Hydrogen peroxide induced metamorphosis of queen conch, *Strombus gigas*: Tests at the commercial scale

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Abstract

The use of natural extracts in production scale culture processes has been problematic because of the temporal and spatial variability inherent in the active constituents of the extracts and the high cost associated with their preparation. Readily available, low cost alternatives to the natural extracts are needed. In the current study, the use of hydrogen peroxide as an alternative to aqueous extracts of the red alga *Laurencia poitei*, the current commercial inducer of queen conch (*Strombus gigas*) metamorphosis, was examined. Exposure of larval conch to 50 μM hydrogen peroxide for 10 h in both small scale and production scale assays induced full larval metamorphosis. The percent metamorphosis induced by hydrogen peroxide was not significantly different from that induced by *L. poitei* extract. In addition, the growth of the newly set juveniles induced by hydrogen peroxide was not significantly different from those induced by the *L. poitei* extract. Thus, hydrogen peroxide provides a low cost and readily available alternative to the natural cue for the commercial scale induction of metamorphosis in queen conch; it also eliminates much of the variability typically associated with the use of the natural inducer.

Keywords: Gastropod; Hydrogen peroxide; Larvae; Metamorphosis; Queen conch; *Strombus gigas*

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1. Introduction

The variability in activity and high cost of production associated with the use of natural extracts in production scale processes has lead to the need for low cost, readily available alternatives to these extracts (Morse, 1984; Davis et al., 1990; Boettcher and Targett, 1996). The induction of metamorphosis, a critical step in the culture of many species, is an area of particular interest for the introduction of alternatives to the currently used natural cues (Morse, 1984; Davis et al., 1990). Neuroactive compounds (i.e. gamma-aminobutyric acid, epinephrine, dopamine, and 3,4-dihydroxyphenylalanine), fatty acids, hydrogen peroxide, and increased ion concentrations (i.e. high K⁺, Cl⁻, Ca²⁺) have been used in small-scale assays to artificially induce the metamorphosis of certain marine invertebrates such as molluscs, echinoderms, and polychaetes (see Morse, 1990; Pires and Hadfield, 1991; Morse, 1992; and Pawlik, 1992 for reviews). These compounds can, in some cases, induce levels of metamorphosis as high as, or higher than, levels induced by the natural metamorphosis cues (see Morse, 1990; Morse, 1992; and Pawlik, 1992 for reviews). They can, therefore, be considered as potential alternatives to natural cues in the induction of metamorphosis of commercially important species. Gamma-aminobutyric acid has been used in the large scale induction of abalone metamorphosis (Morse, 1984) and potassium chloride has been proposed for use in the induction of metamorphosis in a variety of both commercially important and experimental larval invertebrates (Yool et al., 1986; Davis et al., 1990); however, few other alternative compounds have been tested for, or are currently being used, at the commercial level.

Queen conch, *Strombus gigas*, are marine gastropods found in seagrass beds and sandflats throughout the Caribbean (Randall, 1964; Brownell and Stevely, 1981). Their commercial importance in this region is second only to that of the spiny lobster (Hahn, 1989; Appeldoorn, 1994). Currently, there is one commercial facility (Caicos Conch Farm, Turks and Caicos, BWI) and several research facilities involved in the culture of queen conch (Appeldoorn and Rodriguez, 1994). An aqueous extract of the red alga *Laurencia poitei* is used in the commercial induction of conch metamorphosis. The collection and processing of this alga is labor intensive (Davis et al., 1990), and the activity of the extracts produced is variable owing to spatial and temporal variability in the concentration and/or availability of the active compounds in the alga (Boettcher and Targett, 1996). There is a need for a more reliable, readily available alternative for the commercial induction of conch metamorphosis.

The effects of a variety of neuroactive compounds, elevated ion concentrations, and hydrogen peroxide on the induction of metamorphosis of queen conch larvae have been examined in an effort to better understand the processes underlying the induction of metamorphosis in this species (Boettcher and Targett, in press). The most consistent results in terms of induction of metamorphosis were seen with low concentrations of hydrogen peroxide (Boettcher and Targett, in press). Based on these results, the use of hydrogen peroxide as an alternative to the extract of *L. poitei* in the induction of queen conch metamorphosis was examined. Small scale trials with hydrogen peroxide were run to determine optimal exposure time and concentration for the induction of *S. gigas*.
metamorphosis. Protocols were then developed and tested for the use of hydrogen peroxide in the production scale commercial induction of queen conch metamorphosis.

2. Material and methods

Three small scale trials examined the effectiveness of hydrogen peroxide, at varying concentrations and exposure times, as an alternative to aqueous extracts of *Laurencia poitei* for the induction of queen conch metamorphosis. Based on results from these experiments, three large production scale studies were then undertaken in order to design protocols for the use of hydrogen peroxide in the commercial scale induction of queen conch metamorphosis.

2.1. Small scale metamorphosis assays

All metamorphosis assays were carried out at the Caicos Conch Farm, Providenciales, Turks and Caicos, British West Indies (BWI). Small scale assays were run according to the methods described in Boettcher and Targett (1996). Briefly, metamorphosis assays were run as static no-choice experiments with five replicates per treatment and 15 larvae per replicate. Larvae within an experiment were from a single culture. However, different batches of larvae were used in different experiments. Assays were run in 500 ml polyethylene containers with 300 ml ultraviolet sterilized, 10 mm filtered seawater. A positive control (an aqueous extract of *L. poitei* @ 0.01–0.02 g wet weight per milliliter seawater) and negative control (seawater only) were included in each assay. The *L. poitei* for the positive controls was collected off Pine Cay, Turks and Caicos, BWI and the aqueous extract prepared as described for use in the commercial induction processes in Davis (1994a). Fresh extracts were prepared for each experiment. Unless otherwise noted, larvae were exposed to the *L. poitei* extract and to hydrogen peroxide treatments for 5 h, after which time they were transferred to a fresh volume of UV sterilized, 10 mm filtered seawater. A positive control (an aqueous extract of *L. poitei* @ 0.01–0.02 g wet weight per milliliter seawater) and negative control (seawater only) were included in each assay. The *L. poitei* for the positive controls was collected off Pine Cay, Turks and Caicos, BWI and the aqueous extract prepared as described for use in the commercial induction processes in Davis (1994a). Fresh extracts were prepared for each experiment. Unless otherwise noted, larvae were exposed to the *L. poitei* extract and to hydrogen peroxide treatments for 5 h, after which time they were transferred to a fresh volume of UV sterilized, 10 mm filtered seawater. A positive control (an aqueous extract of *L. poitei* @ 0.01–0.02 g wet weight per milliliter seawater) and negative control (seawater only) were included in each assay. The *L. poitei* for the positive controls was collected off Pine Cay, Turks and Caicos, BWI.

Experiments were run at ambient temperature (ca 28–29°C), salinity (ca 39 ppt.), and pH (8.3–8.4); and under natural light conditions (ca 12 h light: 12 h dark). Percent metamorphosis was determined after approximately 24 h, and was calculated as the total number of larvae metamorphosed/total number recovered (Pearce and Scheibling, 1990). Larvae were considered to have undergone metamorphosis when they lost their velar lobes and began to crawl using their foot (Davis, 1994a).

Mean percent metamorphosis for each treatment in each experiment was determined. Mean percent metamorphosis among treatments in each experiment was compared using a Model 1, single factor ANOVA and Tukey's multiple comparison test (α = 0.05). Plots of residuals were used to test for homogeneity of variance and normality of each data set. Treatments in which percent metamorphosis was equal to zero for all replicates were not included in the statistical analyses.

2.1.1. Dose dependency

In the first small scale experiment, the effects of a range of hydrogen peroxide concentrations on larval conch metamorphosis were examined. The treatments in this
experiment were hydrogen peroxide at 10, 25, 50, 75, 100, and 1000 μM. Solutions were prepared from 3% pharmaceutical grade hydrogen peroxide. EM Quant peroxide test strips, a semiquantitative method for detecting peroxides via a reaction with an organic redox indicator (Thomas Scientific, Swedesboro, NJ), were used to monitor the seawater/peroxide solution to verify that it remained at the test level for the duration of the experiment. As described above, positive and negative controls were included in this and all subsequent small scale experiments.

2.2. Exposure time

Optimal exposure time to hydrogen peroxide was examined in two small scale experiments. In the first experiment, the exposure times were 30 min, 1 h, 2 h, 3 h, and 5 h. In the second experiment, the exposure times were 5 h, 7 h, 10 h, and 22 h. Based on the results of the dose dependency experiment, a hydrogen peroxide concentration of 50 μM was used in both exposure time experiments. The concentrations were monitored using EM Quant peroxide test strips. In both of these experiments, the larvae in the positive control were exposed for 5 h. After exposure to hydrogen peroxide or to L. poitei extract, larvae in each treatment were transferred to a fresh volume of UV sterilized 10 mm filtered seawater.

2.3. Production scale metamorphosis assays

Treatments at varying concentrations of hydrogen peroxide and varying exposure times were examined under conditions used for the commercial scale induction of larval conch metamorphosis to assure that the responses of the larvae would parallel their responses in the small scale assays, and to develop protocols for the use of this technique in commercial hatcheries. General techniques for the commercial induction of metamorphosis are based on those described in Davis (1994a) and reflect those currently in use at the Caicos Conch Farm. One day prior to each large scale assay, tests of larval competency were run using procedures similar to those used in the small scale metamorphosis assays described above. Pairs of 500 ml polyethylene containers with 300 ml seawater were prepared for each batch of larvae to be used in the large scale assay, one with the L. poitei extract and one with 50 μM hydrogen peroxide. Fifteen larvae were added to each of the containers, the water changed at 5 h, and metamorphosis monitored after about 24 h. Batches in which at least 75% of the larvae underwent metamorphosis in response to both the hydrogen peroxide and algal extract treatment were used in the full scale assays.

In both the dose dependent and exposure time production scale experiments, competent larvae were transferred from hatchery tanks to polyethylene trays (60 × 60 cm) with polyethylene screen bottoms (mesh size 275 mm) at densities of approximately 1000-2000 larvae per tray. These trays were placed into 250 L tanks. A positive control (L. poitei @ ca 0.02 g wet weight per milliliter seawater) was included in each experiment. Prior to adding the larvae, the appropriate treatment (hydrogen peroxide or L. poitei extract) was added to each tank and mixed well. Two replicate trays per treatment were
run. Percent metamorphosis and survival were monitored at 22 h by determining the range in percent metamorphosis and percent survival for 25–30 animals randomly retrieved from each of the two replicate trays. General behavioral observations of the larvae were monitored on the treatment day and in the week following metamorphosis. The range in percent metamorphosis and percent survival was determined for each treatment in each experiment. Percent metamorphosis among treatments in each experiment was compared using a Model 1, single factor ANOVA and Tukey’s multiple comparison test ($\alpha = 0.05$).

2.3.1. Dose dependency
In production scale experiment one, three concentrations of hydrogen peroxide were examined. The treatments were 50, 70, and 100 $\mu$M (corresponding to 15, 21, and 30 ml of 3% hydrogen peroxide added per tank). EM Quant peroxide test strips were used to monitor peroxide concentrations. Several test strips were used per tank to assure that the solution was homogeneous. As described above, a positive control (L. poitei extract) was included in this and all subsequent production scale assays. Exposure times for the hydrogen peroxide treatments and the L. poitei extract were 5 h. At 5 h the water in each tank was drained, the tank and trays gently rinsed with seawater, and the tanks refilled with seawater. The tanks were then held under static conditions (no flow) for 17 h after which, flow (12 ml s$^{-1}$) was started and the newly set animals fed their normal diet of chitosan settled Chaetoceros gracilis (Davis, 1994a).

2.3.2. Exposure time
In production scale experiment two, exposure times of 3, 5, 7, 10, and 22 h to hydrogen peroxide were examined. Based on the results of the small and production scale dose dependency experiments, the concentration of hydrogen peroxide used in this experiment was 50 $\mu$M and was monitored using EM Quant peroxide test strips. The exposure time for the L. poitei extract was 5 h. After exposure, the water in each tank was changed as described above. The tanks were held under static conditions for 17 h, then flow and feeding were begun as described in the production scale dose dependency experiment.

2.3.3. Flow versus no-flow
Current procedures for the commercial induction of conch metamorphosis using L. poitei extract, call for the flow of seawater to the tanks to begin immediately following the 5 h exposure to the algal cue. Behavioral observations of hydrogen peroxide treated larvae indicated that they do not attach to the bottom of the trays as rapidly as larvae induced to metamorphose by L. poitei extracts, and it was hypothesized that the flow of water may interfere with their attachment process (pers. obs.). A production scale experiment was run to determine if the presence of seawater flow to the tanks immediately after exposure to hydrogen peroxide would affect larval metamorphosis and survival. There were two hydrogen peroxide treatments in this experiment. In both, the larvae were exposed to 50 $\mu$M hydrogen peroxide for 10 h, the tank drained, and the tank and trays gently rinsed and refilled with seawater. In the first hydrogen peroxide treatment the tanks were then held under static conditions for 17 h, after which flow and
feeding were started as described in the production scale dose dependency experiment. In the second, a low flow of seawater (5 ml s⁻¹) to the trays was started immediately following the water change. The concentration of hydrogen peroxide in both treatments was monitored using EM Quant peroxide test strips. Hydrogen peroxide exposure times and concentrations were based on the results of the production scale exposure time and dose dependency experiments. For the positive control, the larvae were exposed to the *L. poitei* extract for 5 h, the trays drained, and the tanks and trays refilled with seawater. The tanks were then immediately exposed to a low flow of seawater (5 ml s⁻¹). At 22 h the seawater flow rate to the hydrogen peroxide flow and *L. poitei* extract treatments was increased to 12 ml s⁻¹. At this time a seawater flow (12 ml s⁻¹) also was started in the hydrogen peroxide no-flow treatment and all larvae in all treatments were fed.

### 2.3.4. Growth of newly set juveniles

The growth rate of juvenile queen conch induced to metamorphose by hydrogen peroxide (50 µM, 5 h, flow) were compared with those induced by *L. poitei* extract (5 h, flow). Siphonal lengths of 25 randomly selected juveniles from five batches each of hydrogen peroxide and *L. poitei* induced conch were determined. At metamorphosis, larvae are approximately 1.1 mm siphonal length. Growth rate was determined by subtracting initial size at metamorphosis from the final size and dividing by the number of days they were allowed to grow. Experiments were run for 19–28 days. Mean growth rates among batches and between hydrogen peroxide and *L. poitei* induced conch were compared using a Model 1, two factor ANOVA (α = 0.05). Survival rates of juveniles were qualitatively monitored.

### 3. Results

#### 3.1. Small scale metamorphosis assays

##### 3.1.1. Dose dependency

The responses of conch larvae to hydrogen peroxide at concentrations of 50 and 75 µM were not significantly different from one another or from the response to *L. poitei* extract (Fig. 1). At hydrogen peroxide concentrations < 25 µM little or no metamorphosis was induced. Hydrogen peroxide at 100 µM induced significantly fewer larvae to metamorphose than concentrations of 50 µM, 75 µM, or *L. poitei* extract. In addition several larvae (1–5) in each replicate of the 100 µM treatment were dead. All larvae in all replicates were dead when treated with hydrogen peroxide at 1000 µM.

##### 3.1.2. Exposure time

In the first experiment examining the effects of exposure times (up to 5 h), exposure to hydrogen peroxide for < 2 h did not induce significant levels of metamorphosis of conch larvae (Fig. 2(a)). At 3 h < 20% metamorphosis was induced. This was significantly lower than the response to the positive control and the response to the 5 h treatment, but was significantly higher than the response to 2 h treatment and the negative control. Treatment for 5 h induced > 60% metamorphosis. This was lower
Fig. 1. Mean percent metamorphosis of queen conch larvae in response to specific concentrations of hydrogen peroxide (10, 25, 50, 75, 100, and 1000 μM), seawater (a negative control), and an extract of Laurencia poitei (a positive control) in a small scale assay. Exposure time was 5 h for the hydrogen peroxide treatments and the positive control. Metamorphosis was monitored at about 24 h. Data are mean ± SD, n = 5. Treatment results with the same letter above the error bar were not significantly different at p < 0.05.

than the response to the positive control, but significantly greater than the response to all other peroxide exposures. In the second experiment examining the effects of exposure times (up to 22 h), the response to L. poitei extract was low (< 50%) (Fig. 2(b)). The responses to hydrogen peroxide at all exposure times were equal to or significantly higher than the response to L. poitei extract. The 22 h exposure induced significantly higher percent metamorphosis than the negative and positive controls and the 5 h and 7 h hydrogen peroxide treatments. The 10 h treatment was significantly higher than the positive and negative controls, but was not significantly different from any of the other hydrogen peroxide treatments.

3.2. Production scale metamorphosis assays

3.2.1. Dose dependency

A concentration of 50 µM hydrogen peroxide induced the highest percent metamorphosis, and yielded the highest survival rate of the peroxide concentrations tested in production scale (Table 1). Metamorphosis and survival in this treatment were not significantly different than those in the L. poitei extract treatment. The percent metamorphosis and survival for the 70 and 100 µM hydrogen peroxide treatments were significantly lower than that for the L. poitei extract, but were not significantly different from the 50 µM treatment.

3.2.2. Exposure time

As in the small scale assays, increasing the exposure time to hydrogen peroxide increased the percent metamorphosis in production scale assays (Table 2). A 3 h
Fig. 2. Mean percent metamorphosis of queen conch larvae in response to hydrogen peroxide (50 μM) for (a) 30 min, 1 h, 2 h, 3 h, and 5 h and (b) 5 h, 7 h, 10 h, and 22 h in small scale assays. A seawater (a negative control) and an extract of Laurencia poitei (a positive control, 5 h exposure) are also included for each experiment. Metamorphosis was monitored at about 24 h. Data are mean ± SD, n = 5. Treatment results with the same letter above the error bar were not significantly different at p < 0.05.

Exposure to hydrogen peroxide gave significantly lower percent metamorphosis than all other treatments (<35%). An increase in response was seen in the 5–7 h range (65–80%) and again in the 10–22 h range (84–92%). The response to 5 h exposure was significantly lower than the responses to the 10 h and 22 h exposures. All exposure times >3 h induced significantly higher percent metamorphosis than that induced by L.
Table 1
Percent metamorphosis and percent survival of queen conch at the production scale in response to three concentrations of hydrogen peroxide and to an extract of *Laurencia poitei*.

<table>
<thead>
<tr>
<th>Hydrogen peroxide</th>
<th>Percent metamorphosis</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>52–70 AB</td>
<td>64–78 AB</td>
</tr>
<tr>
<td>70</td>
<td>35–55 A</td>
<td>48–62 A</td>
</tr>
<tr>
<td>100</td>
<td>38–46 A</td>
<td>45–54 A</td>
</tr>
<tr>
<td><em>Laurencia poitei</em> extract</td>
<td>92–96 B</td>
<td>96–100 B</td>
</tr>
</tbody>
</table>

Exposure time 5 h for all treatments.
Treatment results with the same letter were not significantly different at $p < 0.05$, $n = 2$.

Table 2
Percent metamorphosis and percent survival of queen conch at the production scale in response to hydrogen peroxide (50 µM) at five exposure times and to an extract of *Laurencia poitei*.

<table>
<thead>
<tr>
<th>Hydrogen peroxide</th>
<th>Percent metamorphosis</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22–32 A</td>
<td>100 A</td>
</tr>
<tr>
<td>5</td>
<td>65–72 C</td>
<td>96 A</td>
</tr>
<tr>
<td>7</td>
<td>76–80 CD</td>
<td>96–100 A</td>
</tr>
<tr>
<td>10</td>
<td>84–88 D</td>
<td>84–88 B</td>
</tr>
<tr>
<td>22</td>
<td>46–54 D</td>
<td>77–81 B</td>
</tr>
<tr>
<td><em>Laurencia poitei</em> extract</td>
<td>(5 h)</td>
<td></td>
</tr>
</tbody>
</table>

Treatment results with the same letter were not significantly different at $p < 0.05$, $n = 2$.

*p* & *poitei* extract. Survival in the 22 h exposure treatment was significantly lower than the 3, 5, 7, and 10 h treatments, but not significantly different from that in the *L. poitei* extract treatment. In addition, the conch in the 22 h treatment were slower to respond to external manipulation and were slower moving than the conch in the other treatments.

3.2.3. Flow versus no-flow

Addition of a low flow (5 mL s$^{-1}$) did not appear to affect the ability of larvae to complete metamorphosis or to attach to the bottom of the trays (Table 3). The percent metamorphosis and survival in the seawater flow and static hydrogen peroxide treat-

Table 3
Percent metamorphosis and percent survival of queen conch at the production scale in response to hydrogen peroxide (50 µM, 10 h exposure) with seawater flow and under static conditions, and to an extract of *Laurencia poitei* (5 h exposure, flow).

<table>
<thead>
<tr>
<th>Treatment, flow condition</th>
<th>Percent metamorphosis</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide, noflow</td>
<td>82–85 A</td>
<td>89–92 A</td>
</tr>
<tr>
<td>Hydrogen peroxide, flow</td>
<td>85–88 A</td>
<td>85–88 A</td>
</tr>
<tr>
<td><em>Laurencia poitei</em> extract</td>
<td>77–78 B</td>
<td>92–100 A</td>
</tr>
</tbody>
</table>

Treatment results with the same letter were not significantly different at $p < 0.05$, $n = 2$. 
ments were not significantly different (82–88% metamorphosis and 84–92% survival). The percent metamorphosis in these two treatments was higher than that for the *L. poitei* treated conch (77%). Survival was similar in all three treatments.

3.2.4. Growth of newly set juveniles

There was no significant difference in the growth rate of queen conch juveniles induced to metamorphose by hydrogen peroxide as compared with those induced by *L. poitei* (*P* = 0.48). There was, however, significant variability among batches. There was no interaction between batch and treatment. The growth rate of the hydrogen peroxide induced conch ranges from 0.13 ± 0.03–0.18 ± 0.03 mm per day, and that for *L. poitei* induced conch ranges from 0.12 ± 0.02–0.15 ± 0.03 mm per day. Qualitative observations indicated that, under similar rearing conditions, survival of hydrogen peroxide induced conch were similar to those induced by *L. poitei* extract.

4. Discussion

These experiments demonstrate that the use of hydrogen peroxide is a viable alternative to the use of *L. poitei* extract in the commercial induction of queen conch larval metamorphosis. At a concentration of 50 µM and an exposure time of 10 h hydrogen peroxide induced levels of metamorphosis as high as or higher than those induced by *L. poitei* extract. In addition, survival and growth were comparable with that obtained with the *L. poitei* extract.

Hydrogen peroxide provides a simple, low cost, reliable, and safe method for the commercial induction of larval conch metamorphosis. Hydrogen peroxide is readily available in both reagent grade (30%) and pharmaceutical grade (3%) stock solutions. The cost per batch of larvae, using pharmaceutical grade peroxide is approximately $0.16 (US). This compares with a cost of about $15.00 (US)/batch using *L. poitei* extract and a cost of about $5.00 (US)/batch using potassium chloride which has also been explored as a commercial inducer of queen conch metamorphosis (Davis et al., 1990). Low cost coupled with high activity makes hydrogen peroxide a viable alternative to the use of natural cues in the commercial induction of conch metamorphosis. The availability of easy-to-use peroxide indicator strip tests also allows for concentrations of hydrogen peroxide in the metamorphosis tanks to be readily monitored and adjusted for each batch of larvae run. This offers a tremendous advantage over the highly variable *L. poitei* extracts. Previous studies have shown that variability in the activity of the *L. poitei* extracts was associated with variability in the quantity and/or availability of the cues in plants collected from different locations or at different times (Davis and Stoner, 1994; Boettcher and Targett, 1996). In the current study, the percent metamorphosis induced by the algal extracts ranged from about 45–95%, whereas that for hydrogen peroxide (10 h, 50 µM) was consistently in the 80–95% range. The degree of variability in the response of conch larvae to the *L. poitei* extract seen in this study was similar to that seen in other studies (Davis et al., 1990; Davis and Stoner, 1994; Boettcher and Targett, 1996). The use of hydrogen peroxide eliminated much of the variability associated with the use of the natural inducer. Furthermore, hydrogen peroxide readily
breaks down to oxygen and water and, therefore, does not accumulate in seawater (Nishimura et al., 1988; Waterstrat and Marking, 1995).

The effects of hydrogen peroxide on the induction of metamorphosis in several other larval molluscs have been examined (Pires and Hadfield, 1991, A.A. Boettcher, unpublished data). Low concentrations of hydrogen peroxide (50–200 μM) induced the loss of velar lobes in larvae of the nudibranch *Phestilla sibogae*. However, unlike induction in conch, the nudibranch did not complete metamorphosis. *P. sibogae* larvae were able to complete metamorphosis if they were subsequently treated with the natural inducer, an extract of their coral prey species (Pires and Hadfield, 1991). Small scale trials with the larvae of the abalone *Haliotis rufescens*, using hydrogen peroxide concentrations in the range of 50–200 μM for 4, 24 and 48 h, have shown that hydrogen peroxide has no effect on metamorphosis (A.A. Boettcher, unpublished data). At this time, the effects of hydrogen peroxide on metamorphosis in non-molluscan systems had not been examined. Hydrogen peroxide currently being used for or tested for use in a variety of other capacities in aquaculture, including the induction of spawning of marine molluscs (Morse, 1984), the treatment of microbes in fish culture (Waterstrat and Marking, 1995), and as a source of oxygen for fish transport (Taylor and Ross, 1988). It is also used in combination with ferrous iron as an anti-foulant (Nishimura et al., 1988). At concentrations of about 10 μM hydrogen peroxide, in the presence of ferrous iron, affects the attachment and growth of larval mussels, polychaetes, hydroids, and bryozoans (Nishimura et al., 1988). The mechanisms underlying the induction of metamorphosis by hydrogen peroxide are yet to be examined.

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