

EXHIBIT A: PROJECT NARRATIVE

Impact of urbanization on Chinook salmon, steelhead trout, and their prey: a case study of the American River

Donald Weston – University of California, Berkeley

Daniel Schlenk – University of California, Riverside

Michael Lydy – Southern Illinois University

Tom King – BioAssessment Services, Inc.

Nathaniel Scholz – National Oceanic and Atmospheric Administration (unfunded collaborator)

Kate Macneale – National Oceanic and Atmospheric Administration (unfunded collaborator)

Project purpose

Urbanization along the U.S. west coast has occurred along waterways that have traditionally supported large salmon runs. Whether through construction of barriers to migration, physical habitat modification, or contaminant input, the salmon populations have often undergone dramatic declines as a result. The Bay-Delta system is undergoing similar trends, and its salmonid populations are similarly imperiled. In 2009, only 40,000 fall-run Chinook salmon were counted returning to the Sacramento River, compared to about 200,000 typical through much of the 1970s and 1980s, and a peak of 800,000 in 2002. Winter-run Chinook salmon are listed as endangered, spring-run Chinook salmon are listed as threatened, and the Central Valley steelhead trout population is listed as threatened.

As a result of urbanization, spawning and rearing occurs in waterbodies containing aromatic hydrocarbons, fecal coliforms, pesticides, or other contaminants that are potentially toxic to aquatic life. Degradation of water and/or sediment quality can adversely affect salmonids, through two principal routes. The first is via direct toxicity. Urban streams in the greater Seattle metropolitan area have substantial (> 50%) rates of pre-spawn mortality of returning coho adults, the exact causes of which are unknown, but are believed to have a water quality basis (McCarthy et al., 2008). Secondly, water or sediment toxicity can reduce the abundance of key invertebrate prey for juvenile salmonids, for many urban waterbodies have highly degraded benthic invertebrate assemblages. A reduction in prey availability due to contaminants has clear consequences to the growth of the juvenile fish, and their subsequent survival (Macneale et al., 2010).

The lower American River, extending from Folsom Dam to the river's confluence with the Sacramento River, provides an opportunity for a case study on interactions between urbanization and salmonid species of concern. The American River is one of the Central Valley's major inland production areas for both fall-run Chinook salmon and steelhead, yet passes through the metropolitan area of Sacramento and its suburbs extending eastward to Folsom. The Lower American has, in fact, been referred to as "California's largest urban stream" (Williams, 2001). Years of monitoring using the standard testing species, *Ceriodaphnia dubia* and fathead minnow, have failed to show any toxicity, contributing to the general perception of good water

quality in the river. However, recent testing with the amphipod, *Hyalella azteca*, has provided a disturbing picture (Weston et al., 2010), and suggests a closer look is needed both to protect the salmonid populations of the lower American, and to provide guidance in minimizing the deleterious effects of urbanization in general, elsewhere throughout California.

We have sampled the lower American River on eleven days over the past two winters, each time during or shortly after a rain event, and river water has shown acute toxicity in at least one location on the river on nine of the eleven days. The frequency of toxicity is greater nearer the mouth where the degree of urban influence is the greatest (Table 1).

Table 1. Proportion of days that American River water caused paralysis or mortality to *Hyalella azteca* in lab exposures for 96 h. Each site was sampled on 8-11 days. Data from winters of 2009 and 2010 (Weston et al., (2010) and unpublished data).

	River mouth (Discovery Park)	Eastern Sacramento (Howe Ave.)	Rancho Cordova (Sunrise Blvd.)	Folsom
Frequency of paralysis	55%	50%	10%	12% ¹
Frequency of mortality	36%	40%	10%	12% ¹

¹The single incidence of toxicity was found at a monitoring site, since relocated, that was just downstream of the outfall of Hinkle Creek, and likely reflected creek flow not yet dispersed into the mainstem river.

The cause for this toxicity is pyrethroid insecticides, and specifically the compound bifenthrin. We see a statistically significant correlation between bifenthrin concentration and toxicity, paralysis and mortality appear at the bifenthrin concentration we would expect it to if the compound were responsible, and several lines of evidence from Toxicity Identification Evaluations point to the compound. We commonly find bifenthrin in the river after rain events at concentrations ranging from 2-5 ng/L, a concentration sufficient to cause acute toxicity in *H. azteca* and other sensitive species (e.g., at the threshold of toxicity for the copepod, *Eurytemora affinis*; S. Teh, unpublished data).

The American River passes through 30 miles of highly urbanized lands, from which stormwater runoff is diverted to the river (Figure 1). It has been estimated that the river receives 1.6 billion liters of runoff in an average storm (Armand Ruby Consulting, 2005). We have been sampling this runoff and nearly all of it contains bifenthrin at acutely toxic concentrations. Concentrations in excess of 20 ng/L are routine (in comparison, *H. azteca* paralysis begins at about 2 ng/L bifenthrin), and we have seen concentrations as high as 106 ng/L (Carmichael Creek, January 20, 2010). The pyrethroid cyfluthrin is of secondary concern, with concentrations often about 10 ng/L, in comparison to the 1 ng/L that causes paralysis. The potential for environmental impacts of this runoff on aquatic life of the river is increased by the fact that river flow is dam controlled, and is maintained at its lowest levels during winter months. The volume of water released from Folsom Dam is nearly equivalent to the volume of runoff entering the river in an average storm, resulting in the river being approximately 50% urban runoff by the time it reaches its mouth. Given that this runoff commonly contains pesticides at 10 times the concentrations causing paralysis or mortality to sensitive species, the potential threat to aquatic life in the river is clear.

While the potential for toxicity is evident, and sampling over two winters has repeatedly documented toxicity to a standard testing species, the major unanswered question is what effect

these storm-driven pulses of pesticide toxicity have on the Chinook salmon and steelhead populations that use the lower American River as spawning and rearing habitat. The hypothesis we will test is that storm-driven pulses of urban runoff from the greater Sacramento metropolitan area are introducing sufficient pyrethroids to the lower American River to threaten resident aquatic life, and the observed toxicity to H. azteca represents a food web-mediated threat to juvenile Chinook and steelhead through toxicity to their invertebrate prey.

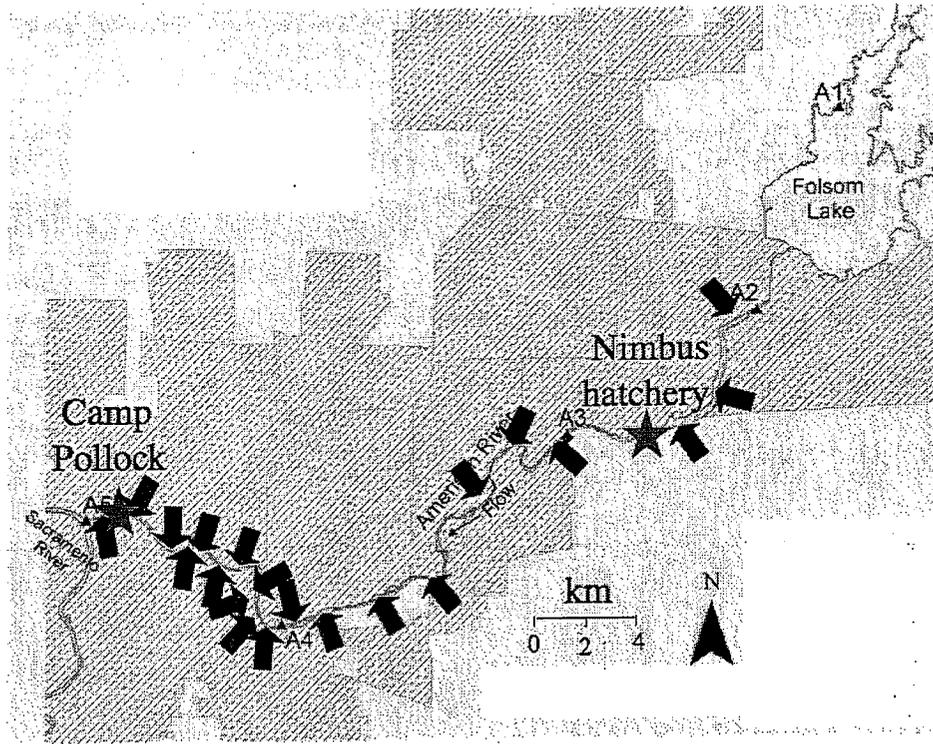


Figure 1. Location of creeks, open drains, and pump stations discharging urban runoff to the Lower American River. Shaded area represents urban land use within Sacramento and suburbs. Camp Pollock and Nimbus Hatchery are the two planned study sites, discussed below. Sites A1-A5 are our historical sampling sites that will provide a temporal perspective on pyrethroid concentrations in the river, but will not be reoccupied in the proposed studies.

Our goals include:

- 1) To determine the sensitivity of several key salmonid prey taxa resident in the American River to selected pesticides, and compare this sensitivity to that of *H. azteca*.
- 2) To determine if urban runoff results in measurable changes in the availability of benthic invertebrate prey in the American River, and to link these changes to contaminant input through a powerful flow-through testing approach.
- 3) To determine if pyrethroids, given their known endocrine disruption potential, are having any direct estrogenic effect on Chinook salmon or steelhead juveniles in the river.

Background and Conceptual Model

The lower American River provides spawning and rearing habitat for two species of special importance to the Delta Science Program and Delta Stewardship Council; fall-run Chinook salmon and steelhead trout. Chinook salmon spawn in the river from October to December, using riffle areas from about Carmichael to the Nimbus Dam. Juveniles emerge in January/February. Some juveniles emigrate shortly after emerging, while others feed in the river until June. The lower American is designated as critical habitat for threatened Central Valley steelhead, which spawn in January/February. Juveniles emerge in March through June and remain in the river until the next winter or spring. Thus, juveniles of both species would be feeding in the river during the rainy season when the availability of prey could be affected by the water quality effects related to urban runoff. There is substantial overlap between the prey utilized by both species, which in the lower American River consists largely of chironomids, mayflies, and caddisflies (Merz and Vanicek, 1996).

In addition to the presence of juvenile salmonids in the river during the months of greatest risk due to runoff, there are two additional factors that compound the potential effects of pyrethroids on aquatic biota of the river. First, river flow in the river is typically at its lowest in winter months, providing less dilution capacity. During the winters of 2007-2010, releases from Folsom Dam have been restricted to 800-1500 cfs for much of the winter, compared to spring/summer peak flows of 5500 cfs. Our studies have measured urban runoff volumes to the river from all the major drains, and found them to total about 1000 cfs in an average storm; thus there is potential for only a 1:1 dilution of any contaminants carried by urban runoff.

Secondly, pyrethroid toxicity is highly temperature dependent, and is atypical in that the compounds become more toxic at colder temperatures. This phenomenon is well established with many fish and invertebrates, and our work with *H. azteca* has shown a doubling of toxicity when temperatures decline from 23°C to 18°C, and a tripling of toxicity at 13°C (Weston et al., 2009a). Lower American River water temperatures range from about 19°C in the summer to about 10°C in the winter. Thus, in the winter months when pyrethroids are present in the river and of concern for juvenile salmonids, they are about three times more toxic than would be the same concentration in summer.

Our basic conceptual model is based on pulses of residential and commercial-use pyrethroids, particularly bifenthrin, entering the American River via urban stormwater runoff. While we recognize that summer irrigation can also lead to entry of pesticides into surface waters, both the concentration of pyrethroids in runoff and the flow rate of runoff are higher in the winter, with the net result that winter inputs are of greater concern (Weston et al., 2009b; Weston and Lydy, 2010).

Upon entry of pesticides into the river, our conceptual model incorporates impacts to the fish species of concern through at least four routes:

Direct mortality to fishes - Arthropods tend to be far more sensitive to pyrethroids in particular, and insecticides in general, than are fish. The 96 h LC₅₀ of bifenthrin for steelhead (*Onchorynchus mykiss*) is 100 ng/L (Pyrethroid Working Group, unpublished data), a value comparable to the highest concentration we have seen in undiluted urban runoff, but 20 times higher than we have observed in the American River. Bifenthrin LC₅₀s of several hundred ng/L are common for a variety of fish species, and the lowest reported fish LC₅₀ we have seen is 40 ng/L (fathead minnow). Therefore we do not expect to see any acute mortality to Chinook or steelhead, and our efforts will be focused elsewhere.

Sublethal effects on fishes - Pyrethroids can cause sublethal effects on fish at concentrations well below acutely toxic concentrations. Esfenvalerate at 200 ng/L causes irregular electrophysiological responses in the olfactory bulb of juvenile coho (Sandahl et al., 2004). The estrogenic activity of pyrethroids is of particular interest in the proposed study. Stereoselective estrogenic activity has been previously identified in the laboratory of a project participant (Schlenk) with bifenthrin enantiomers in the MCF-7 human cell proliferation assay, and *in vivo* in Japanese medaka (*Oryzias latipes*) (Wang et al., 2007). Induction of vitellogenin in rainbow trout has been observed with cypermethrin in concentrations as low as 50 ng/L (Schlenk, unpub. data).

Direct mortality to food organisms – It is difficult to generalize regarding the toxicity of pyrethroids to chironomids, mayflies and caddisflies, since they represent broad taxonomic groups with a similarly broad range of reported LC₅₀s, but some members of these groups can be very sensitive to pyrethroids. Exposure for as little as one hour to 10 ng/L fenvalerate (a pyrethroid less toxic than bifenthrin) caused an increase in caddisfly mortality and delayed emergence (Liess and Schulz, 1996). The 48-96 h LC₅₀s of cypermethrin to the mayfly *Baetis rhodani* and the midge *Chironomus riparius* are 12 and 7 ng/L, respectively (Pyrethroid Working Group, unpublished data), values comparable to the sensitivity of *H. azteca* for which we have already seen toxicity in the American River.

Sublethal effect on food organisms – There have been very few studies of sublethal effects on invertebrate prey, but a study on esfenvalerate effects on caddisfly larvae (Johnson et al., 2008) is intriguing. Exposure to 50 ng/L esfenvalerate for only 16 h caused a statistically significant increase in the number of caddisflies abandoning their case. Moreover, even when returned to clean water, pyrethroid-exposed individuals that had abandoned their case constructed a poorer quality case with less structural strength. Pyrethroids are neurotoxins, and regardless of whether that effect is manifested by paralysis (as we see in *H. azteca*) or case-leaving behavior (as in the caddisfly example), it is possible that urban runoff could result in a temporary increase in food availability for salmon and steelhead as their prey leave the benthic habitat and enter the drift where they are more susceptible to predation. Yet this enhanced prey availability would be short-lived and would deplete the density of benthic invertebrates, thus occurring at the expense of a stable, long-term food supply.

Food web effects on fishes – We hypothesize that pyrethroid effects (described above) can affect the type and abundance of prey. There is already good evidence from the Sacramento River that major changes in the composition and quantity of invertebrate prey affect the growth and perhaps survival of young Chinook salmon (Sommer et al. 2001). It has also been clearly shown that pesticide-related toxicity to prey organisms has, through the inhibition of fish growth, consequences to the productivity of salmon populations and reduces their potential for recovery (Baldwin et al., 2009; Macneale et al., 2010). As a consequence, there is a reasonable expectation that major shifts in the prey community will have major effects on American River juvenile salmonids. There are already enough data on pyrethroids in the American River, and sensitivity of salmonid prey taxa to these pesticides, to consider food web effects possible.

Approach and Scope of Work

We anticipate a three-year study, the focus of which is to conduct flow-through toxicity testing along the banks of the American River. Further detail is provided below, but briefly, we anticipate establishing two testing sites, the first at the Boy Scout's Camp Pollock near the mouth of the American River, and the second at the Nimbus fish hatchery at the dam that creates Lake Natoma. Both sites draw water from the American River, but since the vast majority of urban runoff occurs downstream of the Nimbus hatchery, this location serves as a control against which we could evaluate the impact of runoff to the river. Both locations provide an opportunity for water to be continuously pumped from the adjacent river and routed to exposure systems containing the various test organisms.

The river-side, flow-through systems provide an extremely powerful tool that provides greater ecological relevance than conventional lab-based toxicity testing, while also providing opportunity for experimental manipulations that would not be possible with in situ testing. Among the advantages:

- 1) The test organisms experience a realistic contaminant exposure regime, identical in concentration and duration to resident organisms in the river, rather than being tested with water drawn at a single time point as is typical in laboratory testing and exposed for an arbitrary duration such as 96 h toxicity test.
- 2) Environmental variables that may have an enormously important role in mediating a contaminant's toxic effects, such as temperature or turbidity, would be representative of conditions experienced by resident organisms in the river rather than laboratory conditions.
- 3) Since water from the river is continuously passed through the exposure containers, it reduces any artifacts associated with loss of contaminant to the surfaces of a sampling container (a significant problem with pyrethroids) or contaminant degradation that may occur during sample holding.
- 4) Since a wide variety of conditions change during storm events, beyond merely the pyrethroid input which is of primary interest, it is difficult to ascribe changes observed in bioassessment of the resident macrobenthos to pyrethroids or any other stressor. In a river-side, flow-through system, it is possible to control some of these variables, such as flow rate, to better establish causal relationship.
- 5) It is possible to manipulate water composition in a Toxicity Identification Evaluation context (e.g., passing the flow through activated charcoal to remove dissolved organic compounds), to conduct experiments that would not be possible with test organisms placed directly in the river.
- 6) A river-side structure with flow-through capability offers better protection of the exposure system from vandalism or physical damage than would be provided by placing organisms directly in the river.

Our approach is modeled after a similar system developed by some of our team members (Scholz, Macneale), and used by the National Oceanic and Atmospheric Administration (NOAA) to study the effects of Seattle urban runoff. An installation along a Seattle creek has been used to expose both juvenile salmon and intact macroinvertebrate communities, and to measure the difference in toxicity if the stream water was first passed through particle filters and activated charcoal. Flow-through toxicity testing, without manipulation of water chemistry, has also been used at the DWR water quality monitoring stations at Hood and Stockton (Reece et al., 2009).

Task 1 – Grant management and reporting

UC Berkeley will act as the primary contractor for the proposed work and will issue subawards to the other participating institutions. Dr. Donald Weston of UC Berkeley will serve as the Project Manager. Task 1 includes activities related to contract and technical management of the project, such as preparing subawards, coordinating and monitoring performance of the subawardees, management of project funds, attending meetings with Science Program representatives, and preparing deliverables such as semi-annual reports. This task also includes the Project Manager's presentation of project findings in scientific conferences or to stakeholder groups. Finally, the Project Manager's effort towards preparation of manuscripts for submission to peer-reviewed journals are also included within this task.

Task 2 – Determine pesticide sensitivity of key invertebrate salmonid prey taxa

The scope of task 2 is guided by our desire to address two data gaps that now inhibit efforts to assess the impact of urban pesticides on salmonids in general and American River salmonids in particular. First, data of pesticide sensitivity (e.g., NOECs, LC₅₀s) are critical but are typically available only for standard toxicity testing species, and not necessarily the resident fish species of interest or the invertebrate organisms critical in their diet. Members of our project team have already determined and published on the sensitivity of *H. azteca* to many pyrethroids (Maul et al., 2008a; Weston and Jackson, 2009), but we lack information on key prey taxa in the American River. Secondly, pesticides are often approved and used for decades before adequate data are available even for standard testing species. Most pyrethroids, for example, were in use for 20 years or more with *H. azteca* data only available for one or two compounds, and lacking for most pyrethroid compounds in widespread use.

To address the first issue, we propose to determine basic pyrethroid sensitivity parameters (NOECs, LC₅₀) of three resident species in the river to bifenthrin, the pyrethroid we routinely find in the American River. Our focus will be on taxa shown to be important contributors to the diet of Chinook salmon and steelhead, namely, chironomids, mayflies and caddisflies (Merz and Vanicek, 1996). We will attempt to obtain one representative of each group, though the exact species will be dependent on what is present in the river in sufficient numbers at the time the study is done, and the extent to which they are amenable to laboratory study. The Delta Science Program review panel questioned whether collecting specific taxa from the river is feasible, and whether they could be identified to species. We recognize toxicity testing with resident species is challenging, and unfortunately often not done for that reason, but given its considerable value in establishing ecological relevance, we believe it is worth attempting. Early in the project period we will discuss this approach with experts familiar with American River macroinvertebrates, both those on the project team (T. King) and other outside authorities (J. Merz), to identify the optimal times, places, and species for collection. The taxonomic identity of our selected organisms will be confirmed by sending representative specimens to a specialist on our project team (T. King). Given the taxonomic complications inherent in working with aquatic insect larvae, it may not be possible to identify the precise species with which we are using, and it may be necessary to limit identification to the genus or family level. However, such a practice is common in toxicology of larval forms, and not considered a major obstacle.

While highest priority will be given to resident taxa, should that effort be unsuccessful because target groups are not be present at the times needed or available in sufficient numbers, our alternative approach will be to use representative taxa from commercial suppliers. Members of these broader prey groups (chironomids, mayflies, caddisflies), though not necessarily the species present in the American River, have been successfully used for toxicity testing with pyrethroids (Maul et al., 2008b; Schulz and Dabrowski, 2001; Wendt-Rasch et al., 1999). A chironomid larvae (Chironomus dilutus) is widely available from commercial vendors, and we are also able to obtain burrowing mayfly larvae (Hexagenia sp.) from a commercial source.

To address the second data gap above, the absence of toxicological data for some pesticides even after many years of use, we would like to use this study as an opportunity to acquire basic information on the toxicity of fipronil. Though pyrethroids are our primary focus, and existing data from the American River is more than adequate to justify this concern, we are aware that the pesticide fipronil is an emerging insecticide. Nearly 35,000 lb were used for non-agricultural purposes statewide in 2008, compared to 56,000 lb of bifenthrin, but as recently as 2000 fipronil use was nearly non-existent. It is also noteworthy that based on data collected by UC Davis (L. Oki, unpub. data) and DPR (L.-M. He, unpub. data), fipronil is found in California urban runoff about as frequently as bifenthrin (nearly 100% of samples) and in comparable concentrations (median 5-20 ng/L). There is as yet, no evidence that fipronil is causing toxicity in surface waters, despite its widespread appearance in runoff, but neither are the data available to know whether existing concentrations pose a risk. There are no data on the concentration of fipronil and its degradates that would cause toxicity to H. azteca or the taxa of interest as salmonid prey. All too often toxicological studies lag behind rapidly changing pesticide use patterns. Therefore, we intend to use our study not only to address a known pesticide issue (pyrethroid toxicity), but to proactively address an emerging pesticide that is likely to, at least in part, replace pyrethroids in coming years.

Fipronil toxicology is complicated by the fact that it degrades in the environment to other substances which are of equal or greater toxicity than the parent compound, based in part on studies conducted by members of our project team (Schlenk et al., 2001; Maul et al., 2008b). The degradates include fipronil sulfide, fipronil sulfone, fipronil desulfinyl, and fipronil amide. We propose to determine the sensitivity of H. azteca and the same prey taxa noted above to fipronil and its degradates. LC₅₀ and similar data will be determined to help establish whether fipronil and degradates that our monitoring detects in the river are contributing to toxicity independent of that due to pyrethroids. While testing of fipronil, the sulfone and the sulfide is certain, inclusion of desulfinyl is tentative since obtaining the material at a reasonable cost is uncertain, and our studies will exclude the amide which is not available and nontoxic.

Task 3 – Installation and use of flow-through facilities

Two river-side, flow-through testing facilities will be established. Our primary testing site will be at Camp Pollock, a Boy Scouts' property only 1.5 miles upstream of the river mouth. This location reflects nearly all urban runoff that enters the Lower American River, for only one stormwater pump station is located downstream of this point out of 14 pump stations, two open drains, and seven creeks discharging to the river. A temporary storage building will be installed on the property to house all exposure systems and a pump, with the intake on the Camp Pollock waterfront, to provide flow-through capability.

Our second site will be the Nimbus fish hatchery, operated by the California Department of Fish and Game. Flow-through capability is already present at the hatchery, and we simply need to set up our exposure containers in currently unused tanks. This site will serve as a control, since only a relatively small amount of urban runoff enters the river upstream of this point. There are three creeks that discharge upstream of the hatchery (Alder, Willow, and Hinkle Creeks), but together they make up only 15% of the urban runoff entering the Lower American River, and our sampling has shown two of the three (Alder and Willow) contain few or no pyrethroids. The two-site design is very powerful since our test organisms will be exposed to the same river water at both Nimbus and Camp Pollock, but differ only in the urban runoff inputs that occur in the approximately 20 river miles between them. Even temperature, which one might expect to vary along the length of the river, shows little change between these two sites during winter. In our sampling over past two winters, the water temperature difference between Camp Pollock and Nimbus averaged only 0.8°C, and never exceeded 1.2°C.

Three types of invertebrate toxicity tests will be performed at these sites. First, tests with *H. azteca*, previously used to document American River toxicity in static lab exposures, will be performed in the flow-through systems. Organisms will be taken from cultures at UC Berkeley and placed in the flow-through systems throughout storm events, with monitoring of survival and paralysis. This approach should be useful in extending previous lab toxicity observations to exposure scenarios more realistic in magnitude and duration. Second, similar tests will be done with resident taxa or related species, relying on chironomids, mayflies and caddisflies. The specific taxa chosen, and their source, whether collection of resident species as preferred or purchase from commercial vendors, will be subject to the same considerations discussed previously under Task 2. Third, we will conduct tests with intact macroinvertebrate communities in experimental streams. Doing assessment of community-level changes following runoff events is difficult in the river itself, since a wide variety of variables are changing in uncontrolled fashion during a rain event. For example, changes may be due to physical flow effects independent of contaminant influences. However, in experimental streams within the flow-through system, variables such as flow rate can be controlled, and community effects can be more clearly linked to water quality. NOAA investigators on our project team have done similar studies in which they placed rock substrates in the Cedar River, a river of high water quality used for Seattle's municipal water supply, and after weeks to months of invertebrate colonization of the substrate, the rocks were transferred to the experimental facility alongside an urban creek. We propose a comparable approach, by placing containers of gravel/cobble substrates in the upper watershed of the American River (one of the forks above Folsom Lake) for a few months to permit invertebrate colonization, and then transferring those containers with their intact invertebrate community to our Camp Pollock experimental facility. The material will be placed in elongate tanks, representing experimental streams, with the intact invertebrate community obtained at the substrate source location, and continuously receiving American River water pumped directly from the river. The effect of river water quality on this community, as influenced by rain and runoff, will be monitored in two ways. First, on a daily basis we will capture and quantify organisms leaving these experimental streams in drift, since contaminant effects on substrate abandonment may be as important as direct toxicity. Secondly, we will identify and enumerate surviving organisms remaining on the rock substrate at the conclusion of the exposure period for a given storm (tentatively, about one week).

Our design also allows inference of causality by manipulating the constituents present in the water passing through the exposure systems. One set of exposures will be conducted with river water that has passed through activated charcoal filters, while parallel exposures will be done with untreated river water. Toxicity (or greater drift in the case of the experimental stream experiments) observed in the untreated system, but not in the treated system could be indicative of organic compounds as the cause, including but not limited to pyrethroids. Similar manipulations could be done with and without particle filtration, though in our experience, particle content of the American River is consistently quite low (4 mg/L typical; 15 mg/L maximum during storms). Interpretation of the data from the water treatments, will of course have to take into account ecology of the specific taxa, but when coupled with pesticide chemical data and known thresholds of toxicity, should be helpful in understanding causality. We will also, of course, be able to compare effects of identical treatments at the Camp Pollock site to that at Nimbus, with the presence of runoff in the river being the major difference between these sites.

We anticipate operating our river-side, flow-through facilities in five storm events, tentatively three in the winter of 2011/2012 and two more in the winter of 2012/2013. However, the schedule also allows for fieldwork during the winter of 2013/2014 should it be necessary. The potential for toxicity is likely to be quite variable depending on river flows, which may vary by an order-of-magnitude from one winter to the next. Rainfall is also likely to be a critical but unpredictable variable affecting results. The five events over three years should allow the latitude needed to choose the optimal occasions for sampling based on rainfall and river flow. Approximately two days prior to storm arrival (as weather predictions allow) the test organisms will be set up at each location with flow-through water coming from the river. Tests will be continued a day or two beyond the rain event and subsequent runoff, the duration of which will be storm dependent, but is expected to be on the order of one week. Drift will be measured throughout the rain and runoff event, and all tests will then be taken down and survival or other endpoints recorded at the conclusion of the event. All testing will be accompanied by chemical analysis of the river water for pyrethroids and other constituents to help establish the cause of any observed toxicity (analytical plans and capabilities discussed below),

Task 4 – Endocrine effects in juvenile salmon

As indicated above, pyrethroids have been shown to cause feminization of fish. In rainbow trout this occurs at concentrations about 10 times higher than those typically observed in the American River, but it has not been examined in Chinook salmon, or for either salmon or trout in the complex matrix represented by stormwater runoff. To assess the potential for river water to cause feminization, juvenile Chinook salmon and steelhead trout (obtained from Nimbus hatchery) will be exposed to river water in the flow-through exposure systems. Fish will be maintained in the systems for a couple days prior to a rain event or more as rain predictions allow, exposed for the duration of the storm and subsequent runoff, and sacrificed at the conclusion of the event. Similar exposures will be conducted over three rain events (either all in winter of 2011/2012, or if needed, extending into 2012/2013), and exposures will be done with and without activated charcoal treatment, as for the invertebrate exposures described above.

Feminization will be determined by measurement of vitellogenin protein in plasma in the juvenile fish as previously described (Lavado et al. 2009; Xie et al. 2005). After bleeding the

fish, animals will be dissected and evaluated for gonadal development. Animals showing ovarian growth will be discarded from the study. Laboratory concentration/response studies in both species with waterborne exposures to the estrogen 17 β -estradiol (E2) have been recently conducted, allowing us to express the degree of feminization in terms of the E2 equivalents (EEQ) within the river water. Thus, EEQ estimates can be calculated for each storm event and sample manipulation allowing mass balance comparisons.

The river-side exposures will establish if estrogenic activity is present in the river, but to help interpret data in the context of the pyrethroid concentration needed to induce these effects, the field exposures will be complemented with laboratory exposures to single pyrethroids. Experiments will be conducted in both fish species (Chinook and steelhead) with bifenthrin, the same pyrethroid used in the invertebrate sensitivity tests. Concentrations will bracket measured values in runoff and the river, with approximately five concentrations evaluated. The concentration of bifenthrin that causes measurable vitellogenin production (feminization) in 50% of the population will be calculated for comparisons to field responses.

Task 5 – Analytical chemistry

Data on concentrations of the constituents of interest will be necessary to interpret any toxicity seen in the river during rain and runoff events. Pyrethroids are the primary constituents of interest, and all water samples will be processed for the members of the class most commonly used in urban environments (bifenthrin, lambda-cyhalothrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, permethrin). We are also interested in obtaining data on the emerging insecticide, fipronil. Because of budgetary restrictions, its analysis in river samples has been removed from this study's budget. However, we expect to receive funding from the State Water Board for a fipronil study, and if the funds are awarded, expect to analyze river samples for fipronil and its degradates with funding from that study. Finally, it is known that urban runoff can contain polycyclic aromatic hydrocarbons (PAH) and metals (especially copper) at concentrations of toxicological concern, so these two groups of contaminants will be among the analytes. We will not be conducting any laboratory exposures of PAH and metal sensitivity, as we are for the pyrethroids and fipronil, but PAH and metal data will be useful to interpret results by reference to concentrations previously shown in the literature to cause harm.

Project funding allows for sampling American River water for pyrethroids on six days during each storm event, conducting tests during five rain events, and sampling at both Nimbus and Camp Pollock (total = 60 samples). At Camp Pollock where we expect to frequently find these pesticides, we will also confirm removal of these compounds by the activated charcoal (up to 30 samples). These data would provide an unparalleled demonstration of the day-to-day variation in pesticide concentration that American River biota experience throughout winter storms.

The PAHs and metals will be analyzed from approximately one-third the samples during each storm event (approximately 30 samples total). Finally, in order to report defensible LC₅₀ values from the pesticide sensitivity studies conducted in the laboratory, it is necessary to analytically confirm nominal spiked concentrations, which will generate about 20 samples for fipronil analysis and 12 for pyrethroids.

Water samples will be analyzed for pyrethroid insecticides using proven methods developed in Dr. Lydy's laboratory and used extensively in projects for the Central Valley

Regional Water Quality Control Board. Water samples will be extracted and cleaned up using a standard liquid-liquid extraction technique for detection of pyrethroids at low parts per trillion levels in water (USEPA Method 3510C; Wang et al. 2009). Surrogate standards (decachlorobiphenyl and 4,4'-dibromooctafluoro-biphenyl) will be added into all samples prior to extraction to quantify extraction efficiencies. Pyrethroids will be analyzed on an Agilent GC-ECD following methods by You et al. (2008). Two columns, an HP-5MS (30m x 0.25 mm; 0.25 µm film thickness) and a DB-608 (30m x 0.32 mm; 0.50 µm film thickness) will be used. Five external standards ranging from 5 to 250 ng/ml will be used for calibration. Qualitative identity will be established using a retention window of 1% with confirmation on a second column.

Twenty three PAHs will be examined following the USEPA target analyte list plus pertinent alkyl PAHs, and will include 1-methylnaphthalene, 1-methylphenanthrene, 2,6-dimethylnaphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, anthracene, biphenyl, fluorene, naphthalene, phenanthrene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[e]pyrene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, fluoranthene, indeno[1,2,3-c,d]pyrene, perylene and pyrene. A solid-phase extraction method (USEPA method 3535) will be used for the water extractions and PAHs will be analyzed with an Agilent 6850 GC/5975 XL MS in selected ion monitoring (SIM) mode following USEPA standard protocols (USEPA method 8270C). The instrument will be operated in the splitless mode with 2 µl injections onto an HP-5MS column (30m x 0.25mm; 0.25µm film thickness). Acenaphthene-d₁₀, chrysene-d₁₂, and perylene-d₁₂ will be used as internal standards for PAH quantification. Before extraction, three surrogates, nitrobenzene-d₅, terphenyl-d₁₄, 2,4,6-tribromophenol will added to samples to quantify the extraction efficiency. Prior to the GC/MS analysis, water extracts will be cleaned with silica gel (USEPA method 3630C).

Quality assurance and quality control for the organic analyses will consist of a laboratory control blank, a laboratory control sample, a matrix spike, and a matrix spike duplicate and will be included every 20 samples. A midlevel calibration check standard will be analyzed every 10 samples during the GC analysis. Two or three surrogates will be added to each sample prior to extraction to verify the extraction efficiency of the sample for GC-ECD and GC/MS analysis, respectively. Acceptability limits for percentage recovery of the laboratory control sample, matrix spike, matrix spike duplicate, and surrogates will be within 50-150% and relative percent difference of the results of matrix spike and matrix spike duplicate will be less than 25%.

Water samples for metals analyses will be collected and preserved (0.1% trace metal grade nitric acid) in HPDE bottles, filtered (0.45 µm), and analyzed for copper, cadmium, and zinc on a graphite furnace atomic adsorption spectrometer (Varian AA240 with Zeeman correction). Water samples collected for dissolved organic carbon (DOC) concurrently with the metals samples will be collected in amber bottles pre-cleaned for this analyte and preserved with 0.1% analytical grade hydrochloric acid. DOC concentrations will be determined using catalytic combustion (Shimadzu TOC-VCSN). Quality assurance checks will follow the guidelines established by the US EPA.

Task 6 – Bioenergetic modeling to relate prey quality and abundance to the growth of juvenile salmonids

While our focus is on toxicity to invertebrates of significance as prey for Chinook salmon and steelhead, we are not proposing any direct quantification of salmonid diets under this study.

There are several studies already completed specifically on diet composition of the salmonid species of interest to this study in the lower American River (Merz and Vanicek, 1996; and more recent Fish and Game data now being analyzed (R. Titus, pers. comm.)). In addition, drift and benthic invertebrate data from the lower American River are currently being collected, and are available from many past sampling efforts dating back to the mid-1990's (J. Merz, pers. comm.). We will utilize these existing data sources when interpreting our toxicity findings in light of the ramifications for prey availability to salmonids, but an explicit bioenergetic model has been deleted from the proposal at the request of the Delta Science Program's review panel, and no funding is now included for Task 6.