University of California, Davis

Title of Research Project:

High throughput biomonitoring of Aquatic Invertebrates and Fish

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EXECUTIVE SUMMARY

Runoff can introduce influxes of highly toxic, complex chemical mixtures into nearby aquatic habitats, causing adverse biological effects. These mixtures often include chemicals of concern known to have adverse biological effects in single chemical laboratory exposure experiments. Exposure to multiple stressors occurring in the natural environment often results in complex, nonlinear organismal responses which contrast with results obtained from exposure to single stressors under laboratory conditions. This is especially relevant for mixtures that include new and emerging contaminants of concern, where data on their biological effects may be limited to acute exposures on target and model organisms. Acute single chemical exposure assessments are an integral part of the regulatory framework but are limited in predicting organismal responses to environmentally relevant mixtures.

Multiple testing strategies targeting sensitive endpoints (e.g., gene expression, behavior) can offer a more comprehensive picture of aquatic health in impacted ecosystems. This combined approach will help evaluate species-specific responses and tolerance thresholds to exposure. Differential gene expression and behavioral change of model species are widely used for drug screening in pharmacology and toxicology (Bruni et al., 2016; Hong and Zha, 2019; Kalueff et al., 2016; Rihel et al., 2010), but have not been applied to evaluate the sublethal toxicity of impacted waterways in California's Central Coast (Salinas, CA). By examining changes to gene expression for targeted molecular pathways that are known to coincide with insecticides' mechanisms of activity in aquatic organisms, we can compare the relative degree of subcellular stress induced by target chemicals with responses to environmental mixtures. High-throughput Behavioral assays provide information on ecologically relevant impacts and can link subcellular responses with organism-level changes. Changes in swimming behavior after exposure to various environmental pollutants can act as early indicators of stress. Another well-established monitoring approach is conducting periodic assessments of the biodiversity (bioassessments) present in monitoring sites. Bioassessments generally focus on a sensitive taxonomic group, such as aquatic macroinvertebrates, to understand the biological impacts to changes in habitat quality. Sampling is time- and expertise intensive however and requires taxonomic identification skills. By focusing on a narrow group of organisms, many taxonomic groups are unidentified and unmonitored, which represents a significant knowledge gap in many aquatic systems. By identifying patterns of distribution for a broader range of taxa, we can increase our understanding of the ecosystem-scale impacts of poor water quality and identify high priority areas for conservation. Using multiple testing strategies with sensitive endpoints provides a greater understanding of the consequences of pesticide exposure on invertebrate and fish populations.

The primary goal of our study was to evaluate the effects of agricultural surface water (Salinas, CA) on both sensitive invertebrates (*Daphnia magna, Hyalella azteca)* and a model fish species (*Pimephales promelas*). Two chemicals of emerging concern that are commonly detected at these sites are Imidacloprid (IMI) and Chlorantraniliprole (CHL). We sought to compare the toxicity of single and binary exposures to IMI and CHL with the effects of agricultural surface water (which often contain complex mixtures of chemicals including IMI and CHL at low concentrations). To examine ecologically significant sublethal effects, we determined changes to gene expression in fish (determined by qPCR), and behavioral changes in both fish and invertebrates. To understand sublethal ecologically relevant impacts of

exposure, and to link physiological stress to behavior, we conducted behavior assays in both *D. magna* and *P. promelas*. Lastly, we sought to develop/conduct metabarcoding at key sites across the Salinas watershed to understand the distribution of sensitive taxa used for biomonitoring, such as aquatic macroinvertebrates.

We first developed a suite of novel molecular biomarkers and performed 96h exposure studies on fish and sensitive invertebrates to evaluate the biological effects and toxicity of agricultural runoff from California Department of Pesticide Regulation (CDPR) long-term monitoring sites in and around Salinas, CA. Previous exposure assessments have shown that several of these field sites are highly toxic to invertebrates (Anderson et al., 2018, 2006, 2003). We sampled field water before (May and September 2019) and after (November 2019) a first flush rain event. To capture sublethal effects on invertebrates, we diluted the field water with laboratory control water, using a geometric dilution series. We compared these data with results of single and binary chemical exposures on two chemicals of concern known to be present in our ambient field water samples, Imidacloprid (IMI) and Chlorantraniliprole (CHL). Exposure concentrations represented low and high environmentally relevant values (1.0 µg /L, 5.0 µg/L) for single and binary chemical exposures. Next, we conducted behavior assays in both *D. magna* (hereafter abbreviated as Daphnia) and *P. promelas* (hereafter abbreviated as FHM) to link physiological stress to behavior. Lastly, we collected environmental samples across the watershed and major tributaries and sequenced them, targeting a wide range of taxa using a suite of markers. This provided a more comprehensive picture of biodiversity and connectivity across the watershed.

The chemical analyses of ambient field water collected at CDPR long-term monitoring sites showed repeated detections of two chemicals of concern, IMI and CHL, along with other chemicals. In many of the evaluated samples, both pesticides were present at concentrations that would be expected to affect sensitive species. Several other pesticides of concern exceeded benchmark levels and/or LC50s for sensitive species. Of 47 pesticides analyzed during September 2019, a total of 17 were detected in our surface water samples, and each site contained a minimum of 7 target pesticides. Notably, methomyl a carbamate pesticide, was detected at concentrations more than three-fold above the level expected to impact fish and is likely contributing to the toxicity for these samples (Van Scoy et al., 2013). Of 47 pesticides analyzed during November 2019 (collected within 24h of the first seasonal rain event of the year), 27 were detected in our surface water samples, and each site contained a minimum of 21 target pesticides. Exposure studies conducted on a geometric dilution series revealed high levels of invertebrate mortality during all sampling events, even at low concentrations of field water (as low as 12%). Contrastingly, in initial range-finding single and binary exposures to IMI and CHL, invertebrate mortality was only observed at the highest concentrations (10,000 ng/L). Taken together, exposure to agricultural water containing complex mixtures of chemicals resulted in high levels of invertebrate toxicity but did not result in any fish mortality at any concentration.

Overall, our finding suggests that IMI and CHL may impact neuromuscular health in fish. We demonstrated that CHL activates RyR in FHM and several model invertebrates commonly used in aquatic ecotoxicology. This finding is important for understanding how CHL may impact neuromuscular health in fish. Exposure to agricultural surface waters resulted in invertebrate toxicity that exceeded predictions based on single chemical assessments, and elicited

detoxification responses and impacted neuromuscular function pathways in fish. Our gene expression assays confirm that exposing FHM to agricultural surface water results in changes to pathways involved in detoxification, muscular and neuronal function, Ca^{2+} homeostasis and signaling, and general stress response, and binary IMI/CHL exposure altered RyR function. We found differential expression of FHM genes involved in first line detoxification and general stress pathways (CYP1a and CYP3a) in field water exposures but not in IMI/CHL exposures. Upregulation occurred in field sites that demonstrated high invertebrate mortality. A gene involved in neuronal function (ASPA) was differentially expressed for both field and CHL exposures. To our knowledge, no literature exists on the mechanism by which CHL may affect expression of Aspartoacylase (ASPA). A gene involved in muscular function (SERCA1) was strongly downregulated for field exposures in September 2019. Sarco(endo)plasmic reticulum calcium-ATPase 1 (SERCA1) encodes an ATPase enzyme that regulates calcium channel function. A gene related to cellular Ca^{2+} homeostasis and signaling (RyR2) was altered for both field and IMI/CHL exposures, but not for CHL single exposures. As the Ryanodine receptor (RyR) is the known target of CHL it was surprising that we did not see changes in gene expression when fish were exposed to CHL alone. However, we did find increased RyR2 in the IMI and CHL binary mixtures. The development of molecular biomarkers for use as monitoring and diagnostic tools depend on a fundamental characterization and understanding of the mechanisms underlying a molecular response. Genes with altered expression profiles can be used as biomarkers for environmentally relevant chemical exposures. Specifically, this approach allowed comparisons of the relative degree of subcellular stress induced by CHL and IMI and will help identify species-specific responses and tolerance thresholds to CHL and IMI exposure. In the absence of sublethal endpoints, our findings would have excluded important effects on fish.

Fish and invertebrate swimming behavior were affected by acute (96h) exposure to field water. Fish were hyperactive after exposure to field water, and also showed changes in their ability to respond to light stimuli (photomotor response). Behavior was also a sensitive endpoint for Daphnia at higher concentrations of field water. Photomotor response was shown to be the most sensitive endpoint, following a clear dose-response pattern for the geometric dilution series of field water. Exposure to CHL and IMI resulted in changes to swimming behavior of Daphnia at concentrations lower than their LC50s. Changes in behavior could increase visibility to predators and/or reduce the ability to capture prey, thus altering ecological fitness. This study is the first to address behavior as a sensitive bioassay in urban and agriculturally developed waterways in California's Central Coast (Salinas, CA).

We found that metabarcoding successfully detected a wide range of taxa from environmental DNA samples, including sensitive macroinvertebrates (EPT taxa). We detected EPT taxa from morphology and eDNA, with significant overlap (> 76.67%) between methods. Some taxa identified morphologically were missing from the reference sequence database, highlighting the importance of taxonomic database development. Sequencing detected more benthic macroinvertebrates than morphology when compared at the genus and species levels of taxonomic resolution. For EPT taxa, richness was positively correlated with habitat quality score (California Stream Condition Index; CSCI). Overall taxonomic richness was also positively correlated with CSCI. We found high beta diversity between sites with differing CSCI scores for sensitive taxa, and alpha diversity was positively correlated with habitat quality. Sites in closest proximity to, or hydrologically influenced by, impacted sites (CSCI < 0.63) contained the most

divergent community composition as compared to least impacted reference sites (CSCI > 0.92). Hydrologic distance (waterbody) and CSCI score both accounted for dissimilarity in taxa between sites. Our analyses revealed greater dissimilarity among sites when evaluated at the species level than at the family level. Metabarcoding of eDNA is useful for detecting species of concern and invasive species. As biodiversity continues to decline globally, we must identify hotspots of biodiversity and safeguard them through monitoring and conservation efforts. Biotic indices obtained from eDNA metabarcoding data can be effectively used for watershed-wide, multitrophic assessments of freshwater biodiversity.

Taken together, these findings suggest that ambient field water is more toxic to sensitive species than predicted by two frequently detected chemical components, IMI and CHL. Synergistic effects of multiple stressors is well-documented in previous studies and serves to illustrate the potential hazard of formulating inaccurate conclusions from results of studies of single stressors (Todgham and Stillman, 2013). It is possible that additional chemicals not included in the Pyrethroid screen or LC-screen (e.g., metals) could be contributing to the observed toxicity and sublethal effects on gene expression. CDPR targets prioritized pesticide monitoring candidates based on the current pesticide use report at watershed level, however, and has accumulated over ten years of chemical data for these sites. The high invertebrate mortality levels observed in dilutions of ambient field water as low as 12% suggest that while downstream sites may benefit from lower chemical concentrations due to dilution and other physical processes, ecologically relevant effects may be affecting sensitive species in downstream habitats. Impairment of macroinvertebrate community structure downstream of sampling sites seen in previous studies suggests that multitrophic assessments are crucial to understanding the ecological impacts of runoff on a larger geographical scale (Anderson et al, 2006).

Exposure to agricultural surface waters resulted in invertebrate toxicity that exceeded predictions based on single chemical assessments, elicited detoxification responses and impacted neuromuscular function pathways in fish, and altered swimming behavior in both fish and invertebrates. In the absence of sublethal endpoints, our findings would have excluded important effects on aquatic organisms. By conducting exposures using a dilution series and examining sensitive sublethal endpoints, we obtained a more comprehensive understanding of the effects of agricultural surface water on aquatic life. These findings suggest that while routine chemical screening and acute single chemical toxicity tests are useful tools to understand the short-term impacts of complex mixtures, they may not reflect toxicity or sublethal impacts in natural environments. Future studies should employ multiple testing strategies to improve our ability to predict biological responses to a complex environment.

Graphical Abstract

Central Coast Region, CA

Field Water Dilution series

Chemicals of concern (IMI/CHL)

Environmental DNA Metabarcoding

Mortality ⬆
Differential gene expression ⬆
Swimming behavior ⬆
Response to light stimuli ⬇

Mortality ⬆
Swimming behavior ⬆
Response to light stimuli ⬇

Method Validation
Alpha/Beta diversity
Detection of sensitive taxa
Detection of rare taxa
Detection of invasive taxa