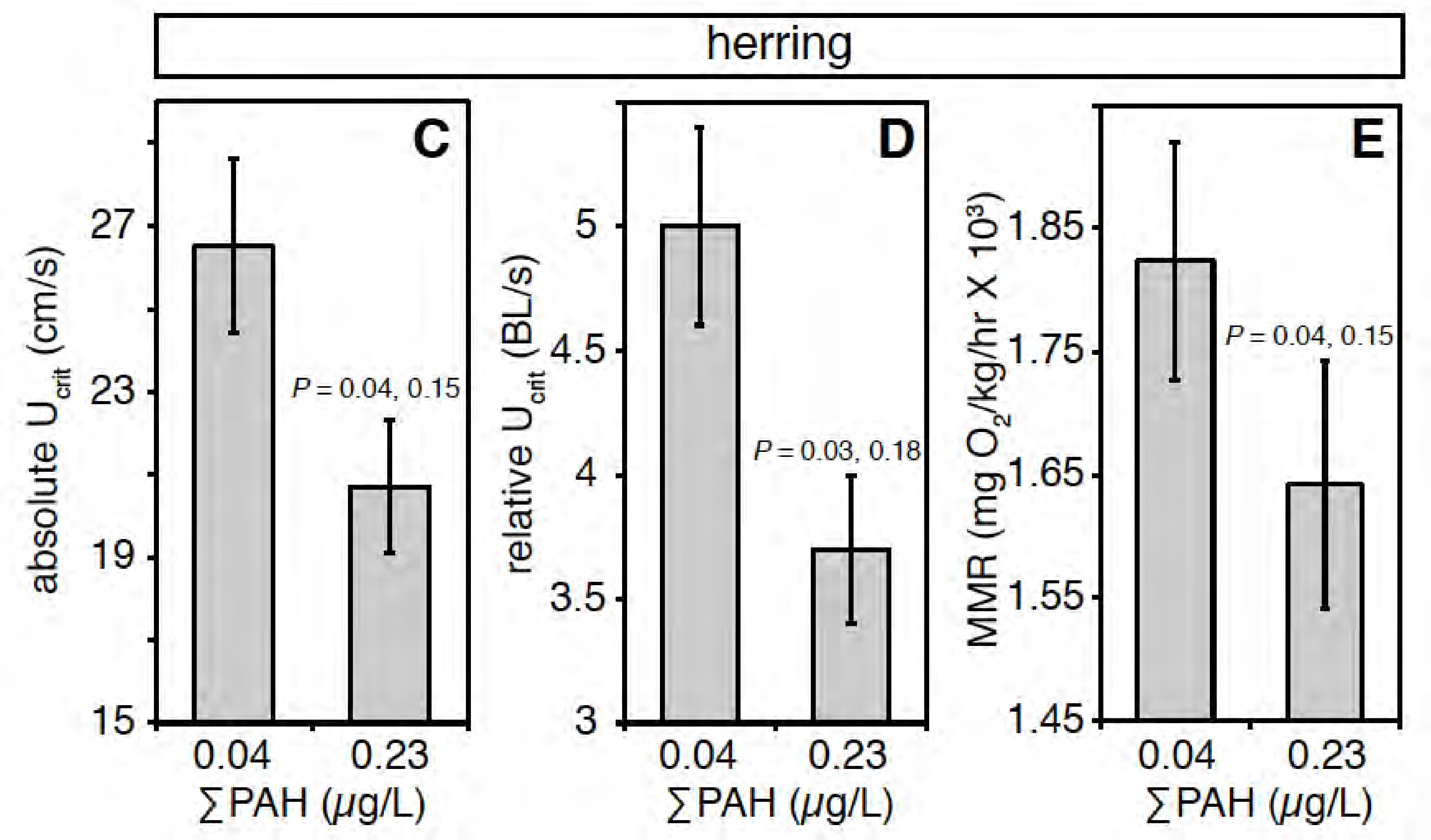
A fish “treadmill” or lap pool



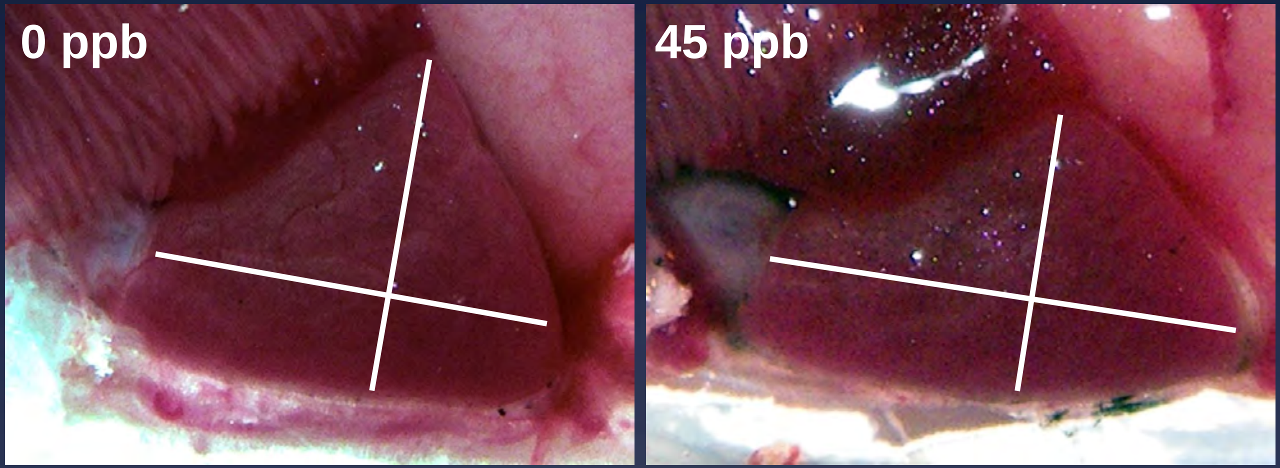
Reduced swimming speed in 9 month old juvenile pink salmon exposed as



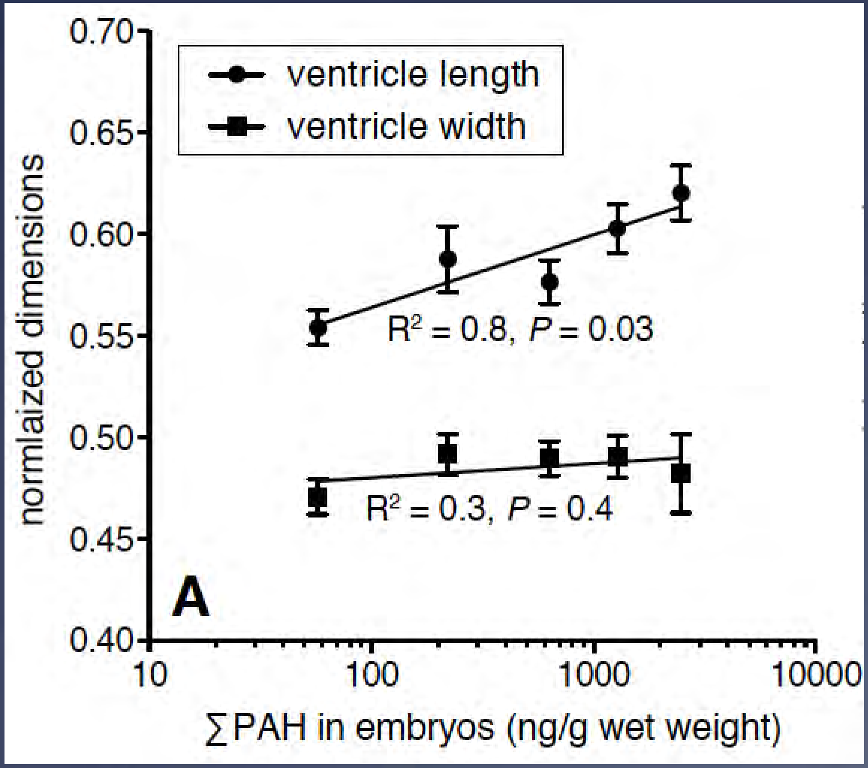
Reduced swimming speed and oxygen consumption in 7 month old juvenile Pacific herring exposed as embryos

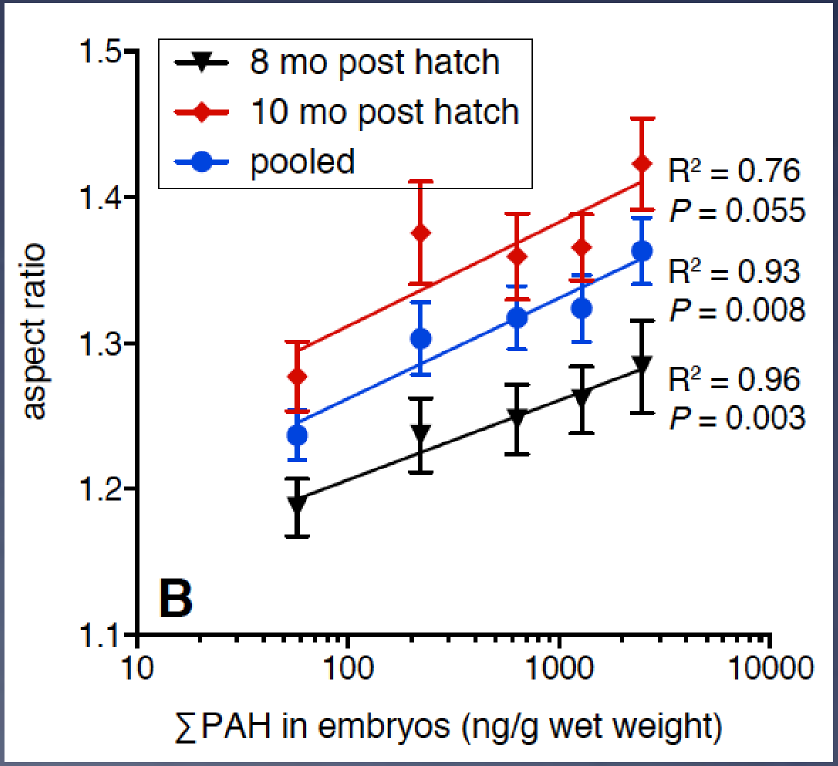


Altered heart shape in juveniles

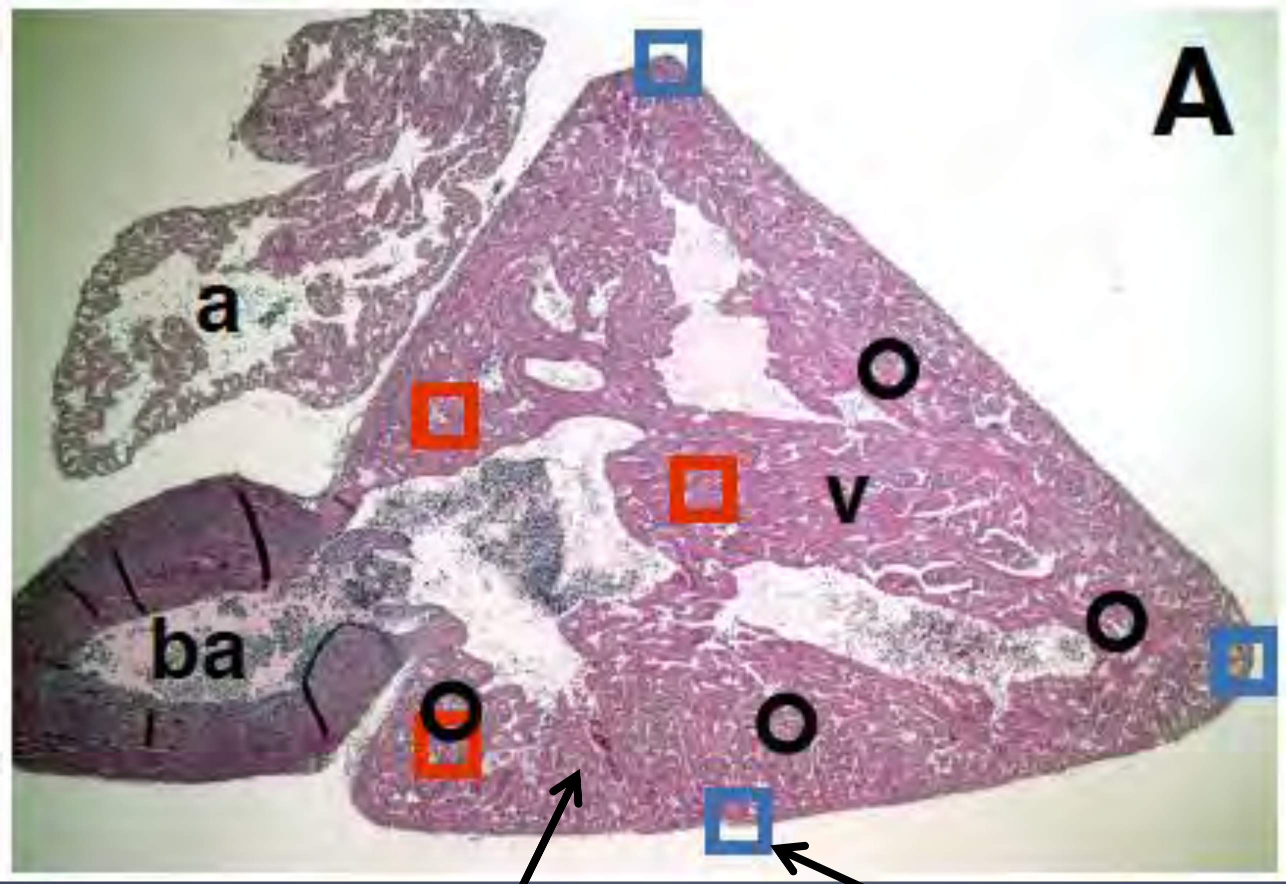


dose-dependent increase in length-width ratio





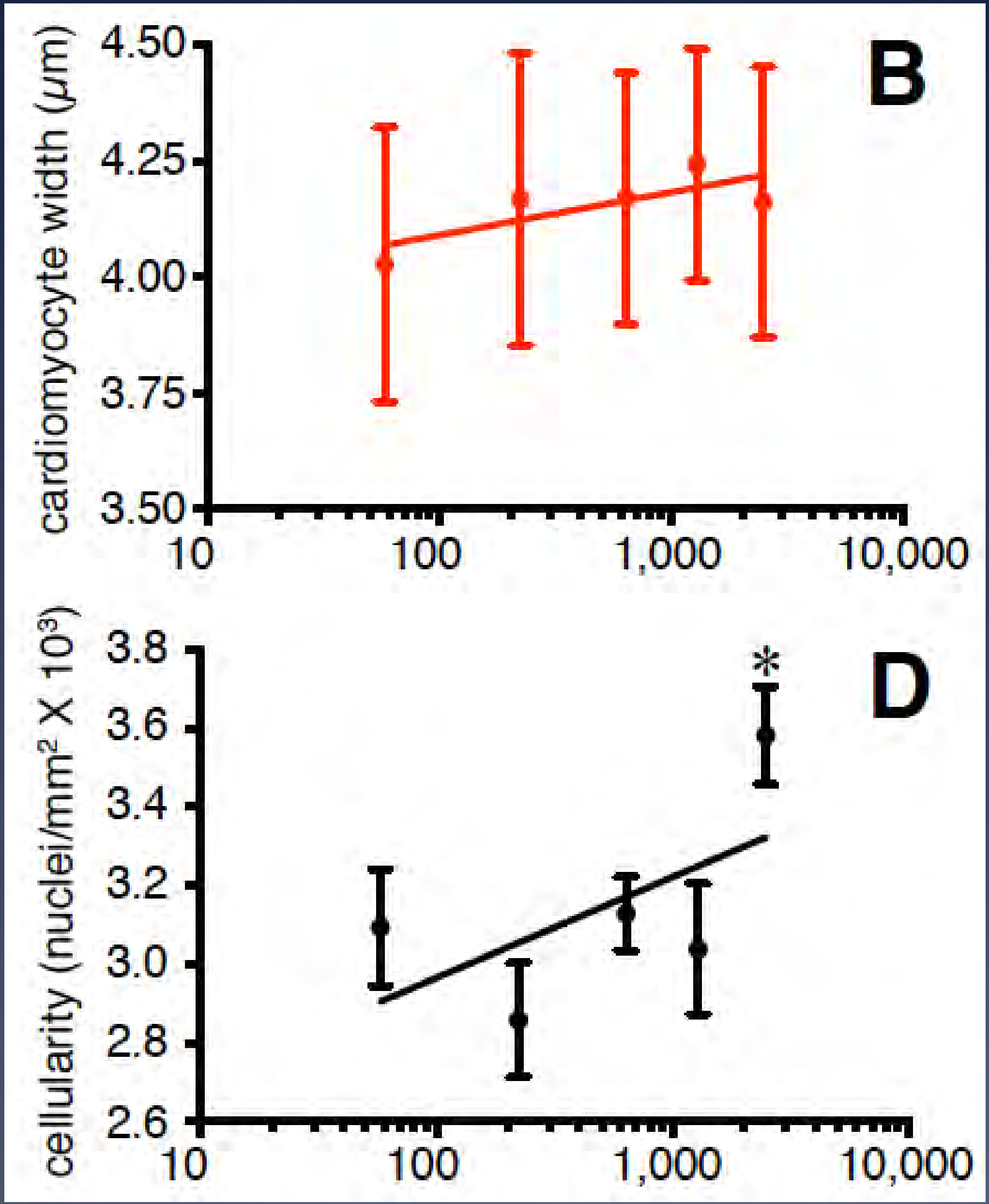
Histological evidence of hypertrophy



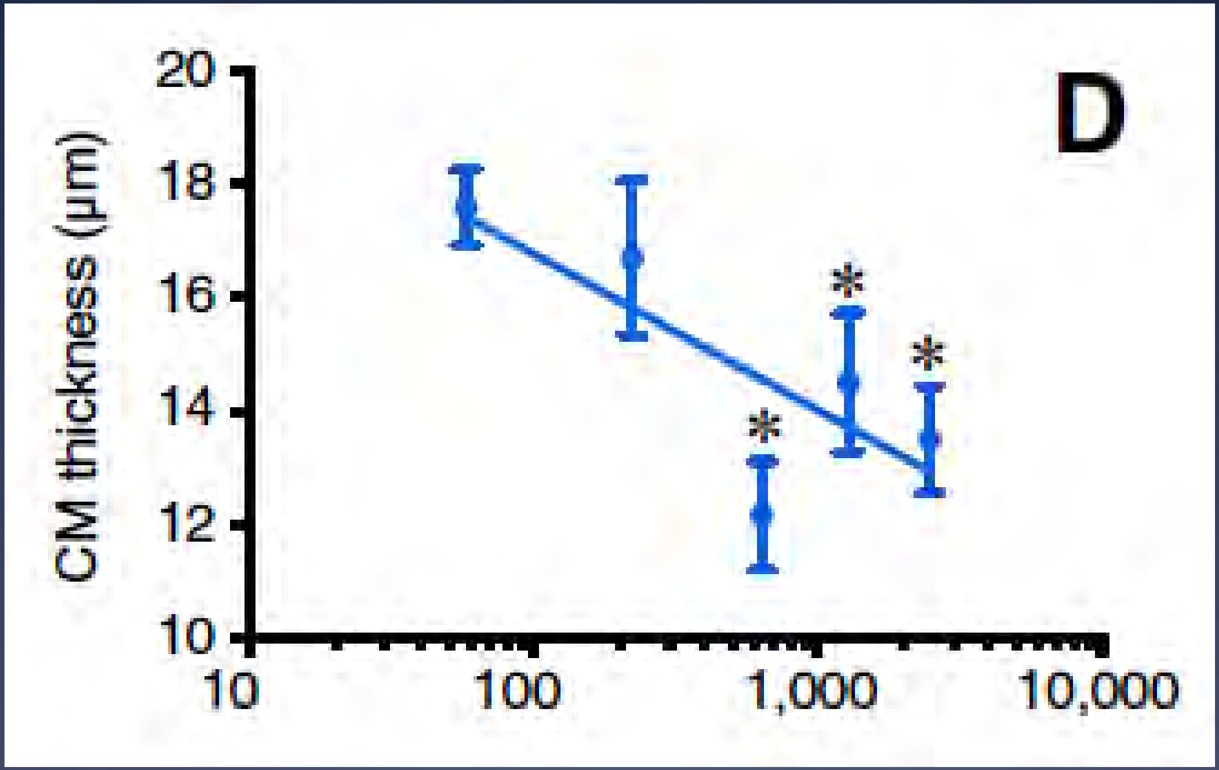
Spongy myocardium

Compact myocardium

Histological signs of hypertrophy



Oil exposure reduced the compact myocardium



"Physiological" hypertrophy causes coordinated growth in both spongy and compact myocardium

Oil exposure cause and effect chain

Cardiotoxic PAH compounds in oil

↓

Abnormal heart rhythm

↓

Alteration in the development of heart shape

↓

Reduced cardiac output

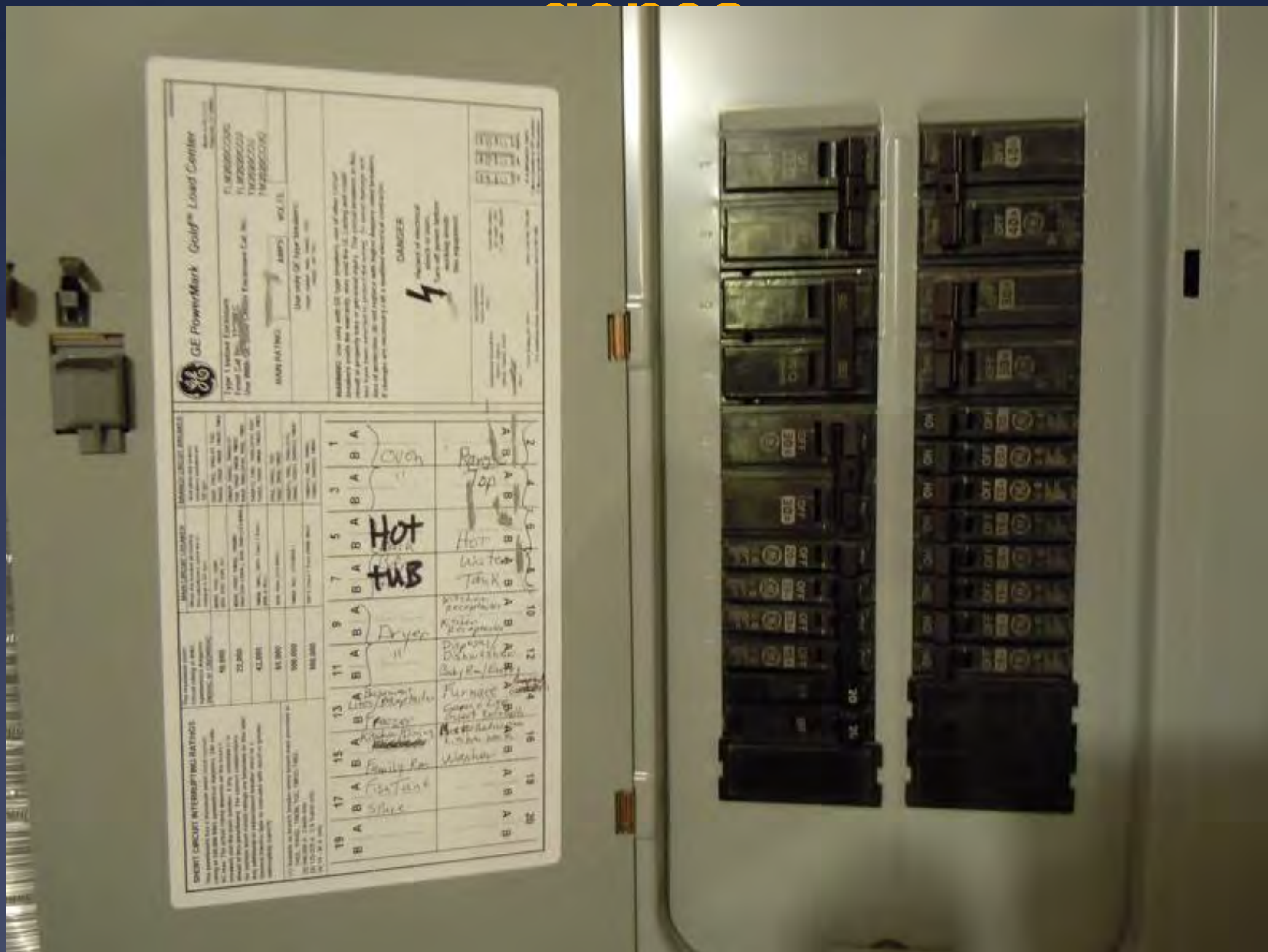
↓

Reduced aerobic capacity

Science Night 2011

Flashback

Anatomy is shaped during embryonic development by “master regulator” genes



Next Generation Sequencing – Illumina RNASeq

DNA Sequencing | Understanding the genetic code with NGS

www.illumina.com/techniques/sequencing/dna-sequencing.html

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Techniques / Sequencing / **DNA Sequencing**

Sequencing

Overview DNA Sequencing RNA Sequencing Methylation Sequencing NGS Library Preparation

Understanding the genetic code

NGS technology enables massively parallel DNA analysis for a deeper understanding of biology

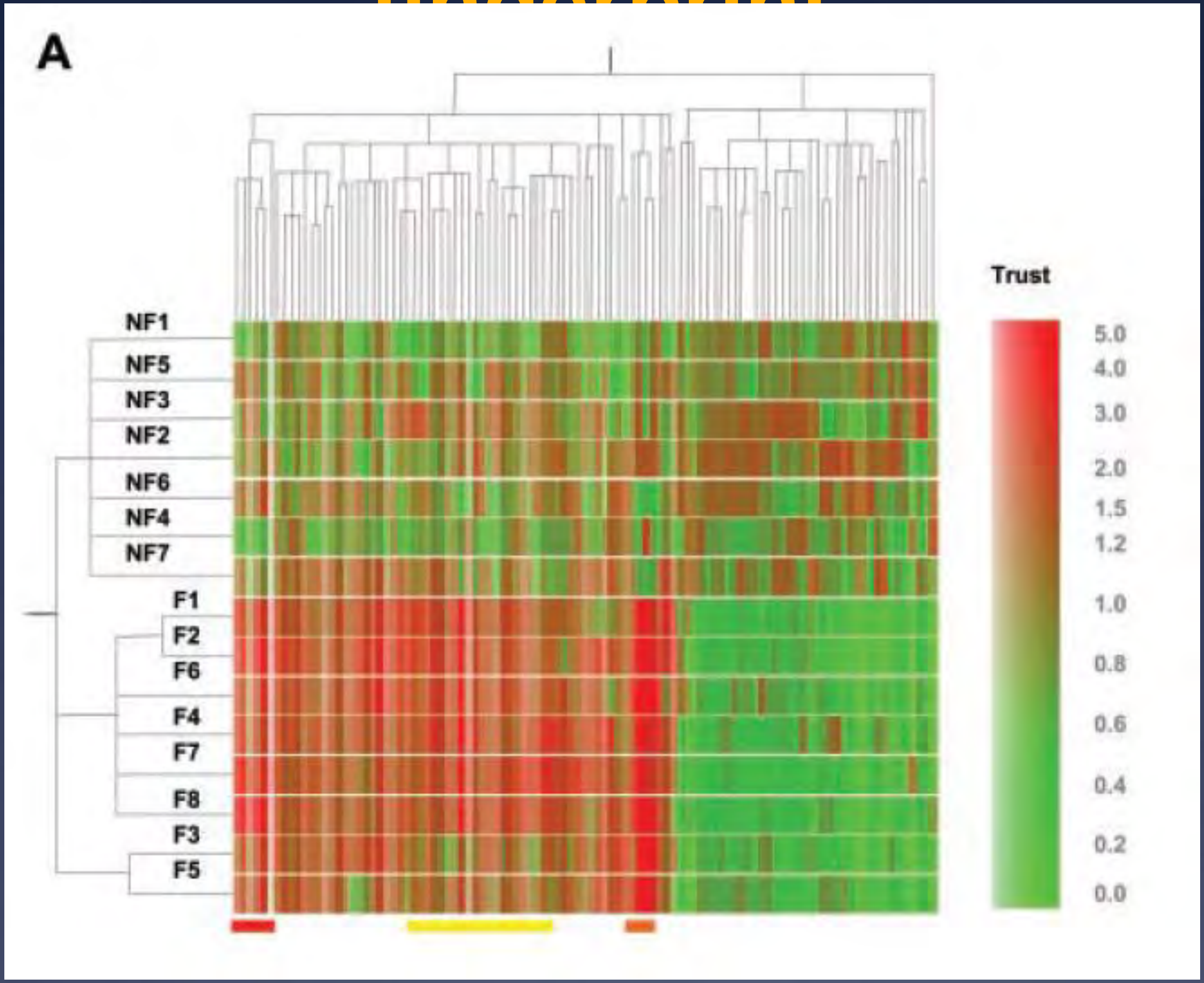
DNA Sequencing

Introduction to DNA Sequencing

Illumina next-generation sequencing (NGS) technology uses clonal amplification and sequencing by synthesis (SBS) chemistry to enable rapid, accurate sequencing. The process simultaneously identifies DNA bases while incorporating them into a nucleic acid chain. Each base emits a unique fluorescent signal as it is added to the growing strand, which is used to determine the order of the DNA sequence.

NGS technology can be used to sequence the DNA from any organism, providing valuable information in response to almost any biological question. A highly scalable technology, DNA sequencing can be applied to small, targeted regions or the entire genome through a variety of methods, enabling researchers to investigate and better understand health and disease.

103-gene human heart failure fingerprint



Tan et al., 2002 PNAS 99:11387

We got: Two master regulators

Table 3: Novel developmental genes altered in oil-exposed hearts

Gene	Difference in 45 ppb (high) vs. control dose	Function
NKX3.2/ZAX	3.1 times higher	Developmental regulator
NKX2.3	3.4 times higher	Developmental regulator

We got: 9 hypertrophy genes

Table 2: Cardiac hypertrophy/cardiovascular genes altered in oil-exposed hearts

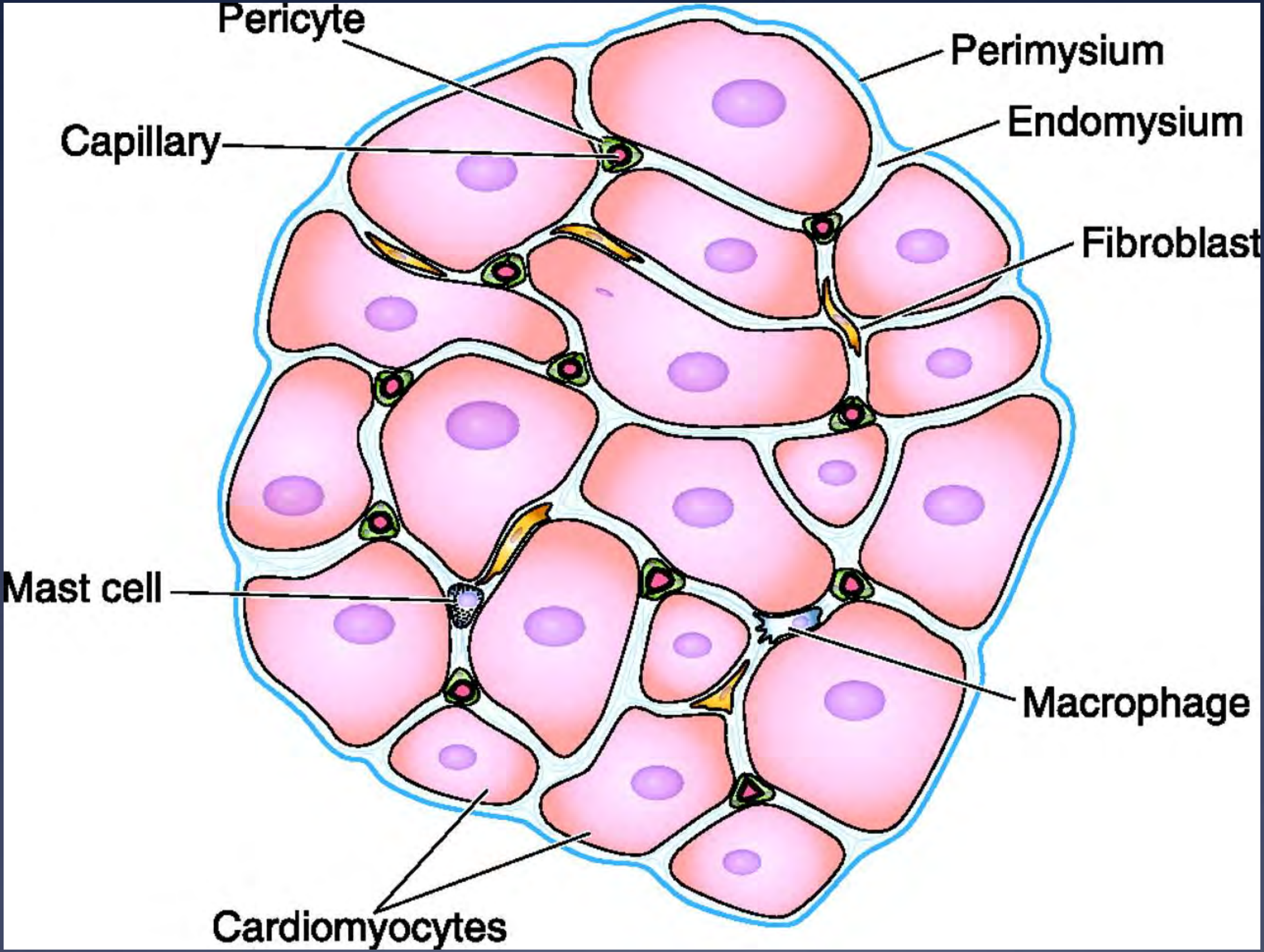
Gene	Difference in 45 ppb (high) vs. control dose
CNTN2	2 times lower
LTBP1	1.3 times lower
HMCN1/FBLN6	1.6 times lower
RNF213	1.4 times higher
APOE	2.3 times higher
NID1	1.6 times lower
LAMA2	1.8 times lower
NOTCH1	1.4 times lower
ARRDC3	1.4 times higher

And the surprise catch: 22 immune and inflammatory

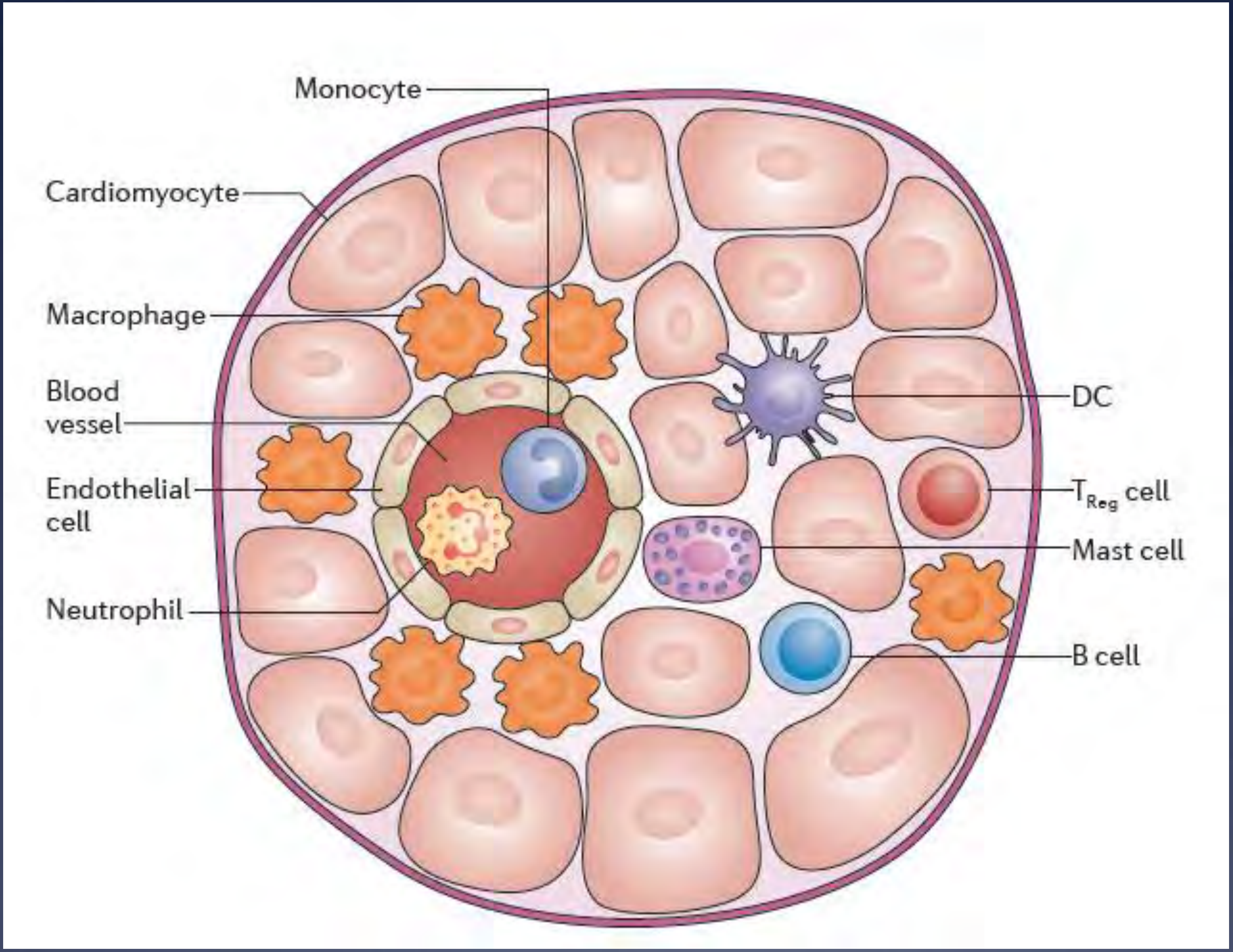
Table 1: Immune system and inflammatory genes altered in oil-exposed hearts

Gene	Difference in 45 ppb (high) vs. control dose
BEST5/RSAD2/viperin	3 times higher
IFIT1	3.4 times higher
IFIT5	2.8 times higher
RTP3	2.1 times higher
NLRC5	1.5 times higher
Tapasin	1.3 times higher
PIGR	1.7 times higher
FGG	2.6 times higher
MHC-I F10alpha	1.5 times higher
CD9	1.5 times higher
CSF1R	1.5 times higher
IL10Rbeta	1.5 times higher
FKBP5	2.9 times higher
PARP14	1.4 times higher
TRIM39	1.4 times higher
STAT1	1.4 times higher
IFI44	1.5 times higher
IRF3/7	1.7 times higher
MX1	1.6 times higher
DHX58/RLR3	1.6 times higher
HERC5	1.7 times higher
CARD6	1.9 times higher

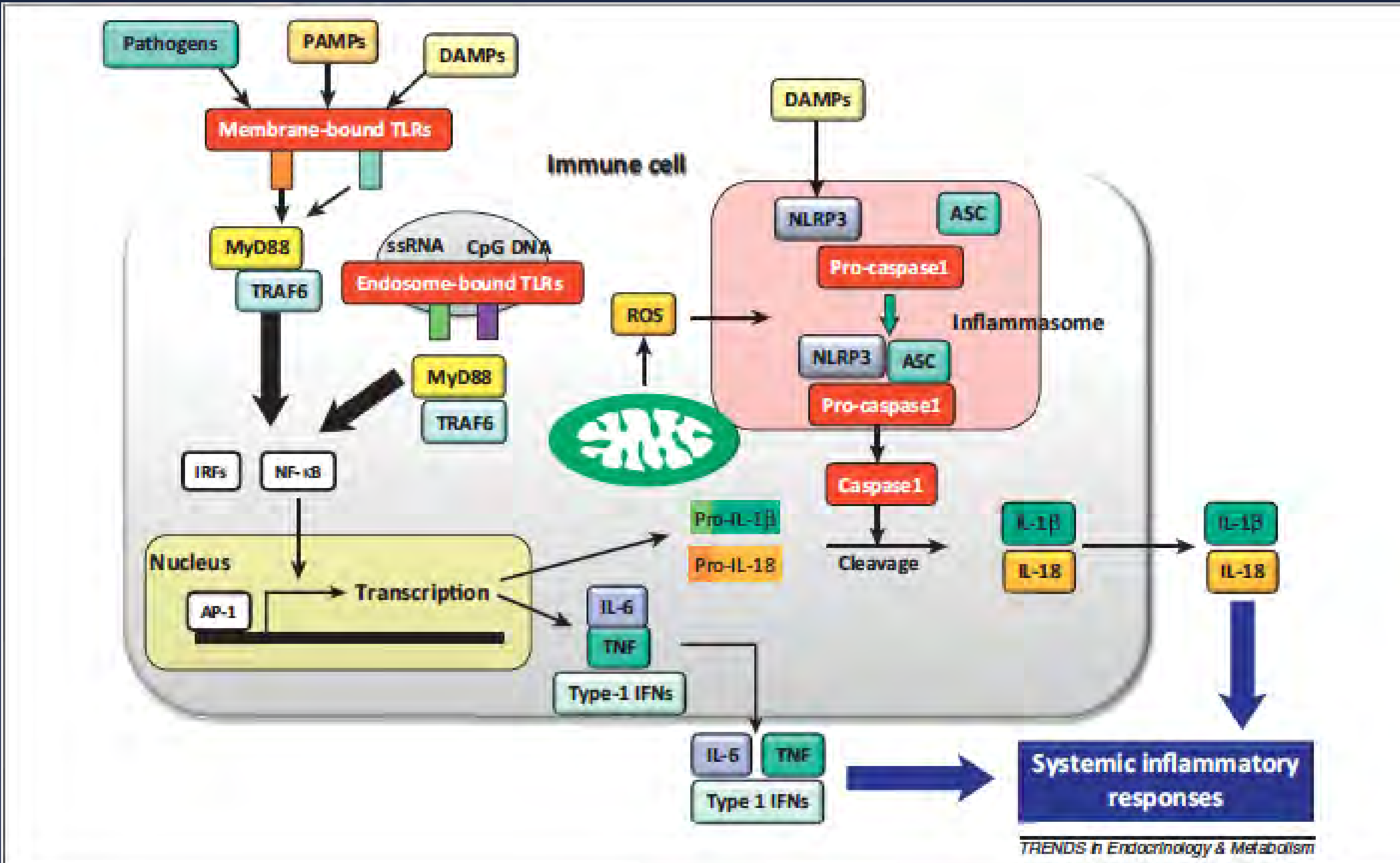
The heart is not just muscle



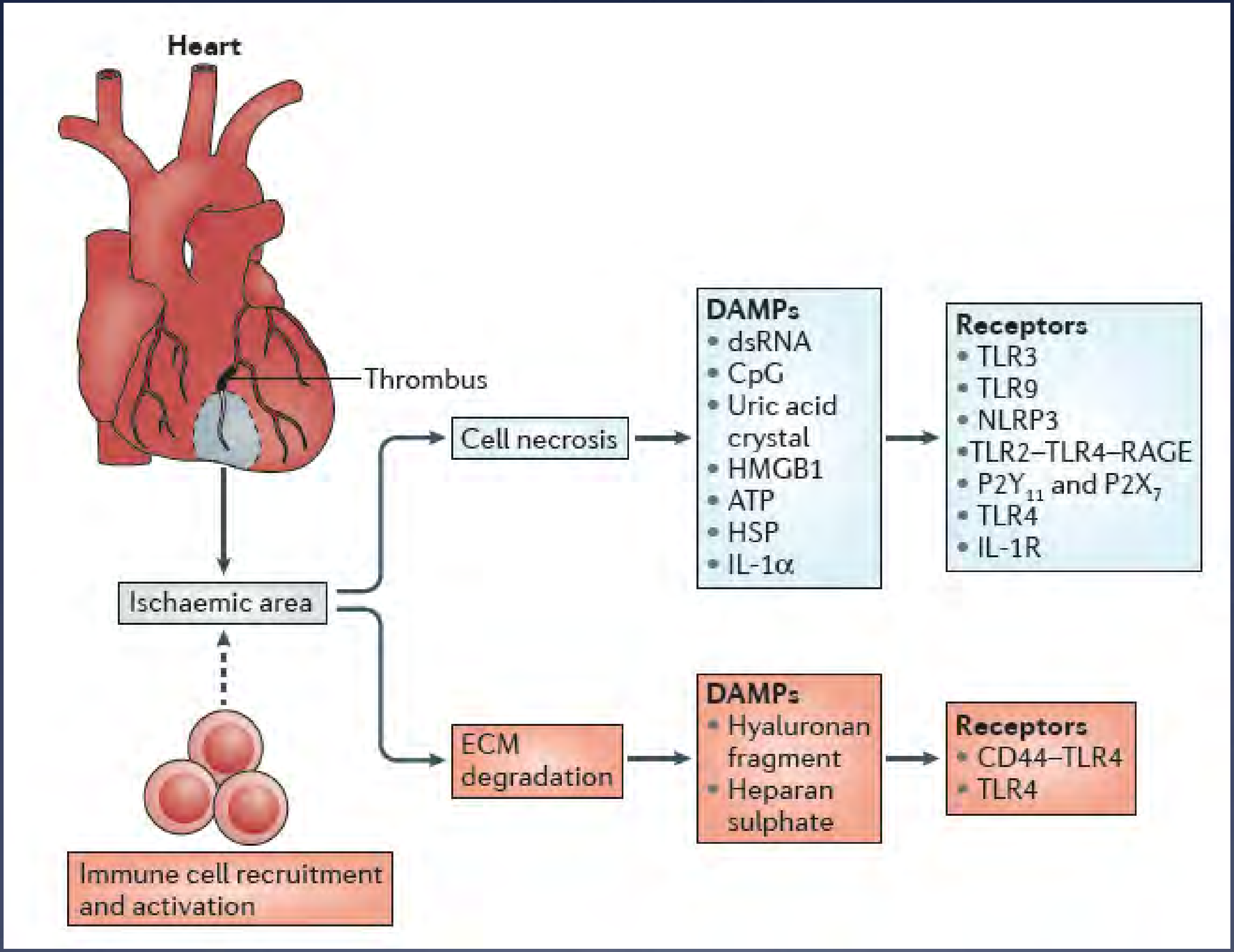
Immune cells in the heart



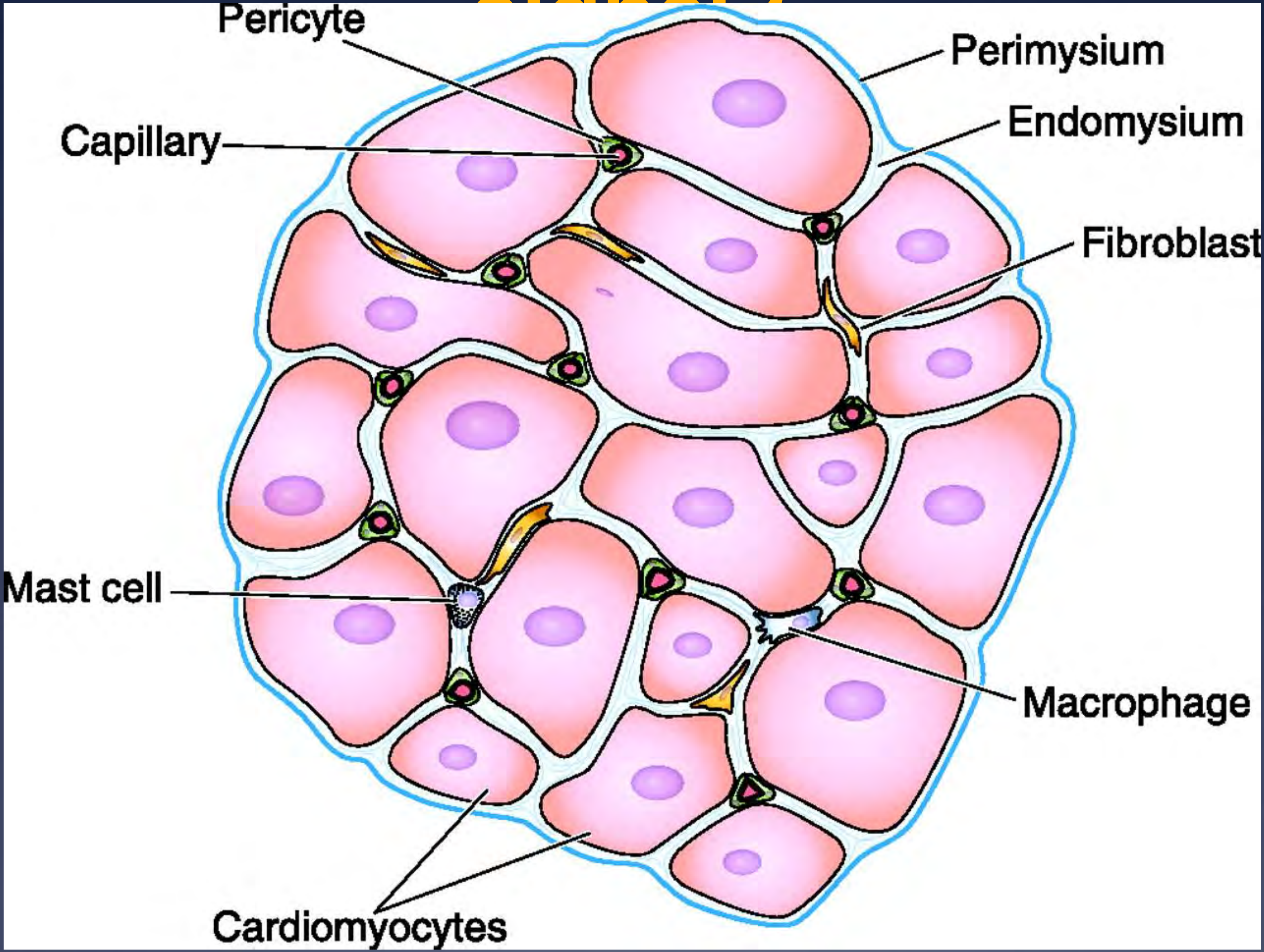
Sensing damaged tissue



Sensing damaged tissue



Which cells are giving the signal?



Oil exposure cause and effect chain

Cardiotoxic PAH compounds in oil

↓

Abnormal heart rhythm

↓

Alteration in the development of heart shape

↓

Reduced cardiac output

↓

Reduced aerobic capacity

↓

Altered bioenergetics (?)

↓

Reduced survival (?)

Next Gen Biomarkers

What's next?

* Validate biomarkers in herring: Anatomical changes in herring and salmon are very similar
* Link effects in individual fish to population and ecosystem levels
* Collect data to populate bioenergetics/recruitment models
* More herring grow-out studies to measure growth, metabolism, expanded cardiorespiratory function, predator avoidance, and more (TBD)
* Interaction between cardiorespiratory performance and infectious disease?