Evaluating Stressors in the San Francisco Estuary using Biomarkers

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Purpose of this Document

The purpose of this document is to briefly summarize the background and status of the San Francisco (SFE) studies and the potential impacts of contaminants, and other stressors such as dissolved oxygen, temperature, salinity and disease, on population health status of fish species in this region with a focus on the potential for using biomarkers within future integrated monitoring efforts.

There is a need to evaluate the use of biomarkers to assess organismal health within monitoring programs such as ambient surface waters, as well as to integrate them in the assessment of the efficacy of restoration programs.

This document, along with relevant reports and peer reviewed journal articles, provides the background for discussion at a planned workshop, the purpose of which is to:

1. Assess the potential application of biomarkers for evaluating stressors and/or adverse effects on SFE species;
2. Identify biomarkers that should be focused on in future SFE research; and,
3. Develop a research framework to determine the role and suitable application of biomarkers within the SFE ecosystem.

Background

In 2005, the Interagency Ecological Program (IEP, the nine agency collaboration that monitors and conducts research in the SFE in fulfillment of the state and federal operation agreements) formed a multi-agency Pelagic Organism Decline (POD) Management Team tasked with designing and managing a comprehensive study to evaluate the causes of the decline and to synthesize and report the results. A Contaminant Work Team (CWT) was formed as part of this broad undertaking. Among other CWT efforts, we are reviewing the role and applicability of biomarkers in assessing physiological stress in the determination of individual, and potentially population, level effects on fishes in the SFE. Scientists working within the Delta have used biomarker technology to assess individual and population health. A subset of those studies will be presented in the workshop.

Additionally, in 2007, the IEP convened a task force to evaluate the appropriate use of biomarkers to assess contaminant effects on four declining fish species in the SFE. The Task Force undertook three stages of assessment. First, the team met via telephone conferences during the summer of 2007 to formulate the scope of the project and outline individual responsibilities. Task Force members then summarized the state of the science on biomarkers, contaminants, and pelagic organism decline species life histories and subsequently prepared a set of synthesis tables for a second phase of work, namely a two day meeting at Fort Mason in San Francisco. The third stage included a written response to the question: “How can biomarkers be used strategically to determine whether contaminants cause significant stress in the POD species, and how can they be integrated into a larger framework of investigation?” Additionally, the Task Force provided strategic recommendations on biomarker implementation (Anderson et al. 2007).

Another review workshop, similar to the one planned herein, was conducted in Amsterdam, on October 9th, 2009, where an expert panel of European scientists gathered to discuss effect-based monitoring strategies and techniques. Questions addressed in this workshop included toxicity profiling (fingerprinting), its application in environmental risk assessment, sampling strategies and sample pretreatment methods, bioassay methods, and uncertainties in toxicity profiling (Hamers et al. 2013), which may be pertinent to ongoing work in the SFE.

We invite the panel to review the expert opinions from these biomarker workshops, in addition to various expert opinions such as Ankley et al. (2009), Ankley et al. (2010), Nichols et al. (2011), Villeneuve et al. (2011), Forbes and Calow (2012), van Straalen and Feder (2012), Hooper et al. (2013) and others.

SFE Ecosystem: monitoring

Monitoring conducted in the freshwater and brackish portions of the SFE (Appendix I) showed an unexpected decline of four pelagic (open-water) fishes (delta smelt; *Hypomesus transpacificus*, longfin smelt; *Spirinchus thaleichthys*, juvenile striped bass; *Morone saxatilis* [introduced sp.], and threadfin shad; *Dorosoma petenense* [introduced sp.]). This decline has collectively become known as the POD.

The summary story that has emerged from POD investigations is that the estuarine ecosystem has undergone a fairly rapid shift from a state that allowed pelagic fishes to thrive, or at least persist, to a state that appears hostile to them. This shift is likely caused by a variety of stressors (Fig. 1) related to water project operations, contaminants, land use, food webs, and changes in climate (Baxter et al. 2010, Brown et al. 2012). There is an urgent need to assess and monitor the resulting stresses on fish populations. In doing so, it is important to note that no one stressor is likely to be solely responsible for this shift, and that they have the potential to - or do influence one another; e.g., potential effects of contaminant mixtures, and flow (Cloern and Jassby 2012).

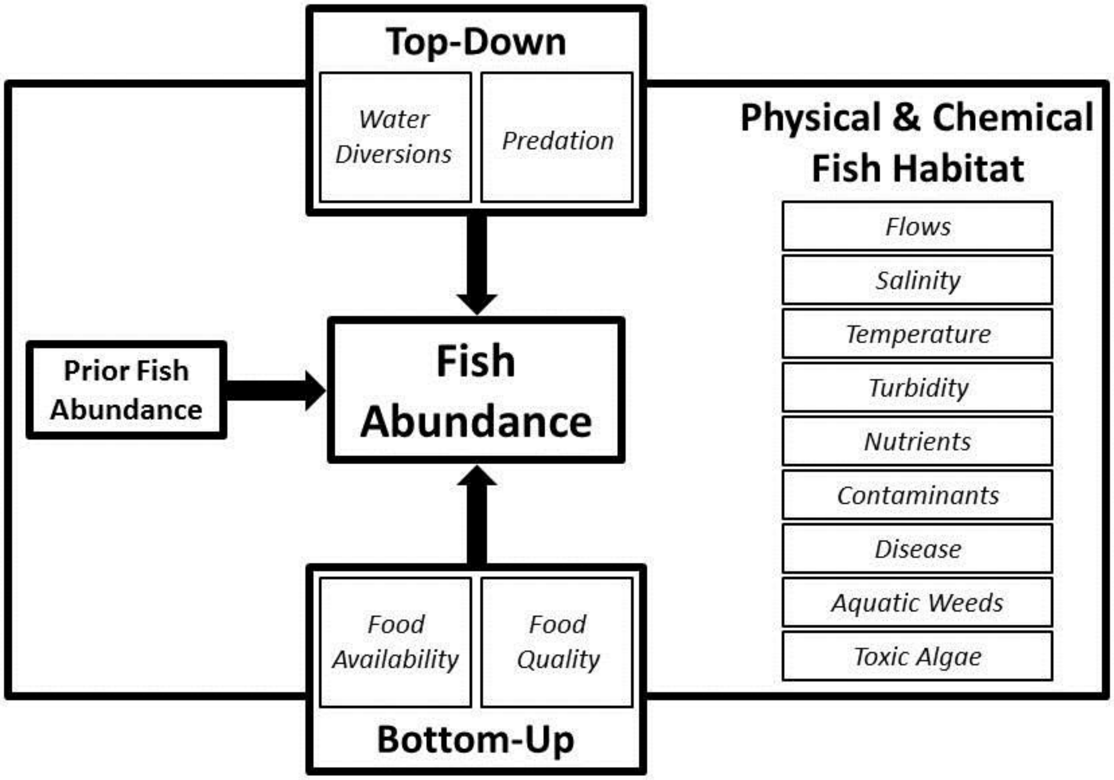

Figure 1*. The basic conceptual model for the pelagic organism decline (Baxter et al. 2010)*

The current interest extends beyond the POD species in the SFE, to include additional listed species of concern; e.g., green sturgeon (*Acipenser medirostris),* white sturgeon (*Acipenser transmontanus*), various salmonid, and other species of interest such as the inland silversides (*Menidia beryllina*), and Sacramento splittails (*Pogonichthys macrolepidotus)*, as informative assessment techniques are needed to evaluate the health status of numerous fish populations. Furthermore, there is a need to establish appropriate surrogate species, suitable for SFE assessments, as current US EPA model toxicity testing organisms; e.g., fathead minnows (*Pimephales promelas*) are not necessarily well suited to this freshwater-to-estuarine environment. Inland silversides are present in the SFE, tolerant of a wide range of salinities, commercially available, and are approved for marine and estuary toxicity testing, by the US EPA[1] .

Biomarkers can be used to establish specific health conditions. Many definitions of what constitutes a biomarker have been postulated, but the broad definition can be expanded to include measurable biological responses that can be used as indicators of organismal health, especially since biomarkers can be applied to assess the effect of numerous stressors beyond contaminants (e.g., environmental variables such as disease, dissolved oxygen, salinity, and temperature), as well to establish nutritional and reproductive conditions. Thus, for the purpose of this exercise (and document) biomarkers will be defined within the context of their applicability to SFE monitoring and research, as biological indicators that potentially measure stressor effects, which may be used predictively and, where possible, in conjunction with responses at higher levels of biological organization.

Questions:

- How can biomarkers help us understand the relative health of organisms and the natural variability in these measured conditions?
- What is the relative importance of biomarkers on individual organisms and population health?

Our underlying goals are to advance our current understanding of the relationships between stressors and biomarker responses, determine the relative importance of various stressors and devise potential measures to mitigate the effects of these stressors.

As mentioned previously, the 2007 report “Biomarkers and the Pelagic Organism Decline” presents an overview and outline of traditional biomarkers, along with recommendations towards their application within the SFE. Much research has been conducted over the past six years, since this publication, and advanced technologies have become more available and at considerably reduced costs.

The key questions, addressed by Anderson et al (2007) can thus be updated to ask:

- How can current biomarker systems be used strategically to determine whether anthropogenic, physicochemical, and/or biological influences are causing significant stress in SFE species?
- What is the relative importance of these stressors to individual and population-level impacts, and thus ecosystem functioning?

In order to interpret stressor effects, addressing these questions must take into consideration geographic, seasonal, and temporal variability of the fish populations, understanding the organismal properties, life stages, and sensitivities. Furthermore, it is essential to incorporate interactions of anthropogenic, physicochemical and

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[1] http://water.epa.gov/scitech/methods/cwa/wet/

biological influences - including routes of exposure and uptake, chemical properties, and concentrations, along with overall health condition.

A primary advantage of using biomarkers, from a contaminants' perspective, is that they can integrate the toxic potency of complex chemical mixtures (along with water physicochemical parameters and possible disease interactions), even when the chemical composition of the mixtures is unknown, or unidentified (Hamers et al. 2013). However, there are also disadvantages to using biomarkers, in that it is not always clear how well they describe the ability of organisms to recover (Nichols et al. 2011, Forbes and Calow 2012), or the relative importance or changes in biomarkers to whole organism and populations (Ankley et al. 2009).

Given the pros and cons, there are a limited number of studies in which biomarkers have been used in complement with monitoring programs. For biomarkers to become applicable in the SFE, baseline criteria are required that quantify the range of response by a given species. Such baseline validation of a biomarker can then complement ongoing population monitoring efforts involving health, abundance and distribution. Hamers et al. (2013) highlight this by stating that "*no environmental quality criteria have been derived for the different endpoints used in effect-based methods, to which the obtained monitoring results can be compared*" and that criteria requires the incorporation of spatio-temporal variability. However, Escher and Leusch (2012) counter-indicate that by using equivalent concentrations (e.g., estradiol or ethylene estradiol (EE2) equivalents) the effect of estrogens can be quantified using in-vitro methods and related to environmental quality standards for estradiol/EE2).

- How can baselines, references or controls be established for field-based assessments?
- How can spatio-temporal variability be incorporated into biomarker data analyses?
- What are the relevant pros and cons that we need to be aware, or cautious, of?

Current and ongoing studies

The following brief summaries are intended to provide the reader with an overview of relevant Delta-specific research that has considered biomarkers, and/or other exposure and effects assessments, which may be considered during the workshop discussions. The studies have been compiled by the stressor(s) being assessed, and the relevant analytical method used.

Biomarker profiling has been, and is being performed at molecular and biochemical levels on delta smelt, longfin smelt, inland silversides, fathead minnows, and striped bass. These include, but are not limited to, microarray and qPCR assessments, vitellogenin and choriogenin, metallothionein, acetyl-cholinesterase (AChE), ethoxyresorufin-O-deethylase (EROD), determination of triglycerides, hepatosomatic (HSI) and gonadosomatic indexing (GSI), that aim to integrate measurements such as growth, reproductive state, and sex ratios.

Contaminants: Many investigations have been conducted on numerous contaminants under laboratory, and ex/situ conditions, (using both vertebrate and invertebrate organisms). Numerous pesticide toxicity studies have been conducted (active ingredients and commercial products, utilizing native, as well as surrogate species (Connon et al. 2009, Eder et al. 2009, Baxa et al. 2010, Beggel et al. 2010, Werner et al. 2010b, Beggel et al. 2011, Connon et al. 2011a, Beggel et al. 2012, Connon et al. 2012b, Durieux et al. 2012, Brander et al. in press). Assessments on ambient water samples have been conducted on specific sites of interest in the SFE (Werner et al. 2008, Werner et al. 2010b, Brooks et al. 2012, Connon et al. 2012b), on treated wastewater and discharges (e.g. Werner et al. 2009, Connon et al. 2011b, Connon and Brander 2012-2015 Grant in progress, Brander et al. in press, Hasenbein et al. in press) creeks (You et al. 2008, Weston and Lydy 2010, Ding et al. 2011), and agricultural runoff (e.g. vegetated ditches Moore et al. 2008, Werner et al. 2010a, Moore et al. 2011) as well as specific population studies on field-caught striped bass (Ostrach et al. 2008, Ostrach and Groff 2009, Durieux et al. 2011, Spearow et al. 2011) and delta smelt throughout the Delta (Teh et al. 2013 Grant in progress-a).

Physicochemical influences: Field data (fish sampling) have been used to determine temperature, salinity, and turbidity ranges in which fish are found, but there has been a lack of studies involving tolerance and adaptation to differing environments. Because of this, numerous behavioral, physiological, and mechanistic studies have recently been, and are being conducted by researchers at UC Davis to assess optimum temperature and salinity, as well as tolerance ranges (e.g., CT/CSmax and CT/CSmin) (Connon et al. 2011-2014 Grant in progress) as well as turbidity effects (Connon et al. 2012-2015 Grant in progress, Hasenbein et al. 2013), in different life stages (larval, juvenile, adult) fish species – focused predominantly on delta smelt and longfin smelt (Fangue and Connon 2013-2014 - Grant in progress). Parallel studies are being conducted on field caught delta smelt, establishing population distribution as determined by salinity and turbidity (Teh et al. 2013 Grant in progress-b)

Low dissolved oxygen (DO) levels can lead to decreased swimming performance, reduced growth, impaired development, and increased susceptibility to predation, parasites, pathogens, and contaminants (Newcomb and Pierce 2010 - Report, Brooks et al. 2012). Low DO has been shown to impact adult Chinook salmon and steelhead, during upstream migration, while juvenile salmonids may be exposed to low DO during downstream migration. Areas within the SFE system are prone to low DO concentrations, some of which have been improved upon. The Stockton Deep Shipping Channel (DWSC), for example was an area in which DO concentrations have dropped as low as 2 to 3 mg/L during warmer periods and low water flows in the San Joaquin River[2]. Wastewater treatment plant upgrades along with aeration treatments, have significantly improved in recent years.

Further studies investigated the effects of temperature and blood oxygen equilibria in hardhead minnow (Kaufman et al. 2013), and salinity preferences of juvenile green sturgeon (Poletto et al. 2013), as well as effects of water diversions, and physical structures, on behavior and swimming performance of Chinook salmon (Mussen et al.

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[2] http://baydeltaoffice.water.ca.gov/sdb/af/index_af.cfm

2012). Many of these fish physiology approaches could be utilized within a biomarker framework to link mechanistic responses with higher levels of biological organization. Moreover, physiological depuration and elimination of contaminants trigger higher oxygen demand. Reviews of the energetic and other indirect interactions between warming climate and multiple stressors, including contaminants, are found in Sokolova et al. (2012) and Noyes et al. (2009).

Biological influences: Pathogen-contaminant interactions have been demonstrated in various studies (e.g., Clifford et al. 2005, Eder et al. 2007, Eder et al. 2008) and health status determined using biomarkers has been correlated to migration success (Miller et al. 2011, Connon et al. 2012a). Immune system effects, and disease, are thus important factors to take into consideration when assessing overall population health.

Studies have been conducted on the dietary effects of microcystin on threadfin shad, *Dorosoma petenense* (Acuña et al. 2012a), and on Sacramento splittail (Acuña et al. 2012b), as well as on estimating the abundance of toxic *Microcystis* (Baxa et al. 2010) and effects on copepods (Ger et al. 2009, Ger et al. 2010) within the SFE, encompassing biological, physicochemical, and toxicity assessments.

Assessments of the effects of anthropogenic, physicochemical, and biological influences, range across different levels of biological organization, but few studies have successfully addressed this in an integrative manner. Plus, there is still lacking integration in the assessments of the interactive effects of multiple stressors (e.g., contaminant mixtures, contaminants and disease). Current studies are addressing this interaction, by utilizing morphometric and nutritional biomarkers to determine condition factors (CF), the hepatosomatic index (HPI) and gonadosomatic index (GSI), in wild caught delta smelt, and compare these, via multivariate analytical methods, to biomarker responses associated with contaminant exposure (e.g., AChE, and EROD), effects of salinity (e.g., Na^+/K^+-ATPase) and other physicochemical influences, and the presence and severity of pathogens including subclinical (carriers) infections with Mycobacterium (Teh et al. 2013 Grant in progress-a).

Quantitative PCR and micorarrays were also used to identify site specific signatures in delta smelt exposed to ambient samples collected as sites of interest throughout the SFE (Connon et al. 2012b) that could potentially be used towards determining contaminant point sources (Hasenbein et al. in press).

Selected recent and current research conducted on SFE species, are presented in table 1, highlighting present expertise. (Further information for the cited literature is provided in this document's annotated reference list).

Table 1: *Select studies, approaches and methodologies recently and/or currently utilized to assess the effect of stressors (C; Contaminant effects, P: Physicochemical influences, B: Biological influences) upon SFE species of interest, as well as surrogate species.*

Species	Stressor	Principal assessment methods	Research references	
Chinook salmon	Entrainment	Behavior/Swimming performance	(Mussen et al. 2012)	P
Chinook salmon	Chlorpyrifos/Esfenvalerate/IHMV	Biochemistry	(Eder et al. 2007)	C/B
Chinook salmon	Chlorpyrifos/Esfenvalerate/IHMV	qPCR	(Eder et al. 2008)	C/B
Chinook salmon	Chlorpyrifos/Esfenvalerate	qPCR/Biochemistry	(Eder et al. 2009)	C
Delta smelt	Esfenvalerate	qPCR/Microarray/	(Connon et al. 2009)	C
Delta smelt	Temperature/Salinity	qPCR/CTmax/min	(Connon et al. 2011-2014 Grant in progress, Teh et al. 2013 Grant in progress-a)	P
Delta smelt	Copper	qPCR/Microarray/ behavior	(Connon et al. 2011a)	C
Delta smelt	Ammonia/um	qPCR/Microarray/ behavior	(Connon et al. 2011b)	C
Delta smelt	Ambient samples	qPCR/Microarray	(Connon et al. 2012b, Hasenbein et al. in press)	C
Delta smelt	Salinity/Turbidity	qPCR/Biochemistry /feeding	(Connon et al. 2012-2015 Grant in progress, Hasenbein et al. 2013)	P
Delta smelt	Ammonia/um ambient/WTP	qPCR/Microarray/ behavior	(Hasenbein et al. in press)	C
Delta smelt	Mycobacterium	qPCR	(Teh et al. 2013 Grant in progress-a)	B
Fathead minnow	Triclosan	qPCR/Biochemistry/behavior	(Fritsch et al. 2013)	C
Fathead minnow	Ambient samples	qPCR/Biochemistry/Growth/Survival	(Deanovic et al. 2013 grant in progress)	C/P/B
Fathead minnow	Bifenthrin, Talstar, Fipronil, Thermidor	qPCR/Swimming performance	(Beggel et al. 2010)	C
Fathead minnow	Bifentrhin	qPCR/Swimming performance	(Beggel et al. 2011)	C
Fathead minnow	Fipronil	qPCR/Swimming performance	(Beggel et al. 2012)	C
Green sturgeon	Salinity	Swimming performance	(Poletto et al. 2013)	P
Hardhead minnow	Temperature	Blood oxygen capacity/Swimming performance	(Kaufman et al. 2013)	P
Inland silversides	Wastewater treatment effluent	Biochemistry	(Brander et al. 2012a)	C
Inland silversides	Bifenthrin and permethrin	Cell assays/ Biochemistry	(Brander et al. 2012b)	C
Inland silversides	Wastewater treatment effluent	qPCR/Biochemistry/cell assays/ histopathology /sex ratio	(Brander et al. in press)	C
Sacramento splittail	Microcystin	Histopathology	(Acuña et al. 2012b)	B
Steelhead	Disease/ health status	qPCR / PCR/ disease / visual condition	(Connon et al. 2012a)	B
Striped bass	Ambient samples	AChE	(Durieux et al. 2011)	C
Striped bass	Beta Napthoflavone	qPCR/Biochemistry	(Durieux et al. 2012)	C
Striped bass	Esfenvalerate /Copper	qPCR	(Geist et al. 2007)	C
Striped bass	Mixtures	Histopathology	(Ostrach et al. 2008, Ostrach and Groff 2009)	C
Striped bass	Mixtures	Biochemistry	(Spearow et al. 2011)	C
Striped bass	Ambient samples	Histopathology	(Teh et al. 2013 Grant in progress-a)	C/P/B
Threadfin shad	Microcystin	Histopathology	(Acuña et al. 2012a)	B
Various	Microcystis/Microcystin	PCR	(Baxa et al. 2010)	B
Yellowfin goby	Henneguya spp	Histopathology/PCR	(Baxa et al. 2013)	B

Conceptual Model

A thorough and balanced understanding of the relationships and exposure pathways, between stressors and organisms, will be critical for interpreting biomarker data in light of their environmental relevance and related stressors. Existing conceptual models for the SFE, have been developed by CALFED Ecosystem Restoration Program (ERP) implementing agencies (DFG, USFWS, and NOAA Fisheries) under the auspices of the Delta Regional Ecosystem Restoration Implementation Plan (DRERIP, please refer to link below[3]) targeted at processes (sediment loads, food web), stressors (contaminants and physicochemistry), habitats (tidal, riparian, floodplains) and fish species of concern.

The Chemical Stressors is part of a suite of conceptual models that collectively articulate the current understanding of important aspects of the Sacramento-San Joaquin River Delta ecosystem. They are designed to structure scientific information such that it can be used to inform sound public policy. DRERIP conceptual models are qualitative models designed to be used by experts to facilitate informed discussions. The Chemical Stressors conceptual model integrates with other DRERIP conceptual models (e.g., contaminant transport, aquatic vegetation, species life history, and food web conceptual models). Incorporated in the Chemical Stressors conceptual model are sub-models pertaining to transport and fate/exposure assessment, bioavailability, bioaccumulation, and toxic effects.

Biomarker monitoring could also improve our understanding of chemical, physicochemical (e.g., salinity, temperature, and flow) and potential biological (e.g., disease) influences (Fig. 2) on organisms and help address questions arising from the abovementiones DRERP conceptual models.

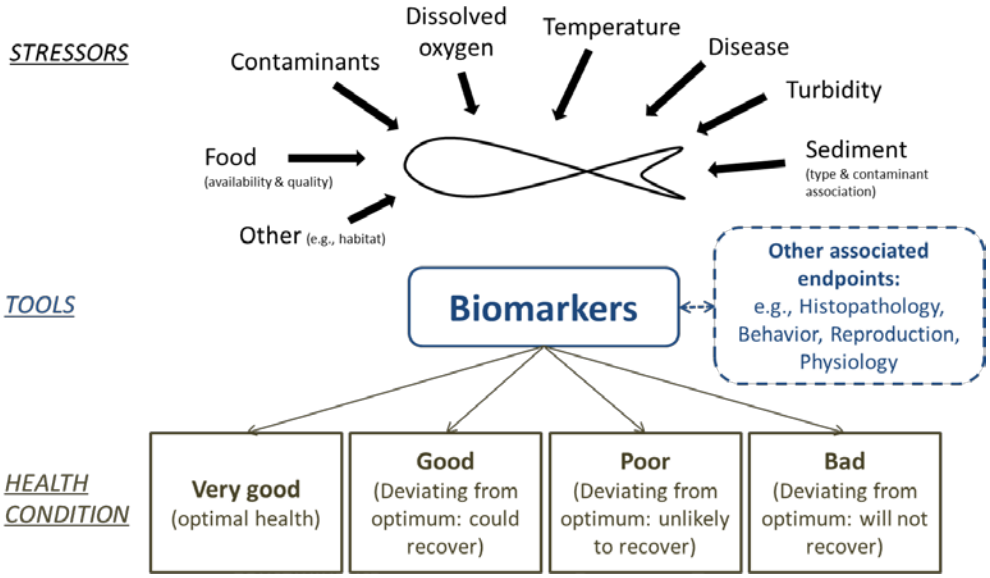

Figure 2. *Conceptual application of biomarkers in the assessment of fish health condition.*

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[3] http://www.dfg.ca.gov/erp/current_models.asp

There are currently no conceptual models available for endocrine disrupting chemicals (EDCs), chemicals of emerging concerns (CECs), pharmaceutical and personal care products (PPCP), nor for physicochemical and biological influences. The role of pathogens and disease has not been a focus of investigation within natural fish populations in the SFE ecosystem, therefore the risks are unknown and have been questioned; however it is an important parameter that is regularly associated with overall stress, and which has, for example, recently been identified as a major cause of bee colony disorder (Pettis et al. 2013).

Within studies where laboratory/hatchery reared organisms are used, the immune system is an important parameter to assess and consider, since a "naïve" individual may be greatly affected by exposure to ambient water samples. As such, the immune system is an important variable to be assessed with the use of biomarkers.

Specific questions to the panel

The overarching objective of this workshop is to discuss and seek guidance on how biomarkers can be used for evaluating stressors and/or adverse effects on SFE fish species. To this end, we request that the expert panel provide guidance on developing a framework to determine the role and suitable application of biomarkers within the SFE ecosystem. Specific questions are:

- What specific information on health condition should be obtained to support biomarker assessments and monitoring? Consider contaminant transport and fate, bioavailability, and bioaccumulation, in conjunction with physicochemical and biological influences.
- What are the current benefits and limitations of the use of single versus multi-biomarker approaches?
- What is the suitability of current biomarkers and/or novel approaches such as genomics, proteomics and metabolomics, to monitoring population health?

Field applications of these techniques are believed to be extremely valuable. However, numerous issues could confound interpretation, including lack of controls, and baselines that indicate what an optimal health condition in the field would be. Furthermore, while field sampling offers critical information on organisms that have survived environmental conditions, it is unable to offer any information on the number and condition of non-surviving individuals. Laboratory studies may provide essential for answering relevant research questions, but organisms that are generally utilized have been reared under stress-free (stress-reduced) conditions. *In-situ* and *ex-situ*[4]

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[4] *In situ* generally refers to caged organisms from a lab culture or field collection that are exposed directly in the field to all ambient conditions prior to making observations of effects.

[4] *Ex situ* generally refers to caged organisms from a lab culture or field collection that are exposed directly to field media (e.g., flow-through surface water) in a field laboratory enclosure where light and temperature or other variables can be controlled.

assessments have been suggested to bridge the gap between the field and the laboratory, but are also somewhat restricted in their use and in the interpretation of their findings. For some species, such as endangered species, these tests are further restricted by take/release permits (Fig. 3a) whilst others such as hatchery-reared salmonid (Fig. 3b) can be tagged and released, and their life history assessed through outmigration to return (Fig. 3b). Other species, such as striped bass, which have no take restrictions, could provide some information on SFE stressors; however, differing life cycles add further complexities to this approach.

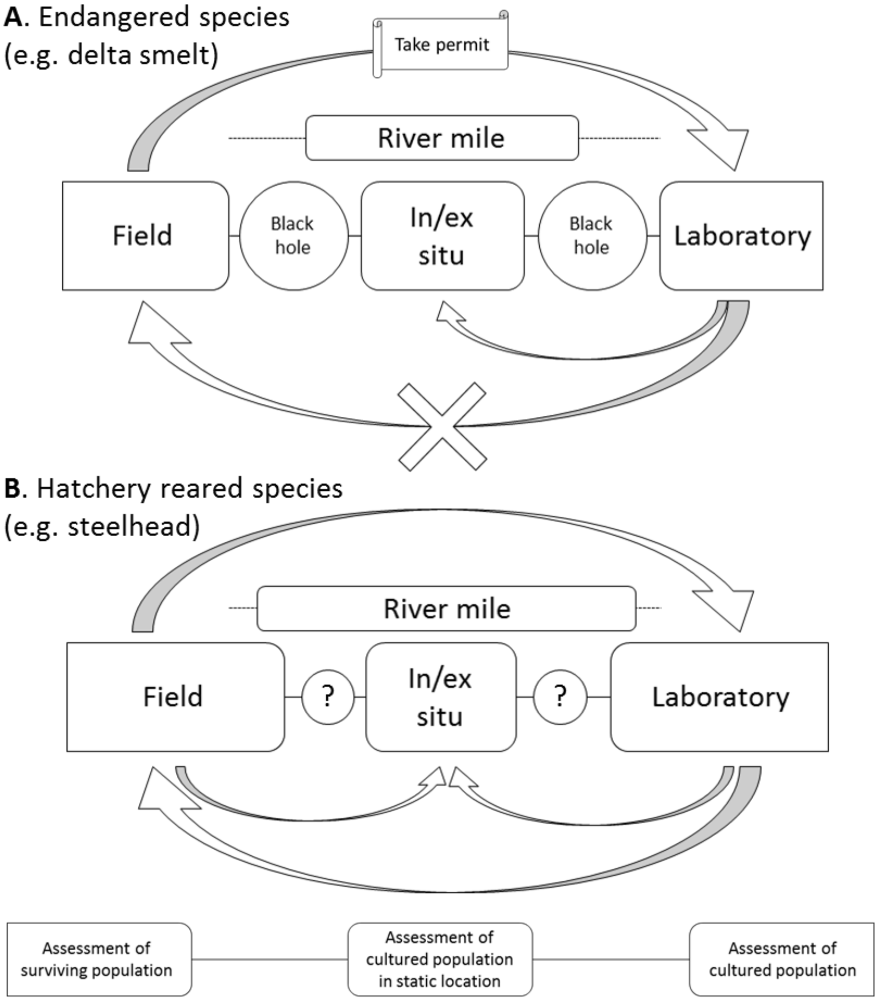

Figure 3. Field vs. laboratory tests: *possible assessments dependent on fish permits. A. An endangered species such as the delta smelt can be collected from the field, and taken to the laboratory for specific assessments, however, laboratory reared organisms cannot be released into the field. Laboratory reared fish, can be used for in/ex-situ exposures (and River mile assessments under controlled conditions). However, because of this, links between laboratory and field based studies, difficult due to a lack of information (black holes). B. Hatchery raised species, assessed in in/ex situ studies, can be used to cross these boundaries, and reduce this lacking information (black holes), thus laboratory and field based studies can be integrated more fully. Field populations generally represent surviving individuals from a population, thus are not fully representative of the direct impact that contaminants may have upon overall population numbers and condition factors. On the other hand,*

laboratory studies generally utilize laboratory-reared individuals that are not necessarily environmentally representative. Placing laboratory-reared individuals in in/ex-situ devices aims to generate more environmental relevance; however, these approaches fix individuals within a specific location (most fish species in the SFE system are not necessarily territorial). River mile studies, that is, a series of strategically located in/ex-situ exposures can offer additional insight into the effect of contaminants and other stressors of interest.

Key stressors of concern include contaminants (agricultural, urban, industrial) salinity, temperature (climate change, riparian shade), turbidity, dissolved oxygen, (nutrients and productivity), food availability and quality, and disease.

- How can biomarker systems be used to assess effects of these stressors, and their interactions within 1) field populations? 2) laboratory studies? and 3) *in-situ*/*ex-situ* exposures?

- How best can field and laboratory based studies be integrated, from a biomarker perspective?

- How do non-lethal vs. lethal sampling limit the use of biomarker assessments?

Sensitivity to any particular stressor or to different stressors can vary among species, life-stages, and environmental conditions; however, model/surrogate species are usually utilized within laboratory studies to interpret and infer responses to exposures. This is particularly true when it is either not possible to conduct studies with, or tools are lacking species of concern. Therefore, it may be useful and necessary to evaluate multiple species and endpoints using biomarkers. As an example, a few species of interest in the Delta can be broadly ranked according to their sensitivity to contaminants as:

1) Sensitive (e.g., Delta smelt; *Hypomesus transpacificus*)
2) Moderately sensitive (e.g., Inland silversides; *Menidia audens*)
3) Tolerant (e.g., rainbow trout; *Oncorhynchus mykiss,* striped bass; *Morone saxatilis*)

Commonly used lab test species (e.g., fathead minnows; *Pimephales promelas,* Japanese medaka; *Oryzias latipes*) are not necessarily comparative to species of interest, but are nevertheless useful for developing models using a knowledge-based perspective.

- Should multiple species or a single species be selected as a model for biomarker investigations? Which species and why?

Many biomarkers have not been validated for use as monitoring tools because they lack specificity. Adverse outcome pathways (AOPs) integrate assessments conducted at the cellular level through pathways that lead to adverse outcomes at higher levels of biological organization. Ankley et al (2010) recommend the use of AOPs to clearly define the longer-term effects of contaminants, thus understanding challenges to physiological mechanisms that lead to detrimental outcomes.

Our overall intention is to evaluate the use biomarkers in the assessment of organismal health within monitoring programs such as ambient surface waters, as well as their integration in the assessment of the efficacy of restoration programs.

- How can AOPs or associations with higher levels of biological organization be integrated into the Delta monitoring approaches?

Finally, the SFE is an extremely complex ecosystem, with numerous variables and multiple interactions. This is confounded with differences in life histories and habitat preferences (and tolerances) amongst the species of interest (Brooks et al. 2012). Further, Anderson et al. (2007) concluded that field chemistry, stressors of potential concern, and general water quality parameters, along with fish health and condition indices, are needed to interpret biomarkers in a weight of evidence approach.

- How best can we integrate life histories, and specific life stages into planned studies?

- What additional information should be collected to aid interpretation of biomarker data?

- What analytical approaches would likely be most useful for interpreting biomarker data and understanding its environmental relevance?

- How do we extrapolate biomarker findings to fundamental fitness parameters such as survival, reproduction?

Required reading and recommended background material

Other than the background presented in this document, we have included the following information:

- Anderson et al. (2007), Biomarkers and the pelagic organism decline: conclusions of the POD Biomarker Task Force, Fort Mason, San Francisco, August 29-30, 2007.

- Hamers et al. (2013), Expert Opinion on Toxicity Profiling – Report from a NORMAN Expert Group Meeting. Integrated Environmental Assessment and Management. 9(2). 185-191.

- Teh et al. (2012) ANNUAL REPORT: (August 2011 – May 2012): Fall Low Salinity Habitat (FLaSH) Fish Health Study: Contrasts in Health Indices, Growth and Reproductive Fitness of Delta Smelt Rearing in the Low Salinity Zone and Cache Slough Regions.

Furthermore, so as to facilitate the panels' access to further information, the references for this document are presented in annotated form so as to include the abstracts of the citations.

URL links to further documentation and unpublished reports are provided either as footnotes within this document, or listed within the reference section below. For further background documentation, that may not be included in this document, please contact Richard Connon, at reconnon@ucdavis.edu.

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References (including abstracts and URL where available)

Acuña, S., D. Baxa and S. Teh (2012a). "Sublethal dietary effects of microcystin producing Microcystis on threadfin shad, Dorosoma petenense." Toxicon **60**(6): 1191-1202 http://www.ncbi.nlm.nih.gov/pubmed/22925841.

> The presence of the toxic cyanobacterium Microcystis in the upper San Francisco Estuary (SFE) since 1999 is a potential but unquantified threat to the health and survival of aquatic organisms such as fish and zooplankton. The microcystins (MCs) predominantly in the LR-form (MC-LR) produced by Microcystis is hepatotoxic and a potential threat to the fishery. Concurrently, in the SFE significant declines in pelagic fish, known as the Pelagic Organism Decline (POD), has been recognized by state and federal agencies since 2000. In 2005, the presence of the toxic algal bloom, Microcystis has been hypothesized as a link to the POD by the Interagency Ecology Program Management Team. This study aims to characterize the toxic effects of Microcystis in one of the POD species, threadfin shad (Dorosoma petenense) by exposure to diets containing Microcystis harvested from the SFE. The diets contained Microcystis with 4.4 (D5) and 10.0 (D10) mug g(-)(1) MC-LR that was fed to threadfin shad for 57 days. The treatments were compared to the control diet, 0 mug g(-)(1) MC-LR (D0). Results showed that ingested Microcystis was localized in the gut by in situ hybridization and MCs were localized in the tissues of the gut, kidney and liver. Condition factor (CF) and liver and gonadal lesions were sensitive to MC exposure. There was a significant inverse relationship between CF and MC-LR with exposed fish exhibiting severe cachexia. Liver lesions of sinusoidal congestion and glycogen depletion significantly increased with increasing MC-LR concentrations, indicating hemorrhaging in the liver and poor nutritional status, respectively. In females, there was a significant increase in severe ovarian necrosis with increasing MC-LR concentration, indicating loss of reproductive potential. The results indicate that MC-LR from Microcystis significantly impairs the health and reproductive potential of threadfin shad has a potential negative effect on populations in the SFE.

Acuña, S., D.-F. Deng, P. Lehman and S. Teh (2012b). "Sublethal dietary effects of Microcystis on Sacramento splittail, Pogonichthys macrolepidotus." Aquat Toxicol **110–111**(0): 1-8 http://www.sciencedirect.com/science/article/pii/S0166445X11003389.

> The presence of the toxic cyanobacterium Microcystis in the upper San Francisco Estuary (SFE) since 1999 is a potential but to date an unquantified threat to the health and survival of aquatic organisms, such as fish and zooplankton. The microcystins (MCs) predominantly in the LR-form (MC-LR) contained in Microcystis is hepatotoxic and a potential threat to the fishery. This study was conducted to determine the effects of dietary exposure of the endemic Sacramento splittail, Pogonichthys macrolepidotus in SFE to Microcystis and its toxin, MC-LR. Juvenile splittail (12.59 ± 0.7 g fish−1) were exposed to five diets for 28 d with MC-LR obtained from: (1) Microcystis harvested from the SFE and (2) a synthetic purified form of MC-LR. Three of the test diets contained 3.55 (D5), 9.14 (D10) and 17.13 (D20) mg MC-LR kg−1 from Microcystis. The other two diets contained either purified MC-LR at 3.89 mg MC-LR kg−1 (D5R) or no MC-LR (D0). The RNA/DNA ratio of fish muscle was significantly lower for all treatments fed test diets containing MC-LR compared to the control diet D0, suggesting Microcystis adversely affected nutritional status. Protein phosphatase

2A expression in the fish from the D5, D10 and D20 treatments were inversely affected by increasing concentrations of MC-LR. Cytoplasmic inclusion bodies and single cell necrosis were more prevalent and greater in severity in the fish exposed to the diets D10 and D20 compared to fish from the D0 treatment and indicate severe liver toxicity in splittail exposed to MC-LR. The sublethal effects on splittail characterized by this study suggest cyanobacterial blooms have the potential to affect splittail nutritional status and health in SFE.

Anderson, S. L., G. Cherr, R. Baxter, D. Nacci, B. Herbold, D. Schlenk, K. Kuivila, E. Gallagher and L. R. Judah (2007). Biomarkers and the pelagic organism decline: conclusions of the POD Biomarker Task Force, Fort Mason, San Francisco, August 29-30, 2007 http://docsfiles.com/pdf_pelagic_organism_decline.html.

Ankley, G. T., D. C. Bencic, M. S. Breen, T. W. Collette, R. B. Conolly, N. D. Denslow, S. W. Edwards, D. R. Ekman, N. Garcia-Reyero, K. M. Jensen, J. M. Lazorchak, D. Martinovic, D. H. Miller, E. J. Perkins, E. F. Orlando, D. L. Villeneuve, R. L. Wang and K. H. Watanabe (2009). "Endocrine disrupting chemicals in fish: developing exposure indicators and predictive models of effects based on mechanism of action." Aquat Toxicol **92**(3): 168-178 http://www.ncbi.nlm.nih.gov/pubmed/19261338.

Knowledge of possible toxic mechanisms (or modes) of action (MOA) of chemicals can provide valuable insights as to appropriate methods for assessing exposure and effects, thereby reducing uncertainties related to extrapolation across species, endpoints and chemical structure. However, MOA-based testing seldom has been used for assessing the ecological risk of chemicals. This is in part because past regulatory mandates have focused more on adverse effects of chemicals (reductions in survival, growth or reproduction) than the pathways through which these effects are elicited. A recent departure from this involves endocrine-disrupting chemicals (EDCs), where there is a need to understand both MOA and adverse outcomes. To achieve this understanding, advances in predictive approaches are required whereby mechanistic changes caused by chemicals at the molecular level can be translated into apical responses meaningful to ecological risk assessment. In this paper we provide an overview and illustrative results from a large, integrated project that assesses the effects of EDCs on two small fish models, the fathead minnow (Pimephales promelas) and zebrafish (Danio rerio). For this work a systems-based approach is being used to delineate toxicity pathways for 12 model EDCs with different known or hypothesized toxic MOA. The studies employ a combination of state-of-the-art genomic (transcriptomic, proteomic, metabolomic), bioinformatic and modeling approaches, in conjunction with whole animal testing, to develop response linkages across biological levels of organization. This understanding forms the basis for predictive approaches for species, endpoint and chemical extrapolation. Although our project is focused specifically on EDCs in fish, we believe that the basic conceptual approach has utility for systematically assessing exposure and effects of chemicals with other MOA across a variety of biological systems.

Ankley, G. T., R. S. Bennett, R. J. Erickson, D. J. Hoff, M. W. Hornung, R. D. Johnson, D. R. Mount, J. W. Nichols, C. L. Russom, P. K. Schmieder, J. A. Serrano, J. E. Tietge and D. L. Villeneuve (2010). "Adverse outcome pathways: A conceptual framework to support

ecotoxicology research and risk assessment." Environmental Toxicology and Chemistry **29**(3): 730-741 http://dx.doi.org/10.1002/etc.34.

> Ecological risk assessors face increasing demands to assess more chemicals, with greater speed and accuracy, and to do so using fewer resources and experimental animals. New approaches in biological and computational sciences may be able to generate mechanistic information that could help in meeting these challenges. However, to use mechanistic data to support chemical assessments, there is a need for effective translation of this information into endpoints meaningful to ecological risk—effects on survival, development, and reproduction in individual organisms and, by extension, impacts on populations. Here we discuss a framework designed for this purpose, the adverse outcome pathway (AOP). An AOP is a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment. The practical utility of AOPs for ecological risk assessment of chemicals is illustrated using five case examples. The examples demonstrate how the AOP concept can focus toxicity testing in terms of species and endpoint selection, enhance across-chemical extrapolation, and support prediction of mixture effects. The examples also show how AOPs facilitate use of molecular or biochemical endpoints (sometimes referred to as biomarkers) for forecasting chemical impacts on individuals and populations. In the concluding sections of the paper, we discuss how AOPs can help to guide research that supports chemical risk assessments and advocate for the incorporation of this approach into a broader systems biology framework. Environ. Toxicol. Chem. 2010;29:730–741. © 2009 SETAC

Baxa, D., A. Stover, M. A. Clifford, T. Kurobe, S. J. Teh, P. Moyle and R. P. Hedrick (2013). "Henneguya sp. in yellowfin goby *Acanthogobius flavimanus* form the San Francisco Estuary." Springer Plus.

> Myxozoan spores were observed in yellowfin goby Acanthogobius flavimanus collected from Suisun Marsh, San Francisco Estuary. The spores formed plasmodia within the gastric and intestinal mucosa that extended into the gut lumen. Although histopathological changes associated with the parasite were not observed, the plasmodia forming myxozoan can block the linings of the intestine and stomach that may affect the perfomance and survival of the yellowfin goby. Morphological features of the spores resembled Henneguya sp. and molecular analysis of the 18S ribosomal DNA (Domain III) confirmed close similarity to H. rhinogobii and H. pseudorhinogobii isolated from the Japanese freshwater goby. However, the yellowfin goby myxozoan is likely an undescribed species based on phylogenetic studies and morphologic features. Detailed descriptions of vegetative and spore stages are currently lacking for proposal to a new species of Henneguya. A specific PCR test was developed, which confirmed a 100% prevalence of the parasite among randomly collected gobies in group 1 (N=30) and group 2 (N=15) at termination of the study at one month in captivity. The myxozoan was also detected from 18 gobies (12%) that died in the first group within two weeks in captivity. Apparently healthy gobies that were examined as controls did not reveal the presence of the myxozoan. While the pathogenesis of the parasite was not confirmed, this study documents the occurrence of a potentially new species of myxozoan in the yellowfin goby and underscores the detection of a parasitic infection in an introduced fish in the San Francisco Estuary. It is likely that the myxozoan

parasite came along with the yellowfin goby upon its introduction into the estuary based on the high similarity score in the Domain III conserved region between the gobiid myxozoans from the two distant locations. While the prevalence of the myxozoan as reported here is restricted to a comparatively small collection site in Suisun slough, the reemergence, identification, and ecological relevance of the parasite on goby populations in the San Francisco Estuary may be investigated in the future using the diagnostic tool developed in this study.

Baxa, D. V., T. Kurobe, K. A. Ger, P. W. Lehman and S. J. Teh (2010). "Estimating the abundance of toxic Microcystis in the San Francisco Estuary using quantitative real-time PCR." Harmful Algae **9**(3): 342-349 http://www.sciencedirect.com/science/article/pii/S1568988310000028.

> Developing an effective and rapid method to identify and estimate the abundance of Microcystis is warranted in the San Francisco Estuary (SFE) in view of expanding cyanobacterial blooms dominated by Microcystis spp. Blooms that occurred in the estuary from July to September 2007 were initially assessed using a standard polymerase chain reaction (PCR) employing primers designed for the conserved Microcystis-specific 16S ribosomal DNA (rDNA) region. The presence of microcystin-producing (MC+) toxic Microcystis was observed in cyanobacterial and water samples as shown by the amplification of the MC toxin synthetase genes mcyB and mcyD by standard PCR. The goal of this study was to develop a real-time quantitative PCR (qPCR) based on the 16S rDNA and mcyD gene sequences of Microcystis found in the SFE to quantify the proportion of toxic Microcystis with mcyD genes among total Microcystis or cyanobacterial population. Cyanobacterial samples collected by diagonal net tows of the water column showed that the ratio of gene copies was dominant for Microcystis among cyanobacteria (28–96%), and Microcystis carrying mcyD genes formed 0.4–20% of the total Microcystis spp. Total Microcystis was also abundant (7.7 × 104 to 9.9 × 107 cells L−1) in ambient surface waters, and the range of Microcystis cell equivalents with mcyD genes (4.1 × 102 to 2.2 × 107 cells L−1) indicated a large variation in the ratio of toxic Microcystis among total Microcystis (0.01–27%). Differences in the proportion of toxic and nontoxic Microcystis, as deduced from the cell equivalents of total Microcystis, were observed across the sampling locations and seasons where concentrations of total MCs (0.007‑10.81 μ g/L) also varied. By revealing trends in the sources and magnitude of toxic and nontoxic Microcystis, qPCR can contribute to rapid risk assessment and prediction of strategies designed to manage the adverse effects of cyanobacterial blooms in the SFE.

Baxter, R., R. Breuer, L. Brown, L. Conrad, F. Feyrer, S. Fong, K. Gehrts, L. Grimaldo, B. Herbold, P. Hrodey, A. Mueller- Solger, T. Sommer and S. K. (2010). Interagency Ecological Program 2010 Pelagic Organism Decline work plan and synthesis of results. Interagency Ecological Program for the San Francisco Estuary http://www.water.ca.gov/iep/docs/FinalPOD2010Workplan12610.pdf.

> Abundance indices for four pelagic fishes in the upper San Francisco Estuary (the Delta and Suisun Bay) rapidly declined to record low levels starting in 2002. These fishes include native delta smelt (listed under federal and California Endangered Species acts) and longfin smelt (listed under the State Endangered Species Act) as well as introduced threadfin shad and juvenile (age-0) striped bass. Three of these species have

also experienced more gradual long-term declines, but the recent rapid collapse of all four species to persistently low levels was unexpected given the relatively moderate hydrological conditions in the first half of this decade. In 2005, the IEP formed a Pelagic Organism Decline Management Team (POD-MT) to evaluate the potential causes of the declines. The POD-MT has developed several conceptual models to guide work plan development and 267 synthesize results. In this report we update previously developed conceptual models with new 268 results and introduce a new conceptual model: 1. The ―basic POD conceptual model was introduced in 2006 and groups the effects of potential drivers of the POD into four categories (previous abundance, habitat, top-down effects, and bottom-up effects); 2. ―Species-specific conceptual models were introduced in 2008 and show how key population drivers presently affect each of the four POD fish species in each season; 3. A new conceptual model posits that the POD represents a rapid ecological ―regime shift that followed a longer-term erosion of ecological resilience. We present this conceptual model as a working hypothesis for future investigations. Much has been learned about individual drivers and their effects on the POD species over the course of the POD investigation. An initial ―triage approach seeking to rule out individual drivers was unsuccessful – we now have evidence that all investigated drivers may have played a role in the POD. As in previous reports, we summarize new evidence for the effects of individual drivers and some of their interactions in the context of the basic POD conceptual model.

Beggel, S., R. Connon, I. Werner and J. Geist (2011). "Changes in gene transcription and whole organism responses in larval fathead minnow (Pimephales promelas) following short-term exposure to the synthetic pyrethroid bifenthrin." Aquat Toxicol **105**(1-2): 180-188 http://www.ncbi.nlm.nih.gov/pubmed/21718662.

The combination of molecular and whole-organism endpoints in ecotoxicology provides valuable information about the ecological relevance of sublethal stressor effects in aquatic ecosystems such as those caused by the use of insecticides and translocation of their residues into surface waters. This study contributes knowledge about the sublethal effects of a common use insecticide, the synthetic pyrethroid bifenthrin, on larval fathead minnow (Pimephales promelas). Transcriptomic responses, assessed by quantitative real-time PCR, combined with individual effects on swimming performance were used to estimate the ecological relevance of insecticide impacts. Significant transcriptomic responses were observed at 0.07 mug L(-1) bifenthrin (lowest observed effect concentration, LOEC) but mostly followed a biphasic rather than a linear dose-response with increasing concentration. Transcript patterns for genes involved in detoxification, neuromuscular function and energy metabolism were linked to an impairment of swimming performance at >/=0.14 mug L(-1) bifenthrin. With increasing treatment concentration, a significant down-regulation was observed for genes coding for cyp3a, aspartoacylase, and creatine kinase, whereas metallothionein was up-regulated. Additionally, bifenthrin induced endocrine responses as evident from a significant up-regulation of vitellogenin and down-regulation of insuline-like growth factor transcripts. Recovery occurred after 6 days and was dependent on the magnitude of the initial stress. During the recovery period, down-regulation of vitellogenin was observed at lowest exposure concentrations. The data presented here emphasize that links can be made between gene transcription changes and behavioral responses which is of great value for the evaluation and interpretation of biomarker responses.

Beggel, S., I. Werner, R. E. Connon and J. P. Geist (2010). "Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (Pimephales promelas)." Sci Total Environ **408**(16): 3169-3175 http://www.ncbi.nlm.nih.gov/pubmed/20434756.

> Toxic effect concentrations of insecticides are generally determined using the technical grade or pure active ingredient. Commercial insecticide formulations, however, contain a significant proportion (>90%) of so-called inert ingredients, which may alter the toxicity of the active ingredient(s). This study compares the sublethal toxicity of two insecticides, the pyrethroid bifenthrin, and the phenylpyrazole fipronil, to their commercial formulations, Talstar and Termidor. Both insecticides are used for landscape treatment and structural pest control, and can be transported into surface water bodies via stormwater and irrigation runoff. We used larval fathead minnow (Pimephales promelas), to determine effects on growth and swimming performance after short-term (24h) exposure to sublethal concentrations of pure insecticides and the respective formulations. Significantly enhanced 7d growth was observed at 10% of the 24h LC(10) (53 microg L(-)(1)) fipronil. Swimming performance was significantly impaired at 20% of the 24h LC(10) (0.14 microg L(-)(1)) of bifenthrin and 10% of the 24h LC(10) of Talstar (0.03 microgL(-)(1)). Fipronil and Termidor led to a significant impairment of swimming performance at 142 microgL(-)(1) and 148 microgL(-)(1) respectively, with more pronounced effects for the formulation. Our data shows that based on dissolved concentrations both formulations were more toxic than the pure active ingredients, suggesting that increased toxicity due to inert ingredients should be considered in risk assessments and regulation of insecticides.

Beggel, S., I. Werner, R. E. Connon and J. P. Geist (2012). "Impacts of the phenylpyrazole insecticide fipronil on larval fish: time-series gene transcription responses in fathead minnow (Pimephales promelas) following short-term exposure." Sci Total Environ **426**: 160-165 http://www.ncbi.nlm.nih.gov/pubmed/22542256.

> The utilization of molecular endpoints in ecotoxicology can provide rapid and valuable information on immediate organismal responses to chemical stressors and is increasingly used for mechanistic interpretation of effects at higher levels of biological organization. This study contributes knowledge on the sublethal effects of a commonly used insecticide, the phenylpyrazole fipronil, on larval fathead minnow (Pimephales promelas), utilizing a quantitative transcriptomic approach. Immediately after 24h of exposure to fipronil concentrations of >/=31 mug.L(-1), highly significant changes in gene transcription were observed for aspartoacylase, metallothionein, glucocorticoid receptor, cytochrome P450 3A126 and vitellogenin. Different mechanisms of toxicity were apparent over the course of the experiment, with short-term responses indicating neurotoxic effects. After 6 days of recovery, endocrine effects were observed with vitellogenin being up-regulated 90-fold at 61 mug.L(-1) fipronil. Principal component analysis demonstrated a significant increase in gene transcription changes over time and during the recovery period. In conclusion, multiple mechanisms of action were observed in response to fipronil exposure, and unknown delayed effects would have been missed if transcriptomic responses had only been measured at a single time-point. These challenges can be overcome by the inclusion of multiple endpoints and delayed effects in experimental designs.

Brander, S. M., B. J. Cole and G. N. Cherr (2012a). "An approach to detecting estrogenic endocrine disruption via choriogenin expression in an estuarine model fish species." Ecotoxicology **21**(4): 1272-1280 http://www.ncbi.nlm.nih.gov/pubmed/22410951.

> A large body of work has established a link between endocrine disrupting compounds (EDCs) and a number of abnormalities in fishes. However, most EDC studies use several standard laboratory denizens to assess impacts, so assumptions about sensitivity are primarily based on these few species. Additionally, existing methods rely on obtaining sufficient plasma to measure EDC biomarkers. Our objectives were (a) to establish a new model species for estuarine fishes, (b) to evaluate endocrine impacts with a highly sensitive and specific biomarker, and (c) to develop a method for the analysis of this biomarker in small fish that do not possess sufficient blood plasma for protein measurement. As such, we created a polyclonal antibody (Ab) to the estrogen-responsive proteins chorion (Ch) and choriogenin (Chg) in Menidia beryllina, found throughout coastal North America and already utilized in EPA Whole Effluent Testing. We then validated the Ab by using it to measure the response to aqueous ethinylestradiol (EE2) through the development an ELISA using Menidia whole body homogenate (WBH). Sensitivity of the Ab to Menidia WBH is greater than that of the commercially available option. ELISA sensitivity, with a detection limit of 5 ng/ml and a working range of 22.6-1370.9 ng/ml, is comparable to ELISAs developed to measure plasma Chg. To our knowledge this is the first ELISA method developed for the detection of Chg using WBH. Including additional model species and methods allowing the evaluation of alternative sample matrices will contribute to an enhanced understanding of inter-species differences in EDC response.

Brander, S. M., R. E. Connon, G. He, J. A. Hobbs, K. L. Smalling, S. J. Teh, J. W. White, I. Werner, M. S. Denison and G. N. Cherr (in press). "From ‘omics to otoliths: responses of an estuarine fish to endocrine disrupting compounds across biological scales." PLOS One.

> Endocrine disrupting chemicals (EDCs) cause physiological abnormalities and population decline in fishes. However, few studies have linked environmental EDC exposures with responses at multiple tiers of the biological hierarchy, including population-level effects. To this end, we undertook a four-tiered investigation in the impacted San Francisco Bay estuary with the Mississippi silverside (Menidia audens), a small pelagic fish. This approach demonstrated links between different EDC sources and fish responses at different levels of biological organization. First we determined that water from our study site, primarily impacted by ranch run-off had only estrogenic activity in vitro, while water sampled from the site receiving urban, limited ranch run-off as well as treated wastewater effluent had both estrogenic and androgenic activity. Secondly, at the molecular level we found that fish had higher expression of estrogen-responsive genes where only estrogenic activity was detected but relatively lower expression where both estrogenic and androgenic EDCs were detected. Thirdly, at the organism level males exposed to both estrogens and androgens had significantly lower mean gonadal somatic indices, higher incidence of severe testicular necrosis and altered somatic growth relative to the site where only estrogens were detected. Finally, at the population level, the proportion of females was significantly lower at the site with measured androgenic and estrogenic activity. Our results suggest that mixtures of androgenic and estrogenic EDCs have antagonistic and potentially additive effects

> depending on the biological scale being assessed, and that mixtures containing androgens and estrogens may produce unexpected effects. In summary, evaluating EDC response at multiple tiers is necessary to determine the source of disruption (lowest scale, i.e. cell line) and what the ecological impact will be (largest scale, i.e. sex ratio)

Brander, S. M., G. He, K. L. Smalling, M. S. Denison and G. N. Cherr (2012b). "The in vivo estrogenic and in vitro anti-estrogenic activity of permethrin and bifenthrin." Environ Toxicol Chem **31**(12): 2848-2855 http://www.ncbi.nlm.nih.gov/pubmed/23007834.

> Pyrethroids are highly toxic to fish at parts per billion or parts per trillion concentrations. Their intended mechanism is prolonged sodium channel opening, but recent studies reveal that pyrethroids such as permethrin and bifenthrin also have endocrine activity. Additionally, metabolites may have greater endocrine activity than parent compounds. The authors evaluated the in vivo concentration-dependent ability of bifenthrin and permethrin to induce choriogenin (an estrogen-responsive protein) in Menidia beryllina, a fish species known to reside in pyrethroid-contaminated aquatic habitats. The authors then compared the in vivo response with an in vitro assay--chemical activated luciferase gene expression (CALUX). Juvenile M. beryllina exposed to bifenthrin (1, 10, 100 ng/L), permethrin (0.1, 1, 10 microg/L), and ethinylestradiol (1, 10, 50 ng/L) had significantly higher ng/mL choriogenin (Chg) measured in whole body homogenate than controls. Though Chg expression in fish exposed to ethinylestradiol (EE2) exhibited a traditional sigmoidal concentration response, curves fit to Chg expressed in fish exposed to pyrethroids suggest a unimodal response, decreasing slightly as concentration increases. Whereas the in vivo response indicated that bifenthrin and permethrin or their metabolites act as estrogen agonists, the CALUX assay demonstrated estrogen antagonism by the pyrethroids. The results, supported by evidence from previous studies, suggest that bifenthrin and permethrin, or their metabolites, appear to act as estrogen receptor (ER) agonists in vivo, and that the unmetabolized pyrethroids, particularly bifenthrin, act as an ER antagonists in cultured mammalian cells.

Brooks, M. L., E. Fleishman, L. R. Brown, P. W. Lehman, I. Werner, N. Scholz, C. Mitchelmore, J. R. Lovvorn, M. L. Johnson, D. Schlenk, S. van Drunick, J. I. Drever, D. M. Stoms, A. E. Parker and R. Dugdale (2012). "Life Histories, Salinity Zones, and Sublethal Contributions of Contaminants to Pelagic Fish Declines Illustrated with a Case Study of San Francisco Estuary, California, USA." Estuaries and Coasts **35**(2): 603-621 http://link.springer.com/article/10.1007%2Fs12237-011-9459-6#page-1.

> Human effects on estuaries are often associated with major decreases in abundance of aquatic species. However, remediation priorities are difficult to identify when declines result from multiple stressors with interacting sublethal effects. The San Francisco Estuary offers a useful case study of the potential role of contaminants in declines of organisms because the waters of its delta chronically violate legal water quality standards; however, direct effects of contaminants on fish species are rarely observed. Lack of direct lethality in the field has prevented consensus that contaminants may be one of the major drivers of coincident but unexplained declines of fishes with differing life histories and habitats (anadromous, brackish, and freshwater). Our review of available evidence indicates that examining the effects of contaminants and other

stressors on specific life stages in different seasons and salinity zones of the estuary is critical to identifying how several interacting stressors could contribute to a general syndrome of declines. Moreover, warming water temperatures of the magnitude projected by climate models increase metabolic rates of ectotherms, and can hasten elimination of some contaminants. However, for other pollutants, concurrent increases in respiratory rate or food intake result in higher doses per unit time without changes in the contaminant concentrations in the water. Food limitation and energetic costs of osmoregulating under altered salinities further limit the amount of energy available to fish; this energy must be redirected from growth and reproduction toward pollutant avoidance, enzymatic detoxification, or elimination. Because all of these processes require energy, bioenergetics methods are promising for evaluating effects of sublethal contaminants in the presence of other stressors, and for informing remediation. Predictive models that evaluate the direct and indirect effects of contaminants will be possible when data become available on energetic costs of exposure to contaminants given simultaneous exposure to non-contaminant stressors.

Brown, L. R., R. Baxter, G. Castillo, L. Conrad, S. Culberson, G. Erickson, F. Feyer, S.Fong, K. Gehrts, L. Grimaldo, B. Herbold, J. Kirsch, A. Mueller-Solger, S. Slater, T. Sommer, K. Souza and v. N. E. (2012). Synthesis of Studies in the Fall Low Salinity Zone of the San Francisco Estuary, September-December 2011 (September 2012) http://deltacouncil.ca.gov/docs/2012-08-24/synthesis-studies-fall-low-salinity-zone-san-francisco-estuary.

In Fall 2011, a large-scale investigation (FLaSH, fall low salinity habitat investigation) was implemented by the Bureau of Reclamation (Reclamation) in cooperation with the Interagency Ecological Program (IEP) to explore hypotheses about the ecological role of low salinity habitat (LSH) in the San Francisco Estuary (SFE), and specifically the importance of fall low salinity habitat to the biology of delta smelt Hypomesus transpacificus, a federal and state listed species endemic to the SFE. This investigation constitutes one of the actions stipulated in the Reasonable and Prudent Alternative (RPA) issued with the 2008 Biological Opinion (BiOp), which called for adaptive management of fall Delta outflow following “wet” and “above normal” water years to alleviate jeopardy to delta smelt and adverse modification of delta smelt critical habitat. The basic hypothesis at the foundation of the RPA is that greater outflows move the low salinity zone (LSZ, salinity 1-6), an important component of delta smelt habitat, westward and that moving the LSZ westward of its position in the Fall of recent years will benefit delta smelt, although the specific mechanisms providing such benefit are uncertain. An adaptive management plan (AMP) was prepared to guide implementation of the RPA (Reclamation 2011) and reduce uncertainty.

Clifford, M. A., K. J. Eder, I. Werner and R. P. Hedrick (2005). "Synergistic effects of esfenvalerate and infectious hematopoietic necrosis virus on juvenile chinook salmon mortality." Environ Toxicol Chem **24**(7): 1766-1772 http://www.ncbi.nlm.nih.gov/pubmed/16050595.

Sublethal concentrations of pollutants may compromise fish, resulting in increased susceptibility to endemic pathogens. To test this hypothesis, juvenile chinook salmon (Oncorhynchus tshawytscha) were exposed to sublethal levels of esfenvalerate or chlorpyrifos either alone or concurrently with infectious hematopoietic necrosis virus

(IHNV). Three trials were performed with fish exposed to concentrations of IHNV between 0.8 x 10(2) and 2.7 x 10(6) plaque-forming units/ml and to 5.0 microg/L of chlorpyrifos or 0.1 microg/L of esfenvalerate. The presence and concentration of IHNV in dead fish were assayed by virus isolation and plaque assay techniques, respectively. Among groups exposed to both esfenvalerate and IHNV, 83% experienced highly significant ($p < 0.001$) mortality, ranging from 20 to 90% at 3 d post-virus exposure, and cumulatively died from 2.4 to 7.7 d sooner than fish exposed to IHNV alone. This trend was not seen in any other treatment group. Virus assays of dead fish indicate a lethal synergism of esfenvalerate and IHNV. Chlorpyrifos had no observed effect on total mortality or IHNV susceptibility. The present results suggest that accepted levels of pollutants may be seemingly nonlethal to fish but, in fact, be acting synergistically with endemic pathogens to compromise survivorship of wild fish populations through immunologic or physiologic disruption.

Cloern, J. E. and A. D. Jassby (2012). "Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay." Reviews of Geophysics **50**(4): RG4001 http://dx.doi.org/10.1029/2012RG000397.

Poised at the interface of rivers, ocean, atmosphere and dense human settlement, estuaries are driven by a large array of natural and anthropogenic forces. San Francisco Bay exemplifies the fast-paced change occurring in many of the world's estuaries, bays, and inland seas in response to these diverse forces. We use observations from this particularly well-studied estuary to illustrate responses to six drivers that are common agents of change where land and sea meet: water consumption and diversion, human modification of sediment supply, introduction of nonnative species, sewage input, environmental policy, and climate shifts. In San Francisco Bay, responses to these drivers include, respectively, shifts in the timing and extent of freshwater inflow and salinity intrusion, decreasing turbidity, restructuring of plankton communities, nutrient enrichment, elimination of hypoxia and reduced metal contamination of biota, and food web changes that decrease resistance of the estuary to nutrient pollution. Detection of these changes and discovery of their causes through environmental monitoring have been essential for establishing and measuring outcomes of environmental policies that aim to maintain high water quality and sustain services provided by estuarine-coastal ecosystems. The many time scales of variability and the multiplicity of interacting drivers place heavy demands on estuarine monitoring programs, but the San Francisco Bay case study illustrates why the imperative for monitoring has never been greater.

Connon, R., J. Geist, J. Pfeiff, A. Loguinov, L. D'Abronzo, H. Wintz, C. Vulpe and I. Werner (2009). "Linking mechanistic and behavioral responses to sublethal esfenvalerate exposure in the endangered delta smelt; *Hypomesus transpacificus* (Fam. Osmeridae)." BMC Genomics **10**(1): 608 http://www.biomedcentral.com/1471-2164/10/608.

The delta smelt (Hypomesus transpacificus) is a pelagic fish species listed as endangered under both the USA Federal and Californian State Endangered Species Acts and considered an indicator of ecosystem health in its habitat range, which is limited to the Sacramento-San Joaquin estuary in California, USA. Anthropogenic contaminants are one of multiple stressors affecting this system, and among them, current-use insecticides are of major concern. Interrogative tools are required to successfully monitor effects of contaminants on the delta smelt, and to research

potential causes of population decline in this species. We have created a microarray to investigate genome-wide effects of potentially causative stressors, and applied this tool to assess effects of the pyrethroid insecticide esfenvalerate on larval delta smelt. Selected genes were further investigated as molecular biomarkers using quantitative PCR analyses. Exposure to esfenvalerate affected swimming behavior of larval delta smelt at concentrations as low as 0.0625 μ g.L-1, and significant differences in expression were measured in genes involved in neuromuscular activity. Alterations in the expression of genes associated with immune responses, along with apoptosis, redox, osmotic stress, detoxification, and growth and development appear to have been invoked by esfenvalerate exposure. Swimming impairment correlated significantly with expression of aspartoacylase (ASPA), an enzyme involved in brain cell function and associated with numerous human diseases. Selected genes were investigated for their use as molecular biomarkers, and strong links were determined between measured downregulation in ASPA and observed behavioral responses in fish exposed to environmentally relevant pyrethroid concentrations. The results of this study show that microarray technology is a useful approach in screening for, and generation of molecular biomarkers in endangered, non-model organisms, identifying specific genes that can be directly linked with sublethal toxicological endpoints; such as changes in expression levels of neuromuscular genes resulting in measurable swimming impairments. The developed microarrays were successfully applied on larval fish exposed to esfenvalerate, a known contaminant of the Sacramento-San Joaquin estuary, and has permitted the identification of specific biomarkers which could provide insight into the factors contributing to delta smelt population decline.

Connon, R. E., S. Beggel, L. S. D'Abronzo, J. P. Geist, J. Pfeiff, A. V. Loguinov, C. D. Vulpe and I. Werner (2011a). "Linking molecular biomarkers with higher level condition indicators to identify effects of copper exposures on the endangered delta smelt (Hypomesus transpacificus)." Environ Toxicol Chem **30**(2): 290-300 http://www.ncbi.nlm.nih.gov/pubmed/21072851.

The delta smelt (Hypomesus transpacificus) is an endangered pelagic fish species endemic to the Sacramento-San Joaquin estuary (CA, USA), and considered an indicator of ecosystem health. Copper is a contaminant of concern in Californian waterways that may affect the development and survival of this endangered species. The experimental combination of molecular biomarkers with higher level effects may allow for interpretation of responses in a functional context that can be used to predict detrimental outcomes caused by exposure. A delta smelt microarray was developed and applied to screen for candidate molecular biomarkers that may be used in monitoring programs. Functional classifications of microarray responses were used along with quantitative polymerase chain reaction determining effects upon neuromuscular, digestive, and immune responses in Cu-exposed delta smelt. Differences in sensitivity were measured between juveniles and larvae (median lethal concentration = 25.2 and 80.4 microg/L Cu(2+), respectively). Swimming velocity declined with higher exposure concentrations in a dose-dependent manner ($r = -0.911$, $p < 0.05$), though was not statistically significant to controls. Genes encoding for aspartoacylase, hemopexin, alpha-actin, and calcium regulation proteins were significantly affected by exposure and were functionally interpreted with measured swimming responses. Effects on digestion were measured by upregulation of chitinase and downregulation of amylase,

> whereas downregulation of tumor necrosis factor indicated a probable compromised immune system. Results from this study, and many others, support the use of functionally characterized molecular biomarkers to assess effects of contaminants in field scenarios. We thus propose that to attribute environmental relevance to molecular biomarkers, research should concentrate on their application in field studies with the aim of assisting monitoring programs.

Connon, R. E., L. S. D'Abronzo, N. J. Hostetter, A. Javidmehr, D. D. Roby, A. F. Evans, F. J. Loge and I. Werner (2012a). "Transcription profiling in environmental diagnostics: health assessments in Columbia River basin steelhead (Oncorhynchus mykiss)." Environ Sci Technol **46**(11): 6081-6087 http://www.ncbi.nlm.nih.gov/pubmed/22587496.

> The health condition of out-migrating juvenile salmonids can influence migration success. Physical damage, pathogenic infection, contaminant exposure, and immune system status can affect survival probability. The present study is part of a wider investigation of out-migration success in juvenile steelhead (Oncorhynchus mykiss) and focuses on the application of molecular profiling to assess sublethal effects of environmental stressors in field-collected fish. We used a suite of genes in O. mykiss to specifically assess responses that could be directly related to steelhead health condition during out-migration. These biomarkers were used on juvenile steelhead captured in the Snake River, a tributary of the Columbia River, in Washington, USA, and were applied on gill and anterior head kidney tissue to assess immune system responses, pathogen-defense (NRAMP, Mx, CXC), general stress (HSP70), metal-binding (metallothionein-A), and xenobiotic metabolism (Cyp1a1) utilizing quantitative polymerase chain reaction (PCR) technology. Upon capture, fish were ranked according to visual external physical conditions into good, fair, poor, and bad categories; gills and kidney tissues were then dissected and preserved for gene analyses. Transcription responses were tissue-specific for gill and anterior head kidney with less significant responses in gill tissue than in kidney. Significant differences between the condition ranks were attributed to NRAMP, MX, CXC, and Cyp1a1 responses. Gene profiling correlated gene expression with pathogen presence, and results indicated that gene profiling can be a useful tool for identifying specific pathogen types responsible for disease. Principal component analysis (PCA) further correlated these responses with specific health condition categories, strongly differentiating good, poor, and bad condition ranks. We conclude that molecular profiling is an informative and useful tool that could be applied to indicate and monitor numerous population-level parameters of management interest.

Connon, R. E., L. A. Deanovic, E. B. Fritsch, L. S. D'Abronzo and I. Werner (2011b). "Sublethal responses to ammonia exposure in the endangered delta smelt; Hypomesus transpacificus (Fam. Osmeridae)." Aquat Toxicol **105**(3-4): 369-377 http://www.ncbi.nlm.nih.gov/pubmed/21820383.

> The delta smelt (Hypomesus transpacificus) is an endangered pelagic fish species endemic to the Sacramento-San Joaquin Estuary in Northern California, which acts as an indicator of ecosystem health in its habitat range. Interrogative tools are required to successfully monitor effects of contaminants upon the delta smelt, and to research potential causes of population decline in this species. We used microarray technology to investigate genome-wide effects in fish exposed to ammonia; one of multiple

contaminants arising from wastewater treatment plants and agricultural runoff. A 4-day exposure of 57-day old juveniles resulted in a total ammonium (NH(4)(+)-N) median lethal concentration (LC50) of 13 mg/L, and a corresponding un-ionized ammonia (NH(3)) LC50 of 147 mug/L. Using the previously designed delta smelt microarray we assessed altered gene transcription in juveniles exposed to 10mg/L NH(4)(+)-N from this 4-day exposure. Over half of the responding genes were associated with membrane integrity and function, however, neurological and muscular function was also affected. Amongst the notable pathways affected by ammonium exposure, directly associated with cellular membranes, are energy metabolism through oxidative phosphorylation, cellular responses to environmental stimuli, highlighted through signal transduction and molecular interactions, cellular processes encompassing transport and catabolism, along with cell motility, development, communication and cell death. To assess these impacts further, key genes were selected as potential biomarkers and investigated using quantitative PCR analysis on fish exposed to 2.5, 5, 10, 20 and 40 mg/L NH(4)(+)-N. Quantitative PCR results indicate biphasic responses, pivoting around the estimated no-observed effect concentration (NOEC; 5.0mg/L NH(4)(+)-N) and below. Genes significantly affected by ammonia exposure include claudin-10, Keratin-15, Septin-3, Transmembrane protein 4, superfamily 4 (membrane), Tropomyosin, Myosin light chain, Calmodulin (muscular), Tubulin cofactor beta (neurological), Sirtuin-6 (development), and Rhesus associated type C glycoprotein 1 (gill- and skin-specific ammonium transporter). The quantitation of the ammonium transporter may highlight the capacity of delta smelt to contend with elevated levels of ammonia, the peak response of which may be indicative of short-term thresholds of tolerance. Our study supports the notion that exposure to ammonia results in cell membrane destabilization, potentially affecting membrane permeability, enhancing uptake and thus synergistic effects of multiple-contaminant exposure.

Connon, R. E., M. Hasenbein, E. B. Fritsch, A. Javidmehr, L. A. Deanovic and I. Werner (2012b). Refinement and Application of Novel Molecular and Biochemical Biomarkers to Determine Sublethal Contaminant Exposure and Effects in Archived Delta Smelt Samples, Interagency Ecological Program.

Gene expression tools developed for delta smelt have been refined and used to investigate whether transcriptomic assessments can be used to determine contaminant exposure, thereby providing information on specific chemical classes that may be responsible for toxic effects on the endangered delta smelt. Transcription profiling assessments were conducted using a suite of 22 genes on archived larval and juvenile delta smelt from laboratory exposures conducted between 2006 and 2009. Organisms tested were exposed to sublethal concentrations of reference contaminants (copper, esfenvalerate, permethrin, chlorpyrifos, and ammonia-wastewater treatment effluent) in years 2006 to 2009, and to ambient water samples (Suisun, Napa, Cache Lindsey, Light 55, Hood and Rough and Ready) during spring of 2008 and 2009. Highest mortality in ambient samples was recorded following exposures to Hood and Rough and Ready, with fish exposed to Suisun ambient samples displaying greatest survival (85-97.9%). Samples from Suisun exposures were therefore used as field controls for transcription assessments. Both Hood and Rough and Ready sites consistently displayed the greatest change in transcription and genes that responded significantly indicate predominant effects on fish development. Transcription profiling successfully differentiated

> responses between sites, highlighting similarities between Cache Lindsay and Light 55, as well as between Hood and R&R, suggesting similar effects from contaminant exposures at respective sites. Effects of salinity differences between treatments, as well as larval/juvenile age were less significant than were site specific responses. Profile comparisons with reference contaminants through principal component analysis, suggest that copper is not likely to be a key factor on the determined toxicity, however pesticide profiles correlated predominantly with samples from Cache Lindsay, Light 55, Hood and Rough and Ready. A site-specific study conducted on samples from Hood, was conducted in comparison with exposures to effluent samples from the Sacramento Regional Wastewater Treatment Plant (SRWTP) and water from the Sacramento River at Garcia Bend (SRGB). Results indicate that profiles from Hood are similar to SRWTP effluent and significantly different from SRGB, however, SRGB samples were significantly different to laboratory controls, suggesting that SRWTP effluent is not solely responsible for the responses determined at Hood, and that contaminants upstream of the effluent discharge may be contributing to mortality. Transcription profiling is a useful tool that should be integrated into toxicity identification evaluation (TIE) assessments, and will prove useful in identifying sources of contaminants when used in carefully designed river-mile monitoring studies.

Deanovic, L. A., K. M. Jeffries, K. Callinan, R. E. Connon and S. J. Teh (2013 grant in progress). Testing the toxicity toolbox, California State Water Resources Control Board, California State Water Resources Control Board.

Ding, Y., D. P. Weston, J. You, A. K. Rothert and M. J. Lydy (2011). "Toxicity of sediment-associated pesticides to Chironomus dilutus and Hyalella azteca." Arch Environ Contam Toxicol **61**(1): 83-92 http://www.ncbi.nlm.nih.gov/pubmed/21052987.

> Two hundred sediment samples were collected and their toxicity evaluated to aquatic species in a previous study in the agriculturally dominated Central Valley of California, United States. Pyrethroid insecticides were the main contributors to the observed toxicity. However, mortality in approximately one third of the toxic samples could not be explained solely by the presence of pyrethroids in the matrices. Hundreds of pesticides are currently used in the Central Valley of California, but only a few dozen are analyzed in standard environmental monitoring. A significant amount of unexplained sediment toxicity may be due to pesticides that are in widespread use that but have not been routinely monitored in the environment, and even if some of them were, the concentrations harmful to aquatic organisms are unknown. In this study, toxicity thresholds for nine sediment-associated pesticides including abamectin, diazinon, dicofol, fenpropathrin, indoxacarb, methyl parathion, oxyfluorfen, propargite, and pyraclostrobin were established for two aquatic species, the midge Chironomus dilutus and the amphipod Hyalella azteca. For midges, the median lethal concentration (LC(5)(0)) of the pesticides ranged from 0.18 to 964 mug/g organic carbon (OC), with abamectin being the most toxic and propargite being the least toxic pesticide. A sublethal growth endpoint using average individual ash-free dry mass was also measured for the midges. The no-observable effect concentration values for growth ranged from 0.10 to 633 mug/g OC for the nine pesticides. For the amphipods, fenpropathrin was the most toxic, with an LC(5)(0) of 1-2 mug/g OC. Abamectin, diazinon, and methyl parathion were all moderately toxic (LC(5)(0)s 2.8-26 mug/g

OC). Dicofol, indoxacarb, oxyfluorfen, propargite, and pyraclostrobin were all relatively nontoxic, with LC(5)(0)s greater than the highest concentrations tested. The toxicity information collected in the present study will be helpful in decreasing the frequency of unexplained sediment toxicity in agricultural waterways.

Durieux, E. D., R. E. Connon, I. Werner, L. S. D'Abronzo, P. S. Fitzgerald, J. L. Spearow and D. J. Ostrach (2012). "Cytochrome P4501A mRNA and protein induction in striped bass (Morone saxatilis)." Fish Physiol Biochem **38**(4): 1107-1116 http://www.ncbi.nlm.nih.gov/pubmed/22252335.

The striped bass (Morone saxatilis) supports a valuable recreational fishery and is among the most important piscivorous fish of the San Francisco Estuary. This species has suffered a significant decline in numbers over the past decades, and there is indication that contaminants are important contributors. Polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aromatic hydrocarbons (PHAHs) including PCBs and dioxins are widespread in the estuary, they typically bioaccumulate through trophic levels, reaching highest levels in top predators and are known to affect the fish health and development. The aim of this study was to investigate the dynamics of cytochrome P4501A (Cyp1a) induction simultaneously at different levels of biological organization (RNA transcription and protein synthesis) as a biomarker of exposure to PAHs and PHAHs. We utilized beta-naphthoflavone (BNF) as a model PAH to induce Cyp1a responses in juvenile striped bass in both dose-response and time-response assessments and determined Cyp1a mRNA and protein levels. Significant responses were measured in both systems at 10 mg BetaNuF kg(-)(1), a concentration used for time-response studies. Messenger RNA levels peaked at 6 h post-injection, while protein levels increased progressively with time, significantly peaking at 96 h post-injection; both remaining elevated throughout the duration of the test (8 days). Our data suggest that rapid induction of gene transcription following exposure and subsequent cumulative protein synthesis could provide a useful means of identifying temporal variants in exposure to Cyp1a inducers in Morone saxatilis. The potential application of this combined Cyp1a gene and protein biomarker in this species for field studies is discussed.

Durieux, E. D., T. B. Farver, P. S. Fitzgerald, K. J. Eder and D. J. Ostrach (2011). "Natural factors to consider when using acetylcholinesterase activity as neurotoxicity biomarker in Young-Of-Year striped bass (Morone saxatilis)." Fish Physiol Biochem **37**(1): 21-29 http://www.ncbi.nlm.nih.gov/pubmed/20582624.

Acetylcholinesterase (AChE) activity is one of the most common biomarkers of neurotoxicity used in aquatic organisms. However, compared to its extensive use as biomarker, the effects of natural factors on AChE activity remain unclear especially in estuarine fishes. The aim of this study was to evaluate the effects of natural factors on AChE activity of striped bass (Morone saxatilis) juveniles. Brain AChE activity was measured in YOY (Young-Of-Year) individuals collected monthly from August 2007 to January 2008 at 12 different sites in the San Francisco Estuary system. The spatio-temporal variability of AChE was analyzed relative to water temperature and salinity as well as fish size. AChE activity was highly positively correlated with water temperature and to a lesser extent negatively with fish size while no relationship was detected with salinity. Taking into account these natural factors when using AChE as a biomarker

> will help to determine and understand the effects of neurotoxic contaminants on fish in estuarine systems.

Eder, K. J., M. A. Clifford, R. P. Hedrick, H. R. Kohler and I. Werner (2008). "Expression of immune-regulatory genes in juvenile Chinook salmon following exposure to pesticides and infectious hematopoietic necrosis virus (IHNV)." Fish Shellfish Immunol **25**(5): 508-516 http://www.ncbi.nlm.nih.gov/pubmed/18691654.

> Impairment of fish immune function as a consequence of polluted aquatic environments can result in changes in susceptibility to disease. In this study, we investigated the effects of two widely used insecticides, chlorpyrifos (CP) and esfenvalerate (EV), and a pathogen, infectious hematopoietic necrosis virus (IHNV), singly and in combination, on survival and cytokine (Mx protein, IL-1beta, TGF-beta and IGF-1) expression in juvenile Chinook salmon. Fish were exposed for 96 h to sublethal concentrations of CP (3.7 microg L(-1)) or EV (0.08 microg L(-1)), allowed to recover in clean water for seven days, then exposed to IHNV (6.4x10(5)TCID(50)mL(-1)) for 1.5h. Mortality was recorded daily, and spleen and anterior kidney samples were collected on day 4 (after CP or EV treatment), day 20 and day 60 (after CP or EV treatment and subsequent IHNV exposure) of the experiment. Significant mortality after 60 days was observed following exposure to EV (17%) or IHNV (20%), and prior insecticide exposure did not synergize the acute effects of pathogen treatment. By day 4, exposure to CP as well as EV led to a significant decrease of Mx protein and IL-1beta expression; by day 20, EV-exposed fish significantly overexpressed IL-1beta. Mx protein transcription was up-regulated in spleen and kidney of all IHNV-exposed fish groups by day 20. All but one treatment (EV) led to significantly decreased IGF-1 transcription in spleen on days 20 and 60, whereas a short-term increase was seen after CP exposure (day 4). In kidney, decreases of IGF-1 transcription were less pronounced. TGF-beta transcription was up-regulated in CP/IHNV and EV/IHNV exposure groups. Our results indicate that CP and EV alter the expression of cytokines, but this did not negatively affect the ability of fish to survive a subsequent exposure to IHNV. Induced TGF-beta transcription indicated that the combined stressors affected fish in a synergistic manner, but the consequences are unknown. Increased transcription of Mx protein was a reliable indicator of virus exposure.

Eder, K. J., H. R. Kohler and I. Werner (2007). "Pesticide and pathogen: heat shock protein expression and acetylcholinesterase inhibition in juvenile Chinook salmon in response to multiple stressors." Environ Toxicol Chem **26**(6): 1233-1242 http://www.ncbi.nlm.nih.gov/pubmed/17571690.

> Rapid expression of heat shock protein (hsp) families in response to a variety of stressors has been demonstrated in many organisms, including fish. The present 60-d challenge study was designed to compare hsp induction in juvenile Chinook salmon following exposure to individual pesticides, virus, and both stressors combined. Heat shock protein expression patterns over time were monitored and related to the extent of virus infection and mortality. Acetylcholinesterase (AChE) inhibition and recovery in response to applied stressors were measured in brain. High enzyme inhibition levels have been correlated with imminent mortality, and other sublethal physiological effects have been observed in fish concurrent with depressed AChE activity. Mortality was elevated considerably in fish exposed to 0.08 microg/L of the pyrethroid esfenvalerate

(EV). Mortality due to infectious hematopoietic necrosis virus (IHNV) was lower in groups previously treated with pesticides; however, these fish died sooner than individuals exposed to virus only. Both pesticides, EV and the organophosphate (OP) chlorpyrifos (CP), as well as virus exposure, induced hsp expression, but highest hsp levels were observed after the combined treatments, suggesting an additive effect between virus and pesticides. Highest virus titers were accompanied by strongest hsp induction, indicating a connection between virus concentration and hsp expression. In conclusion, the measurement of hsp expression appears to be a very sensitive, integrative indicator of stress. Esfenvalerate and IHNV did not affect AChE activity, and exposure to 3.7 microg/L CP led to significantly inhibited AChE for at least 20 d. The time required for complete recovery of AChE activity raises concern about deleterious behavioral effects.

Eder, K. J., C. M. Leutenegger, H. R. Kohler and I. Werner (2009). "Effects of neurotoxic insecticides on heat-shock proteins and cytokine transcription in Chinook salmon (Oncorhynchus tshawytscha)." Ecotoxicol Environ Saf **72**(1): 182-190 http://www.ncbi.nlm.nih.gov/pubmed/18573527.

This study investigated sublethal, molecular effects of two current-use insecticides, chlorpyrifos (CP) and esfenvalerate (EV) in juvenile Chinook salmon. Heat-shock protein (hsp60, hsp70, hsp90) expression was quantified by Western blotting in muscle, liver and gill, and transcription of four cytokines (TGF-beta, IL-1beta, IGF-1, Mx-protein) was measured by real-time TaqMan PCR in anterior kidney and spleen. Expression of hsp was increased in muscle and liver at 1.2 and 7.2 microg/L CP, and at 0.01 and 0.1 microg/L EV, respectively. Transcription of IL-1beta and TGF-beta was elevated in kidney at 1.2 microg/L CP, while EV had no effect. No changes in cytokine transcription were observed in the spleen. Our results show that these insecticides cause cellular effects at environmental concentrations, and that hsps are sensitive indicators of sublethal exposure to CP and EV. In addition, CP may exert immunotoxic effects by altering the transcription of important mediators of the fish immune system.

Escher, B. and F. Leusch (2012). Bioanalytical Tools in Water Quality Assessment. London, UK, IWA Publishing.

Fangue, N. A. and R. E. Connon (2013-2014 - Grant in progress). Developing molecular tools to assess the mechanisms of tolerance and resistance to environmental stressors in longfin smelt (Spirinchus thaleichthys), Interagency Ecological Program.

Forbes, V. E. and P. Calow (2012). "Promises and problems for the new paradigm for risk assessment and an alternative approach involving predictive systems models." Environ Toxicol Chem **31**(12): 2663-2671 http://www.ncbi.nlm.nih.gov/pubmed/23165997.

The need for cost-effective risk assessment of chemicals is leading to the development of a reductionist paradigm that tries to assess impacts on humans and ecosystems from molecular changes. However, the biggest challenge for this paradigm comes from the emergence of properties that arise out of the interactions of the parts that are not included and yet which are key for assessing likely impacts. Although identifying key events and adverse outcome pathways can shed light on the involvement of important metabolic processes in toxicity, this does not mean that particular molecular initiating

> events are likely to be robust or accurate predictors of impacts that matter. There are even greater challenges for the new paradigm applied to ecological systems than to human health because of the need to link across more levels of biological organization. The present study argues for a predictive systems approach that makes the linkages through systems models in a mechanistic way that allows for emergence and that also has the potential for reducing the costs and use of animals in ecological risk assessments.

Fritsch, E. B., R. E. Connon, I. Werner, R. E. Davies, S. Beggel, W. Feng and I. N. Pessah (2013). "Triclosan impairs swimming behavior and alters expression of excitation-contraction coupling proteins in fathead minnow (Pimephales promelas)." Environ Sci Technol **47**(4): 2008-2017 http://www.ncbi.nlm.nih.gov/pubmed/23305567.

> Triclosan (TCS), a high volume chemical widely used in consumer products, is a known aquatic contaminant found in fish inhabiting polluted watersheds. Mammalian studies have recently demonstrated that TCS disrupts signaling between the ryanodine receptor (RyR) and the dihydropyridine receptor (DHPR), two proteins essential for excitation-contraction (EC) coupling in striated muscle. We investigated the swimming behavior and expression of EC coupling proteins in larval fathead minnows (Pimephales promelas) exposed to TCS for up to 7 days. Concentrations as low as 75 mug L(-1) significantly altered fish swimming activity after 1 day; which was consistent after 7 days of exposure. The mRNA transcription and protein levels of RyR and DHPR (subunit CaV1.1) isoforms changed in a dose and time dependent manner. Crude muscle homogenates from exposed larvae did not display any apparent changes in receptor affinity toward known radioligands. In nonexposed crude muscle homogenates, TCS decreased the binding of [(3)H]PN20-110 to the DHPR and decreased the binding of [(3)H]-ryanodine to the RyR, demonstrating a direct impact at the receptor level. These results support TCS's impact on muscle function in vertebrates further exemplifying the need to re-evaluate the risks this pollutant poses to aquatic environments.

Geist, J., I. Werner, K. J. Eder and C. M. Leutenegger (2007). "Comparisons of tissue-specific transcription of stress response genes with whole animal endpoints of adverse effect in striped bass (Morone saxatilis) following treatment with copper and esfenvalerate." Aquat Toxicol **85**(1): 28-39 http://www.ncbi.nlm.nih.gov/pubmed/17767966.

> Changes in the gene transcription of stress response genes in resident fish can be powerful biomarkers for the identification of sublethal impacts of environmental stressors on aquatic ecosystems. In this study, we tested the effects of two reference toxicants, copper (Cu) and the pyrethroid insecticide esfenvalerate [(S)-alpha-cyano-3-phenoxybenzyl-(S)-2-(4-chlorophenyl)-3-methylbutyrate], on lethal (mortality) and sublethal endpoints (growth, swimming behavior, transcription levels of stress response genes) in juvenile (81-90-day-old) striped bass (Morone saxatilis). We established cellular stress response markers for proteotoxicity (HSP70, HSP90), phase I detoxification mechanism (CYP1A1), metal-binding (metallothionein), as well as immune-function and pathogen-defense (TGF-beta, Mx-protein, nRAMP). Quantitative real-time TaqMan PCR was used to examine tissue-specific changes in the transcriptome of liver, spleen, white muscle, anterior kidney and gills after 7-day Cu exposures and 24-h esfenvalerate exposures. On the transcriptome level, exposure to Cu

showed strongest effects on the transcription of metallothionein in spleen tissue, causing a 4-fold increase of mRNA at 42ppb total Cu and a 10-fold increase at 160 ppb Cu. Exposure to Cu also caused significant tissue-specific changes in gene transcription for immune-system related genes. Esfenvalerate exposure had tissue-specific effects on the transcription of HSP70, HSP90 and CYP1A1. The most significant effects were detected in liver tissue after exposure to 0.64 microg/L esfenvalerate. Our results show that the stress response at the transcriptome level is a more sensitive indicator for Cu and esfenvalerate exposures at low concentrations than swimming behavior, growth or mortality. The accuracy of studies on quantitative changes in the transcriptome can benefit from an initial evaluation or the inclusion of several different tissues and the use of multiple housekeeping genes.

Ger, K. A., S. J. Teh, D. V. Baxa, S. Lesmeister and C. R. Goldman (2010). "The effects of dietary Microcystis aeruginosa and microcystin on the copepods of the upper San Francisco Estuary." Freshwater Biology **55**(7): 1548-1559 http://dx.doi.org/10.1111/j.1365-2427.2009.02367.x.

1. Increasing blooms of Microcystis aeruginosa have unknown impacts on the copepods Eurytemora affinis and Pseudodiaptomus forbesi, which are the dominant zooplankters and key prey species for endangered larval fish in the upper San Francisco Estuary. 2. Laboratory feeding experiments were designed to measure the effect of Microcystis on copepod survival and to distinguish the effects of toxicity and nutrition. In a series of survival tests, copepods were fed a mixed diet of algae plus one of two strains of Microcystis, either producing (MC+) or lacking microcystin (MC−). 3. Microcystis significantly reduced survival even when it was a small proportion of the diet, indicating that toxicity was the major cause of mortality. Contrary to expectation, however, the MC+ strain did not result in higher mortality, suggesting that non-MC metabolites of Microcystis can be toxic to copepods. 4. Across treatments, survival of P. forbesi was greater than that of E. affinis, although the two copepods responded differently to both the ratio and the strain of Microcystis in their food. Survival of P. forbesi was greater on the MC+ strain and was inversely proportional to the ratio of dietary Microcystis (MC+ or MC−). In contrast, survival of E. affinis declined similarly across treatments and was not related to the proportion or strain of dietary Microcystis. Results indicate that the copepod P. forbesi can coexist with Microcystis while the other copepod E. affinis cannot. 5. Regardless of species, dietary Microcystis caused significant mortality to copepods, and it may cause adverse impacts to the potentially food-limited zooplankton community of the San Francisco Estuary. These impacts may not be related to the cellular MC concentration because Microcystis contains other metabolites that negatively affect copepods.

Ger, K. A., S. J. Teh and C. R. Goldman (2009). "Microcystin-LR toxicity on dominant copepods Eurytemora affinis and Pseudodiaptomus forbesi of the upper San Francisco Estuary." Science of the Total Environment **407**(17): 4852-4857 http://www.sciencedirect.com/science/article/pii/S0048969709005191.

This study investigates the toxicity and post-exposure effects of dissolved microcystin (MC-LR) on the dominant copepods of the upper San Francisco Estuary (SFE), where blooms of the toxic cyanobacteria Microcystis aeruginosa coincide with record low levels in the abundance of pelagic organisms including phytoplankton, zooplankton, and fish. The potential negative impact of Microcystis on the copepods Eurytemora affinis and Pseudodiaptomus forbesi has raised concern for further depletion of high quality fish food. Response of copepods to MC-LR (MC) was determined using a 48-h standard static renewal method for acute toxicity testing. Following exposure, a life table test was performed to quantify any post-exposure impacts on survival and reproduction. The 48-h LC-50 and LC-10 values for MC were 1.55 and 0.14 mg/L for E. affinis; and 0.52 and 0.21 mg/L for P. forbesi. Copepod populations recovered once dissolved MC was removed and cultures returned to optimal conditions, suggesting no post-exposure effects of MC on copepod populations. Dissolved microcystin above 0.14 mg/L proved likely to have chronic effects on the survival of copepods in the SFE. Since such high concentrations are unlikely, toxicity from dissolved microcystin is not a direct threat to zooplankton of the SFE, and other mechanisms such as dietary exposure to Microcystis constitute a more severe risk.

Hamers, T., J. Legler, L. Blaha, K. Hylland, I. Marigomez, C. A. Schipper, H. Segner, A. D. Vethaak, H. Witters, D. de Zwart and P. E. Leonards (2013). "Expert opinion on toxicity profiling - report from a NORMAN expert group meeting." Integr Environ Assess Manag http://dx.doi.org/10.1002/ieam.1395.

This paper describes the outcome and follow-up discussions of an expert group meeting (Amsterdam, October 9(th) , 2009) on the applicability of toxicity profiling for diagnostic environmental risk assessment. A toxicity profile was defined as a toxicological "fingerprint" of a sample, ranging from a pure compound to a complex mixture, obtained by testing the sample or its extract for its activity towards a battery of biological endpoints. The expert group concluded that toxicity profiling is an effective first tier tool for screening the integrated hazard of complex environmental mixtures with known and unknown toxicologically active constituents. In addition, toxicity profiles can be used for prioritization of sampling locations, for identification of hot spots, and - in combination with Effect-Directed Analysis (EDA) or Toxicity Identification and Evaluation (TIE) approaches - for establishing cause-effect relationships by identifying emerging pollutants responsible for the observed toxic potency. Small volume in vitro bioassays are especially applicable for these purposes, as they are relatively cheap and fast with costs comparable to chemical analyses, and the results are toxicologically more relevant and more suitable for realistic risk assessment. For regulatory acceptance in the EU, toxicity profiling terminology should keep as close as possible to the European Water Framework Directive (WFD) terminology, and validation, standardization, statistical analyses and other quality aspects of toxicity profiling should be further elaborated. Integr Environ Assess Manag © 2013 SETAC. Copyright © 2013 SETAC.

Hasenbein, M., L. M. Komoroske, R. E. Connon, J. Geist and N. A. Fangue (2013). "Turbidity and Salinity Affect Feeding Performance and Physiological Stress in the Endangered Delta Smelt." Integrative and Comparative Biology http://icb.oxfordjournals.org/content/early/2013/08/05/icb.ict082.abstract.

Coastal estuaries are among the most heavily impacted ecosystems worldwide with many keystone fauna critically endangered. The delta smelt (Hypomesus transpacificus) is an endangered pelagic fish species endemic to the Sacramento–San Joaquin Estuary in northern California, and is considered as an indicator species for ecosystem health. This ecosystem is characterized by tidal and seasonal gradients in water parameters (e.g., salinity, temperature, and turbidity), but is also subject to altered water-flow regimes due to water extraction. In this study, we evaluated the effects of turbidity and salinity on feeding performance and the stress response of delta smelt because both of these parameters are influenced by water flows through the San Francisco Bay Delta (SFBD) and are known to be of critical importance to the completion of the delta smelt's life cycle. Juvenile delta smelt were exposed to a matrix of turbidities and salinities ranging from 5 to 250 nephelometric turbidity units (NTUs) and 0.2 to 15 parts per thousand (ppt), respectively, for 2 h. Best statistical models using Akaike's Information Criterion supported that increasing turbidities resulted in reduced feeding rates, especially at 250 NTU. In contrast, best explanatory models for gene transcription of sodium–potassium-ATPase (Na/K-ATPase)—an indicator of osmoregulatory stress, hypothalamic pro-opiomelanocortin—a precursor protein to adrenocorticotropic hormone (expressed in response to biological stress), and whole-body cortisol were affected by salinity alone. Only transcription of glutathione-S-transferase, a phase II detoxification enzyme that protects cells against reactive oxygen species, was affected by both salinity and turbidity. Taken together, these data suggest that turbidity is an important determinant of feeding, whereas salinity is an important abiotic factor influencing the cellular stress response in delta smelt. Our data support habitat association studies that have shown greater delta smelt abundances in the low-salinity zone (0.5–6.0 ppt) of San Francisco Bay, a zone that is also understood to have optimal turbidities. By determining the responses of juvenile delta smelt to key abiotic factors, we hope to aid resource managers in making informed decisions in support of delta smelt conservation.

Hasenbein, M., I. Werner, L. A. Deanovic, J. Geist, E. B. Fritsch, A. Javidmehr, C. Foe, N. A. Fangue and R. E. Connon (in press). "Transcriptomic profiling permits the identification of pollutant sources and effects in ambient water samples." Science of the Total Environment.

Contaminant exposure is one possible contributor to population declines of endangered fish species in the Sacramento-San Joaquin Estuary, California, including the endangered delta smelt (Hypomesus transpacificus). Herein we investigated transcriptional responses in larval delta smelt resulting from exposure to water samples collected at the Department of Water Resources Field Station at Hood, a site of concern, situated upstream of known delta smelt habitat and spawning sites and downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP). Microarray assessments indicate impacts on energy metabolism, DNA and RNA processing, the immune system, development and muscle function. Transcription responses of fish exposed to water samples from Hood were compared with exposures to 9% effluent samples from SRWTP, water from the Sacramento River at Garcia Bend (SRGB), upstream of the effluent discharge, and SRGB water spiked with 2 mg/L total ammonium (9% effluent equivalent). Results indicate that transcriptomic profiles from Hood are similar to 9% SRWTP effluent and ammonium spiked SRGB water, but significantly different from SRGB. SRGB samples however were also significantly different from laboratory controls, suggesting that SRWTP effluent is not solely responsible for the responses determined at Hood, that ammonium exposure likely enhances the effect of multiple-contaminant exposures, and that the observed mortality at Hood is due to the combination of both effluent discharge and contaminants arising

> from upstream of the tested sites. This study demonstrates that transcriptomic responses of fishes can be valuable endpoints for the detection of pollutants and their sources in surface waters at sublethal and even non-detectable concentrations.

Hooper, M. J., G. T. Ankley, D. A. Cristol, L. A. Maryoung, P. D. Noyes and K. E. Pinkerton (2013). "Interactions between chemical and climate stressors: a role for mechanistic toxicology in assessing climate change risks." Environ Toxicol Chem **32**(1): 32-48 http://www.ncbi.nlm.nih.gov/pubmed/23136056.

> Incorporation of global climate change (GCC) effects into assessments of chemical risk and injury requires integrated examinations of chemical and nonchemical stressors. Environmental variables altered by GCC (temperature, precipitation, salinity, pH) can influence the toxicokinetics of chemical absorption, distribution, metabolism, and excretion as well as toxicodynamic interactions between chemicals and target molecules. In addition, GCC challenges processes critical for coping with the external environment (water balance, thermoregulation, nutrition, and the immune, endocrine, and neurological systems), leaving organisms sensitive to even slight perturbations by chemicals when pushed to the limits of their physiological tolerance range. In simplest terms, GCC can make organisms more sensitive to chemical stressors, while alternatively, exposure to chemicals can make organisms more sensitive to GCC stressors. One challenge is to identify potential interactions between nonchemical and chemical stressors affecting key physiological processes in an organism. We employed adverse outcome pathways, constructs depicting linkages between mechanism-based molecular initiating events and impacts on individuals or populations, to assess how chemical- and climate-specific variables interact to lead to adverse outcomes. Case examples are presented for prospective scenarios, hypothesizing potential chemical-GCC interactions, and retrospective scenarios, proposing mechanisms for demonstrated chemical-climate interactions in natural populations. Understanding GCC interactions along adverse outcome pathways facilitates extrapolation between species or other levels of organization, development of hypotheses and focal areas for further research, and improved inputs for risk and resource injury assessments.

Kaufman, R., R. Coalter, N. Nordman, D. Cocherell, J. Cech, Jr., L. Thompson and N. Fangue (2013). "Effects of temperature on hardhead minnow (Mylopharodon conocephalus) blood-oxygen equilibria." Environmental Biology of Fishes: 1-9 http://dx.doi.org/10.1007/s10641-013-0116-8.

> Habitat perturbations, including dam construction with consequent temperature changes and the introduction of non-native species to California’s mid- to low-elevation streams, have negatively influenced some native fish populations’ historic distribution and abundance. Populations of hardhead, Mylopharodon conocephalus (Cyprinidae), have experienced such population declines, but environmental temperature effects on this large (to 60 cm SL), native species are poorly documented. We measured temperature effects on in vitro blood-oxygen affinity and equilibrium curve shape, key dynamics of the species’ oxygen-transport system, derived from blood collected from wild-caught hardhead. Over an 11–30 °C temperature range, the half-saturation value (P50, an inverse measure of affinity) increased with the temperature from 0.51 to 1.80 kPa for low-PCO2 (“arterial”) treatments and from 2.02 to 2.92 kPa for high-PCO2 (“venous”) treatments. The apparent heat of oxygenation (temperature effect) was

> higher at temperatures > (absolute value) 19 °C. Therefore, hardhead's blood has a decreased ability to bind oxygen at its gills at temperatures ≥25 °C, compared to that at temperatures ≤19 °C. The hardhead's Bohr factors (Φ), non-bicarbonate buffer values (β), nucleoside triphosphate (NTP) concentrations, blood oxygen capacities (CBO2), and mildly sigmoid-shaped oxygen equilibrium curves showed no relationship with temperature. Overall, their blood-oxygen equilibria suggest that hardhead can tolerate moderate hypoxia and temperature variations in its environment and that they have some capacity for sustained, high-aerobic activity.

Miller, K. M., S. Li, K. H. Kaukinen, N. Ginther, E. Hammill, J. M. R. Curtis, D. A. Patterson, T. Sierocinski, L. Donnison, P. Pavlidis, S. G. Hinch, K. A. Hruska, S. J. Cooke, K. K. English and A. P. Farrell (2011). "Genomic Signatures Predict Migration and Spawning Failure in Wild Canadian Salmon." Science **331**(6014): 214-217 http://www.sciencemag.org/content/331/6014/214.abstract.

> Long-term population viability of Fraser River sockeye salmon (Oncorhynchus nerka) is threatened by unusually high levels of mortality as they swim to their spawning areas before they spawn. Functional genomic studies on biopsied gill tissue from tagged wild adults that were tracked through ocean and river environments revealed physiological profiles predictive of successful migration and spawning. We identified a common genomic profile that was correlated with survival in each study. In ocean-tagged fish, a mortality-related genomic signature was associated with a 13.5-fold greater chance of dying en route. In river-tagged fish, the same genomic signature was associated with a 50% increase in mortality before reaching the spawning grounds in one of three stocks tested. At the spawning grounds, the same signature was associated with 3.7-fold greater odds of dying without spawning. Functional analysis raises the possibility that the mortality-related signature reflects a viral infection.

Moore, M. T., D. L. Denton, C. M. Cooper, J. Wrysinski, J. L. Miller, K. Reece, D. Crane and P. Robins (2008). "Mitigation assessment of vegetated drainage ditches for collecting irrigation runoff in California." J Environ Qual **37**(2): 486-493 http://www.ncbi.nlm.nih.gov/pubmed/18268312.

> Widespread contamination of California water bodies by the organophosphate insecticides diazinon and chlorpyrifos is well documented. While their usage has decreased over the last few years, a concomitant increase in pyrethroid usage (e.g., permethrin) (replacement insecticides) has occurred. Vegetated agricultural drainage ditches (VADD) have been proposed as a potential economical and environmentally efficient management practice to mitigate the effects of pesticides in irrigation and storm runoff. Three ditches were constructed in Yolo County, California for a field trial. A U-shaped vegetated ditch, a V-shaped vegetated ditch, and a V-shaped unvegetated ditch were each amended for 8 h with a mixture of diazinon, permethrin, and suspended sediment simulating an irrigation runoff event. Water, sediment, and plant samples were collected spatially and temporally and analyzed for diazinon and permethrin concentrations. Pesticide half-lives were similar between ditches and pesticides, ranging from 2.4 to 6.4 h. Differences in half-distances (distance required to reduce initial pesticide concentration by 50%) among pesticides and ditches were present, indicating importance of vegetation in mitigation. Cis-permethrin half-distances in V ditches ranged from 22 m (V-vegetated) to 50 m (V-unvegetated). Half-

distances for trans-permethrin were similar, ranging from 21 m (V-vegetated) to 55 m (V-unvegetated). Diazinon half-distances demonstrated the greatest differences (55 m for V-vegetated and 158 m for V-unvegetated). Such economical and environmentally successful management practices will offer farmers, ranchers, and landowners a viable alternative to more conventional (and sometimes expensive) practices.

Moore, M. T., D. L. Denton, C. M. Cooper, J. Wrysinski, J. L. Miller, I. Werner, G. Horner, D. Crane, D. B. Holcomb and G. M. Huddleston, 3rd (2011). "Use of vegetated agricultural drainage ditches to decrease pesticide transport from tomato and alfalfa fields in California, USA." Environ Toxicol Chem **30**(5): 1044-1049 http://www.ncbi.nlm.nih.gov/pubmed/21309027.

Irrigation and storm water runoff from agricultural fields has the potential to cause impairment to downstream aquatic receiving systems. Over the last several years, scientists have discovered the benefit of using edge-of-field practices, such as vegetated agricultural drainage ditches, in the mitigation of pesticides and sediment. After demonstrating this practice's feasibility in California, field trials were initiated to document irrigation runoff pesticide mitigation in California alfalfa and tomato fields. In the alfalfa field, chlorpyrifos concentration was decreased by 20% from the inflow to the ditch outflow. Thirty-two percent of the measured chlorpyrifos mass was associated with ditch plant material. In the tomato field, permethrin concentration was decreased by 67% and there was a 35% reduction in suspended sediment concentration from inflow to the ditch outflow. When surface water was not present in the ditch systems, the sediment was a significant repository for pesticides. Based on the field trials, vegetated agricultural drainage ditches can be successfully used as part of a suite of management practices to reduce pesticide and sediment runoff into aquatic receiving systems.

Mussen, T. D., D. Cocherell, Z. Hockett, A. Ercan, H. Bandeh, M. L. Kavvas, J. J. Cech and N. A. Fangue (2012). "Assessing Juvenile Chinook Salmon Behavior and Entrainment Risk near Unscreened Water Diversions: Large Flume Simulations." Transactions of the American Fisheries Society **142**(1): 130-142 http://dx.doi.org/10.1080/00028487.2012.720633.

Abstract Juvenile Chinook Salmon Oncorhynchus tshawytscha from California's Central Valley must pass thousands of unscreened water diversion pipes during their out-migration to the Pacific Ocean. The number of fish that become entrained into (drawn through) these diversions at different hydraulic and environmental conditions is currently unknown. We tested the ability of juvenile Chinook Salmon to avoid entrainment into a 0.46-m-diameter unscreened water diversion pipe while swimming in a large-river-simulation flume. Fish swimming experiments were conducted at 0.15, 0.38, and 0.61 m/s sweeping velocities (simulating the river current) with 0.42 and 0.57 m3/s water diversion rates during the day and at 0.15 and 0.61 m/s with a diversion rate of 0.57 m3/s in turbid water and during the night. The number of fish entrained during day experiments ranged from 0.8% (SE, 0.3) to 8.5% (SE, 0.3). The percentage of pipe passage events resulting in fish entrainment nearly doubled at the 0.57 m3/s water diversion rate (1.7%) compared with that at 0.42 m3/s (0.9%). In clear water conditions during the day, more fish became entrained at the higher water diversion rate (0.57 m3/s) and slower sweeping velocity (0.15 m/s), with fish entrainments starting 38.6 cm (SE, 1.6) from the center of the pipe inlet, where fish experienced an increased velocity gradient and a mean resultant velocity of 0.74 m/s. Fish entrainment was strongly influenced by the number of pipe passages per experiment, rather than by swimming orientation or time spent in the flume. More fish were entrained at the faster sweeping velocity (0.61 m/s) in turbid water during the day and at night, indicating that juvenile Chinook Salmon may use nonvisual guidance (e.g., lateral line system) to avoid water diversions in slower currents. These results help to provide a scientific basis for protecting out-migrating juvenile Chinook Salmon exposed to unscreened water diversions. Received February 24, 2012; accepted August 7, 2012

Newcomb, J. and L. Pierce (2010 - Report). Adverse Effects on Salmon and Steelhead and Potential Beneficial Effects of Raising Dissolved Oxygen Levels with the Aeration Facility. California Department of Water Resources.

Nichols, J. W., M. Breen, R. J. Denver, J. J. Distefano, 3rd, J. S. Edwards, R. A. Hoke, D. C. Volz and X. Zhang (2011). "Predicting chemical impacts on vertebrate endocrine systems." Environ Toxicol Chem **30**(1): 39-51 http://www.ncbi.nlm.nih.gov/pubmed/20963851.

Animals have evolved diverse protective mechanisms for responding to toxic chemicals of both natural and anthropogenic origin. From a governmental regulatory perspective, these protective responses complicate efforts to establish acceptable levels of chemical exposure. To explore this issue, we considered vertebrate endocrine systems as potential targets for environmental contaminants. Using the hypothalamic-pituitary-thyroid (HPT), hypothalamic-pituitary-gonad (HPG), and hypothalamic-pituitary-adrenal (HPA) axes as case examples, we identified features of these systems that allow them to accommodate and recover from chemical insults. In doing so, a distinction was made between effects on adults and those on developing organisms. This distinction was required because endocrine system disruption in early life stages may alter development of organs and organ systems, resulting in permanent changes in phenotypic expression later in life. Risk assessments of chemicals that impact highly regulated systems must consider the dynamics of these systems in relation to complex environmental exposures. A largely unanswered question is whether successful accommodation to a toxic insult exerts a fitness cost on individual animals, resulting in adverse consequences for populations. Mechanistically based mathematical models of endocrine systems provide a means for better understanding accommodation and recovery. In the short term, these models can be used to design experiments and interpret study findings. Over the long term, a set of validated models could be used to extrapolate limited in vitro and in vivo testing data to a broader range of untested chemicals, species, and exposure scenarios. With appropriate modification, Tier 2 assays developed in support of the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program could be used to assess the potential for accommodation and recovery and inform the development of mechanistically based models.

Noyes, P. D., M. K. McElwee, H. D. Miller, B. W. Clark, L. A. Van Tiem, K. C. Walcott, K. N. Erwin and E. D. Levin (2009). "The toxicology of climate change: Environmental contaminants in a warming world." Environment International **35**(6): 971-986 http://www.sciencedirect.com/science/article/pii/S0160412009000543.

> Climate change induced by anthropogenic warming of the earth's atmosphere is a daunting problem. This review examines one of the consequences of climate change that has only recently attracted attention: namely, the effects of climate change on the environmental distribution and toxicity of chemical pollutants. A review was undertaken of the scientific literature (original research articles, reviews, government and intergovernmental reports) focusing on the interactions of toxicants with the environmental parameters, temperature, precipitation, and salinity, as altered by climate change. Three broad classes of chemical toxicants of global significance were the focus: air pollutants, persistent organic pollutants (POPs), including some organochlorine pesticides, and other classes of pesticides. Generally, increases in temperature will enhance the toxicity of contaminants and increase concentrations of tropospheric ozone regionally, but will also likely increase rates of chemical degradation. While further research is needed, climate change coupled with air pollutant exposures may have potentially serious adverse consequences for human health in urban and polluted regions. Climate change producing alterations in: food webs, lipid dynamics, ice and snow melt, and organic carbon cycling could result in increased POP levels in water, soil, and biota. There is also compelling evidence that increasing temperatures could be deleterious to pollutant-exposed wildlife. For example, elevated water temperatures may alter the biotransformation of contaminants to more bioactive metabolites and impair homeostasis. The complex interactions between climate change and pollutants may be particularly problematic for species living at the edge of their physiological tolerance range where acclimation capacity may be limited. In addition to temperature increases, regional precipitation patterns are projected to be altered with climate change. Regions subject to decreases in precipitation may experience enhanced volatilization of POPs and pesticides to the atmosphere. Reduced precipitation will also increase air pollution in urbanized regions resulting in negative health effects, which may be exacerbated by temperature increases. Regions subject to increased precipitation will have lower levels of air pollution, but will likely experience enhanced surface deposition of airborne POPs and increased run-off of pesticides. Moreover, increases in the intensity and frequency of storm events linked to climate change could lead to more severe episodes of chemical contamination of water bodies and surrounding watersheds. Changes in salinity may affect aquatic organisms as an independent stressor as well as by altering the bioavailability and in some instances increasing the toxicity of chemicals. A paramount issue will be to identify species and populations especially vulnerable to climate–pollutant interactions, in the context of the many other physical, chemical, and biological stressors that will be altered with climate change. Moreover, it will be important to predict tipping points that might trigger or accelerate synergistic interactions between climate change and contaminant exposures.

Ostrach, D. and J. Groff (2009). The Role of Contaminants, within the Context of Multiple Stressors, in the Collapse of the Striped Bass Population in the San Francisco Estuary and its Watershed (Final report). Department of Water Resources, Contract # 4600004664 http://www.science.calwater.ca.gov/pdf/workshops/POD/POD_yr2_Ostrach_090522_report_final_djo.pdf.

Ostrach, D. J., J. M. Low-Marchelli, K. J. Eder, S. J. Whiteman and J. G. Zinkl (2008). "Maternal transfer of xenobiotics and effects on larval striped bass in the San Francisco Estuary." Proc Natl Acad Sci U S A **105**(49): 19354-19359 http://www.ncbi.nlm.nih.gov/pubmed/19033204.

> Aquatic ecosystems around the world face serious threats from anthropogenic contaminants. Results from 8 years of field and laboratory investigations indicate that sublethal contaminant exposure is occurring in the early life stages of striped bass in the San Francisco Estuary, a population in continual decline since its initial collapse during the 1970s. Biologically significant levels of polychlorinated biphenyls, polybrominated diphenyl ethers, and current-use/legacy pesticides were found in all egg samples from river-collected fish. Developmental changes previously unseen with standard methods were detected with a technique using the principles of unbiased stereology. Abnormal yolk utilization, brain and liver development, and overall growth were observed in larvae from river-collected fish. Histopathological analyses confirmed and identified developmental alterations. Using this methodology enabled us to present a conclusive line of evidence for the maternal transfer of xenobiotics and their adverse effects on larval striped bass in this estuary.

Pettis, J. S., E. M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose and D. Vanengelsdorp (2013). "Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae." PLOS One **8**(7): e70182 http://www.ncbi.nlm.nih.gov/pubmed/23894612.

> Recent declines in honey bee populations and increasing demand for insect-pollinated crops raise concerns about pollinator shortages. Pesticide exposure and pathogens may interact to have strong negative effects on managed honey bee colonies. Such findings are of great concern given the large numbers and high levels of pesticides found in honey bee colonies. Thus it is crucial to determine how field-relevant combinations and loads of pesticides affect bee health. We collected pollen from bee hives in seven major crops to determine 1) what types of pesticides bees are exposed to when rented for pollination of various crops and 2) how field-relevant pesticide blends affect bees' susceptibility to the gut parasite Nosema ceranae. Our samples represent pollen collected by foragers for use by the colony, and do not necessarily indicate foragers' roles as pollinators. In blueberry, cranberry, cucumber, pumpkin and watermelon bees collected pollen almost exclusively from weeds and wildflowers during our sampling. Thus more attention must be paid to how honey bees are exposed to pesticides outside of the field in which they are placed. We detected 35 different pesticides in the sampled pollen, and found high fungicide loads. The insecticides esfenvalerate and phosmet were at a concentration higher than their median lethal dose in at least one pollen sample. While fungicides are typically seen as fairly safe for honey bees, we found an increased probability of Nosema infection in bees that consumed pollen with a higher fungicide load. Our results highlight a need for research on sub-lethal effects of fungicides and other chemicals that bees placed in an agricultural setting are exposed to.

Poletto, J. B., D. E. Cocherell, A. P. Klimley, J. J. Cech and N. A. Fangue (2013). "Behavioural salinity preferences of juvenile green sturgeon Acipenser medirostris acclimated

to fresh water and full-strength salt water." Journal of Fish Biology **82**(2): 671-685 http://dx.doi.org/10.1111/jfb.12023.

> To quantify the salinity preference of juvenile green sturgeon Acipenser medirostris, two groups of A. medirostris [140 days post hatch (dph); total length (LT) 38·0–52·5 cm] were acclimated to either near fresh water (mean ± s.e. salinity = 3·2 ± 0·6) or full-strength salt water (34·1 ± 1·2) over 8 weeks. Following acclimation, the two groups were divided into experimental and control groups, where experimental A. medirostris from both freshwater and saltwater acclimations were individually introduced (200–220 dph) into a rectangular salinity-preference flume (maximum salinity gradient: 5–33). Control A. medirostris were presented with only their acclimation water (fresh water or salt water) on both sides of the flume. It was demonstrated that A. medirostris acclimated to both salt water and fresh water spent a significantly greater amount of time on the side of the testing area with the highest salinity concentration ($P < 0·05$ and $P < 0·001$, respectively) while control A. medirostris spent an equal amount of time on each side of the flume. These findings indicate that juvenile A. medirostris are not only capable of detecting salt water within the first year of their lives but perhaps are actively seeking out saline environments as they move through a watershed. Establishing A. medirostris salinity preferences provides a better understanding of the early life history of this threatened species, shedding light on possible outmigration timing.

Sokolova, I. M., M. Frederich, R. Bagwe, G. Lannig and A. A. Sukhotin (2012). "Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates." Mar Environ Res **79**(0): 1-15 http://www.sciencedirect.com/science/article/pii/S0141113612000712.

> Energy balance is a fundamental requirement of stress adaptation and tolerance. We explore the links between metabolism, energy balance and stress tolerance using aquatic invertebrates as an example and demonstrate that using key parameters of energy balance (aerobic scope for growth, reproduction and activity; tissue energy status; metabolic rate depression; and compensatory onset of anaerobiosis) can assist in integrating the effects of multiple stressors and their interactions and in predicting the whole-organism and population-level consequences of environmental stress. We argue that limitations of both the amount of available energy and the rates of its acquisition and metabolic conversions result in trade-offs between basal maintenance of a stressed organism and energy costs of fitness-related functions such as reproduction, development and growth and can set limit to the tolerance of a broad range of environmental stressors. The degree of stress-induced disturbance of energy balance delineates transition from moderate stress compatible with population persistence (pejus range) to extreme stress where only time-limited existence is possible (pessimum range). It also determines the predominant adaptive strategy of metabolic responses (energy compensation vs. conservation) that allows an organism to survive the disturbance. We propose that energy-related biomarkers can be used to determine the conditions when these metabolic transitions occur and thus predict ecological consequences of stress exposures. Bioenergetic considerations can also provide common denominator for integrating stress responses and predicting tolerance limits under the environmentally realistic scenarios when multiple and often variable stressors act simultaneously on an organism. Determination of bioenergetic sustainability at the

organism's level (or lack thereof) has practical implications. It can help identify the habitats and/or conditions where a population can survive (even if at the cost of reduced reproduction and growth) and those that are incapable of supporting viable populations. Such an approach will assist in explaining and predicting the species' distribution limits in the face of the environmental change and informing the conservation efforts and resource management practices.

Spearow, J. L., R. S. Kota and D. J. Ostrach (2011). "Environmental contaminant effects on juvenile striped bass in the San Francisco Estuary, California, USA." Environ Toxicol Chem **30**(2): 393-402 http://www.ncbi.nlm.nih.gov/pubmed/21038432.

The decline of pelagic organisms in the San Francisco Estuary (SFE) (California, USA) is attributed to several factors, including water diversions, invasive species, and exposure to environmental toxicants. The present study evaluated the effects of environmental contaminants on liver vitellogenin, metallothionein, 7-ethoxyresorufin-O-deethylase (EROD), and benzyloxyresorufin O-deethylase (BROD) activity in juvenile striped bass (Morone saxitilis) in the SFE. Analysis of juvenile striped bass liver extracts revealed site-specific elevations of vitellogenin, metallothionein, and EROD biomarkers across the estuary. Although some striped bass in the estuary showed EROD activity similar to unhandled hatchery controls, several sites in the estuary showed significantly higher EROD activity that was in the range of beta-naphthoflavone (BNF)-injected, positive controls. Overall, EROD activity averaged 283% higher in estuary fish than in hatchery controls. Chemical analyses of extracts from semipermeable membrane devices (SPMDs) deployed in the estuary for one month showed elevated polyaromatic hydrocarbon (PAH) levels. Semipermeable membrane devices extract injections-induced metallothionein and BROD in striped bass livers. These data show that environmental exposures are impacting EROD and other biomarkers in the SFE striped bass population. Previous studies in our laboratory have associated poor larval development with maternal transfer of environmental contaminants. Further studies are needed to monitor contaminant exposures by the use of biomarkers and to integrate them into a more effective pelagic species recovery plan in the SFE.

Teh, S., Acuna S, J. A., K. T., B. D., J. Hobbs, S. S., C. D. and R. Baxter. (2013 Grant in progress-a). Fall low salinity habitat (FLaSH) fish health study: contrasts in health indices, growth and reproductive fitness of delta smelt and other pelagic fishes rearing in the low salinity zone and cache slough regions, Report to the Ecosystem Restoration Program, California Department of Fish and Wildlife, Water Brabch, Sacramento, California.

Teh, S., D. Baxa, A. Javidmehr, S. Acuna and T. Kurobe (2013 Grant in progress-b). Studies Assessing Factors that Influence the Spawning Migration Behavior and Reproductive Condition of Delta Smelt (Hypomesus transpacificus) in the Sacramento-San Joaquin Delta. Subtitle: Investiagting the relationship of health and reproductive condition in delta smelt (hypomesus transpacificus) and turbidity levels in the Sacramento-San Joaquin Delta in 2010 and 2011. Annual report to the Science Division, Bay-Delta Office, U.S. Bureau of Reclamation, California.

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> Nico M. van Straalen is a professor of Animal Ecology at VU University, Amsterdam, where he teaches evolutionary biology, animal physiology, ecology, and environmental toxicology. His research interests concern the evolution of toxicant tolerance in soil-living invertebrates, and the molecular mechanisms involved. His laboratory developed genomics resources for the collembolan Folsomia candida, a model species for soil toxicity testing. With Dick Roelofs he wrote a textbook “An Introduction to Ecological Genomics” (OUP, 2nd Ed., 2012). In the past Nico van Straalen has contributed to risk assessment methodology by introducing the species-sensitivity distribution approach. He served in the founding board of SETAC-Europe. Martin E. Feder is Faculty Dean of Academic Affairs, Division of The Biological Sciences and The Pritzker School of Medicine, at The University of Chicago. His research focuses on understanding adaptation to the environment from both a mechanistic and evolutionary perspective. His laboratory investigates the heat-shock protein Hsp70, its encoding genes, and its regulation in Drosophila as a model system for understanding evolutionary adaptation. Martin Feder chaired the first Gordon Research Conference on Evolutionary and Ecological Functional Genomics, in 2003

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> Effects of bisphenol A (BPA) on ovarian transcript profiles as well as targeted end points with endocrine/reproductive relevance were examined in two fish species, fathead minnow (Pimephales promelas) and zebrafish (Danio rerio), exposed in parallel using matched experimental designs. Four days of waterborne exposure to 10 ?g BPA/L caused significant vitellogenin induction in both species. However, zebrafish were less sensitive to effects on hepatic gene expression and steroid production than fathead minnow and the magnitude of vitellogenin induction was more modest (i.e., 3-fold compared to 13?000-fold in fathead minnow). The concentration?response at the ovarian transcriptome level was nonmonotonic and violated assumptions that underlie proposed methods for estimating hazard thresholds from transcriptomic results. However, the nonmonotonic profile was consistent among species and there were nominal similarities in the functions associated with the differentially expressed genes, suggesting potential activation of common pathway perturbation motifs in both species. Overall, the results provide an effective case study for considering the potential application of ecotoxicogenomics to ecological risk assessments and provide novel comparative data regarding effects of BPA in fish.

Werner, I., L. A. Deanovic, D. Markiewicz, M. Stillway, N. Offer, R. E. Connon and S. M. Brander (2008). Pelagic Organism Decline (POD): Acute and Chronic Invertebrate and Fish Toxicity Testing in the Sacramento-San Joaquin Delta 2006-2007. Final Report, Interagency Ecological Program,.

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> The current study investigated the potential of vegetated drainage ditches for mitigating the impact of agricultural irrigation runoff on downstream aquatic ecosystems. Water column toxicity to larval fathead minnow (Pimephales promelas),and the amphipod Hyalella azteca was measured for 12 h or less at the ditch inflow and outflow, using custom-built in situ exposure systems. In addition, water and sediment samples were subject to standard toxicity tests with Ceriodaphnia dubia and H. azteca, respectively. No acute toxicity to larval fathead minnow was observed; however, runoff was highly toxic to invertebrates. Passage through a 389- to 402-m section of vegetated ditch had a mitigating effect and reduced toxicity to some degree. However, runoff from an alfalfa field treated with chlorpyrifos remained highly toxic to both invertebrate species, and runoff from a tomato field treated with permethrin remained highly toxic to H. azteca after passage through the ditch. Predicted toxic units calculated from insecticide concentrations in runoff and 96-h median lethal concentration (LC50) values generally agreed with C. dubia toxicity measured in the laboratory but significantly underestimated in situ toxicity to H. azteca. Sediments collected near the ditch outflow were toxic to H. azteca. Results from the current study demonstrate that experimental vegetated ditches were unable to eliminate the risk of irrigation runoff to aquatic ecosystems. In addition, protective measures based on chemical concentrations or laboratory toxicity tests with C. dubia do not ensure adequate protection of aquatic ecosystems from pyrethroid-associated toxicity. Environ. Toxicol. Chem. 2010;29:2859–2868. © 2010 SETAC

Werner, I., L. A. Deanovic, M. Stillway and D. Markiewicz (2009). Acute Toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt - 2009. FINAL REPORT, The California State Water Resources Control Board.

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Weston, D. P. and M. J. Lydy (2010). "Urban and agricultural sources of pyrethroid insecticides to the Sacramento-San Joaquin Delta of California." Environ Sci Technol **44**(5): 1833-1840 http://www.ncbi.nlm.nih.gov/pubmed/20121184.

> While studies have documented the presence of pyrethroid insecticides at acutely toxic concentrations in sediments, little quantitative data on sources exist. Urban runoff, municipal wastewater treatment plants and agricultural drains in California's Sacramento-San Joaquin River Delta were sampled to understand their importance as contributors of these pesticides to surface waters. Nearly all residential runoff samples were toxic to the amphipod, Hyalella azteca, and contained pyrethroids at concentrations exceeding acutely toxic thresholds, in many cases by 10-fold. Toxicity identification evaluation data were consistent with pyrethroids, particularly bifenthrin and cyfluthrin, as the cause of toxicity. Pyrethroids passed through secondary treatment

> systems at municipal wastewater treatment facilities and were commonly found in the final effluent, usually near H. azteca 96-h EC(50) thresholds. Agricultural discharges in the study area only occasionally contained pyrethroids and were also occasional sources of toxicity related to the organophosphate insecticide chlorpyrifos. Discharge of the pyrethroid bifenthrin via urban stormwater runoff was sufficient to cause water column toxicity in two urban creeks, over at least a 30 km reach of the American River, and at one site in the San Joaquin River, though not in the Sacramento River.

You, J., S. Pehkonen, D. P. Weston and M. J. Lydy (2008). "Chemical availability and sediment toxicity of pyrethroid insecticides to Hyalella azteca: application to field sediment with unexpectedly low toxicity." Environ Toxicol Chem **27**(10): 2124-2130 http://www.ncbi.nlm.nih.gov/pubmed/18419174.

> Tenax extraction is a simple, inexpensive approach to estimate the bioavailability of hydrophobic organic contaminants from sediment. In the present study, a single-point Tenax extraction was evaluated regarding its correlation with the acute toxicity to Hyalella azteca using field-collected sediments in California, USA. Pyrethroids were believed to be the primary contributor to the observed toxicity, and a significant correlation existed between the expected toxicity (given pyrethroid concentrations) and the mortality at most sampling sites. A small subset of sites, however, showed unexpectedly low toxicity to H. azteca despite high concentrations of pyrethroids. These samples were evaluated by Tenax extraction with the expectation that this procedure, which qualifies bioavailable instead of total pyrethroid concentration in sediment, would better explain the anomalously low toxicity. The term bioavailable toxic unit was proposed to link sediment toxicity with chemical availability, and the toxicity in the 17 selected sediments was better explained using Tenax extraction. The r2 value of the regression between sediment toxicity and toxic unit for the 17 sediments increased from 0.24 to 0.60 when the Tenax-extractable concentration was used in place of the total concentration. Results also showed that adsorption to sand particles might play a controlling role in pyrethroid bioavailability and, in turn, sediment toxicity to benthic invertebrates.

Appendix I:

Summary of Fish Monitoring Surveys in the SFE and Delta

<u>20mm Survey</u>

- Postlarval-juvenile delta smelt distribution and relative abundance
- Stations throughout the delta and downstream to the eastern portion of San Pablo bay and Napa River
    - 41 stations sampled/survey; 46 in wet year
    - 8 stations added in 2008
- Biweekly mid-March through early July
- Three 10 minute stepped oblique (bottom to top) tows per station

<u>Smelt Larva Survey</u>

- Longfin smelt larvae to assess vulnerability of longfin smelt larvae to entrainment in south Delta export pumps
- Delta, Suisun Bay, and Suisun Marsh
    - 35 survey locations
- First two weeks in January and repeats every other week through the second week in March
    - 4-day surveys at a time
- Single 10 minute oblique tow per station

<u>Spring Kodiak Trawl</u>

- Relative abundance and distribution of spawning delta smelt
- From San Pablo Bay upstream to Stockton on the San Joaquin River, Walnut Grove on the Sacramento River, and the Sacramento Deep Water Ship Channel
    - 40 stations
- January to May
    - 4-5 days a month
- Single 10 minute surface tow between 2 boats

<u>San Francisco Bay Study</u>

- Effects of freshwater outflow on the abundance and distribution of fish in SF Estuary
- Dumbarton Bridge in South San Francisco Bay, to just wet of Alcatraz Island in Central San Francisco Bay, throughout San Pablo and Suisun bays, north to the confluence Steamboat and Cache sloughs on the Sacramento River, and east to Old River Flats on the San Joaquin River.
    - 35 original stations
    - 7 stations added in 1988, 4 stations in 1991, 6 stations in 1994
    - Total of 52 stations
- Monthly year-round

<u>Midwater Trawl</u>

- Single Oblique tow per station

- Samples pelagic fishes, is towed with the current at a standard engine rpm for 12 minutes and retrieved obliquely such that all depths are sampled equally.

Otter Trawl

- The otter trawl, which samples demersal fishes, shrimp, and crabs, is towed against the current at a standard engine rpm for 5 minutes then retrieved.

Open water Survey (Discontinued)

- Included a plankton net that sampled larval fish and crustaceans - discontinued in 1989.

Fall Midwater Trawl

- Determine the relative abundance and distribution of age-0 striped bass and other upper estuary pelagic species (including delta smelt, longfin smelt, American shad, and threadfin shad)
- Range from San Pablo Bay upstream to Stockton to the San Joaquin River, Hood on the Sacramento River, and the Sacramento Deep Water Ship Channel
    - 122 stations (100 stations for index, 22 for delta smelt distribution)
- Monthly September through December
- Single oblique tow per station

Summer Townet Survey

- Relative distribution and abundance of young of the year (age-0) striped bass in the Delta. The Delta smelt index was developed in 1990
- Ranging from eastern San Pablo Bay to Rio Vista on the Sacramento River and to Stockton on the San Joaquin River, stations in Cache Slough and Sacramento Deep Water Ship Channel
    - 32 historic stations (31 contribute to indices)
- Initially biweekly once mean size reached 20 mm; now biweekly early June through late August
- 2 or 3 oblique tow station per station

Full details of these and other studies and surveys can be accessed at http://www.dfg.ca.gov/delta/data/