Final Report

The Acute and Chronic Effects of Pesticides on the Calanoid copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, of the San Francisco Estuary

Submitted to:

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**Background**

Over the past decade, a major change in pesticide use in California has been the switch from organophosphorus insecticides to pyrethroid insecticides for both agricultural and home use (TDCEnvironmental 2005, 2008a 2008b). While organophosphorus pesticides are still used and identified in delta waters, use of organophosphate insecticides on orchards began decreasing in the mid–1990s as pyrethroid insecticides were promoted by various state agencies and programs in California as alternatives (Epstein et al. 2001, Zhang and Wilhoit 2005). In 2006 alone, 198,495 kg of Chlorpyrifos, approximately 18,249, 18,181, and 33,682 kg of bifenthrin λ-cyhalothrin and permethrin (pyrethroid insecticides) were applied to agricultural lands (CADPR, 2010; Kuivila and Hladik 2008), and approximately 54,000 kg of fipronil was used in California primarily for structural pest control (CADPR, 2010).

Levels detected in the environment of the five pesticides are typically in the low part per trillion ranges (ng/L). The detection limits for analytical chemistry for the five pesticides investigated are near the levels found in the environment, especially for λ-cyhalothrin and bifenthrin. In a study from 2008 thru 2010, Werner et al (2010) detected chlorpyrifos (≤10ng/L), permethrin (≤35ng/L), and bifenthrin (≤117ng/L) in the San Francisco Estuary (SFE). A study of sediments in the central valley of California detected chlorpyrifos (≤98.6. ng/g), permethrin (≤158.8 ng/g), bifenthrin (≤32.2ng/g), and λ-cyhalothrin (≤11.7 ng/g) (Weston and Zhang 2008). Fipronil, an emerging pesticide of concern, was not examined in either of these studies therefore the levels in the water and sediment is uncertain. Additionally, a summary report of water in the Sacramento-San Joaquin delta from 2003 to 2010 shows that chlorpyrifos was found in 45.3% (242/528, # of samples detected/# of samples measured), fipronil 16.9% (82/486), permethrin 0% (0/482), λ-cyhalothrin 0% (0/332) and bifenthrin 0% (0/63) (Orlando 2013). Despite the lack of detections of pyrethroids in water, these pesticides are still detected in sediment samples collected from the San Francisco Estuary. In the same report, measuring sediment from 2003 thru 2010, chlorpyrifos was found in 0% (0/5# of samples detected/# of samples measured), fipronil 0% (0/5), permethrin 32.4% (24/74), λ-cyhalothrin 35.1 % (26/74) and bifenthrin 79.7 % (59/74) (Orlando 2013). Given the prevalence of chlorpyrifos, fipronil, permethrin, λ-cyhalothrin and bifenthrin in SFE waterways, it is important to understand the ecological implication of each of these chemicals.

As the shift from OP to PY pesticides occurred in the SFE, pelagic fish abundance declined remarkably. At the same time, populations of these pelagic fishes’ prey items (i.e. zooplankton) crashed as well. Two significant members of the SFE zooplankton community are the calanoid copepods *Eurytemora affinis* (Poppe 1880) and *Pseudodiaptomus forbesi* (Poppe and Richard 1890). These two species are a critical food source in the estuary of pelagic fishes which are in significant decline (Kimmerer 2004, Mueller-Solger et al. 2006). Over the past few decades, scientists have witnessed a significant decline in *E. affinis* and *P. forbesi* populations in the SFE (Bennett 2005). While predation, competition, changes in hydrology and toxic algal blooms are postulated to contribute to their population decline, one factor associated with the decline
of *E. affinis* and *P. forbesi* abundance is pesticide exposure, which is the main focus of this study.

There are three tasks in this project. Task 1 is to develop techniques to mass cultures *E. affinis* and *P. forbesi* for use in experiments; Task 2 is to determine baseline toxicity values of five pesticides to *E. affinis* and *P. forbesi*; and Task 3 is to determine sensitivity of copepods to different physicochemical stressors.

**Task 1. Maintaining of Mass Culture of Copepods**

Because *E. affinis* and *P. forbesi* are the preferred test species and are not commercially available, maintaining the integrity, health, and adequate population of the copepod cultures under selected pH and temperature conditions is extremely important. Therefore, it is critical to establish optimum culture conditions to ensure that high quality and quantity of the copepods will be available for the acute and chronic bioassays. Copepods were collected from Rio Vista and Suisun Marsh in the San Francisco Estuary using a 174µm zooplankton tow net in June 2007. Two brood stocks of *E. affinis* and *P. forbesi* at 2 ppt and 5ppt salinity were acclimated and maintained in aerated 120L tanks with standard moderately hard fresh water (pH 7.8 at 20±1°C). Water quality parameters were monitored weekly and maintained as follows: alkalinity (80 mg/L), dissolved oxygen (>8 mg/L), and ammonia (<1 µg/L) (Hach, USA). An equal biovolume of the instant algae (IA) mix was given as food at 500 µg C.L⁻¹ day⁻¹. The IA diet is comprised of highly nutritious and pure concentrated forms of the phytoplankton *Nannochloropsis* and *Pavlova* (Instant Algae, Reed Mariculture, USA). Approximately 50% of the total culture medium was replaced weekly with aerated and pH/temperature acclimated medium. The culture system was maintained under a natural photoperiod (16L:8D) and covered with a semi-transparent black tarp to reduce exposure to light.

**Materials and Methods**

**Copepod mass culture protocol**

In our copepod mass culture system, density and water quality conditions are essential in maintaining healthy mass cultures. After the initial two week start period of the copepod mass culture, the mass cultures must be diluted, concentrated or refreshed once or twice per week to maintain mass cultures.

The density of the mass culture is monitored weekly to maintain approximately 100 adult copepods per 1L. To check density, use a clean 1L beaker and take sub-samples from the top, side (if applicable) and bottom of the mass culture. Estimate the number of copepods in each sample, record and determine the average number of adult copepods per liter. If the culture has more than 100 adults per 1 L, dilute with new culture medium as necessary. In general, a good dilution is 10-25% of the total mass culture volume. If a higher percentage dilution is necessary, spread across multiple days. To do a water dilution, use white construction buckets (approximately 20L) and open drain on bottom of mass culture allowing culture to pass through into the bucket. Empty bucket and repeat
as many times necessary until mass culture is at the appropriate level based off of density. Add reconstituted water to the tank to bring the culture back to proper level.

If the density is less than 100 adults per L, the mass culture will be concentrated. To concentrate the copepods in the mass culture, wet a mesh sieve (20-45um) with DI water. Place the sieve in a white bucket and use the drain on the bottom of the conical tank. Slowly open the drain and allow 0.5L of culture to drain directly into a bucket. This will help to remove any detritus accumulated at the bottom of the tank. Next, place mesh sieve (20-45um) directly under drain and allow 10-20L of the culture to slowly pass through, collecting copepods in mesh and allowing filtered water to pass through into bucket. After filtering water, gently pour back copepods into mass culture. Discard filtered water remaining in bucket. Repeat as necessary to complete appropriate concentration. Refill mass culture with RO water to get mass culture to desired volume and copepod density.

Harvesting copepods for experiments

Approximately one week prior to desired experiment start date, check mass cultures for densities and age classes. To do this, concentrate approximately one liter of culture by placing mesh sieve (20-45um) under bottom drain, collecting copepods in the mesh and allowing filtered water to drain into bucket. Pour concentrated copepods collected in mesh sieve (20-45um) into petri dish and inspect under dissection microscope. Estimate the number of individuals in each major age class (nauplii, copepodite, adult and gravid females).

If experiments are to be conducted with copepodites (juveniles), check for high levels of late stage nauplii. Over the course of the week, these nauplii will grow into juveniles (depending on conditions of the culture) and be of proper age for experiments. In a healthy culture system, this same process can be repeated for other age classes (copepodites → adults, adults → gravid females, gravid females → nauplii), as each copepod stage lasts approximately one week (varies on water quality).

Copepods to be used for experiments should only be collected from cultures that are in need of a concentration. Following protocol similar to a concentration, place mesh sieve (20-45um) under bottom drain and allow 10-20L of culture to filter through, collecting copepods in mesh and filtered water in bucket. Place mesh sieve (20-45um) in a 1 L beaker with some extra culture water. This will increase the water volume in the mesh, maintain temperature and ultimately reduce stress to the organisms. Drain concentrated copepods into petri dishes and count number of copepods desired for experiment using a dissection scope (bottom lighted) or light box. To count copepods, hand-select copepod using a 5mL disposable pipette and place into single droplet on petri dish. Repeat this step on same petri dish until desired number of copepods (usually 20) for each replicate is achieved. Under a dissection scope, double-check each droplet to ensure copepods are active and are of appropriate age class. Using a separate 5mL disposable pipette, suction solution from treatment replicate and rinse copepods on petri dish into the same treatment replicate. Repeat as necessary to conduct full-scale experiment. Any leftover copepods (not of the correct age class) should be replaced into mass culture system.
Special considerations: Copepods should not be left out on light box or dissection scope display for longer than 15 minutes. This will cause unnecessary stress and cause poor experimental results. Furthermore, harvested copepods in mesh should not be left out longer than one hour. If more time is needed to count copepods, reduce the volume of mass culture harvested and repeat harvesting more frequently.

Task 2. Toxicity testing of pyrethroid insecticides, organophosphate insecticides, and fipronil to E. affinis and P. forbesi

Materials and Methods

Acute toxicity tests were conducted using modified EPA static renewal guidelines (US EPA 2002). Tests were conducted in 600mL glass beakers of moderately hard water prepared to 2psu using Instant Ocean (Spectrum Brands, Madison, WI, USA). The pesticides were obtained from ChemService (West Chester, PA, USA), and added to waters using a methanol solvent, with solvent controls in all tests. Water parameters were maintained at salinity: 2.03 ± 0.07 ppt, alkalinity: 95.24 ± 11.23 mg/L CaCO₃ hardness: 502.86 ± 45.73, pH: 8.00 ± 0.104, and conductivity: 3312.86 ± 303.49. Four replicate beakers were used at each of the six test concentrations, except for chlorpyrifos where there were three replicates per treatment. Twenty 16 + 2 day old copepods were added to each beaker. Tests were conducted at 20°C, under a light cycle of 16 hours light: 8 hours dark. For the pyrethroid pesticides, an 80% water change was conducted at the 48 hour interval, during which, deceased and paralyzed copepods were enumerated. For Chlorpyrifos and Fipronil, an 80% water change was conducted at 24 hour intervals, during which deceased and paralyzed copepods were enumerated. Copepods were fed daily with 400 (E. affinis) and 500 (P. forbesi) μgC/L/day of Instant Algae (IA) (equal volumes of Nannochloropsis and Pavlova from Reed Mariculture, Campbell, CA, USA). After 96 hours, surviving copepods were enumerated.

Analytical chemistry

Composite samples were collected from water at test initiation and at 48 hours of exposure. Samples were sent to California Department of Fish and Game Water Pollution Control Laboratory (Rancho Cordova, CA, USA) and analyzed for the chemical of concern. Samples were analyzed so that results could be presented in terms of detected rather than nominal concentration.

Statistical Analysis

All statistics were conducted in R studio (version 0.97.248). All dose response curves were created using the drc package (version 2.3-0). The log-logistic model was selected to describe the dose response curves because six other models were compared using AIC and LL with 3 parameters worked well with all data sets. In some cases (e.g., E. affinis and bifenthrin) other models better fit the data, however, the better fit model was not significantly different from the LL.3 model.
Results

The study indicates that *E. affinis* and *P. forbesi* of the San Francisco Estuary can be cultured and used in large scale experiments. Additionally, modified EPA standardized acute toxicity tests with *E. affinis* and *P. forbesi* indicate that these species are sensitive to key pesticides at concentrations detected in the environment.

Figures 1-5 show the dose response curves of *E. affinis* and *P. forbesi* exposed to the five pesticides. The pyrethroid pesticides, bifenthrin and lambda-cyhalothrin were the most toxic of the five pesticides investigated with 96hLC$_{50}$ values of 15.43 and 19.40 ng/L for *E. affinis*, and 25.39 and 16.80 ng/L for *P. forbesi*, respectively. The organophosphorus pesticide, chlorpyrifos was the least toxic of the pesticides investigated, with 96hLC$_{50}$ values of 716.44 ng/L for *E. affinis* and 1080.13 ng/L for *P. forbesi*, respectively. The 96hLC$_{50}$ values of permethrin for *P. forbesi* and *E. affinis* were 46.58 and 107.93 ng/L. The 96hLC$_{50}$ values of fipronil for *E. affinis* and *P. forbesi* were 136.21 and 200.95 ng/L. A comparative toxicity of the five pesticides between *E. affinis* and *P. forbesi* is shown in Figure 6. Table 1 summarizes comparative 96hLC$_{50}$ values of this study to other test species.

![Figure 1: 96 hours dose response curves of chlorpyrifos to *E. affinis* and *P. forbesi*. The 96hLC$_{50}$ values are 716.44 ng/L for *E. affinis* and 1080.13 ng/L for *P. forbesi*, respectively. The y-axis is the percent of animals that survived.](image-url)
Figure 2: 96 hours dose response curves of fipronil to *E. affinis* and *P. forbesi*. The 96hLC₅₀ values are 136.21 ng/L for *E. affinis* and 200.95 ng/L for *P. forbesi*. The y-axis is the percent of animals that survived.

Figure 3: 96 hours dose response curves of permethrin to *E. affinis* and *P. forbesi*. The 96hLC₅₀ values are 107.93 ng/L for *E. affinis* and 46.58 ng/L for *P. forbesi*. The y-axis is the percent of animals that survived.
Figure 4: 96 hours dose response curves of \( \lambda \)-cyhalothrin to \textit{E. affinis} and \textit{P. forbesi}. The 96hLC\textsubscript{50} values are 19.4 ng/L for \textit{E. affinis} and 16.8 ng/L for \textit{P. forbesi}, respectively. The y-axis is the percent of animals that survived.

Figure 5: 96 hours dose response curves of bifenthrin to \textit{E. affinis} and \textit{P. forbesi}. The 96hLC\textsubscript{50} values are 15.43 and 25.39 ng/L for \textit{E. affinis} and \textit{P. forbesi}, respectively.
Figure 6: Summary of the comparison of the 96 hour median lethal concentrations of chlorpyrifos, fipronil, permethrin, λ-cyhalothrin, and bifenthrin to *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Error bars indicated 95% confidence intervals. Values were calculated using the LL.3 model in the drc package for R.
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Species</th>
<th>96hLC₅₀ (ng/L)</th>
<th>95% CI</th>
<th>Temperature (°C)</th>
<th>Age (days)</th>
<th>Citation</th>
<th>Notes</th>
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<tr>
<td>Chlorpyrifos</td>
<td>P. forbesi</td>
<td>1080.13</td>
<td>(789.72, 1398.38)</td>
<td>20±1</td>
<td>16±2</td>
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<td>E. affinis</td>
<td>716.44</td>
<td>(574.59, 825.4)</td>
<td>20±1</td>
<td>16±2</td>
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<td></td>
<td>H. azteca</td>
<td>42.7</td>
<td>(33-49.2)</td>
<td>20</td>
<td>14-21</td>
<td>Anderson and Lydy 2001</td>
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<td></td>
<td>C. dubia</td>
<td>54</td>
<td>(40, 71)</td>
<td>24-25</td>
<td>&lt;1</td>
<td>Bailey et al. 1997</td>
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<td>P. forbesi</td>
<td>200.95</td>
<td>(144.62, 257.29)</td>
<td>20±1</td>
<td>16±2</td>
<td>Lesmeister (Aquatic Health Program)</td>
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<tr>
<td></td>
<td>E. affinis</td>
<td>136.21</td>
<td>(40.23, 232.19)</td>
<td>20±1</td>
<td>16±2</td>
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<td>H. azteca</td>
<td>540</td>
<td>(NA)</td>
<td>22.6-23.7</td>
<td>7-14</td>
<td>Lizotte et al. 2009</td>
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<td></td>
<td>C. dubia</td>
<td>143,000</td>
<td>(126400, 163430)</td>
<td>22</td>
<td>&lt;1</td>
<td>Qin et al. 2011</td>
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<td>P. forbesi</td>
<td>46.58</td>
<td>(54.479, 81.498)</td>
<td>20±1</td>
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<td>E. affinis</td>
<td>107.93</td>
<td>(92.97, 122.90)</td>
<td>20±1</td>
<td>16±2</td>
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<td>H. azteca</td>
<td>21.1</td>
<td>NA</td>
<td>23</td>
<td>7 to 14</td>
<td>Anderson et al. 2006</td>
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<td>C. dubia</td>
<td>510</td>
<td>(465, 557)</td>
<td>21</td>
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<td>H. azteca</td>
<td>2.2</td>
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<td>25</td>
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<td>Callinan (Aquatic Health Program)</td>
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<td></td>
<td>C. dubia</td>
<td>300</td>
<td>(150, 550)</td>
<td>25</td>
<td>&lt;1</td>
<td>Mokry and Hoagland 1990</td>
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<td>P. forbesi</td>
<td>25.39</td>
<td>(21.52, 29.26)</td>
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<td>16±2</td>
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<td></td>
<td>E. affinis</td>
<td>15.43</td>
<td>(12.84, 18.01)</td>
<td>20±1</td>
<td>16±2</td>
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<td></td>
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<td>(20.1, 32.9)</td>
<td>23</td>
<td>7 to 14</td>
<td>Holzer 2011</td>
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<td>C. dubia</td>
<td>51</td>
<td>(34, 68)</td>
<td>21</td>
<td>&lt;1</td>
<td>Yang et al. 2006</td>
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<td></td>
<td>C. dubia</td>
<td>390</td>
<td>(290, 490)</td>
<td>22</td>
<td>&lt;1</td>
<td>Qin et al. 2011</td>
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Table 1: Comparison of 96hLC₅₀ values of E. affinis and P. forbesi to two commonly used EPA standardized test species (Hyalella azteca and Ceriodaphnia dubia). All values highlighted in red are results from the Aquatic Health Program. 96hLC₅₀ values for H. azteca and C. dubia were collected from http://cfpub.epa.gov/ecotox/quick_query.htm and their primary citations. The only values for C. dubia and λ-cyhalothrin are for 48 hours.
Task 3. Tolerance of P. forbesi to temperature and salinity

Climate change is projected to increase the mean temperature and salinity of the San Francisco Estuary (SFE), potentially imperilling organisms in the SFE (Cloern et al. 2011). To determine whether the projected increases in temperature and salinity pose a threat to *Pseudodiaptomus forbesi*—a key member of the SFE food web—we tested the tolerances of *P. forbesi* to a range of temperatures and salinities in a laboratory experiment. We fit a beta-binomial model to these data, with the eventual aim to link our model to climatic models of temperature and salinity. These data are immediately applicable to determining the optimal salinity and temperature to use to mass-culture *P. forbesi*.

**Methods**

Tolerance of *P. forbesi* juveniles to a range of salinities at 20 °C for 96 hours was tested to determine the concentration at which *P. forbesi* juveniles experience 50% morality (96hLC₅₀). Juveniles were selected because past experiments have shown that juveniles are more vulnerable than adults to changes in water quality. *P. forbesi* were cultured at 5 ppt Instant Ocean (Spectrum Brands, Madison, WI, USA) dissolved in ‘moderately hard synthetic freshwater’ (USEPA 2002). To simulate the salt-water intrusion into the SFE, Instant Ocean was used to produce 5 nominal salinities: 5, 10, 15, 20, and 25ppt. To begin the experiment, 4- 600mL beakers filled with 500mL of water at each concentration, producing 20 beakers that ranged in concentration from 5-25ppt. Four replicates of each salinity were used. One hour after feeding the copepod culture, 20 juvenile copepods were randomly selected from the culture and placed into each of the beakers. The beakers were then placed into a 20 °C water bath and air was bubbled into each beaker. Copepods were fed 400 μgC/L/day of Instant Algae (equal volumes of *Nannochloropsis* and *Pavlova* from Reed Mariculture, Campbell, CA, USA) during the 4 day experiment. At 48 and 96 hours after the start of the experiment, mortalities were assessed. Any missing individuals were considered as dead (juvenile *P. forbesi* decompose quickly at 20°C).

The 96hLC₅₀ value was used as one of the two salinities in a second experiment to determine how changes in temperature affect *P. forbesi* at different levels of salinity. The second salinity was 5 ppt Instant Ocean (the salinity at which we raise the copepods). To begin the experiment we randomly assigned target temperatures to water baths. Water temperatures in the experiment ranged from 5.3-33.2 °C. We conducted a water renewal at 48 h and quantified mortality as previously described. Water temperature in each water bath was measured each morning and evening during the experiment.

**Results**

A linear beta-binomial model with a logistic link function was fit to the salinity mortality data (Fig. 7). The naïve posterior of the model was sampled 10,000 times, and then was used each of the models to calculate the nominal salinity at which 50% mortality occurred (i.e., plugged 0.5 into ‘y’ and solved for ‘x’ for each of the models). We averaged the results of these calculations to derive the 96hLC₅₀, and used the data to calculate a 95% CI around the LC₅₀. The 96hLC₅₀ for salinity was 14.79 (95%CI=13.78028 to 15.77421) at 20°C (Fig. 7). Figure 8 shows the selected
temperature/mortality model of \( a + b \times \text{temp} + c \times \text{ppt} + d \times \text{temp}^2 \), with a beta-binomial distribution of error. Results indicate \( P. \text{forbesi} \) response to temperature and salinity is additive and non-linear. Mortality increased at both low and high temperatures at both levels of salinity (5 and 14.79 ppt). Our result suggests that the ideal temperature for \( P. \text{forbesi} \) survival is between 15 and 17 °C.

Fig. 7 Mortality of \( P. \text{forbesi} \) as a function of nominal salinity. The solid line is the model averaged predictions and the dashed lines are the 95% confidence interval of the model.
Fig. 8 The selected temperature/salinity model of mortality is $a + b \times \text{temp} + c \times \text{ppt} + d \times \text{temp} \times \text{temp}$, with a beta-binomial distribution of error. Circles are 14.79 parts per thousand (ppt) Instant Ocean salt, squares are 5 ppt Instant Ocean salt. The dashed lines are the 95% confidence intervals for the selected model.
Summary of Results

1. We have developed a culture technique with which we can raise sufficient copepods to conduct large scale experiments of the resident calanoid copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*.

2. *E. affinis* and *P. forbesi* are sensitive to pesticides at environmentally relevant concentrations.

3. There are species specific differences in sensitivity to pesticides:
   a. *E. affinis* is more sensitive to chlorpyrifos, fipronil, and bifenthrin
   b. *P. forbesi* is more sensitive to permethrin and λ-cyhalothrin
   c. *E. affinis* is more sensitive to fipronil than permethrin
   d. *P. forbesi* is more sensitive to permethrin than fipronil

4. The orders of most to least toxic pesticides for *E. affinis* are: bifenthrin, λ-cyhalothrin, fipronil, permethrin and chlorpyrifos.

5. The orders of most to least toxic pesticides for *P. forbesi* are λ-cyhalothrin, bifenthrin, permethrin, fipronil and chlorpyrifos.

6. *E. affinis* and *P. forbesi* are least sensitive to organophosphate chlorpyrifos and most sensitive to pyrethroids pesticides.

7. Our data indicate that the optimal temperature for *P. forbesi* is ~15-17 °C.

Discussion and Conclusion

This study is the first of its kind to develop culture techniques to conduct large scale experiments of the resident calanoid copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, of the San Francisco Estuary. Unlike standardized test species such as *Ceriodaphnia dubia* and *Daphnia magna*, the resident zooplankton species, *E. affinis* and *P. forbesi* are not currently commercially available. Moreover, in addition to the inherent issue of prior exposure to contaminants which will complicate the interpretations of laboratory results; shifts in seasonal field abundance of these calanoid copepods may also prevent conducting year-round experiments of wild caught copepods. Therefore, the development of consistent and standardized laboratory raised mass cultures is essential and will allow researchers to conduct toxicity testing on *E. affinis* and *P. forbesi* year round.

This study is also the first of its kind to determine baseline acute toxicity values of pesticides to two species of resident zooplankton species in the San Francisco Estuary. Results of this study show that *E. affinis* and *P. forbesi* are sensitive to neurotoxic pesticides, especially the bifenthrin and λ-cyhalothrin, at environmentally relevant concentrations. Comparative toxicity of *E. affinis* and *P. forbesi* to organophosphorus and pyrethroid insecticides are summarized in Table 1. Our results indicate that *E. affinis* and *P. forbesi* 96hLC50 (716 and 1080 ng/L) values for chlorpyrifos are greater than 20-fold above *H. Azteca* (43 ng/L) and *C. dubia* (54 ng/L). The 96hLC50 value of *P. forbesi* for permethrin is greater than 2-fold above *H. Azteca* and 9-fold below *C. dubia*. The 96hLC50 values of *E. affinis* and *P. forbesi* for λ-cyhalothrin are greater than 8-fold above *H. Azteca* and 17-fold below *C. dubia*. The 96hLC50 values of *E. affinis* and *P. forbesi* for bifenthrin are comparable to *H. Azteca* and are greater than 2-fold below *C. dubia*. 
The toxicity of Fipronil, an emerging pesticide of concern, to \textit{E. affinis} and \textit{P. forbesi} is between pyrethroid and Chlorpyrifos, i.e., the orders of most to least toxic are pyrethroid> fipronil> chlorpyrifos. Currently, there is little published fipronil LC50 data available for comparison to the copepods used in this study. Our results indicate \textit{E. affinis} and \textit{P. forbesi} 96hLC50 (~136-201 ng/L) values of this study are greater than twofold below \textit{H. Azteca} (~540 ng/L) and aquatic midges (420 ng/L) and greater than 100-fold below reported freshwater crustacean (i.e. \textit{Ceriodaphnia dubia}, \textit{Daphnia pulex}, \textit{Procambarus clarkii}, \textit{Procambarus zonangulus}) acute (48–96 h) LC50s for this compound (10,300–19,500 ng/L) (Gunasekara et al. 2007).

Of the five pesticides tested, \textit{E. affinis} is more sensitive to bifenthrin, fipronil, and chlorpyrifos while \textit{P. frobesi} is more sensitive to permethrin and λ-cyhalothrin. In addition, the data indicate both copepods species are less sensitive to chlorpyrifos when compared to \textit{H. azteca} and \textit{C. dubia}. Both copepods are more sensitive to pyrethroid pesticides and fipronil than to organophosphate pesticides. This raises the possibility that the major change in pesticide use from organophosphorus insecticides to pyrethroid insecticides and fipronil in California in the last decade may have contributed to the decline in zooplankton abundance in the SFE.

A linear beta-binomial model with a logistic link function was selected to evaluate salinity/temperature effects on \textit{P. forbesi} survival. Results indicate salinity effects on \textit{P. forbesi} mortality are reduced at temperature between 15-17 °C.

**Recommendations**

Several issues are noted in the current study:

1. Fipronil is an effective toxicant towards arthropods and poses significant risk to non-target crustacean species. The metabolites of fipronil have also been suggested to be more toxic than the parent compound. Furthermore, bioaccumulation of fipronil in fish suggests a potential adverse effect to aquatic organisms in higher trophic levels and to the health of aquatic ecosystems (US EPA 1996). The current study indicates \textit{E. affinis} and \textit{P. forbesi} are most sensitive to fipronil. Additional study evaluating the toxicity of fipronil metabolites to copepods and the chronic effects of fipronil to fish growth and reproduction are needed.

2. Additional study is needed to determine the tolerance of \textit{E. affinis} to temperature and salinity. Once we have these data, we will link the statistical model to climatic models and use it to predict how the distributions of both copepod species will be affected by climate change. The model will also be used to determine optimal temperatures and salinities for culturing \textit{E. affinis} and \textit{P. forbesi}. Eventually, we anticipate testing whether changes in salinity and temperature are likely to interact with pesticides to affect copepods.
References


Holzer, B. 2011. Determination of critical body residue values for three current use pesticides in Hyalella azteca: Predictive techniques versus direct tissue measurement. Oklahoma State University, Stillwater, OK.


