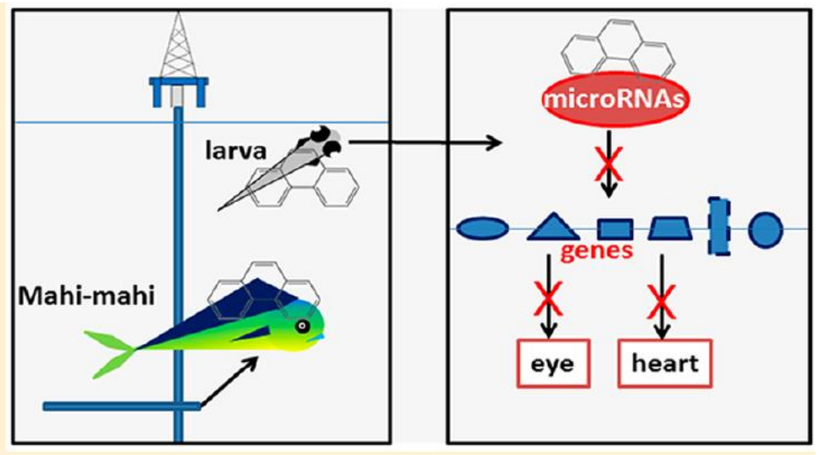
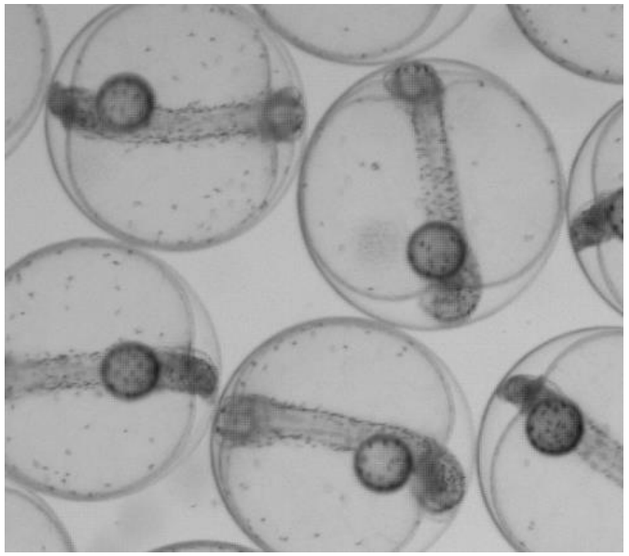
Potential of microRNA 133b as an initiating event for oil-induced cardiotoxicity in developing fish

Justin Greer, Subham Dasgupta, David Volz, Daniel Schlenk

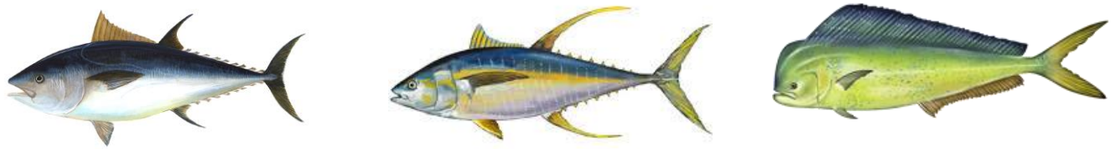




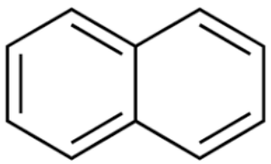
RECOVER

Crude oil and PAHs

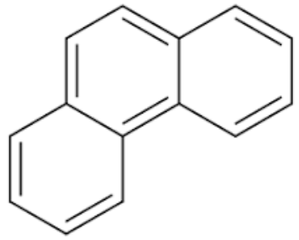
- Oil is a pervasive environmental pollutant in marine ecosystems
- Large anthropogenic input from oil spill
  - *Deepwater Horizon* – 210 million gallons
- Potential for acute exposure of early life stage fish
- Oil toxicity in marine organisms primarily mediated by polycyclic aromatic hydrocarbons (PAHs)



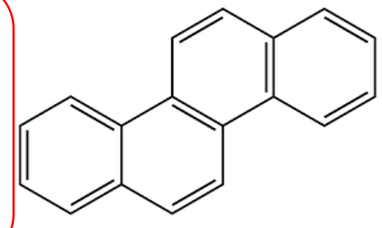
Naphthalene



Phenanthrene



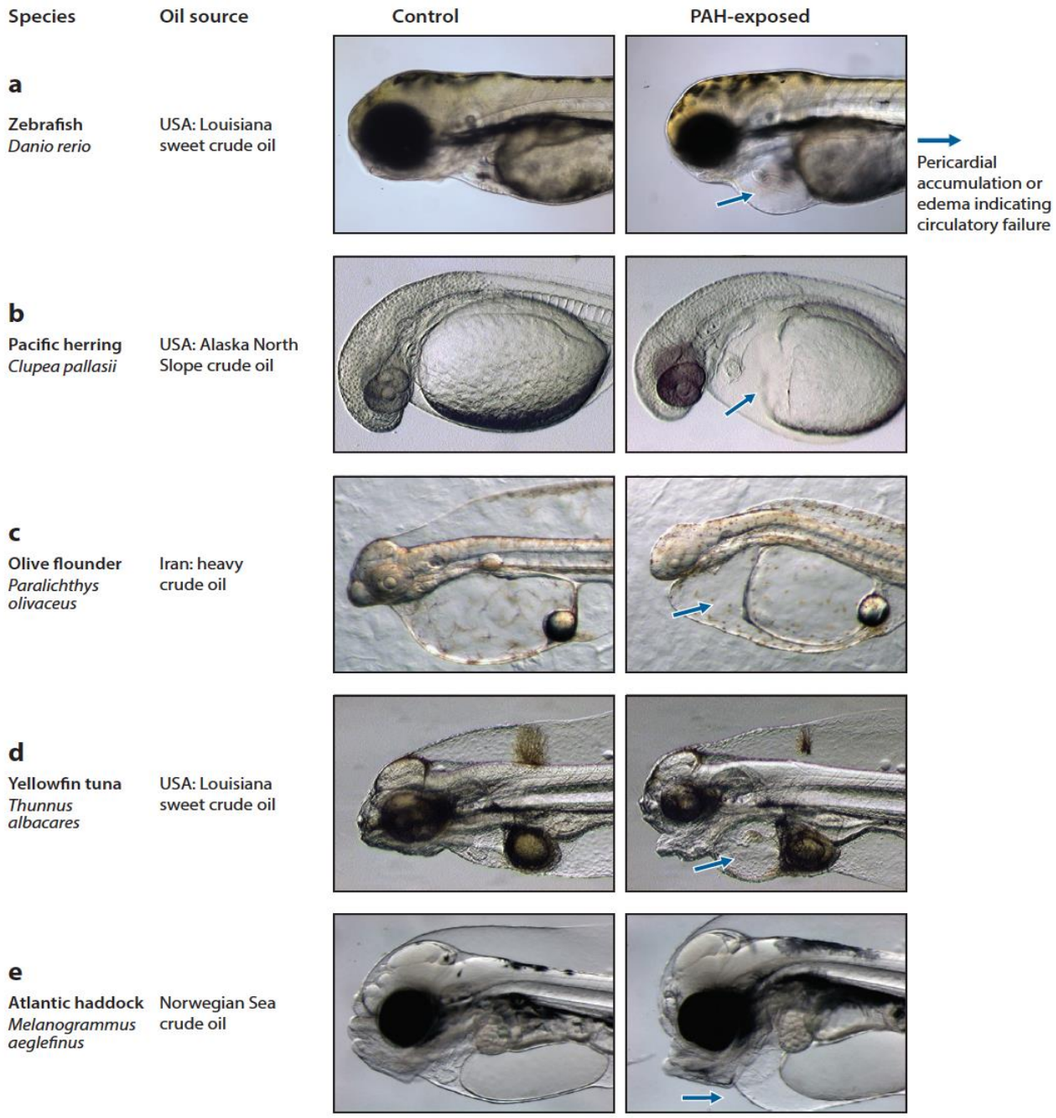
Chrysene



RECOVER

Developmental toxicity of crude oil

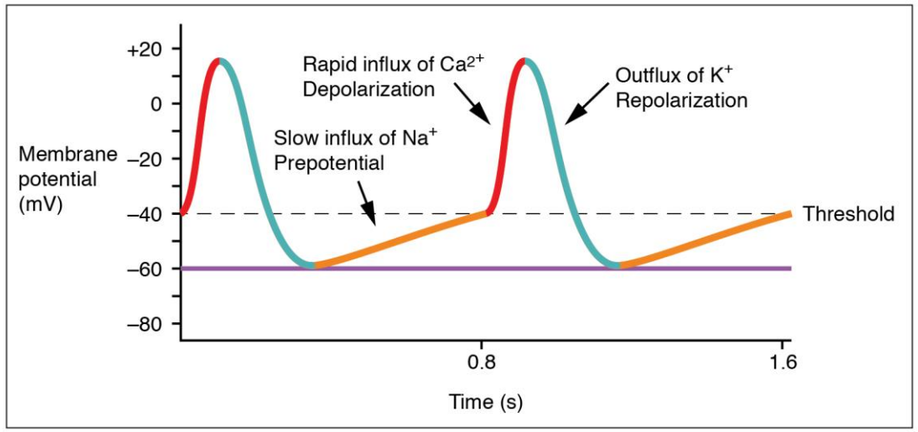
- Mechanisms of oil toxicity are early life stage fish are well-conserved
- <u>Cardiotoxicity</u>
  - Pericardial Edema
  - Reduced atrial contractility
  - Arrhythmia
- Reduced swimming performance in juveniles
- Craniofacial malformations
- Reduced eye size and visual acuity

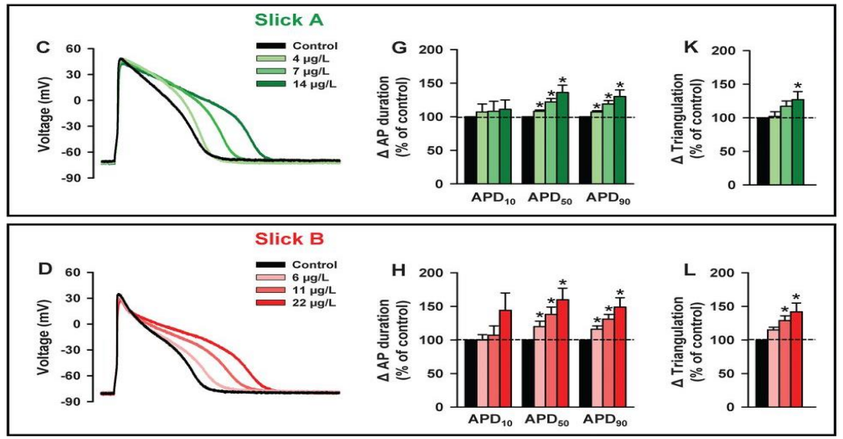


Schultz & Incardona 2015

RECOVER

Cardiomyocyte physiology and oil-induced effects





Brette *et al.* 2014

- Proper cardiomyocyte function relies on tightly coordinated ion fluxes of Na^+, K^+, and Ca^{2+}

- Elongation of cardiomyocyte action potentials following oil exposure through dysregulation of Ca^{2+} and K^+ fluxes that are essential for proper cardiac function

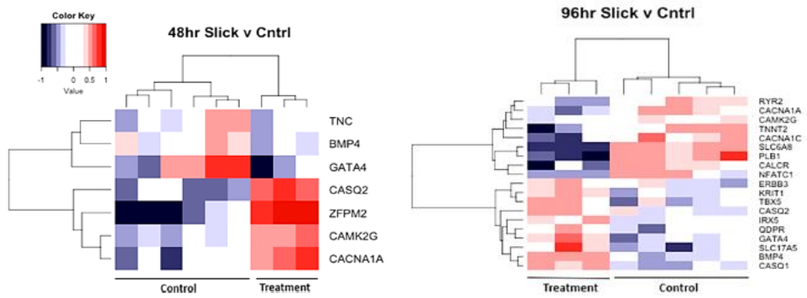
- Suggest deficits in function of Ca^{2+} and K^+ ion channels

RECOVER

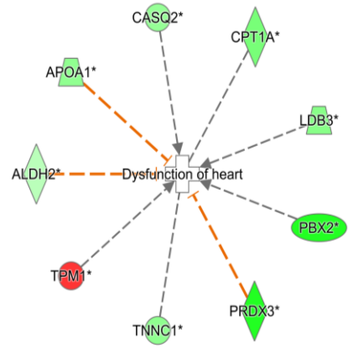
Molecular correlates of cardiac physiology

- <u>Downregulation</u> of cardiac Ca^{2+} and delayed-rectifier K^{+} ion channel genes in oil-exposed embryos and larvae

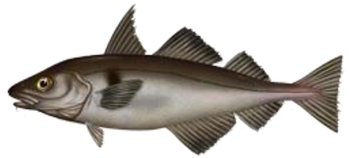
- Correspond to changes in cardiac physiology observed in tuna cardiomyocytes

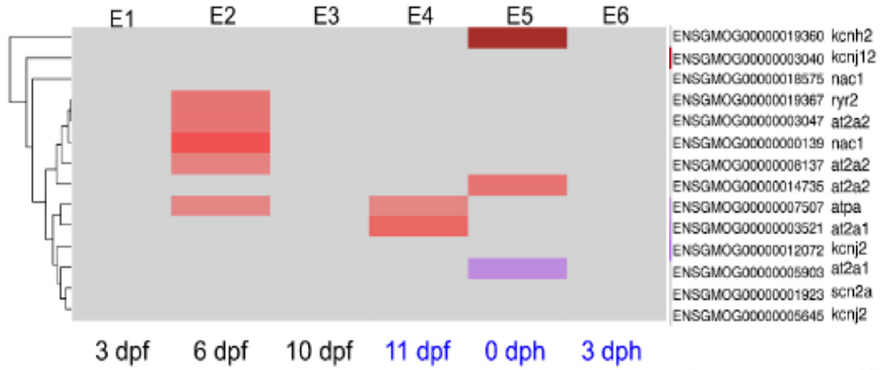


Xu *et al.* 2016



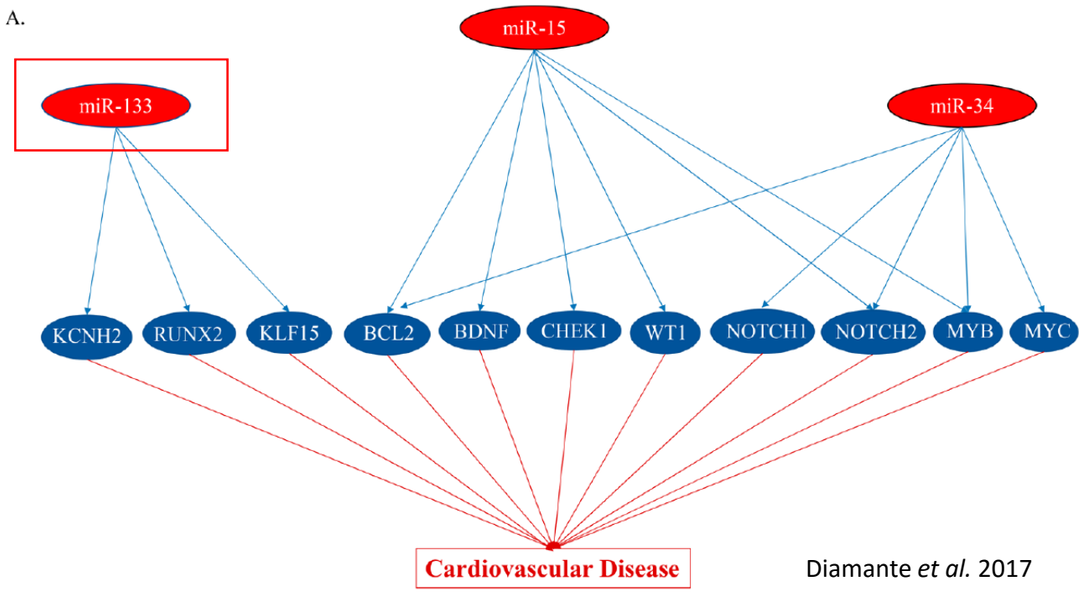
Greer *et al. in review*





Sørhus *et al.* 2017

Role of microRNAs in regulation of gene expression



Diamante *et al.* 2017

- MicroRNAs (miR) are small RNA molecules that bind to target RNAs, leading to reduced expression
  - a single miR can target hundreds of different genes
- Oil exposure in mahi-mahi embryos upregulates cardiac specific miR133
- Pathway analysis tools show that the miR133 upregulation may inhibit expression of cardiac Ca^{2+} and K^{+} ion channel genes, ultimately leading to physiological and phenotypic effects

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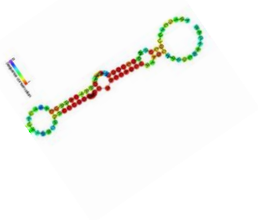
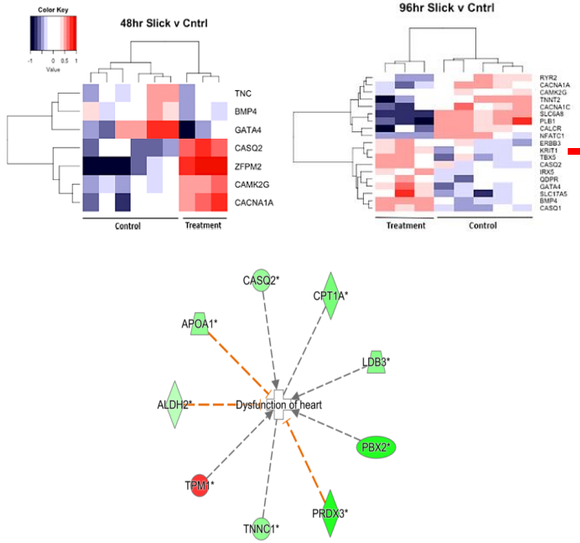
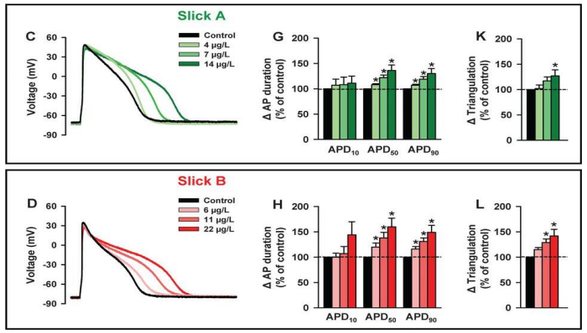
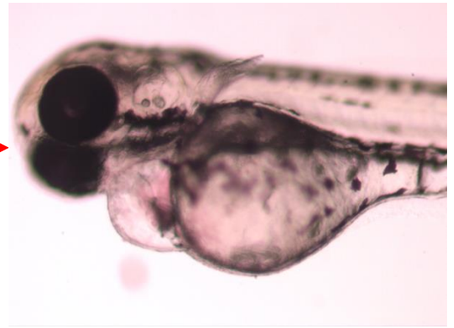
Hypothesis

miR 133b









- Increased abundance of miR133b during early development is one of the molecular initiating events leading to cardiotoxicity of oil-exposed embryos/larvae

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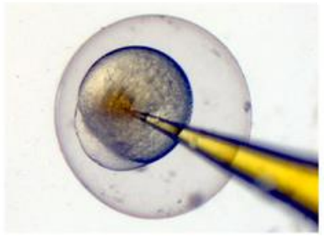
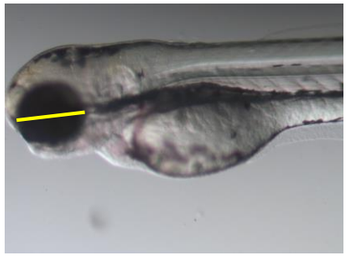
Methods

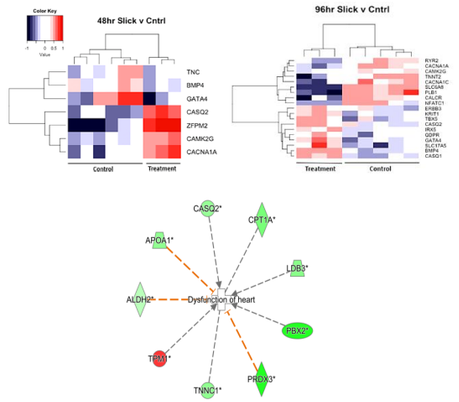


Microinjected at 1-8 cell stage with 75µM miR133b or negative control miR



Phenotypic assessments - 72hpf

1. Pericardial area
2. Heart rate
3. Eye Size
4. Mortality



Gene expression analysis – 5 and 72 hpf

- Targeted Ca^{2+} and K^{+} ion channel genes consistently downregulated in other studies

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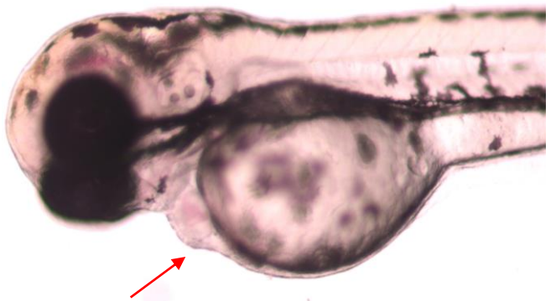
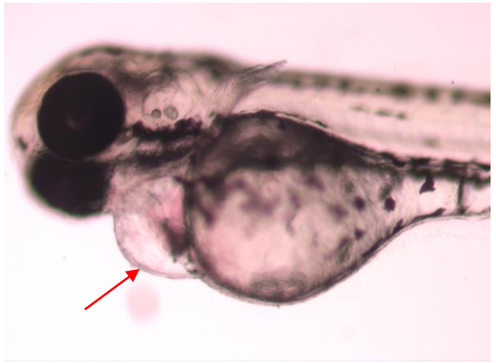
Phenotypic effects of miR133b



Negative control miR



miR133b

phenanthrene

McGruer *et al*. in prep

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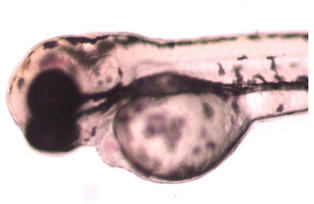
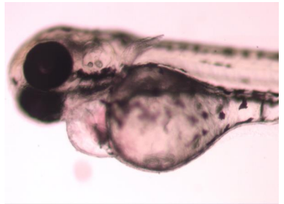
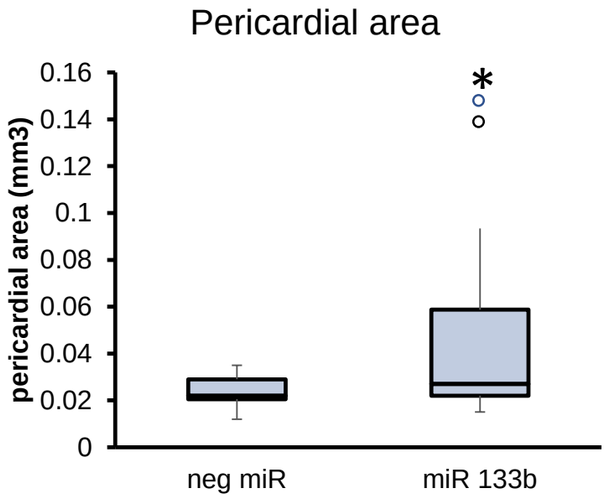
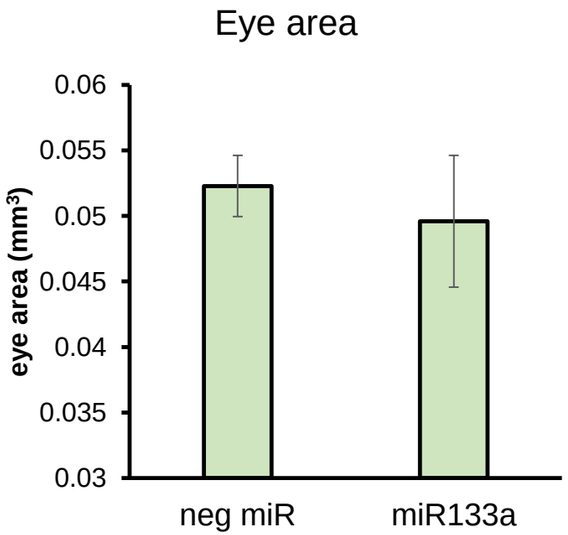
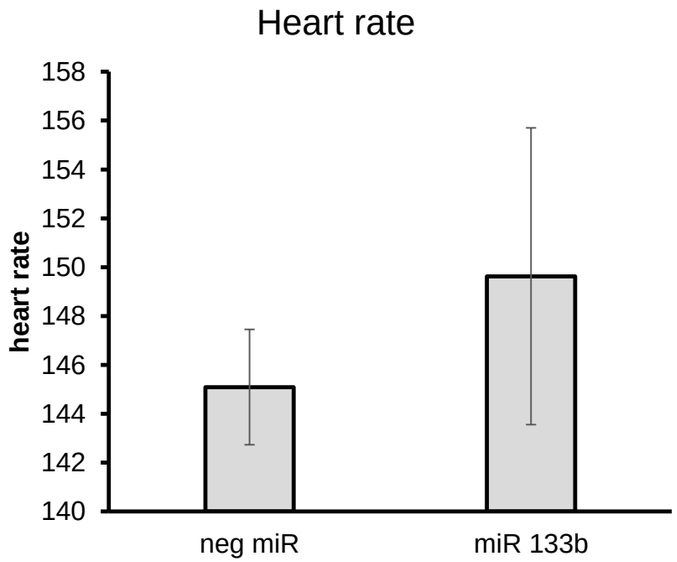
Phenotypic measurements











- Significantly increased pericardial area at 72 hpf
- Gross observations of reduced circulation in some embryos
- No changes in eye area, heart rate, or mortality

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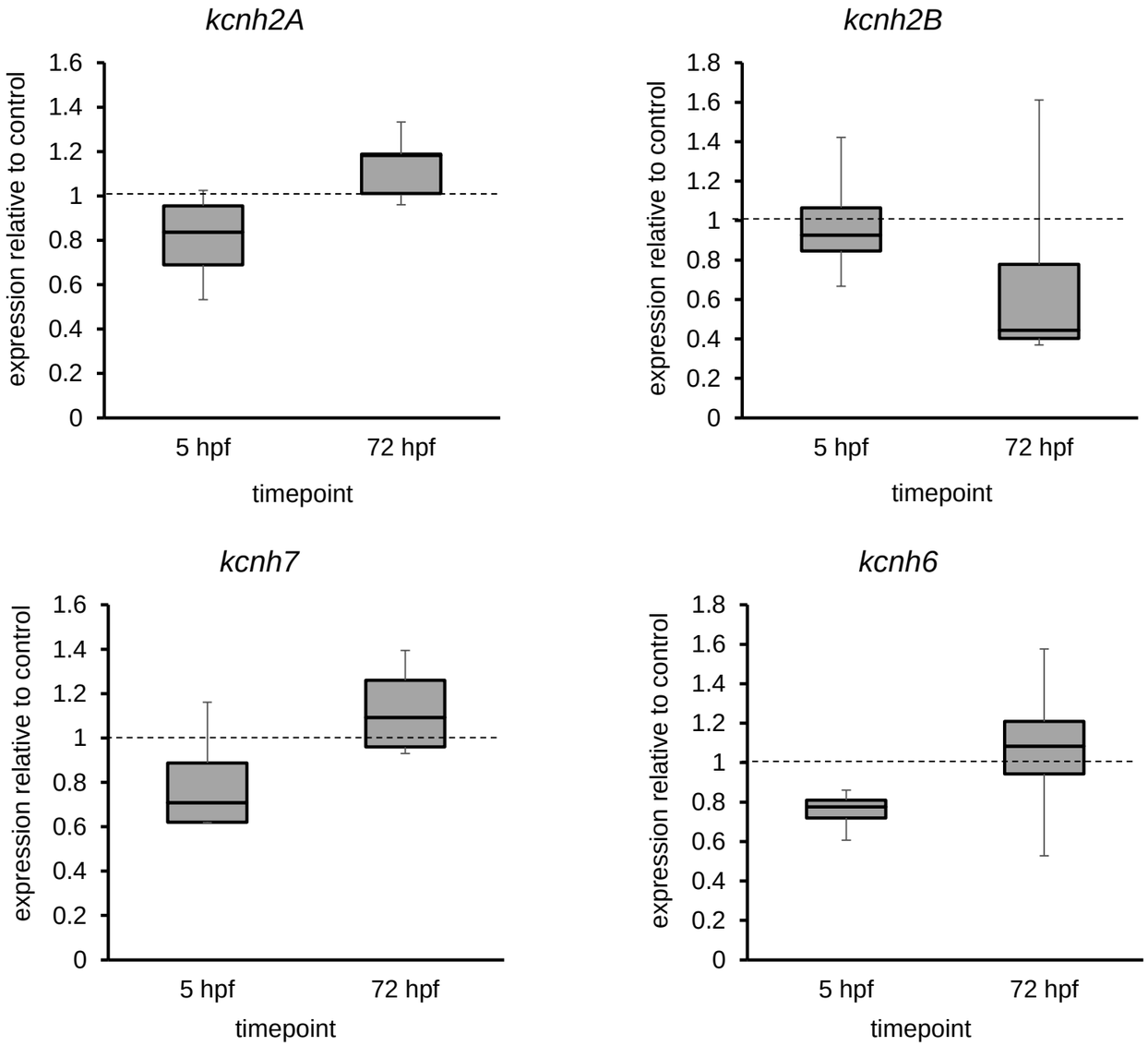
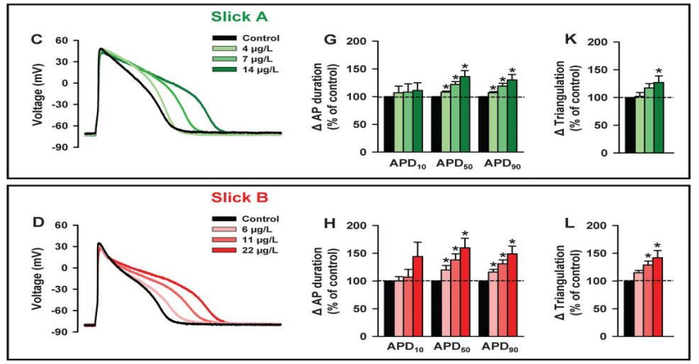
qPCR – Delayed rectifier K^+ genes





Brette *et al.* 2014

- Gene expression analysis on four I_{Kr} genes

Gene	Primary location of expression
kcnh2A	Brain and muscle
kcnh2B	Brain
kcnh7	unknown
kcnh6	heart

- miR 133b appears to target cardiac-specific delayed I_{kr} channel *kcnh6* at 5 hpf

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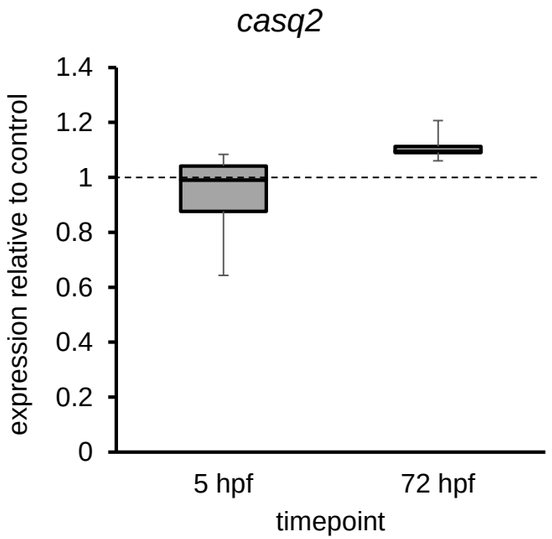
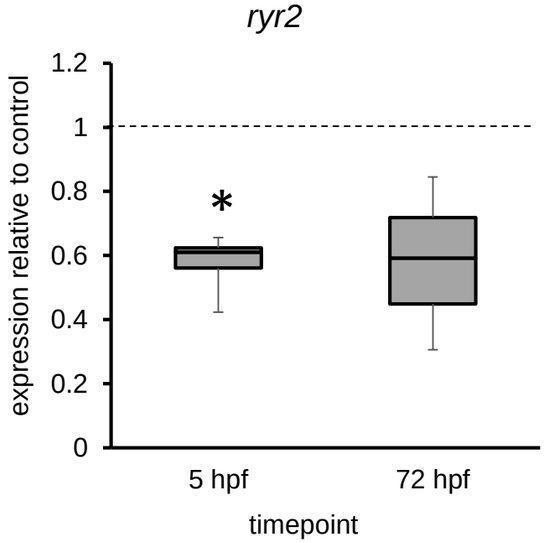
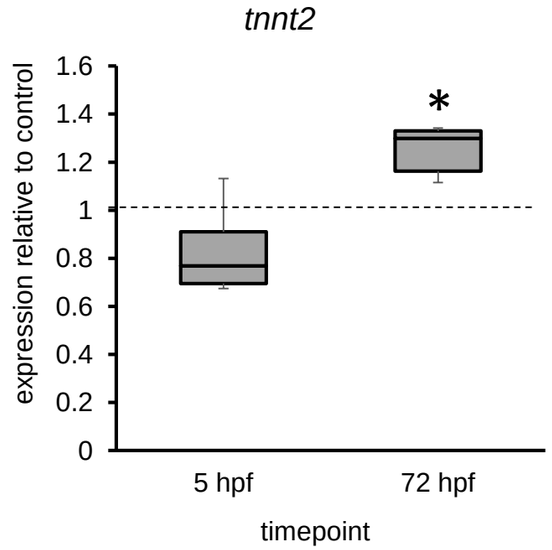
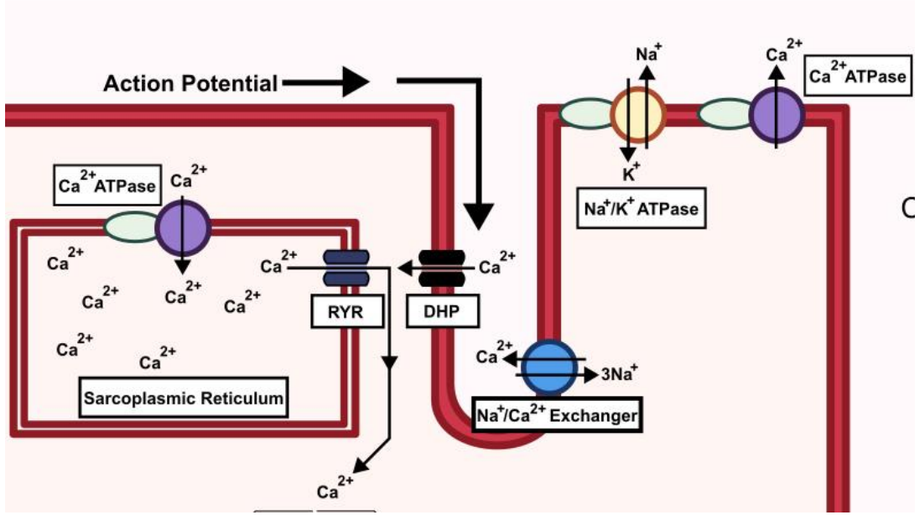
qPCR – Ca^{2+} cycling and homeostasis genes







- Ryanodine receptor 2 is the primary mediator of calcium-induced calcium release, a key (and unique) component of cardiac physiology
- Also downregulated in mahi and Atlantic haddock embryos



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Conclusions and Future Studies

- Upregulation of miR133b following oil exposure may be a key molecular initiating event for cardiotoxicity during early development in fish
- miR133b induced pericardial edema and reduced circulation in zebrafish embryos at 72 hpf
- No observable effects outside of the heart at 72 hpf
- mir133b appears to target cardiac-specific I_{Kr} and Ca^{2+} genes
  - reduced expression of *kcnh6* and *ryr2* at 5 hpf, which may initiate improper cardiac development
- Moving forward…

  Does inhibition of miR133 rescue cardiotoxic effects of phenanthrene during early life stages?

RECOVER

Acknowledgments

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