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EFFECT OF THE POLLUTANTS LEAD, ZINC, HEXADECANE AND OCTOCOSANE ON TOTAL GROWTH AND SHELL GROWTH IN THE AKOYA PEARL OYSTER, *PINCTADA IMBRICATA*

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ABSTRACT Pearl oysters (*Pinctada imbricata*) were held in the laboratory and exposed to various levels of the heavy metals lead and zinc and the aliphatic hydrocarbons hexadecane and octacosane for 2 months. Individual oysters were followed over the course of the experiment, allowing specific calculation of total oyster growth (wet weight) and shell growth. Significant reductions in total oyster growth were observed when oysters were exposed to high concentrations (270μg L^{-1}) of either zinc or lead. Exposure to the aliphatic hydrocarbons had no effect on total oyster growth. High concentrations of lead completely halted shell growth, the first demonstration of pollutant induced cessation of biomineralization in pearl oysters. Conversely, exposure to moderate levels of lead and the long-chain hydrocarbon octacosane resulted in significant increases in shell width growth. The results from this study indicate that *P. imbricata* is relatively tolerant of the selected pollutants and could be deployed within a remediative context in moderately polluted coastal areas.

KEY WORDS: *Pinctada*, oyster, pollution, biomineralization, pearl, metals, shell, aquaculture

INTRODUCTION

The Akoya pearl oyster, *Pinctada imbricata* (Röding 1798), has a broad global distribution and is found in temperate and tropical waters. This species has been farmed in Japan for over 100 y for its small yet lustrous pearls. But Japanese production of high quality Akoya pearls has fallen from 118,000 kg in 1993 to 63,000 kg in 1996, with continuing production declines (Miyazaki et al. 1999). This decline has in part been attributed to deteriorating water quality (Tomaru et al. 2001). It is well known that bivalve molluscs accumulate many pollutants within their tissue and shell, a fact that has led to their use as biomonitors of hydrocarbons (Sericano et al. 1995) and heavy metals (Bourgoin 1990, Phillips & Rainbow 1993) pollution in marine and estuarine waters. Pearl oysters are no exception to this, and have been used as biomonitors of heavy metals (Bou-Olayan et al. 1995) and hydrocarbons (Fowler et al. 1993). Whereas it is known that pearl oysters accumulate hydrocarbons and heavy metals in their tissues on exposure and that these compounds can exert toxic effects on other bivalve molluscs (Kennedy et al. 1996), there is little information regarding the tolerance of pearl oysters to pollutants.

The tolerance of *P. imbricata* to particular pollutants is of further interest because pearl aquaculture has recently been proposed as a coastal remediation technology (Gifford et al. 2004). Pearl oysters have a high filtration rate, concentrate pollutants and nutrients within their tissues, and they yield a valuable product that is not bound for human consumption. However, the success of a pearl oyster remediation system would rely on the profitability of pearling operations to make the coastal remediation commercially viable. Therefore, it is necessary to investigate the oyster's tolerance limits, for both general pearl oyster health and pearl quality.

Relatively little is known about the effects of pollutants on shell biomineralization in molluscs. In the Pacific oyster, *Crassostrea gigas,* exposure to tributyltin results in the production of a gelatinous substance within the shell and shell deformity (Alzieu et al. 1986), whereas exposure of the mussel, *Mytilus californianus*, to barium results in abnormal shell calcification (Spangenberg & Cherr 1996). In one of the few studies to investigate the effects of dietary pollutant exposure on shell biomineralization, high (500 μg g^{-1}) dietary concentrations of lead resulted in a 25% reduction in shell mass in juvenile garden snails (*Helix aspersa*), yet shell size was unaffected by lead (Beeby et al. 2002). Similarly, transplanted *Crassostrea gigas* exposed to high concentrations of cadmium, copper and zinc in Chesapeake Bay had significantly thinner shells than control oysters (Frazier 1976). High concentrations of cadmium have also been shown to inhibit shell growth in *Crassostrea virginica* (200 μg L^{-1}, Shuster & Pringle 1969) and *Mytilus edulis* (500 μg L^{-1}, Sturesson 1978). Exposure of *C. gigas* to lead for 4 months led to significant differences in the amino acid profile of the shells (Almeida et al. 1998). Shell length growth in *M. edulis* is significantly reduced by exposure to the heavy metals zinc, mercury, copper, and cadmium but not lead and nickel (Strömgren 1982), and various hydrocarbon mixtures (Strömgren 1986, Strömgren et al. 1986). Given that high levels of pollutants alter shell production in many species of molluscs, it is possible that exposure to pollutants could alter shell growth in pearl oysters.

This study investigates the effects of pollutants on total growth and shell growth in pearl oysters. The oysters were exposed to either the essential metal zinc or the nonessential metal lead. These metallic pollutants are both common estuarine pollutants arising from urban, industrial and agricultural applications. Oysters were also separately exposed to either of the aliphatic hydrocarbons hexadecane and octacosane, common estuarine pollutants arising from urbanization and recreational and industrial boating. Furthermore, the hydrocarbons were chosen to represent differing hydrophobicity, which in turn has resulted in differing compartmentalization of pollutants between the shell and soft tissue of molluscs in previous work in our laboratory (Walsh et al. 1995). It was hypothesized that high concentrations of lead, zinc, hexadecane and octacosane would reduce both total and shell growth in the Akoya pearl oyster.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the NSW Fisheries Port Stephens Fisheries Centre from June to August 2003 according to

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the protocols of the American Society for Testing and Materials (ASTM) E 729–96 (1996) for static renewal tests. Fifty-one individually aerated 8 L aquaria were divided across two large water baths maintained at 22°C for the course of the experiment. Oysters used were from the same spawning batch from the NSW Fisheries oyster hatchery. Each aquarium contained a single oyster, allowing individual changes in oyster physiology to be monitored. After a 48-h acclimatization period and byssal attachment to the wall of the aquarium, exposure to the pollutant commenced. For the metal treatments, exposure was through the addition of sufficient $Pb(NO_3)_2$ or $ZnSO_4$ solution calculated so as to give final concentrations of background +10, 30, 90, 270 μg L^{-1} of Pb and Zn. For the aliphatic hydrocarbons, sufficient pollutant dissolved in chloroform (at a concentration so as to ensure that the maximum concentration of chloroform in any aquaria was 0.2 ml L^{-1}) was added to give the required concentrations of background +30, 90, 270, 810 ng L^{-1}. The chloroform/hydrocarbon pollutant stocks were stored at −15°C and the metal solutions were stored at 4°C for the duration of the experiment.

Water was changed thrice weekly and the oysters were fed daily a mixture of the algae *Chaetoceros muelleri* and Tahitian *Isochrysis* aff. *galbana* and *Pavlova lutheri*. There were three replicate aquaria for each treatment. A control treatment with three replicates was also run. Replacement water was heated to 22°C prior to water changes and the salinity and temperature were monitored daily. Temperature and salinity throughout the experiment remained within the range 22 ± 0.5°C and 33 ± 0.5 g kg^{-1}, respectively.

Parameters Monitored

Before commencing, excess moisture was drained from the surface of the oysters and the total oyster mass was determined to the nearest 0.1 g, then shell length and shell width measured to the nearest mm using vernier calipers. After 2 months, the same parameters were measured.

Statistical Analysis

Differences in shell length, shell width and total oyster mass were analyzed using 1-way ANOVA. Normality and homogeneity of variance were verified using Levennes test and via graphing residuals, and the data were natural log transformed where necessary. The significance level was taken at $P < 0.05$. Posthoc analysis was conducted with pairwise Tukey HSD test. For the Gaussian correlation analyses, as well as testing for normality and homogeneity of variance, a power analysis was also conducted. All statistical analyses were carried out using SPSS v.10.1 and figures were compiled using SigmaPlot v 6.

RESULTS

Total Oyster Growth (Increase in Wet Weight)

No oyster mortalities occurred during the experimental period and all oysters increased in total wet weight over the 2-month period. Oysters cultured in the control treatment increased total wet weight by, on average (±SE), 45 ± 8%.

Exposure to the higher concentrations of zinc and lead resulted in reductions in oyster growth as compared with control oysters (Fig. 1a, b). Oysters exposed to the highest experimental lead treatment had significantly reduced growth ($F = 3.94$, $P = 0.036$, Fig. 1a), compared with control oysters. Whereas there was no significant difference in the total growth of oysters exposed to the highest zinc treatment and control oysters, a significant decrease in total growth was observed for the 270 μg L^{-1} zinc treatment compared with the 30 μg L^{-1} treatment ($F = 5.14$, $P = 0.016$ Fig. 1b).

Exposure to the aliphatic hydrocarbons hexadecane and octacosane at 30–810 ng L^{-1} did not reduce total body mass after 2 months (Fig. 1c, d).

Shell Growth

Exposure of the oysters to 270 μg L^{-1} of lead significantly reduced shell length growth ($F = 5.14$, $P = 0.016$ Fig. 2a) as compared with the control treatment. The mean length of shells actually decreased by 2 ± 1% (mean ± SE) because of shell weakening at the distal margin. Visually, shells from oysters cultured in the high lead treatment lacked the imbricate processes at the distal margin evident in oysters grown in other treatments, indicating shell stress (Fig. 3). Although there was no impact on the measured shell length in the 90 μg L^{-1} treatment, Figure 3 shows that these oysters also lacked the imbricate processes. These two highest lead concentrations resulted in shells that were weaker and more brittle than control oysters. Exposure to zinc did not significantly affect shell length growth over the 2-month period (Fig. 2b). Exposure to hexadecane and octacosane at 30–810 ng L^{-1} did not have any significant effects on shell length (Fig. 2c, d).

Shell width growth was significantly greater in oysters exposed to 30 and 90 μg L^{-1} lead, yet significantly decreased in oysters exposed to 270 μg L^{-1} lead compared with control treatments ($F = 4.6$, $P = 0.02$, Fig. 4d). Exposure to the longer chain hydrocarbon, octacosane, also resulted in increased shell width growth (Fig. 4d), but no significant differences to shell width were observed for oysters exposed to zinc and hexadecane (Fig. 4b, c).

Relationships Between Concentration of Pollutants and Shell Growth

For lead, zinc and octacosane, clear associations were apparent between the concentration of the pollutant and the percentage increase in shell width (Fig. 5). Moderate additions of these pollutants above the background level caused increases in the shell width growth. However, exposure to the higher experimental treatments resulted in reduced levels of shell width growth. For hexadecane, there was no relationship between pollutant concentration and shell width growth.

In contrast to shell width, whereas high concentrations of lead inhibit shell length growth, there was no relationship between concentration of pollutant and shell length growth for all 4 experimental treatments.

DISCUSSION

Heavy metals inhibit growth in a variety of mollusc species (Manley et al. 1984, Wikfors et al. 1994, Din & Ahamad 1995, Keppler & Ringwood 2001). In this study, total oyster mass was reduced in *P. imbricata* oysters exposed to high concentrations of lead and zinc. The dose response was not linear, with no obvious effects on oyster growth at 10–90 μg L^{-1} for lead and zinc; this indicates that *P. imbricata* is relatively tolerant of the individual effects of lead and zinc, at least up to concentrations of approximately 90 μg L^{-1}. Effects on total oyster mass were observed at the higher concentration (270 μg L^{-1}). Typically, the concentration of dissolved lead in low-moderately human impacted estuaries is below 10 μg L^{-1} (Barnes et al. 1982, Dassenakis et al. 1997). As

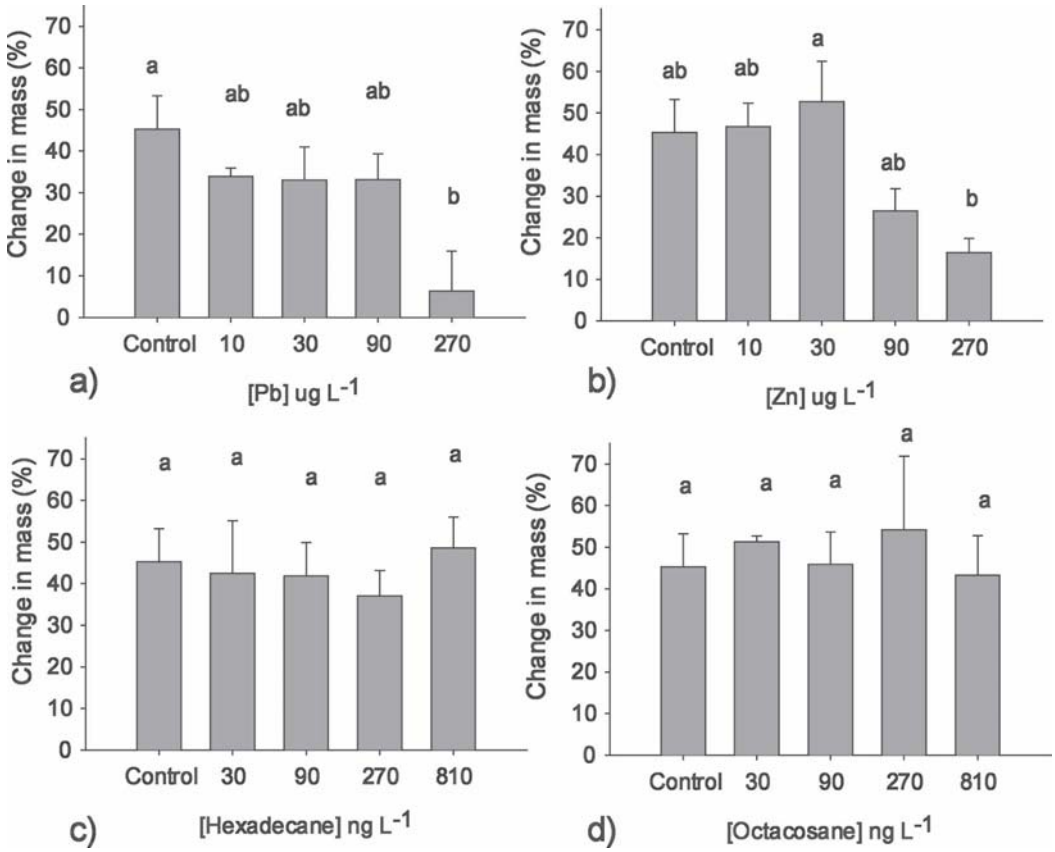

Figure 1. Mean change in mass (%) of *Pinctada imbricata* cultured under five different concentrations of lead, zinc, hexadecane and octacosane. Treatments identified as statistically similar are denoted by identical letters as determined by Tukey HSD pairwise comparison test. Error bars show SE (n = 3).

such, this study provides important information on the zinc and lead tolerance limits of the commercially important Akoya pearl oyster. However, caution is necessary in extrapolating these laboratory results to field situations, because possible synergistic or antagonistic effects among pollutants may exist. Furthermore, the potential for size dependent changes of metal impacts on oyster health must also be considered.

Exposure to either of the aliphatic hydrocarbons used in this study did not significantly effect total oyster growth. Organic contaminants are known to adversely affect bivalve molluscs in a variety of ways including reduced feeding (Widdows et al. 1990), induction of stress proteins (Cruz-Rodriguez & Chu 2002), inhibition of oogenesis (Chu et al. 2000) and increased respiration (Widdows et al. 1990). However, most of these studies involve either haloginated hydrocarbons, such as polychlorinated biphenyls (PCB), or polycyclic aromatic hydrocarbons (PAH), with very little toxicological information regarding aliphatic hydrocarbons available. The results from this study indicate that dissolved concentrations of aliphatic hydrocarbons in the high ng L^{-1} range do not impact total growth of *P. imbricata.*

Shell structure of *P. imbricata* exposed to 270 μg L^{-1} lead was visibly altered, with a complete lack of imbricate processes (frills) around the shell margin. Little information exists on the effects of pollutants on shell growth (Shuster & Pringle 1969, Frazier 1976, Strömgren 1982, Almeida et al. 1998, Beeby et al. 2002), and this is the first study to investigate the effects of pollutants on biomineralization in an economically important pearl oyster. Lead exposure has previously been shown to reduce shell thickness in the common garden snail *Helix aspersa* (Beeby et al. 2002), and alter amino acid composition of the shell of *Crassostrea gigas* (Almeida et al. 1998). In our study, shell growth was completely impeded by exposure to 270 μg L^{-1} of lead. Shuster and Pringle (1969) reported complete inhibition of shell growth by *Crassostrea virginica* exposed to similar concentrations of cadmium, whereas Strömgren (1982) observed cessation of shell growth in *Mytilus edulis* when exposed to copper and mercury. These results could be because of reduced activity of the enzyme carbonic anhydrase, an enzyme essential for shell and pearl production (Wilbur & Jodrey 1955, Freeman 1960, Miyamoto et al. 1996). Lead has previously been shown to inhibit levels of carbonic anhydrase in both anemones and corals (Gilbert & Guzman 2001), cadmium inhibits carbonic anhydrase in estuarine crabs (Vitale et al. 1999, Skaggs & Henry 2002) and eels (Lionetto et al. 1998), whereas silver, copper and zinc all reduce levels of carbonic anhydrase in crabs (Skaggs & Henry 2002).

Whereas high concentrations of lead significantly reduced shell growth, exposure to 30 and 90 μg L^{-1} of lead had the opposite effect, significantly increasing growth in shell width. Whereas active metal incorporation into shell matrix has previously been described (Bertine & Goldberg 1972, Sturesson 1976, 1978, Al-Aasm et al. 1998), this is the first reported case of an increase in shell growth in response to moderate metal challenge. Exposure to octacosane also resulted in a significant increase in the growth of shell widths. In contrast, hexadecane did not significantly increase shell width growth. These results complement the earlier work of Walsh et al. (1995), who observed that longer chain hydrocarbons

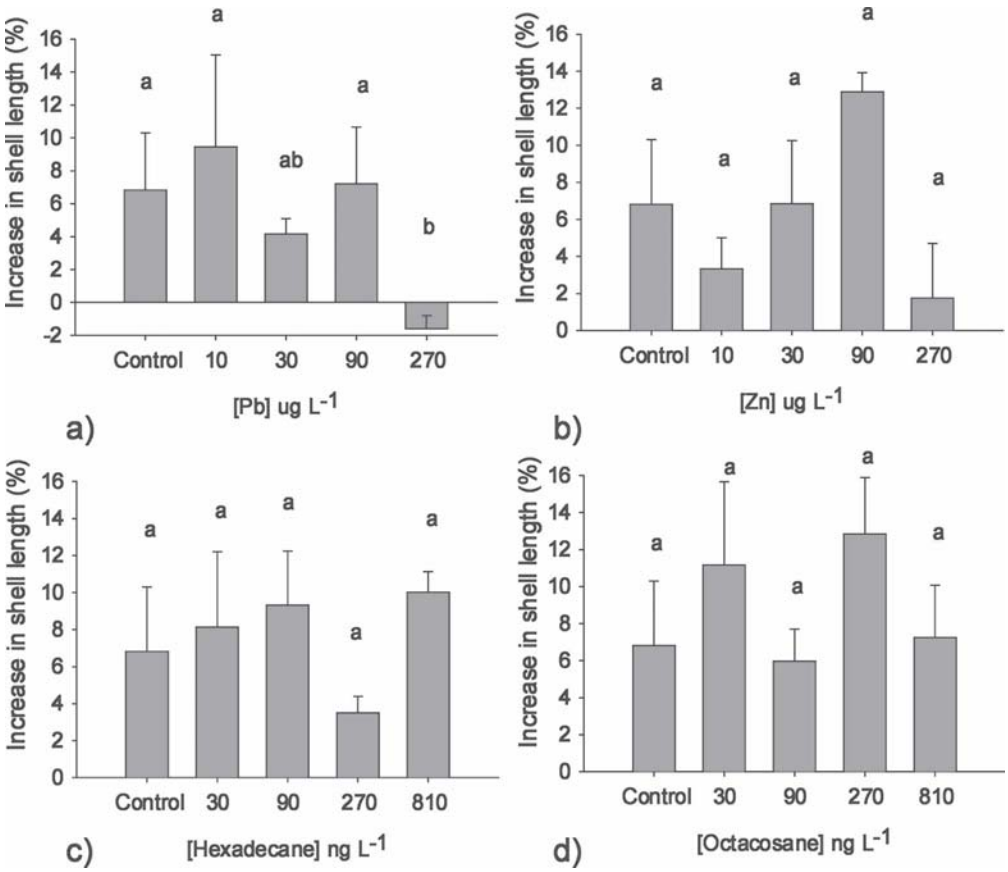

Figure 2. Mean change shell length (%) of *Pinctada imbricata* cultured under five different concentrations of lead, zinc, hexadecane and octacosane. Treatments identified as statistically similar are denoted by identical letters as determined by Tukey HSD pairwise comparison test. Error bars show SE (n = 3).

(those compounds resistant to cellular degradation systems) preferentially accumulated in the shell of the gastropod *Austrocochlea constricta*, whereas shorter chain hydrocarbons were preferentially accumulated in the soft tissue of the organism. These authors proposed that the smaller aliphatic hydrocarbons might be more easily detoxified via cellular enzymatic systems within the soft tissue, whereas more hydrophobic and recalcitrant compounds could be actively partitioned into the shell matrix and thereby removing them from metabolically active tissue (and subsequently the food chain). The observed gaussian response of shell width to three of the four pollutants (Fig. 4) tested was consistent with this theory, although this result is highly dependent on the growth of control oysters and in this experiment the control group consisted of only 3 oysters. Further studies are continuing to investigate the capacity of these animals to partition heavy metal and the more hydrophobic organic pollutants into the shell matrix.

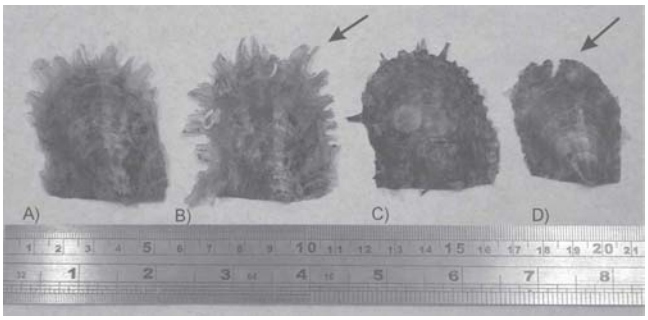

Figure 3. Oysters cultured under different concentrations of lead; a) 10 µg L^{-1} b) 30 µg L^{-1} c) 90 µg L^{-1} and d) 270 µg L^{-1}. Note the imbricate processes (marked in b) are absent around the shell margins for the oysters cultured under 90 and 270 µg L^{-1}.

In contrast to the results of this study, Strömgren et al. (1986) observed significant reductions in shell growth of the mussel *M. edulis* exposed to high concentrations of a microencapsulated mixture of n-alkanes. Concentrations used in our study were, however, approximately 1,000 times less than those used by Strömgren et al. (1986) and were the likely cause of the observed differences. Interestingly, Strömgren et al. (1986) found no difference in the effect of the aromatic and the n-alkane hydrocarbon mixtures on shell growth. Typically, aromatics are viewed as being more toxic than aliphatic hydrocarbons (Anderson et al. 1974). Given that very little is known about the effects of hydrocarbons on shell production (Strömgren 1986, Strömgren et al. 1986) further research in this area is clearly warranted.

Recently, pearl aquaculture has been proposed as a coastal bioremediation technology (Gifford et al. 2004). This is because of the fact that pearl oysters have high filtration rates (Pouvreau et al. 1999), concentrate pollutants within their tissue and shell (Al-Madfa et al.1998, Bou-Olayan et al. 1995), have a high protein content (Suzuki 1957, Numaguchi 1995), are found native in many areas of the world (Colgan & Ponder 2002) and the highly valued pearl product is not bound for human consumption. Because the mechanism of pearl production is similar to that of shell production in the oyster, any effects observed on shell growth would likely be mirrored in pearl formation. Therefore, the inhibition of

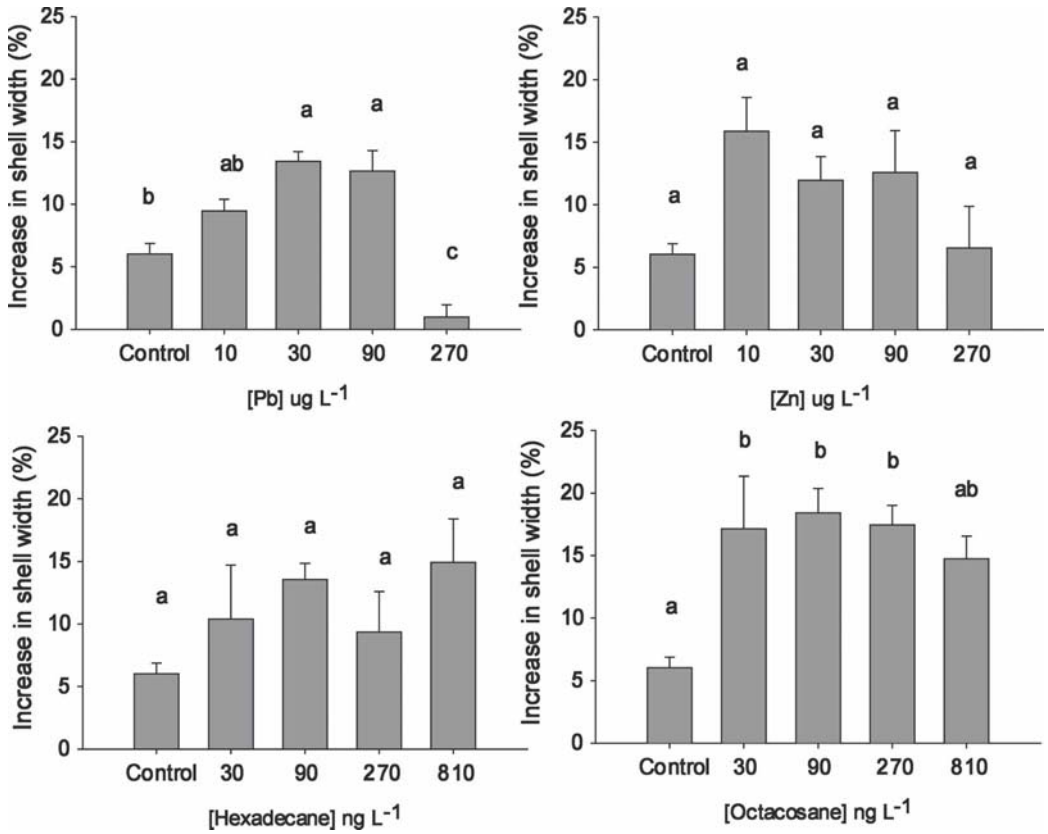

Figure 4. Mean change in shell width (%) of *Pinctada imbricata* cultured under five different lead, zinc, hexadecane or octacosane treatments. Treatments identified as statistically similar are denoted by identical letters as determined by Tukey HSD pairwise comparison test. Error bars show SE (n = 3)

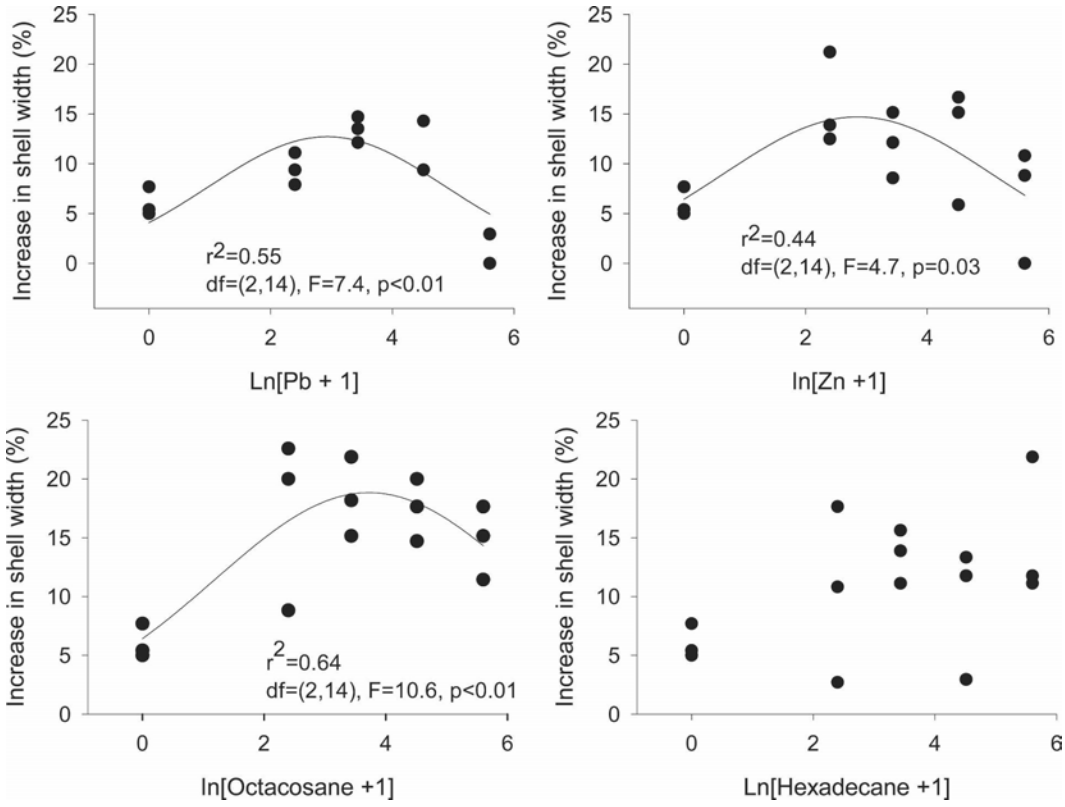

Figure 5. Relationships between concentration of pollutant and oyster shell width.

shell growth by high (270 μg L^{-1}) concentrations of lead in this study demonstrates that pearl aquaculture would be unlikely to succeed in areas suffering from high concentrations of dissolved lead. However, moderate concentrations (10–90 μg L^{-1}) of dissolved lead had the opposite effect, stimulating shell width growth. Furthermore, whereas high (270 μg L^{-1}) zinc concentrations did not significantly effect shell growth, a finding similar to Mai et al. (2003) who found that dietary zinc did not effect biomineralization in the abalone *Haliotis discus hannai*, they did reduce total oyster growth. Therefore, pearl aquaculture would unlikely succeed in areas with high dissolved zinc concentrations. The concentrations of aliphatic hydrocarbons used in this study did not negatively affect any of the measured oyster parameters, and in fact stimulated shell width growth at 30–270 ng L^{-1}. Therefore careful evaluation of potential sites would be required to balance metal remediation requirements and pearl quality/oyster health outcomes.

CONCLUSION

Exposure to high (270 μg L^{-1}) concentrations of dissolved lead reduced total oyster growth in *P. imbricata*. Importantly, high (270 μg L^{-1}) concentrations of lead significantly reduced shell growth and altered the visible appearance of the shell, the first demonstrated case of pollutant-impeded biomineralization in pearl oysters. However, intermediate concentrations (10–90 μg L^{-1}) of lead and zinc actually stimulated shell width growth. Exposure to the aliphatic hydrocarbons hexadecane and octacosane had no effect on the total oyster growth. However, exposure to moderate (30–270 ng L^{-1}) concentrations of the long chain hydrocarbon octacosane significantly increased shell width growth. The results of this study demonstrate the general tolerance of *P. imbricata* to the pollutants lead, zinc, hexadecane and octacosane.

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