Appendix 1: CURRENT-USE PESTICIDES – these are intended as examples of major classes or uses of current-use pesticides and do not necessarily represent the most important or most toxic pesticides. Environmental levels are listed as either a range of maximum concentrations (major river to small sloughs) or typical maximum concentrations in bed sediments in major stream or river reach (i.e. not edge of field) but are not intended to be comprehensive.

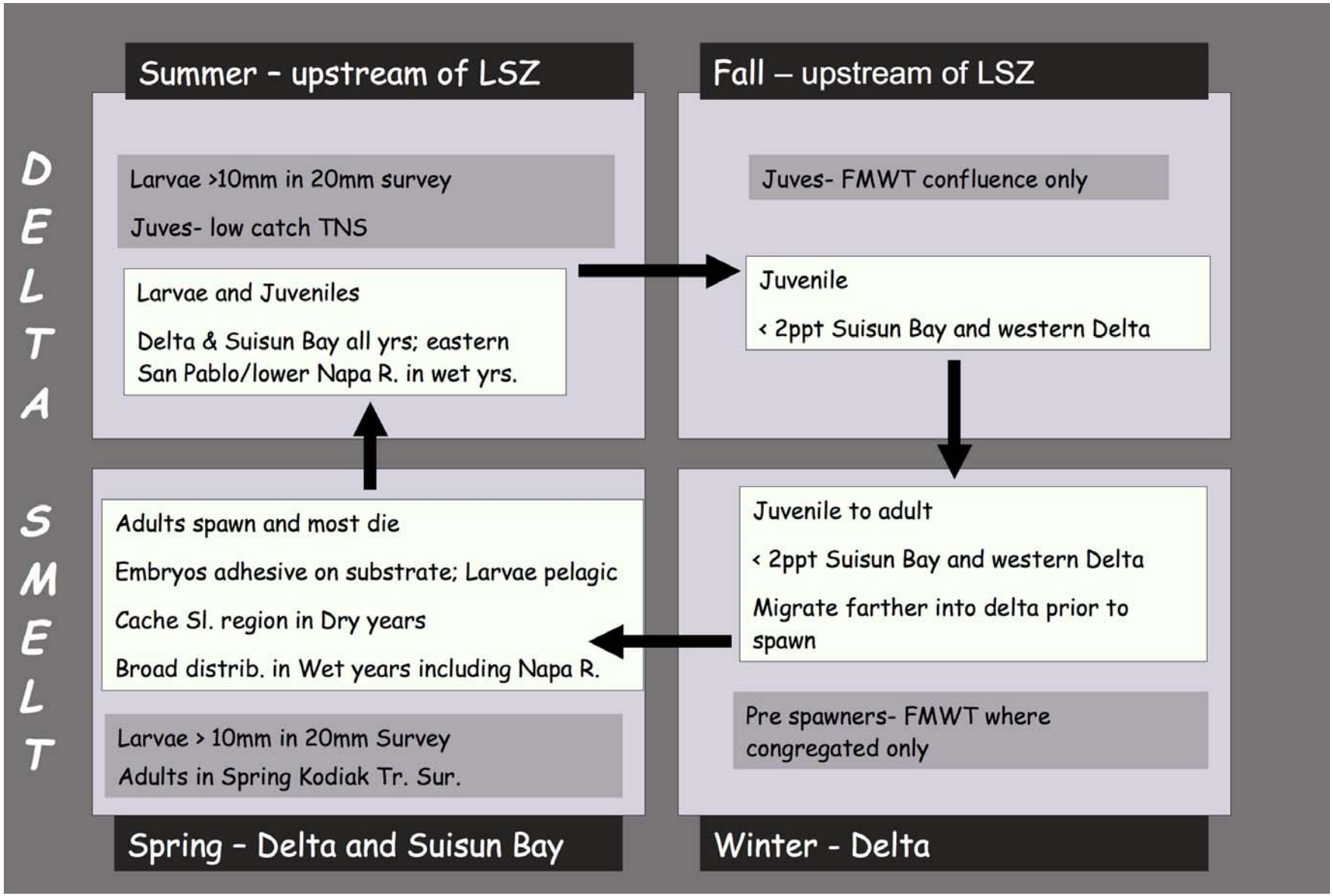
CONTAMINANT(S)	SOURCES AND ENVIRONMENTAL CONCENTRATIONS
Pyrethroid Insecticides (bifenthrin, cypermethrin, esfenvaerate, lambda-cyhalothrin, permethrin)	• Increasing use in both agricultural and urban areas • Primarily associated with sediments • Typical bed sediment maximum concentrations are 5-10 ppb but concentrations in tailwater ponds or near storm drain outfalls can reach 500 ppb (Weston et al., 2004, 2005)
Organophosphate Insecticides (chlorpyrifos, diazinon, malathion)	• Decreasing use • Primarily dissolved • Typical water maximum concentrations are 15 – 800 ng/L (CV RWQCB technical reports, 2006, 2007; Calanchini and Johnson, 2007; Lu, 2004)
Carbamate Insecticides (carbaryl, carbofuran)	• Decreasing use • Primarily dissolved • Typical water maximum concentrations are 10 – 900 ng/L (CV RWQCB technical reports, 2006, 2007)
Rice Herbicides (molinate, thiobencarb, propanil)	• Changing use (molinate and thiobencarb decreasing, propanil increasing) • Primarily dissolved • Maximum water concentrations In smaller creeks are up to 10 ug/L but lower in mainstem rivers (Orlando and Kuivila, 2004)
Other Herbicides (diuron, hexazinone, simazine)	• No major changes in use • Primarily dissolved • Diuron and simazine are ubiquitos with maximum water concentrations of 1 – 2 ug/L. while hexazinone is more localized (Green and Young, 2006; Orlando and Kuivila, 2006)

Appendix 2: LEGACY ORGANOCHLORINES – these have been identified from USEPA 303(d) listing or proposed TMDLs and are primarily of human-health concern for consumption of fish.

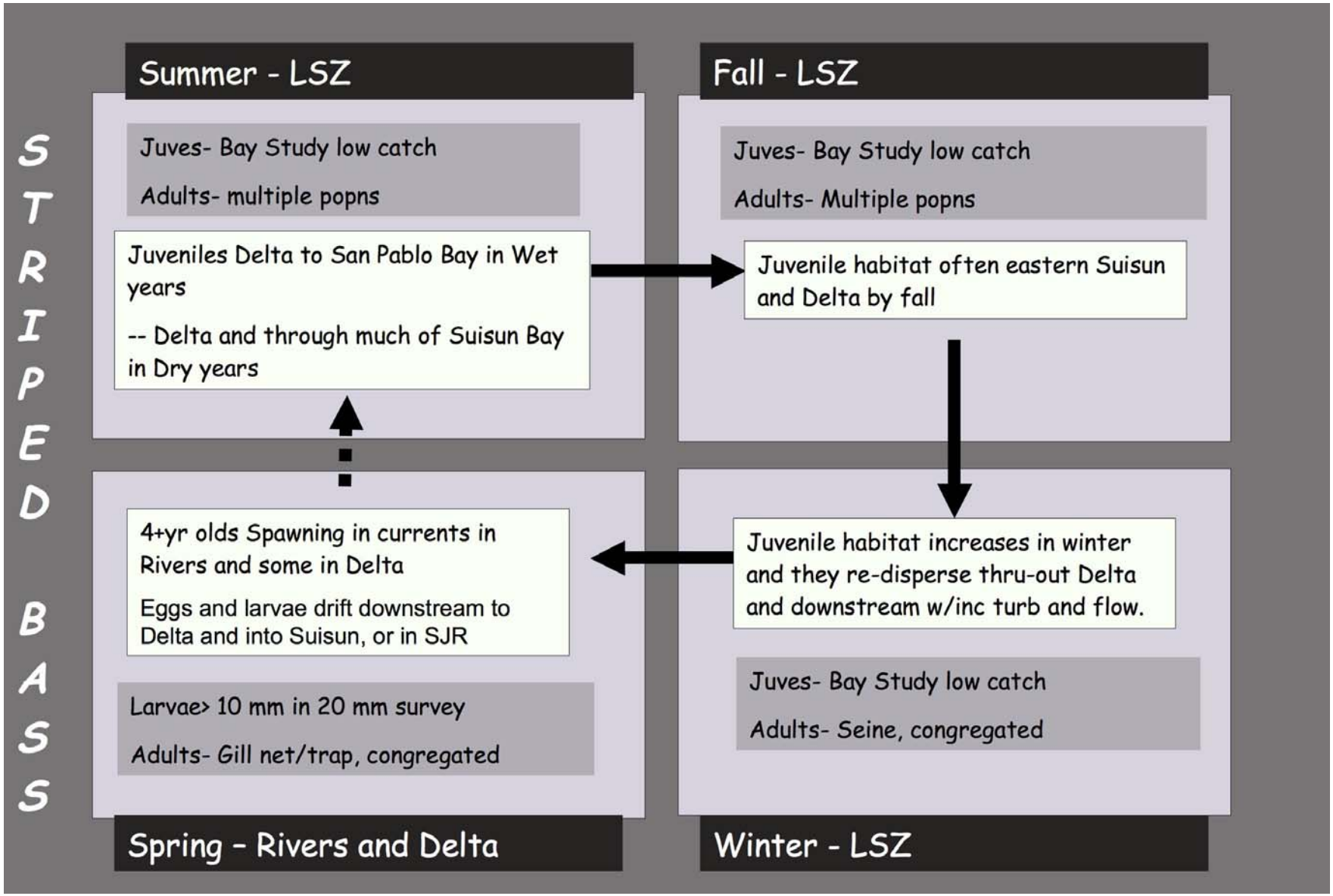
CONTAMINANT(S)	SOURCES AND ENVIRONMENTAL CONCENTRATIONS
Dieldrin	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • Fish tissue concentrations > 2 ng/g in 15% of samples; median concentration white croaker = 1.85 (Davis et al., 2006) • Fish consumption and endocrine disruption concerns
Chlordane	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • Median concentration in sturgeon was 11 ppb (Davis et al., 2006) • Fish consumption concerns
∑DDTs	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • Maximum concentration in white croaker = 65 ppb (Davis et al., 2006) • Fish consumption and endocrine disruption concerns
PAHs	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • Mean concentration in sediments in North Bay and Delta = 96 & 31 mg/kg TOC, respectively (Oros and Ross, 2004) • Potential impacts on early life stages of fish (SFEI, 2007)
PCBs	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • In sediments, concentrations in 68% of samples exceeded 2.5 ppb (SFEI, 2006) • In white croaker, concentrations ranged from 239 to 530 ppb and averaged 342 ppb (SFEI, 2006) • Fish consumption concerns
Dioxins and Furans	• Highest concentrations near urban areas • Primarily associated with sediment and tissue • Concentrations in sport fish well above threshold for concern; entire Bay included on US EPA 303(d) List (SFEI, 2007) • Fish consumption concerns

Appendix 3: TRACE METALS - these have been identified from USEPA 303(d) listing or proposed TMDLs and may not be at levels to cause biological effects.

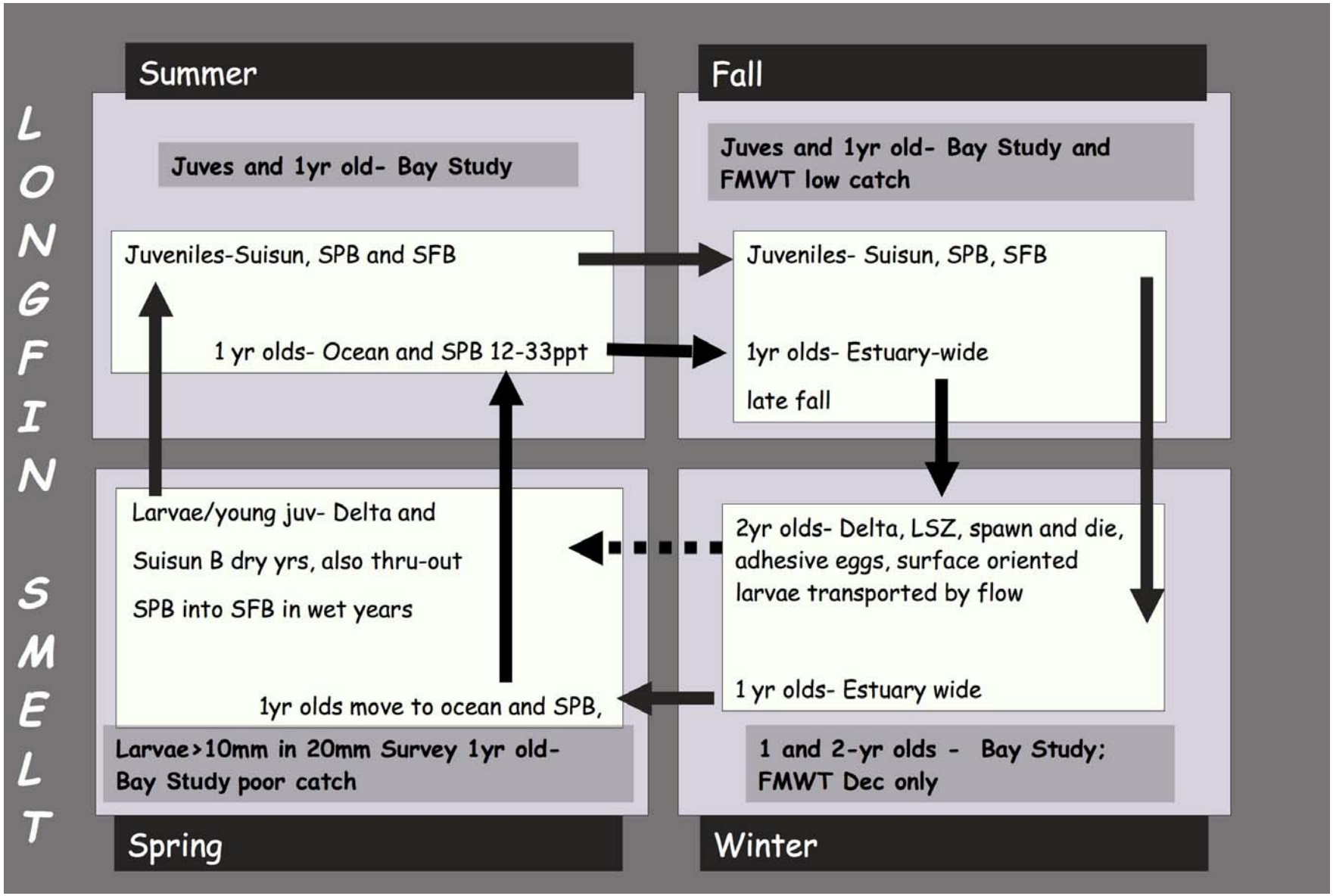
CONTAMINANT(S)	SOURCES AND ENVIRONMENTAL CONCENTRATIONS
Mercury	• Primarily associated with sediment and tissue • Elevated concentrations in *Menidia beryllina* = 116-169 ppb in Cosumnes River, Yolo Bypass, and upper San Joaquin River (SFEI, 2006; 2007) • Primarily fish consumption concerns
Selenium	• Present in water, sediment and tissue • On US EPA 303(d) list for San Francisco Bay (SFEI, 2007) • Selenium concentrations may be high enough to cause deformities, growth impairment, and mortality in early life-stages of Sacramento splittail and white sturgeon (Stewart et al., 2004; SFEI, 2007)
Nickel	• Currently on US EPA 303(d) list for San Francisco Bay but will likely be delisted in 2008 (SFEI, 2007)
Copper	• Both dissolved and associated with sediment • Sources include urban, mining, agriculture, and use as an aquatic herbicide • Removed from US EPA 202(d) list in San Francisco Bay in 2002 (SFEI, 2007) • In the past five years, none of the 159 water samples analyzed had a dissolved copper concentration above site-specific objectives for the Bay (SFEI, 2007)



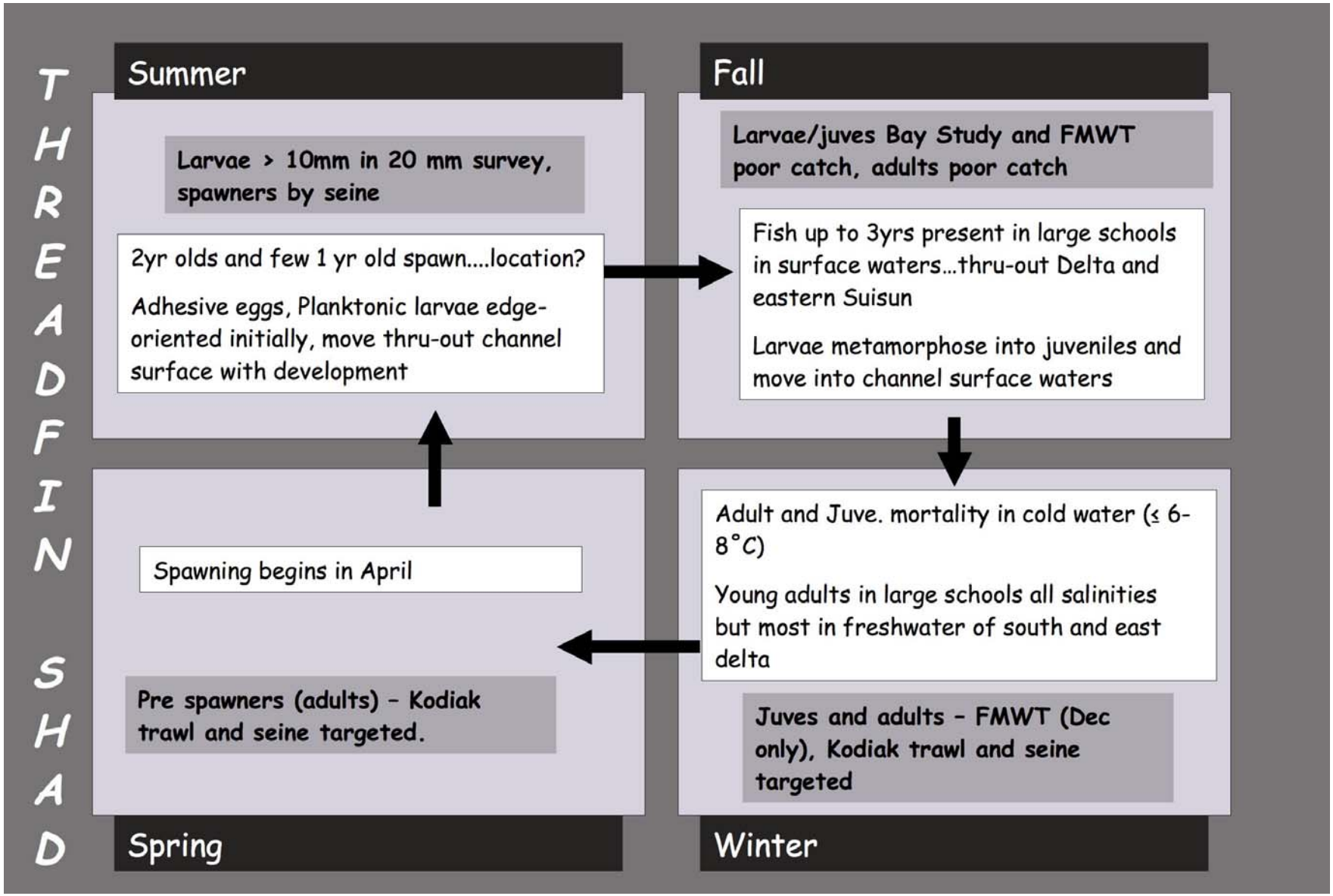
Appendix 4: Geographic and seasonal distribution of delta smelt age/size classes.



Appendix 5: Geographic and seasonal distribution of striped bass age/size classes



Appendix 6: Geographic and seasonal distribution of longfin smelt age/size classes



Appendix 7: Geographic and seasonal distribution of threadfin shad age/size classes

Appendix 8: Biotransformation Enzymes; CYP1A

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Cytochrome P450 1A (CYP1A) aka EROD	Enzyme that metabolizes planar aromatic compounds such as PAHs, PCBs, Dioxins. Expression of mRNA, protein and catalytic activity (EROD) is regulated by substrate bioavailability to aryl hydrocarbon receptor.	qPCR for mRNA ELISA or western immunoblots for CYP1A protein EthoxyresorufinO-de-ethylase (EROD) catalytic activity Each can be measured in microsomes of liver, gill, intestine, skin EROD is simplest method All three endpoints require cyropreservation of tissue samples at -80deg C Immunohistochemistry can be carried out with fixed tissues Embryo, larvae and tissue homogenates can be used as well as whole embryos/larvae for EROD in 96-well fluorometer	Expression indicates bioavailablity of planar aromatic hydrocarbons. Has been linked to adverse developmental effects in salmonid eggs Has been linked to PAH-induced liver lesions and immune suppression	Simple specific method for exposure to PAHs, PCBs, and Dioxins Sequence (qPCR) and catalytic activity (EROD) conserved in vertebrates Commercially available antibodies for ELISA	Catalytic Activity can be inhibited by substrates in high concentrations or metals Sensitive to hormonal impacts (spawning females have impaired expression) Response may be diminished in non-migratory species residing in chronically contaminated settings. Although kits for protein expression are available; plate-reading spectrophotometer or fluorometer is required for EROD; isolation of microsomes also increase signal:noise Only useful in fish; not useful in invertebrates	Excellent indicator of exposure (Stegeman and Hahn 1994; Bucheli and Fent 1995), but limited use as effects indicator Success stories for effects are NOAA Hepatic lesions (Myers et al. 2003) Blue-sac disease-developmental toxicity in ovo by chlorinated hydrocarbon/TEQs (Cook et al. 1997; Nacci et al. 2005)

Appendix 9: Metallothionein

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Metallothionein	Small cytosolic metal-binding protein with 30% sulfhydral residues which also participate in radial scavenging	qPCR for mRNA ELISA or western immunoblots for protein (SDS Page gels not appropriate) DTNB assay for SH groups can be used but is not specific for MT Can be measured in liver, gill, kidney of juveniles or whole tissue homogenates of embryo and larvae (unfixed) Requires cryopreservation Ultracentrifugation enhances signal:noise ratio	Expression indicates bioavailablity of metals and/or acute cellular stress. Should be used in addition to other HSPs or Oxidative stress indicators to determine metal contributions DTNB method should try to eliminate GSH or cysteine contamination if possible	Provides information regarding bioavailability of metals in controlled settings (i.e. aquaculture or point source areas—mining). Good indicator of acute stress, but false negative potential also present. Simple measurements via DTNB or pPCR	Not always selective for metal exposure	Useful in known metal contaminated sites, but not useful in unknown areas. (Olsson 1996, Van der Oost 2003)

Appendix 10: (Acetyl)cholinesterase inhibition

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
AchE	Enzyme necessary for the catabolism of the neurotransmitter acetylcholine. Inhibition occurs primarily by organophosphate and carbamate insecticides	Easily measured using DTNB spectrophotometric method. Enzyme assay using brain, blood cell or plasma of juvenile fish. Homogenates of embryo and larvae (not fixed) also active Requires cryopreservation	Inhibition of enzyme relative to control/reference/ base-line indicates bioavailability of inhibiting compounds Potential to estimate neurological effects and altered behavior	Relatively specific indicators of bioavailable OP or carbamate in fish Non-destructive sampling possible in blood Relationships to behavior have been observed in salmonids Threshold values can be obtained for specific species	Kinetics of inhibitors vary (carbamates are reversible) Metals may interfere with DTNB Requires accurate baseline which is tissue and species specific Need specific inhibitors to rule out other esterases (Butryl, pseudo E) which may occlude results	Excellent indicator of exposure of OP/Carbamates and also linkage to effects Should be used as a Tier 1 endpoint (Scholz et al. 2006; (Fulton and Key 2001)

Appendix 11: Stress Proteins (HSP70)

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Stress Proteins aka Heat Shock/stress Proteins (HSP)	Cytosolic chaperone protein induced during proteolysis May provide cytoprotection	qPCR for mRNA Western immunoblots for protein (most standard) Can be measured in any tissue of juvenile fish as well as homogenates of embryos or larvae (not fixed). Requires cyropresevation	Expression indicates acute cellular stress	Provides information regarding acute cellular stress	Not specific for any class of compound. No consistent relationship to higher order effects Relationship to exposure unclear	Not consistently effective in field settings; kinetics of stress response limits overall use (van der Oost et al. 2003)

Appendix 12: Glutathione (GSH) parameters

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Analysis of glutathione (GSH) parameters, including GSH, oxidized glutathione (GSSG), GSH biosynthetic enzymes	Certain metals and organic chemicals, as well as complex mixtures may increase GSH biosynthesis in target tissues.	1.GSH-tissue extracts analyzed by a number of methods, including spectrophotometry, HPLC 2. GSH biosynthetic enzyme activities can be analyzed by spectrophotometry, HPLC methods with slight modifications. Also, mRNA expression can be measured using quantitative PCR methods	Used in the laboratory in rodent and fish cell studies to determine if chemicals alter redox status and cause cellular injury through oxidative damage Fish GSH levels sometimes measured in field studies as a biomarker of pollutant exposure and effects related to oxidative damage	If analyses are performed carefully can provide information linking chemical exposures and cellular level effects (i.e. oxidative damage) Can provide, to some degree, health status measurements if used with other physiological markers Measurements are specific Changes in GSH biosynthetic enzymes by enzymatic activity or mRNA can provide mechanistic information on chemical modes of action	Accurate GSH and oxidized glutathione levels necessitate careful sample handling or artifacts can be produced which bias results. GSSG, the oxidized formed of GSH, is rapidly exported out of cells. As a result, often increased GSSG levels are not observed in cells even under oxidative stress, thus leading to false negatives. GSH levels are tightly regulated in most cells, and induction of GSH is typically not sustained during chemical exposures. Accordingly, the timing of analysis during exposures can be critical. GSH biosynthetic enzyme analysis and RNA level requires carefully developed PCR technique specific to species of interest. Induction responses in aquatic animals not well understood. In other species, GSH levels are strongly affected by dietary status. Little is known about the effects of environmental variables on GSH levels in fish	May have limited utility for the POD in field studies due to; 1) potential effects of dietary stress and other environmental influences, 2) lack of validation studies in fish exposed to multiple stressors, (ie salinity, DO etc), 3) lack of basic understanding of the regulation of GSH in target species. Has potential if used as part of a suite of biochemical and physiological indices to assess tissue or fish health if appropriate controls are used. (Gallagher and Di Giulio 1991; Winston 1991; Gallagher, Hasspieler et al. 1992; Dickinson, Levonen et al. 2004; Chen, Shertzer et al. 2005; Iles and Liu 2005; Lee, Kang et al. 2006; Farombi, Adelowo et al. 2007)

Appendix 13: Antioxidant Enzyme Parameters

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Enzymes include superoxide dismutase (SOD), glutathione peroxidases (GPXs), catalase, and peroxisomal enzymes	Early studies in lower organisms (*Drosophila*) showed that exposure to conditions or agents that induce oxidative stress, a common mode of toxicity, could markedly elevate the expression of protective antioxidant enzyme activities Numerous studies in rodents and some in fish species show that chemical exposures can sometimes increase activities of antioxidant enzymes	Enzymatic activities are analyzed by a number of methods, including spectrophotometry, HPLC using subcellular fractions from target tissues (ie liver, gills) that contain enzymes Also, mRNA expression can be measured by isolating total mRNA from target tissues and using quantitative PCR methods	Used in the laboratory in rodent and fish studies to determine if a chemical or drug acts through oxidative damage, and to determine adaptive response of the organism to chemical exposure Also used to determine the protective potential of some drug or chemical agents that induce a protective antioxidant response in tissues Have been sometimes employed in field studies as a biomarker to detect pollution-mediated oxidative damage in aquatic animals	Analyses are very specific and provide specific information measuring and organisms cellular response against oxidative stress Increases in antioxidant enzyme activity or mRNA may reflect and adaptive response to an early disease state. Can provide important information about mechanisms of action of chemical exposures. Activity measurements need to be carefully optimized, but for many enzymes are not technically challenging.	Studies in fish indicate assays are extremely variable with respect to dose-response effects on antioxidant enzymes. Not well understood if all antioxidant enzymes in fish species are actually inducible (ie the activity is increased) under oxidative stress. Little investigation of regulation of these genes compared to other species. Peroxisomal enzymes (fatty acid oxidases, catalase) are induced under very specific pathogenesis in rodents. Evidence to date indicates that fish do not respond similarly to rodents, and there are species differences among fish. Antioxidant enzyme analysis at the RNA level requires carefully developed PCR assays. In rodents, antioxidant enzyme expression and activity may be affected by dietary status. Temperature and DO can affect antioxidant parameters in fish,. Otherwise, little is known about the effects of diet and other environmental variables on fish antioxidant enzymes.	Limited utility for the POD in identifying chemical stressors in the field due to; 1) lack of basic lab studies in POD species on these parameters, 2) potential effects of nutrition and environmental influences, 3) lack of validation studies in fish exposed to multiple stressors, (ie salinity, DO etc), 4) inconsistencies among species with regards to regulation of antioxidant genes. Has potential if used as part of a suite of biochemical and physiological indices to assess tissue or organism health if appropriate controls are used. (Conway, Tomaszewski et al. 1989; Winston 1991; Kelly, Havrilla et al. 1998; Cattley and Roberts 2000; Malek, Sajadi et al. 2004; Lushchak and Bagnyukova 2006; Valavanidis, Vlahogianni et al. 2006; Farombi, Adelowo et al. 2007; Prieto, Pichardo et al. 2007)

Appendix 14: Vitellogenin/Choriogenin

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Vitellogenin/ Choriogenin	Egg yolk precursor and oocyte membrane/envelope proteins under estrogen receptor control in the liver. Transported to the gonad in the blood. Occurrence in male or juvenile animals indicates estrogenic activity	qPCR for mRNA in liver of juveniles ELISA or western immunoblots for protein Protein can be measured in plasma/liver of fish Alkaline labile phosphate in plasma also potential endpoint for Vtg Homogenates of embryo and larvae (fixed) can be used to measure protein via immunochemical and qPCR Note that fixed tissues cannot be used for qPCR unless preserved in RNA later Cryopreservation necessary	Expression indicates feminization and in vivo estrogen receptor activation	Excellent indicators of bioavailable estrogenic activity which may occur as a result of ER binding by xenoestrogen, but also enhancement of endogenous estrogen concentrations Non-destructive sampling possible in blood of sexually dimorphic species	Relationships to higher order effects such as reproduction or population change inconclusive in the field	Excellent indicator of in vivo estrogenic activity (can be coupled with in vitro assays to determine xenoestrogen vs. endogenous estrogen disruption). (Vethaak et al. 2006 Hiramatsu et al. 2005 Sumpter and Johnson 2005; Kidd et al. 2007)

Appendix 15: Immune Function

Assay	Biological Basis	Technique/Matrix	Application	Strengths	Weaknesses	Summary and References
Immune function, general, e.g., plasma/ lymphoid lysosyme, Cox-2, Total IgM	Changes in activity or concentration of components of the immune system linked to increased disease susceptibility; affected seasonality, condition, environmental and certain environmental chemicals	Pathologies identified in fixed tissues suggest immune impairment; blood and lymphoid tissues (anterior kidneys and spleen) sampled from live larvae (>= 5 cm) and adults provide suitable tissue for functional analyses	Used in field studies to identify site differences, fish condition, and potentially, pollution effects Lab studies required to validate specific chemical effects, and develop species-specific correlations with disease vulnerability.	Has been recommended for use or follow up for biological effects of pollution by other international efforts (i.e., ICES, Hagger et. al. 2006) Can be conducted on small fish using relatively small sample numbers (e.g., Frederick et al. 2007, using *F. heteroclitus*) Can, to some degree, integrate interactive effects of multiple stressors, including chemical exposures Correlated with processes important for population persistence	Some techniques require creation of species-specific tools (i.e., IgM antibodies) although some cross species (i.e., lysozyme, Beckham et al. 2005) Non-specific to chemical exposures May be un-interpretable without comparable information from controlled conditions and additional validation providing supporting information, such as effects of season and nutrition on endpoints.	Immune function is biologically and ecologically important, and can be impaired by exposure to chemicals. Impairment to immune function can be inferred from high evidence of disease (lesions) and parasitism but cannot be linked causally to chemical exposures without additional information. Several biomarkers can be assessed using small samples of blood/lymphoid tissue from field-collected fish (e.g., Frederick et al. 2007) and have general applicability for many fish species (e.g., ICES report; Hagger et al. 2006). Specific techniques may require parallel lab/field validation or may only be interpreted comparatively among populations for the POD issue due to lack of validation for smelt and shad, although striped bass are relatively well-studied.

Appendix 16: DNA Damage

Assay	Biological Basis	Technique/Matrix	Application	Strengths	Weaknesses	Summary and References
DNA Damage	Changes in DNA integrity such as strand breaks, or chemically-altered DNA bases has been linked with poor fertility and embryo-larval success; affected by certain pollutants (classic genotoxicants and oxidative stressors) but also some cells are affected by environmental or seasonal factors Bulky DNA adducts provide record of chemical agents of exposure and effect.	Single cells, such as nucleated blood cells, or dissociated tissue cells which can be sampled from live larvae (>= 5 cm) and adults provide suitable tissue for analyses ^{32}P post-labeling, quantifies bulky DNA adducts sensitively, requiring small samples	DNA integrity used in field studies to identify site differences, fish condition, and potentially, genotoxic pollution effects; Lab studies required to validate species sensitivities and differentiate chemical from non-chemical effects. Bulky DNA adducts document bioeffective PAH exposures	DNA integrity has been recommended for follow up to measure biological effects of pollution by other international efforts (i.e., ICES, Hagger et al. 2006) Methods are fairly species-independent, and can be conducted easily (low tech) on small fish using relatively small sample numbers Can, to some degree, integrate interactive effects of multiple stressors, including chemical exposures Correlated with processes important for population persistence DNA adducts are recommended for use or follow up for biological effects of pollution by other international efforts (i.e., ICES, Hagger et al. 2006)	DNA integrity measures are non-specific to chemical exposures, and may be un-interpretable without comparable information from controlled conditions and additional validation providing supporting information, such as effects of season and nutrition on endpoints. DNA adduct method is highly specialized (and hazardous), therefore targeted application may be appropriate.	DNA integrity is biologically and ecologically important, and can be impaired by exposure to chemicals. DNA damage can be linked causally to chemical exposures, but may also reflect preparative artifacts or natural factors. Several biomarkers can be assessed using small samples of cells from blood or tissues, including those preserved from field-collected fish. Methods are easy, inexpensive and have general applicability for many fish species (e.g., ICES report; Hagger et al. 2006). Specific techniques may require parallel lab/field validation or may only be interpreted comparatively among populations for the POD issue due to lack of validation for these species. DNA adducts can be measured sensitively using standardized techniques (Hagger et al. 2006), and document specific chemical exposures, which can be linked to adverse biological effects.

Appendix 17: Ion Regulation

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Na-K ATPase activity Na-H pump Mitochondrial-rich cells (chloride cells) Blood ionic strength/ion composition Salinity challenge organisms and assess survivorship	Organisms in estuaries and salmon juveniles smolting experience and must adapt to large changes in salinity. Adaptive mechanisms include changes in Na-K ATPase and chloride cells particularly in the gills/respiratory tissues. Metals are known to affect ion channels in specific ways and may alter ion regulation. Cu inhibits Na uptake, divalent metals may block Ca channels, and metals may use ion channels for uptake. Can also ne competitive inhibitors. While contaminants may not specifically induce adaptive mechanisms, they may alter the normal response to salinity change and therefore this indicator may be very important to organisms survival	Determine Na-K ATPase activity using spectrophotometry or fluorometry with colored or fluorescent substrates in absence and presence of oubain, a specific Na-K ATPase inhibitor. Can determine Na-K ATPase activity from other ATPases in this way. Histology for mitochondria-rich cells. Mitotracker for quantitating mitochondria Salinity challenge: Can juveniles transition to smolt in 48 hr. salinity exposure	Has been used in the laboratory and field, but must know salinity changes and have good reference samples. Correlate % change in activity with contaminant exposure. Used in field-collected organisms. Europe with flatfish, crabs. Some studies found no differences in activity, others have.	Relatively easy to conduct enzyme assays for Na-K ATPase Relates to physiological adaptations that in turn relate to survival Activity should relate to amount of enzyme and/or presence or absence of inhibitors from water Could include companion measures of Na-K ATPase protein using commercial antibody to determine mechanism. Could also conduct recovery experiment by lab depuration or reciprocal transplant Can conduct assay with extracts of water from sites to confirm direct role of water-bourne contaminants rather than physiological response Measurement techniques sensitive Can be expensive to conduct thorough study Enzyme activity stable for months if stored properly Salinity challenge highly relevant	Not a specific biomarker for exposure. Rather indicator of physiological health and how it is perturbed by contaminants In estuary could be highly variable in moving organisms or in organisms at tidal boundary Affected by temperature and other factors that also affect osmoregulation. Should be clear seasonal, monthly, and daily changes for boundary organisms. Data interpretation sometimes confusing as basis for increase or decrease in activity not always relatable to exposure. Salinity challenges good organismal endpoint, but may not mimic actual adaptive change scenario (eg. days vs. weeks??) Life stage dependent: different life stages have different ionic regulation abilities	Could be useful for estuarine species. Stress of first rain coupled with contaminants? Could use outplants, where references are well understood. (-Hamann et al. 1999. . Biomarkers, 4:4, 290-302. -de la Torre et al. 2005. Chemosphere., 59:577-83. -Sancho et al., 2003. Ecotox. Env. Safety., 56:434-41. - de la Torre et al. 1999. Environ. Toxicol., 14:313-19 -Stagg et al., 1992. Mar. Env. Res., 33:255-66. -Playle, 1998. Sci. Total Env., 219:147-63.)

Appendix 18: Multixenobiotic Resistance (MXR)

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Western blot/ELISA; immunocyto-chemistry PCR/QPCR; FISH	Highly conserved ABC efflux transporters, primarily Pgp and Mrp family proteins are present across phyla. Commercial antibodies typically cross-react with fish, perhaps less so with inverts. Antibodies targeted to conserved domains (eg. ATP binding domain). Proteins may exist in cytoplasm but not yet be inserted in plasma membrane = no increase in activity. Using probes targeted to conserved regions usually successful.	Compare protein levels between animals from different sites. Can assess single tissues, blood cells, or even whole animal extract. Same for mRNA levels. Histologically quantitate protein and or message in tissues long after collections.	Has been used in the laboratory and field. Some groups use as part of routine biomonitoring as you can store tissues for later analyses. Easier than dye assays with respect to interpretation and sampling issues. More complex if lab does not conduct these analyses. Can be used simultaneously with assessments of other biomarkers such as HSPs, metallothioneins, P450s, etc. Little done with quantitative immunocytochemistry (more all or nothing), but useful for tissue analyses and relates to cancer.	Once validated for organism of interest, reliable but should be used in conjunction with physiological endpoint (dye accumulation). Should relate to levels of contaminants bioaccumulated Measurement techniques very sensitive Expensive to conduct Stability of samples a real plus for long term routine use of method	Assesses total protein in tissues, not just active protein. Some organisms may have stored protein and mobilize when needed. Pumping activity could change w/o protein (or mRNA) levels changing Efflux part of general stress response. Other stresses may cause upregulation. Good to look at other stress responses simultaneously. Protein levels relatively stable (>48hrs ??) over time following exposure. mRNA not and probably less usable. Harder to relate mRNA to efflux activity-needs ground-truthing. Affected by temperature. Clear seasonal increases and decreases in efflux activity shown in mussels. Need reference station that is same physical environment. Data interpretation straightforward but interference from environmental conditions could swamp contaminant signal if lab and reference organisms are not carefully assessed. Perhaps outplants ideal here.	Excellent for fish tissues Could use outplants, including bivalves, as sentinels coupled to this method. Field collected bivalves may be used for exposure assessments for fish using other techniques for MXR (see below). (-Bard. 2000. Aquat. Toxicol., 48:357-89. -Kurelec et al. 2000. Ecotoxicol., 9:307-27. -Moore et al., 2004. Mut. Res., 552:247-68. -Minier et al. 1999. Biomarkers, 4:6, 442-54. -Bresler et al., 1999. Helgol. Mar. Res., 53:219-43. -Minier et al., 2006. Mar. Ecol. Prog. Ser., 322:143-54. -Hamdoun et al., 2002. Aquat. Toxicol., 61:127-40. -Hamdoun et al., 2006. Develop. Biol., 276:452-62.)

Appendix 19: Microarray Analysis

Assay	Biological Basis	Technique/ Matrix	Application	Strengths	Weaknesses	Summary and References
Microarray analysis	Changes in tissue gene expression are often linked to mechanism of action of a chemical, or exposure to certain environmental chemicals	Total RNA from target organ (example: fish liver) hybridized to cDNA or oligonucleotide-specific array platforms	Used in the laboratory to understand pathways of chemical injury Sometimes employed in field studies to identify site differences and pollution effects	Provides an enormous amount of data pertaining to the expression genes in a target organ of an organism Changes in dramatically-altered genes (up- or down-regulated) can lead to identification of new biomarkers, if carefully validated in controlled laboratory studies Can uncover new routes of toxicity of a chemical Can, to some degree, integrate interactive effects of multiple chemical exposures	Expensive, high cost of microarrays and reagents as well as specialized equipment. Need to conduct follow-up quantitative PCR analysis on a subset of genes to validate results obtained by microarray analysis High level of technical expertise needed, especially with bioinformatics/statistical analysis, as well as QA/QC issues associated with processing of arrays Arrays need to be developed or validated for target species of interest if the array platform is for another species due to differences in gene sequences Data interpretation issues: disease states, developmental stage, can modify gene expression Extensive variability in gene expression is often observed in wild fish, power analyses may lead to cost-prohibitive experimental designs. Genes that are important in toxicity of a chemical may not necessarily be altered by chemical exposure (false negatives). Expression of some genes may be altered by nonspecific stress (false positives) Gene expression profiles for chemicals still being developed. Accordingly, may have less utility to define chemical exposures Assumes knowledge of a target organ prior to initiating experiment	Limited utility for POD field studies due to; 1) lack of validated or available microarray platforms for smelt, striped bass, and shad, 2) Potential problems in sampling adequate numbers of fish , 3) expense, 4) need for parallel laboratory studies with multiple stressors (i.e. salinity, DO etc) Has potential utility for future studies in the lab or *in situ* if arrays are developed and validated for POD species (Williams, Gensberg et al. 2003; Malek, Sajadi et al. 2004; van der Meer, van den Thillart et al. 2005; von Schalburg, Rise et al. 2005; Sheader, Williams et al. 2006; Larkin, Villeneuve et al. 2007)