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POLICY AND PROGRAM ANALYSIS

Review of and Recommendations for Monitoring Contaminants and their Effects in the San Francisco Bay–Delta

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ABSTRACT

Legacy and current-use contaminants enter into and accumulate throughout the San Francisco Bay–Delta (Bay–Delta), and are present at concentrations with known effects on species important to this diverse watershed. There remains major uncertainty and a lack of focused research able to address and provide understanding of effects across multiple biological scales, despite previous and ongoing emphasis on the need for it. These needs are challenging specifically because of the established regulatory programs that often monitor on a chemical-by-chemical basis, or in which decisions are grounded in lethality-based endpoints. To best address issues of contaminants in the Bay–Delta, monitoring efforts should consider effects of environmentally relevant mixtures and sub-lethal impacts that can affect ecosystem health. These efforts need to consider the complex environment in the Bay–Delta, including variable abiotic (e.g., temperature, salinity) and biotic (e.g., pathogens) factors. This calls for controlled and focused research, and the development of a multi-disciplinary contaminant monitoring and assessment program that provides information across biological scales. Information gained in this manner will contribute toward evaluating parameters that could alleviate ecologically detrimental outcomes. This review is a result of a Special Symposium convened at the University

of California–Davis (UCD) on January 31, 2017[1] to address critical information needed on how contaminants affect the Bay–Delta. The UCD Symposium focused on new tools and approaches for assessing multiple stressor effects to freshwater and estuarine systems. Our approach is similar to the recently proposed framework laid out by the U.S. Environmental Protection Agency (USEPA) that uses weight of evidence to scale toxicological responses to chemical contaminants in a laboratory, and to guide the conservation of priority species and habitats. As such, we also aimed to recommend multiple endpoints that could be used to promote a multi-disciplinary understanding of contaminant risks in Bay–Delta while supporting management needs.

INTRODUCTION

A recent decadal synthesis of contaminant research in the San Francisco Bay–Delta (Bay–Delta; Fong et al. 2016) concluded that contaminants are present at concentrations that can cause both acute lethality as well as sub-lethal toxicity to aquatic organisms. Chemical pollution is therefore likely to play a significant role in the currently degraded state of the Bay–Delta ecosystem (Healey et al. 2016). As an indication of risk to delicate food webs, acute toxicity has been documented in both tributaries and mainstem rivers of the Delta (Deanovic et al. 2014; Hasenbein et al. 2014; Weston et al. 2014; Weston and Lydy 2014; Weston et al. 2015a; Deanovic et al. 2018; Weston et al. 2018). Moreover, environmental surveillance efforts have measured a diversity of contaminants in Bay–Delta habitats at concentrations known to negatively affect the health of fish and invertebrates (Fong et al. 2016; Healey et al. 2016; Jabusch et al. 2018). Collectively, this has raised concerns that poor water quality conditions are limiting the recovery of Delta Smelt and other high-priority species for conservation (Hobbs et al. 2017). Previous reviews have repeatedly emphasized that contaminants have been an area of critical uncertainty that needed focused research (CALFED 2000; SBDS 2008; Johnson et al. 2010; Brooks et al. 2012; Mount et al. 2012; Scholz et al. 2012; NRC 2013; IEP MAST 2015; Luoma et al. 2015). Nevertheless, despite previous needs assessments, much remains unknown about the presence and fate of contaminants in the Bay–Delta, as well as their effects across broad scales of biological organization (Fong et al. 2016; Healey et al. 2016). This review:

1. highlights current challenges that limit our understanding of contaminant effects in the Bay–Delta,
2. outlines key developments in toxicological tools and approaches for effect-based analyses as discussed in the Special Symposium, convened at the University of California–Davis (UCD) on January 31, 2017,
3. details advances in the field of analytical chemistry, and
4. provides recommendations for incorporation into contaminant monitoring efforts.

CHALLENGES IN REGULATORY FRAMEWORKS

Regulatory toxicology uses data from standard endpoints to determine risk associated with a given chemical exposure. Standardized acute toxicity tests, such as those used for National Pollutant Discharge Elimination System (NPDES) permitting programs, for example, incorporate an initial battery of screening tests that use organisms across three trophic levels (i.e., they incorporate algae in addition to fish and invertebrates; USEPA 1994, 2002). If toxicity is detected, then more in-depth tests are conducted toward characterizing a sample's toxicity. These data are traditionally derived from acute toxicity assays for mortality (various model species), growth, or reproduction (fish and invertebrates), and inhibitory concentration (IC50) for growth (vascular plants and algae) (Code of Federal Regulations – 40CFR Part 158: Subpart G 158.630 and 158.660). Most notably, median lethal or effect concentrations (LC50s, EC50s) have been the standard toxicological metrics for decades, and they are usually based on continuous exposures that last 24, 48, or 96 hours. Toxicants in the environment, however, more

1 *https://marinescience.ucdavis.edu/engagement/past-events*

commonly occur at sub-lethal concentrations; i.e., at concentrations that don't kill organisms outright, but may present intermittent or chronic exposures. While traditional laboratory-derived LC50s and EC50s are good tools to assess comparative toxicity of different contaminants or toxicity between different species, they may have little to no ecological relevance to conditions in nature—beyond providing a conservative threshold when used for risk assessment purposes. Ecological risk assessments for the purpose of regulatory decision-making typically focus on individual chemicals, with few water quality objectives (described below) that include interactive effects between chemicals in mixtures (i.e., synergism, additivity, antagonism; Figure 1) or the possible influence of other environmental stressors (e.g., temperature, salinity, hypoxia).

Interactions between contaminants and other components of aquatic systems are often highly complex, necessitating a multi-disciplinary monitoring and assessment approach (e.g., Macneale et al. 2010; Nilsen et al. 2019). Moreover, while field methods to assess biological integrity continue to evolve (e.g., in estuarine and coastal systems; Borja et al. 2008), it remains challenging to attribute observed declines in the abundance and diversity of taxa to specific stressor categories. Stressors include habitat attributes that may be chemical (e.g., toxicants), physical (e.g., temperature), or biological (e.g., pathogens) in nature, or multiple combinations of these. This points to the importance of controlled studies to delineate causal drivers for species losses, as well as technological advances that are making it increasingly possible to identify cause-and-effect relationships for common stressors and stressor combinations. Interactions between contaminants and multiple other biotic and abiotic stressors are, more often than not, likely to result in habitat compression (Gustafson et al. 2015; Hasenbein et al. 2018); i.e., an increased loss of suitable habitat as a result of a species' inability to contend with further environmental change. Furthermore, in the light of global climate change, the use and dispersion of pesticides is predicted to increase, to combat the forecasted increase in the distribution of insect pests and weeds (McDonald et al. 2009; Kattwinkel et al. 2011). This will further affect organisms, populations, and ecosystems, as well as complicate contaminant concentration or effect monitoring—and suggests that current regulatory practices greatly underestimate ecosystem effects.

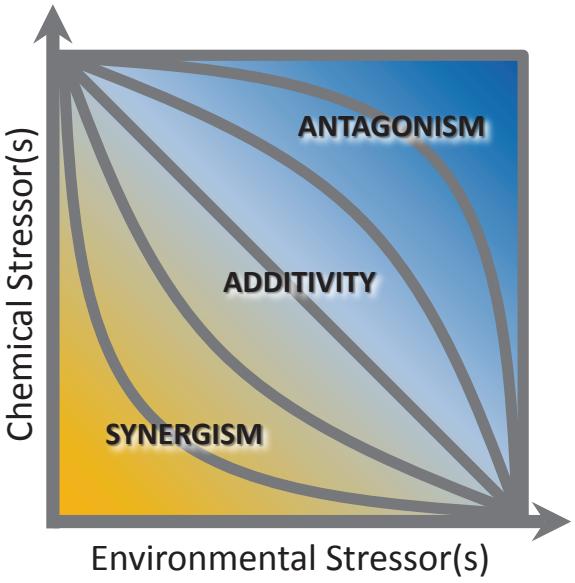

Figure 1 Isobologram depicting potential synergistic, additive and antagonistic effects resulting from the interaction of contaminants (e.g., pesticides) and environmental stressors (e.g., temperature). Highest values on the X and Y axes correspond to equivalent responses (e.g., equal percentage mortality) resulting from exposure to each respective stressors. These points are connected by *lines* that represent equal effect; *isoboles*, depicting their interaction.

Contaminant monitoring efforts conventionally involve laboratory analyses of field-collected water, sediment, or tissue samples. Historically, this approach has worked particularly well to identify environmentally persistent and bioaccumulative chemicals such as polychlorinated biphenyls (PCBs; e.g., Greenfield and Allen 2013), dichlorodiphenyltrichloroethane (DDT) and other chlorinated pesticides (e.g., Connor et al. 2007), and polybrominated diphenyl ethers (PBDEs; e.g., Sutton et al. 2015). Mercury similarly persists in Bay–Delta food webs and remains at high concentrations in several economically important sport fish, creating an enduring concern for the health of humans and piscivorous wildlife (see Davis et al. 2012a; Davis et al. 2012b). Focused chemical monitoring efforts

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in the Bay–Delta have successfully detected mercury, PCBs, and dioxins, which have been classified as contaminants of high concern (SFEI 2013; SFEI 2015). Elevated concentrations of selenium—sourced from agricultural drainage and effluents from oil refineries, which accumulate through the food web—are known to affect reproduction, growth, and development in fishes (Stewart et al. 2004; Linares–Casenave et al. 2015). Outside of this so-called "legacy pollution," urbanization and increasing human populations have led to increases in water pollution caused by chemicals of emerging concern (CECs): chemicals that are increasingly being detected in surface waters at low concentrations, and for which there is concern about their detrimental effects on aquatic life: e.g., pharmaceuticals and personal care products. Heavy metals continue to pose a threat to aquatic life, and are detected at concentrations that affect the functioning of fishes' nervous systems, affecting behavior and olfaction, and risk of predation (Brooks et al. 2012; Grossman 2016); responses to exposure are known to be altered by exposure temperature, as well as prior thermal acclimation history of aquatic species (Hallman and Brooks, 2015). The introduction of novel stressors such as microplastics and synthetic fibers act as new vectors for many pollutants, and present their own direct physical and chemical effects once ingested (Sutton et al. 2016; Maruya et al. 2018). With an ever-changing and growing list of threats to aquatic systems, monitoring strategies must also change.

New contaminants appear on the market faster than toxicologists are able to evaluate their ecological effects. Researchers continuously play catch up on a seemingly never-ending treadmill; referred to here as a "*pesticycle*." We use the term pesticycle to describe the dynamic nature of required pesticide assessments; as pesticides change, their toxicity needs to be evaluated—and technological approaches to evaluate them need to be developed as well. (These are sometimes multi-decadal cycles). Evaluations attempt to determine if—and if so, *which* replacement products, or pesticide active ingredients—are likely to have the greatest ecological effect, since restrictions are imposed on those that have been established to cause environmental harm. Pesticides, after registration, are required by California law to be continuously evaluated for actual or potential significant adverse effects to the environment, as more toxicological and environmental data become available (CDPR 2017). A primary emphasis put forward by the European Union—such as the Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH)[2] and the Water Framework Directive (WFD)[3]—is that applied ecotoxicology needs to become more proactive, rather than reactive testing being conducted, i.e., following a precautionary principle (Miller 2019). Post-registration monitoring is required to establish environmental relevance and protect species of conservation concern (Vijver et al. 2017). Monitoring (collecting field data), however, is by definition a reactive (or retrospective) approach, as contaminants must already be in the system to be detectable (Vijver 2019). A proactive (or prospective) approach that involves extensive lab testing of a chemical, with significant ecological relevance, is needed before it is used and introduced into the environment. This, however, is a seemingly impossible task, because of the number of synthetic chemicals that enter the market each year.

EFFECT-BASED TOOLS FOR USE IN MONITORING FRAMEWORKS

Agencies are beginning to recognize the limitations of some regulatory frameworks, especially in the light of the challenges highlighted above. For example, the Water Quality Control Plan (Basin Plan) for the Central Valley Water Quality Control Board[4], for example, attempts to address issues regarding the presence of multiple contaminants, stating that evaluations should determine "additive or synergistic effects of multiple pollutants." The Basin Plan provides guidance on how to determine additivity of limited chemical concentrations, but little

2 *http://ec.europa.eu/environment/chemicals/reach/reach_en.htm*

3 *http://ec.europa.eu/environment/water/water-framework/index_en.html*

4 *https://www.waterboards.ca.gov/centralvalley/water_issues/basin_plans/sacsjr_201805.pdf*

guidance on interpreting effects analyses that include dozens to hundreds of chemicals acting on an organism. During the 50 years since René Truhaut coined the word "ecotoxicology" (1969, detailed in Truhaut 1977), a multitude of scientific advances have worked toward understanding contaminant effects beyond mortality. Advances include high throughput, non-targeted analytic techniques and approaches to determine effect-based toxicity. Here, effect-based evaluations lend themselves to weight of evidence and validation of the correlations between exposure and effect, better than a systematic monitoring of the effect on a contaminant-by-contaminant basis. Evaluation and management of contaminants are hoped to integrate the latest state-of-the-art approaches toward establishing monitoring programs to evaluate complex contaminant mixtures, and potential synergistic, additive — or antagonistic — effects on populations, communities, and ecosystems as a whole. Establishing comprehensive monitoring systems, combined with hypotheses-driven research, are needed to determine the sources and magnitude of anthropogenic effects on aquatic biota, which will be critical in informing management decisions and conservation efforts. Below, we highlight several advancements and important studies that could lend themselves to building a more comprehensive plan.

High Throughput Screening for Chemical Prioritization

New and emerging contaminants of concern are continuously driving new monitoring efforts worldwide (Brack et al. 2017). Regulatory processes have historically addressed the acute toxicity of contaminants as determined using low-throughput approaches, at high costs; not population-level effects. Millions of dollars are spent on evaluations per single chemical, and it takes years for the needed regulatory information to be gathered, resulting in only a small fraction of new compounds ever being tested for their toxicity (Krewski et al. 2010). With few exceptions, these regulations do not consider knowledge of action mechanisms, which modern effect-based techniques are able to provide. This calls for utilizing existing state-of-the-art technology, and promoting further development, advancing the way in which toxicological assessments are being conducted. This, in fact, has been the aim of the Toxicity Testing in the 21st Century (Tox21) strategy established in 2007 (Krewski et al. 2010). Tox21 is a US federal collaboration among Environmental Protection Agency (EPA), National Institutes of Health (NIH), the National Institute of Environmental Health Sciences (NIEHS), including National Center for Advancing Translational Sciences (NCATS) and the National Toxicology Program (NTP), as well as the Food and Drug Administration (FDA). Tox21 efforts share their mission with that of European Union partners, such as EU-ToxRisk, an integrated program that drives mechanism-based toxicity testing and risk assessment for the 21st century (Daneshian et al. 2016). The concept is to move away from single toxicity tests toward high-throughput screening, which allow for the rapid execution of multiple toxicity assessments that incorporate *in vitro* and/or *in vivo* screening (e.g., using cell lines and/or fish embryo tests), with an understanding of toxicity pathways. High-throughput screening also incorporates computational toxicology to either prioritize screening or predict adverse outcomes that connect effects at various biological scales[5,6]. At a minimum, the priority should be to establish a hierarchy of testing that first uses high-throughput screening, to then prioritize fewer chemicals which may need additional scrutiny (e.g., life cycle testing) and assessment across biological scales (e.g., Brander et al. 2015). Then, data obtained from such testing can be used to discern what environmental conditions may alleviate observed detrimental outcomes. The Tox21 committee states that prioritization should be based on risk assessment needs, i.e. testing should be based on chemical use and likelihood of exposure and geared toward science-policy decisions. They further indicate that while thorough testing on all chemicals is impractical, emerging tools and approaches hold great promise for rapid screening of a multitude of chemicals. As to strategies, Tox21 highlights that these will need to be evaluated relative to the value of

5 *https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21*

6 *http://www.eu-toxrisk.eu/page/en/about-eu-toxrisk.php*

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knowledge provided, in terms of depth, breadth, animal welfare, and conservation (Krewski et al. 2010). It is important to show linkages between the *in vitro* assays and *in vivo* endpoints to be able to use these technologies to assess risk.

More thorough, proactive testing could include an evaluation of the proposed products' uses to determine what other chemicals would likely be found concurrently and at what concentrations. This should not be limited to pesticides and their so-called inert ingredients (i.e., active ingredient and formulations), and if possible, should include toxicity evaluations at ecologically relevant temperatures. Appropriate, sensitive species should be determined based on the chemical's proposed application method (e.g., spray vs. granule), mode of action, and types of species that would be potentially exposed. This information would be combined to devise a suite of laboratory tests to be performed before the chemical is used—and therefore before it is introduced into the environment. These tests should include evaluation of sub-lethal effects on biological indicator performance beyond growth and reproduction (e.g., behavior) (Nilsen et al. 2019). Contaminants need to be evaluated as a syndrome; as symptoms that occur together, rather than isolated from biotic and abiotic interactions, so as not to underestimate their ecological effect (Vijver 2019).

Adverse Outcome Pathways and Systems Biology

Mechanistic analyses can be evaluated and confirmed through integration of endpoints measured across levels of biological organization, from cellular-level to whole organism-level effects, with implications for population-level effects (Figure 2). This integration can be achieved through the adverse outcome pathway (AOP) framework (Figure 3), defined as

> *"a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event (e.g., a molecular interaction between a xenobiotic [a chemical that is foreign to the body] and a specific biomolecule) and an adverse outcome at a biological level of organization relevant to risk assessment" (Ankley et al. 2010).*

This framework establishes a basis upon which to test multiple hypotheses that offer greater environmental relevance. This construct can use data from a multitude of studies to evaluate outcomes reported at different levels of biological organization. This, in turn, serves to determine specific endpoints for the evaluation of contaminants, where the effect upon a species of concern may need to be further confirmed. By adopting a systems biology approach, information gained in this manner can be expanded to evaluate the effect upon an ecosystem as a whole. Systems biology is a multi-disciplinary approach that integrates biology, bioinformatics, chemistry, and toxicology, among other fields, providing tools to integrally infer, link, and quantify how systems (populations, communities, and ecosystems) change in response to environmental alterations (Garcia–Reyero and Murphy 2018). In this approach, interacting networks that respond to perturbations are assessed to discover, understand, and predict the emerging properties of the system (Weston and Hood 2004; Garcia–Reyero and Perkins 2011), through examining the dynamics of cellular to organismal functions, rather than their individual components (Kitano 2001; Kitano 2002; Garcia–Reyero and Murphy 2018). Effects on reproductive systems determined at the cellular level (e.g., induction or inhibition of vitellogenin, testosterone, and/or estrogen), can, for example, be mathematically modeled as surrogates of fecundity so effects at higher levels of biological organization (e.g., reproductive output) and population consequences can be inferred (Perkins et al. 2011; White et al. 2017). In fact, endocrine disruption-related AOPs are some of the best understood and validated evaluation processes (e.g., Miller et al. 2007). It has been proposed that AOPs be incorporated into the USEPA's Endocrine Disruptor Screening Program (EDSP), which evaluates potential contaminant action via estrogenic, androgenic, and thyroid hormone pathways (Browne et al. 2017). This program is based on a tiered approach by which *in vitro* hormonal tests provide an initial screening; *in vivo* assays then confirm hormonal disruption and subsequent effects on development

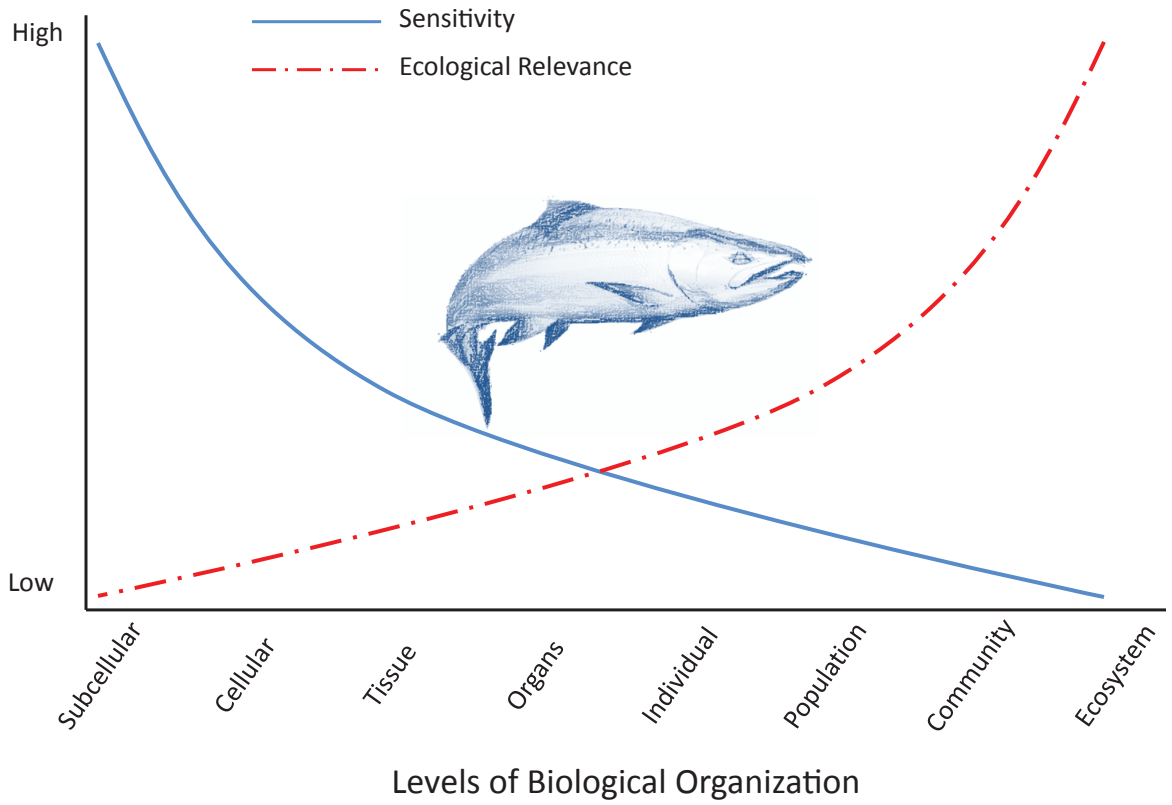

Figure 2 The sensitivity and ecological relevance of toxicological assessments vary depending on the level of biological organization at which tests are conducted. Evaluation of links across multiple levels of biological organization provide for a comprehensive effect-based assessment of perturbations within an ecosystem.

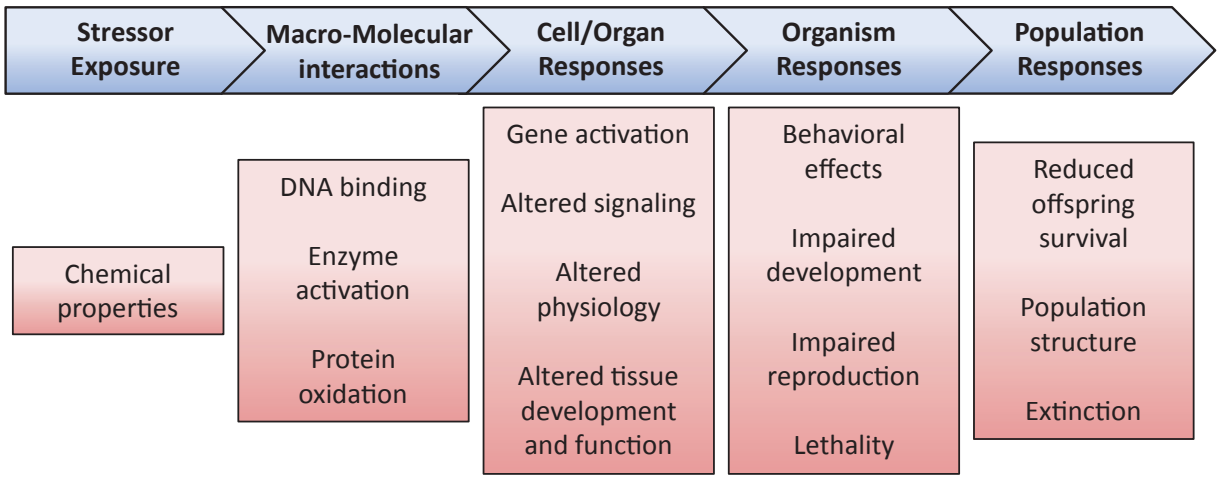

Figure 3 Adverse Outcome Pathways (AOP): Exemplar schematic representation of causally linked events at multiple levels of biological organization (adapted from Ankley et al. 2010)

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and reproduction. Thus, AOPs directly complement and integrate a systems biology approach, and by understanding differential sensitivities of species within a particular habitat, detrimental effects on community and ecosystem properties can be evaluated.

Progress in Effect-Based Analyses

Numerous studies have used these endpoints to evaluate how contaminants affect individuals and populations (Hellou, 2011; Melvin and Wilson 2013; Hasenbein et al. 2015; Johnson and Sumpter 2016), highlighting direct relevance, for example, between impaired behavior and predation risks (e.g., fish with an impaired nervous system are as good as dead; Bertucci et al. 2018) or contaminant effects on development and resulting sex ratios (e.g., how many males vs. females does a population need; White et al. 2017). Beside mortality (acute toxicity), morphology (development), behavior, and reproduction are easily understood effect-based endpoints. In fact, developmental deformities and impaired behavior have been highlighted as important population–relevant endpoints in determining adverse effects that follow exposure to endocrine-mimicking compounds (Marty et al. 2017). Advances in image analysis software allow for detailed morphological and behavioral evaluations (as reviewed in: Teixidó et al. 2018; Xia et al. 2018). What is less understood is how different contaminant classes affect development, behavior, and reproduction (i.e., by which mechanisms do contaminants alter organismal health). To support not only chemical regulations (e.g., pesticide-use regulations) but also ecosystem-management efforts (Kiani et al. 2016; Gouveia et al. 2019), the mechanisms that underlie organismal sensitivity (or tolerance) to contaminants must be understood. In fact, one of the core missions of the field of ecotoxicology is to understand the mechanisms by which contaminants perturb normal organismal performance (as reviewed in Connon et al. 2012). To help drive this mission, future research should focus on the development of rapid throughput assays for a wide list of molecular targets, to define mechanisms and build predictive models that describe potential organismal or population-level outcomes. Using multi-endpoint, ideally high-throughput, effect-based assays — that also incorporate the need for a mechanistic evaluation of how contaminants perturb normal performance — can provide the weight of evidence needed to more adequately characterize risk.

'Omic Techniques

'Omic approaches allow us to better understand what sensitivity differences mean, and the mechanisms by which contaminants may contribute to population declines over single or multiple generations (Connon et al. 2012; Connon et al. 2018). The use and application of 'Omics as high-throughput screening approaches have rapidly increased in recent years (reviewed in Martyniuk and Simmons 2016). They are broad spectrum, interrogative, and non-biased ways to detect tolerance to — or changes in — responses of exposed individuals at the level of the genes (genomics and epigenomics), messenger RNA transcripts (Transcriptomics), proteins (proteomics), and metabolites (metabolomics) (Figure 4). Together, these describe the phenotype of the organism, resulting from interactions with the environment in which they live. Thus, alterations in phenotype can be evaluated at the 'Omic level, following carefully designed exposure studies. Transcriptome-wide approaches can now be applied to species where no prior genomic sequence information is available, allowing for direct, comparative studies to be performed across multiple species of conservation concern, since there is a high level of gene conservation across taxa. Similarly, other approaches and tools such as large-scale proteomics have expanded in ecotoxicological research for nearly 2 decades, having originated with biological assays such as western blotting and enzyme-linked immunosorbent assays ELISAs, and enzyme activity or receptor binding assays (e.g., Martyniuk and Denslow 2009; Martyniuk et al. 2012; Halden et al. 2015). Evaluation of metabolic outcomes, i.e., endogenous metabolites detected within a biological sample, are now conducted in large-scale metabolomics studies, usually evaluating the outcome of transcription and protein activities. Metabolites, however, are often produced through biotransformation processes by Phase I (oxidation reduction and hydrolysis) and Phase II (increased

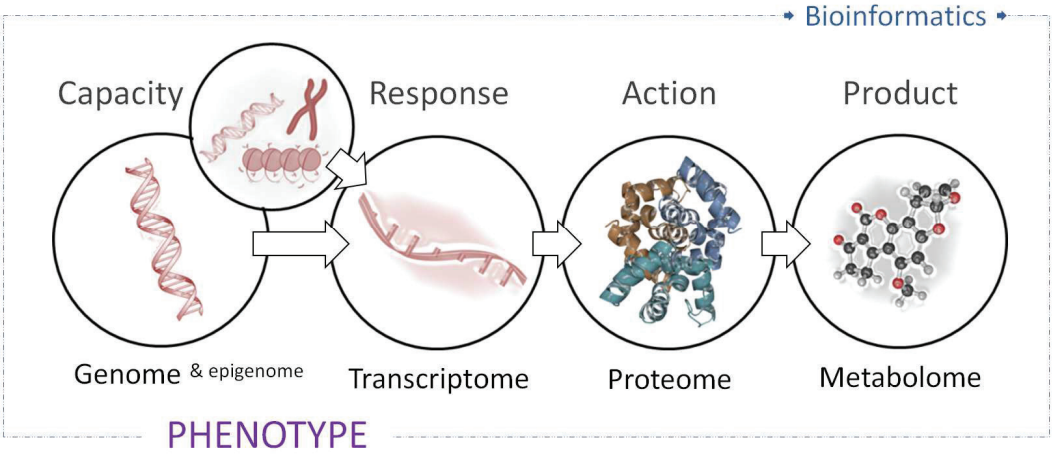

Figure 4 Simplified representation of information that can be gathered at different 'Omic levels. While each 'Omic technique can provide specific information about mechanisms by which contaminant exposure can affect an organism, bioinformatics analyses and integration of these approaches can provide extensive knowledge on the capacity of an organism to contend with contaminant exposures and resulting mechanisms of action as defined by the phenotype.

solubility and elimination) enzymes that occur in the absence of modulation of transcription and translation (Martyniuk and Simmons 2016). The increased use of such technologies is seen across many facets of research. The 'Omics movement is now beginning to probe the epigenome (Vandegehuchte and Janssen 2011; Head et al. 2012; Brander et al. 2017) and the whole genome through re-sequencing methods (Bentley 2006), enabling investigators to better understand multi-generational effects and sensitivity differences between populations, which are described below in more detail. As with all 'Omics techniques, the costs of these assessments are rapidly decreasing, and such approaches are highly valuable both to generate detailed mechanism-based, adverse-outcome pathways, and to develop biomarkers of effect, for use in contaminant monitoring and risk assessments (Monsinjon and Knigge 2007; Martyniuk and Simmons 2016) in laboratory or field settings (Perkins et al. 2017).

Given the number of new chemicals that arrive on the market each year, and the associated pesticycle, researchers should aim, when possible, to evaluate larger numbers of chemicals using high-throughput approaches such as fish embryo toxicity (FET) tests (Roper and Tanguay 2018). However, in recent years, it has become evident that responses measured from short-term exposures are not necessarily informative over the longer term, especially in terms of transgenerational effects that result from either indirect exposure (e.g., maternal transfer) or epigenetic change. One of the earliest examples of transgenerational effects studies, conducted by (Anway et al. 2005), demonstrated how pesticide-exposed pregnant rats produced male offspring with lower sperm number and viability—an effect that persisted to the 4th generation in unexposed organisms. More recently, transgenerational effects have been demonstrated in Zebrafish (*Danio rerio*), Medaka (*Oryzias latipes*), and Inland Silversides (*Menidia beryllina*) (DeCourten and Brander 2017; Alfonso et al. 2019; Cleary et al. 2019). These generational effects may be related to changes in the epigenome (information placed on top of the genome) representing epigenomics. Epigenomics is a relatively new component of the 'Omics tool-box, and, as such, still requires validation for use in regulation. Epigenomics evaluates the heritability of genetic changes that do not involve alterations in the DNA sequence but rather modifications to the genetic expression capacity, thus playing a pivotal role in controlling the expression of genes (Head et al. 2012; Brander et al. 2017). Epigenetic tags, such as methyl or acetyl groups, are laid down on top of DNA or the histones around which DNA is wrapped. Epigenetic modifications

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are vital during embryonic development for cellular differentiation, but epigenetic modifiers also respond to environmental stressors (Vandegehuchte and Janssen 2011; Head et al. 2012; Brander et al. 2017). As such, the methylation status of cytosines in DNA regulatory regions, as well as methyl or acetyl groups added to the lysine tails of histones, mediate how transcription factors interact with the DNA, because they control how tightly the DNA is configured.

Recently published research highlights the importance of epigenetic tags in evaluating responses to aquatic pollutants, and demonstrates that epigenetic mechanisms are important both within the lifetime of an organism, as well as in subsequent generations, since epigenetic modifications to gametes can be inherited through multiple generations (Corrales et al. 2014; Head 2014; Bhandari 2016; Voisin et al. 2016). The number of epigenome-focused studies is rapidly growing in the field of ecotoxicology (Head et al. 2012; Brander et al. 2017). Recent studies have shown increased methylation on genes specific to development and reproduction. For example, in a study using Zebrafish exposed to the polycyclic aromatic hydrocarbon benzo(a) pyrene during embryonic development, Gao et al. (2018) reported a high level of methylation of genes involved in the brain–pituitary–gonadal axis, corresponding significantly with suppressed ovarian development and reproductive capability. Similarly, reproductive failure was associated with DNA hyper-methylation in *Chironomus riparius* after exposure to the endocrine-disrupting compound, Bisphenol A (BPA) (Lee et al. 2018).

In addition to epigenetic mechanisms, multi-generational effects may also be observed as a result of evolutionary changes, when some highly adaptable species are able to evolve tolerance to particular contaminants (Medina et al. 2007; Whitehead et al. 2017). This results in differences in sensitivity to contaminants across populations (Brady et al. 2017), and potentially leads to decreased genetic diversity and fitness costs in the adapted populations (Oziolor et al. 2017; Major et al. 2018). Evolutionary toxicology is a discrete field aimed at evaluating the effects of chemical pollutants on the genetics of natural populations, which is critical when species of conservation concern are evaluated (Bickham 2011). Several examples now exist of adaptation to pollution (Amiard–Triquet 2011; Reid et al. 2016), including within the Bay–Delta (e.g., resistance to pesticides in the crustacean *Hyalella azteca* [Weston et al. 2013; Major et al. 2018]). However, these selection events may influence organism fitness (Meyer and Di Giulio 2003; Janssens et al. 2014; Heim et al. 2018), and overall genetic diversity (Bickham et al. 2000). Other population-level effects to mutation rate and gene flow can erode the capacity of the population to contend with other environmental stressors (i.e., phenotypic plasticity), resulting in either adaptation or extirpation. In addition, adaptation of contaminant exposure in one species is highly correlated with other community-wide effects (Klerks 2002), suggesting that when one species is rescued through evolution, there are likely many other species within the same community that are not able to adapt, and their populations decline. Identifying the presence of a pesticide-resistant population (discussed later, see "Resistance as an Indication of Impairment"), without knowing that it is resistant, for example, may suggest a lack of effect, thus negatively affecting monitoring efforts.

Because genetic changes in evolutionary toxicology often arise in critical proteins and in target sites for the chemical pollutants, studying the genetic differences in adapted populations may also uncover the molecular initiating events of contaminants and help inform AOPs. As in other examples above, 'Omics tools are highly applicable to the field of evolutionary toxicology. RADseq (Restriction site Associated DNA Sequencing) has been developed as a powerful tool for interrogating a genome and identifying large numbers of genetic variations in natural populations (Catchen et al. 2017; McKinney et al. 2017); however, RADseq data alone may miss many loci under selection in studies of local adaptation. Genome-wide evaluations are better able to detect these subtle changes (Lowry et al. 2017; Therkildsen and Palumbi 2017). In addition, applying transcriptomics alongside

genome-wide scans for genetic differences can detect fitness trade-offs by highlighting altered biological pathways that result from genetic changes in adapted populations (Ozioler et al. 2017). By exposing the effects of contaminants on populations over evolutionary time-scales, evolutionary toxicology and technologies such as RADseq, genome re-sequencing, and transcriptomics have great potential to identify affected areas and inform regulations (Lowry et al. 2017; Oziolor et al. 2017). Resources applied to evaluating the effects of contaminants on evolutionary fitness could inform long-term effects upon aquatic ecosystems populations. As highlighted by Dobzhansky (1973) "*nothing in biology makes sense except in the light of evolution*."

'Omic Tools and Data Sets of Direct Relevance to the Bay–Delta

A legacy of conventional toxicology is the reliance on a few, well-studied, model (or sentinel) species in determining chemical toxicity. While it is important to use model organisms from a regulatory perspective, and toward providing a broad understanding of the effects of specific chemicals, the field of ecotoxicology is rapidly expanding to incorporate species of concern. This is important to highlight because model species are used because of their ease of culture under laboratory conditions. This does not, in fact, adequately serve the goals of environmental risk assessment when it comes to evaluating effects on species of conservation concern. As mentioned above, multiple 'Omic approaches are now directly available to be applied to these species of concern (e.g., endemic species, or sport fishes), to conduct toxicological assessment of specific ecosystems, such as the Bay–Delta. Contaminant research conducted for species of conservation concern, such as the Delta Smelt (*Hypomesus transpacificus*) and Longfin Smelt (*Spirinchus thaleichthys*) has necessitated project-specific system constructions to accommodate short studies, which are based on grant-to-grant funding sources. New model species specific to the Bay–Delta may be required, and advances in effect-based analytical technologies make this a viable option. Appropriate evaluation of contaminant effects on Bay–Delta species of conservation concern would, however, thus require an initial investment into readily accessible experimental facilities that could be modified and tailored to the experimental requirements of each species.

Transcriptomes and genomes of most model aquatic species used in ecotoxicology assessments have been sequenced and annotated, and are readily available via the following publicly available repositories:

- **fish**—e.g., *P. promelas* (Saari et al. 2017; Gust et al. 2018); Rainbow Trout (*Oncorhynchus mykiss*, Berthelot et al. 2014)
- **invertebrates**—e.g., *Daphnia* spp. (Colbourne et al. 2005; Orsini et al. 2016), *Hyalella azteca* (Christie et al. 2018; Poynton et al. 2018), *Chironomus* spp. (Herrero et al. 2017; Mantilla et al. 2018)
- **phytoplankton**—e.g., *Raphidocelis subcapitata* (Suzuki et al. 2018)

Below, we present a summary of 'Omic tools and data sets available for fish, invertebrates, and algal species associated with the Bay–Delta.

Fish Species

Transcriptomes and genomes of several Bay–Delta fish species have been made available over the past decade, and provide a foundational resource for toxicological assessment studies that are directly ecologically relevant to their ecosystem. The Delta Smelt, an endemic species to the Bay–Delta, is protected under both Federal and California State Endangered Species Acts (ESAs); listed as threatened in 1993 (USFWS 1993) and as endangered in 2010 (CDFW 2018). Delta Smelt are very nearly extinct, and ongoing conservation efforts remain intensive (Hobbs et al. 2017). A refuge population was first established in 1993 at the UC Davis Fish Conservation and Culture Laboratory. The goal was a captive and genetically managed broodstock that could (1) hedge against near-term extinction, (2) provide fish for controlled laboratory studies, and (3) eventually supplement the wild population

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through controlled re-introductions into natural habitats (Lindberg et al. 2013; Finger et al. 2018). Delta Smelt are semi-anadromous and, as their name implies, confined in their range to the Delta (Moyle 2002; Sommer and Mejia 2013). Following controlled manipulations of water quality, transcriptome data have been analyzed to assess the effects of contaminants (pesticides, pharmaceuticals, heavy metals, ammonium, and unknown constituents in ambient water samples) as well as the physiological stress associated with salinity and thermal extremes (Connon et al. 2009; Connon et al. 2011a; Connon et al. 2011b; Hasenbein et al. 2014; Komoroske et al. 2014; Jeffries et al. 2015a; Komoroske et al. 2015; Komoroske et al. 2016). The functional response pathways associated with changes in gene expression have been used to develop suites of biomarkers that are associated with impairments to growth and development, behavior, and reproduction (Hook et al. 2014; Connon et al. 2018). These ensembles of molecular health indicators hold promise in terms of diagnosing the subtle but important effects of a broad range of water-quality stressors for this sensitive species.

Similarly, Longfin Smelt were also listed in 2009, under the California ESA as a threatened species (CDFW 2018). Recently, a transcriptomic approach was used to evaluate their critical thermal tolerance range (Jeffries et al. 2016). Anadromous Longfin Smelt are distributed from Alaska to California, and the Bay–Delta provides habitat for the southernmost distinct population segment for the species. Little is known about their habitat requirements or their vulnerability to contaminants. Abundance of both Delta Smelt and Longfin Smelt are currently at historical lows in the Bay–Delta (Hobbs et al. 2017). Similar to Delta Smelt, efforts are currently underway to establish a captive broodstock for Longfin Smelt, in part to support expanded environmental health research for the latter.

The Inland Silverside (*Menidia beryllina*) has long been a focus for ecotoxicological research, both in the lab and in the field. A non-native species in the Bay–Delta, Inland Silversides were introduced to the San Francisco Estuary in the late 1960s (Moyle 2002). They have recently served as a useful euryhaline model for the study of endocrine disruption and other categories of sub-lethal effects in the Bay–Delta and beyond (Brander et al. 2013; Brander et al. 2016a; DeCourten and Brander 2017; Mehinto et al. 2018). *M. beryllina* are part of the EPA's Whole Effluent Toxicity (WET) Testing Program (NPDES WET; USEPA 2002) and have been shown to be very sensitive to toxicants compared to other model fish species (Clark et al. 1985), particularly at elevated salinities. They are found in estuarine and brackish habitats throughout coastal North America (Middaugh and Hemmer 1992); are available commercially; and can be reared, spawned, and cultured through multiple generations in the laboratory (Middaugh et al. 1987; Brander et al. 2016a; DeCourten and Brander 2017). Furthermore, they tolerate fluctuating salinities and are widely distributed throughout the Bay–Delta. *M. beryllina* also have relatively high site fidelity (Gleason and Bengtson 1996), and thus contaminant exposure and response profiles for field-collected fish are more likely to reflect local habitat conditions. Given that the bioavailability of many chemicals—and hence toxicity—can vary, depending on the salinity at which an organism is exposed (Bosker et al. 2017; Saranjampour et al. 2017), this is of great value, because exposures can be conducted across a salinity gradient that represents the entire estuary. In 2013–2014, the transcriptome of *M. beryllina*, along with two other *Menidia* species (*M. audens* and *M. menidia*), was sequenced at the UC Davis Genome Center (Jeffries et al. 2015b; Brander et al. 2016b). More recently (2017–2018), the *M. beryllina* genome was sequenced, assembled, and annotated at the same facility. Ultimately, the genome is currently being used to inform both RNAseq (transcriptomic) and DNA methylation (epigenetic) analyses. Researchers have documented biologically significant effects of endocrine-disrupting chemical (EDC) exposure on *Menidia* in laboratory experiments and in wild populations (Duffy et al. 2009; Brander et al. 2012a; Brander et al. 2012b; Brander et al. 2013; Adeyemo et al. 2015; Mehinto et al. 2018). Modeling approaches in *M. beryllina* demonstrate that the production of a specific egg protein (choriogenin, an egg membrane protein) and corresponding reductions in egg fertilization,

as measured via spawning assays, can be linked to population declines. The sequenced genome provides information needed to evaluate responses at the epigenomic level. Transcriptomic and proteomic scale data also exists for this species (Brander et al. 2016a; Cole et al. 2016), and behavioral assays comparable to those used for Zebrafish have recently been validated (Frank et al. 2019). This allows for the development of high-throughput assays that evaluate a diversity of responses, which can be used in a species of concern to rapidly provide answers to a wide range of questions.

Recent studies using *M. beryllina* indicate that parental exposure to environmentally detected concentrations of EDCs such as bifenthrin and ethinylestradiol, at picomolar concentrations, result in organism-level effects on embryonic development in exposed offspring (DeCourten and Brander 2017; Decourten et al. 2019a). Ongoing work will determine whether these effects are a result of exposure of the parents during germ cell migration, or whether deleterious responses can be attributed to changes in DNA methylation and/or maternal transfer. Findings from these initial multi-generational studies highlight the importance of evaluating effects across multiple generations, since the effects of low-dose exposures in parents may not be observed until the F1 (exposed as primordial germ cells) or F2 generation (not directly exposed). For this reason, after identifying potential effects on hormonal systems, we should strive to acquire in-depth, high-coverage genomic information across Bay–Delta fish and invertebrate model species. Having accurately annotated genomes allows for DNA methylation to be linked back to the specific genes influenced by these epigenetic tags, and will serve to provide further knowledge on the effect of multiple stressors in field vs. laboratory-maintained species; e.g., wild vs. cultured Delta Smelt. Furthermore, running tests across multiple generations (to the F2 or F3 generations, for example), should be a long-term goal of any contaminant and environmental stressor monitoring program. Maternal effects, following exposure to contaminants, were shown to affect Striped Bass (*Morone saxatilis*) (Ostrach et al. 2008), another species of interest in the Bay–Delta. These laboratory studies, conducted at environmentally realistic concentrations and exposure duration, along with field evaluations of sex ratios (e.g., Brander et al. 2013; White et al. 2017) add to the weight of evidence (Fong et al. 2016), indicating that this is a problem in the Bay–Delta.

Much toxicological research has been conducted on salmonid species, particularly using the Rainbow Trout (*Oncorhynchus mykiss*) model (USEPA 2002). The anadromous form of *O. mykiss*, Central Valley Steelhead distinct population, is federally ESA listed (NMFS 2006). Furthermore, the genomes and transcriptomes of several salmonids have been sequenced (e.g., Tomalty et al. 2015; Christensen et al. 2018; Healy et al. 2018). To our knowledge, however, no mechanistic ecotoxicological studies relevant to the Bay–Delta have been conducted with these species. In other systems, such as the Fraser River watershed in British Columbia, Canada, transcription profiles were used to evaluate contaminant impacts on Sockeye (*O. nerka*) and Chinook (*O. tshawytscha*) Salmon (Veldhoen et al. 2010). One of the highlights of this study was the similarity of gene responses of true female fish with that of males that were sex changed as a result of contaminant exposure. Other studies using high-throughput sequencing of Coho Salmon (*O. kisutch*) determined that estrogens may alter processes associated with reproductive timing (Harding et al. 2013). The data generated from these salmonid studies can be used to develop biomarkers to evaluate how CECs affect priority species in the Bay–Delta, including federally endangered Sacramento River winter-run Chinook, threatened Central Valley spring-run Chinook, and threatened Central Valley Steelhead.

Besides SFBD-relevant fish species listed above, `Omic data also exist for the Sacramento Splittail (*Pogonichthys macrolepidotus*) (Jeffries et al. 2019; Mundy et al. 2019) and the Striped Bass (Reading et al. 2012; Chapman et al. 2014; Li et al. 2014), as well as for the federally threatened Green Sturgeon (*Acipenser medirostris*), the proteome of which was investigated in the context of selenium exposure (Silvestre et al. 2010). The rapid development and use of 'Omic approaches

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for species of interest (and/or concern) within the Bay–Delta opens the door to broader effect-based assessments for numerous contaminants and multiple stressors. This, in turn, would allow for better interpretation; in-depth studies of effects at higher levels of biological organization (development, behavior, reproduction); and subsequent risk assessment in the Bay–Delta.

Invertebrate Species

Not only have 'Omic approaches been used on Bay–Delta-relevant fish species, but invertebrate species, such as *Hyalella azteca*, have been extensively used to evaluate fish food sources affected by contaminants. The *H. azteca* transcriptome has been sequenced as a component of toxicological studies (e.g., Poynton et al. 2013; Christie et al. 2018) and has been made publicly available. The genome sequencing for *H. azteca* has recently been completed[7], and is also currently available, providing a foundational resource for toxicogenomic studies with this species (Poynton et al. 2018;). The availability of the genome has dramatically improved annotations of chemically induced response transcripts, providing a more comprehensive description of the processes affected by several different chemical stressors (Poynton et al. 2018). Overall, the genome enables detailed investigations in gene, transcript, and protein regulation, as well as in how chemical exposures influence their expression. In addition, the most complete set of peptide hormone targets for any amphipod has been predicted from the *Hyalella* transcriptome (Christie et al. 2018). These peptides play important roles in the amphipod endocrine system through hormonal neurotransmitters (peptidergic signaling), regulating molting, growth, and development. Studying their regulation will increase our understanding of endocrine disruption in *H. azteca* (Vandenbergh et al. 2003; Janer et al. 2005) and other amphipods (Sundelin et al. 2000; Ford et al. 2004; Ford et al. 2005), and provide an important study model for endocrine disruption in benthic communities caused by both aqueous- and sediment-associated chemicals (Hyne 2011).

Extensive 'Omic approaches have been developed and applied using model invertebrate species such as Daphnia spp. (Colbourne et al. 2005; Orsini et al. 2016) and *Chironomus* spp. (Herrero et al. 2017; Mantilla et al. 2018). A multitude of data exists on publicly available repositories for these species that have evaluated contaminants relevant to the Bay–Delta (Poynton et al. 2007; Shaw et al. 2007; Connon et al. 2008; Heckmann et al. 2008; Orsini et al. 2016; Herrero et al. 2017; Mantilla et al. 2018). The advantages of using invertebrate species in ecotoxicological assessments include the diversity of animals for test systems, short generation times, ease of culture of some species, and fewer legal/conservation issues (deFur 2004), plus they offer direct insight into how contaminants affect food webs. To our knowledge, however, no mechanistic studies that use the approaches described above have been conducted on Daphnid or Chironomid species in the context of the Bay–Delta.

Phytoplakton Species

Numerous phytoplankton species have been used in freshwater and marine algal toxicity tests. One of the ecotoxicological standards for which 'Omic data exists (Suzuki et al. 2018) is *Raphidocelis subcapitata* (= *Selenastrum capricornutum*; = *Pseudokirchneriella subcapitata*). Advances in 'Omic technologies, as detailed above, now provide the capacity with which to obtain genomic information from a multitude of species for which there has been no prior genomic data. This enhances our ability to conduct focused studies on multiple phytoplankton species of interest in the Bay–Delta, not only to assess contaminant effects, but also to evaluate and mechanistically understand how ammonia/um (a nutrient at adequate levels) affects phytoplankton growth. Phytoplankton cell membranes, for example, have specific transporters for specific nitrogen sources — e.g., ammonium transporters (Amt), nitrate transporters (Nrt), and urea transporters (Urt) — with numerous isoforms of each transporter within each cell type (Berg et al. 2008). These transporters likely have differential affinities to each nitrogen source. Kang and Chang (2014) have investigated gene sequence diversity among phytoplankton species, which significantly differentiates responses between

7 *https://www.ncbi.nlm.nih.gov/genome/16496*

chlorophytes and diatoms. Furthermore, Kang et al. (2011) have identified similar diversities in nitrate transporters, strongly differentiating a diatoms, chlorophytes, cyanobacteria, and haptophytes, which are likely to alter sensitivity between these species. These results support potential preferential requirements for one or another nitrogen source (Gonzalez–Ballester et al. 2004). These physiological characteristics could be used to better understand the mechanisms behind preferential use of ammonium vs. nitrate across various algal species in the Delta, and to help determine postulated thresholds for ammonium inhibition for diatoms in the Bay–Delta (Dugdale et al. 2012; Glibert et al. 2014; Senn and Novick 2014).

'Omic approaches, applied across multiple phytoplankton species, can therefore help algal bloom occurrences, species distribution, and habitat requirements — as well as the impact of contaminants — to be better understood; for example, herbicides that are directly applied to surface waters to control invasive aquatic vegetation such as Brazilian waterweed (*Egeria densa*), water hyacinth (*Eichhornia crassipes*), and water primrose (*Ludwigia* spp.) (Ta et al. 2017) on primary production. Conducting mechanistic studies across trophic levels is of particular importance in a system that has been described as food limited (Kimmerer et al. 2018), because this will provide information needed to address the cause, rather than treating the symptom.

IN SILICO AND META-ANALYSES: DATA-DRIVEN BIOINFORMATICS APPLICATIONS FOR DETERMINING ADVERSE OUTCOMES

In silico toxicology uses computational methods to analyze, simulate, visualize, or predict effects that result from exposure to contaminants (Raies and Bajic 2016), thus helping predict adverse outcomes. The recent increase in 'Omics-driven research focused on understanding effects from contaminant exposure, along with strict requirements for archiving this data in publicly accessible repositories worldwide (e.g., Gene ExpressionOmnibus[8], Sequence ReadArchive[9]) provides a stage for robust in silico approaches to predict risk. The ability to leverage these databases has infinite potential for using data-driven, statistical machine-learning procedures, which are useful for toxicity evaluations across species (Berger et al. 2013; Wang et al. 2016; Vijver et al. 2017; Campos and Colbourne 2018; Connon et al. 2018). These data sets should be mined and meta-analyses conducted to help identify characteristic differences and commonalities in responses to particular chemical groups and the functional pathways affected. Such meta-analytical approaches can be used to confirm and validate effects, as well as rapidly identify chemicals for which further information is required (Raies and Bajic 2016). Furthermore, the use of species homologies to discern responses across species could guide the development and validation of novel toxicity tests, including biomarkers and systematic processes, with which to better evaluate adverse effects that affect aquatic ecosystems, thus scaling up from population-level effects.

An example of such an approach is presented by Wang et al. (2016), who describe how a meta-analysis approach can be used to develop data-driven bioinformatics applications to determine adverse outcomes. Using human connectivity mapping (CMap), an in silico approach developed for biomedical research, the researchers evaluated over 3,500 Zebrafish and Fathead Minnow (*Pimephales promelas*) transcriptome profiles. CMap determines similarities in the underlying mechanisms of action of chemicals by connecting chemicals and disease-based transcriptomic profiles (Sandmann et al. 2014), aiding the development of biomarkers focused on stressors of concern. Wang et al. (2016) successfully determined connectivity between the mode of action of chemicals and gene expression profiles across both species, associated with the same or similar chemicals. Using this approach, researchers were able to confirm the identities of several estrogenic chemicals, a polycyclic aromatic hydrocarbon, and a neurotoxin present

8 *https://www.ncbi.nlm.nih.gov/geo/*

9 *https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?)*,

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in surface waters near several wastewater treatment plants.

To further confirm or develop adverse outcomes with ecological significance, meta-analytical approaches can, of course, extend beyond the 'Omics level to incorporate and synthesize the multitude of published effect-based data obtained at multiple levels of biological organization (tissue, organ, whole organism, population, and communities). Inclusion of multiple species (non-model species, and or species of concern) can offer greater ecological relevance, and provide risk assessments across trophic levels (Vijver et al. 2017). Multiple data sets also exist for a multitude of stressors, besides contaminants, and could be used to differentiate among effects attributable to environmental stressors, contaminant exposure, and their respective interactions. This multi-species toxicological approach has been described as phylogenetic toxicology, which uses comprehensive genomic data sets to gain knowledge of toxicity processes (Colbourne et al. 2015). The ultimate goal of phylotoxicity evaluations would be to make regulatory-relevant, experimentally derived predictions of toxicity, based on the underlying mechanistic basis of chemically induced adverse events. The application of high-throughput toxicity testing with data-rich genomics assays allows researchers to explore commonalities (high homologies) and differences in responses across taxa (phytoplankton, invertebrate, and vertebrate species) to help identify shifts in responses to exposure, as well as toxicity pathways and co-responsive molecular networks specific to different chemical classes.

EVALUATING SPECIES RICHNESS

Loss of biodiversity is commonly used as a proxy to characterize community-level effects of environmental stressors (Hajibabaei et al. 2012; Taberlet et al. 2012). Traditional biomonitoring is limited by costly and time-consuming taxonomic identification, access to specimens, and cryptic species or life stages. These limitations can obscure the changes to ecosystem function and biodiversity that indicate the early stages of stressor effects. Genomic techniques such as environmental DNA (eDNA; the detection of a species' genetic material present in the environment) and eDNA meta-barcoding (the characterization of eDNA from multiple species in a single sample) can enhance traditional biomonitoring techniques, and greatly expand our understanding of community dynamics and ecosystem functioning, revealing the site-specific meta-genomic distribution of biodiversity across a wide range of taxa (Mächler et al. 2014; Brandon–Mong et al. 2015; Deiner et al. 2017). Meta-barcoding is particularly useful to accurately identify cryptic species, damaged specimens, specimens at all life stages, and taxa present at very low densities (Creer et al. 2016), and can be used to identify a wide variety of taxa simultaneously from pooled tissue or environmental eDNA samples (Emilson et al. 2017). Such meta-data can be used to evaluate species richness variation throughout Bay–Delta regions at population and community levels, including across trophic levels — e.g., phytoplankton and invertebrate species distribution — and to investigate habitat use by various fish species. Such results could be contrasted with high-throughput chemical analyses to determine the potential contribution of contaminants, as well as with multiple drivers of environmental change. Meta-barcoding has also been successfully used to evaluate invertebrate communities and fish present in different habitats (e.g., Andújar et al. 2018; Stat et al. 2018). Studies that use DNA meta-barcoding are increasing rapidly, and this high-throughput, community-focused approach has been effectively incorporated into ecotoxicological studies. Environmental DNA samples, for example, were successfully used to characterize differences between freshwater invertebrate communities as driven by multiple contaminants that originated from different land-use types, and by using community data to identify primary contaminants responsible for toxicity (Xie et al. 2017). For such an approach to be effectively used in the Bay–Delta, however, a reference genetic database specific to the system would first need to be developed. While a multitude of genetic sequences exist in publicly available repositories, data for cryptic and unknown species would require sequencing; such databases should, therefore,

be developed in collaboration with experienced taxonomists (Porter and Hajibabaei 2018).

RESISTANCE AS AN INDICATION OF IMPAIRMENT

The presence of pesticide-resistant organisms in the Bay–Delta is significant evidence that pesticides have directly affected, and continue to affect, the ecosystem. As such, the detection of pesticide-resistant organisms can be used as an effect-based endpoint, likely associated with pesticide use in a particular location. In the presence of strong selective pressures, some organisms are able to adapt to the contaminants, which results in resistant populations, thus providing compelling evidence that contaminants have affected the aquatic community. Widespread resistance to insecticides has been documented in the epibenthic amphipod *H. azteca* throughout California (Weston et al. 2013, 2018; Major et al. 2018) with frequencies equivalent to or greater than the populations of insect pests the insecticides target directly. It follows that the selective pressure on aquatic invertebrates at least compares to that which the target insects experience during insecticide application.

The resistant *H. azteca* populations experience trade-offs in the form of reduced fecundity, and decreased tolerance to other stressors (Weston et al. 2013; Heim et al. 2018). In addition, there is concern about bioaccumulation and trophic transfer of insecticides to fish predators (Corcellas et al. 2015; Muggelberg et al. 2017); however, the ecological consequences of this genetic adaptation likely go beyond the species level and are still unknown (Medina et al. 2007). The ability of *H. azteca* to be "rescued" through adaptation (Bell 2013) is related to its large population size, short generation time, and high sensitivity to the insecticides. For other susceptible arthropod species (e.g., Ephemeroptera– Plecoptera–Tricoptera [EPT] taxa) it is completely unknown if they also develop resistance, or undergo local extinction because of their inability to adapt. However, it is clear that the presence of resistant *H. azteca* indicates the continual effects of pesticides in the Bay–Delta. The resistant populations also act as a unique effect-based monitoring tool, within an *in situ* context. When placed in impaired waterways alongside non-resistant animals, the survival of resistant *H. azteca* relative to non-resistant *H. azteca* can provide a "biological toxicity identification evaluation (TIE)" (Weston et al. 2018) that can identify if an insecticide is responsible for toxicity. Because the mechanism of resistance is highly specific to insecticide class, the biological TIE becomes an excellent indicator for causality. Although these characteristics are not unique to *H. azteca*, many epibenthic invertebrate species are not likely to be as adaptable, because of their longer generation times and smaller populations. More research is needed to understand the evolutionary potential of other species within the macroinvertebrate community of the Bay–Delta, which species are likely to adapt, and at what cost, and which are likely to decline because of contaminant toxicity.

APPROACHES USED TO UNDERSTAND EFFECTS OF CHEMICAL MIXTURES AND INTERACTING STRESSORS

Mixture toxicity has been extensively studied in salmonids, particularly current-use insecticides that target acetylcholinesterase (AChE) in the central and peripheral nervous system. This enzyme hydrolyzes the neurotransmitter acetylcholine at synapses, and the toxic blockade of AChE disrupts cell–cell signaling and a wide range of behaviors in fish and other animals. Two major classes of insecticides, the organophosphates and the carbamates, inhibit AChE. Organophosphates include diazinon, chlorpyrifos, malathion, and ethoprop; carbamates include carbaryl and carbofuran. These and related agrochemicals are widely used in the Central Valley, and they are commonly detected in freshwater habitats (Aggarwal et al. 2013). What is more important, they frequently co-occur with other insecticides that share the anticholinesterase mechanism of toxicity. Exposures to single chemicals disrupt olfactory-mediated migratory and predator-avoidance behaviors in Chinook Salmon (diazinon; Scholz et al. 2000) as well as swimming and feeding in juvenile Coho Salmon (chlorpyrifos; Sandahl et al. 2005). Moreover, individual-based and

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population-scale modeling has been used to link decreases in juvenile feeding to reduced ration, reduced somatic growth, smaller size at seaward migration, increased size-selective mortality, and decreases in population abundance and intrinsic growth rate over time (Baldwin et al. 2009). Significantly, co-exposures to certain binary combinations of organophosphates and carbamates yielded synergistic and pronounced AChE inhibition in the brains of juvenile salmon (Laetz et al. 2009), and these synergistic effects on brain chemistry were subsequently extended to impaired swimming behavior (Laetz et al. 2013). These effects need to be evaluated across multiple species to determine whether differences exist between salmonid species, as well as across other fish in the Bay–Delta. Lastly, in the context of interactions between chemical and non-chemical stressors, synergistic neurobehavioral toxicity in salmon is exacerbated by increasing surface water temperature. Specifically, the degree of the synergistic inhibition of AChE by a diazinon and ethoprop mixture (each at exposure concentrations below a part per billion) doubled when water temperatures were raised from 12 °C to 18 °C (Laetz et al. 2014).

Mixtures of chemicals can have additive or even synergistic effects, not revealed with single chemical exposures. Within these mixtures, the most relevant chemicals to potentiate toxicity at low doses—from the standpoint of real-world exposure—are those that act on specific cellular targets. Two examples are EDCs and transporter-interfering chemicals (TICs). EDCs can act as hormone mimics or antagonists, at multiple levels within the endocrine system (Windsor et al. 2018). They can have unanticipated effects by priming or sensitizing the organism to subsequent exposures, particularly when exposures occur during certain developmental stages. TICs are a more recently described class of chemicals that can act directly to enhance the toxicity of chemicals by interfering with xenobiotic transporters (Guseman et al. 2016; Nicklisch et al. 2016). This interference leads to enhanced uptake of chemicals within the mixture that would otherwise be eliminated, thus enhancing differences in effect response as a result of interactive effects. Although the complete molecular mechanisms that underlie additive and synergistic effects (Pivcevic and Zaja 2006) are complex, the presence of TICs could specifically affect the net uptake and accumulation of certain environmental chemicals of interest (Figure 5). For instance, the environmental chemicals and endocrine disruptors BPA, perfluorooctanoic acid (PFOA), and zearalenone (ZEN) have been shown to be transported by breast cancer resistance protein (BCRP) (Mazur et al. 2012; Dankers et al. 2013; Bruyere et al. 2017) and inhibition of BCRP could, in turn, promote EDC and toxic substrate accumulation. Therefore, drug transporter inhibition is another key event—along with non-monotonic dose–response, synergism of single non-lethal concentrations of chemicals, and interaction with other environmental stressors—necessary to predict toxicity and assess the risks of environmental chemicals. The Bay–Delta provides a critical test case to explore these effects.

Exposures to environmentally relevant concentrations of EDCs and other stressors (e.g., altered temperature, hypoxia) are an established threat to ecological health (Kidd et al. 2007; Thomas et al. 2007; Brander 2013; DeCourten and Brander 2017; DeCourten et al. 2019b). Commonly encountered chemicals such as pyrethroids are known to interfere with endocrine function (Brander et al. 2016a) and such effects are demonstrated in a number of Bay–Delta fish (Connon et al. 2009; Brander et al. 2013; Jeffries et al. 2015a; Weston et al. 2015b; Brander et al. 2016a). In fact, modeling approaches demonstrate that exposure to endocrine-active compounds such as bifenthrin can result in altered fecundity and subsequent population decline, especially when considered an additional stressor on top of the altered sex ratios already present in some areas of the Bay–Delta (White et al. 2017). Over the past decade in the Bay–Delta, studies using effect-based analysis for estrogenic activity identified a group of alkylphenol ethoxylates (APEs) and alkylphenols (APs)—along with the persistent herbicide, diuron, and the pyrethroid insecticide, bifenthrin (Schlenk et al. 2012). When the compounds were administered as individual compounds at concentrations observed in the Bay–Delta, estrogenic effects were not

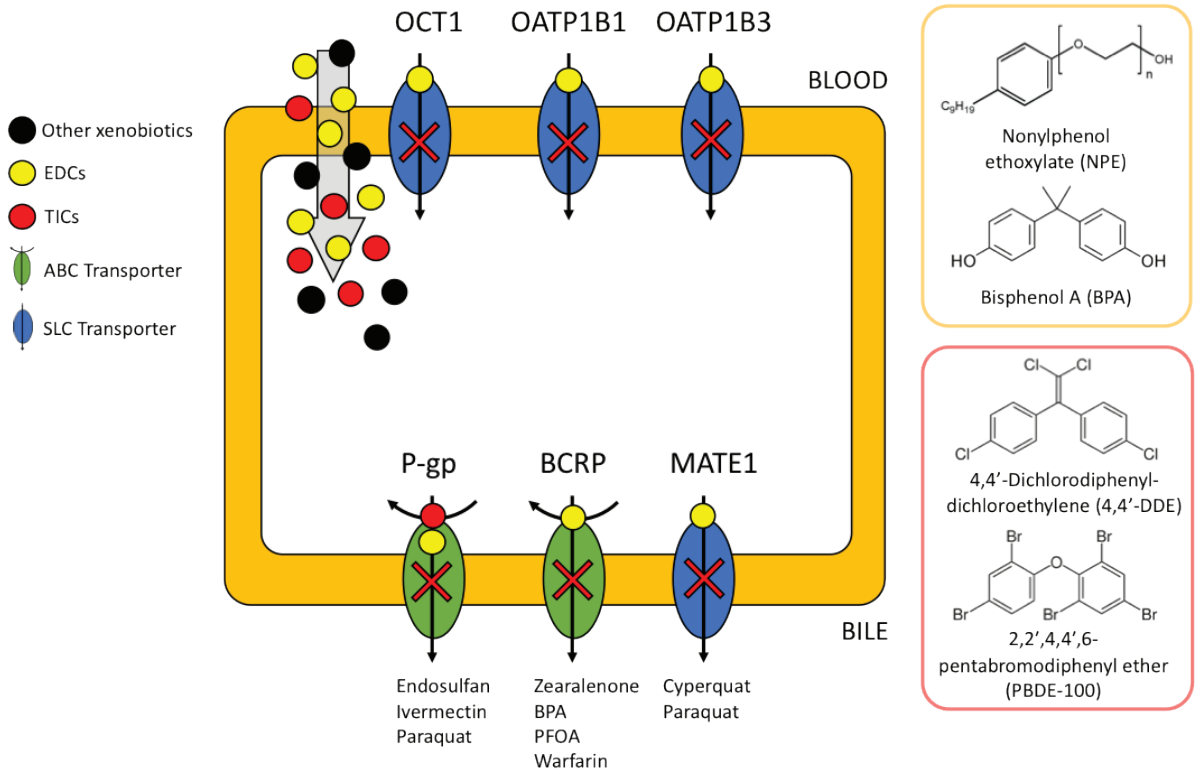

Figure 5 Schematic interactions of xenobiotics, including EDCs and TICs, with common drug transporters in hepatocytes. Inhibition of ABC and SLC transporters can lead to higher accumulation of both the inhibiting environmental chemical and other toxic substrates otherwise expelled from the cells. (According to the results from Kleinow et al. 2004; Dankers et al. 2013; Nicklisch et al. 2016; Bruyere et al., 2017; Chedik et al. 2018)

observed in isolated hepatocytes or in whole-fish exposures. However, when the compounds were combined in representative mixtures, significant estrogenic activity was observed in whole animals, suggesting a complex interaction. Using qualitative adverse-outcome pathways, attempts have been made to identify how the compounds interact with one another, and how climate change and other environmental stressors may enhance or diminish the endocrine responses in resident fish. Alkylphenol ethoxylates and alkylphenols enhanced the estrogenic activity of diuron in male Fathead Minnows (Crago et al. 2015). Subsequent studies in male tilapia also indicated the induction of cytochrome P450 by nonylphenol ethoxylates (NPE) and nonylphenols NPs, presumably enhancing the formation of more active metabolites of diuron such as 3,4-dichlorophenyl-N-methylurea (DCPMU), which was more potent than the parent compound, diuron, in males and females (Felicio et al. 2016; Pereira et al. 2016). Anti-androgenic activity was also noted in males, in addition to the estrogenic responses that indicate multiple targets that result in overall feminization (Pereira et al. 2015). Alkylphenol ethoxylates and alkylphenols APs also enhanced the estrogenic activity of bifenthrin in Fathead Minnow (Crago et al. 2015); however, this interaction's mechanism is unclear. Recent studies in a number of fish species indicate that bifenthrin may alter upstream steroid biosynthesis through dopaminergic and estrogen-receptor signaling (Crago and Schlenk 2015), although direct ligand activation of nuclear estrogen receptors (ERs) may not appear to be involved (Bertotto et al. 2018). However, the role of bifenthrin metabolites in activating nuclear ERs is still being investigated (DeGroot and Brander 2014). In addition, studies that evaluate how climate change affects this mixture reported that not only are alterations observed in the hypothalamic–pituitary–gonadal (HPG) axis, but

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the hypothalamic–pituitary–thyroid (HPT) axis may also play a significant role. Tying together the individual AOPs for the individual compounds with and without APE/AP predictions can be made to justify in vivo mixture interactions. It will be important to quantitatively link key events to predict dose-dependent changes with the mixtures under the modeled climate changes proposed for the Bay–Delta (Figure 6).

Transporter interfering chemicals can act through a variety of mechanisms to alter function of xenobiotic transporters, thereby affecting net balance of xenobiotic uptake and elimination (Smital et al. 2004; Epel et al. 2008). One mode of action for TICs is through direct binding to the ligand-binding domain (Nicklisch et al. 2016) of major xenobiotic efflux pumps such as P-glycoprotein (aka ABCB1) and subsequent inhibition of the transporter's efflux function. Similar TIC effects have been reported for interactions of environmental chemicals with solute-carrier transporters (Bain et al. 1997; Kleinow et al. 2004; Fardel et al. 2012; Bircsak et al. 2013). In larger species, such as Yellowfin Tuna, TIC lipid levels as high as 3.3 µM have been reported (Nicklisch et al. 2017), which is well within the range of individual and pollutant mixture IC50 values toward P-glycoprotein (Nicklisch et al. 2016). Chemicals that act as TICs include organochlorine pesticides, brominated flame retardants, and polychlorinated biphenyls, all of which have been found to accumulate in fish, including several species endemic to the Bay–Delta (Greenfield et al. 2002; Hunt et al. 2008), at levels that could impair transport function.

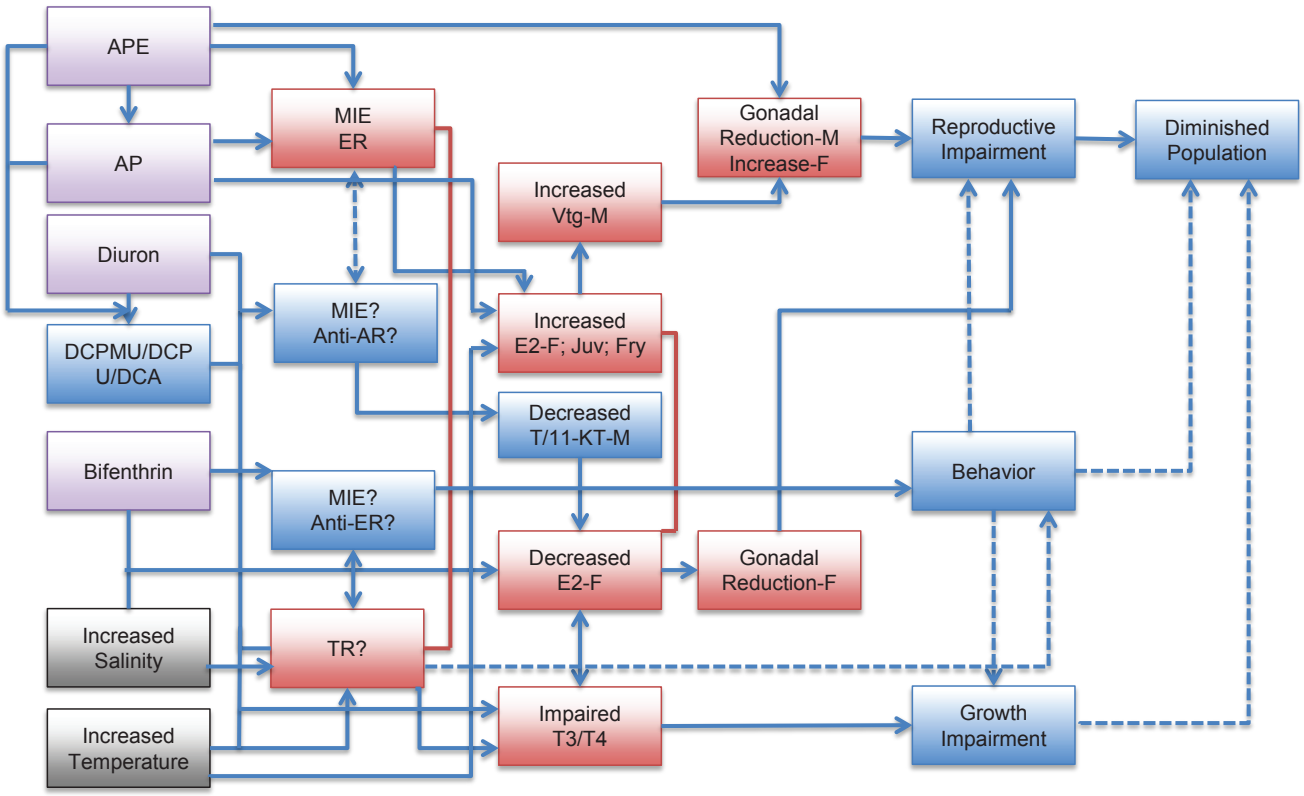

Figure 6 Potential Adverse Outcome Pathway for mixtures of estrogenically active contaminants in the Sacramento River, California that integrate temperature and salinity.

PROGRESS IN ANALYTICAL CHEMISTRY ALLOWS FOR MORE COMPREHENSIVE MONITORING

Chemical analysis is an important component of water quality assessments because toxicants must be identified to develop appropriate management actions. Traditional targeted approaches consist of screening for specific compounds of interest or concern. Monitoring data that are based on insufficiently low detection limits are of little use and can convey a false sense of safety for the environmental risk (Hollender et al. 2018; Werner and Young 2018). This approach can also underestimate the degree of exposure of aquatic organisms (Moschet et al. 2014). There are two strategies in place to overcome this issue and to obtain the required sensitivity in detection limits: (1) use of specific detection methods with enhanced sensitivity for target chemical classes (e.g., negative chemical ionization mass spectrometry for pyrethroids; Parry and Young 2013); and (2) improving signal strength by sampling larger volumes or concentrating the compounds via adsorptive methods such as solid phase extraction or passive sampling (Moschet et al. 2014; Moschet et al. 2015).

Beyond the conventional lists of regulated priority pollutants and pesticides, suspect and non-target screening methods are increasingly in use to identify toxic constituents in complex environmental mixtures (Ruff et al. 2015; Singer et al. 2016; Hollender et al. 2017). A case example is urban stormwater runoff, which contains myriad chemicals that originate from motor vehicle tires, exhaust, brake pads, crank case oil, and other sources. Untreated urban runoff is highly toxic to Coho Salmon and their invertebrate prey (McIntyre et al. 2015; Spromberg et al. 2016) as well as to Zebrafish, a model for investigating developmental toxicology (McIntyre et al. 2014). Recently, liquid chromatography coupled with high-resolution quadrupole time-of-flight mass spectrometry (HRMS) was used to screen highway runoff and the tissues of salmon exposed to runoff, with the goal of identifying common chemical features. Several thousand distinct features were detected in runoff (Du et al. 2017). Subsequent analyses that incorporated water samples from urban streams where spawning Coho Salmon consistently die prematurely in response to toxic stormwater (Scholz et al. 2011) has narrowed the list of causal toxic agents to fewer than 100 chemicals (Peter et al. 2018). Moreover, the HRMS approach revealed the presence of a family of (methoxymethyl) melamine compounds in urban waterways, the first such detections in North American surface waters (Peter et al. 2018). These contaminants were subsequently linked to urban stormwater-related mortality syndrome in Coho Salmon, until then known to be the causing agent. Overall, advances in analytical chemistry, combined with advances in effects-based research, have considerable potential for the study of complex mixtures and the resolution of previously unidentified toxic contaminants that may be widely distributed in Bay–Delta habitats.

To monitor polar and non-polar organic pollutants such as pesticides, pharmaceuticals, and industrial chemicals, passive sampling has been shown to be an alternative to ambient water samples (Mills et al. 2011; Moschet et al. 2014). Because of relatively easy handling during deployment and extraction, passive samplers can serve as a cost-effective and robust monitoring tool. However, for a proper quantification, robust sampling rates (R_s) are critical for all sampler types and compounds. R_s are substance-specific, and an intense discussion is underway about whether or not R_s can be predicted from physicochemical properties. In addition, there is no standard calibration method yet (Mills et al. 2011). Moschet et al. (2015) proposed an *in situ* calibration for substances with relatively constant river concentrations that can be quantified accurately in the field if substance specific sampling rates (R_s) are determined. The substance-specific R_s can then be used in future monitoring studies in rivers that have similar environmental conditions (i.e., flow velocity, temperature, pH). It is important to note, however, that concentrations sampled by passive samplers are average concentrations over a certain period of time, whereas the traditional grab samples or composite samples taken at certain time-points represent measurement of concentrations, for example of short-term pulses after runoff events.

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Although targeted monitoring approaches are constantly improving in terms of compound coverage (e.g., 100 to 300 compounds for many current multi-residue methods) and sensitivity (low ng/L concentrations are now routine method detection limits for many constituents in surface water), this approach may underestimate exposure and risk toward aquatic organisms by neglecting unanticipated constituents, including novel compounds and environmental transformation products (Kern et al. 2009; Moschet et al. 2017). High-resolution mass spectrometry (HRMS), as described in Moschet et al. (2017), can provide comprehensive analytical information by screening for a high number of suspects (>2,000). High-resolution approaches such as these rely on databases that contain chemical formulas, which enable analysts to presumptively identify compounds without the need for analytical reference standards. The simultaneous use of both liquid and gas chromatography high-resolution mass spectrometry (e.g., quadrupole time of flight or QTOF) allows comprehensive chemical contaminant profiles to be determined, limited only by compounds missing from chemical libraries.

Chemical characterization alone, however, is in most cases not expected to provide the necessary data to inform management decisions on contaminant toxicity (Krewski et al. 2010). This is especially problematic since contaminants occur as complex mixtures with varying modes of action, which likely cause synergistic effects and can be toxic at relatively low water concentrations (Cedergreen 2014). Besides sophisticated algorithms, which require extensive prior chemical-by-chemical and concentration-by-concentration knowledge of mechanisms by which contaminants affect biological performance, effect-based assessments continue to be necessary to determine environmental impact.

THE WAY FORWARD

Several recommendations that address scientific challenges and knowledge gaps as they relate to the Bay–Delta were put forward by Fong et al. (2016). These included the need for long-term and comprehensive monitoring, identification of specific monitoring endpoints, broader spatial and temporal coverage, and diversified testing—along with greater synthesis, analyses, integrated monitoring efforts, and use of adaptive management. A key point is the need for access to toxicological data in a timely fashion. The authors specifically highlighted that a dedicated research program is required to evaluate the effects of contaminants on Bay–Delta species of concern. We don't intend to repeat recommendations made by Fong et al. (2016), but rather to add to these recommendations by highlighting specific effect-based tools and how they could be used to evaluate the health of the Bay–Delta in the light of recent scientific development and the vast expertise shared by co-authors and contributors to the Special Symposium.

Because of the complex mixtures of contaminants, which not only interact with each other but also with multiple other anthropogenic and environmental stressors, we strongly emphasize the need for effect-based assessments as a step toward understanding effects upon species of concern. Sub-lethal effect assessments need to be the focus of ecotoxicological studies, because sub-lethal effects that affect fitness and inhibit behaviors essential for survival (e.g., foraging and predator avoidance), are among the greatest threats to aquatic organisms. Numerous endpoints are likely to be affected by chemical exposure; thus, without mechanistic data, modes of action cannot be determined. Mechanistic studies will provide knowledge on how specific stressors (chemical or environmental) may affect a species of concern. While mechanisms of action are expected to be somewhat similar across species (phylogenetic toxicology), specific response differences will determine levels of sensitivity needed to determine a true impact on that species. Vast data sets resulting from evaluations conducted on model species can guide endpoints to be evaluated in a species of concern. In many cases in silico analyses may be sufficient to determine such impact; however, these impacts need to be evaluated within a fundamental niche context, because the mode of action of many contaminants is likely to vary under different habitats or different environmental conditions (low vs. high temperature, salinity)

(e.g., Hasenbein et al. 2018). We therefore recommend that contaminant effects be evaluated under various thermal and salinity regimes, as applicable to the tolerance levels of the species of conservation concern for which these measures are needed.

Because it is not feasible to directly sample all the places and times of interest that are required for comprehensive contaminant assessment and management, monitoring programs must be properly designed and the monitoring data generated by many monitoring programs must be appropriately interpreted (Wang et al. 2019). With limited resources, a monitoring design that combines long-term monitoring sites with survey sites will provide more information on the ecosystem's overall health (Fong et al. 2016). Long-term monitoring sites can be those sites that integrate pollution signals from many land use/source types or sites with unique source type, such as effluent of wastewater treatment. These sites could potentially be developed to include facilities for evaluations across multiple generations (particularly when using invertebrate species). Survey sites can be randomly distributed in space or a stratum of land use/source combination (Garrett et al. 2017; Van Metre et al. 2017) ongoing Delta RPM research). The selection of long-term and survey sites should thus be based on statistical comparisons of watershed characteristics, such as land use and demographics. The same consideration of watershed characteristics applies to the interpretation of monitoring data that are intermittent and irregular in spatial/temporal coverage. To understand the driving forces behind pollution, a hybrid model that links the large array of watershed characteristics with historical and current conditions and monitored pollutant concentrations was proposed. Such models can thus interpolate or extrapolate the relationship between the driving forces and observed concentrations to other space- and time-related factors not directly monitored, allowing for a comprehensive evaluation of the system's overall health (Wang et al. 2019)

When ambient water samples are evaluated, the use of both targeted and non-targeted chemical analyses alongside effect-based assessments is encouraged, because this will facilitate the identification of chemicals responsible for observed toxicity. Toxicity identification evaluation (TIE) studies would need to be conducted on additional samples from sites of interest. TIE processes are laborious and can be expensive; thus, for pesticide evaluations that incorporate species for which resistance has been identified (e.g., *H. azteca* as described above), they would provide weight of evidence as well as aiding identification evaluations. The combined use of clades with varying degrees of pesticide resistance (e.g., to organophosphate vs. pyrethroid insecticides), for which survival of the resistant species versus mortality of controls could be evaluated, would effectively help identify contaminants responsible for observed toxicity.

For effects that take longer to manifest (e.g., xenobiotic-DNA adduct formation, cancer), and predominantly in long-lived species, histopathological studies have shown that it is possible to determine cause and effect (Myers et al. 2003; Schwacke et al. 2014; Smith et al. 2017). Studies such as these were able to fulfill the select criteria for causality in risk assessment, including: "*(1) strength of association, (2) consistency of association, (3) specificity of association, (4) toxicological and biological plausibility, (5) temporal sequence/timing (i.e., exposure precedes disease, effect decreases when the cause is decreased or removed), (6) dose-response or biological gradient, and (7) supportive experimental evidence*" (Myers et al. 2003). Lacking life history data, it is unlikely that field-caught organisms that are exposed to highly variable contaminant loadings (contaminant mixtures) and multiple stressors will provide an appropriate evaluation of short-term effects on species of concern. Such studies would not provide sufficient information to determine cause and effect. Effect-based studies that integrate multiple stressors are more suitably conducted either under controlled laboratory conditions (preferable for mechanistic evaluations), or in the field, by conducting either *in situ* (e.g., caged deployment), or *ex situ* exposures (e.g., river water pumped through strategically placed piers or riverbank-based laboratories). Such

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effect-based studies, in combination with non-targeted chemical analysis, could provide a means by which to assign probability of effect by a specific contaminant or contaminants. We suggest the integration and expansion of continuous monitoring technology. The California Department of Water Resources manages the water quality monitoring for the Interagency Ecological Program's Environmental Monitoring Program (IEP EMP). The CDWR has numerous active continuous water quality monitoring sites throughout the Delta, geared toward providing a better understanding of Bay–Delta ecology, and providing information pertinent to the management and conservation of the Bay–Delta. Use of existing field stations as well as the development of new continuous monitoring stations is strongly encouraged as facilities in which to conduct *ex situ* exposures and sample for contaminants. Such monitoring stations provide the capacity to conduct real-time water quality monitoring as well as serve as experimental stations for flow-through exposures, so the effect of multiple stressors on ecosystem health can be evaluated.

Integrative field-based approaches, whereby assessments are conducted across different taxa — for example, via meta-barcoding — will more appropriately fill data gaps in relation to species distribution, habitat use, and long-term community and ecosystem impacts. Studies such as these could easily be combined with effect-based ambient water toxicity testing, to determine habitat quality throughout the Bay–Delta and to identify potential contaminant sources. This would provide a framework under which much-needed research and monitoring of agricultural runoff and storm drains from urban land could be evaluated (as reviewed in Fong et al. 2016).

A major challenge for ecotoxicological assessments is trying to keep up with the pesticycle. It takes many years to evaluate the effects of pesticides and develop sensitive analytical chemistry approaches for their detection. More robust toxicological assessments need to be used proactively, before chemicals are allowed to be registered, to determine their potential impacts to aquatic ecosystems. The effects of contaminants and their interaction with multiple stressors need to be understood to determine how to reverse habitat compression and invest in the restoration of habitats suitable for species of concern. This would necessitate not only determining what causes adverse effects, but also investigating and testing what could potentially alleviate ecological repercussions. Evaluating the extent of stress relief could be tested, for example, in conjunction with cool refugia in laboratory studies, or in pools and riffles in the field (2019 communication between M. Brooks and REC, unreferenced, see "Notes"). Indeed, one of the contributors to this Special Symposium indicated that there is a need to question what it will take to have sustainable fish populations in the Bay–Delta, correctly pointing out that "*if we can't answer that, then that is where the science needs to go*." Scientifically and societally, however, we first need to acknowledge that certain chemicals are already eliciting adverse effects from Bay–Delta fish populations. Based on this knowledge, we can develop interdisciplinary and applied scientific approaches to mitigate ecological risk to these aquatic communities and maintain the ecological integrity of the Bay–Delta.

Tools used in regulatory programs have not kept pace with those available to assess contaminant effects. Because of this, regulatory programs are not including relevant information in their assessments, which points to the lack of better integration and collaboration among regulatory body needs, legislators, and researchers. Although some steps have been taken to evaluate additivity and synergism of chemicals, broader assessments that include information ranging from changes in organism response to effects on species distribution are not yet being employed. This review serves as a summary of tools that could be used to advance this effort. Standardization and documentation of these methods could be a next step toward their use in regulatory programs.

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