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10   **Title:** Disinfection of almaco jack (*Seriola rivoliana* Valenciennes) eggs: evaluation of three

11   chemicals

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13   **Running Title:** Egg disinfection of *Seriola rivoliana*

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33 **Abstract**

34   Almaco jack (*Seriola rivoliana* Valenciennes) is an excellent candidate for aquaculture

35 due to its fast growth rate and high market value. While *S. rivoliana* have adapted well to

36 captivity, survival at early life stages can be improved to increase profitability during production.

37 A wide range of variables cause larval mortalities but high bacterial loads in rearing tanks are

38 often correlated with these losses. The aim of this study was to investigate the effect of egg

39 disinfection on bacterial load and hatch rate of *S. rivoliana*.

40   Disinfectants tested included: formalin (F100 and F200; 100 and 200 mg/L, respectively,

41 for 60 minutes), hydrogen peroxide (HPO; 300 mg/L for 10 minutes), and peracetic

42 acid/hydrogen peroxide (PAA/HPO; 15.7 mg/L/39.6 mg/L for 1 minute). Concentrations and

43 contact times were determined based on current use in marine aquaculture and preliminary trials.

44   Eggs treated with HPO and F100 had significantly higher hatch rates than the untreated

45 control group. All treatments significantly decreased total *Vibrio* counts compared to untreated

46 eggs, however total bacterial counts were only decreased following treatments with PAA/HPO

47 and F200. To prevent egg mortality due to bacterial overgrowth, consideration should be given to

48 the use of surface disinfection using HPO or F100. Future studies should investigate the use of

49 peracetic-based products at lower doses.

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1 INTRODUCTION

Aquaculture contributes greatly to global food security and nutrition (FAO, 2016). The United States is the second largest consumer of seafood, but ranks 16th in aquaculture production, resulting in a seafood trade deficit of over $15 billion USD a year (National Marine Fisheries Service, 2018). Approximately half of the seafood imported by the United States is raised in aquaculture (National Marine Fisheries Service, 2018); thus, future expansion of the US aquaculture industry will decrease dependence on foreign seafood and improve the national seafood trade balance.

Seriola spp. are widely recognized as commercial food fish species with large potential for aquaculture production due to their fast growth, high commercial demand, filet quality, high market value, and adaptability to intensive culture conditions (Fernández-Palacios, Schuchardt, Roo, Hernández-Cruz, & Izquierdo, 2015; Roo, Fernández-Palacios, Schuchardt, Hernández-Cruz, & Izquierdo, 2015; Sicuro & Luzzana, 2016); these factors have stimulated global interest in *Seriola* spp. culture in recent years. According to FAO, global production of farmed *Seriola* spp. totaled over 165,000 tons and was valued at $1.13 billion USD in 2017 (FAO, 2019). The Gulf of Mexico Fishery Management Plan recognized *Seriola* as an important marine finfish for aquaculture development and listed almaco jack (*Seriola rivoliana* Valenciennes; also referred to as longfin yellowtail, highfin amberjack, and yellow kingfish) as one of the seven fish species most likely to be cultured offshore in the Gulf of Mexico (Gulf of Mexico Fishery Management Council & NOAA, 2009).

Seriola rivoliana are currently being produced commercially; however, improvements in egg hatching protocols would increase commercial production and the economic viability of this species. A common constraint impacting marine finfish culture is high mortality during early development. External pathogens can be transferred from broodstock to eggs, and egg surfaces are easily colonized by environmental bacteria (Stuart, Keller, & Drawbridge, 2010). Commercial aquaculture hatcheries use intensive egg incubation techniques that can promote bacterial overgrowth, resulting in mortalities arising from hypoxia, developmental deficiencies, infectious disease, and/or egg lysis (Hansen & Olafsen, 1999). Chemical surface disinfection of fish eggs is a common biosecurity practice to reduce egg mortality and improve rearing success (El-Dakour, Saheb, & Al-Abdul-Elah, 2013). Chemical therapeutants are frequently used in freshwater aquaculture to treat disease but less so in tropical marine hatcheries (De Swaef, Van

87   den Broeck, Dierckens, & Decostere, 2016; Verner-Jeffreys, Nakamura, & Shields, 2007),

88   despite similar microbial management concerns. Therefore, investigations into the effectiveness

89   of egg disinfection protocols for use in saltwater environments are needed.

90   Therapeutic products used in food fish aquaculture must be approved by the Food and

91   Drug Administration (FDA). Povidone iodine is a compound on the FDA Low Regulatory

92   Priority list for the disinfection of fish eggs (Bowker & Trushenski, 2019) and is generally used

93   in salmon and trout hatcheries, but has been demonstrated to lower hatch rates in *S. rivoliana*

94   (Chalupnicki, Ketola, Starliper, & Gallagher, 2011; Stuart et al., 2010; Wagner, Oplinger, Arndt,

95   Forest, & Bartley, 2010). Currently in the United States, formalin (37% formaldehyde) products

96   and a 35% hydrogen peroxide (HPO) product are approved for treating fish eggs against the

97   fungal disease saprolegniasis in aquaculture. The FDA has approved formalin products for all

98   finfish eggs, while the HPO product is only approved for freshwater-reared finfish eggs.

99   Formalin and HPO products are used at varying concentrations and times in both freshwater and

100   marine aquaculture (De Swaef et al., 2016).

101   *Fish egg disinfection is a common practice in freshwater fish hatcheries, while it is still

102   developing as a practice in marine hatcheries. Freshwater fish produce demersal eggs that are

103   susceptible to fungal growth and are also relatively large, ranging up to 7-8 mm in salmonid

104   species and often have thick chorions and the zona radiata that can provide mechanical

105   protection from the outside environment (Helfman, Collette, Facey, & Bowen, 2009). This added

106   protection in freshwater species allows for higher chemical concentrations to be used for

107   disinfection and even have them applied daily to combat fungal growth (DeSwaef et al., 2016).

108   Comparatively, marine species generally produce pelagic eggs ranging from 0.5 to 5.5 mm in

109   diameter (Helfman et al., 2009) and may require lower therapeutant concentrations due to higher

110   sensitivity to their environment. Formalin has been used successfully to disinfect rainbow trout

111   (*Oncorhynchus mykiss*) eggs (Bailey & Jeffrey, 1989; Cline & Post, 1972), white seabass

112   (*Atractoscion nobilis*), California halibut (*Paralichthys californicus*), and California yellowtail

113   (*Seriola lalandi*) (Stuart et al., 2010). HPO has also been used successfully in marine

114   aquaculture, including on red drum (*Sciaenops ocellatus*) eggs (Douillet & Holt, 1994) and

115   almaco jack eggs and juveniles (Mansell, Powell, Ernst, & Nowak, 2005; Verner-Jeffreys,

116   Nakamura, & Shields, 2007).

117   *Peracetic acid (PAA) products are registered by the Environmental Protection Agency

118   (EPA) for use in agriculture, food processing, and medical facilities as disinfectants but use in

119   aquaculture industries is new. Peracetic acid-based products contain peracetic acid along with

120   hydrogen peroxide to maintain the chemical stability; however, PAA is considered the active

121   component (U.S. EPA Office of Wastewater Management, 2012). The European Chemical

122   Agency (ECHA) has approved PAA as a biocide to be used as a disinfectant in veterinary

123   hygiene and medicine; it has been adopted by halibut hatcheries to disinfect eggs (Brown, 2010).

124   Recent research in the United States with PAA focuses on fungus control in channel catfish

125   (*Ictalurus punctatus*) hatcheries (Straus, Meinelt, Farmer, & Mitchell, 2012) as well as with

126   Atlantic cod (*Gadus morhua*) (Brown, King, & Skonberg, 2004) as an alternative disinfectant as

127   it is less environmentally persistent compared to some other approved chemicals used in the

128   industry.

129   Increased bacterial colonization often leads to egg mortality. There are approved

130   treatments available to minimize this problem. Consequently, the purpose of this study was to

131   determine optimal disinfection protocols for *S. rivoliana* eggs, and ultimately to reduce bacterial

132   loads and increase hatchability.

133

134   **2 MATERIALS AND METHODS**

135   **2.1 Broodstock collection and maintenance**

136   All procedures were performed in accordance with the ethical standards of the institution

137   as outlined in Mote Marine Laboratory's Animal Welfare Assurance (A4219-01). All

138   experimental protocols were approved by Mote Marine Laboratory's Animal Care and Use

139   Committee (IACUC Approval No. 17-10-KM1). Broodstock *S. rivoliana* were collected using

140   hook and line in the Gulf of Mexico about 120 miles offshore from Madeira Beach, Florida, in

141   the Spring of 2017 (License # SAL-16-010-SCR). Twenty-three *S. rivoliana* were used to

142   establish broodstock populations at Mote Aquaculture Research Park in Sarasota, Florida and

143   were divided into two separate, indoor, photoperiod (12H light) and temperature (26°C)

144   controlled recirculating tank systems (A, B). Tank A housed 7 females and 5 males and Tank B

145   housed 7 females and 4 males. Each tank system consisted of a green, fiberglass tank with a

146   diameter of 4.6 m and a depth of 1.5 m, an egg collector tank and filtration equipment for solids

147   removal and biofiltration, a protein skimmer, and two UV sterilizers. The total tank system

148   volume was 28 m^3. Salinity was maintained at 35 ± 0.920 g/L. Fish were fed a daily diet of squid

149   (50%) and herring (50%) at 3% of the total tank biomass. Broodstock in both tanks were photo-

150   thermally conditioned to induce daily spawning using the environmental parameters stated

151   above. Eggs were skimmed from the surface into a 300 μm mesh bag, harvested and then

152   volumetrically counted.

153

154   **2.2 Egg collection and disinfection**

155   Eggs were collected the morning following a spawning event (blastula stage) and

156   estimates of total fecundity and total fertilization rate were assessed volumetrically using Class A

157   glassware and standard methods described previously (Hauville, Zambonino-Infante, Gordon

158   Bell, Migaud, & Main, 2016). Eggs collected into 10 mL aliquots (n = 3) were examined

159   microscopically (4x objective; BX53 Upright Microscope, Olympus Corporation, Tokyo, Japan)

160   to determine fertilization rate. Unfertilized and dead eggs were removed, and a final count of

161   fertilized eggs was obtained by counting the total number of eggs present in 10 mL aliquots (n =

162   3). Viable eggs were volumetrically distributed at 1,000 eggs/L in each of five 10 L treatment

163   cones (Artemia International LLC, Fairview, TX) containing source water (ozonated 35 g/L

164   saltwater).

165   Treatment concentrations and exposure times (Table 1) were based on previous uses in

166   experimental and commercial finfish aquaculture. Treatment order varied for each spawn to limit

167   the effect of time to exposure. Formalin (Parasite-S, Syndel USA, Ferndale, WA) concentrations

168   (F100 and F200, for 100 and 200 mg/L, respectively) and contact time (60 min) were based on

169   recommendations from Stuart et al. (2010) and commercial producers working with *Seriola* spp.

170   (Neil A. Sims, CEO of Kampachi Farms, Inc, personal communication). The HPO concentration

171   (35% Perox-aid at 300 mg/L, Syndel USA, Ferndale, WA) and contact time (10 min) was taken

172   from previous studies on treating gill flukes in *Seriola lalandi* juveniles (Mansell et al., 2005)

173   and recommendation by Roy Yanong, DVM (personal communication). A peracetic acid-based

174   product (5.6% PAA, 26.5% HPO) has been used commercially for Atlantic cod at concentrations

175   of 180 mg/L for one minute (Brown et al., 2004), but preliminary trials with the peracetic-acid

176   based product used in the present study (Peroxy-Serve MPS, Zep Inc., Atlanta, GA; 15.5% PAA,

177   5.5% HPO) indicated that these concentrations were lethal for *S. rivoliana* eggs. The

178   concentration was decreased until signs of mortality were no longer observed, resulting in a

179 treatment of 1 ml disinfectant in 10 L seawater. Thus, the administered concentration was

180 calculated at 15.7 mg/L for one minute in preliminary trials for this species. Sodium bicarbonate

181 buffer (20g) was added to the PAA treatment to neutralize the acidity giving an average pH of

182 8.01. Control eggs received no disinfectant treatment but were stocked into cones to minimize

183 effects from differential handling. Three spawns were collected from one set of broodstock and

184 two were collected from the other, totaling five spawns collected on five separate spawning

185 dates. Egg disinfections were carried out on each spawn in which all treatments were tested

186 through temporal replicates (n = 5).

187

188 **2.3 Hatch rates and malformations**

189 Following disinfection treatment, eggs were collected and immediately rinsed in source

190 water to remove remaining disinfectant. Control eggs were also rinsed. Eggs from each treatment

191 were stocked at 100 eggs/L into replicate 1 L beakers (n = 4) of ozonated and UV sterilized

192 saltwater. The beakers were set in a randomized pattern in 3 water-bath tables and kept at a

193 temperature of 26°C. Hatch rate and detectable developmental malformations in post-hatch

194 larvae were assessed by microscopic examination 36 hours post-fertilization.

195

196 **2.4 Bacterial counts**

197 Following each trial, eggs from each treatment were collected with a sieve and gently

198 rinsed with sterile seawater. Four replicates of ten eggs each were collected aseptically from each

199 treatment with a 1 mL pipet and placed into sterile microcentrifuge tubes. Excess water was

200 removed and 250 µL sterile saltwater was added. Eggs were homogenized using 1.5 mL pellet

201 mixer pestles (VWR 47747-358, VWR International, Atlanta, GA) and vortexed for 30 s. Initial

202 homogenate and a 1:10 dilution were plated in duplicate on two different culture media. Tryptic

203 soy agar (TSA) was used to obtain total bacterial counts and thiosulfate-citrate-bile salts-sucrose

204 agar (TCBS) was used to obtain total *Vibrio* counts. Colony counts were determined visually

205 following incubation at 26°C for 7 days. Bacterial counts were expressed as colony forming units

206 (CFU)/egg.

207

208 **2.5 Statistical analysis**

209   The hatch and bacterial counts did not significantly differ among the tank sources, so the

210   data were pooled to examine the treatment effects. Percent hatch, percent malformation of

211   hatched larvae, and CFU/egg were examined using a General Linear Model to test *a priori*

212   hypotheses regarding the effect of treatment compared to the control. This was based on the null

213   hypotheses that each of the four different treatments had no effect on the hatch rate,

214   malformations or bacterial load of the *S. rivoliana* eggs. Because quality can be variable among

215   spawning events, spawn date was included as a fixed factor. The results of treatment are

216   presented relative to the control and the results for spawn are presented relative to Spawn 5, such

217   that the effects of those factors are set to zero by the model. Percent hatch exhibited a non-

218   normal distribution with a slight negative skew, and although a square transformation did slightly

219   improve normality, this transformation did not influence the equality of variance or the

220   conclusions of the analysis compared to the non-transformed data. Therefore, to maintain the

221   interpretability of the model parameter estimates, no transformation was applied. The CFU/egg

222   of both the total bacterial counts and total *Vibrio* counts had a non-normal distribution with a

223   strong positive skew and was log-transformed using the natural log to improve the homogeneity

224   of variance. Because data included zero counts, a value of 0.001 CFU/egg was added to all

225   values so that the natural log could be taken. Significance was assessed at $\alpha = 0.05$. The

226   statistical package used was XLStat (version 2018.5; Addinsoft, Long Island City, NY).

227

228   **3 RESULTS**

229   **3.1 Hatch rates and deformities**

230   The average spawn size was 322,133 eggs with an average fertilization rate of 58.6%

231   (Table 2). Throughout the trial period, hatch rates ranged from 28 to 98%. After adjusting for the

232   influence of spawn date, the HPO and F100 treatments resulted in significantly higher hatch rates

233   ($\alpha = 0.002$, $P < 0.001$, respectively) than the control (66 ± 3.48% hatched) at 76 ± 2.83% and 78

234   ± 2.62%, respectively (Figure 1, Table 3). There were no significant differences between the

235   treatments and the control in malformations of hatched larvae (Figure 2, Table 4), after adjusting

236   for the influence of spawn date, with all treatments having less than 10% detected

237   malformations.

238

239   **3.2 Bacterial counts**

240   Total bacterial counts were highly variable and ranged from 1 to 460 CFU/egg in the

241   control. After controlling for the influence of spawn date, the PAA and F200 treatments

242   significantly reduced total bacterial counts ($\alpha = 0.001$, $\alpha = 0.003$, respectively) compared to the

243   control (Figure 3, Table 5). Total *Vibrio* counts in control eggs ranged from 0 to 80 CFU/egg. All

244   treatments significantly decreased the total *Vibrio* counts ($P < 0.002$) as compared to the control

245   (Figure 4, Table 6) after adjusting for differences among spawns. On average, disinfection

246   reduced *Vibrio* concentrations by 12.9%.

247

248   **4 DISCUSSION**

249   This study demonstrated the effectiveness of four disinfection protocols on bacterial load

250   and the hatch rate of *S. rivoliana* eggs. There was a significant effect of treatment on hatchability

251   of the eggs. Treatment with F100 for 60 minutes increased the hatch rate by 12% as compared to

252   controls. These results are nearly identical to those of Stuart et al. (2010), who demonstrated that

253   formalin at the same concentration and exposure time increased hatch rates in *Seriola lalandi* by

254   9%, without negatively impacting survival or larval size at first feed. El-Dakour et al. (2013)

255   showed that 250 mg/L formalin treatment for 20 minutes on sobaity seabream eggs was an

256   effective method to improve hatchability and larval survival; however, concentrations exceeding

257   200 mg/L reduced the hatchability and survival of the larvae. The parent compound

258   formaldehyde is an extremely reactive chemical that interacts with proteins, DNA, and RNA, and

259   has been reported to be effective against bacterial, fungal, parasitical, and viral pathogens (De

260   Swaef et al., 2016), but is a known carcinogen (United States Department of Health and Human

261   Services, 2010).

262   In this study, HPO treatment also significantly improved the hatch rates of *S. rivoliana*.

263   HPO at a concentration of 300 mg/L for 10 minutes increased hatch rates by 10% as compared to

264   controls. Verner-Jeffreys et al. (2007) reported that HPO at a stronger dose (500 mg/L or greater)

265   for 5 minutes should be employed in surface disinfection of *S. rivoliana* eggs and caused no

266   significant differences in survivability of the larvae in a 3-day survival study compared to

267   untreated larvae illustrating that HPO treatments have not shown negative effects during early

268   stages of development as they used a higher concentration for a shorter time compared to the

269   current study. HPO at concentrations ranging from 50 to 250 mg/L did not improve the

270   hatchability of sobaity seabream eggs, although a higher concentration of HPO may in fact

271 improve hatchability (El-Dakour et al., 2013). HPO is a strong oxidizing agent that does not

272 leave a residue (De Swaef et al., 2016). It is considered environmentally friendly as it easily

273 decomposes to harmless compounds of oxygen gas and water making it safer for personnel to

274 use in hatcheries compared to formalin products.

275   PAA and F200 reduced total bacterial load as measured on TSA. Despite this

276 observation, hatch rates were not increased in the PAA and F200 treatment groups. PAA works

277 synergistically with HPO and is used as a bactericide, virucide, and fungicide, and remains

278 effective in the presence of organic material (De Swaef et al., 2016). Similar to HPO, PAA will

279 decompose into harmless, non-toxic compounds including water, oxygen, and carbon dioxide

280 (Kitis, 2004). However, PAA has better membrane penetrating characteristics as it is not broken

281 down by catalase enzymes produced by microorganisms (Liu, Straus, Pedersen, & Meinelt,

282 2015). Marchand et al. (2012) found that PAA-based products reduced the growth of both

283 bacterial (*Flavobacterium columnare*) and fungal (*Saprolegnia parasitica*) fish pathogens in

284 their *in vitro* study. Brown et al. (2004) saw no bacterial growth on Atlantic cod eggs using a

285 much higher concentration of PAA (180 mg/L PAA/ 780 mg/L HPO) than the present study.

286 Additionally, the PAA disinfectant used by Brown et al. (2004) has a much higher ratio of

287 HPO:PAA than the disinfectant used in this study and it should be noted that HPO enhances the

288 toxicity of PAA-based products (Liu et al., 2015). Despite the lower HPO:PAA ratio in Peroxy-

289 Serve MPS, the concentrations used in Brown et al. (2004) were lethal to *S. rivoliana*.

290 Additionally, sodium bicarbonate was added to buffer the low pH caused by PAA; without this

291 addition, the pH of the treatment cone containing PAA decreased by about 2 pH units.

292 Sensitivity of embryos to different disinfectants can vary greatly between species (Brown et al.,

293 2004) and while this study did not assess survival and saw no significant malformations it is

294 possible these disinfectants at the tested concentrations caused unseen damage.

295   No link was found between reduced total bacterial counts and increased hatch rates in the

296 present study. Eggs in high density can be heavily overgrown with bacteria within hours of

297 fertilization (El-Dakour et al., 2013), but the bacterial load can vary significantly between

298 cohorts of eggs (Bergh, 1999), as well as between eggs within the same cohort (Verner-Jeffreys,

299 Nakamura, & Shields, 2006). The high variation in total bacteria loads of eggs can be due to

300 occasional eggs having higher numbers of associated bacteria compared to others in the same

301 cohort (Verner-Jeffreys et al., 2006). Another explanation for this high variation is that there are

302 different bacterial communities at the time of egg collection. Holmefjord and Lein (1990) found
303 that naturally spawned Atlantic halibut eggs had an increased bacterial growth on the egg surface
304 compared to strip spawned eggs. The naturally spawned eggs come in direct contact with the
305 broodstock tank water which can harbor large amounts of microflora. These variations were
306 demonstrated in the present study as well, since eggs within the control harbored anywhere from
307 1 to 460 CFU/egg. A trend was evident towards reduced total bacteria counts in HPO and F100
308 treatments which also had higher hatch rates, and these trends warrant further investigation.
309 Although PAA and F200 significantly reduced total bacteria counts, they did not demonstrate
310 improved hatch rates.

311   It may not be desirable to remove too many bacteria, as some bacteria present in these
312 systems are likely commensal or even mutualistic, playing a role in competitive exclusion of
313 potential pathogens. A disruption in the balance of the microbial community can select for
314 opportunistic pathogenic bacteria (De Swaef et al., 2016) and increase the egg's susceptibility to
315 infection and disease. Thus, in addition to total bacterial counts, it may be informative to identify
316 the specific bacterial groups that are being removed by the disinfectants in order to better
317 decipher the impacts of disinfection on hatchability.

318   As egg surfaces are ideal substrates for bacterial colonization, hatched larvae are exposed
319 to potentially high abundances of opportunistic pathogens, including those of the genus *Vibrio*.
320 This genus of Gram-negative motile rods contains numerous species that are naturally found in
321 marine water and occur in association with animal surfaces and internal organs. *Vibrio* can cause
322 skin lesions (i.e., ulcers) and systemic disease (Noga, 2010). Bergh (1999) postulates that
323 infection from *Vibrio anguillarum* causes significant mortalities in Atlantic halibut larvae post-
324 hatch, but this bacterium may be successfully removed through application of an egg surface
325 disinfectant.

326   Although all disinfectants in this study significantly reduced *Vibrio* on the eggs, only
327 F100 and HPO significantly increased hatch rates, suggesting as previously stated for the total
328 bacterial load that the bacterial species being removed may be more important to larval survival
329 than the reduction of all *Vibrio*. Future studies should identify pathogens to *S. rivoliana* and
330 determine the most effective disinfectant to limit infection.

331   Batch immersion of eggs following collection from the broodstock tank and before
332 transfer to the larval rearing tanks is a practical, straightforward method of providing biosecurity

333   in the hatchery. This study did not observe increased malformations of newly hatched larvae

334   compared to untreated larvae, but further work should be conducted to ensure there are no long-

335   term toxic effects associated with the treatments on hatched larvae. This study only evaluated a

336   single exposure time per treatment and future studies should be conducted to find the optimum

337   exposure time as the timing of egg disinfection can affect the toxicity of the chemical treatments

338   (Verner-Jeffreys et al., 2007).

339   In summary, to prevent egg mortality of *S. rivoliana* associated with bacterial

340   overgrowth, consideration should be given to the use of surface disinfection with HPO or

341   formalin. This data demonstrated that the use of HPO at 300 mg/L or formalin at 100 mg/L is

342   effective at reducing *Vibrio* and increasing hatch rates of *S. rivoliana*. Further work should be

343   carried out to refine egg disinfection protocols, especially with PAA as it is still a relatively new

344   chemical used in aquaculture, for *S. rivoliana* aquaculture.

345

350

351   **DATA AVAILABILITY STATEMENT**

352   The data that support the findings of this study are available from the corresponding author upon

353   reasonable request.

354

355   **CONFLICT OF INTEREST STATEMENT**

356   The authors declare they have no conflict of interest.

357

358   **ETHICS STATEMENT**

359   The authors confirm that the ethical policies of the journal, as noted on the journal's author

360   guidelines page, have been adhered to and the appropriate ethical review committee approval has

361   been received. The US National Research Council's guidelines for the Care and Use of

362   Laboratory Animals were followed.

363

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460

Table 1. Treatment concentrations and exposure times (n = 5).

Abbreviation	Chemical	Dose (mg/L)	Duration (min)
Control	None	NA	NA
F100	Formalin	100	60
F200	Formalin	200	60
HPO	Hydrogen Peroxide	300	10
PAA	Peracetic Acid/Hydrogen Peroxide	15.7/39.6 (20g NaHCO$_3$ buffer)	1

Table 2. Spawn and fertilization rates of individual trials. All treatments were tested during each trial.

Trial	Total Eggs	Viable Eggs	Fertilization Rate
1	445,667 ± 33,160	227,667 ± 17,250	51.7 ± 8.1%
2	196,667 ± 17,308	109,333 ± 17,308	56.8 ± 14.2%
3	71,667 ± 5,312	41,566 ± 6,600	58.0 ± 1.5%
4	400,667 ± 15,965	221,667 ± 4,111	55.4 ± 2.9%
5	496,000 ± 21,119	352,333 ± 35,975	71.1 ± 6.9%
Average	322,133	188,467	58.6%

Table 3. Hatch rate results from a general linear model examining the influence of treatment and spawn date. The treatment effects are presented relative to the control, while the spawn date effects are presented relative to the last spawn such that the effect of those factors are set to zero by the model.

Source	Effect on Average CFU/egg	Standard Error	t	P
Control	0	0	-	-
HPO	0.108	0.034	3.199	0.002
PAA	0.003	0.034	0.081	0.936
F100	0.126	0.034	3.731	0.000
F200	0.020	0.034	0.599	0.551
Spawn 1	-0.101	0.034	-2.989	0.004
Spawn 2	0.160	0.034	4.750	<0.001
Spawn 3	0.027	0.034	0.790	0.431
Spawn 4	-0.162	0.034	-4.813	0.001
Spawn 5	0	0	-	-

Table 4. Deformed hatch rate results from a general linear model examining the influence of treatment and spawn date. The treatment effects are presented relative to the control, while the spawn date effects are presented relative to the last spawn such that the effect of those factors are set to zero by the model.

Source	Effect on Average CFU/egg	Standard Error	t	P
Control	0	0	-	-
HPO	-0.016	0.023	-0.687	0.494
PAA	0.017	0.023	0.734	0.465
F100	0.008	0.023	0.351	0.727
F200	0.014	0.023	0.611	0.543
Spawn 1	0.012	0.023	0.506	0.614
Spawn 2	0.014	0.023	0.591	0.556
Spawn 3	0.052	0.023	2.233	0.028
Spawn 4	0.035	0.023	1.496	0.138
Spawn 5	0	0	-	-

Table 5. Results of the natural log transformed data of the total bacteria loads from a general linear model examining the influence of treatment and spawn date. The treatment effects are presented relative to the control, while the spawn date effects are presented relative to the last spawn such that the effect of those factors are set to zero by the model.

Source	Effect on Average CFU/egg	Standard Error	t	P
Control	0	0	-	-
HPO	-0.761	0.537	-1.416	0.160
PAA	-1.825	0.530	-3.446	0.001
F100	-0.930	0.523	-1.780	0.079
F200	-1.645	0.529	-3.111	0.003
Spawn 1	-3.572	0.530	-6.743	<0.001
Spawn 2	1.347	0.537	2.507	0.014
Spawn 3	-1.371	0.529	-2.592	0.011
Spawn 4	0.173	0.523	0.331	0.741
Spawn 5	0	0	-	-

Table 6. Results of the natural log transformed data of the total Vibrio loads from a general linear model examining the influence of treatment and spawn date. The treatment effects are presented relative to the control, while the spawn date effects are presented relative to the last spawn such that the effect of those factors are set to zero by the model.

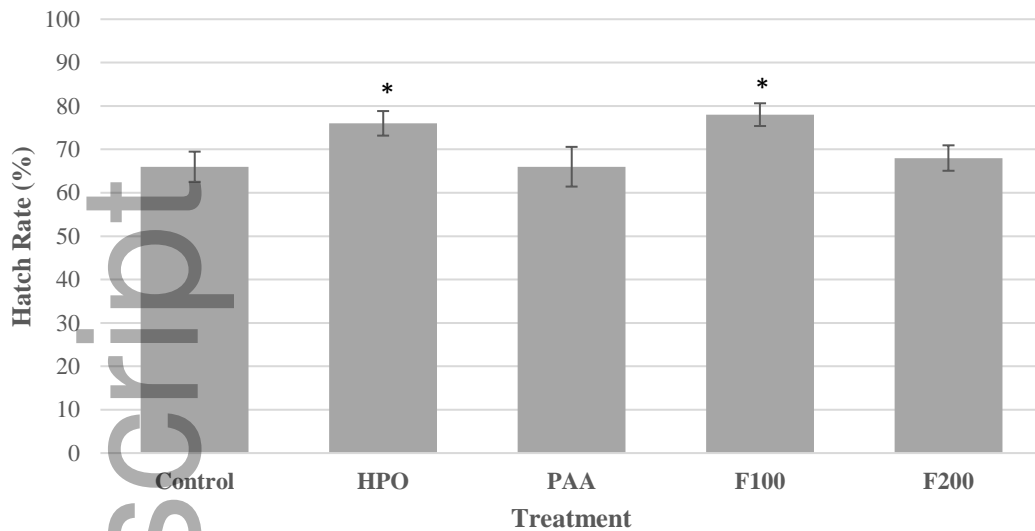| Source	Effect on Average CFU/egg	Standard Error	t	P
Control	0	0	-	-
HPO	-2.851	0.915	-3.116	0.002
PAA	-5.548	0.915	-6.064	<0.001
F100	-4.279	0.915	-4.676	<0.001
F200	-3.705	0.915	-4.050	0.000
Spawn 1	-1.449	0.915	-1.584	0.117
Spawn 2	3.179	0.915	3.475	0.001
Spawn 3	-0.552	0.915	-0.604	0.548
Spawn 4	1.264	0.915	1.381	0.171
Spawn 5	0	0	-	-

Figure 1. Mean (+/- SE) hatch rates for S. rivoliana eggs treated with disinfectants (n = 5). Asterisks denote significant differences from the control determined by general linear models (α = 0.05).
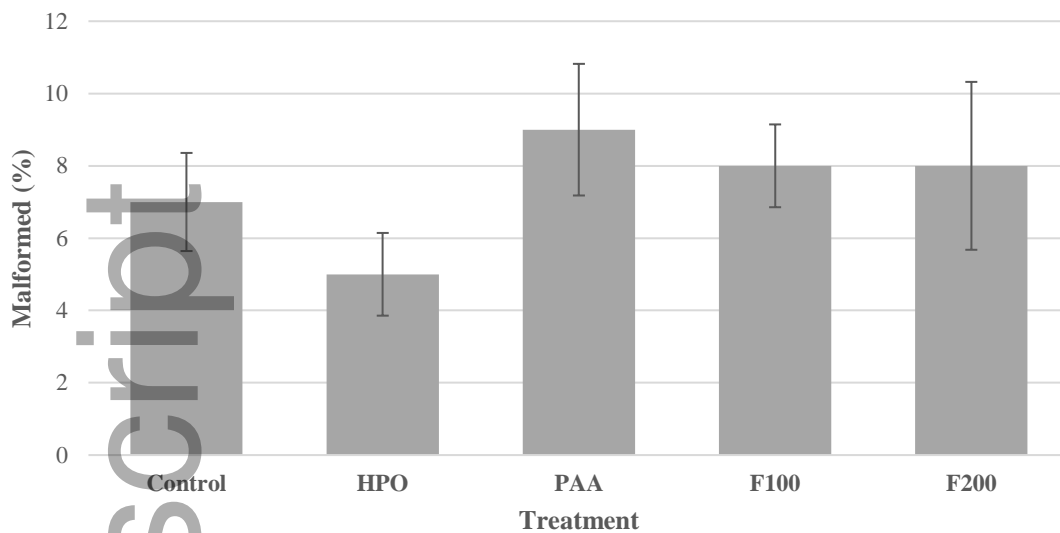
Figure 2. Mean (+/- SE) percent of malformation in hatched S. rivoliana larvae (n = 5). There were no significant differences from the control determined by general linear models.
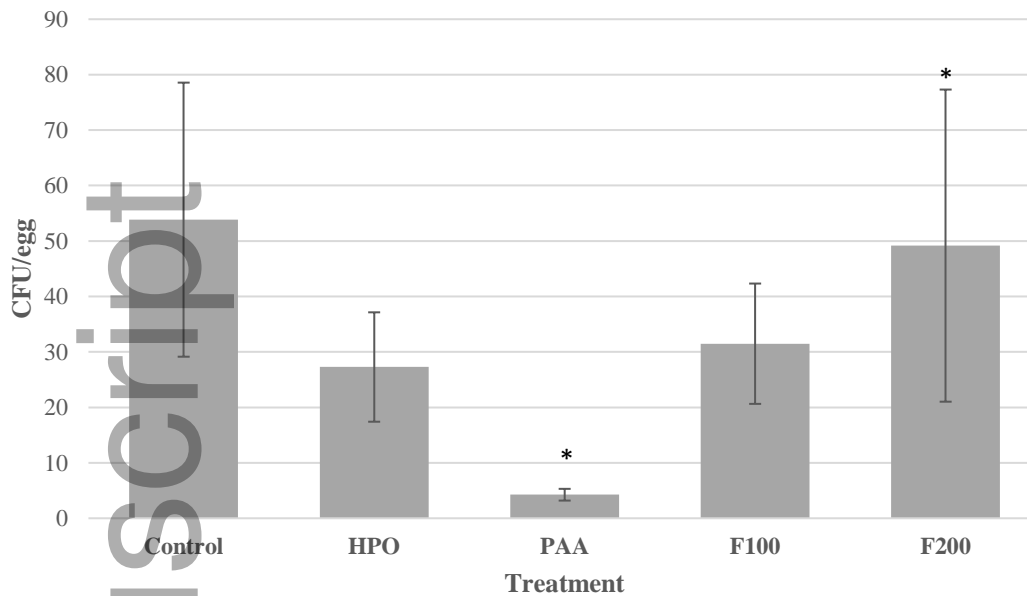
Figure 3. Mean (+/- SE) total bacteria counts as determined from TSA agar (n = 5). Asterisks denote significant differences from the control determined by general linear models on natural-log-transformed data (PAA, α = 0.001; F200, α = 0.003; Table 3).
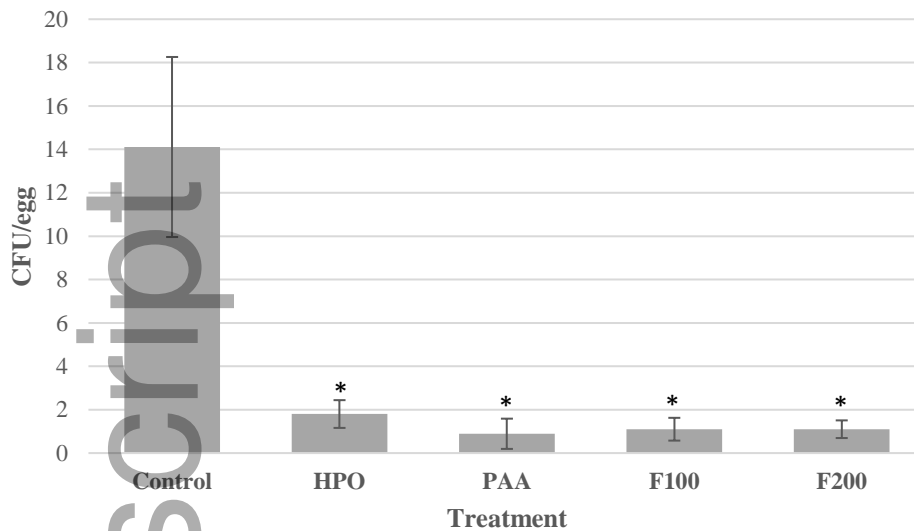
Figure 4. Mean (+/- SE) total Vibrio counts as determined from TCBS agar. Asterisks denote significant differences from the control determined by general linear models on natural-log-transformed data ($\alpha = 0.05$).