Cell Assay Technology for CEC Screening in Ambient Waters

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Science Advisory Panel for CECs in California's Aquatic Ecosystems

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Recommendation of the 2012 CEC Expert Panel

Develop and apply bioanalytical tools (or cell assays) to:

- Supplement existing chemical-by-chemical monitoring
- Expand the coverage of analytes and screen for unexpected chemicals
- Better understand mixture effects on aquatic life

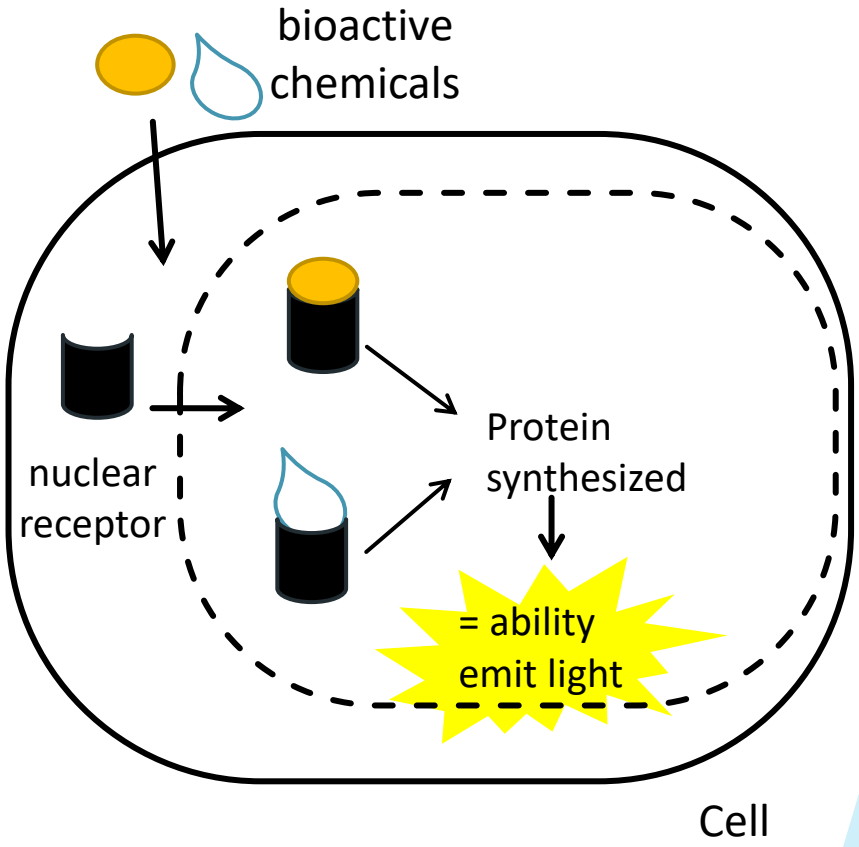
What are cell assays?

Rapid, high-throughput methods to screen for multiple chemicals simultaneously

Mammalian cells engineered to track effects of chemicals on key biological pathways

Currently used to assess CECs effects in other sectors (e.g. cosmetic, pharmaceutical industries), and for chemical registration

How do they work?



Sample extracts and cells incubated in multiwell plates

Combined measure of known and unknown chemicals with same mode of action

Light intensity is proportional to the concentration of bioactive chemicals

- Expressed relative to a known chemical (BEQ, ng/L)

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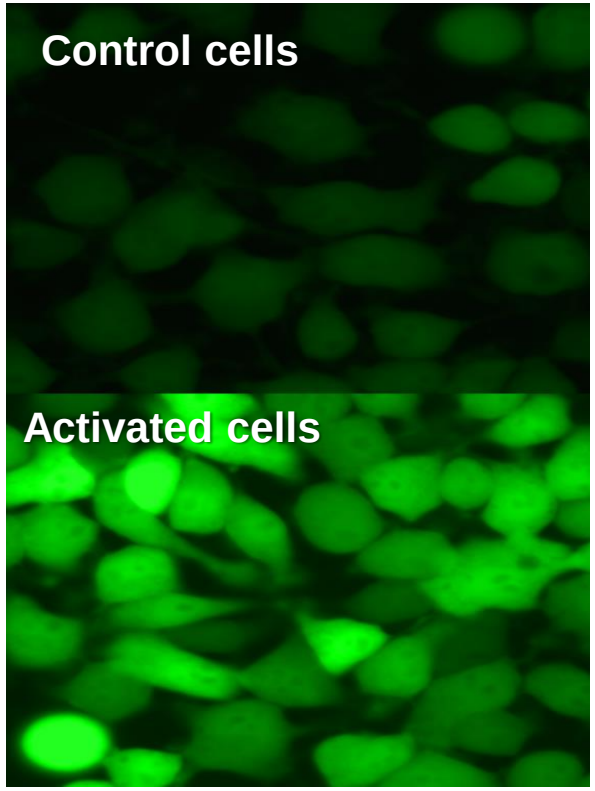

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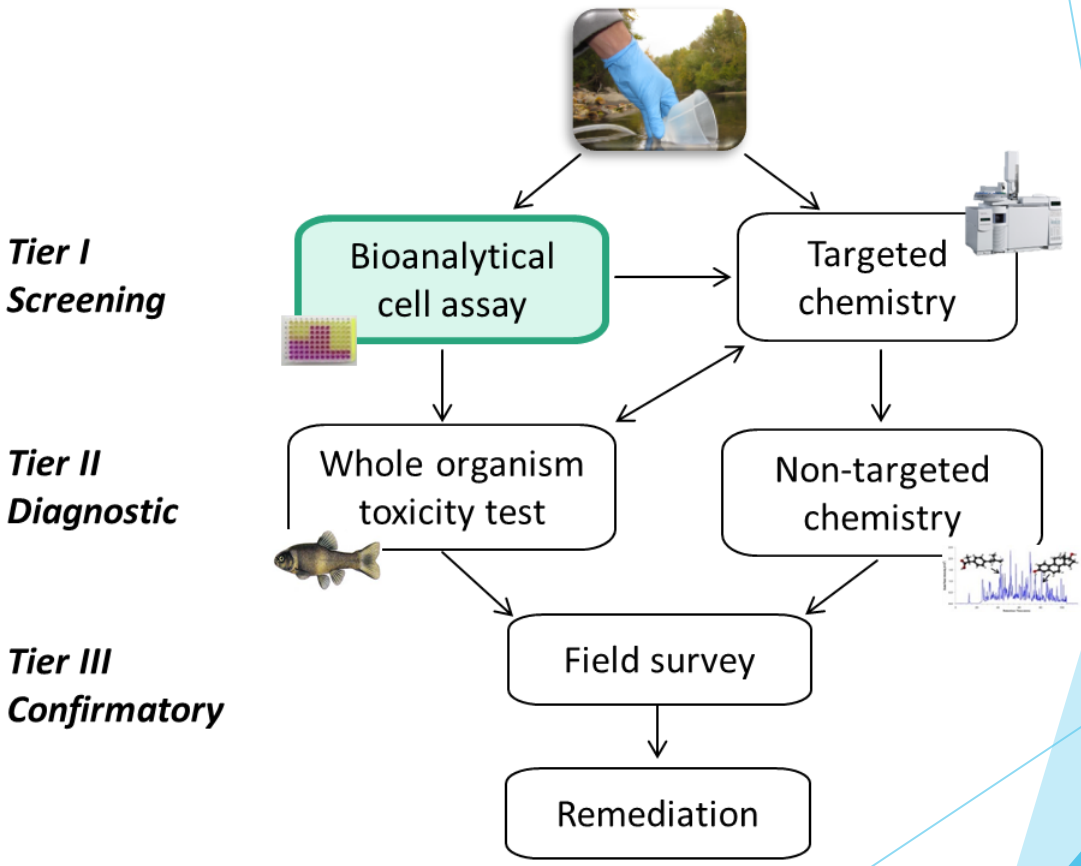
Proposed framework to streamline current practices

Tiered approach developed with the experts to:

- Promote discovery of new/unexpected chemicals
- Detect early signs of toxicity and protect aquatic organisms



Advancing the use of cell assays for ambient monitoring

Stage 1 -  Identify relevant cell assay endpoints

Stage 2 -  Optimize cell assay protocols

Stage 3 -  Conduct pilot studies in different habitats

Stage 4 -  Develop data interpretation framework

Stage 5 -  Develop documentation, training for end-users

Promising cell assay endpoints

Stage 1 - 	- Thyroid receptor (TR)
Stage 2 - 	- Progesterone receptor (PR) for progestins
Stage 3 - 	- Peroxisome proliferated-activated receptor (PPAR) - Androgen receptor (AR)
Stage 4 - 	- Glucocorticoid receptor (GR) for anti-inflammatory steroids
Stage 5 - 	- Estrogen receptor alpha (ERα) for synthetic estrogens, alkylphenols - Aryl hydrocarbon receptor (AhR) for dioxin-like chemicals

Pilot testing of cell assays in CA habitats

Various matrices tested

- Wastewater influents and effluents
- Ambient waters (river, streams, runoff)
- Freshwater and marine sediment

Cell bioassay endpoints

- Estrogen receptor alpha (ERα)
- Glucocorticoid receptor (GR)
- Aryl hydrocarbon receptor (AhR)

Key questions addressed

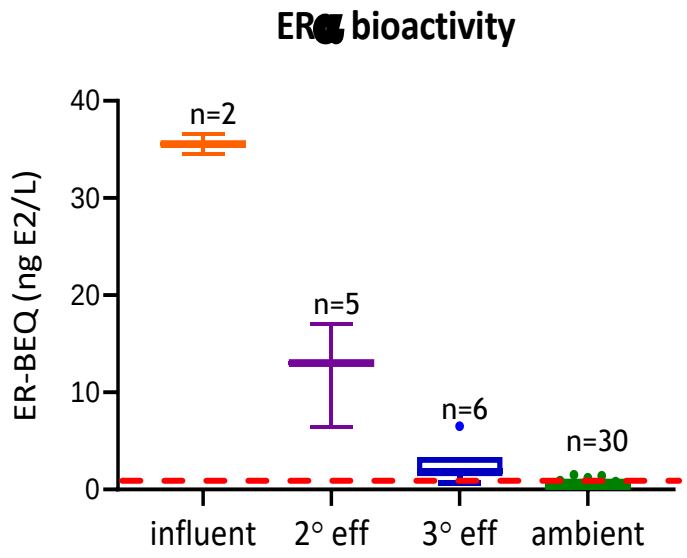
What is the sensitivity of cell bioassays?
Can they discriminate between "clean" and contaminated samples?

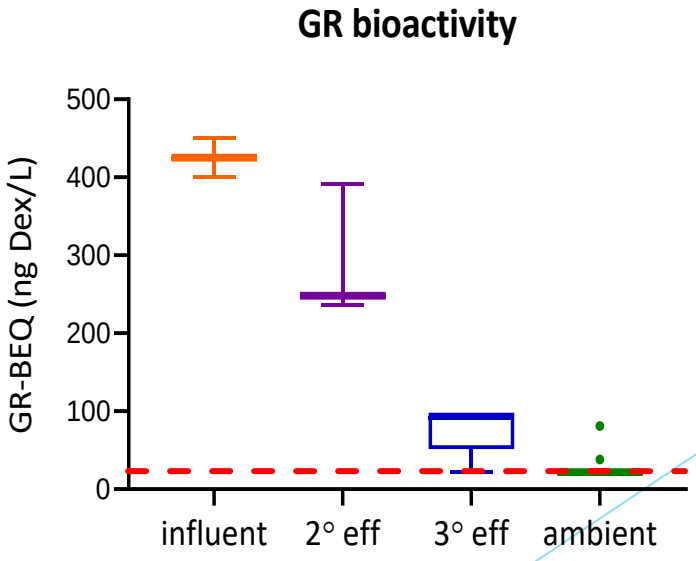
Do bioactivity patterns make sense?
Are cell bioassay data in agreement with other lines of evidence?

Can we explain the responses?

Bioscreening of wastewater and receiving waters

- 8 wastewater treatment plants and 20+ sites in receiving waters
- Patterns consistent with monitoring data
- Majority of receiving waters had little to no bioactivity
- But uncertainties remain regarding levels of concern





Evaluating levels of concern to aquatic life

- Series of lab and field exposures conducted with different species and life stages
- ER-induced toxicity evaluated at various levels of biological organization

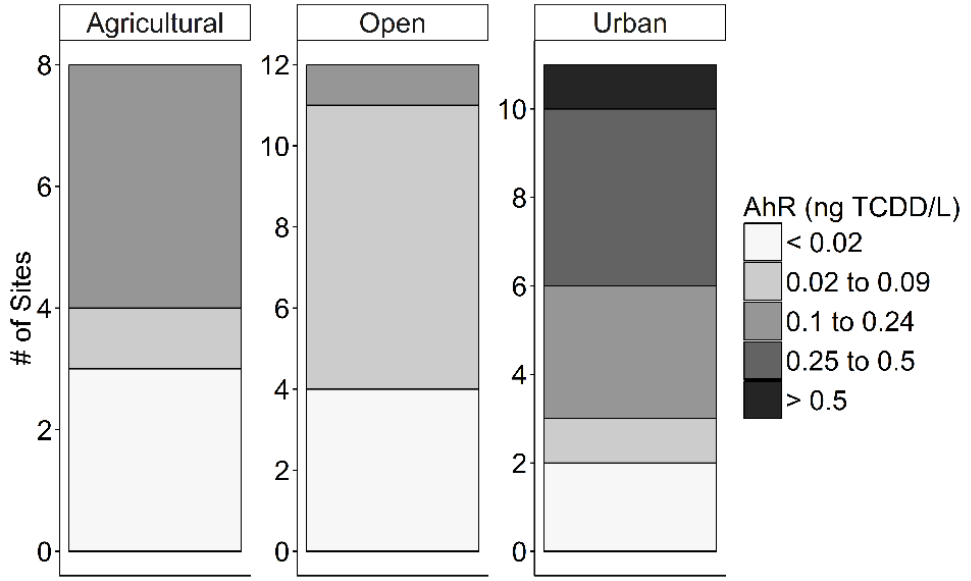
ERa cell bioactivity	Altered genes/ proteins levels	Tissue damage	Organism/ population effects
BEQ <0.5 ng/L	✗	✗	✗
BEQ: 1 – 5 ng/L	✓ vitellogenin, choriogenin	✗	✗
BEQ >10 ng/L	✓✓ vitellogenin, choriogenin	✓ Reduced gonad maturation	✓ Skewed sex ratio

Bioscreening of urban and agricultural streams

- Study conducted as part of the Stormwater Monitoring Coalition (SMC) program
- 31 sites in 5 counties of southern California

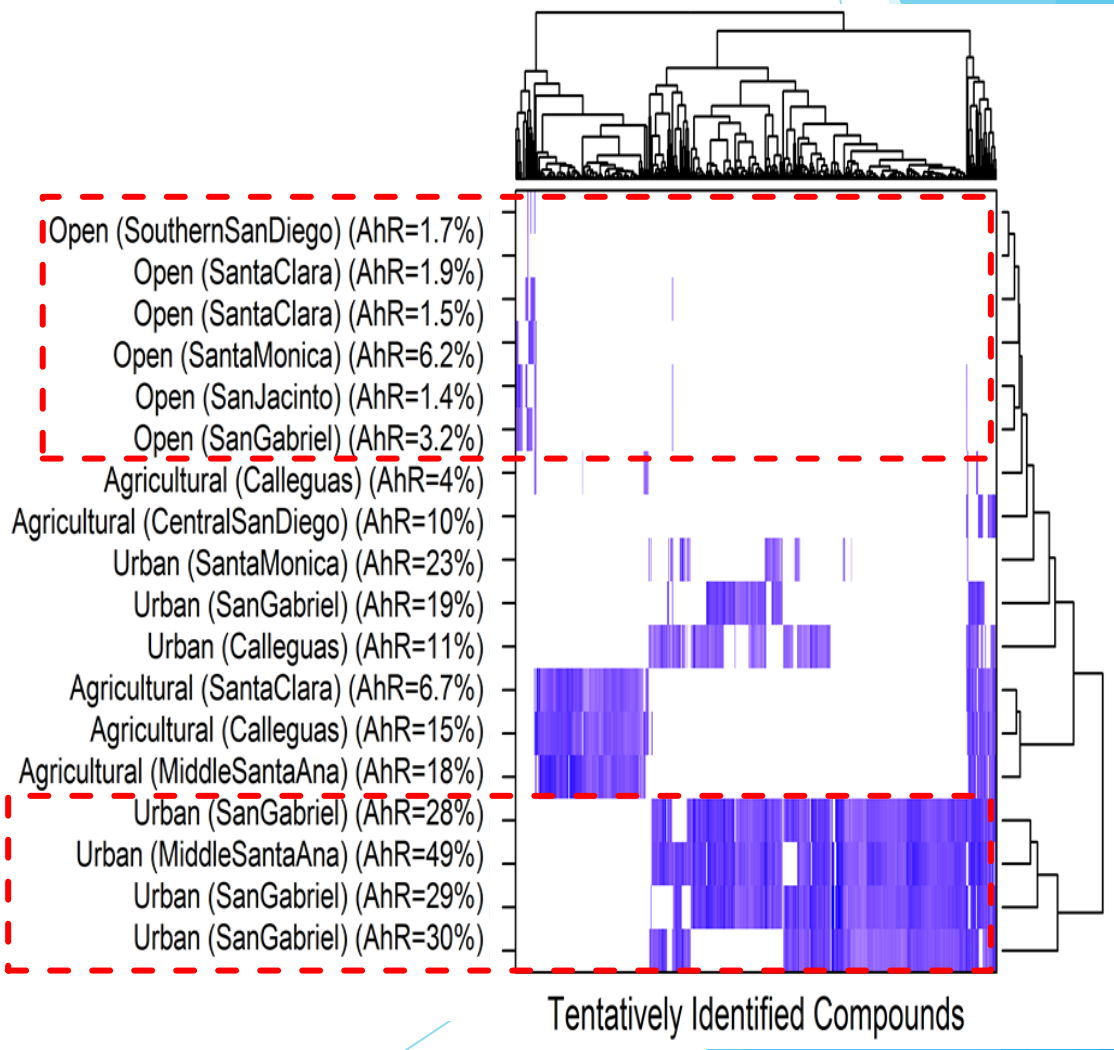
- Very little ER and GR bioactivity

- But widespread AhR responses, with highest levels found in urban runoff



Promising tools to monitor unknown chemicals

- Targeted analyses could not explain the AhR responses
- But non-targeted (GCxGC-TOF/MS) analyses were in agreement with AhR bioscreening data

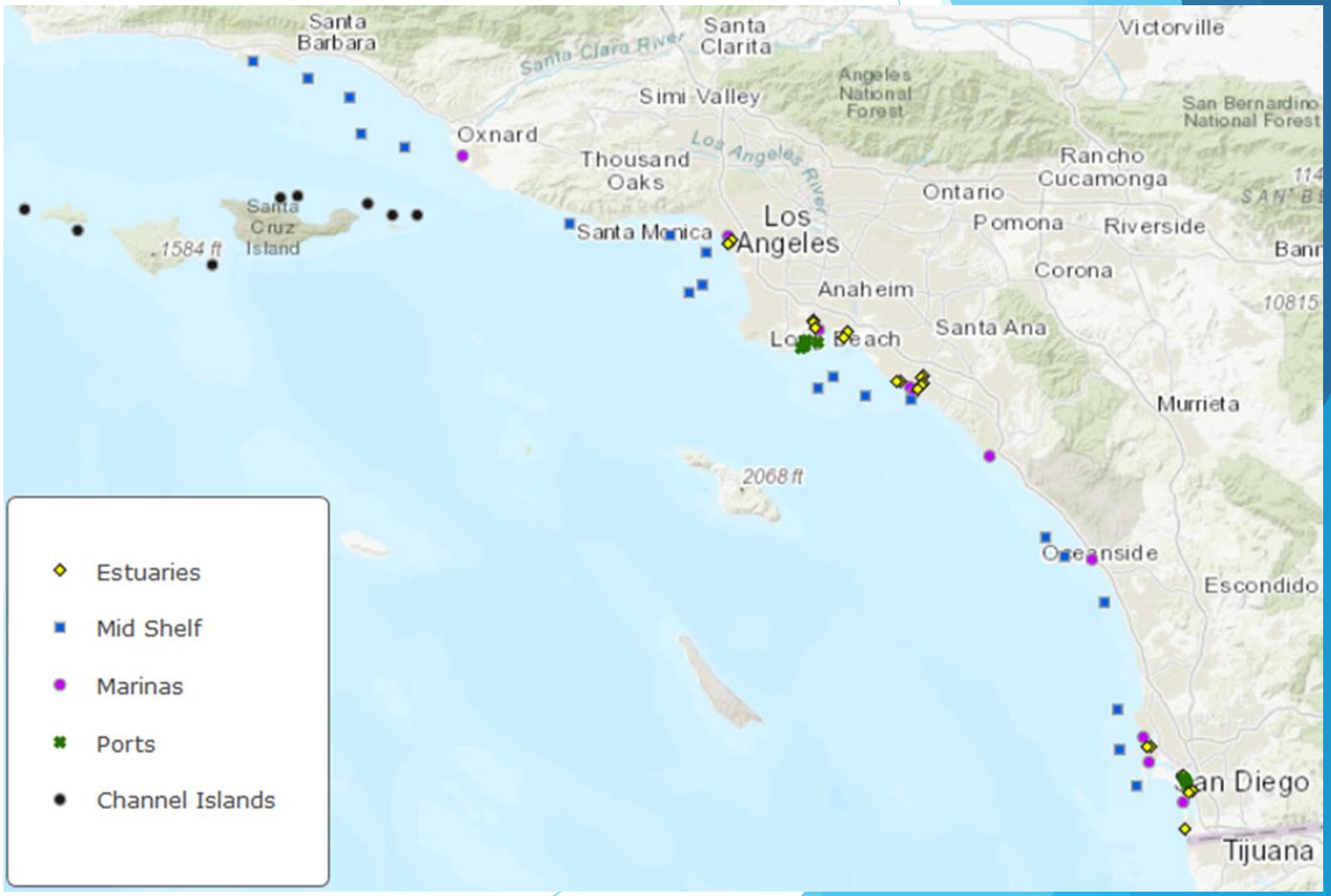


Bioscreening of coastal and marine sediment

Part of Bight 18 regional survey which aims to assess:

- Sediment quality
- Benthic communities
- Fish health and bioaccumulation

Cell assays and NTA incorporated to screen for unregulated/ bioactive chemicals



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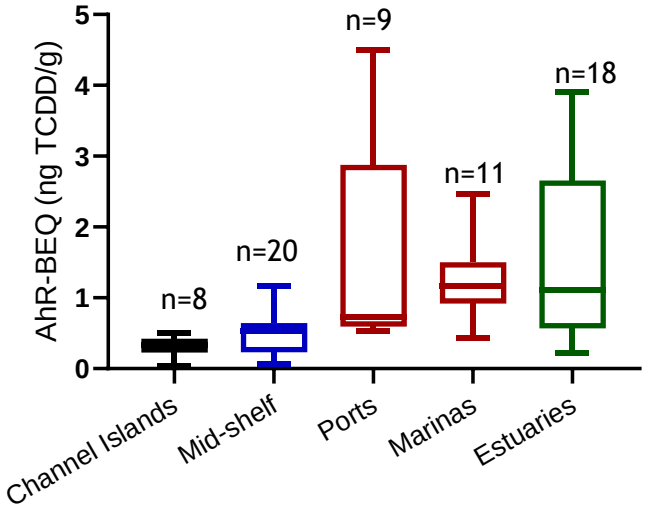
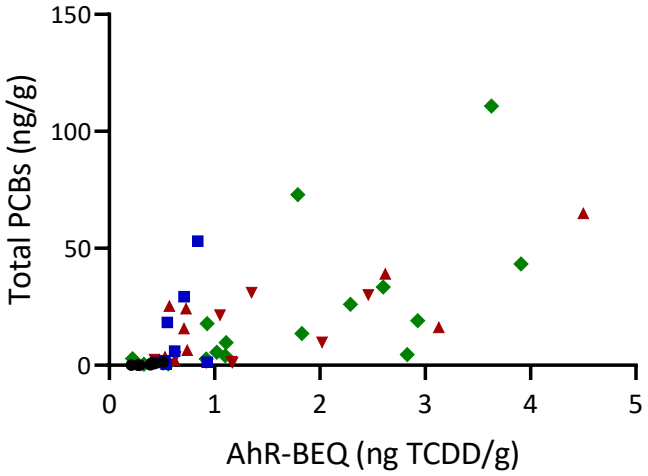
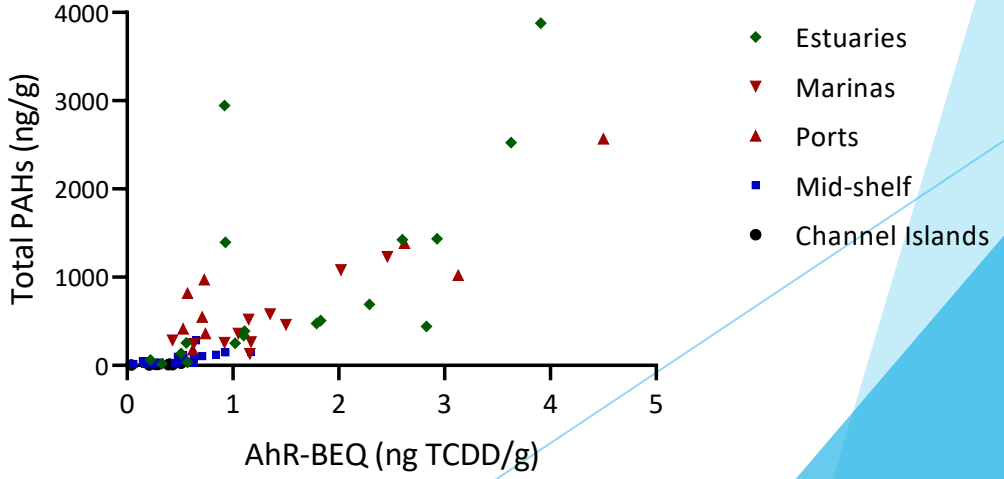


- Consistently low levels in Channel Islands and mid-shelf
- Highest AhR bioactivity observed in estuaries and ports
- Positive correlation between AhR responses and ΣPAHs





Lessons learned

Data are encouraging for the handful of endpoints available

- Protocols can be standardized for reproducible results
- Responses are indicative of water quality

Useful for site prioritization and guide further testing as part of a tiered monitoring approach

Promising to predict toxicity and protect sentinel species

Next steps

Optimize new cell assay endpoints and QA parameters

- New project started with SWB and WRF to develop protocols applicable to various matrices (recycled, fresh and marine)

Develop data interpretation for stage 3 & 4 cell assays (GR, PPAR)

- Identify bioactive contaminants
- Establish relationship between bioactivity and higher order effects
- Develop bioscreening thresholds

Questions?

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