KERO-TR-99-01
June 1999

TECHNICAL REPORT

EFFECTS OF ACROLEIN AND OTHER PESTICIDES ON WATER QUALITY AND AQUATIC BIOTA IN TULE LAKE NATIONAL WILDLIFE REFUGE

by

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June 30, 1999

INTRODUCTION

A strategic junction in the Pacific Flyway, the Klamath Basin has historically received the largest concentration of migratory waterfowl in North America, currently about 3 million waterfowl. The basin is also heavily used by threatened bald eagles (*Haliaeetus leucocephalus*), including approximately 95 nesting pairs (Isaacs et al. 1997), and from 300 to 900 overwintering individuals (Dr. David Mauser, U.S. Fish and Wildlife Service, unpublished). A large fraction of the eagles forage in Lower Klamath and, to a lesser extent, Tule Lake National Wildlife Refuges (NWRs).

Two endangered species of fish, the Lost River (*Deltistes luxatus*) and shortnose (*Chasmistes brevirostris*) suckers, are also restricted to the Klamath Basin. The smallest known populations of these two suckers, estimated at 250 adult fish, reside in Tule Lake Sump 1A, in the Tule Lake NWR. Tule Lake populations are largely isolated from other populations as a result of the Anderson-Rose Dam, the only remaining spawning habitat for this population segment, to the north, and irrigation pumps at the outlet of Tule Lake. Although the low population size of suckers at Tule Lake has been primarily attributed to the loss of deep water habitat, poor quality of spawning habitat at the Dam, and the introduction of non-native fish species (U.S. Fish and Wildlife Service 1993; Littleton 1993), poor water quality and inputs of pesticides may also play a role.

Both poor upstream water quality in the Lost River and the shallow water conditions of Tule Lake have, for many years, contributed to low dissolved oxygen, high concentrations of ammonia, and other adverse water quality conditions in the lake (Sorenson and Schwarzbach 1991; Dileanis et al.1996). More than 50 different pesticides can be used by refuge farmers and additional, more toxic pesticides, such as acrolein (2-propenal) and parathion, are used on irrigation canals and private agricultural lands upstream of Tule NWR. The effects of both current and historic pesticide use on aquatic species are not completely understood. In two instances, applications of acrolein (commercially known as Magnacide-H) upstream or in an adjacent canal preceded fish die-offs (Littleton 1993; Snyder-Conn, unpublished), but a causal relationship with the acrolein applications was not clearly demonstrated in either case. Other recent pesticide studies, including those on the pesticide acrolein, also have not established any clear link between acute or chronic toxicity of refuge drainwaters waters to refuge aquatic biota and pesticides (Sorenson and Schwartzbach 1991; Winchester 1994; Dileanis et al.1996), although some of these studies did relate toxicity to poor conventional water quality parameters.

Acrolein is used by irrigation districts, including Tulelake Irrigation District, to clear canals of submergent and emergent vegetation. The herbicide causes rapid disintegration of plant material into very small fragments that can effectively pass

through pumps and irrigation equipment. The effects of the disintegration on water quality include potential increases of turbidity and nutrients downstream, with possible indirect effects on other water quality parameters. Acrolein is an extremely volatile compound with a half-life ranging from about 14 hours to 92 hours in water depending on pH, water temperature, and turbulence. Acrolein metabolites include an array of compounds: oxalic acid, malonic acid, glycidol, 3-oh propionic acid, lactic acid, glycerol, 1,3-propanediol, propiolic acid, glyceric acid, bicarbonate, propionic acid, and propanol (Haag 1988). Many of these compounds are themselves either volatile or common in natural waters and therefore difficult to assess directly.

It is known that acrolein is an extremely hazardous substance, with chronic and acute toxicity concentrations to freshwater aquatic life occurring in the parts-per-billion range (see review by Eisler 1994). For example, the median lethal concentration of acrolein after a 96-hr exposure (LC_{50}) to fathead minnows is 14 μg/L (Holcombe et al. 1987 in Eisler 1994), and the LC_{50}s for Lost River and shortnose juvenile suckers are 77.0 and 46.5 ug/L, respectively, with all suckers dying after a 24-hr exposure to 65 ug/L (Little 1997). Common invertebrate species, including *Daphnia magna* (water fleas), which are abundant in Tule Lake, are also immobilized or die at 51-80 μg/L (48-hr) (EPA 1980 and Holcombe et al. 1987, in Eisler 1994). Amphibians appear even more sensitive. For example, the clawed frog, *Xenopus laevis*, has a 96-hr LC_{50} of 7 μg/L (Holcombe et al. 1987 in Eisler 1994).

Despite the potential toxicity of acrolein from upstream, off-refuge applications, studies in refuge waters have generally not demonstrated concentrations sufficient to produce toxicity to refuge aquatic life (Winchester 1994; Dileanis et al. 1996). This may be attributable to effective flow stoppages (as required by the pesticide use label), shunting of most of the treated water onto agricultural lands for irrigation, the rapid dissipation of acrolein before reaching the refuge, and/or to the poor stability of preserved samples.

The objective of this study was to as to evaluate the potential impacts of acrolein and other pesticides on water quality and aquatic invertebrates and fish, with a primary focus on the potential effects of acrolein applications in waters upstream of the refuge on aquatic biota in Tule Lake NWR. This study consisted of water quality monitoring (water temperature, dissolved oxygen, conductivity, pH, and turbidity), nutrient monitoring (total nitrogen, total phosphorus, and ammonia), and pesticide monitoring of water samples (acrolein and organophosphate and carbamate pesticides) following two periods of multiple acrolein applications. Prior to initiation of the study, we verified the USGS's sample preservation methods for acrolein by spiking Klamath Basin (Lost River) water for various time intervals (Sandstrom and Snyder-Conn, in prep.). To evaluate the impacts of ambient water quality and potential pesticide concentrations on aquatic biota, we employed 96-hr *in situ* toxicity tests using the waterflea *Daphnia magna*, fathead minnows *Pimephales promelas,* and the pulmonate snail, *Planorvella pierosoma subcrenaum*.

MATERIALS AND METHODS

During the two 96-hour study periods described above, in situ toxicity tests, water quality monitoring, nutrient monitoring, pesticide sampling, and invertebrate monitoring were conducted to evaluate whether pesticides, including acrolein, were reaching refuge waters and affecting survival of aquatic biota or water quality. The methods employed during these studies are detailed below.

In Situ Toxicity Tests

Bioassay Organisms

Daphnia magna neonates, fathead minnow larvae *(Pimephales promelas)*, and the pulmonate snail (*Planorvella pierosoma subcrenaum)* were used for in situ (on site) toxicity tests. *Daphnia* and the fathead minnows were air-expressed by overnight mail from Aquatic Bio-Systems, Inc. as less than 24-hour-old young. Pulmonate snail specimens were collected from an upstream location at the Crystal Springs boat ramp and varied in both size and age (0.2-1.5 cm diameter). The taxonomic identity of the snails was verified by Dr. Terrence Frest (Deixis Consultants, Seattle, WA). At the time of field testing, the test organisms were 48-72 hr old. At this time, the *Daphnia* were approximately 150-200 µm in length and the minnows were about 1-2 mm in length.

Acclimation and Transport Procedures

Organisms were held in 3.8-liter acrylic aquaria, after gradual acclimation to the aquaria water over a period of 35-40 minutes. First, organisms were floated for 15-20 minutes in open containers to allow exposure to oxygen and for temperature acclimation prior to introduction into upstream Lost River water into the shipment containers. Then small amounts of water from the holding tanks were added every 5 minutes for an additional 20 minutes for acclimation to the water quality of the receiving water. Specimens were then held for approximately 24 hours in aquaria before placement in the field to enable culling of individuals which appeared unhealthy or dead.

The organisms were held in aquarium water which had been filtered through 75-µm nylon mesh. The Lost River source water was from a location upstream of the study sites. It was collected within 24 hours of expected organism deliveries for use in the aquaria. *Selenastrum capricornutum* microalgae (3.0×10^7 cells/ml x 20 ml) were added to provide a green water culture for both the *Daphnia* and minnow larvae to regulate water quality and provide food for both species at that

age. Twenty-four hour *Artemia* nauplii were also fed *ad libertum* to the minnow larvae to supplement their diet. Aeration was provided by an air pump and air

stone with larger pores set near the surface to provide light agitation, while protecting organisms from damaging exposure to air bubbles. The temperature of aquaria was maintained between 19.5-20.7°C in a temperature-controlled environment to avoid overheating and to regulate temperature.

Artemia were hatched in an aerated 18.8-liter, plastic carboy in a 20-ppt saline solution maintained at 28°C. Fifty milliliters of *Artemia* cysts were added to the saline solution. Post-hatch *Artemia* were fed 2.5 g activated Baker's yeast (Fleischman's) initially, with quantities adjusted thereafter based on water quality. Water exchanges were made as needed in the *Artemia* culture.

Organisms for the in situ toxicity tests were transported from holding aquaria into PVC flow-through containers (described below) using a Hach 1-10 mL Ten Sette pipette with sterile plastic tips. The opening of the plastic tip was widened prior to use to reduce potential injuries to the test organisms. Specimens were transported to small waxed cups as an intermediate stage for checking counts of organisms. Twenty organisms (*Daphnia* or fathead minnows) or 10 pulmonate snails were added to each container. Care was taken to avoid exposing *Daphnia* to air or to the water/air interface where tiny air bubbles could get trapped under their carapace, inducing anomalous floating behavior. Flow-through containers were randomized so that organisms placed in each container and destined for the various study locations were subject to sorting any identified bias.

The flow-through containers were kept in 2-liter, cleaned, open containers filled with the same source waters as the aquaria during transport. These containers were in turn placed in coolers, each with a single ice pack to prevent overheating and the related stress mortality during physical transport to and from the study sites. All containers were secured within the coolers to prevent excess movement. Care was also taken during loading, unloading, and driving to minimize movement.

The study design originally called for deployment and removal of two replicate containers per test species from each study location at 24 hours, 48 hours and 96 hours. This was the accomplished design during the second study period. However, due to high rates of transport mortality in *Daphnia* and fathead minnows received from the supplier (prior to field deployments) during the first study period, only one *Daphnia* container was removed from the reference site after 24 hours, and there were no fathead minnows were removed at this time

interval from either the refuge border or Tule Lake sites. However, two replicate containers of each of the three species were deployed and removed at both the 48-hour and 96-hour periods at each of the study sites.

Flow-Though Containers

In situ tests occurred in flow-through containers constructed using 5.1 cm (inner diameter) polyvinyl chloride (PVC) compressor couplings, about 0.5 liters in volume (with the rubber seals removed) (Figure 2). Both ends of the coupling were covered with monofilament nylon mesh (105 µm for *Daphnia*, 250 µm for fathead minnows, and 500 µm for snails)(Aquatic Eco-Systems, Apopka, FL), fastened by tightening the endcaps. Mesh size was selected to ensure containment of organisms while maximizing flow and protecting specimens from potential predators. The screening also allowed accessability of prey organisms. Three plastic cable ties were attached to each container, forming a loop for attachment to the *in situ* structure. Both couplings and 2-liter containers were labeled for site and species with a permanent marker prior to transport and deployment in the field.

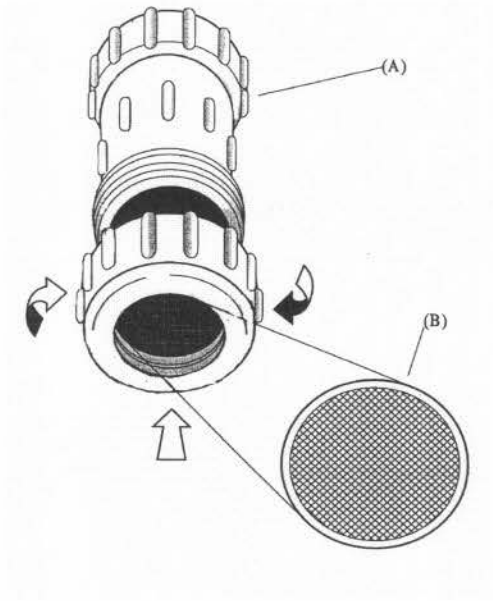


Figure 2. Flow-through, in situ toxicity test cannister, made with a 5-cm PVC compressor coupling and Nitex screening on both ends. Mesh sizes 105, 250, and 500 µm where used for *Daphnia*, fathead minnow, and snail tests respectively. (A) PVC coupling (B) Nitex screen mesh.

Field Deployment of Flow-Through Containers

Prior to deployment, the test organisms were briefly acclimated to the site water by allowing submerging the 2-liter containers holding the flow-though containers in the site water. The flow-through containers were hooked by plastic loops onto S-hooks, which were in turn clamped onto "H-" or "T-" shaped, stainless steel perforated sheet metal posts (1.8 m) already planted at each study site. Rigid right angles were maintained with 0.95 cm nuts, bolts, and 0.3 m pieces of sheet metal at approximately 45° angles. The posts were implanted in the sediment as deeply as needed to ensure stability and maintain appropriate depth for the organisms. Because acrolein is less dense than water and volatile, the flow-through containers were placed in the upper 30 cm of the water column. The horizontal bar on which the containers were clamped was aligned with the current to reduce stress on the posts and organisms.

Daphnia and fathead minnow containers were aligned along the length of each structure. Six snail containers were also attached in tandem (pairs) to the downstream side of the post bars with fishing line. The snail containers were held upright in a square (25.4 cm x 25.4 cm) of closed-cell foam board with the top 1/5 of the coupling out of water. This provided the pulmonate snails with air to breath at the water/air interface. Both the structure and the trailing snail containers were covered with a olive green/brown cotton cloth for camouflage to reduce the potential for vandalism or interference with the study.

During the first study period, insufficient numbers of *Daphnia* and fathead minnows survived the aquarium acclimation period for performing replicate toxicity tests at all sites and time intervals. Several flow-through containers were also lost after deployment (probably as a result of inadequate clamping of the S-hooks to the posts or vandalism). These losses limited the results for the first study period, except for the pulmonate snails, for which there were two replicates at each site and time interval. During the second study period, we obtained replicate results for in situ tests with all fathead minnows and snail containers at all sites and time intervals, but were missing flow-through containers with *Daphnia* at two sites at the 96-hour time period.

Water Quality Monitoring

Concurrent with the 96-hour toxicity tests at the reference, refuge border, and Tule Lake study sites, temperature, conductivity, dissolved oxygen (DO), pH, and depth were measured. During the first sample period, we used a Hydrolab H_20 Water Quality Multiprobe with a Surveyor 3 display logger for instantaneous, on-site readings at all sample sites. During the second study period, this equipment was used only at the refuge border site, and we continuously deployed a Hydrolab Datasonde 3 and Datasonde 4 units at the upstream reference and

Tule Lake sites, recording water quality measurements hourly.

Cleaning and calibrations of all Hydrolab units were performed immediately prior to each study period according to the manufacturer's recommendations (Hydrolab 1991). For cleaning, all probes and exposed casings were swabbed with cotton Q-tips and 95% isopropyl alcohol, and rinsed regularly with de-ionized (DI) water. The DO membrane and electrolyte fluid were replaced prior to each calibration and the pH probe was covered and soaked in reference electrolyte when not in use.

Conductivity calibrations were performed using a 700 μS/cm standard, similar to conductivities in the study area. The pH was then calibrated with both pH 7.02 and 10.05 standard (high ionic strength) buffers at 20°C. The dissolved oxygen probe was calibrated based on the air saturation, temperature, and barometric pressure. All units were calibrated within 48 hours of expected field use.

Calibration, programming, and downloading of all units were performed through an IBM PC hyperterminal (ProCom). Drift was recorded within 48 hours after each run was completed. Potential drift was also evaluated by comparing between the Hydrolab used for instantaneous measurements at all sites and the Hydrolabs deployed continuously at the reference and lake sites during the second study period. These drift evaluations indicated that the instruments performed well, with no significant drift, and that drift corrections of the data were unnecessary.

Flow rates at each site were measured and recorded with a Global Flow Probe FP101 flowmeter using integrated measurements over a one minute time interval. Latitude and longitude coordinates were taken with a hand-held, global positioning system (GPS) unit. Turbidity was measured with a Hach 2100P turbidimeter on three replicate samples collected every 24 hours at each site. These measurements were made on site immediately following sample collection. The turbidimeter was calibrated with four Hach Stablecal turbidity standards immediately prior to this study. Daily calibration checks of the turbidimeter was performed with a fresh 4.34 NTU reference standard, which most closely approximated turbidity values at the sites. Total settleable solids (TSS) were also measured daily at each site using the Imhoff cone method (Greenberg 1995).

Nutrient Monitoring

To evaluate potential changes in nutrient concentrations as a result of the acrolein applications, three replicate water samples each were collected at the reference, refuge border, and Tule Lake study sites: (1) at the beginning of each

study period and (2) after approximately 24 hours. Unfiltered samples for nutrient analysis (ammonia, total persulfate nitrogen [TN], and total phosphorus) were collected in 10-mL test tubes using a Hach 10-mL Ten Sette pipette with sterile new pipette tips at each sample site/sample period.

Both ammonia and total nitrogen samples were immediately preserved on site with 40% sulfuric acid to a pH <2; no preservative was used for the phosphorus samples. All of the nutrient samples were immediately chilled in ice following collection, refrigerated, and then submitted by air courier to Dr. Jack Jones, University of Missouri (Columbia, Missouri) for analysis within 28 days.

Ammonia samples were analyzed by the phenate colorimetric method (Stainton et al. 1977), and total nitrogen (TN) was determined by the persulfate method with second derivative spectroscopy (Crumpton et al. 1992). Total phosphorus was analyzed by the Method 4500-P, using ascorbic acid reduction after persulfate oxidation (American Public Health Association 1989). A blank and a set of three standards (0.3 - 1.0 mg/L), constituting at least 10% of all samples in a batch, were run with each set of samples analyzed. Linear equations subsequently developed had r^2 values $\geq$ 0.99.

Invertebrate Monitoring

Free-swimming, benthic, and epibenthic macroinvertebrates from each site were sampled at 24- and 48-hour intervals to compare the reference and pesticide-exposed sites and to determine site differences potentially related to toxicity. The invertebrates were sampled with a 1.5 meter D-kick net (WildCo, NC) by skimming the sediment in approximately one meter, S-shaped motions. Six replicate samples per site per time period were composited and rinsed thoroughly with water to remove excess sediment. Algae and other extraneous matter were removed by hand. The remaining invertebrates were placed in labeled Whirlpak bags and preserved in 50% isopropyl alcohol. Snails $\geq$4 mm and other invertebrates $\geq$5 mm in size were identified to the lowest possible taxon (usually genus). Both relative abundance and diversity patterns were examined at the sites. The Shannon-Wiener H' statistic was used to compare the taxonomic diversity among the sites.

Pesticide Monitoring

Acrolein

The protocol for preservation of acrolein was first evaluated in a preliminary study of time stability and preservation at different pH concentrations under an Intra-Agency Agreement with Dr. Mark Sandstrom, National Ambient Water

Quality Laboratory, U.S. Geological Survey (USGS, Arvada, CO). Lost River water was provided for this spiking study, because it is the source of water for the J Canal and Tule Lake and we wanted to confirm that the technique would work without chemical interference or significant loss of acrolein from study site water. The preliminary study confirmed that greater than 75% recovery of acrolein could reliably be obtained using the prescribed preservation method for up to 13 days of storage following sample collection. The results will be published separately (Sandstrom and Snyder-Conn, in prep.), but the procedures adopted are briefly described below.

Sampling and equipment cleaning during acrolein sampling followed USGS protocol, including use of a prototype stainless steel volatile organic sampler (Shelton 1997). Four 40-mL amber purgeable vials precleaned for organics were submerged in the sampler for four minutes or more immediately below the surface at each site on the beginning dates of each sample period, allowing all oxygen to flush from the samples. Three of the vials were then pH-adjusted to pH 4 with hydrochloric acid at each site. (The exact amount of acid required to achieve this pH was determined with the fourth vial, sacrificed for this purpose at each site.) A field spike, field blank, and a trip blank were also similarly preserved during each study period. Preserved samples were immediately chilled in ice, refrigerated, and shipped by air courier within 48 hours to USGS for analysis. Analysis occurred within seven days of the sample collections.

Sampling occurred on July 30 and August 13, at the beginning of the first and second sample periods, respectively. Analysis of acrolein was by purge and trap gas chromatography/mass spectroscopy as described by Connor et al. (1998). The reported method limit of detection for the volatile organics, was 1.4 ug/L. Recoveries from the field spikes were 106 and 113%, indicating little, if any, acrolein loss during sample storage.

Other Pesticides

Subsurface composite grab water samples (950-mL) were collected at the refuge border (46B Drain) and Tule Lake (Pump 10) sites on July 30, 1997 (first sample period) and at pumps 24 and 10 on the J Canal at Tule Lake, on August 13, 1997 (second sample period) for pesticide analysis. The samples were collected in certified (Series 200) amber glass IChem containers with teflon lid liners precleaned for organics. Samples were immediated chilled in ice after collection, refrigerated, and then shipped by air courier to the a U.S. Fish and Wildlife Service laboratory, Patuxent Analytical Control Facility (Laurel, MD), for analysis. Samples from the two dates were analyzed for 23 organophosphate and 6 carbamate pesticides. Analysis was performed by gas chromatography using a flame photometric detector for organophosphate determinations and a nitrogen phosphorus detector for carbamate determinations (Patuxent Wildlife Research Center 1989). Megabored capillary columns were used for the GC separations.

RESULTS

Water Quality

Water quality measurements were made instantaneously, twice daily, over a 96-hour period at each site during the first study period. During the second study period, twice daily measurements were made at the refuge border site and hourly measurements were continously collected with Hydrolabs deployed at the reference and Tule Lake sites. All data are provided in Appendix A.

Temperatures fluctuated greatly at Tule Lake (Table A-1 and Figure 3) within a six-degree Celsius range during the continuous monitoring of the second study period. The J Canal reference site experienced smaller, more regular fluctuations of about two degrees Celsius. Water temperatures at the reference site routinely peaked at about 11:00 PM and reached minima near 9:00 AM, whereas the lake site reached maximum temperatures near 5:00 PM and minima around 8:00 AM. Although we lacked continuous data for the Pump 24 site, temperatures at this site appeared to more closely resemble those of Tule Lake.

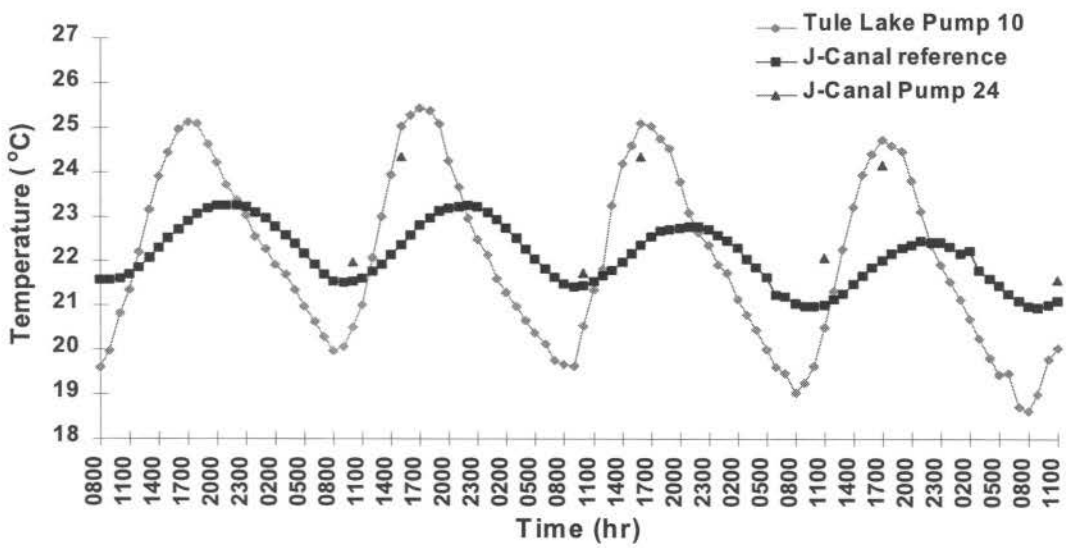


Figure 3. Comparison of J-Canal reference site, Tule Lake Pump 10, and J-Canal Pump 24 temperatures from August 13 - August 17, 1997.

A comparison of the instantaneous data for all three sites during the first study period

showed significant differences between the J-Canal reference site and Drain 46B (P = 0.014 for morning and P = 0.048 for afternoon temperatures); between Drain 46-B and Tule Lake Pump 10 (P = 0.004 for afternoon temperatures); and between Tule Lake Pump 10 and the J-Canal reference site (P = 0.003 for morning and 0.028 for afternoon temperatures). During the second 96-hr study, there were significant differences only for the afternoon temperatures (reference site and Pump 24, P = 0.006; and the reference site and Tule Lake Pump 10, P = 0.011). There was no significant difference observed between J-Canal Pump 24 and the Tule Lake Pump 10 sites. However, paired t-test results of the continuous water quality data showed no significant differences in temperature between the J-Canal reference site and Pump 10 at Tule Lake.

Very different dissolved oxygen (DO) concentrations occurred at the three study sites, (Table A-1 and Figure 4). During the first study period, dissolved oxygen concentrations were usually lower than at other sites and often less than 2 mg/L even in the afternoon and early evening. In contrast, both the 46B Drain and Tule Lake showed fairly regular diurnal variation, with low morning concentrations. The dissolved oxygen concentrations were slightly higher at the reference site during the second study period, ranging from 2.3-4.6 mg/L, whereas the lake site displayed a more extreme, and irregular pattern (range 0.3 - 9.6 mg/L). The highest DO concentrations at the reference site were consistently observed near 12:00 am and the lowest at 9:00 AM, differing from the lake site with peaks at various morning hours.

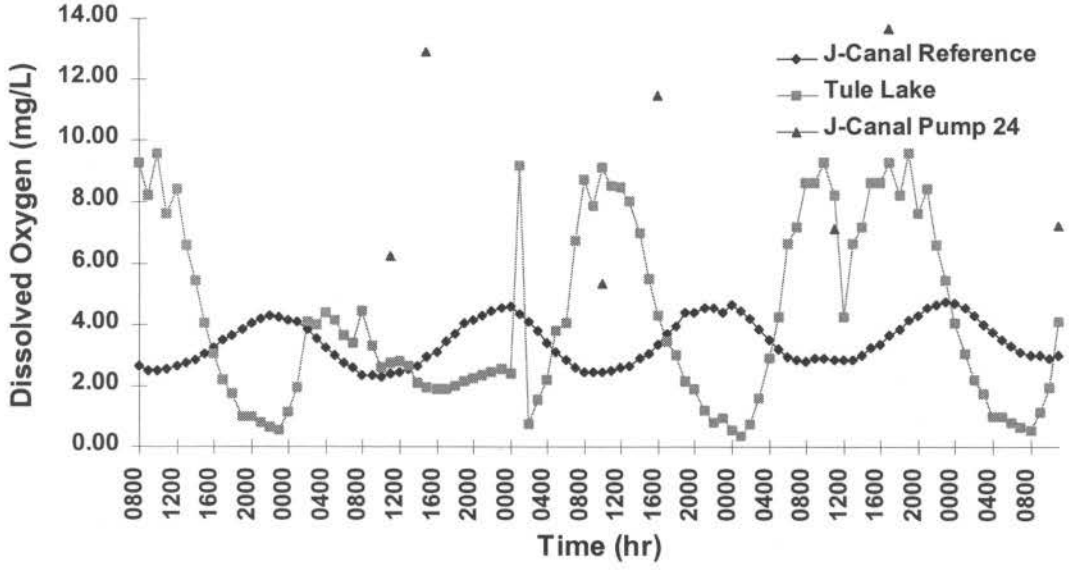


Figure 4. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 dissolved oxygen levels from August 13 - August 17, 1997.

A paired t-test of the continuous water quality data at the reference site and Tule Lake

Pump 10 showed highly significant differences ($P < 0.01$) for both morning and evening DO concentrations. Instantaneous readings during the first study period were significant in the morning only between Drain 46-B and the J-Canal reference site ($P = 0.027$). Differences in afternoon DO readings between Drain 46-B and the J-Canal reference site and between the J-Canal reference site and Tule Lake Pump 10 were also highly significant ($P \leq 0.01$), but differences between Tule Lake Pump 10 and Drain 46-B were not significant. During the second study period, differences in instantaneous morning DO concentrations between Pump 24 and the J-Canal reference site and between the J-Canal reference site and Tule Lake Pump 10 were highly significant ($P \leq 0.01$). Afternoon readings were also significantly different ($P = 0.004$ and 0.044, for the two respective site comparisons).

During the first study period, both the reference and 46B Drain sites remained within the range of pH 7 to pH 9, while higher pH values were attained at Tule Lake Pump 10. However, all instantaneous, first study period morning pH concentrations among sites were significantly different, as were afternoon differences between Drain 46-B and J-Canal reference site and Tule Lake Pump 10 and the J-Canal reference site. During the second study period (Figure 5), the reference and lake sites ranged from 6.99 - 7.22 and 7.83 - 9.08, respectively. Although both sites exhibited diurnal variation, the J Canal reference site experienced only minor fluctuations, while the lake site varied more than a full pH unit. Paired t-tests for the second sample period showed highly significant differences in both morning and afternoon pH concentrations between the J-Canal reference site and Pump 24 and between the J-Canal reference site and Tule Lake Pump 10.

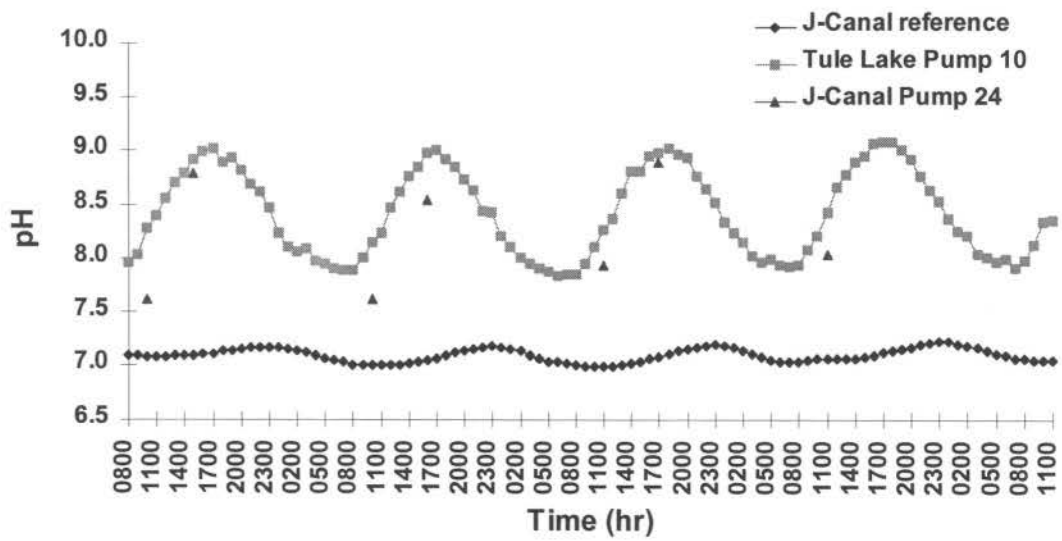


Figure 5. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 pH concentrations from August 13 - August 17, 1997.

Specific conductance varied little at each site during either study period, as exemplified in Figure 6 (the second study period). The reference site displayed small gradual changes, whereas the lake site experienced frequent, small fluctuations. The most notable differences in conductivity were between the reference site, which ranged from 186 - 218 μS/cm, and Tule Lake, which ranged from 366-473 μS/cm ($P<0.001$) during the second study period. The refuge border site (Pump 24) closely resembled the reference site.

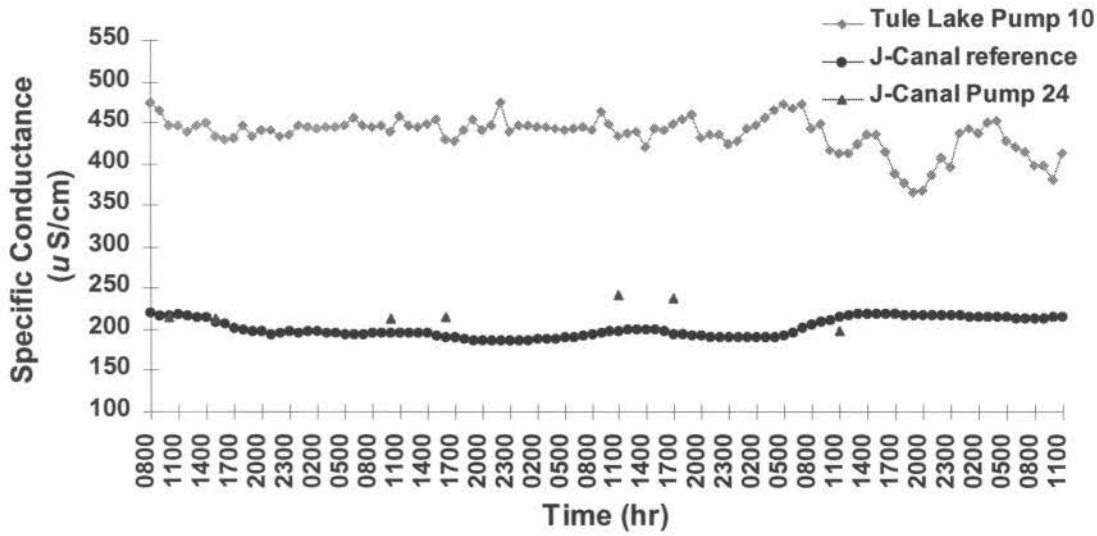


Figure 6. Comparison of J-Canal reference, Tule Lake Pump 10, and J-Canal Pump 24 specific conductance levels from August 13 - August 17, 1997.

Daily turbidity measurements were taken at each site during both study periods, but there were generally no patterns of statistically significant differences among sites. The only significant difference was between the J-Canal reference and refuge border site during the second study period ($P = 0.04$). In general, turbidities remained below 5 NTU; however, occasionally one of three replicate samples showed a turbidity slightly greater than 10 NTU, indicating some "patchiness" within sites.

Immobility/Mortality

In situ toxicity test results for *Daphnia* and fathead minnows appear in Appendix B. All pulmonate snails survived at all sites during both study periods; therefore, these results are not appended.

Daphnia results are summarized in Figures 7 and 8. Rates of death/immobilization were highly variable both within and among sites and time intervals, and did not follow

any consistent pattern. During both study periods, more *Daphnia* died or were immobilized at the reference site in flow-through containers removed after only 24 hours than in containers removed later, suggesting influences related to transport and unrelated to site water quality or pesticide exposure. During the first period, very low

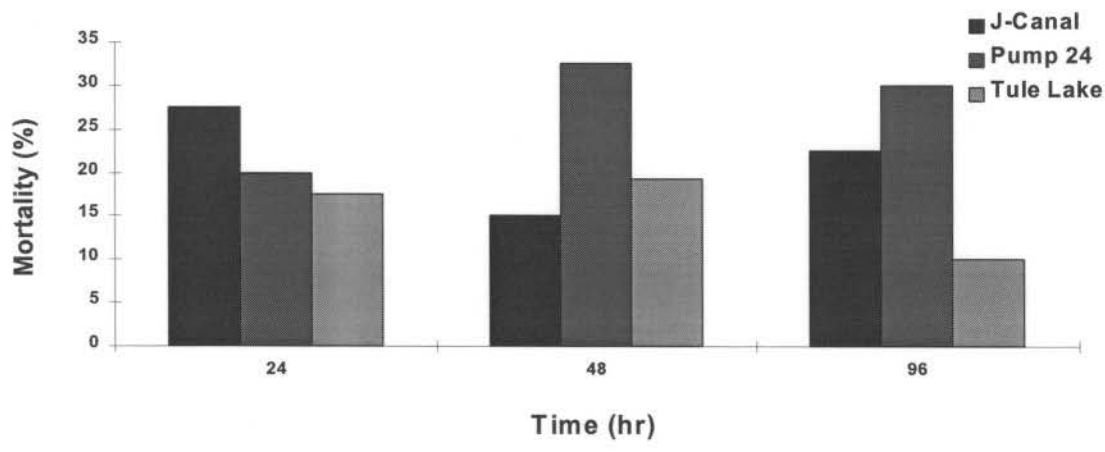


Figure 7. Percent dead/immobilized *Daphnia* from July 30 - August 2, 1997 at the reference (J Canal), refuge border (46B Drain), and Tule Lake study sites at 24-, 48-, and 96-hours

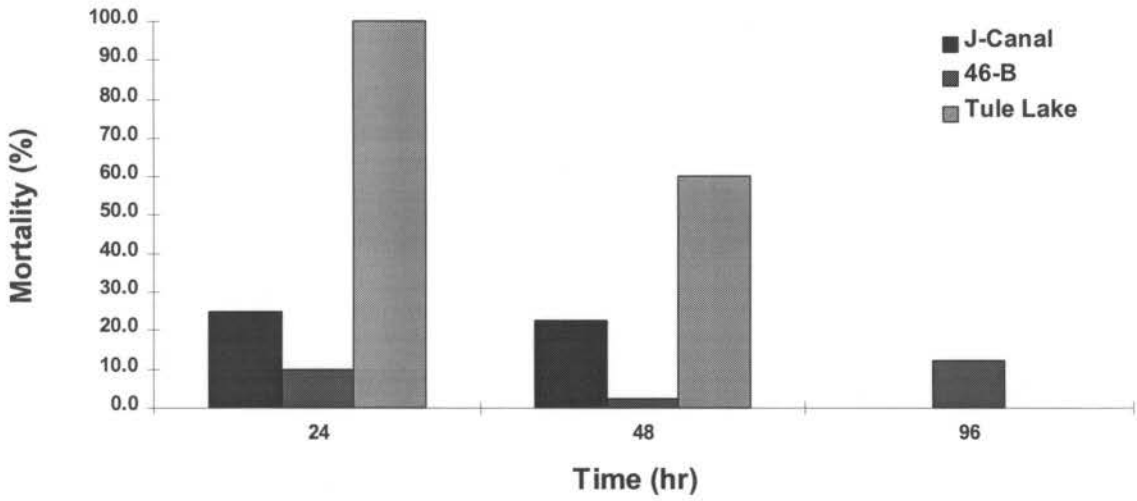


Figure 8. Percent dead/immobilized *Daphnia* from August 13 - August 17, 1997, at the J-Canal reference, J-Canal Pump 24, and T*ule Lak*e study sites at 24-, 48-, and 96-hours.

rates (<15%) of death and immobilization of *Daphnia* (less than those at the reference site), occurred at the 46B Drain at the refuge border. Only slightly higher rates occurred at the refuge border site during the second study period. During this period, rates of mortality/ immobilization were relatively low (≤ 35%) at all sites and there were no significant among-site differences.

High numbers of fathead minnows died during acclimation, culturing and transport prior to deployment procedures during the first study period. Therefore, insufficient data were available to compare fathead minnow mortality among sites during this period. However, data from the study are shown in Appendix B (Table B-1). During the second sample period, improved culture and transport technique and/or healthier minnows resulted in replicated mortality data for all the sites and time intervals (Figure 9). Except for 100% mortality at Tule Lake after the 96-hr interval, rates of mortality were similar among sites and exposure times. Accordingly, there were no significant statistical differences among the three sites over time using a Friedman rank analysis of variance. Very few fish died at the refuge border site during any time interval, but the fathead minnows at the Tule Lake site exhibited a gradual increase in mortality over the first 48 hours, followed by an abrupt increase to 100% mortality by the 96-hour interval. As with the *Daphnia*, the fathead minnows experienced greater mortality after 24 hours at the reference site than after 48 hours, again suggesting that early deaths at this site may not have been related to in situ exposure conditions.

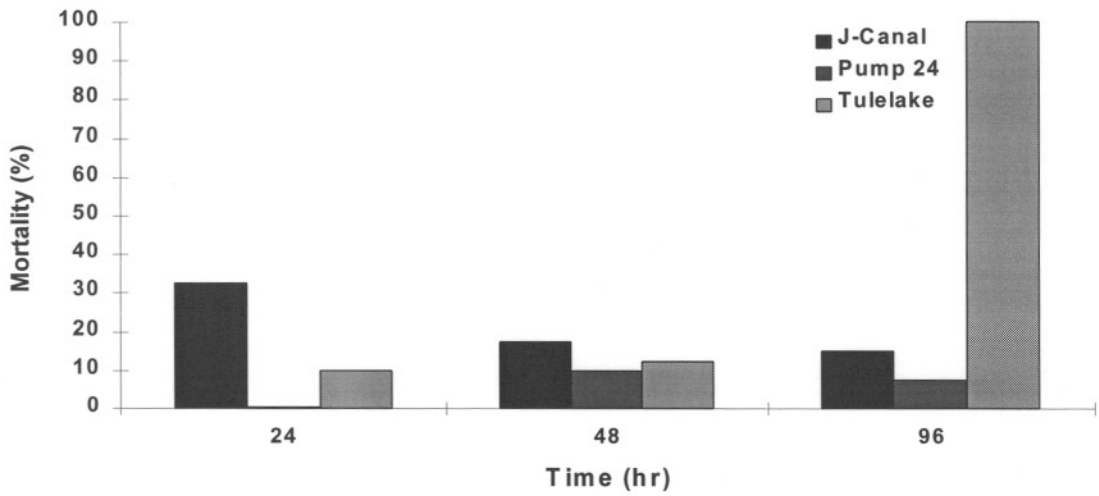


Figure 9. Percent mortality of fathead minnows from August 13 - August 17, 1997, at the J-Canal reference, J-Canal Pump 24, and T*ule Lak*e study sites at 24-, 48-, and 96-hours.

Pesticides

No acrolein was detected in any water sample, except for expected concentrations in field spikes. Nor were any organophosphate or carbamate pesticides detected in

samples from either study period (Appendix C).

Nutrients

Nutrient data are presented in Appendix D. Figures 10 and 11 provide comparisons of ammonia and nitrogen concentrations and phosphorus concentrations at the sites.

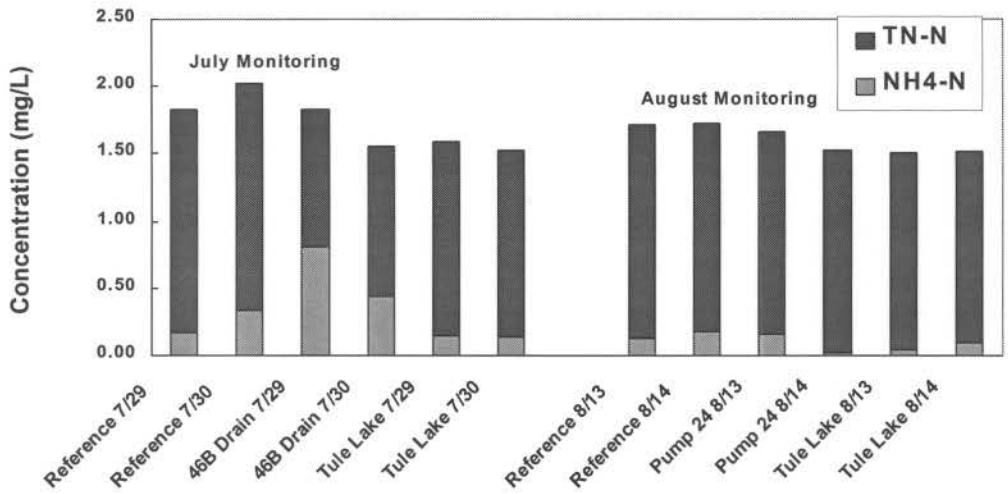
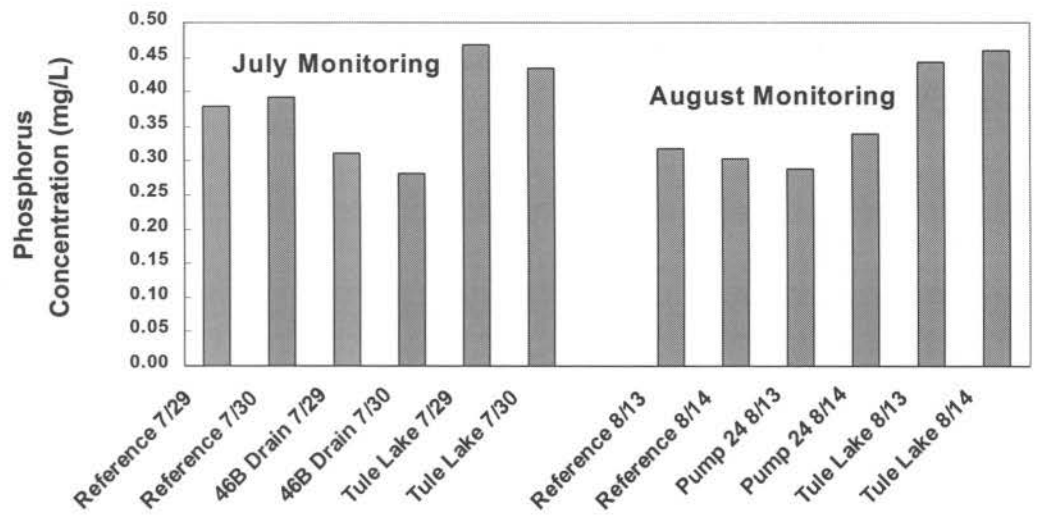


Figure 10. Comparison of total ammonia and total nitrogen concentrations during the July and August study periods in 1997.

Figure 11. Comparison of total phosphorus concentrations during the first and second study periods in 1997.

Except at the reference sites, total nitrogen tended to decline slightly or remain similar over time at each site. The highest nitrogen concentrations occurred at the reference site and the lowest concentrations occurred at the Tule Lake site. Ammonia concentrations, although constituting part of the total nitrogen, did not parallel total nitrogen concentrations. Instead, higher concentrations of ammonia occurred at the 46B Drain during the first study period. Other ammonia concentrations differences were smaller and did not follow any particular trend with time. Very high total phosphorus concentrations were observed at all of the sites, with Tule Lake concentrations exceeding those of the other sites. The refuge border and reference concentrations were quite similar.



Macroinvertebrates

Macroinvertebrate abundance and diversity patterns were compared during each study period for the J Canal reference site, refuge border site (either the 46B Drain or the Pump 24 at the J Canal), and Tule Lake at Pump 10. Results are summarized in Appendix E. From 52 to 303 macroinvertebrates 5 mm or greater in size were found per composite sample, and from 6 to 13 species occurred per site on a given date. The Shannon-Wiener Diversity Index, H', was extremely very low (<1) at all the sites. Dominant taxa included pulmonate snails, nematodes, trichopterans, and odonates in late July with ephemeropterans, coleopterans, odonates, trichopterans common in the mid-August samples. There were no consistent patterns of increase or decrease in the number of individuals or species within or among the sites.

DISCUSSION

Almost 1000 km of irrigation canals and drains occur in the Klamath Basin, and much of this system is systematically treated with the aquatic pesticide, acrolein, to limit the clogging of the pumps and waterways by aquatic vegetation. Fish and Wildlife Service biologists and members of the the public have identified a serious concern regarding the downstream effects of this pesticide in many locations throughout the West, where fish kill incidents are not uncommon following acrolein applications (Eisler 1994; EPA 1996). A special concern is impacts of acrolein applications to Tule Lake NWR aquatic resources, including two species of endangered suckers present in Sump 1A of Tule Lake. This study was designed to evaluate potential effects of acrolein applications, with a secondary purpose of evaluating the effects of any other pesticides detected in the irrigation water.

To evaluate the potential effects of acrolein treatments on refuge resources, we examined water quality, nutrient concentrations, and caged and local aquatic biota during two 96-hour periods. Acrolein applications occurred at eight locations on the J Canal upstream of the refuge during the first study period and at five locations during the second study period. In contrast to two previous studies of acrolein effects in the refuge (Winchester et al. 1994, Dileanis et al. 1996), all possible precautions were taken to assure that pesticide applicators were unaware of the study. Among other precautions, no stations were located immediately downstream of the application sites in visual range of applicators and applicators were not informed of the study in advance. Also, in situ equipment was camouflaged. Thus, results should be representative of the impacts of current, normal acrolein application schedules and practices on refuge waters and biota several miles downstream of application sites, the defined objective of the study. However, the findings cannot be extrapolated to assess the effects of applications on water quality or biota immediately downstream of application sites.

An important achievement during this study was the validation of our sampling and preservation methods for acrolein, another possible limitation of previous studies. Using Lost River water (source water for the J Canal), we were able to confirm through spiking experiments, that local irrigation drainwater with acrolein could be reliably preserved and stored for at least 13 days, with acrolein recovery rates exceeding 75%. Field spikes during the actual study confirmed even higher rates of acrolein recovery.

Despite good method validation, no acrolein was detected during the two study periods in waters at the reference site, the refuge border sites, or in Tule Lake Sump 1A, indicating that detectable levels of acrolein (at or above the method detection limit of 1.4 ug/L (parts per billion) were probably reaching refuge waters. Nor were any other

pesticides detected in the study. However, our analyses were restricted to carbamate and organophosphate pesticides, and did not include herbicides other than acrolein, or certain non-carbamate-based fungicides.

Although this study disclosed very poor water quality both upstream and within the refuge, we were unable to relate these conditions to acrolein applications. Similarly poor water quality and high nutrient concentrations have been identified previously upstream and within the refuge (Sorenson and Schwarzbach 1991; Kaffka et al. 1995; Dileanis et al. 1996). These and other researchers (Boyer 1993; Bennett 1994; Coyle, USGS, unpublished) found high rates of aquatic toxicity during in situ studies. Also associated with the poor water quality upstream and within the refuge were high rates of anomalies in frogs and fish (Boyer 1993; Littleton 1993). Although relationships with poor water quality, including low dissolved oxygen, high pH, and toxic unionized ammonia were apparent in the previous research, none of the above studies demonstrated a relationship between toxicity or anomaly rates and the presence of pesticides.

Macroinvertebrate abundance and diversity patterns also appeared unrelated to pesticide applications during the study periods. Instead, abundance and diversity varied almost as much within and between sample sites, and taxonomic diversity (evaluated using the Shannon-Wiener H' Index) remained quite low at the upstream reference, as well as at the refuge sites. Our results were quite similar to those of Bennett (1994) and Dileanis et al. (1996). In all of our studies, the most likely causes of low diversity appear to be the very poor water quality in both upstream and refuge waters.

There were no mortalities among the pulmonate snails placed at either the reference or test sites during either study period, and mean rates of *Daphnia* immobilization/death remained relatively low (< 35% and often $\leq$ 20%) at both the reference and refuge border sites during both study periods. Higher rates of immobilization/death in *Daphnia* were encountered at Tule Lake only during the first study period. However, during this period, the pattern (greater mortality in flow-through containers removed after 24 hours than after 48 hours) indicated that these test organisms probably were probably subject to transport or other effects unrelated to exposures on site.

There were inadequate healthy fathead minnows for first study period comparisons. However, during the second study period, fathead minnow mortality rates were below those at the reference site, and remained less than 20% throughout the study at the refuge border site (Pump 24 at the J Canal). Also, mortalities were quite low (<15%) at Tule Lake after 24 and 48 hours, but rose to 100% at 96 hours.

The 96-hour total die-off of fathead minnows in the flow-through containers

corresponded to a kill of approximately 15 lake fish (including chubs, fathead minnows, and Sacramento perch) and invertebrates, including snails and odonates, at this pump site on the same date. This mortality incident was relatively abrupt and appeared confined to the Tule Lake Pump 10 area, based on spot checks along the J Canal upstream of the pump, and checks of Pumps 11 and 12, north of Pump 10 on Tule Lake. At most of these other sites, small, live, healthy fish were observed, and no dead fish were found. The most likely explanation for the die-off of the fathead minnows (but not the *Daphnia*), may be related to the very low oxygen concentrations (< 1 mg/L), which occurred during the study period, including a prolonged period of low oxygen on August 13 after the 48 hour period. Such low oxygen conditions resulted in 100% fathead minnow mortality yet low *Daphnia* mortality in another study elsewhere in the Basin, where pesticide use did not occur (Snyder-Conn, in prep.).

It is also possible that low oxygen conditions could have been aggravated by an application of the herbicide Rodeo (glyphosate) by the County to segments of the northern and western shoreline of Tule Lake Sump 1A. The glyphosate was sprayed to treat emergent purple loosestrife on August 14, 1997. The applications could have directly affected lake dissolved oxygen through the decay process, or there may have been additional stress on the biota related to the very slight toxicity of glyphosate to aquatic organisms. Unfortunately, we did not test for glyphosate during our study and did not conduct a survey of the western shoreline for dead fish, because we were unaware of spraying activity until much later. Therefore, we are uncertain whether glyphosate was even present at the Pump 10 site, much less involved the die-off. Laboratory results concerning median lethal concentrations of glyphosate to *Daphnia* and fathead minnows during 48- and 96-hour exposures have produced highly variable results, with the herbicide being more toxic to fathead minnows in some bioassays and more toxic to *Daphnia* in others (Mayer and Ellersieck 1986). Therefore, the toxicity data do not shed any additional light on the possible glyphosate involvement. Based on all the known circumstances, including lack of dead fish at Pump Sites 11 and 12 closer to the spray zone, it would appear that low oxygen concentrations alone best explain the die-off event.

CONCLUSIONS

1. Validation studies confirmed that irrigation waters spiked with acrolein were properly collected and preserved for analysis using protocols adopted in this study.

2. No acrolein and no organophosphate or carbamate pesticides were detected at any of the study sites during this study, indicating that best management practices during applications may be normally sufficient to prevent entry of acrolein or other pesticides into refuge waters at detectable concentrations under mid-summer conditions.

3. Waters within and upstream of the refuge exhibited very poor water quality, experiencing conditions of high temperature and low dissolved oxygen. These conditions appeared unrelated to acrolein applications.

4. Nutrient concentrations, including total nitrogen and total phosphorus, were also very high both upstream and within Tule Lake NWR. Downstream concentration changes appeared unrelated to acrolein applications.

5. Patterns of mortality of caged snails, *Daphnia*, and fathead minnows in refuge waters did not appear related to acrolein or other pesticide applications during the study periods. Invertebrate abundance and diversity was low at all sites and there were obvious trends related to the acrolein applications during the study periods.

6. A fathead minnow die-off in both our flow-through containers and in adjacent Tule Lake waters at Pump 10 occurred sometime between August 13 and August 15. The die-off was probably related to the very low dissolved oxygen concentrations experienced in Tule Lake during this time period.

ACKNOWLEDGMENTS

We thank Marcus Horton, formerly of the Klamath Falls Fish and Wildlife Office, for technical assistance and good humor during the field studies; Doug Laye and Ron Larson, Klamath Falls Fish and Wildlife Office, for their peer review, including constructive editorial and technical comments; Mike Neuman, Bureau of Reclamation, for providing the site map; and Leanne Mitchel for her speedy proofing of the final manuscript.

LITERATURE CITED

American Public Health Association. 1989. Standard methods for the examination of water and wastewater. 17th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.New York, v.p.

Bennett, J. K. 1994. Bioassessment of irrigation drainwater effects on aquatic resources in the Klamath Basin of California and Oregon. Ph.D. dissertation, University of Washington. 195 pp.

Boyer, R. 1993. Evaluation of water quality in relation to frogs at Klamath Basin national wildlife refuges. M.S. Thesis. University of Washington, Seattle. 90 pp.

Connor, B.F., D.L. Rose, M.C. Noreiga, L.K. Murtagh, and S.R. Abney. 1998. Determination of 86 volatile organic compounds in water by gas chromatography/mass spectrometry, including detections less than reporting limits. U.S. Geological Survey Open-File Report 98-829. 78 pp.

Crumpton, W.G., T.M. Isenhart, and P.D. Mitchell. 1992. Nitrate and organic N analyses with second derivative spectroscopy. Limnol. Oceanogr. 37:907-913.

Dileanis, P., S. E. Schwartzbach, J. Bennett, and others. 1996. Detailed study of water quality, bottom sediment, and biota associated with irrigation drainage in the Klamath Basin, California and Oregon, 1990-1992. U.S. Geological Survey Water-Resources Investigations Report 95-4232, Sacramento, CA. 68 pp.

Eisler, R. 1994. Acrolein hazards to fish, wildlife, and invertebrates: a synoptic review. National Biological Survey Biological Rep. 23. 29 pp.

EPA. 1980. Ambient water quality criteria for acrolein. U.S. Environmental Protection Agency. EPA 440/5-80-016. 94 pp.

EPA. 1996. Fish kills from algicides. Pesticides Section, U.S. Environmental Protection Agency. Pesticide monitoring in the Northwest:1-6.

Greenberg, A. E. 1995. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C. 1100 pp.

Haag, W. R., C. D. Yao, T. Pettit, and T. Mill. 1988. Estimation of hydrolysis rate constants for acrolein (Magnacide-H herbicide, Magnacide-B microbiocide) in the environment, SRI International, Menlo park, CA. 54 pp.

Holcombe, G.W., G.L. Phipps, A.H. Sulaiman, and A.D. Hoffman. 1987. Simultaneous multiple species testing: acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish and invertebrate families. Arch. Environ. Contam. Toxicol. 16:697-710.

Hydrolab Corporation. 1991. DataSonde® 3 multiparameter water quality datalogger operating manual. Hydrolab Corp., Austin, TX. 45 pp.

Isaacs, F.B., R.G. Anthony, and D.P. Anderson. 1997. Bald eagle nest locations and history of use in Oregon and the Washington portion of the Columbia River Recovery Zone, 1971-1997. Oregon Cooperative Wildlife Research Unit, Oregon State University, Corvallis. 18 pp. + tables, figures and an appendix.

Kaffka, S.R., T.X. Lu, and H.L. Carlson. 1995. An assessment of the effects of agriculture on water quality in the Tulelake region of California. University of California Intermountain Research and Extension Center Research Progress No. 108. 85 pp.

Little, E.E. 1997. Acute toxicity of acrolein to Lost River and shortnose suckers. Final Report submitted to the Bureau of Reclamation, Klamath Falls Area Office, on February 7, 1997. 4 pp.

Littleton, T. M. 1993. Water quality and fishes of the Lower Klamath and Tule Lake National Wildlife Refuges. Masters Thesis, University of Washington. 53 pp.

Mayer, F.L., Jr., and M.R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Fish Wildlife Service Resour. Publ. 160. 570 pp.

Patuxent Wildlife Research Center. 1989. Analytical chemistry group SOP: organophosphate/carbamate scanning method (0-25.00). U.S. Fish and Wildlife Service, April 28, 1989. n.p.

Shelton, L.R. 1997. Guidelines for collecting volatile organic compounds in stream water for the National Water Quality Assessment Program. U.S. Geological Survey Open-File Report 97-401. Sacramento, CA. 31 pp.

Smith, A. M., J. Mao, R. A. Doane, M. F. Kovacs, Jr. 199-. Metabolic Fate of ^{14}C-acrolein under aerobic and anaerobic aquatic conditions. 33 pp.

Snyder-Conn, E. 1996. CA-acrolein effects on aquatic ecosystems in Tule Lake NWR. U.S. Fish and Wildlife Service Contaminants Assessment. 16 pp.

Sorenson, S.K., and S.E. Schwarzbach. 1991. Reconnaissance investigation of the water quality, bottom sediment, and biota associated with irrigation drainage in the

Klamath Basin, California and Oregon, 1988-89. U.S. Geological Survey Water-Resources Investigations Report 90-4203. 64 pp.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The chemical analysis of fresh water. Fisheries and Environment Canada. Misc Spec. Pub. No. 25, 2nd Edition.

U.S. Fish and Wildlife Service. 1993. Recovery plan: Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chamistes brevirostris*). U.S. Fish and Wildlife Service, U.S. Department of the Interior, Region 1, Portland, OR. 108 pp.

Winchester, W. D. 1994. Acrolein herbicide monitoring, Tulelake area. California Regional Water Quality Control Board, North Coast Region, Santa Rosa, CA. 11 pp.

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VII. BUDGET

VII.A. Previously Allocated Expenditures

FY 1996....	
Operational	$ 0
Analytical (PACF + non-PACF)	$ 0
FY 1997	
Operational	$ 16,550
Analytical (PACF + non-PACF)	$ 25,970

VII.B. Funds Requested for Next FY (this proposal)

OPERATIONAL	
Personnel Costs (salaries, benefits and overhead)	$ 14,050
Travel	$ 1000
Supplies	$ 500
Equipment	$ 500
Other (specify): (e.g., cooperative agreement, contracts, etc.)	$ ____
Sub-total for Operational Costs	**$ 16,050**
ANALYTICAL	
PACF (carbamate and organophosphate pesticides)	$ 6,200
Non-PACF (non-routine analytical - acrolein, nutrients)	$ 19,770
Sub-total for Analytical	**$ 25,970**
Total Funds Requested for FY 1997	**$ 42,020**
GRAND TOTAL OF PROJECT COSTS TO DATE	**$ 42,020**

VII.C. Estimated Future Costs

FY 1998

Operational (Report Writing)	$ 5,000
Analytical (PACF + non-PACF)	$ _____

FY 199.....

Operational	$ _____
Analytical (PACF + non-PACF)	$ _____

VIII. APPROVALS

Reviewed by: _____ Date: 7-8-96
Manager, Tule Lake NWR

Submitted by: Elaine Snyder-Conn PhD by pers Date: 7/7/96
Contaminant Specialist, Field Office

Approved by: Donald W. Hoffeck Date: 7/26/96
Environmental Contaminants Coordinator

Acting ARD Donald W. Hoffeck 7/26/96