

# VIRGIN RIVER RESOURCE MANAGEMENT AND RECOVERY PROGRAM

## 2011 ANNUAL REPORT

**Project Title:** Development and Optimization of Spawning and Intensive Culture Techniques for Woundfin

**Project Number:** V.09.02

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**Project Title:** Development and Optimization of Spawning and Intensive Culture Techniques for Woundfin

**Project Summary:** (Explain project goals and objectives and how the project fulfills recovery element, fills data gaps, or provides stepping stone to recovery.)

The underlying purpose of this multi-year research project conducted at the Bozeman Fish Technology Center (BFTC) in collaboration with Dexter National Fish Hatchery and Technology Center (DNFHTC), Wahweap State Fish Hatchery (Wahweap), and Bubbling Ponds State Fish Hatchery (Bubbling Ponds) is development of methods for increasing production of woundfin at conservation propagation facilities to help meet requirements of the restocking program for recovery of the species. The goals of this project are: (1) establish intensive culture techniques for future application at conservation propagation facilities, and (2) investigate current issues related to existing pond culture practices at conservation propagation facilities and recommend changes for improving survival of embryos and larvae.

Through 2011, twelve research tasks (objectives) had been identified to achieve the dual goals of this project. Tasks 1–5 were completed during years 1 and 2 of the project and accomplishments were summarized in monthly and annual reports:

1. Successfully rear woundfin obtained from DNFHTC captive stock in test aquaria at the BFTC.
2. Describe the reproductive cycle of woundfin.
3. Determine optimal diets for larval and juvenile woundfin.
4. Develop tool(s) to determine sexual dimorphism and assess spawning readiness.
5. Refine intensive conditions for successful woundfin spawning to maximize spawning success and condense the spawning season.

This annual report summarizes progress and accomplishments on tasks 6–12 during year 3 of the project:

6. Determine dietary protein and energy needs of juvenile woundfin cultured at three temperatures.
7. Optimize thermal regime to maximize spawning potential.
8. Determine ultraviolet (UV) effects on embryo survival.
9. Determine predation rates on embryos and larvae by adult woundfin.
10. Develop hormonal injection regime for ovulation and spermiation of woundfin.
11. Initial funding to begin development of broodstock diet formulations to increase fecundity and larval survival and performance.
12. Cooperate and coordinate with DNFHTC, Wahweap, and Bubbling Ponds to ensure the necessary transfer of technology and information.

**Project Status/Anticipated/Expected Date of Completion:** (One paragraph describing product status, due date, and anticipated date of completion)

Task 6. The experimental phase has been completed. Results were compiled in a poster presentation at the Aquaculture America 2012 conference in Las Vegas, NV, and the poster will be provided to the Virgin River Resource Management and Recovery Program (VRRMRP) office in St. George, UT. A manuscript is in preparation for submission to a peer-reviewed journal with expected completion date of May 2012.

Task 7. The thermal regime experiment was conducted at BFTC, DNFHTC, and Bubbling Ponds. All facilities maintained the same water temperature profiles and photoperiod. Fish did successfully spawn at BFTC and DNFHTC but not at Bubbling Ponds. At the BFTC, 2.7% more eggs were spawned in the treatment group compared to the control. At DNFHTC, significantly more eggs were spawned in the treatment group compared to the control group. DNFHTC did apply the ramping temperature to their main production and reported that the maturation cycle was compressed. Three times more young-of-year woundfin were produced in 2011 compared to the average production of other years. The increase in production may be contributed to a combination of the application of the thermal regime as well as removal of larval fish into a separate pond from the spawning pond. DNFHTC will incorporate the ramping phase of the temperature profile in future years. Full analyses for this experiment are underway and will be presented in the March 2013 annual report.

Task 8. An evaluation of the effect of ultraviolet-B (UV-B) radiation on woundfin embryo and larval survival was completed at the BFTC during the 2011 spawning season. Woundfin embryos and larvae were exposed to UV-B concentrations that simulated levels documented in a living stream used for woundfin culture at Bubbling Ponds (0.06 mW/cm<sup>2</sup>). We used a dose response experiment and four UV-irradiance levels: 0.060 mW/cm<sup>2</sup>, 0.030 mW/cm<sup>2</sup>, 0.015 mW/cm<sup>2</sup>, and 0.000 mW/cm<sup>2</sup> to approximate 100, 50, 25, and 0% of the ambient irradiance levels documented at Bubbling Ponds. Complete (100%) mortality occurred in both embryo (during blastulation, gastrulation, neurulation)

and larval woundfin (immediate post-hatch to 8 weeks post-hatch) exposed to UV-B levels that were 25, 50, and 100% of the ambient irradiance levels. We also began exploring the efficacy of three potential UV-B mitigation strategies (Aquashade®, shadecloth, and dissolved organic carbon (DOC) amendments) that may assist in attenuating UV-B in outdoor culture ponds. A manuscript has been submitted to Aquaculture and is under review. The final results of this task are presented in Appendix 1.

Task 9. We experimentally measured rates of predation on embryos and larvae by adult woundfin at the BFTC in 2011. Predation was a significant source of mortality on both embryos ( $W = 210$ ,  $P < 0.001$ ) and larvae ( $W = 45$ ,  $P = 0.004$ ). There was a median of three (95% C.I. 1 - 4) embryos predated per tank ( $n=8$  trials). No predation difference between eyed and non-eyed embryos was detected ( $W = 32$ ,  $P = 0.07$ ). There was a median of one (95% C.I. 0.5 - 2) larvae predated per tank ( $n=5$  trials). There was a significant difference between the number of mixed unpigmented and pigmented ( $n=4$  trials) and unpigmented ( $n=1$  trials) larvae predated ( $W = 26$ ,  $P = 0.02$ ). There was a median of one (95% C.I. 0 - 2) mixed unpigmented and pigmented larvae predated per trial, while there was a median of three (95% C.I. 2 - 4) unpigmented larvae predated per trial. To examine the potential effects of predation, we assumed 100% and 50% of the predation rate determined at the BFTC, and a conservative 40-day window of opportunity for adults to predate on both embryos and larvae. We also assumed that fish only predate on embryos and larvae during daylight hours; which is approximately 15 hours for the propagation facilities. As a result of these assumptions, the estimated annual embryo loss may be 105,000 to 210,000 embryos and larvae at DNFHTC. Similarly, the estimated annual loss may be 1,800 to 3,600 embryos and larvae at Bubbling Ponds and 12,000 to 24,000 embryos and larvae at Wahweap. A manuscript has been submitted to Journal of Fish and Wildlife Management and is under review.

Task 10. An initial hormonal induction study with woundfin was conducted at the BFTC during the 2011 spawning season. Three hormones (common carp pituitary extract (CPE), lutenizing hormone releasing hormone, and human chorionic gonadotropin) were screened at each of two doses to determine their efficacy in induction of ovulation and spermiation. Common carp pituitary extract (20 ug/g) resulted in the highest ovulatory success and mean number of eggs released per female. Follow-up experiments will be conducted during the 2012 spawning season. After completion of those experiments, all data analysis will be complete and a manuscript for publication will be written and submitted. This data will be presented in the March 2013 annual report.

Task 11. The experimental phase is underway. Diets were formulated and manufactured, and feeding began on 6 September 2011. Culture temperatures are being adjusted to induce spawning the last week of May 2012. Data collection is expected to be completed by July 2012 with data analysis and reporting finalized by January 2013.

Task 12. Monthly reports were submitted to the VRRMRP office and to the conservation propagation facilities. Bozeman FTC researchers were in regular contact with conservation propagation facility managers throughout the spawning season and post-spawning season.

**Accomplishments/Recommendations/Results:** (Highlight important findings of the project and summarize important results. If project report or findings result in recommendations for future work or direction of recovery efforts include a summary.)

Task 6. After 8 weeks, fish mass ranged from 1.94 to 2.37 g with the greatest growth rates occurring at 28°C and 45% dietary protein. Dietary fat concentration had no effect on growth of woundfin at either dietary protein concentration or across culture temperatures. Dietary protein concentration affected feed conversion ratio with fish consuming 45% dietary protein having lower feed conversion than fish consuming 35% protein diets. Neither dietary lipid concentration nor culture temperature affected feed conversion ratios of woundfin. Condition of fish was not influenced by dietary protein or lipid concentration, but fish reared at 28°C had the highest condition factor. The rate of ammonia excretion was elevated with increasing culture temperature and with increasing dietary protein concentration but was not influenced by dietary fat concentration. These data indicate that woundfin should be cultured on a 45% protein diet with lower dietary lipid levels (~8 %) to ensure maximum growth rates and minimal feed conversion ratios across the range of culture temperatures normally observed in spring through fall.

Task 7. The thermal regime experiment was conducted at BFTC, DNFHTC, and BPSFH. All facilities maintained the same water temperature profiles and photoperiod. In January, 30 woundfin (1:2 sex ratio using body conformation as an indicator of sex) were placed into each of two tanks (60 fish total). The water temperature was decreased (no more than 1°C per day) to 8-9°C depending on the facility. One tank was randomly assigned as the control tank and the other tank as the treatment tank. The control tank had a naturally vernalized water temperature profile throughout the spring (8-22°C), while the treatment tank had a temperature profile to entrain the maturation cycle (8-16°C followed by a quick ramp from 16-22°C).

During the last full week of May, the water temperatures were ramped in the treatment tank from 16 to 22°C (1°C/day). The following Monday (May 30) after the water temperature reached 22°C, spawning substrate was placed in both the control and treatment tanks by 2 PM. On Tuesday (May 31) morning, the substrate tray was removed, rinsed of feces, and placed in a separate incubation/hatching tank (22°C). The control and treatment substrate trays were kept in separate incubation/hatching tanks. Fish were fed several pinches of feed in the morning after removal of the substrate tray. Tanks were cleaned prior to addition of the fresh spawning substrate by 2 PM each day. Fish were placed on a normal feeding regime over the weekend, and the spawning regime was repeated the next week.

Fish did successfully spawn at BFTC and DNFHTC but not at Bubbling Ponds. At the BFTC, 2.7% more eggs were spawned in the treatment group compared to the control. At DNFHTC, significantly more eggs were spawned in the treatment group compared to the control group. DNFHTC did apply the ramping temperature to their main production and reported that the maturation cycle was compressed. Three times more young-of-year woundfin were produced in 2011 compared to the average production of other years. The increase in production may be contributed to a combination of the application of the thermal regime as well as removal of larval fish into a separate pond from the spawning pond. . They will incorporate the ramping phase of the temperature profile in future years. Full analyses for this experiment are underway.

Task 8. See Appendix 1.

Task 9. Predation was a significant source of mortality on both embryos ( $W = 210$ ,  $P < 0.001$ ) and larvae ( $W = 45$ ,  $P = 0.004$ ). If possible, substrate trays should be removed from ponds as soon as possible and hatched out in a separate pond from adults. As well, larvae should be removed from ponds as soon as possible and grown out in a separate pond from adults to avoid predation.

*Task 10.* An initial hormonal induction study with woundfin was conducted at the BFTC during the 2011 spawning season. Three hormones (common carp pituitary extract (CPE), lutenizing hormone releasing hormone, and human chorionic gonadotropin) were screened at each of two doses to determine their efficacy in induction of ovulation and spermiation.

- Treatment 1: LHRH 0.01  $\mu\text{g/g}$  (10  $\mu\text{g/kg}$ ),
- Treatment 2: LHRH 0.05  $\mu\text{g/g}$  (50  $\mu\text{g/kg}$ ),
- Treatment 3: hCG 1 IU/g on two consecutive days,
- Treatment 4: hCG 2 IU/g
- Treatment 5: CPE 5  $\text{ug/g}$
- Treatment 6: CPE 20  $\text{ug/g}$

Common carp pituitary extract (20  $\text{ug/g}$ ) resulted in the highest ovulatory success (83%) and mean number of eggs released per female (500 eggs/female). Follow-up experiments will be conducted during the 2012 spawning season. After completion of those experiments, all data analysis will be conducted and a manuscript for publication will be written and submitted. This data will be presented in the March 2013 annual report.

Task 11. Progress on this task is limited at this point in time with an expected completion date of January 2013.

Task 12. Monthly reports were submitted to the VRRMRP office and to the conservation propagation facilities. Bozeman FTC researchers were in regular contact

with conservation propagation facility managers throughout the spawning season and post-spawning season.

**Budget:**

Funds Provided: \$59,953

Funds Expended: \$59,953 (Total anticipated expenditures by the end of the fiscal year)

Remaining Balance: \$0

APPENDIX 1.

IN REVIEW: AQUACULTURE

Effects of Ultraviolet-B Radiation on Survival of Woundfin (*Plagopterus argentissimus*)  
Embryos and Larvae with Application to the Conservation Propagation Program

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**Abstract.** Endangered woundfin (*Plagopterus argentissimus*) embryos and larvae were exposed to artificial ultraviolet-B (UV-B) radiation to directly examine the effects of UV-B on survival. The experiment was part of a project assisting the Virgin River Resource Management and Recovery Program's efforts to increase production of this endangered fish. The UV-B radiation used in this experiment was administered in treatments of 0.060 mW/cm<sup>2</sup>, 0.030 mW/cm<sup>2</sup>, and 0.015 mW/cm<sup>2</sup> to simulate 100%, 50%, and 25% of the ambient irradiance levels documented in outdoor tanks and living streams at Bubbling Ponds State Fish Hatchery (Bubbling Ponds), Arizona, USA. Embryos and larvae were exposed for 14.5 h followed by 9.5 h of darkness, in correspondence with the daylight h at Bubbling Ponds. No embryos survived UV-B treatments; survival among control (UV-B free) treatments varied among females indicating that there may be important parental effects that influence embryo survival. Larval mortality was also 100% for individuals exposed to any of the three UV-B treatments. In contrast to embryo trials, larval survival in UV-B free treatments exceeded 80%. These experiments provide evidence that woundfin embryos are sensitive to even low levels of UV-B. Susceptibility of larvae to UV-B appears to be a function of age at exposure with older larvae exhibiting significantly lower levels of mortality during the initial days of exposure. Preliminary experiments with UV-B mitigation strategies indicated that shade cloth, Aquashade®, and elevated dissolved organic carbon can aid in the attenuation of UV-B, and these strategies may assist hatchery managers in implementing UV-B mitigation measures during periods when woundfin are most susceptible.

*Keywords:* woundfin, embryo, larvae, UV-B, ultraviolet radiation, *Plagopterus argentissimus*

## 1. Introduction

The steady and significant depletion of stratospheric ozone has produced a measurable increase in the levels of ultraviolet-B (280-320 nm; UV-B) radiation entering earth's freshwater habitats (Cracknell and Varotsos, 2009). Measurements of elevated UV-B have been accompanied by studies documenting the deleterious effects of UV-B on susceptible life stages of aquatic organisms (Häder et al., 1995; Bancroft et al., 2007; Dong et al., 2007; Romansic et al., 2008; Calfee et al., 2010). Studies conducted with fish embryos and young larvae show increased mortality and impaired development as a result of exposure to ambient and elevated UV-B (Hunter et al., 1979; Kouwenberg et al., 1999; Häkkinen et al., 2002; Weigand et al., 2004; Dong et al., 2007), and combined, these factors may limit recruitment to adult populations (Béland et al., 1999). For example, recent evidence shows that Atlantic cod (*Gadus morhua*) embryos are particularly susceptible to UV-B radiation during gastrulation (Kouwenberg et al., 1999).

The principal effect of UV-B on fish and other aquatic organisms is the formation of pyrimidine dimers that manifest as lesions and alter the structure of DNA (Douki, 2010). Specifically, pyrimidine dimers inhibit replication and transcription of the DNA (Buma et al., 2003) and can lead to deformities and/or increased mortality (Bancroft et al., 2007). Because of the harmful and potentially lethal effects of UV-B, many organisms have physiological strategies that assist in repairing or preventing UV-B induced damage (Häkkinen et al., 2002; Wiegand et al., 2004; Bancroft et al., 2007) or have altered activity patterns (e.g. seeking UV-B free refugia or modifying periods of activity) to minimize exposure to elevated UV-B (Ylönen et al., 2004; Garcia et al. 2009). Despite the documented effects of UV-B, studies to date have been limited to a relatively small number of aquatic vertebrates.

Woundfin (*Plagopterus argentissimus*) is an endangered species of minnow within the Cyprinidae family. The woundfin is native to the lower Colorado River Basin, but the combined effects of habitat loss, flow alteration, and competition with invasive species has reduced the woundfin's distribution to a fraction of its historic range (Chen et al., 2011). Woundfin are now restricted to reaches of the mainstem Virgin River (Chen et al., 2011) and are now listed as endangered; a designation that provides protection under the Endangered Species Act (USFWS 2000). Recovery efforts for the federally endangered woundfin include captive propagation at three rearing facilities: Dexter National Fish Hatchery and Technology Center (DNFHTC), New Mexico, Bubbling Ponds State Fish Hatchery (Bubbling Ponds), Arizona, and Waheap State Fish Hatchery (Waheap), Utah. The principal goal of the recovery program was to successfully rear woundfin at these facilities for repatriation of woundfin to its former range. The Virgin River Resource Management and Recovery Program has a restocking program currently underway that call for as many as 100,000 to 200,000 woundfin to be produced annually (S. Meismer, pers. comm.).

The culture practices and conditions for woundfin rearing at the production facilities are conducive to exposing embryos and larvae to UV-B. In fact, much of the production of woundfin at the aforementioned facilities is done through the use of outdoor ponds or living streams. These habitats lack the physical complexity of natural habitats and are low in dissolved organic carbon (DOC); organic debris and DOC aid in blocking or attenuating UV-B (Bukaveckas. and Robbins-Forbes., 2000; Williamson et al., 1996). At the Bubbling Ponds, few viable woundfin larvae have been found following the spawning season in outdoor ponds and living streams. Given the reported effects of UV-B on developing embryos and larvae, we hypothesized that UV-B may contribute to poor survival at the conservation propagation facilities. To test this

hypothesis, we examined how varying levels of UV-B affect captive-reared woundfin embryo and larval survival. Ambient UV-B radiation at Bubbling Ponds during the woundfin spawning season was documented in 2011, and we used these ambient levels as a guide for our study. Accordingly, three UV-B irradiance levels were included to better understand what UV-B levels are lethal to woundfin.

## **2. Materials and Methods**

### **2.1 UV-B Exposure Chamber**

The UV-B treatments were established using a four chambers exposure system. This system was composed of an aluminum water bath (35.5 cm x 26.2 cm x 121.9 cm) separated into four discrete chambers using sheets of 2 mm fiberglass reinforced plastic (FRP) that were secured to the water bath with a silicone sealant. The system was located in a quarantine room at Bozeman Fish Technology Center (BFTC), and therefore, the system had a dedicated water system. Water flowed through the system at a rate of 7.5 L/min at a constant temperature of approximately 22° C. At the initiation of the experiments source water pH was 8.7, NO<sub>3</sub>-N concentration was 0.01 mg/L, NH<sub>3</sub>-N concentration was below detection levels, and the dissolved oxygen concentration was 6.9 mg/L. Temperature loggers (iButton, Maxim, Sunnyvale, CA, USA) were also used to document water temperatures within each of the four exposure chambers.

Treatments were designed to simulate ambient UV-B levels (approximately 0.060 mW/cm<sup>2</sup>) measured at Bubbling Ponds facility during spawning. Measurements of UV-B were taken at a depth of 60 cm where embryos have been detected in previous years. Treatments levels in this experiment were 0.060 mW/cm<sup>2</sup>, 0.030 mW/cm<sup>2</sup>, 0.015 mW/cm<sup>2</sup>, and 0 mW/cm<sup>2</sup>

and designed to simulate 100%, 50%, 25% and 0% of the ambient UV-B levels documented at Bubbling Ponds. A UV-B free treatment was used as a control in all trials. Ultraviolet-B measurements were made using a 2100 PMA (personal measuring assistant) meter and a 2102 UV-B detector (Solar Light, Philadelphia, Pennsylvania, USA).

The three different UV-B irradiance levels were achieved by suspending one UV-B-313-EL light bulb (Q-Lab, Cleveland, Ohio, USA) and one Verilux Instant Sun Full Spectrum model F40T12SUN light bulb (Verilux Inc., Stamford, Connecticut, USA) in a common ballast established at different distances from test embryos or larvae held in incubation cups. The cups were constructed using a 5 cm diameter PVC coupler with a nylon screen (500  $\mu$ m) inserted 3.8 cm from the bottom of the coupler. The control chamber consisted of two Verilux full spectrum light bulbs suspended at a distance equal to the 50% UV-B treatment. The order of treatments was randomized between trials by reestablishing unique irradiance levels in each chamber. The photoperiod was set at 14.5 h light and 9.5 h darkness and maintained with an automatic timer. This photoperiod corresponded to the average number of daylight h during spawning season at the Bubbling Ponds rearing facility. We documented variation in irradiance levels for the entire 14.5 hr irradiance period once for each of the four UV-B treatments.

For both embryo and larval trials, animals were checked daily for mortality. Accordingly, incubation cups were removed from the system and checked for mortalities under a dissecting microscope. The number of mortalities was recorded for each incubation cup. Nonviable embryos and larvae were removed, and living woundfin were placed back into their respective cups and treatment chambers. Every 24 h, the cups were inspected and mortalities counted, until there were no embryos or larvae remaining in the treatment chambers.

## 2.2 Spawning

Woundfin embryos and larvae used in this experiment were progeny from the 2008 and 2009 year class females (origin DNFHTC) maintained in indoor tanks at the Bozeman Fish Technology Center in Bozeman, MT, USA. Before the start of each embryo trial, thirty fish (20 females and 10 males) were hormonally injected with 20 µg/g Carp Pituitary Extract (CPE; ARGENT Chemical Laboratories®, Redmond, Washington, USA). The fish were then strip spawned 24 h post injection. The eggs were immediately fertilized, and fertilization success was assessed (presence of first cleavage) within 30 min.

## 2.2 Embryo Trials

A total of 240 embryos collected from three different females (80 embryos each per female) were placed into 24 uniquely labeled 5 cm incubation cups (n = 10 embryos per cup) at each trial. Duplicate cups from each female were placed into one of four treatment chambers. For embryo trials 1 and 2, we ran the experiment out 5 days. In trials 3, 4, and 5 we carried out the experiments until days 9, 11, and 10, respectively. We chose to use a more protracted observation period in later trials to more carefully assess mortality of control embryos over a longer period. Because no mortality was seen in control embryos past 5 days, all data are reported up to 5 days.

## 2.3 Larval Trials

Embryos not used for embryo trials were placed in 10 cm incubation jars and allowed to hatch. After hatching, hatchlings were put into 61 cm diameter fiberglass tanks with 20 cm of water. The tanks contained hatchlings from multiple females and therefore contained a mixture

of progeny. Some of the larvae were exposed to UV-B just 2 days after hatching (Trials 1 and 2). For trials 3, 4, and 5 larvae were 5-6 days, 27-30 days, and 41-44 days post hatch, respectively. For the larval trials, we lowered water levels to 5.4 cm inside the exposure chambers to prevent larvae from swimming out of the incubation cups. Because of the reduction in water levels, we were unable to produce a treatment that was 25% of the ambient UV-B levels documented at Bubbling Ponds. Treatment levels for larval trials were limited to 100%, 50%, and 0% of the ambient intensity. The number of incubation cups was also reduced to three per exposure chamber resulting in a total of nine cups. Five larvae were housed in each of the incubation cups, necessitating 45 larvae to conduct a trial. Observations of mortality were conducted by shining a LED light with a red filter over the tops of the cups and documenting the number that were either motionless or lying on the mesh at the bottom of the cup. Mortalities were removed daily and the number of survivors was recorded. In the final two larval trials, we produced a second 0% treatment using multiple sheets of acetate to shield the larvae from UV-B emitted from the combination of UV-B-313-EL and full spectrum light bulbs. This 0% UV-B control contrasted with the other 0% UV-B irradiance produced using two full spectrum bulbs. These final two larval trials required an additional 3 incubation cups and total of 60 larvae, due to the addition of the second control chamber. These trials allowed us to compare survival among the two different 0% UV-B treatments and assess whether bulb type (full spectrum versus UV-B bulbs) contributed the results in our 0% UV-B treatments.

## 2.4 UV-B Mitigation Strategies

We examined the ability of three different UV-B mitigation strategies, 90% black knitted shade cloth, Aquashade® aquatic dye, and dissolved organic carbon (DOC; introduced as

sucrose [ $C_{12}H_{22}O_{11}$ ]) to attenuate UV-B. We briefly describe the experiments for each strategy below. All experiments were conducted outside in 3.5 m<sup>3</sup> tanks (external dimensions 1.2 m x 2.4 m x 1.2 m) that were filled to a depth of 60 cm to mimic water depths present at Bubbling Ponds rearing facility and using source waters described above.

#### 2.4.1 Aquashade®

Prior to introduction, UV-B measurements were taken in untreated source waters at the surface and at a depth of 60 cm in order to obtain a ratio between the two measurements. Aquashade was then introduced in aliquots that would increase the concentration the dye in the tank by 1 mg/L. After its introduction, Aquashade was mixed for 5 min and allowed to dilute evenly for approximately 1 hr. After 1 hr, UV-B measurements were taken at a depth of 60 cm and at the surface. The surface reading was used to document any possible changes in ambient UV-B that occurred over the course of 1 hr. By using the surface UV-B measurement and the ratio of UV-B absorbed in 60 cm of untreated water, we were able to calculate UV-B levels at a 60 cm depth in untreated water. This step was important because experiments were conducted outside and UV-B levels were subject to natural fluctuations. After readings were recorded for each Aquashade concentration, the concentration was raised 1 mg/L, and the steps were repeated until we reached a final concentration of 9 mg/L. This experiment was completed in triplicate for concentrations ranging from 1 to 4 mg/L and only once for the remaining concentrations up to 9 mg/L.

#### 2.4.2 Dissolved Organic Carbon

Natural levels of DOC in aquatic environments lead to the attenuation of UV-B in freshwater (Morris et al., 1995; Williamson et al., 1996). Sucrose ( $C_{12}H_{22}O_{11}$ ) was introduced to manipulate DOC in source waters. Although sucrose alone does not reflect the natural complexity of DOC in the environment, it provided an opportunity to examine how the intentional manipulation of DOC may influence UV-B levels in outdoor rearing facilities. Dissolved organic carbon levels were manipulated by increasing concentrations in DOC between background levels in source water to background + 6.5 mg/L DOC as  $C_{12}H_{22}O_{11}$ . The approach for measuring and estimating UV-B attenuation followed those described for treatments of Aquashade. The entire experiment was repeated twice.

#### 2.4.3 Shadecloth

We characterized the reduction in UV-B provided by 90% black knitted shade cloth (EnviroCept Greenhouse & Supply, Benton City, WA, USA). Shade cloth was floated at the water surface and held in place during measurements. As described previously, measurements were completed at a depth of 60 cm and at the surface both before and after the treatments were imposed to assist in documenting natural changes in UV-B in addition to quantifying the treatment effect.

#### 2.5 Data Analysis

We used one-way analysis of variance to detect differences in the survival of embryos or larvae among UV-B treatments for each trial and across trials to examine differences in mortality for individual exposure days. For some trials, limited sample sizes or lack of within group variation prevented us from using inferential statistics. In these instances, descriptive statistics

were used to summarize survival by treatment. All percentage data was arcsine square-root transformed prior to analysis (French and Lindley, 2000). We used correlation and regression analysis to examine the relationship between larval age and survival during each 24-hr exposure period. Finally, a non-linear exponential decay function was used to model the attenuation of UV-B with different concentrations of Aquashade and DOC.

### **3. Results**

#### **3.1 Environmental Conditions**

Water temperatures averaged 21.1 °C (range: 18.5 to 24.5 °C) across treatments, and the mean measured water temperature differed by less than 0.2°C among the four exposure chambers (21.2, 21.0, 21.1, and 21.0°C). More than 90% of the 535 total readings from each chamber were  $\leq 22^\circ\text{C}$ . Time series plots of experimental chamber temperatures showed no indication of a UV-B treatment effect; however, these plots did indicate that there was variation in source water temperature over the course of the 10 trials. While this variation was measurable, it would have been experienced by all organisms regardless of the UV-B treatment.

The 100% UV-B treatment had a mean ( $\pm 1$  SD) irradiance of  $0.0563 \pm 0.0006$  mW/cm<sup>2</sup> (range 0.0541 – 0.0583 mW/cm<sup>2</sup>) which was slightly lower than our target treatment of 0.060 mW/cm<sup>2</sup>. The 50 and 25% treatments had mean irradiance levels of  $0.0297 \pm 0.0006$  mW/cm<sup>2</sup> and  $0.0164 \pm 0.0003$  mW/cm<sup>2</sup>, respectively. Although some variation in irradiance levels were documented over the 14.5 hr dosing period, the coefficients of variation (CV) for the mean irradiance levels were low and similar across treatments (Figure 1); CV's were 1.64 (25%), 2.02 (50%), and 1.01 (100% UV-B), respectively.

### 3.2 Embryo Results

Across all trials, embryo exposure to any level of UV-B resulted in complete mortality (Table 1). Survival of control treatments (0% UV-B) varied markedly by female and ranged from 0.0% to 95.0% across all trials; mean embryo survival was 35.4% in control treatments. Across all trials and treatments, mortality was highest ( $P < 0.001$ ,  $F = 35.576$ ) on day 2 of UV-B exposure ( $76.5 \pm 6.1\%$ ; mean  $\pm$  1 SD; Figure 2). In contrast, mean mortality for day 1 and 3 of UV-B exposure was  $11.5 \pm 6.6\%$  and  $3.0 \pm 1.3\%$ , respectively. Although trials 1 and 2 were carried out for only 5 days, trials 3, 4, and 5 were run for 9, 11, and 10 days, respectively. Results from these latter trials indicate that no additional mortality occurred after 5 days in the control group.

### 3.3 Larval Results

Exposure to any level of UV-B resulted in 100% mortality of larval woundfin (Table 2). Across all trials, larval mortality differed by day ( $P = 0.021$ ,  $F = 3.691$ ), and mean mortality tended to be highest on days 2 and 3 ( $22.7 \pm 9.9\%$  and  $21.0 \pm 5.7\%$ ). Despite these patterns of mortality, larval mortality was documented on all 5 days of UV-B exposure (Figure 3). For the 50% UV-B treatments, no mortality was documented within the first 24 h of exposure and only 1.7% of total mortality was documented on day 2; peak mortality was seen on day 4. In contrast, mortality was greatest ( $P < 0.001$ ,  $F = 23.33$ ) on days 2 ( $49.3 \pm 12.0\%$ ) and 3 ( $49.3 \pm 10.9\%$ ) of 100% UV-B treatments, and by day 5, we documented complete mortality of all individuals exposed to 100% UV-B treatments.

Susceptibility of larvae to UV-B appears to be a function of age at exposure. Larvae used in these trials varied in age from 2 days in trials 1 and 2 to 41- 44 days in trial 5. For the 100%

UV-B treatments, larval age explained  $\geq 78\%$  of the variation in day 2 and day 3 mortality. For example, on day 2 of the experiment, documented mortality averaged 66.7% for 2-day old larvae to 6.7% for 43.5 day old larvae in the 100% UV-B treatments ( $R = -0.887$ ,  $P = 0.045$ ).

### 3.4 UV-B Mitigation Strategies

All three UV-B mitigation strategies reduced incoming levels of UV-B. A single treatment of 90% black knitted shade cloth reduced UV-B levels at a depth of 60 cm to  $7.1 \pm 0.4\%$  (mean  $\pm 1$  SD) of pretreatment levels. In addition, UV-B levels were inversely related with concentration of both Aquashade® aquatic dye ( $P < 0.001$ ;  $R^2 = 0.967$ ) and DOC ( $P = 0.002$ ;  $R^2 = 0.480$ ; Figure 4). At a depth of 60 cm, an Aquashade® concentration of 9.0 mg/L attenuated approximately 95% of pre-treatment UV-B. In contrast, DOC concentrations attenuated approximately 20 to 25% of UV-B at concentrations  $\geq 4$  mg/L (Figure 4).

## 4. Discussion

There is evidence that UV-B has detrimental effects on early life stages of fish (Hunter et al., 1979; Kouwenberg et al., 1999; Häkkinen et al., 2002; Weigand et al., 2004; Dong et al., 2007). Specifically, UV-B increased mortality in northern anchovy (Hunter et al., 1979; Béland et al., 1999; Kouwenberg et al., 1999), mackerel (Hunter et al., 1979), bluegill sunfish (Gutiérrez-Rodríguez and Williamson, 1999), and zebrafish (Dong et al., 2007) and elicited avoidance behavior in whitefish larvae (Ylönen et al., 2004). We show here that embryos and larvae of the endangered woundfin are sensitive to UV-B, and that all developing woundfin exposed to UV-B during our trials experienced complete mortality. In contrast, UV-B free treatments produced using full spectrum lights (embryos and larvae) or by shielding woundfin

with acetate film (larvae only) resulted in significantly higher levels of survival. Our work also demonstrates that larvae differ in their sensitivity to UV-B; susceptibility of larvae to UV-B appears to be a function of age at exposure. Here, we show that the youngest larvae (2 days post hatch) experienced an order of magnitude greater mortality than 43.5 day old larvae after just 2 days of UV-B exposure. This finding is consistent with work with other fish species (Kouwenberg et al., 1999; Wiegand et al., 2004). For example, goldfish embryo sensitivity to UV-B increased with increased cumulative exposure and embryos that were 25 h post fertilization were the most vulnerable to even a short (2 to 4 hr) exposure to elevated UV-B. For zebrafish, the hatching success, incidence of malformations, and mortality varied significantly among embryonic stage classes. Interestingly, a greater incidence of malformations and higher mortality occurred when embryos were exposed more than 3 h post fertilization.

Recent evidence shows that zebrafish embryos raised in outdoor ponds were more tolerant to UV-B than laboratory-raised embryos (Dong et al. 2007). One explanation for the documented differences in zebrafish survival is individual variability in the expression of screening pigments. Screening pigments such as melanin can reduce the effects of UV-B radiation on fish and amphibians (Häkkinen et al., 2002; Garcia et al., 2009). However, Dong et al. (2007) found no measurable differences in zebrafish embryo pigmentation that would explain differences in mortality. The woundfin UV-B experiments summarized here were conducted with both F1 and F2 embryos and larvae. Although larval woundfin have visible dorsal melanophores within a few days of hatching (Darrel Snyder, *personal communication*), both developmental stages were vulnerable to UV-B. Larval woundfin raised in outdoor ponds at the aforementioned facilities experience phytoplankton blooms (i.e. DNFHTC) and therefore may have less pigmentation than larval woundfin raised indoors, a phenomenon observed in

razorback sucker (Robert Muth, *personal communication*). Alternatively, in outdoor ponds or living streams that are clear (i.e. BPSFH) and have low DOC woundfin larvae may have more pigmentation than conspecifics raised indoors. Because pigmentation may offer photoprotection, future work should attempt to quantify the pigmentation levels for woundfin used in experiments.

Our results show that the survivable level of UV-B radiation for developing woundfin embryos is  $< 0.015 \text{ mW/cm}^2$  or  $< 25\%$  of the ambient levels present at Bubbling Ponds. Further work is needed to document the actual survivable level of UV-B in order to decrease the negative effects of UV-B radiation on production. In addition, current levels of UV-B at other conservation propagation facilities should be documented to better understand UV-B factors limiting production elsewhere. Given our results, future work should examine whether reductions in UV-B radiation lead to increases in woundfin survival and production in the captive rearing facilities described above. Reduction in UV-B radiation could be accomplished in a number of ways including the creation of UV-B free refugia (Ylönen et al., 2004) in individual rearing ponds.

We report on the efficacy of three approaches for reducing UV-B exposure. These UV-B mitigation strategies included the use of Aquashade, 90% black knitted shade cloth, and the manipulation of DOC concentrations. Both shade cloth and Aquashade® treatments were capable of producing a  $\geq 90\%$  reduction in ambient UV-B levels and may provide opportunities to create UV-B free refugia. However, it is unknown what levels of UV-B reduction are needed to increase survival of woundfin embryos and larvae at each rearing facility.

In summary, our work shows that woundfin embryos and larvae are vulnerable to UV-B levels  $> 0.015 \text{ mW/cm}^2$  at temperatures ranging from 18.5 to 24.5 °C. This work will help raise

awareness about the effects of UV-B on early developmental stages of the federally listed woundfin but also provide insight on UV-B mitigation strategies that could be deployed in outdoor rearing facilities.

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### **Literature cited**

Bancroft, B. A., Baker, N. J., Blaustein, A. R., 2007. Effects of UV-B radiation on marine and freshwater organisms: a synthesis through meta-analysis. *Ecology Letters*. 10, 332-345.

Bèland, F., Browman, H. I., Rodrigue, C. A., St.-Pierre, J. F. 1999. Effect of Solar Ultraviolet Radiation (280-400 nm) on the eggs and larvae of Atlantic cod (*Gaudus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*. 56, 1058-1067.

Bukaveckas, P.A., Robbins-Forbes, M., 2000. Role of dissolved organic carbon in the attenuation of photosynthetically active and ultraviolet radiation in Adirondack lakes. *Freshwater Biology*. 43, 3, 339-354.

Buma, A.G.J., Boelen, P., Jeffrey W.H., 2003. UVR-induced DNA damage in aquatic organisms. In: *UV Effects in Aquatic Organisms and Ecosystems* (eds Helbling, E.R. & Zagarese, H.). Royal Society of Chemistry, Cambridge, UK, pp. 291-327.

Calfee, R.D., Little, E.E., Pearl, C.A., Hoffman, R.L., 2010. Effects of simulated solar UV-B radiation on early developmental stages of the Northwestern Salamander (*ambystoma gracile*) from three lakes. *Journal of Herpetology*. 44, 572-580.

Chen Y., Childs, M.R., Keeler-Foster, C., 2011. Evaluation of Woundfin Augmentation Efforts in the Virgin River by Estimation of Admixture Proportions. *Transactions of the American Fisheries Society*. 140, 3, 598-604.

Cracknell, A.P., Varatsos, C.A., 2009. The contribution of remote sensing to the implementation of the Montreal Protocol and the monitoring of its success. *International Journal of Remote Sensing*. 30, 3853-3873.

Dong, Q., Svoboda, K., Tiersh, T.R., Monroe, W.T., 2007. Photobiological effects of UVA and UV-B light in Zebrafish embryos: Evidence for a competent photorepair system. *Journal of Photochemistry and Photobiology*. 88, 137-146.

Douki, T., 2010. "Thymine cyclobutane dimers: the most frequent and persistent DNA lesions in skin exposed to both UV-B and UVA." *Expert Review of Dermatology* 5.6 (2010): 649+. *Academic OneFile*. Web. 7 July 2011.

French, D., Lindley D., 2000. Exploring the data *in* Statistics in Ecotoxicology. (T Sparks, editor). John Wiley & Sons, Limited and critical life stages. Chichester, UK.

Garcia, T.S., Paoletti, D.J., Blaustein, A.R., 2009. Correlated trait response: comparing amphibian defense strategies across a stress gradient. *Canadian Journal of Zoology*. 87, 41-49.

Hader, D.P., Worrest, R.C., Kumar, H.D. 1995. Effects of solar ultraviolet radiation on aquatic ecosystems. *Ambio* 24:174-180.

Häkkinen, J., Vehniäinen, E., Ylönen, O., Heikkilä, J., Soimasuo, M., Kaurola, J., Oikari, A., Karjalainen, J., 2002. The effects of increasing UV-B radiation on pigmentation, growth and survival of coregonid embryos and larvae. *Environmental Biology of Fishes*. 64, 451-459.

Hunter, R.J., Taylor, J.H., Moser, H.G. 1979. Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, *Engraulis mordax*, and the Pacific mackerel, *Scomber japonicus*, during the embryonic stage. *Photochemistry and Photobiology*. 29,325–338.

Kouwenberg J.H.M., Browman, H.I., Cullen, J.J., Davis, R.F., St.-Pierre, J.F., Runge, J.A., 1999. Biological Weighting of ultraviolet (280-400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (*Gadus morhua*) eggs. *Marine Biology*. 134, 269-284.

Morris D.P., Zagarese, H., Williamson, C.E., Balseiro, E.G., Hargreaves, B.R., Modenutti, B., Moeller, R., Queimalinos, C., 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography*. 40, 1381-1391.

Palen, W.J., Schindler, D.E., Adams, M.J., Pearl, C.A., Bury, R.B., Diamond, S.A. 2002. Optical characteristics of Natural Waters protect Amphibians from UV-B in the U.S. Pacific Northwest. *Ecology* 83, 2951-2957.

Romansic, J.M., Waggener, A.A., Bancroft, B.B., Blaustein, A.R., 2009. Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (*Rana cascada*) larvae. *Hydrobiologia*. 624, 219-233.

USFWS (U.S. Fish and Wildlife Service). 2000. Endangered and threatened wildlife and plants; designation of critical habitat for the woundfin and Virgin River chub. *Federal Register* 65:17(26 January 2000):4140–4156.

Wiegand M.D., Young, D.L.W., Gajda, B.M., Thuen, D.J.M., Rittberg, D.A.H., Huebner, J.D., Loadman, N.L., 2004. Ultraviolet light-induced impairment of goldfish embryo development and evidence for photorepair mechanisms. *Journal of Fish Biology*. 64, 1242-1256.

Williamson C.E., Stemberger, R.S., Morris, D.P., Frost, T.M., Paulsen, S.G., 1996. Ultraviolet radiation in North American lakes: Attenuation estimates from DOC measurements and implications for plankton communities. *Limnology and Oceanography*. 41, 1024-1034.

Ylönen, O., Huuskonen, H., Karjalainen, J., 2004. UV avoidance of coregonid larvae. *Ann. Zool. Fennici* 41, 89-98.

Table 1. Mean percent survival of woundfin embryos following 5 days of exposure to four levels of the ambient UV-B radiation.

	Trial				
Treatment (% UV-B)	1	2	3	4	5
0%	11.3	4.8	46.5	53.3	42.9
25%	0.0	0.0	0.0	0.0	0.0
50%	0.0	0.0	0.0	0.0	0.0
100%	0.0	0.0	0.0	0.0	0.0
ANOVA results	--	--	P = 0.028, F = 5.209	P = 0.011, F = 7.255	P < 0.001, F = 168.976
-- Summarized using embryos from only two females. Because of the limited number of replicates, inferential statistics were not used.					

Table 2. Mean woundfin larval survival following exposure to two levels of UV-B and two different UV-B controls (full spectrum light or acetate blocked UV-B). All experiments were carried out over a 5-day exposure period. Larvae were 2 days old at the start of Trials 1 and 2, 5-6 days old for Trial 3, 27-30 days old for Trial 4, and 41-44 days old for Trial 5.

Treatment	Trial				
	1	2	3	4	5
0% UVB(full spectrum lights)	80.0	86.7	100.0	100.0	100.0
0% UV-B (acetate blocked UV-B)*	NA	NA	NA	100.0	100.0
50% UV-B	0.0	0.0	0.0	0.0	0.0
100% UV-B	0.0	0.0	0.0	0.0	0.0
ANOVA results	--	P < 0.001, F = 169	**	**	**

NA – not applicable

-- Summarized using larvae from a single female. Because of the limited number of replicates, inferential statistics were not used.

\* A second 0% UV B treatment was produced by using multiple sheets of acetate (9) which effectively blocked 100% of the UV-B radiation emitted from a UV-B-313-EL light bulb and full spectrum bulb pairing used in all > 0% UV-B treatments.

\*\* F statistics were not generated because there was no within group variation (i.e. there was no variation in survival among experimental units within a given UV-B treatment)

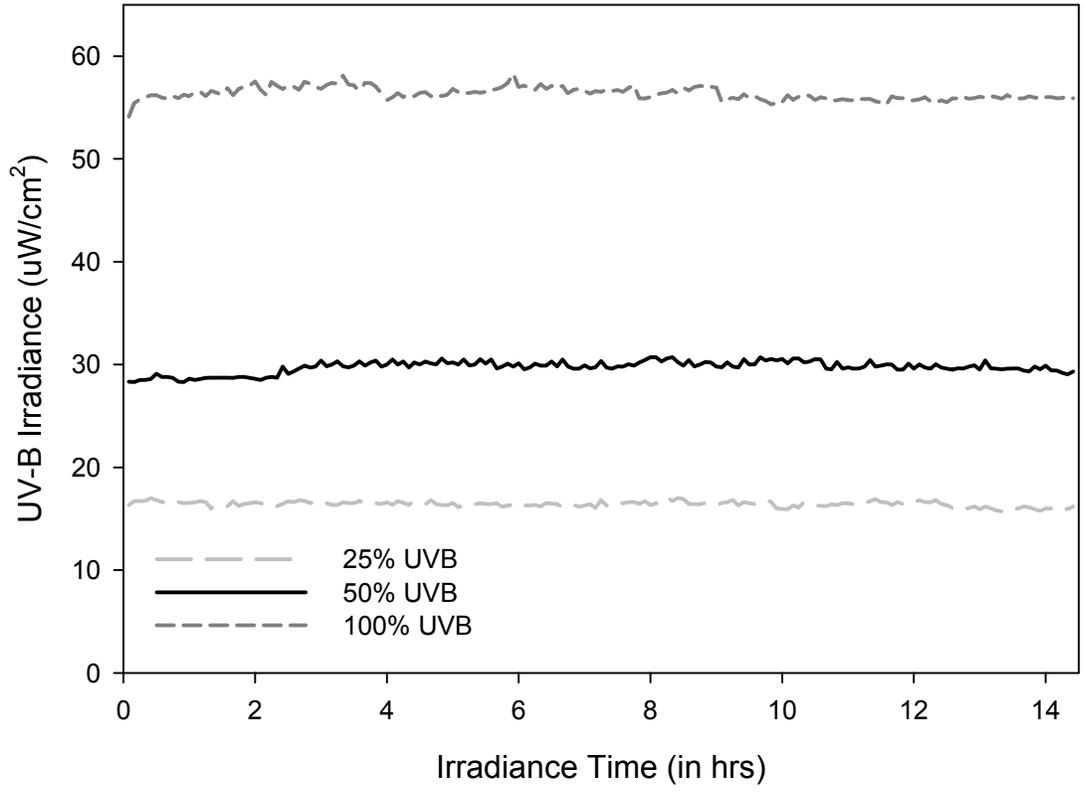


Figure 1. Ultraviolet-B irradiance levels summarized over 14.5 hr irradiance period for each treatment level.

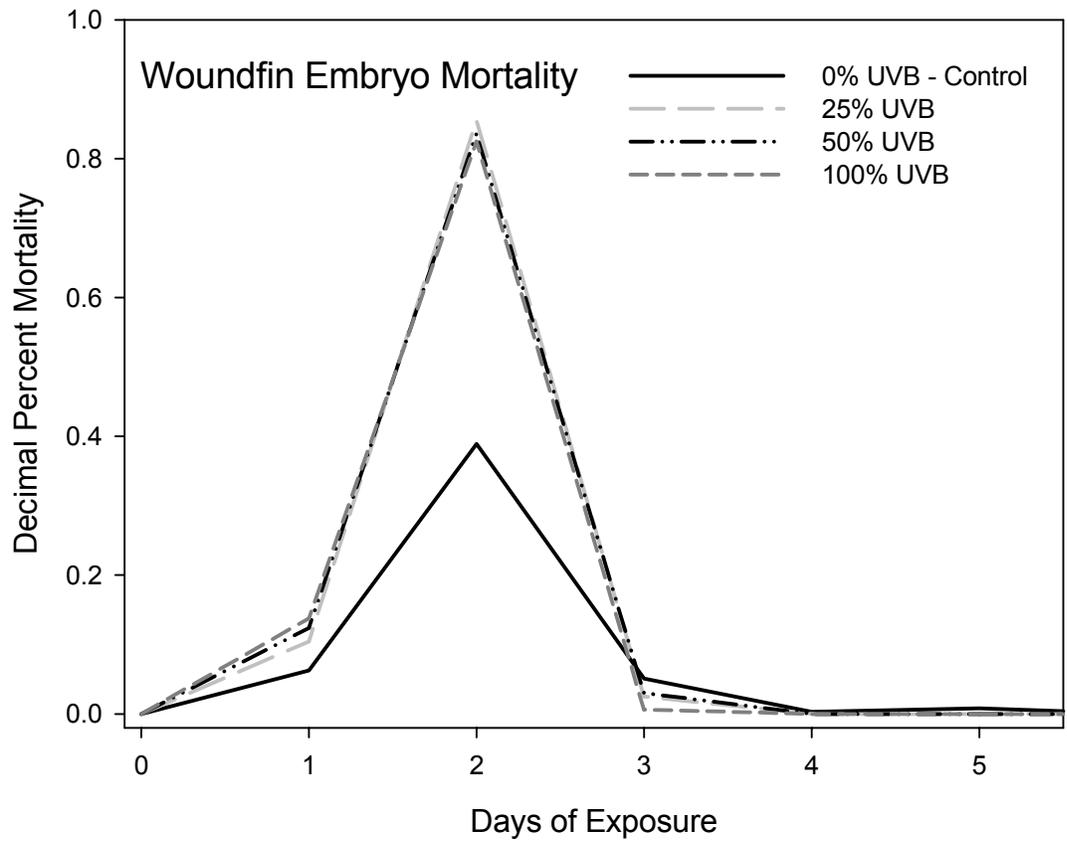


Figure 2. Woundfin embryo mortality summarized by UV-B treatment over a 5-day exposure period. Embryo mortality is expressed as decimal percentage.

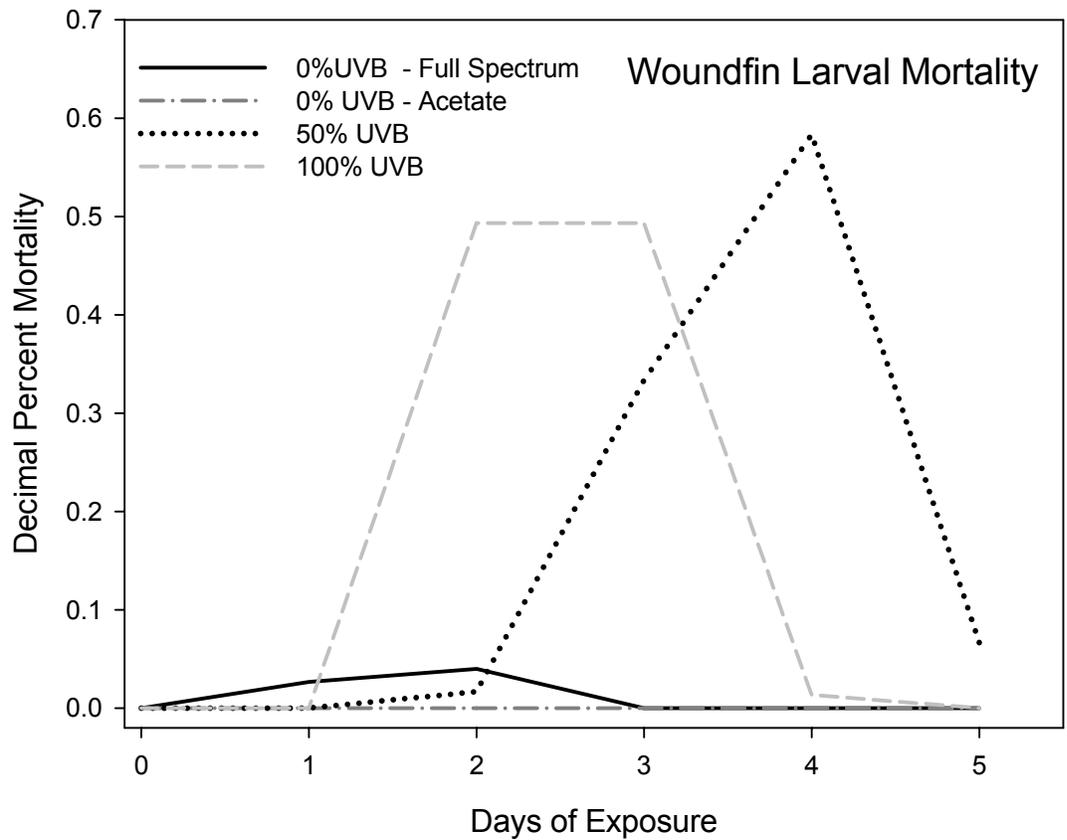


Figure 3. Woundfin larval mortality summarized by UV-B treatment over a 5-day exposure period. Larval mortality is expressed as decimal percentage.

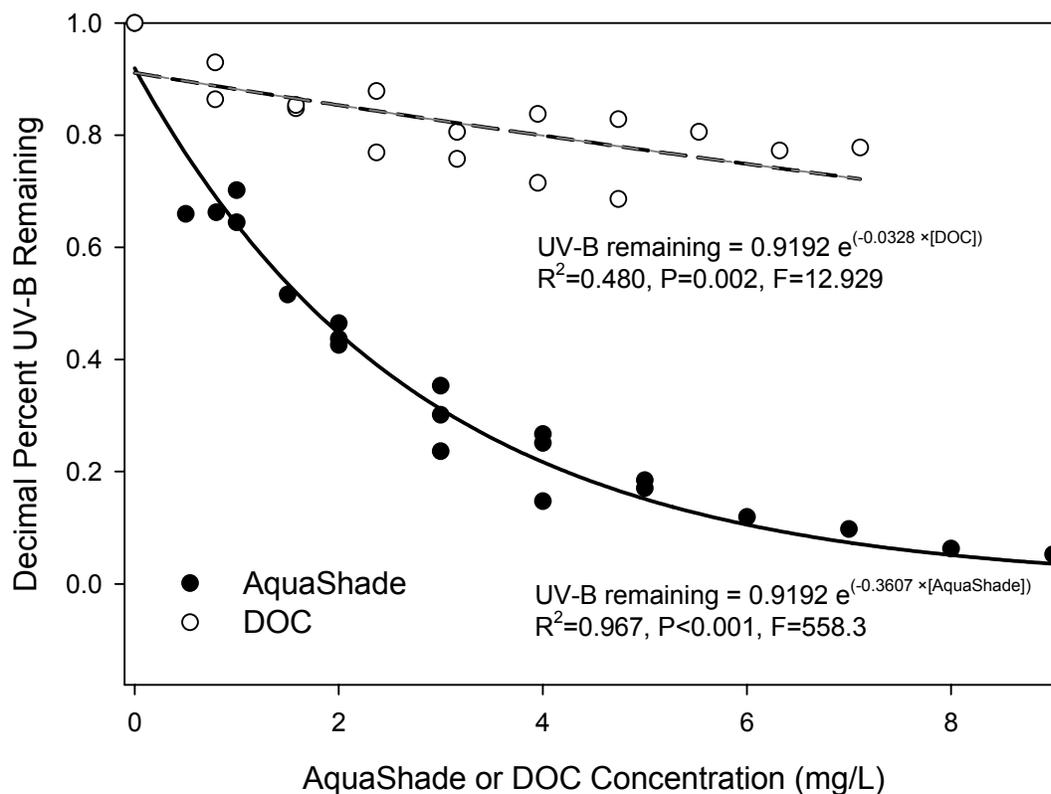


Figure 4. Ultraviolet-B irradiance expressed as a function of AquaShade® and dissolved organic carbon concentrations. Dissolved organic carbon concentrations were manipulated using sucrose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>).