Investigating chemical-microbiota interactions in zebrafish

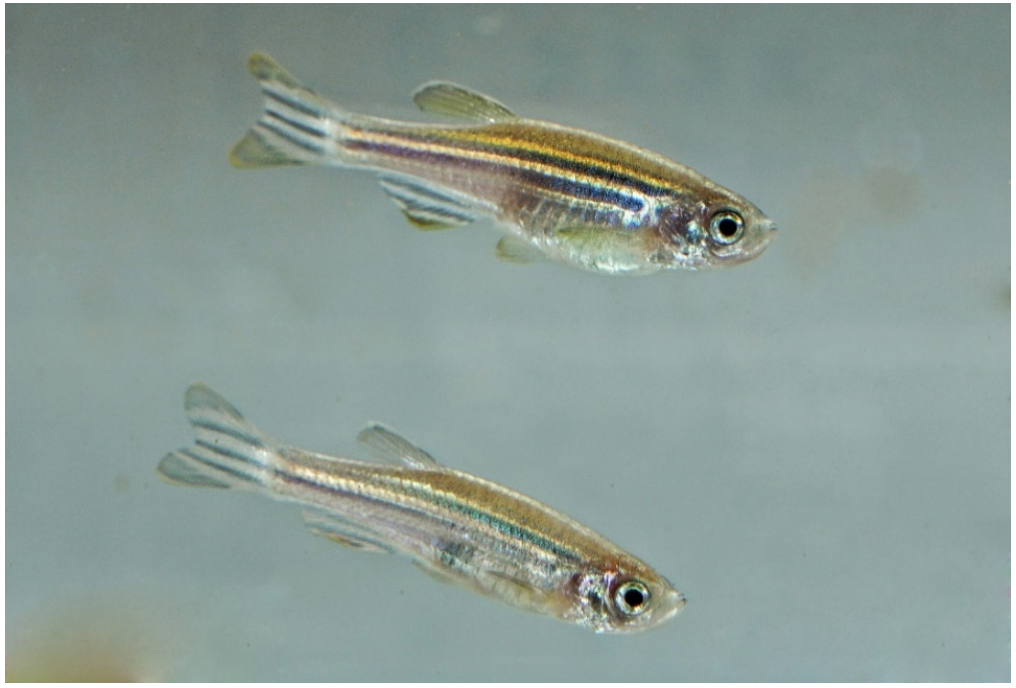

Image credit: Chuck Gaul, US EPA

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U.S. EPA/ORD/NHEERL/ISTD

EPA's Computational Communities of Practice
April 26, 2018

This presentation does not necessarily reflect EPA policy
No conflicts of interest to disclose

Outline

- Background
- Triclosan case study
- Estradiol case study
- BPA and BP replacements case study
- Summary
- Challenges

Microbiota

- Consists of all the bacteria, viruses, and fungi external to the body
- Colonization begins at birth and continues throughout life
- Required for development of host organs and systems

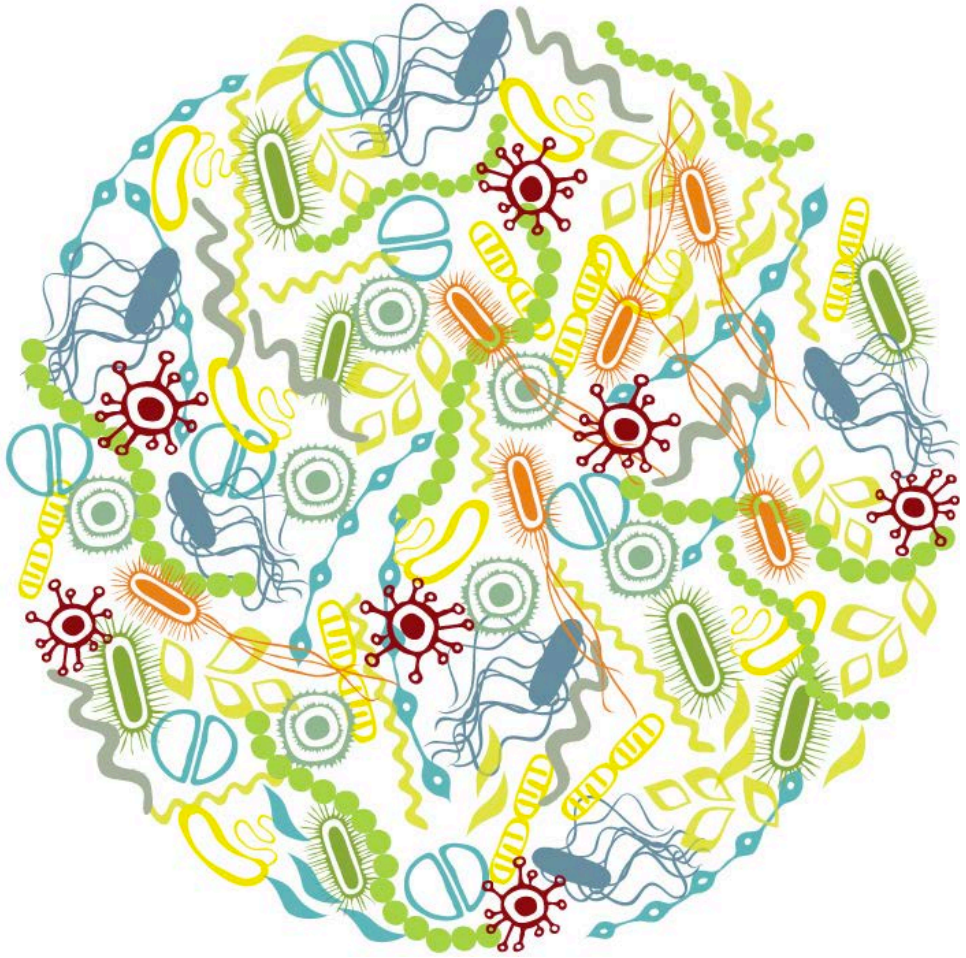
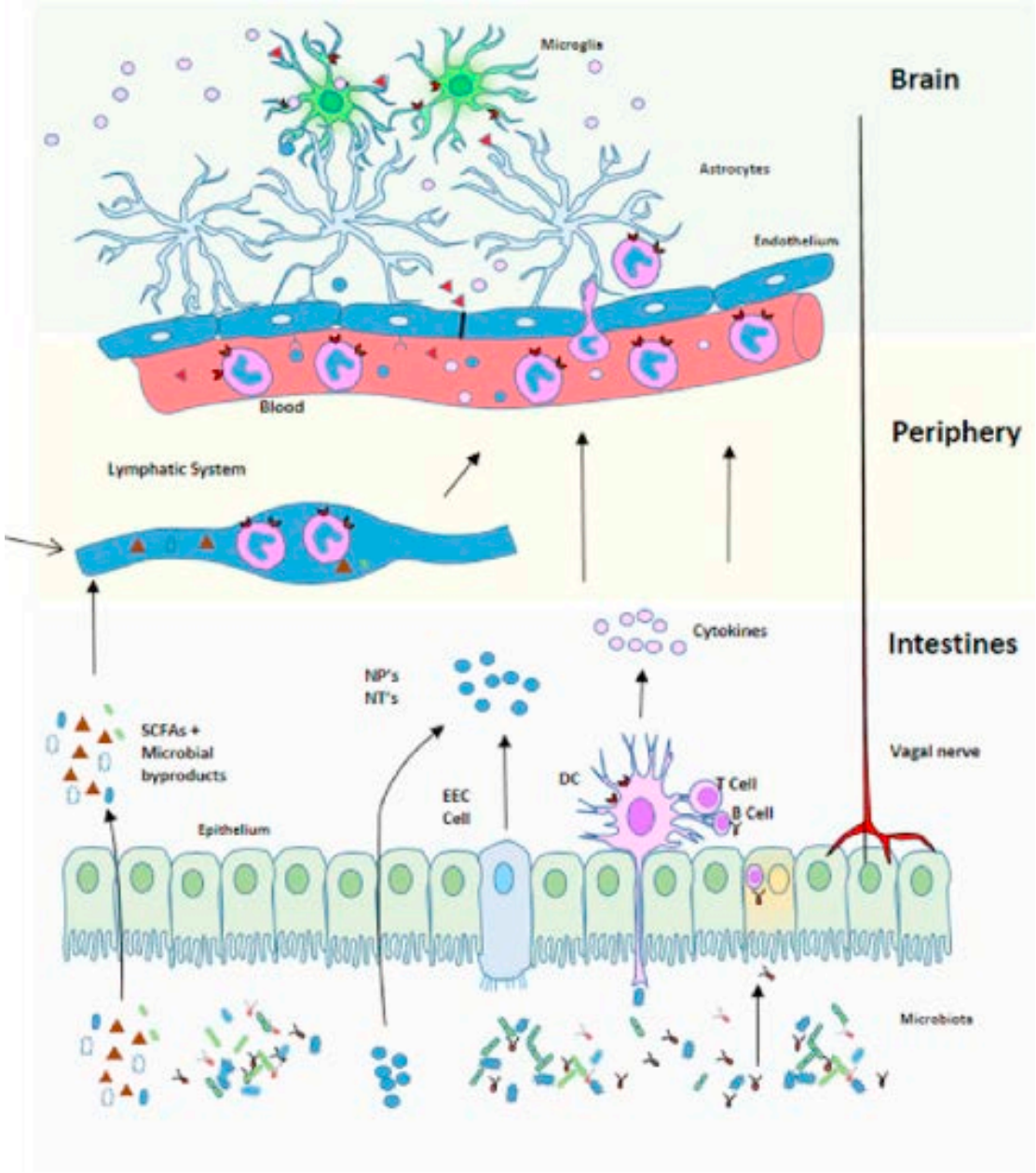


Image source: http://www.umassmed.edu/microbiome/microblog1/publications/

Microbiota-gut-brain axis

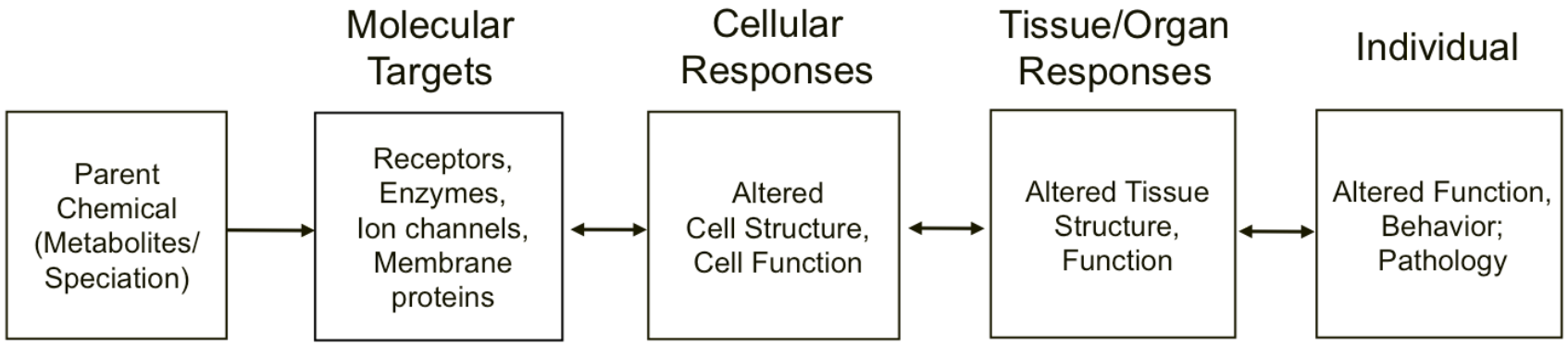
- Bidirectional communication
- Colonization status modifies neurodevelopmental events
- Imbalances in gut microbiota composition are associated behavioral disorders
- Microbiota has not be assessed as a modifying factor for the developmental neurotoxicity (DNT) of environmental chemicals



Source: Rea et al. 2016

Microbiota-chemical interactions

Adverse outcomes
- Cancer
- Developmental neurotoxicity (DNT)
- Immunotoxicity
- Pulmonary toxicity
- Reproductive toxicity



Adapted from: http://www.oecd.org/chemicalsafety

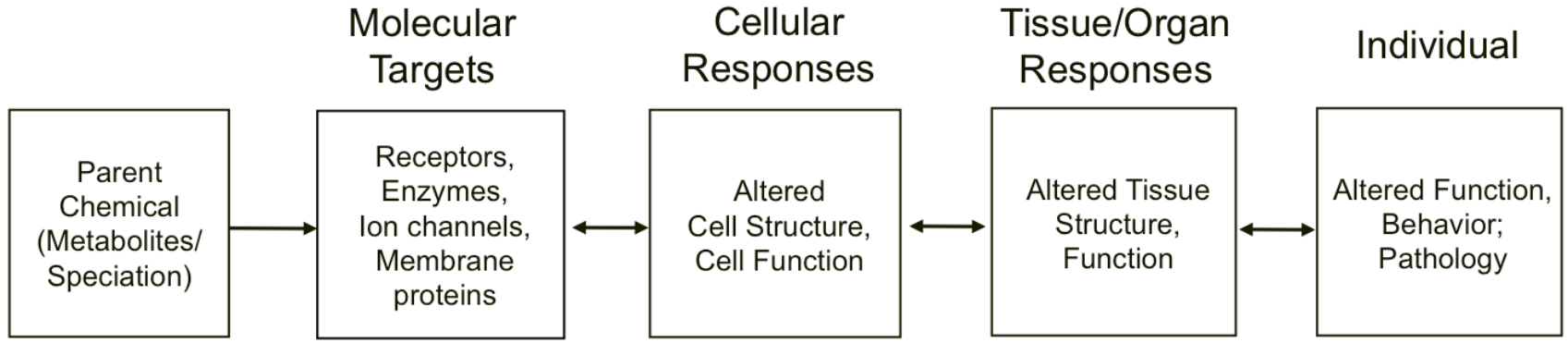
Microbiota-chemical interactions

1. Toxicokinetic hypothesis

Microbiota

Parent chemical

- **Bioactivation**
- **Detoxification**



EPA

Adverse outcomes

- Cancer
- Developmental neurotoxicity (DNT)
- Immunotoxicity
- Pulmonary toxicity
- Reproductive toxicity

Adapted from: http://www.oecd.org/chemicalsafety

Microbiota-chemical interactions

Adverse outcomes
- Cancer
- Developmental neurotoxicity (DNT)
- Immunotoxicity
- Pulmonary toxicity
- Reproductive toxicity

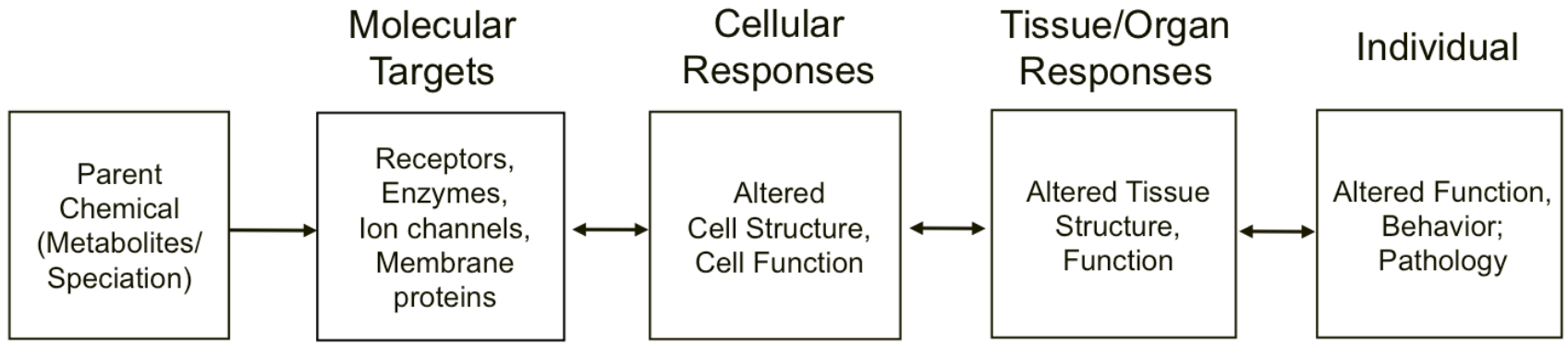
1. Toxicokinetic hypothesis

Microbiota

Parent chemical

2. Toxicodynamic hypothesis

- **Bioactivation**
- **Detoxification**



Microbiota

Adapted from: http://www.oecd.org/chemicalsafety

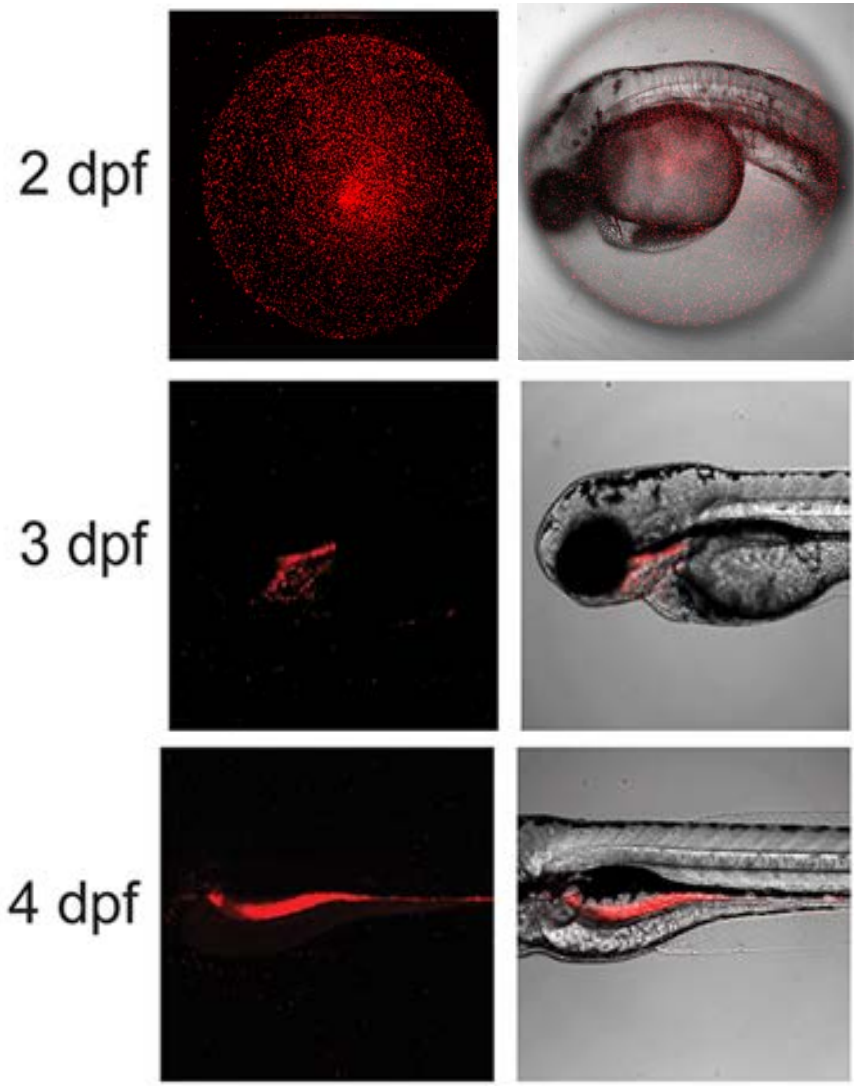
Hypothesis

Host-associated microbiota:

1. Modify the toxicity of environmental chemicals via biotransformations; and/or
2. Is a target of chemical exposures during sensitive windows of early development.

Zebrafish as a model system for microbiota research

- External and rapid development
- Majority of genes conserved with humans
- Complex resident microbiota
- Control colonization status
- Methods for rearing axenic (microbe-free) zebrafish through early development
- Simple conventionalization (add microbes to axenic embryos)

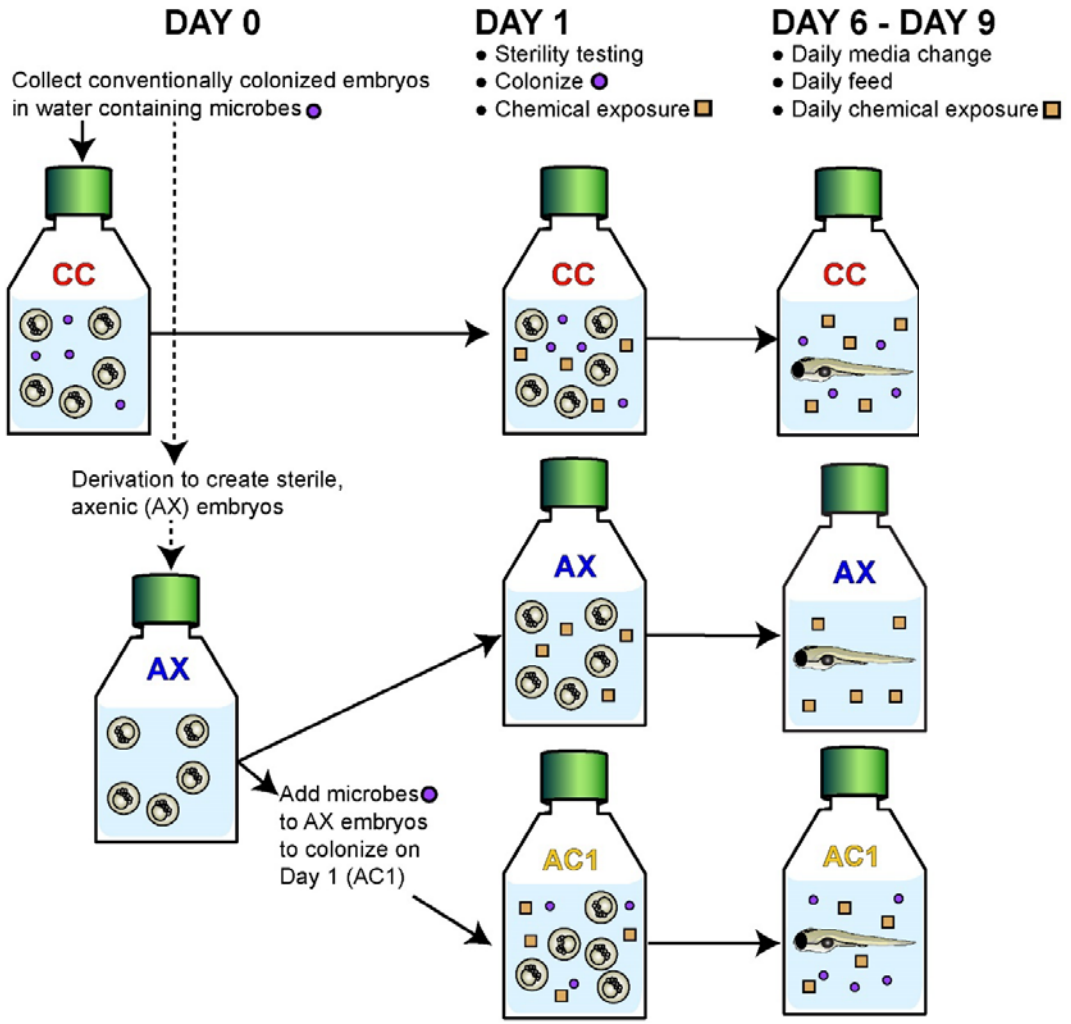


days post fertilization (dpf)

Phelps et al. 2017, Scientific Reports

*A. Veronii:*dTomato, gift from K. Guillemin, University of Oregon

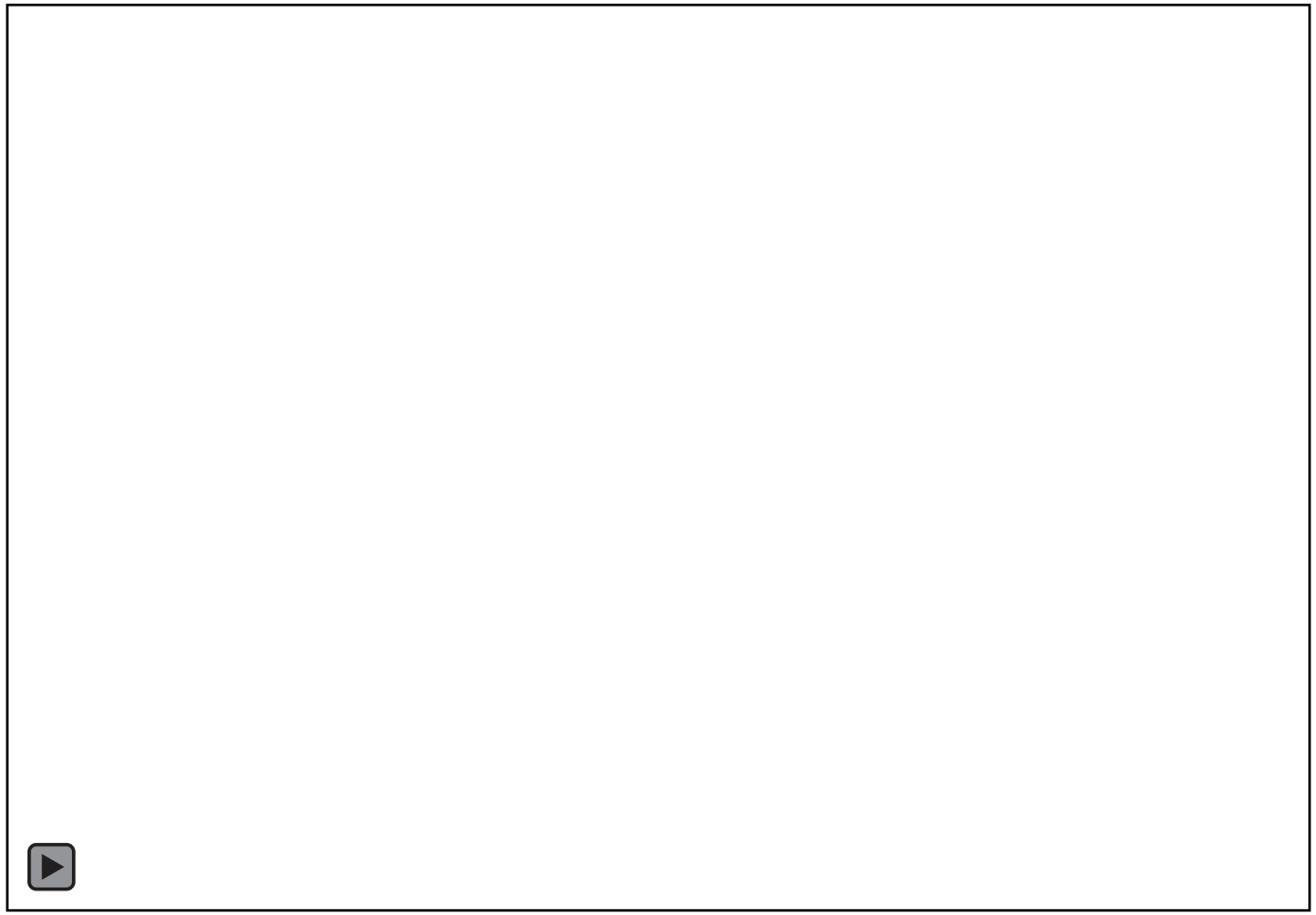
Does microbiota modify the toxicokinetics or toxicodynamics of xenobiotic exposures?



- **CC = conventionally colonized**
- **AX = Axenic or microbe-free**
- **AC1 = Axenic larvae colonized on day 1**

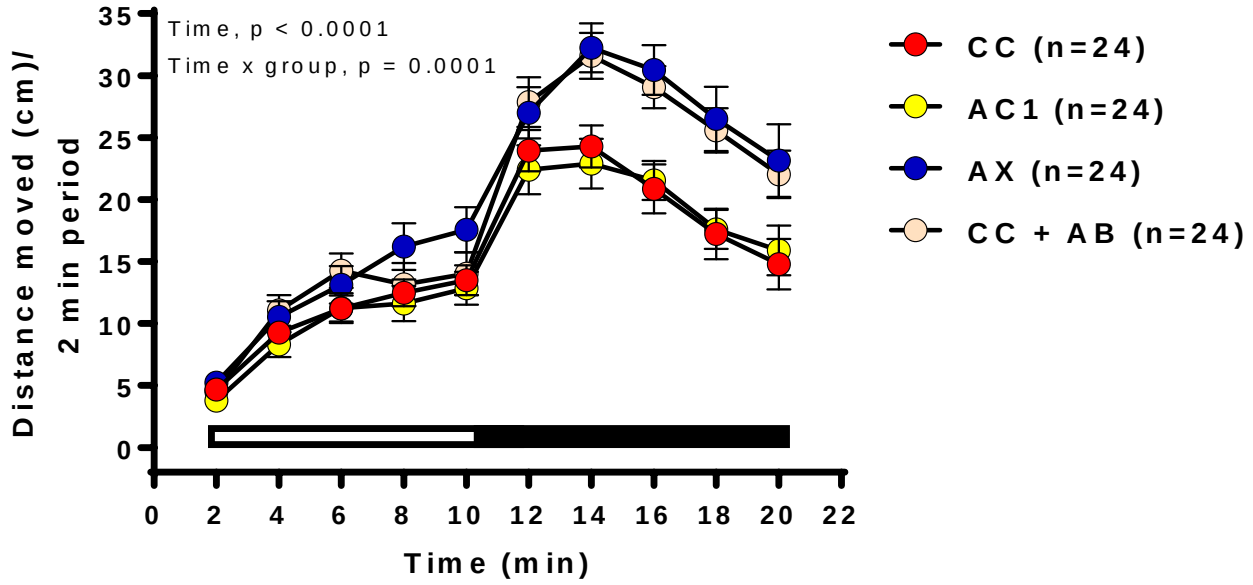
Phelps et al. 2017, Scientific Reports

Microbiota & DNT: Zebrafish neurobehavioral toxicity assay



Developmental antibiotic exposure mimics AX hyperactivity phenotype at 10 dpf

A.



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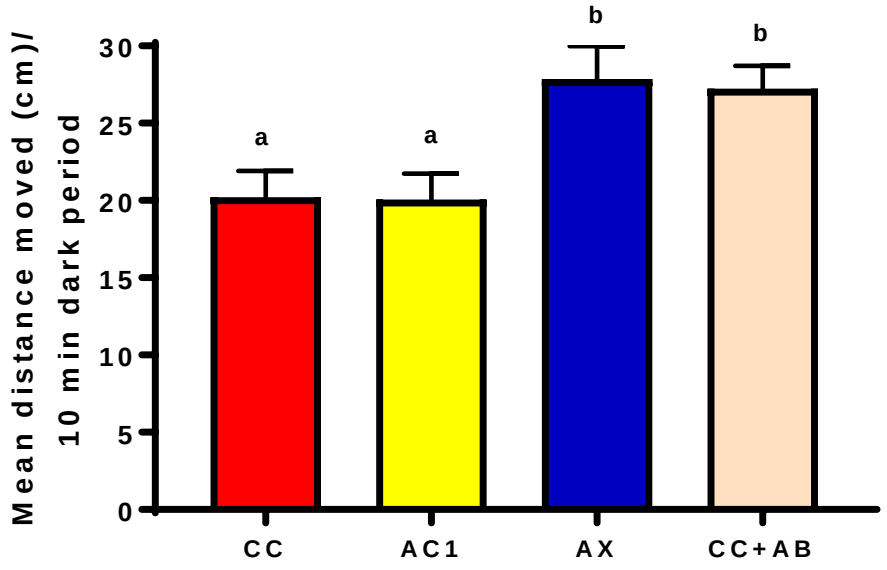
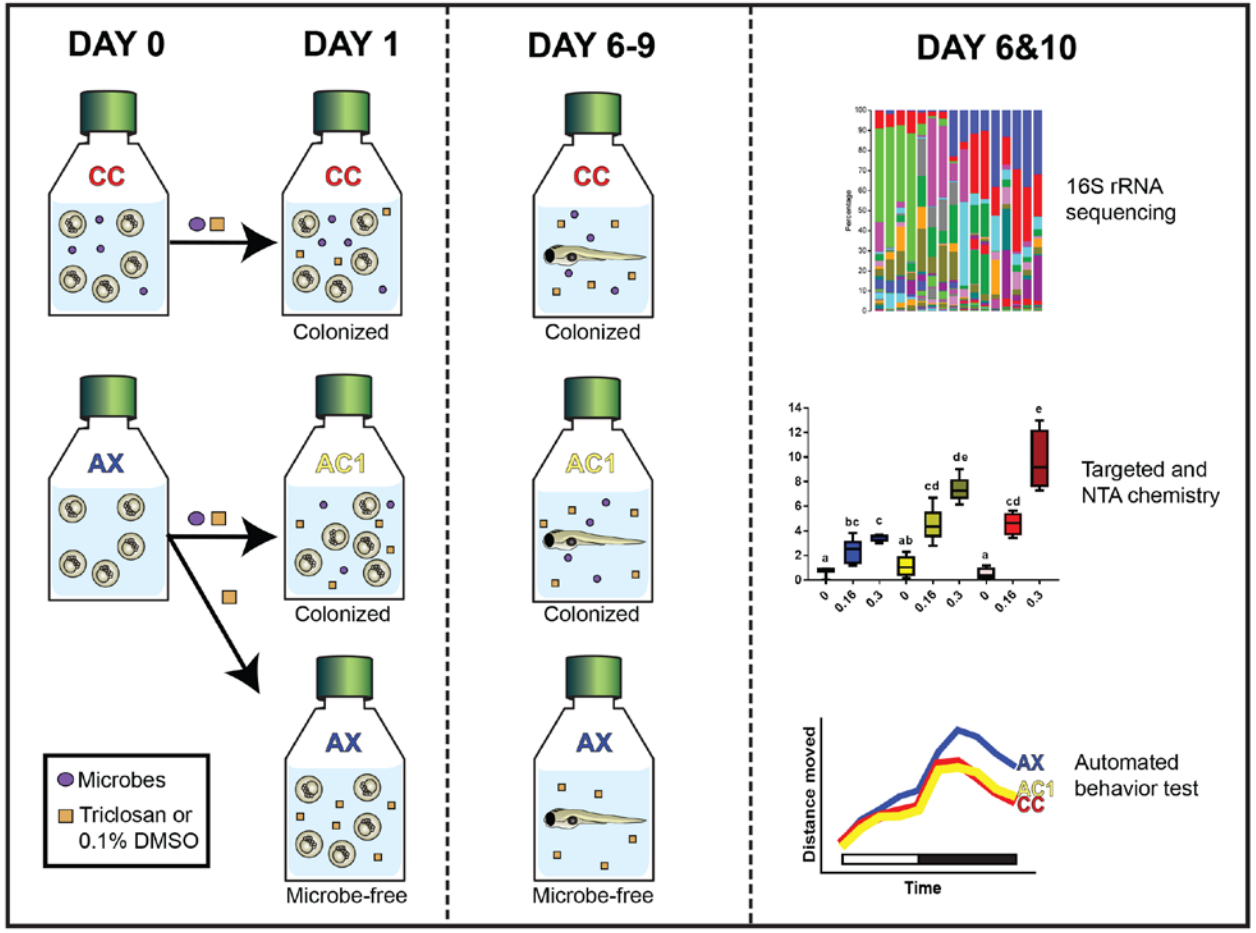


- AB = amphotericin B (0.25 ug/mL), kanamycin (5 ug/mL), and ampicillin (100 ug/mL)

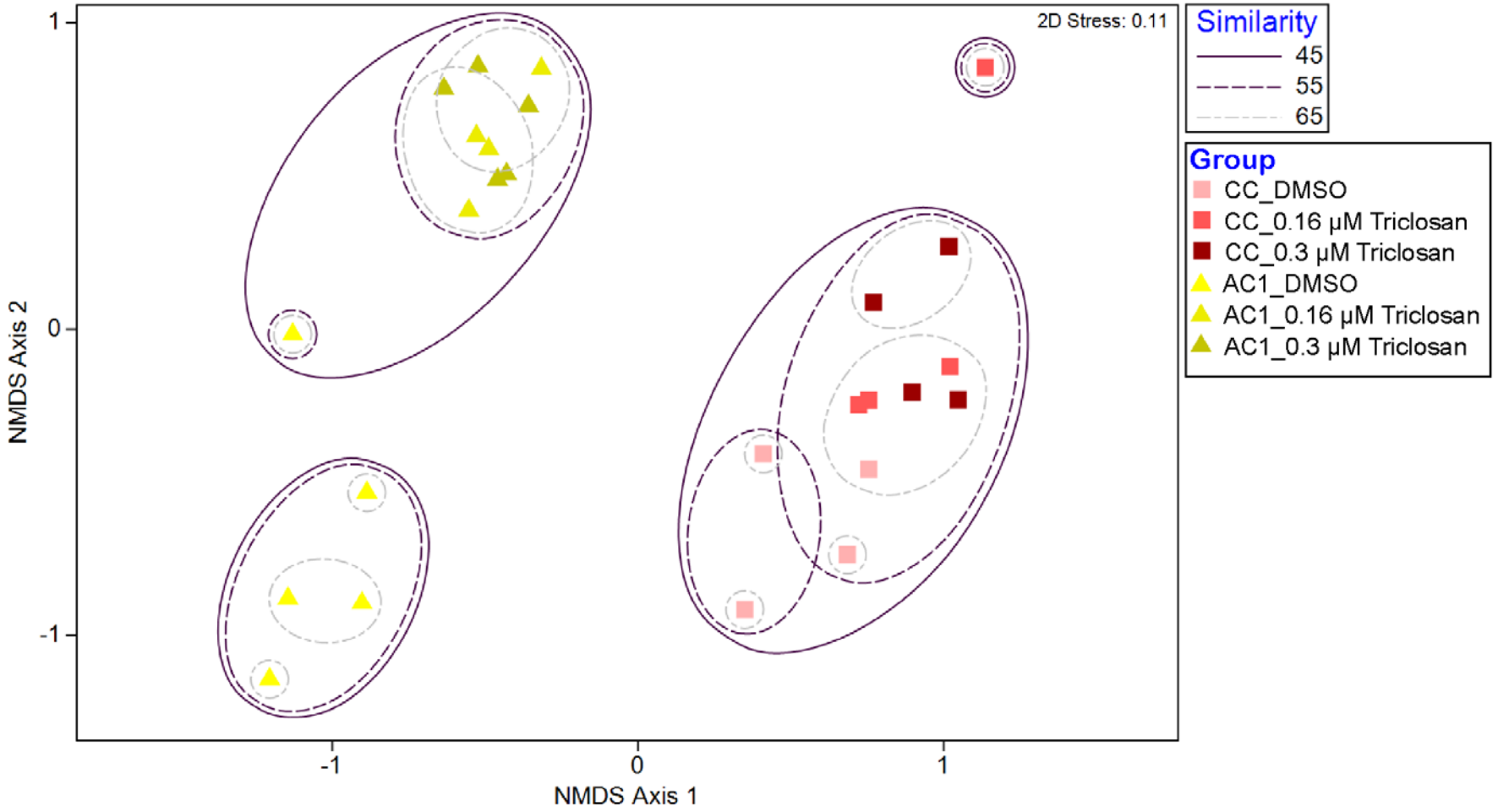
Phelps et al. 2017, Scientific Reports

Examine microbiota-chemical interactions: Triclosan case study

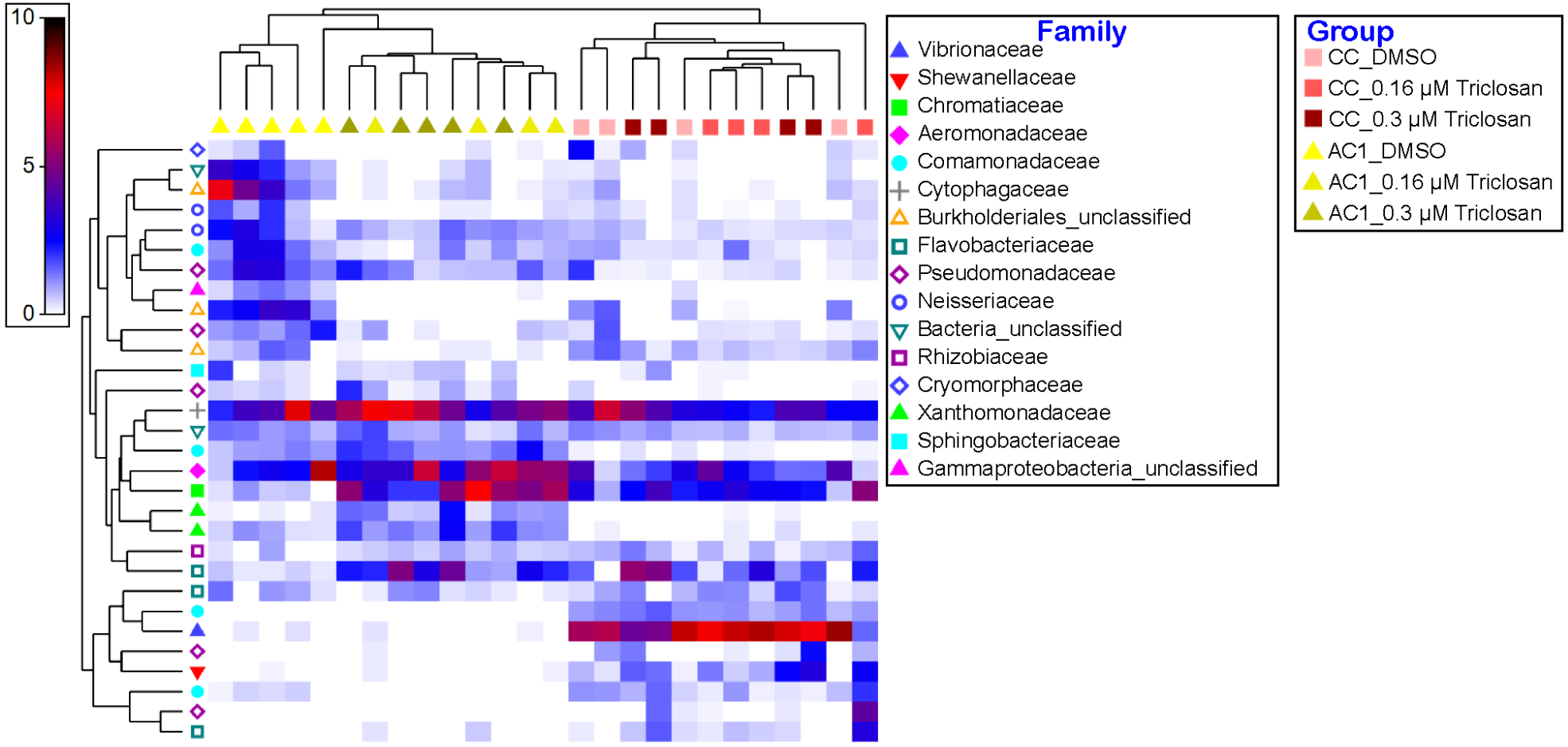


Phelps et al., In preparation

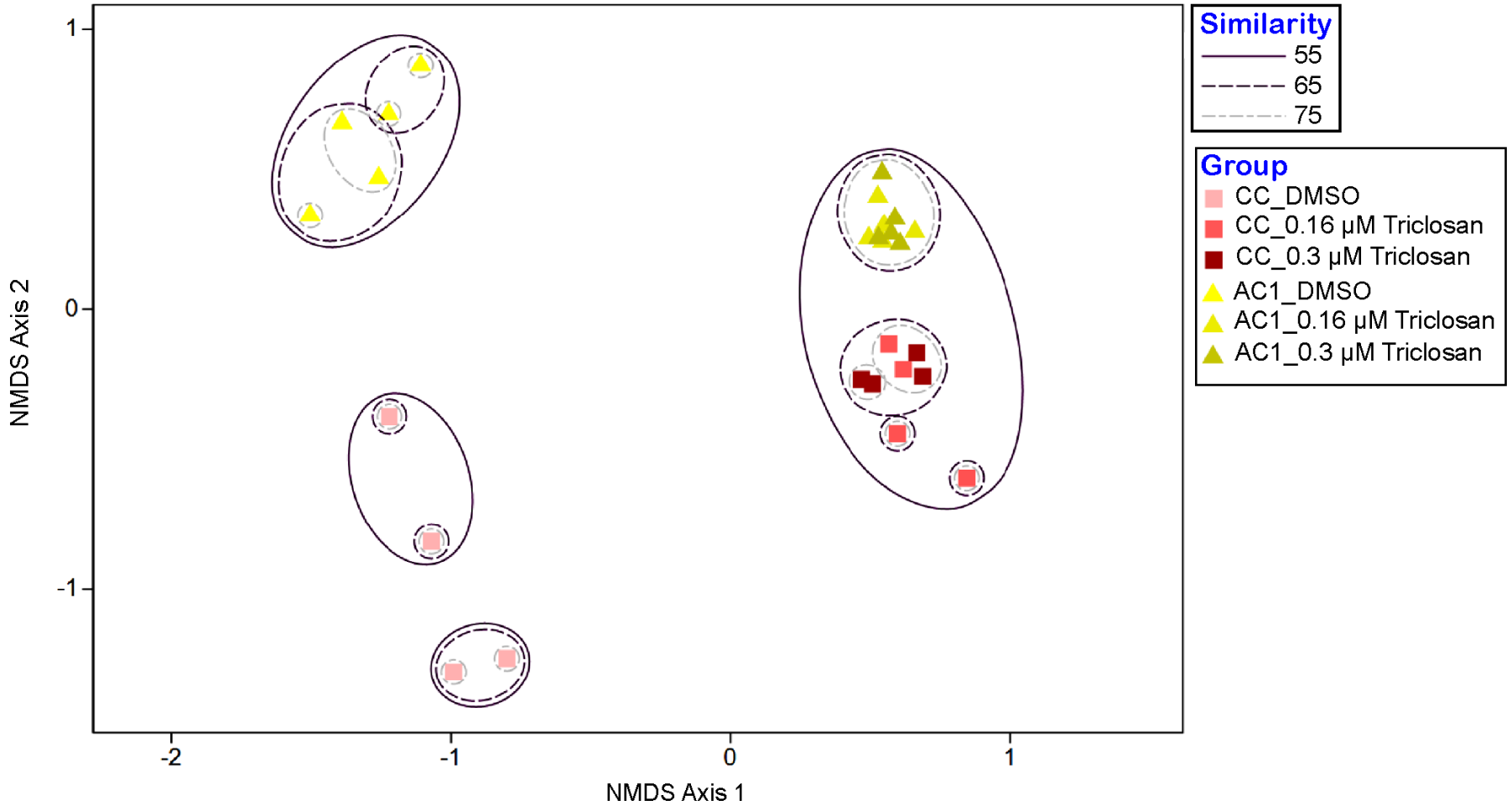
Chemical dependent effects on host-associated microbiota begin to emerge at 6 dpf



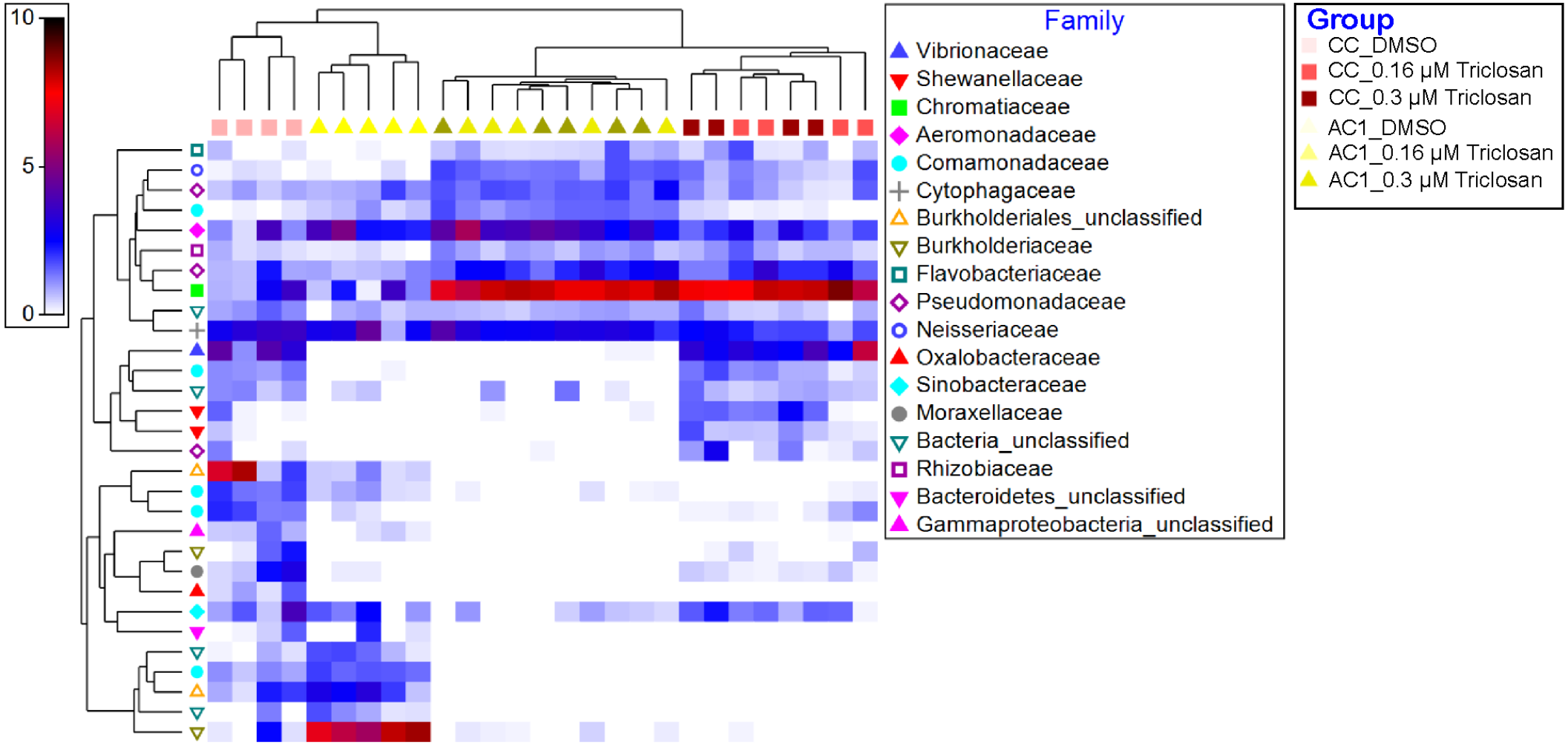
Chemical dependent effects on host-associated microbiota begin to emerge at 6 dpf



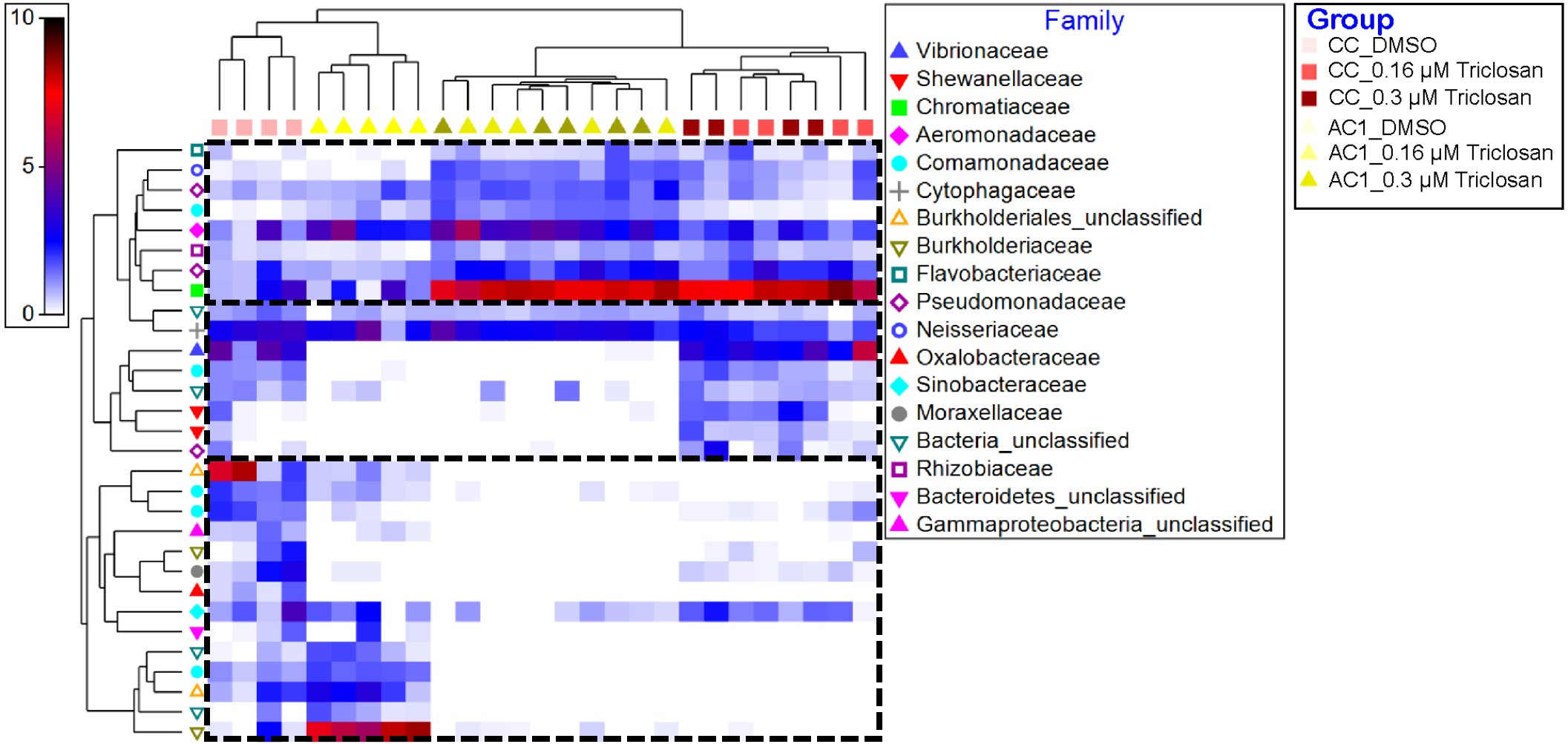
Widespread changes in microbiota coalesce at 10 dpf



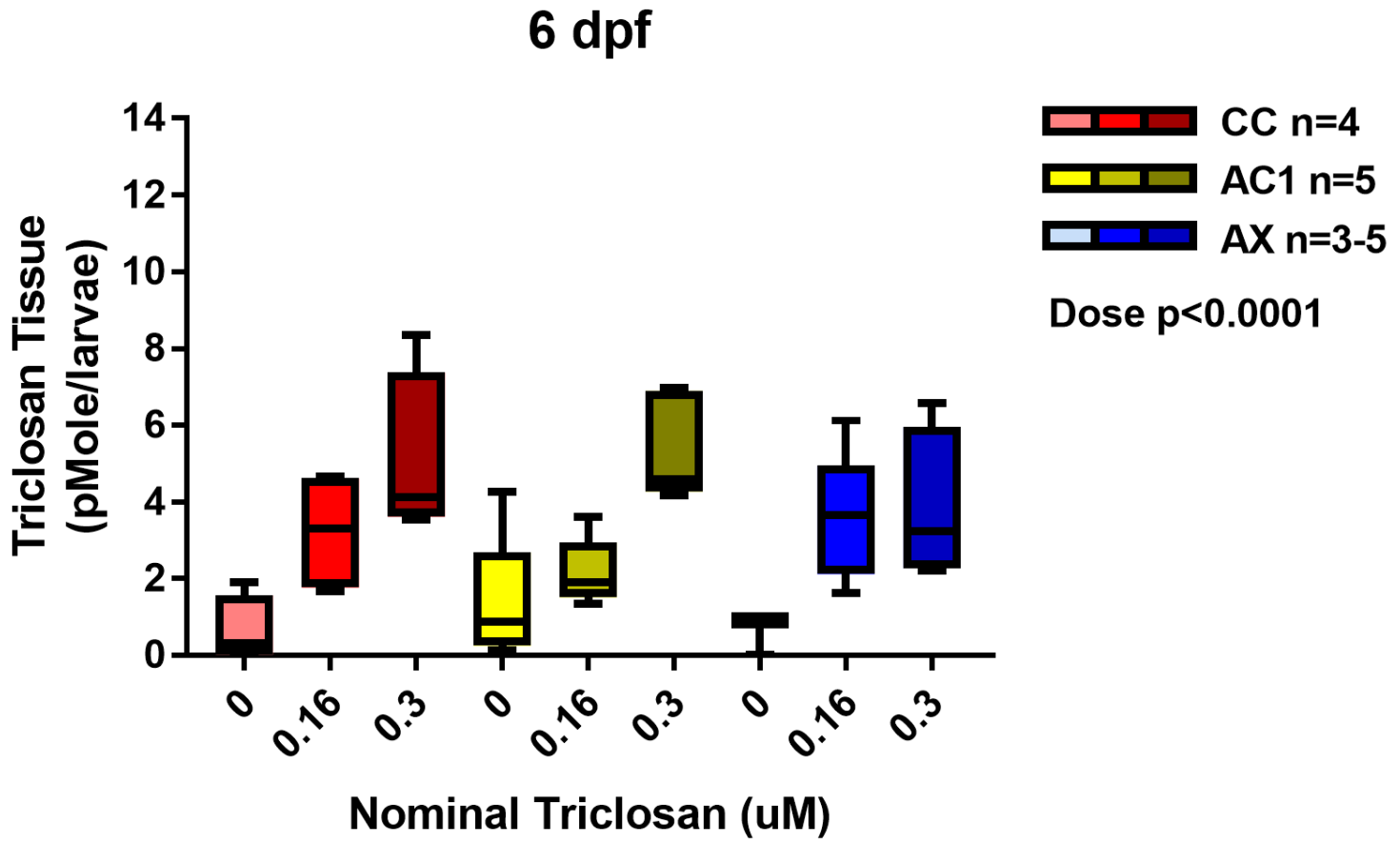
Triclosan exposure changes relative family-level taxonomy



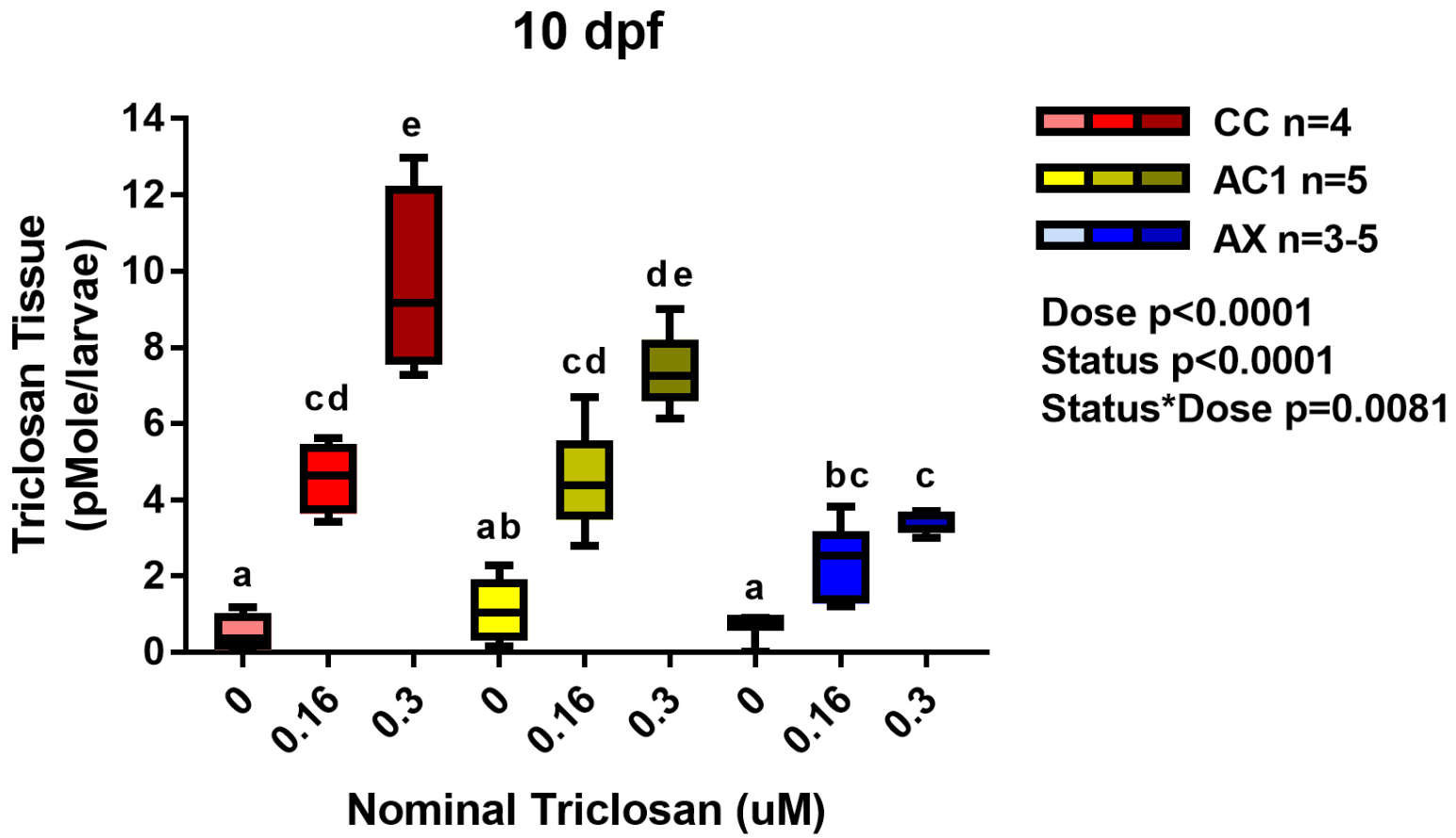
Triclosan exposure changes relative family-level taxonomy



No status-dependent differences in parent tissue dose observed at 6 dpf

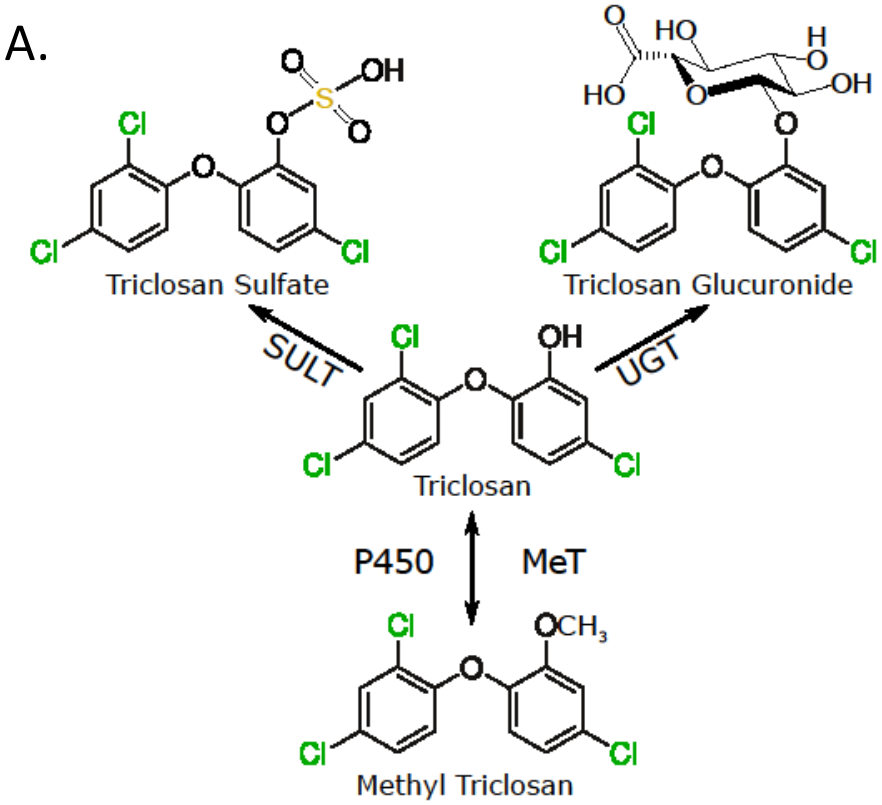


Colonized zebrafish contain higher concentrations of triclosan at 10 dpf



Do triclosan resistant microbes biotransform triclosan?

A.



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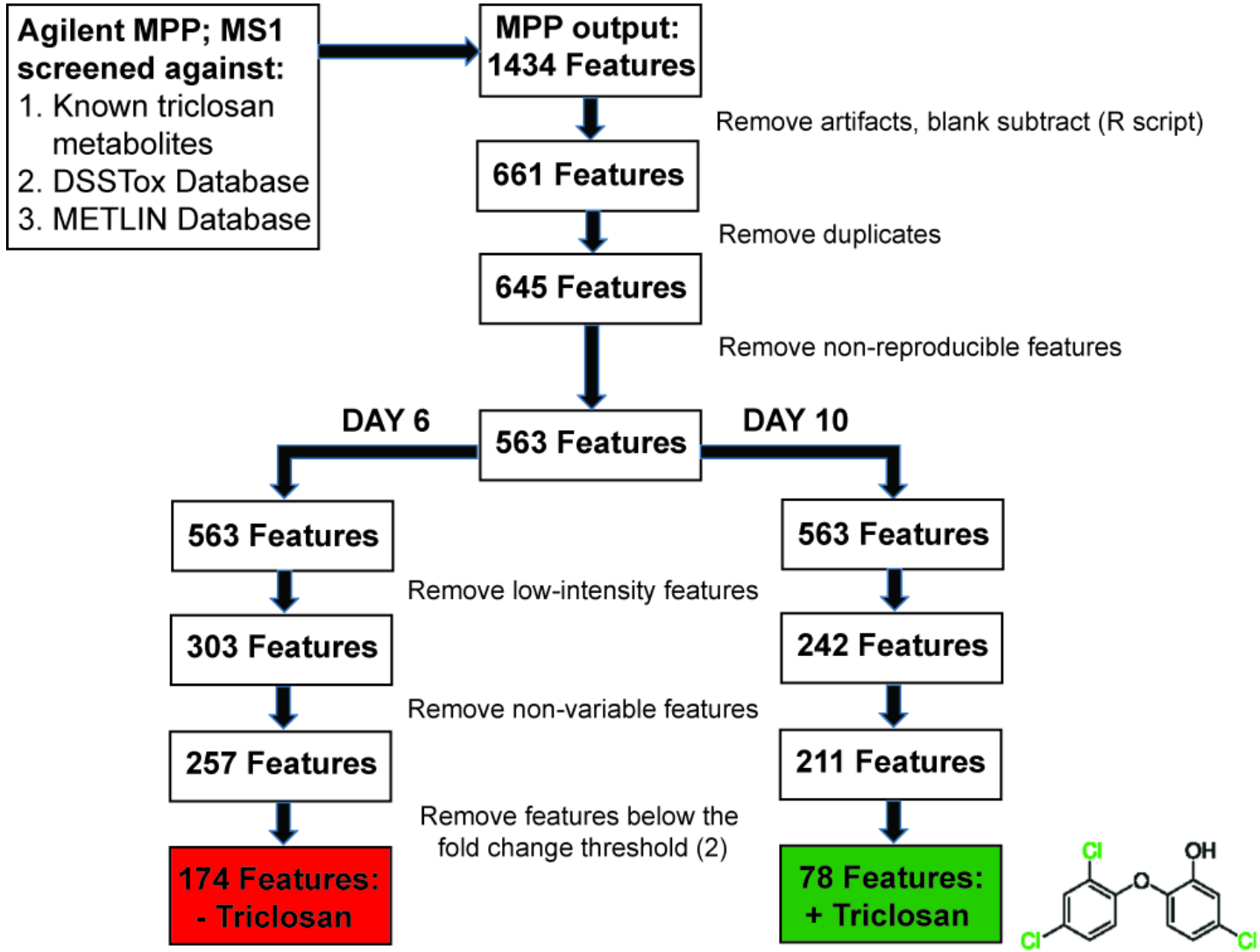
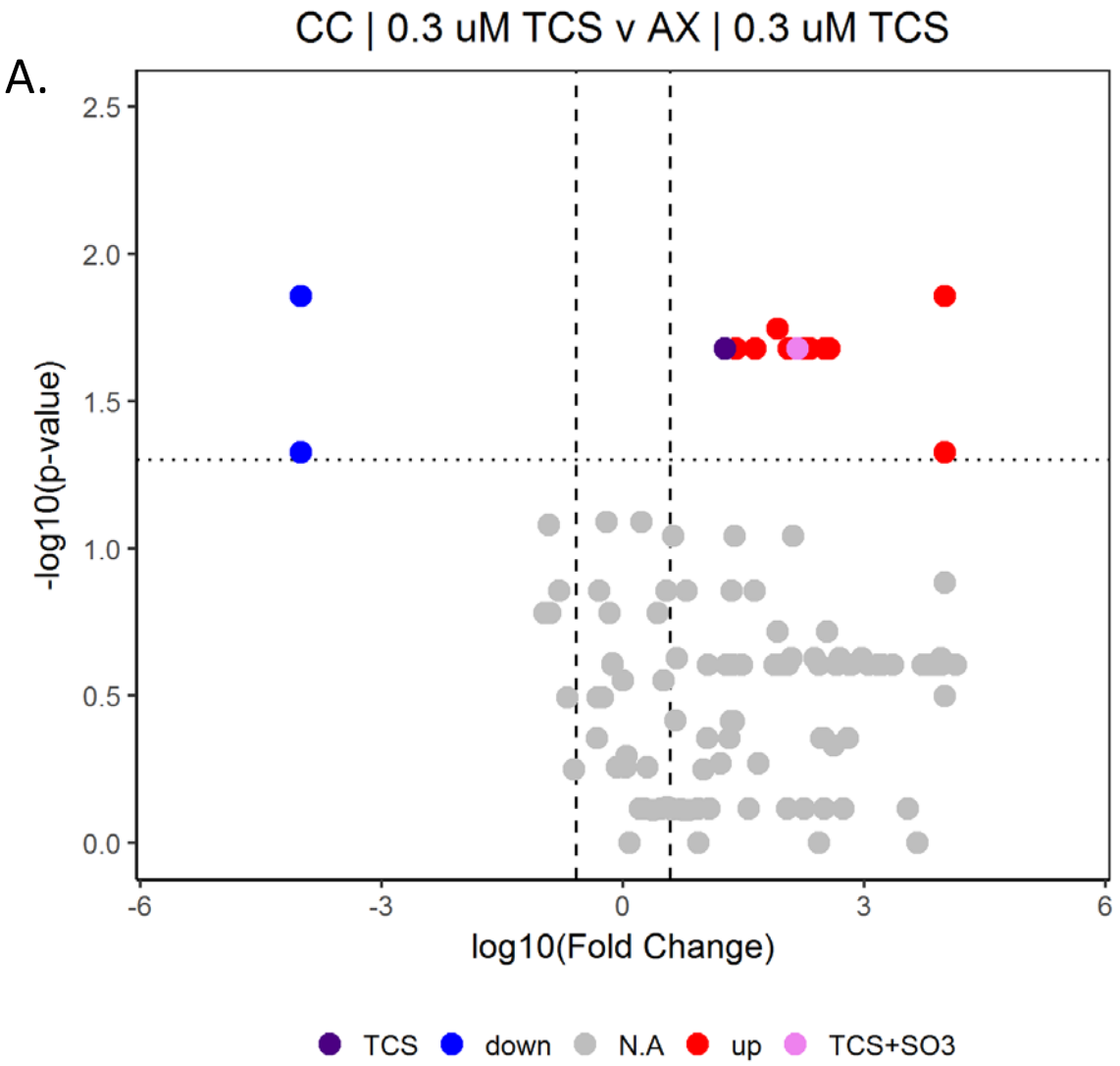


Microbial colonization changes 78 features ≥ 2 fold

A.



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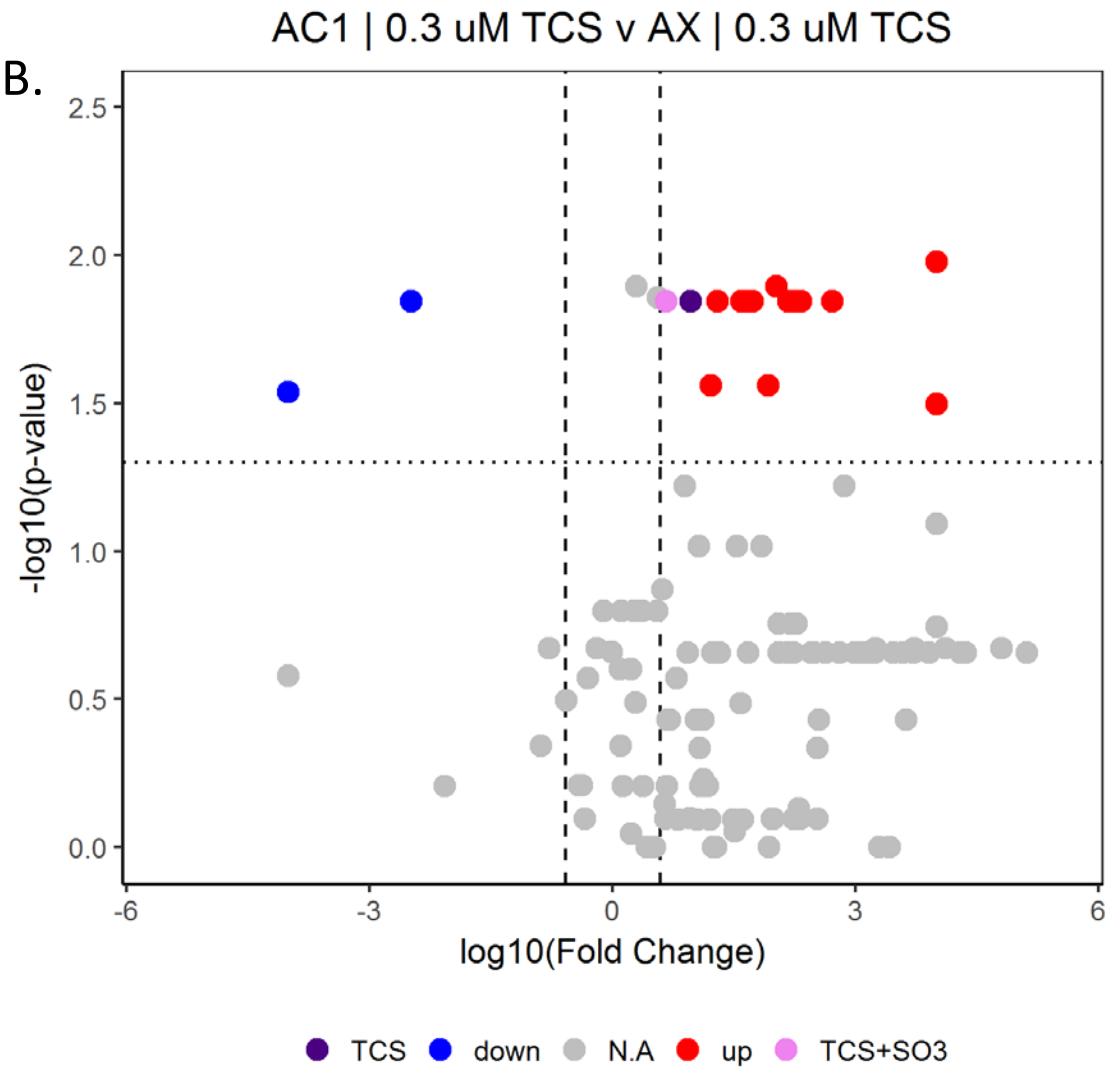
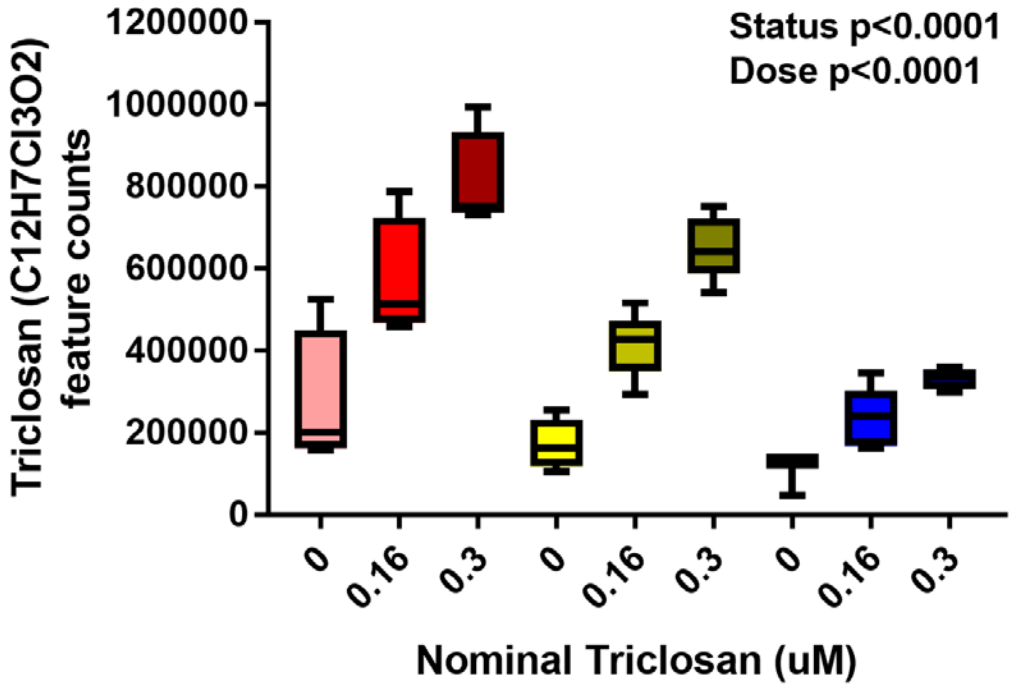


Colonized larvae contain higher concentrations of parent triclosan by NTA

A.



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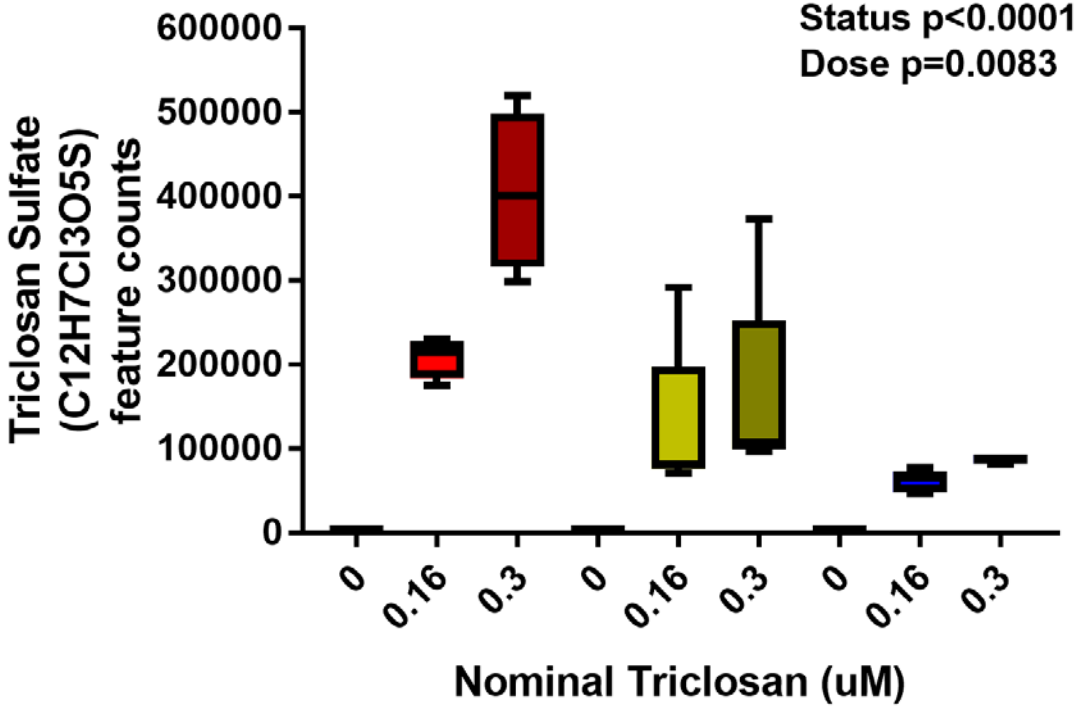
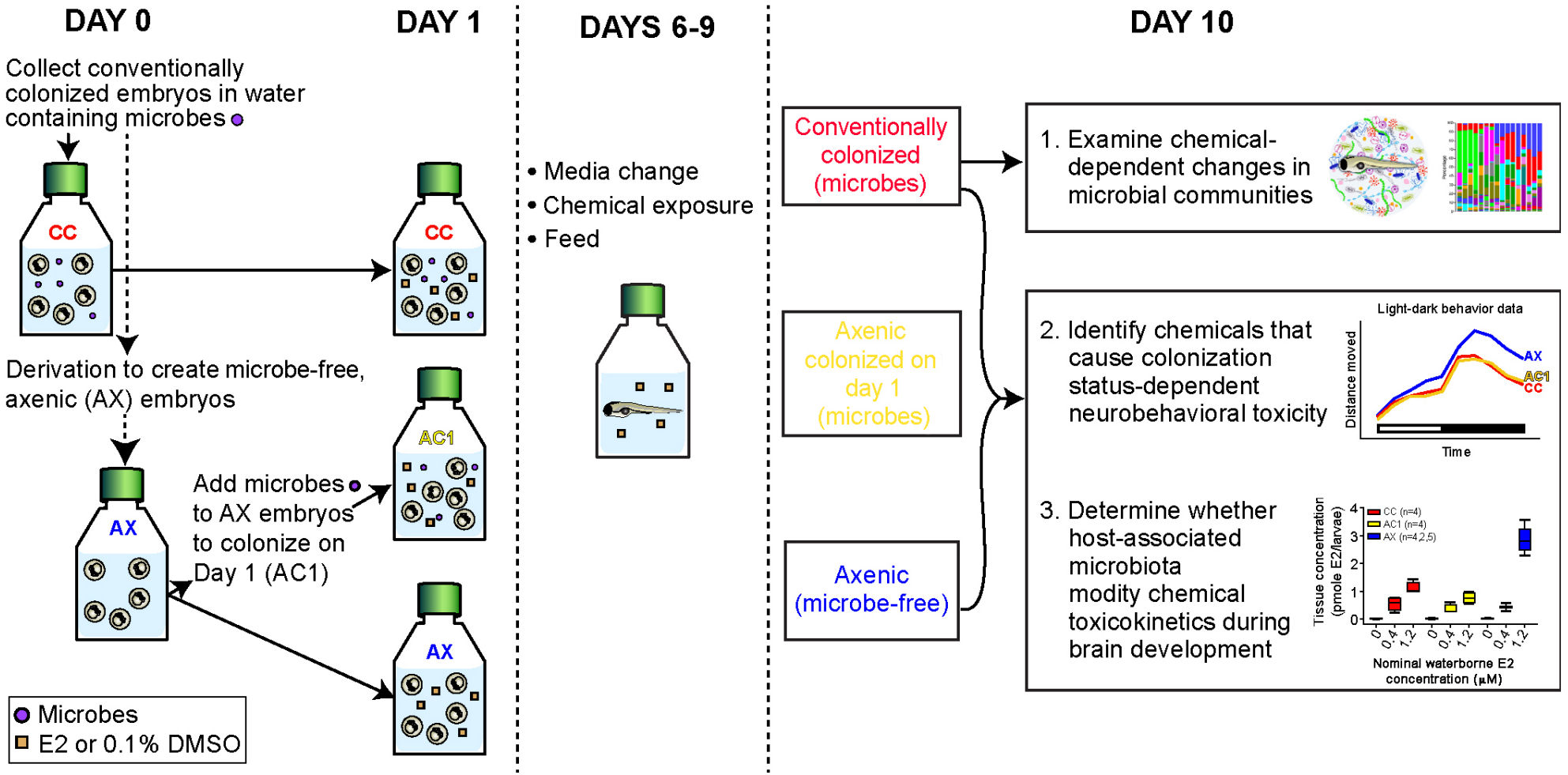


CC n=4
AC1 n=5
AX n=3-5

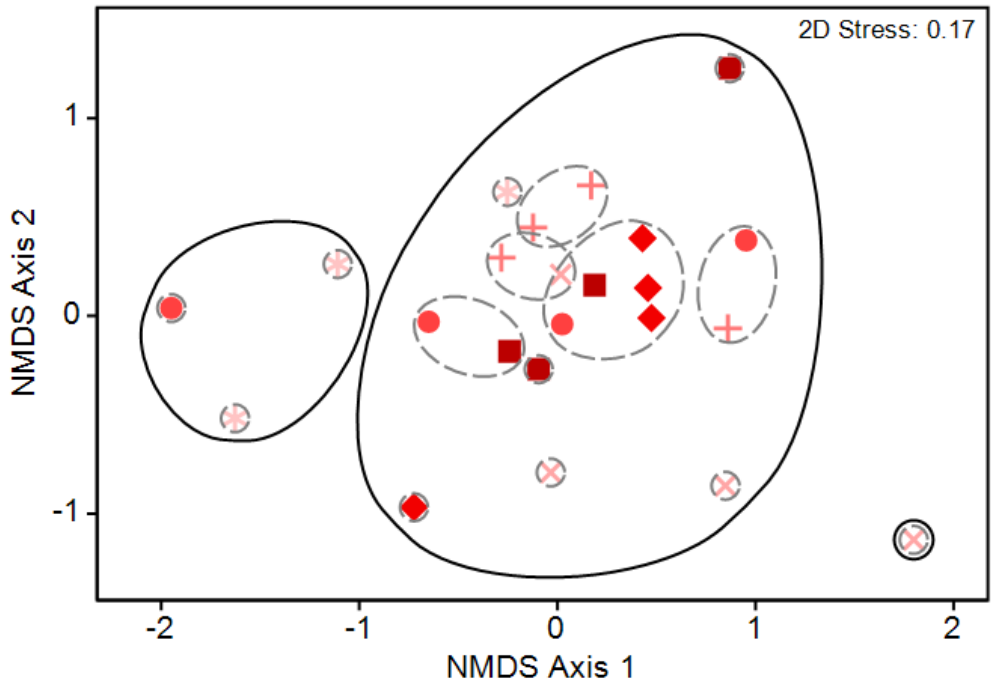
Link microbiota to phenotype: 17-β estradiol (E2) case study



Catron et al., In preparation

Exogenous E2 exposure does not affect microbial community structure

A.



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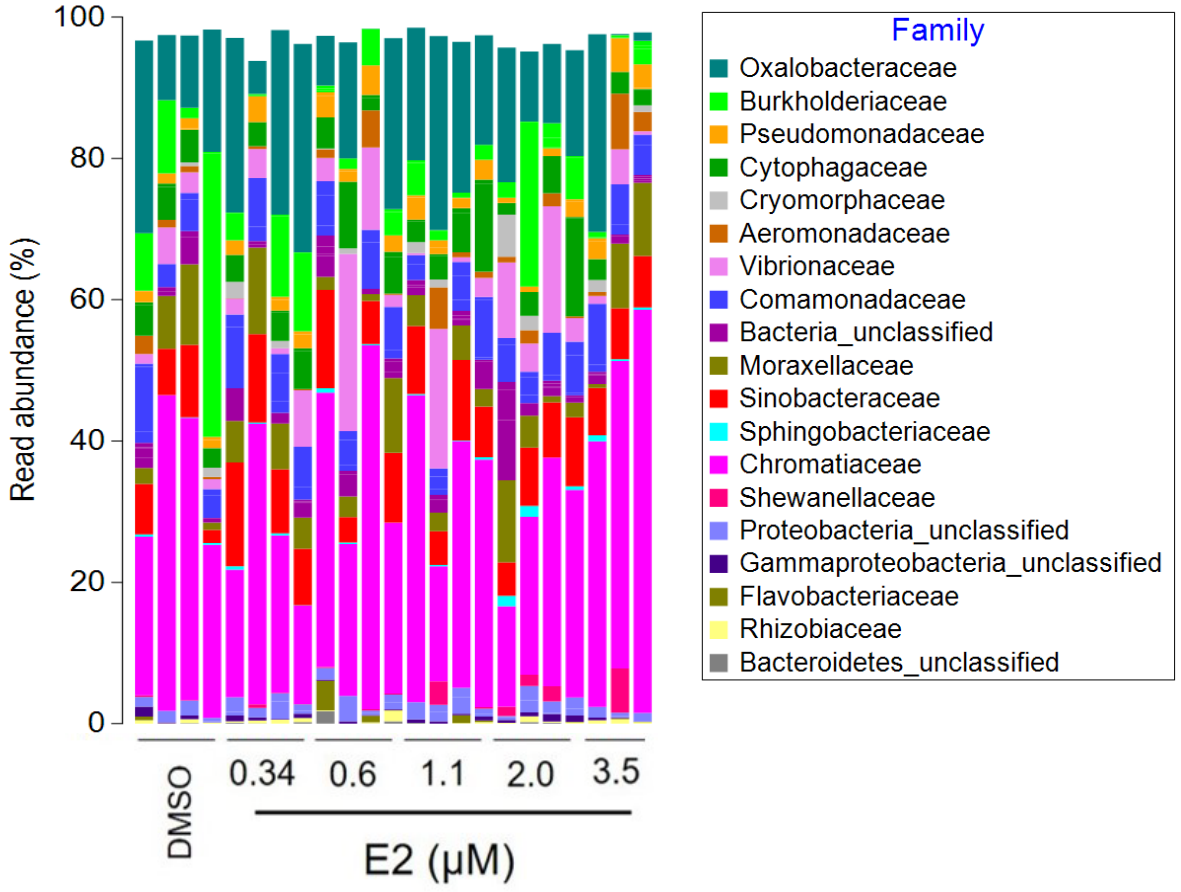
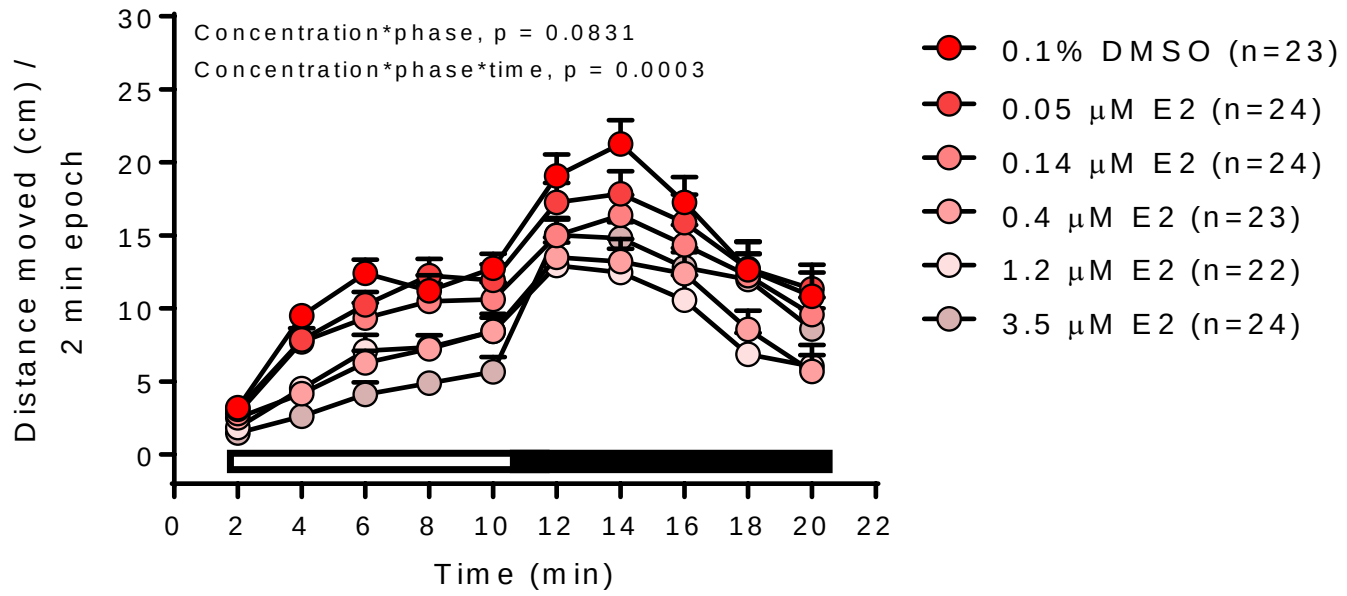


E2 exposures triggers behavioral hypoactivity in colonized zebrafish

A.



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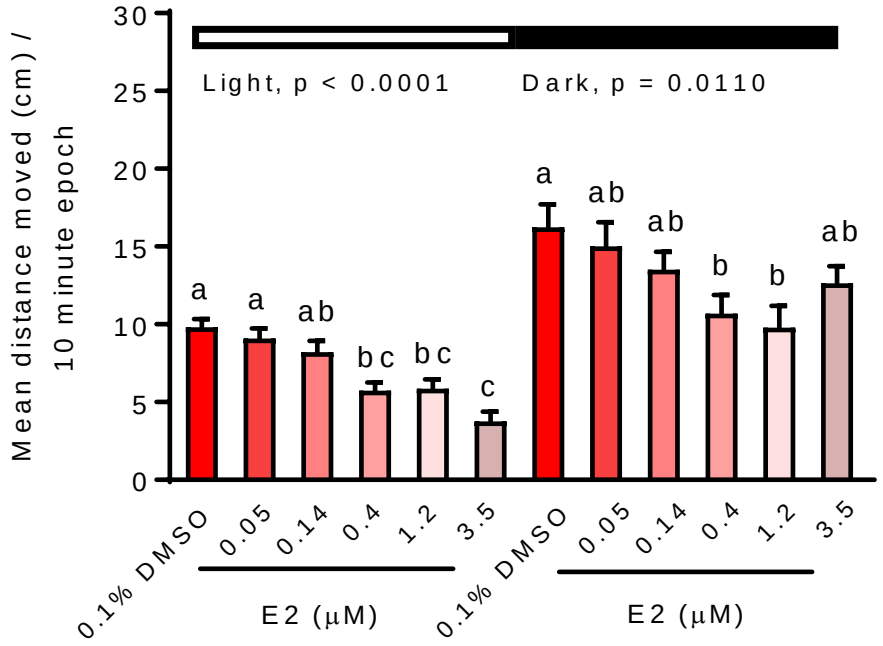
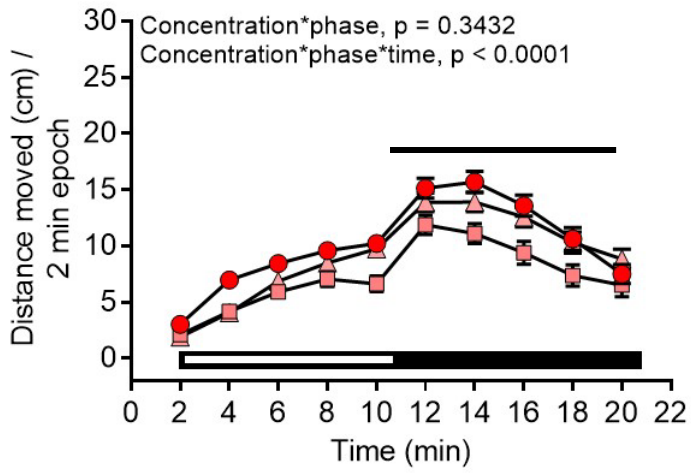
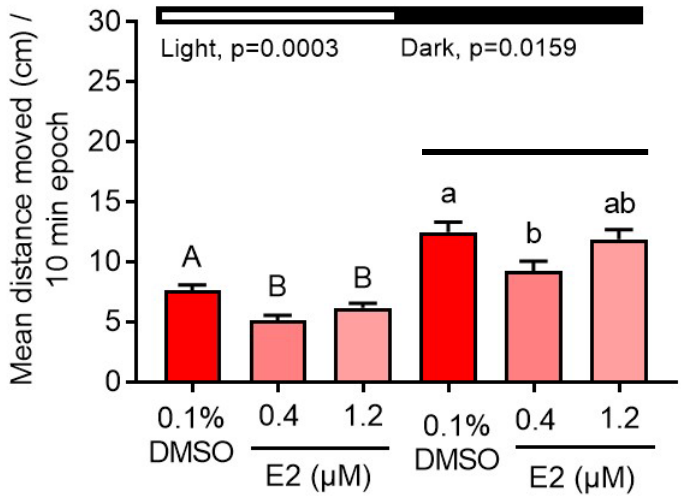
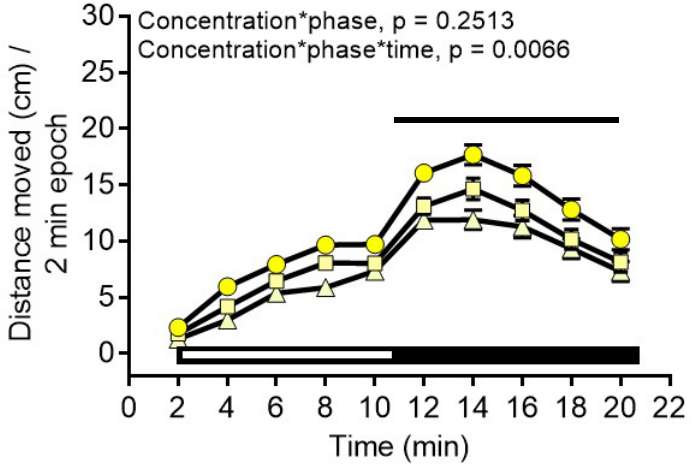


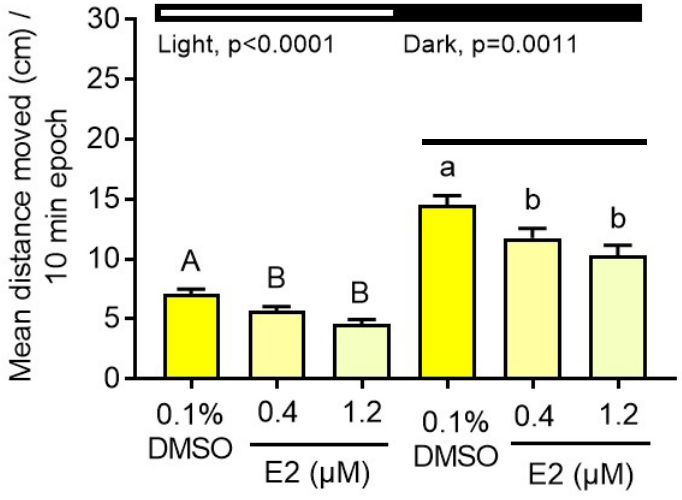
CC = colonized



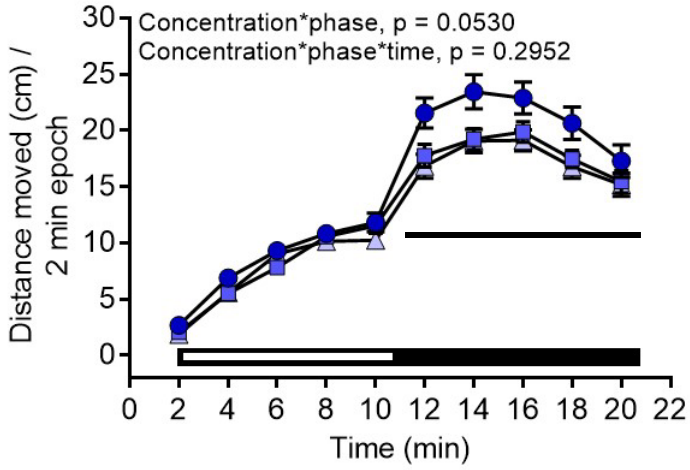


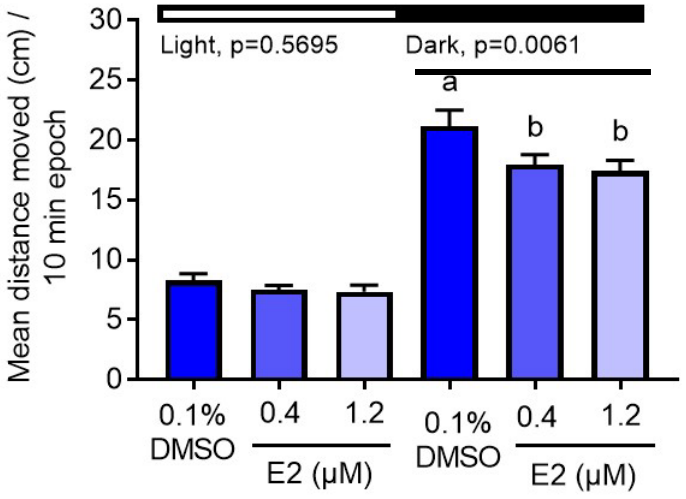
AC1 = colonized



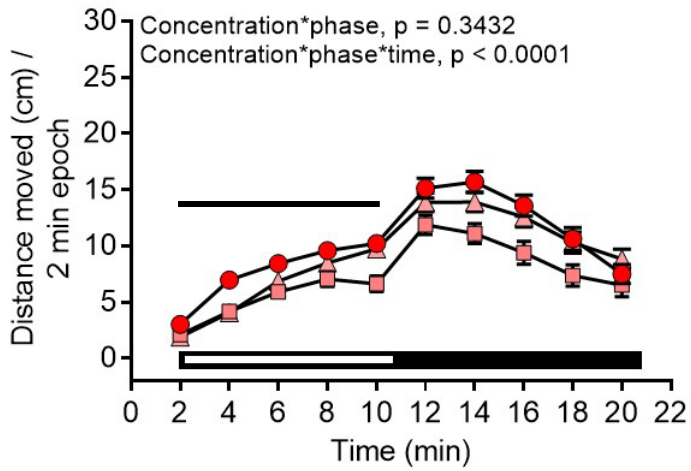


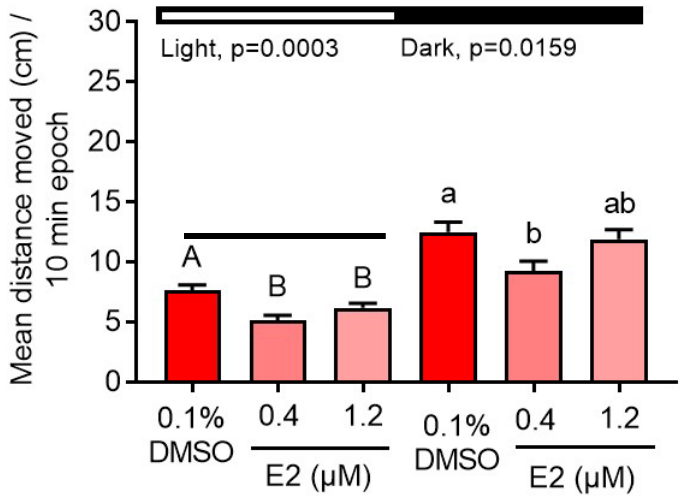
AX = microbe-free



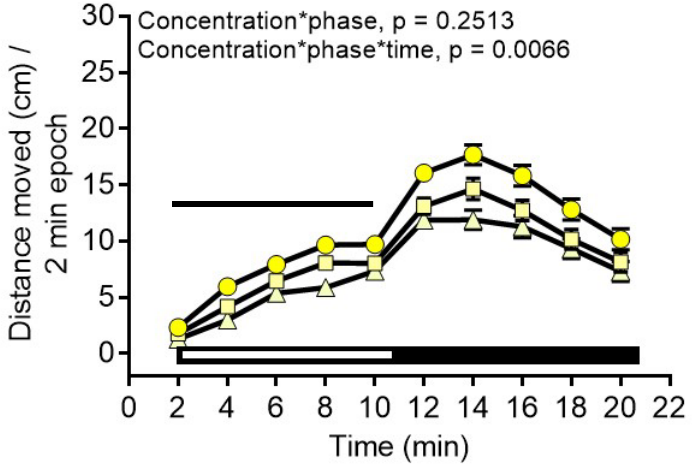


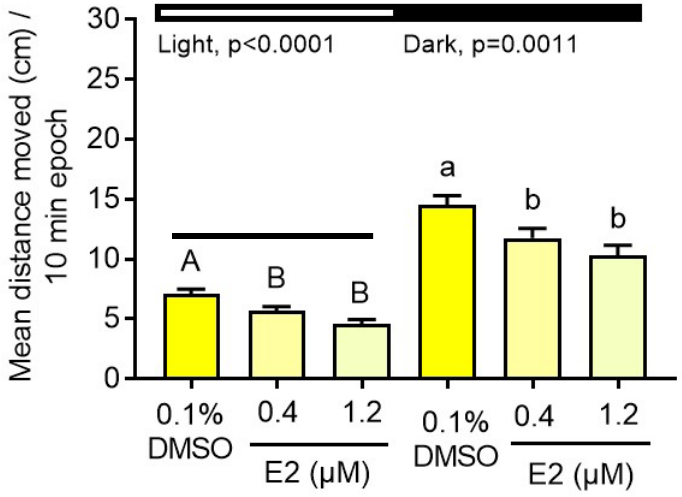
CC = colonized



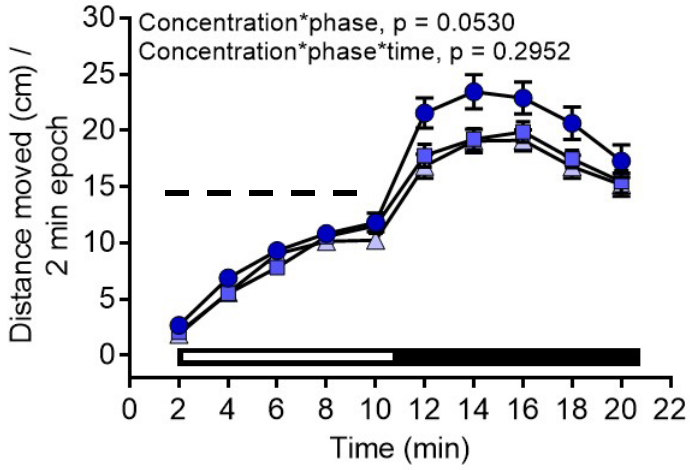


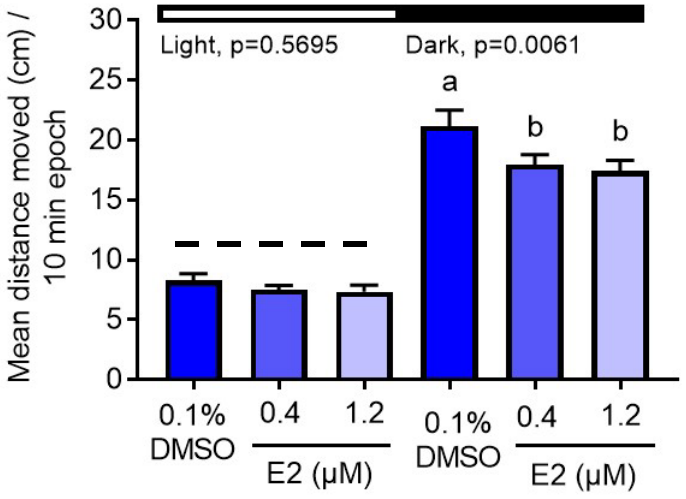
AC1 = colonized



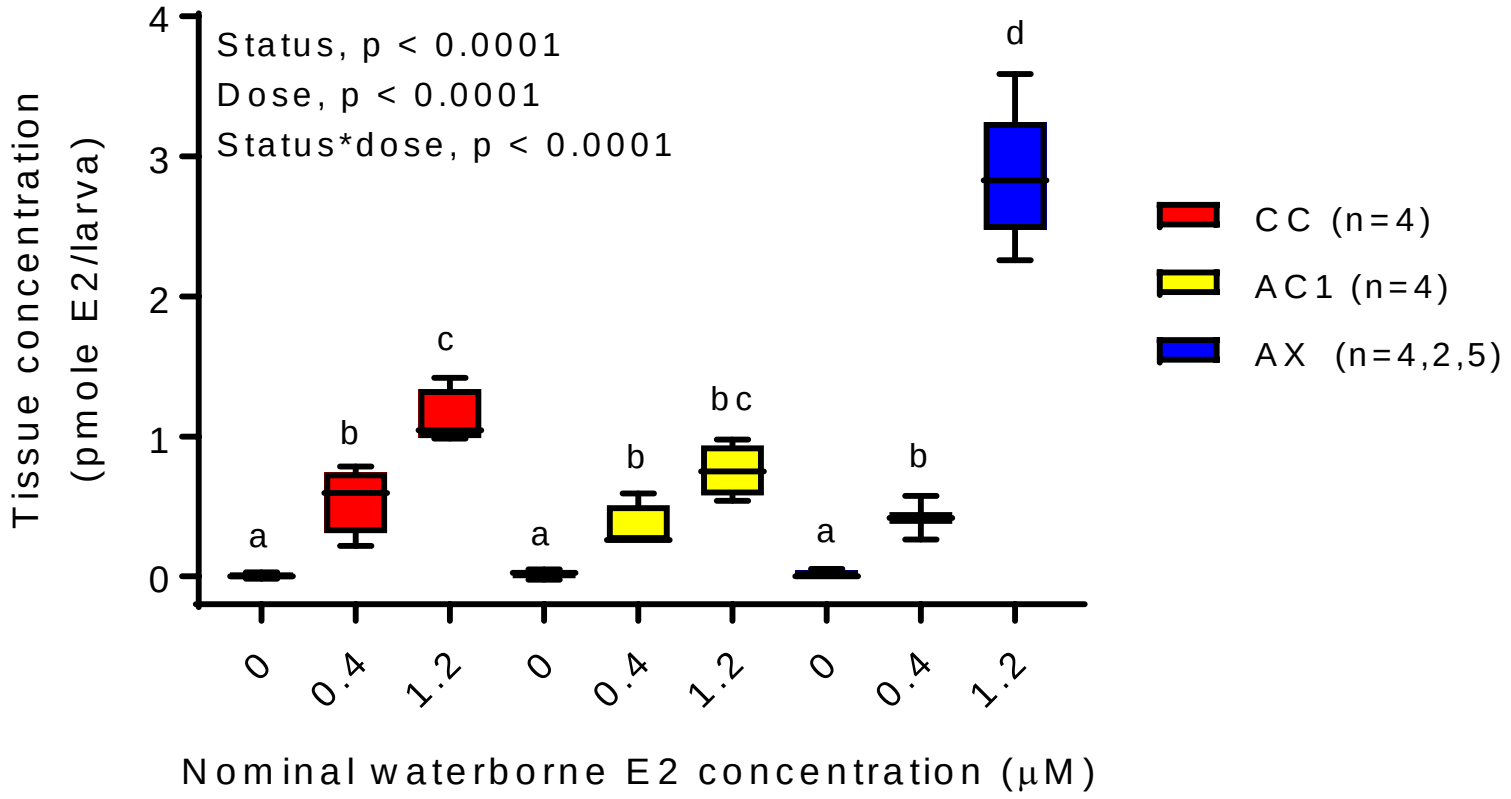


AX = microbe-free

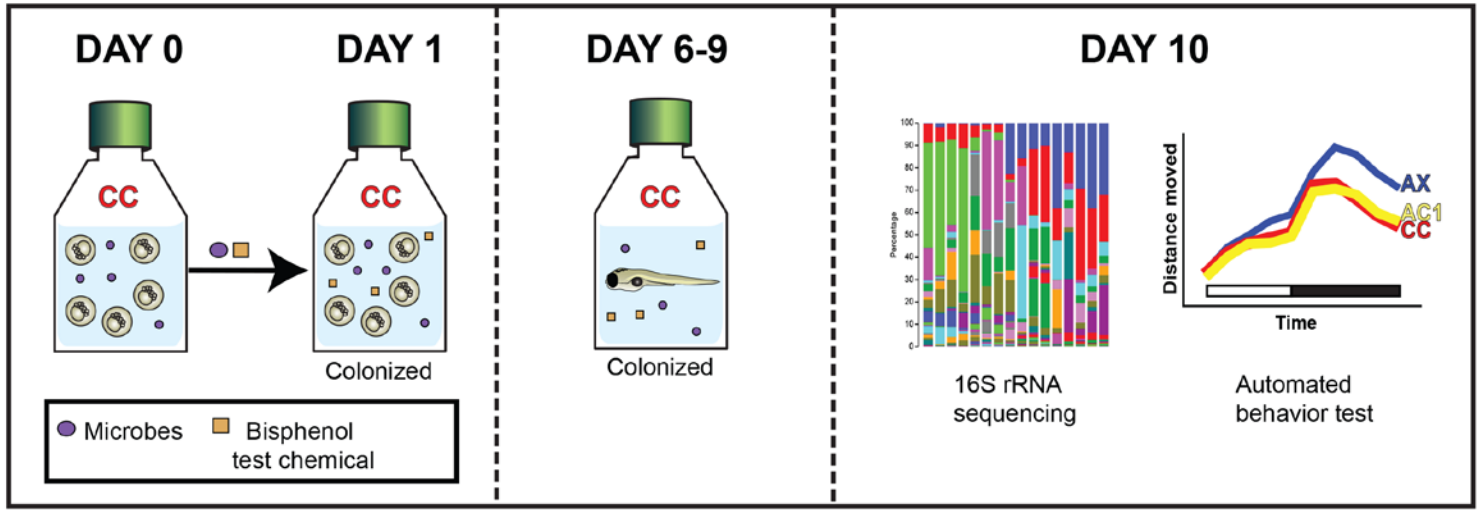


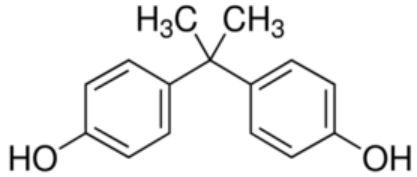


Microbe-free zebrafish contain higher concentrations of parent compound

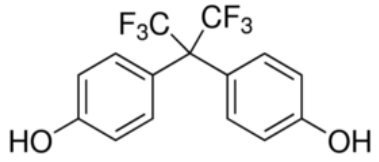


Examine chemical-dependent changes in microbial communities: Bisphenol A (BPA) and BPA replacement compounds case study

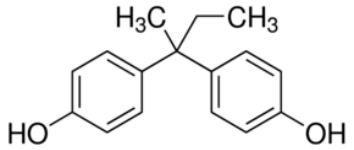




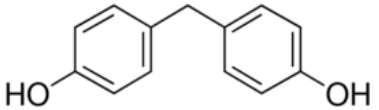
Bisphenol A (BPA)



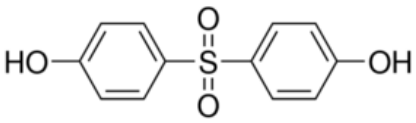
Bisphenol AF (BPAF)



Bisphenol B (BPB)

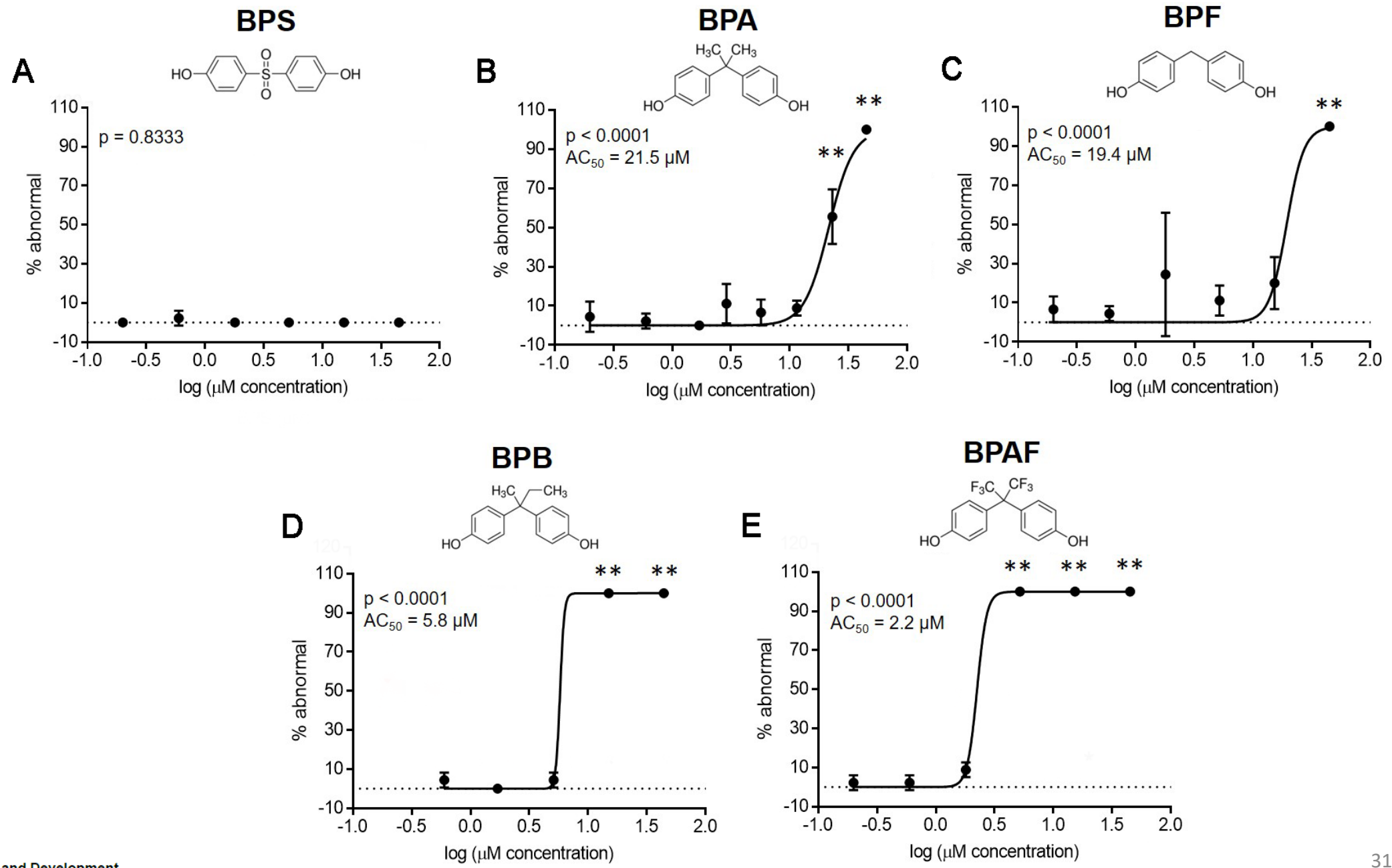


Bisphenol F (BPF)

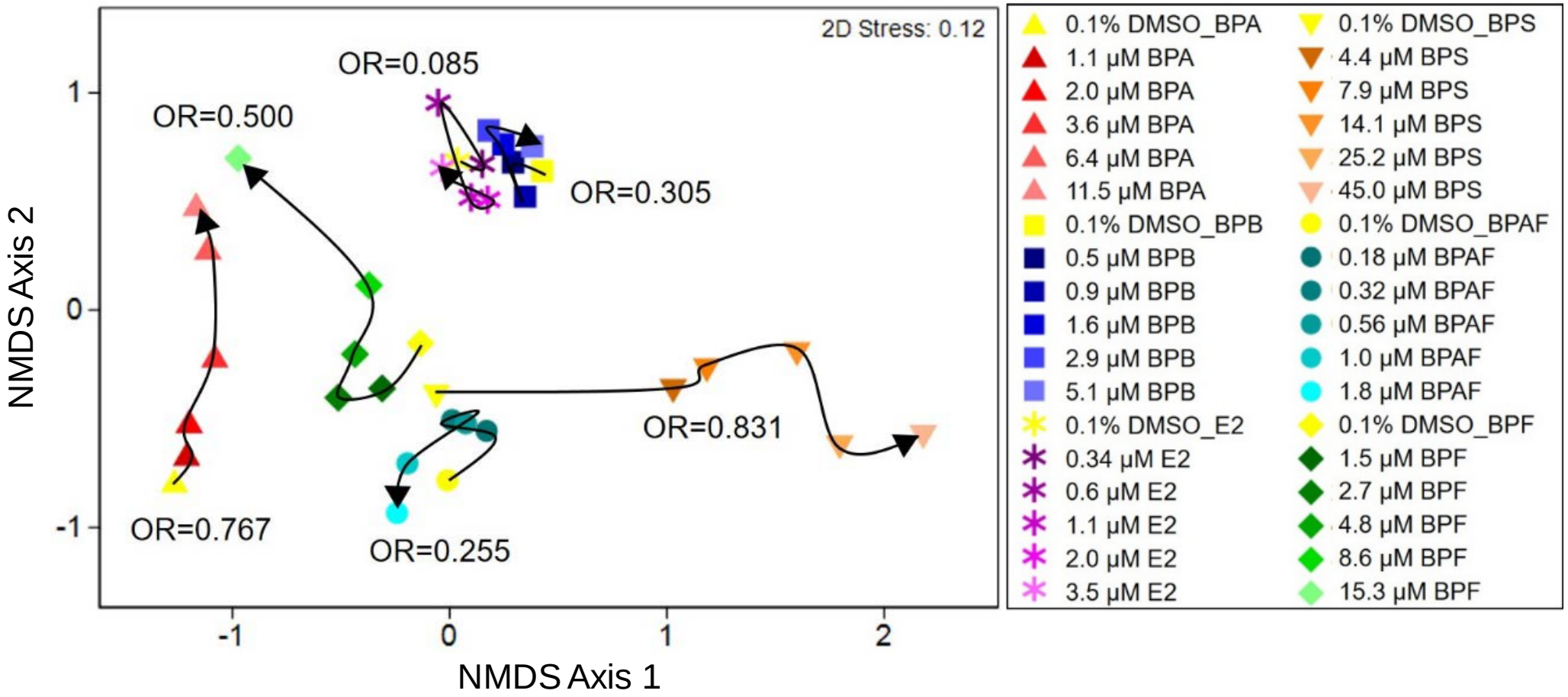


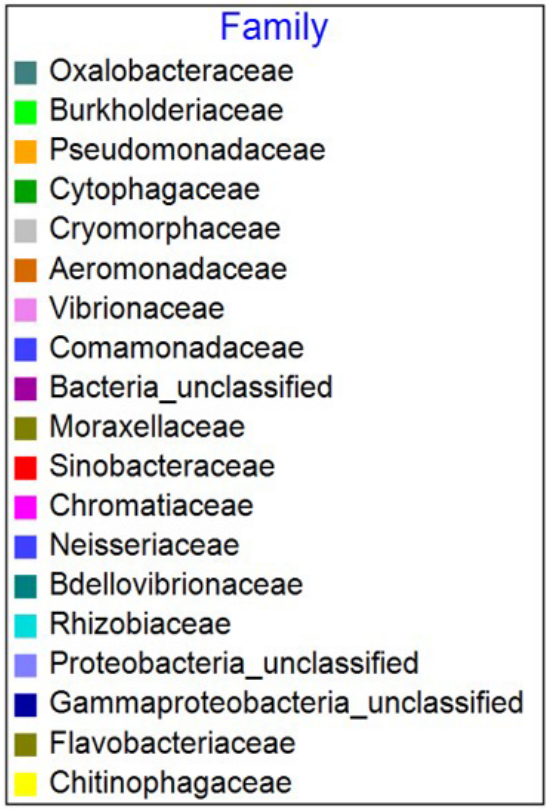
Bisphenol S (BPS)

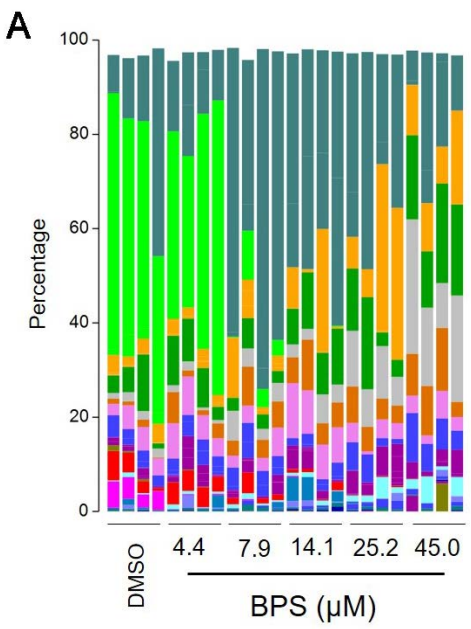
Catron et al., Under revision

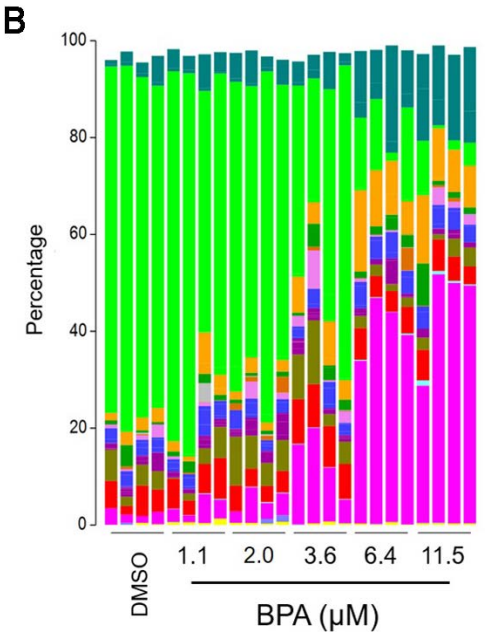

BPS
A
HO
OH
p = 0.8333
% abnormal
log (μM concentration)
BPA
B
H3C CH3
HO
OH
p < 0.0001
AC50 = 21.5 μM
**
% abnormal
log (μM concentration)
BPF
C
HO
OH
p < 0.0001
AC50 = 19.4 μM
**
% abnormal
log (μM concentration)
BPB
D
H3C CH3
HO
OH
p < 0.0001
AC50 = 5.8 μM
**
% abnormal
log (μM concentration)
BPAF
E
F3C CF3
HO
OH
p < 0.0001
AC50 = 2.2 μM
**
% abnormal
log (μM concentration)

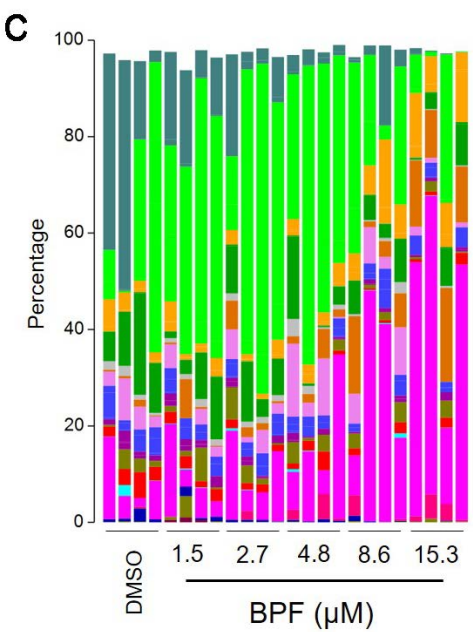
BPS, BPA, or BPF exposure disrupted global microbial community structure

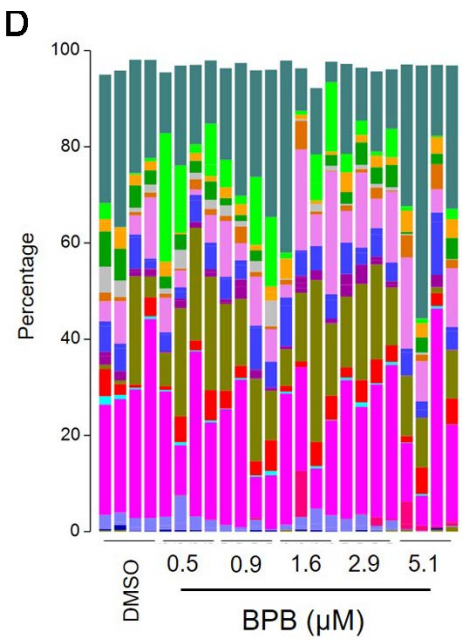


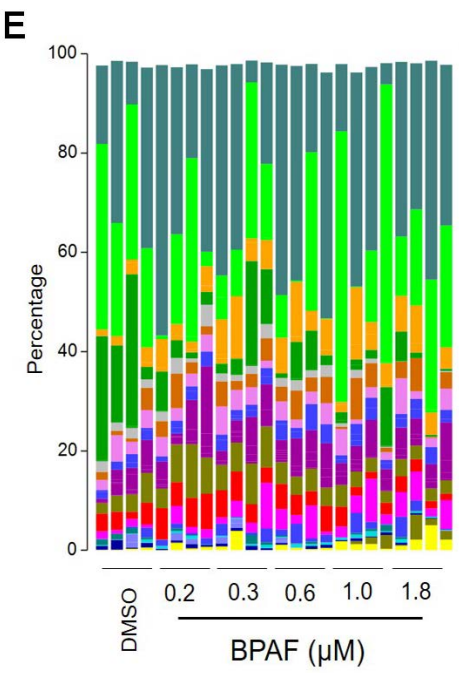

Family
Oxalobacteraceae
Burkholderiaceae
Pseudomonadaceae
Cytophagaceae
Cryomorphaceae
Aeromonadaceae
Vibrionaceae
Comamonadaceae
Bacteria_unclassified
Moraxellaceae
Sinobacteraceae
Chromatiaceae
Neisseriaceae
Bdellovibrionaceae
Rhizobiaceae
Proteobacteria_unclassified
Gammaproteobacteria_unclassified
Flavobacteriaceae
Chitinophagaceae

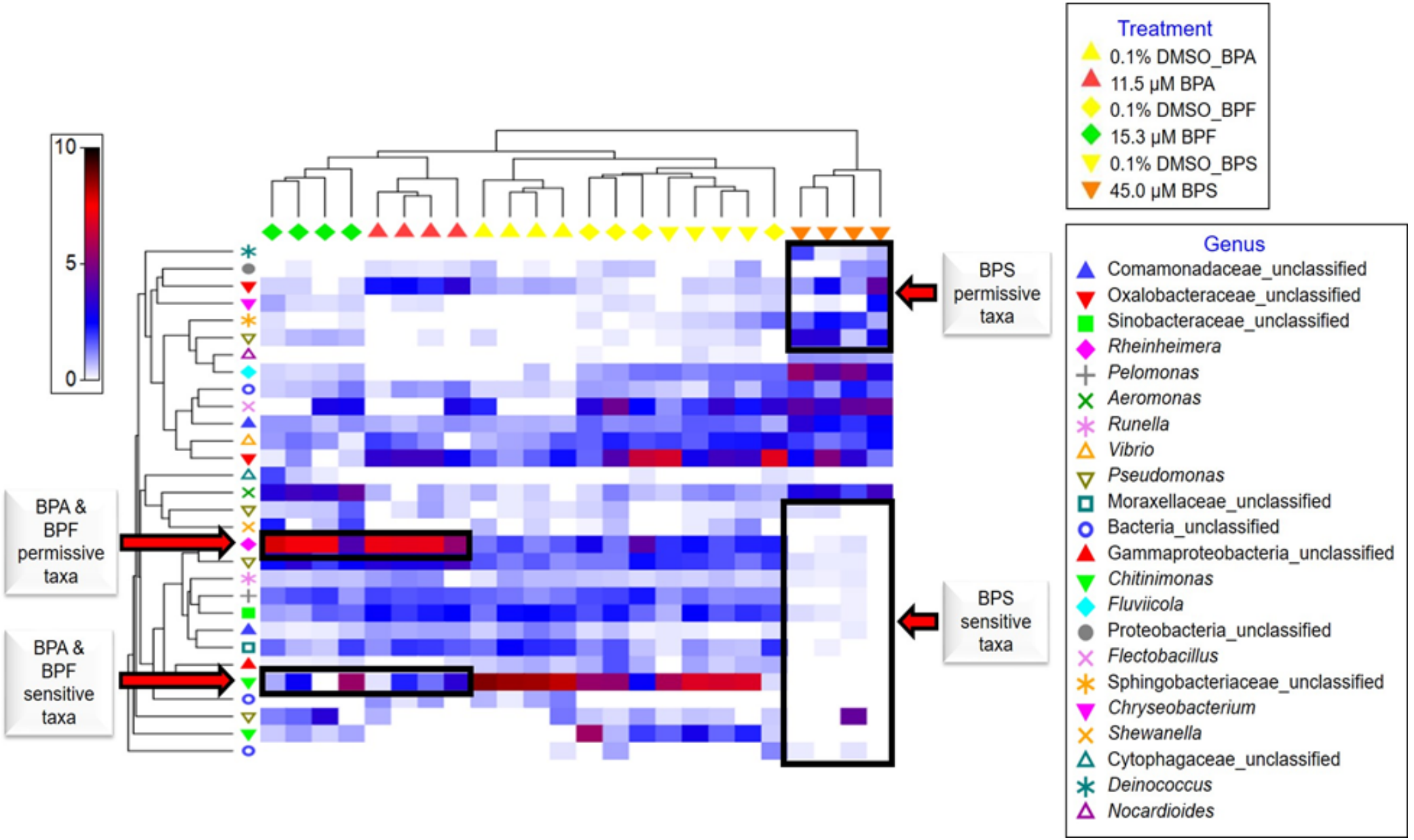

A
Percentage
0
20
40
60
80
100
DMSO
4.4
7.9
14.1
25.2
45.0
BPS (μM)


B
Percentage
0
20
40
60
80
100
DMSO
1.1
2.0
3.6
6.4
11.5
BPA (μM)

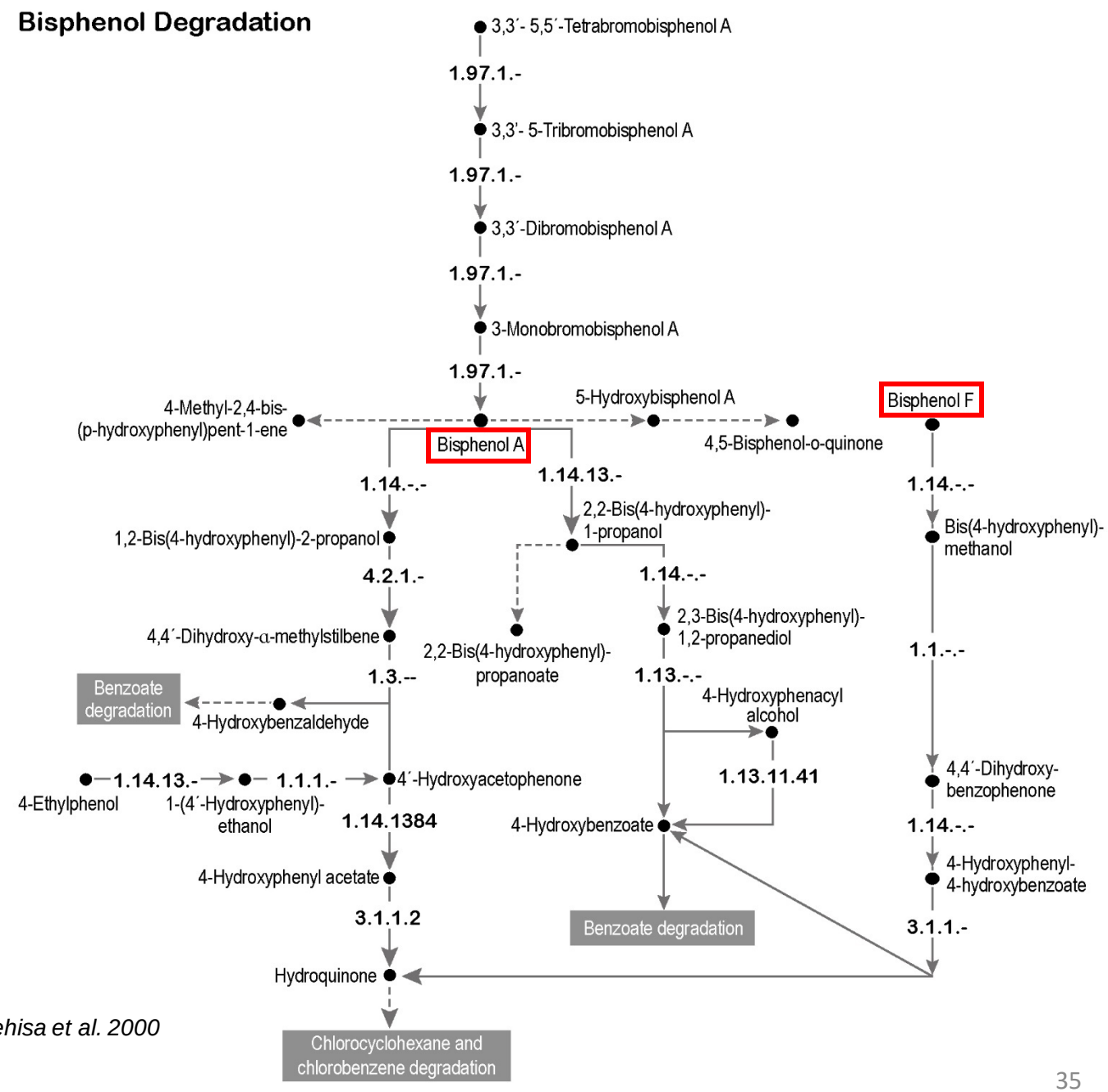

C
Percentage
0
20
40
60
80
100
DMSO
1.5
2.7
4.8
8.6
15.3
BPF (μM)


D
Percentage
0
20
40
60
80
100
DMSO
0.5
0.9
1.6
2.9
5.1
BPB (μM)


E
Percentage
0
20
40
60
80
100
DMSO
0.2
0.3
0.6
1.0
1.8
BPAF (μM)


10
5
0
BPA & BPF permissive taxa
BPA & BPF sensitive taxa
BPS permissive taxa
BPS sensitive taxa
Treatment
0.1% DMSO_BPA
11.5 µM BPA
0.1% DMSO_BPF
15.3 µM BPF
0.1% DMSO_BPS
45.0 µM BPS
Genus
Comamonadaceae_unclassified
Oxalobacteraceae_unclassified
Sinobacteraceae_unclassified
Rheinheimera
Pelomonas
Aeromonas
Runella
Vibrio
Pseudomonas
Moraxellaceae_unclassified
Bacteria_unclassified
Gammaproteobacteria_unclassified
Chitinimonas
Fluviicola
Proteobacteria_unclassified
Flectobacillus
Sphingobacteriaceae_unclassified
Chryseobacterium
Shewanella
Cytophagaceae_unclassified
Deinococcus
Nocardioides

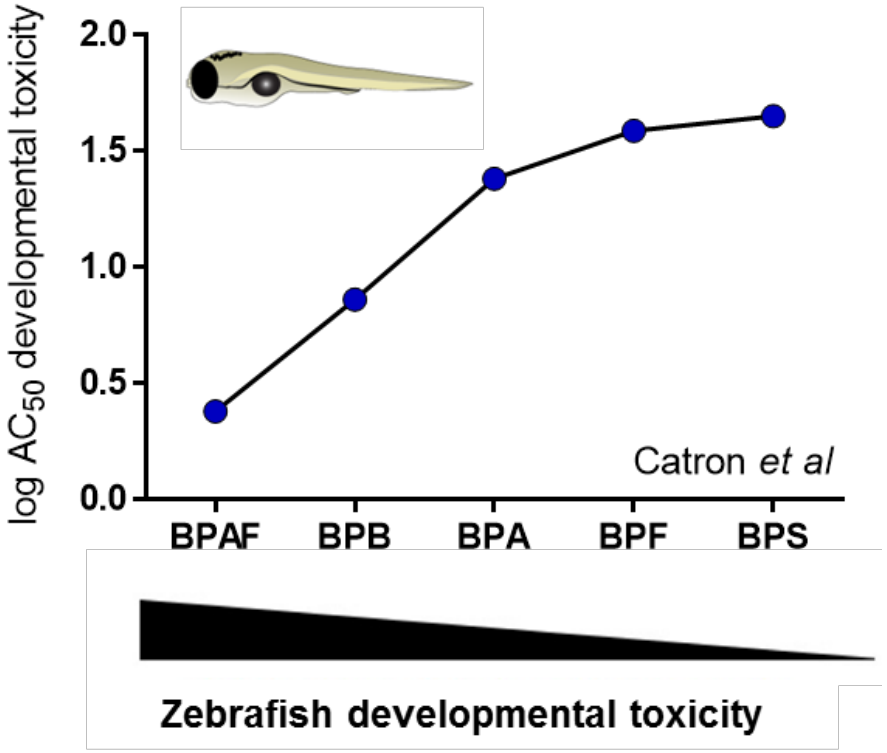
Predicted microbial functions by linear discriminant analysis (PICRUSt)



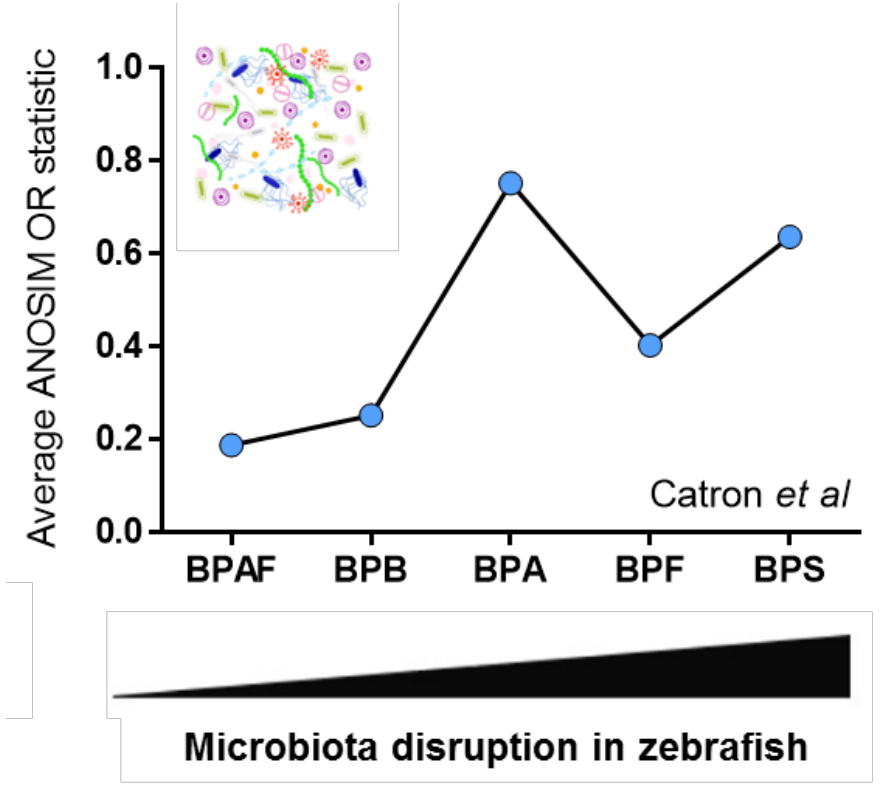
Recreated from: http://www.genome.jp/kegg-bin/show_pathway?map00363; *Kanehisa et al. 2000*

Differential chemical effects: Host developmental toxicity *vs.* microbiota disruption

A.



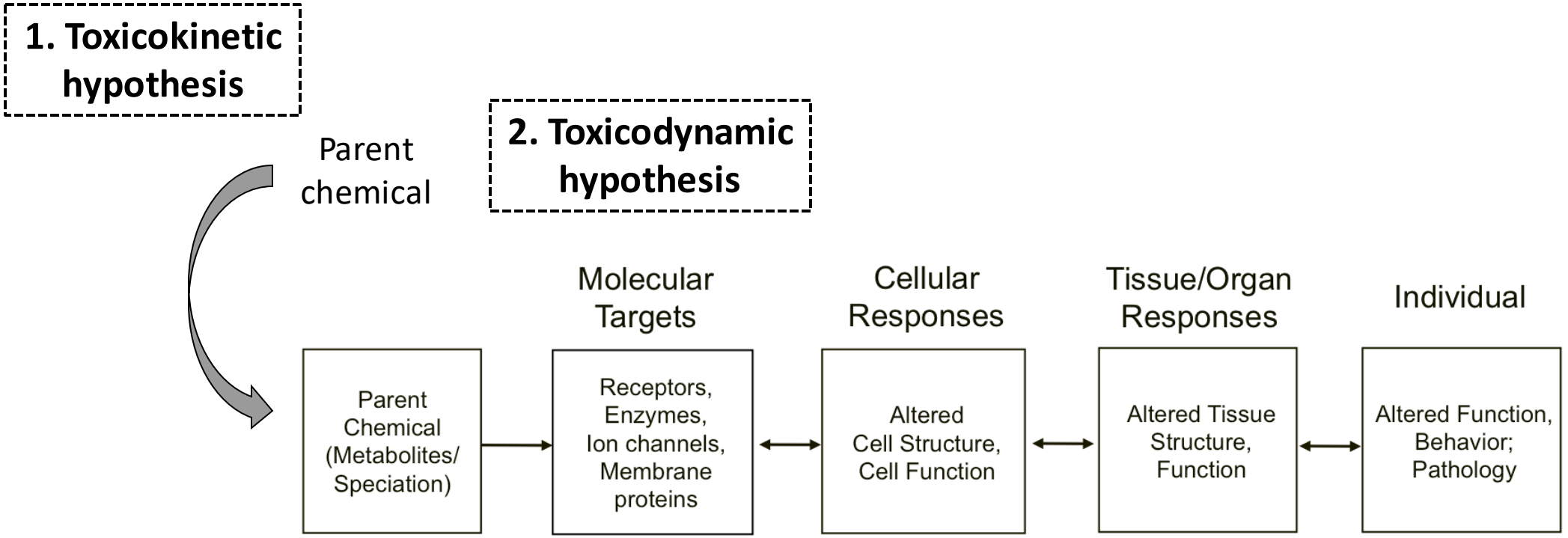
B.



Summary

1. We developed an experimental system to test whether microbiota affects the kinetics and/or dynamics of xenobiotic exposures
2. Axenic zebrafish are hyperactive
3. Antibiotic exposure phenocopies hyperactivity in colonized zebrafish
4. Triclosan resistant taxa increase host parent tissue dose and perform a sulfation reaction
5. Exogenous E2 exposure triggers hypoactivity in the light period in colonized zebrafish, possibly via a bioactivation event
6. Inverse relationship between host toxicity and microbiota disruption

Microbiota-triclosan interaction take home



TOXICOKINETIC

- Biotransformation; triclosan: *Phelps et al.* In preparation.
- Biotransformation; estradiol (E2): *Catron et al.* In preparation.

TOXICODYNAMIC

- Antibiotics: *Phelps et al.* Scientific Reports. 2017.
- Bisphenol compounds: *Catron et al.* Submitted.

Outstanding questions

- Do chemical-induced compositional changes affect other aspects of development or predispose the organism to future insults?
- Do microbiota-mediated biotransformations broadly affect chemical toxicity?

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