



Induced spawning of the blood ark *Anadara tuberculosa*, using hydrogen peroxide

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Abstract. A great number of mollusc species have been induced to spawn reliably using physical, biological, and chemical methods, or a combination of them. The addition of hydrogen peroxide (H₂O₂) to the water is one of the most effective of the techniques tested. Although successfully used on some species, the technique has not worked in others. The blood ark *Anadara tuberculosa* or Piangua is a valuable commercial species along the eastern tropical Pacific seascape. One of the major limitations for the culture of the species is a lack of improved induced-spawning protocols. In this study we report a methodology to induce spawning of the Piangua with the addition of H₂O₂ that is easy to implement and practice. Thirty Pianguas (size = 45 to 58 mm in length) were randomly selected and individually placed in plastic containers filled with 250 mL of the experimental aqueous solutions; then randomly assigned into six treatments with five replicates of one Piangua each. Aqueous H₂O₂ solutions were prepared with brackish water (10 PSU) and to contain approximate 5-10, 25, 50, 150, and 294 millimoles of H₂O₂ per liter; the control group did not receive any H₂O₂. The trials were conducted at a constant temperature of 25°C. After three hours of immersion in different solutions of H₂O₂, the solutions were exchanged to just brackish water; each trial was terminated after four-hour periods. During the trial periods continuous observations were made to detect the release of the gametes. The specimens that did not spawn were dissected to establish their stage of sexual maturity. Spawning was observed when immersed in concentrations of H₂O₂ ranging from 25 to 150 mM; mortality was observed when immersed in the 294 mM solution. The Pianguas that did not spawn were those that were not ripe. It is possible to spawn the Piangua blood ark species with the addition of H₂O₂ to the water as has been done previously with other molluscs. This method is inexpensive and simple to apply.

Key Words: Piangua, aquaculture, Colombia, propagation.

Introduction. *Anadara tuberculosa* is the bivalve mollusc of greatest importance in the diet and economy of artisanal fishermen and the rural population that lives along the coasts of the eastern-tropical Pacific, between Mexico and northern Peru (Gracia & Díaz 2002; Borda & Cruz 2004; Lucero-Rincón et al 2013). On the Pacific coast of Colombia this bivalve is traditionally called Piangua; in Ecuador Concha Prieta. Pianguas live in the mangrove swamp estuaries, and are an entirely wild-caught resource. In Colombia, the Pianguas are collected among the roots of the mangrove, mainly by low-income, local women of Afro-American descent, that market them directly to users or use intermediaries because the larger markets are geographical far away from where they are caught (Lucero-Rincón et al 2013; Palacios 2012). The high demand, coupled with being a wild-caught animal, results in the resource becoming increasingly overexploited in many parts of their range, and the mangroves showing symptoms of deterioration (Gracia & Díaz 2002).

The aquaculture of the Piangua would be an excellent alternative food production system for agricultural development and food security in the region. In addition, it would be a sustainable activity that would favor the protection and conservation of mangrove areas. At present, there is no captive production of Piangua seed in Colombia and it is still incipient in other countries (Velasco & Barros 2008; Vásquez et al 2009; MAGAP - Concepto Azul 2015).

The development and standardization of induced spawning techniques are necessary in most aquaculture practices since it provides for the continuous production of 'seed' and larvae for grow-out. In bivalve molluscs, ripe broodstock are induced to spawn using physical, biological, and chemical methods, or a combination of all three (Morse et al 1977a, b; Morse 1984; Helm et al 2004; Argüello-Guevara et al 2013).

Of these methods, the dilution of hydrogen peroxide (H_2O_2) in water stands out as an effective technique to induce the spawning of molluscs (Morse et al 1977b; Morse et al 1978; Morse 1984). The mode of action of H_2O_2 is related to the stimulation of prostaglandin biosynthesis (Morse et al 1977a, b). Prostaglandins can act as hormones and regulate the reproductive system in molluscs (Morse et al 1977a, b).

The possibility of using H_2O_2 to induce the spawning of the Piangua would be very convenient since the product is easy to obtain, inexpensive, and simple to apply; as it has been demonstrated in other mollusc species (Morse et al 1978; Morse 1984). In addition, H_2O_2 is a chemical that in handling and use is generally recognized as safe (GRAS) in terms of safety, health, and the environment.

Blood ark clams, including Piangua are typically artificially spawned using thermo-stimulation techniques (e.g., Sturmer et al 2009; Vásquez et al 2009). Although H_2O_2 has been mentioned as a chemical method to spawn them, in those studies no results or the findings were reported. The objective of this study was to establish a practical methodology for the induced spawning of the Piangua with the simple addition of H_2O_2 . This methodology would also serve as a guide to develop a viable protocol to induce the spawning of other mollusc species that have been difficult to reproduce in Colombia and elsewhere.

Material and Method. During the months of July and August 2016, random samples of the blood ark *Anadara tuberculosa*, with sizes between 45 and 58 mm in length, were collected in the mangrove forests near Buenaventura, Colombia; 44 mm is considered the minimum size for first reproduction in Piangua (Borda & Cruz 2004). In the experimental design, 30 Pianguas were randomly selected (from the original initial sample), and placed individually in plastic containers. Again randomly choosing five Pianguas per experimental group; for a total of six treatments, including a control that did not receive any type of H_2O_2 manipulation. A solution in water of 30% H_2O_2 was used as reference and basis to calculate the molar concentration of H_2O_2 of the final solution in each treatment. H_2O_2 solutions were prepared with approximate concentrations of 5-10, 25, 50, 150, and 294 H_2O_2 in milliMolarity (mM) (Stoklosa & Ansel 1986). The amount of H_2O_2 calculated for each treatment was diluted in approximately 250 mL of water with low salinity (10 practical salinity units, PSU) and a temperature of 25°C, in which the Pianguas were submerged. Individual Pianguas were submerged for three hours into their respective H_2O_2 test solution concentration. At the end of this time period, the test solution was poured off and replaced with fresh brackish water (10 PSU). The test animals remained for another hour in brackish water (no H_2O_2) at which time the trial period ended. During the trial period, continuous observations were made to detect the expulsion of the gametes. The specimens that did not spawn were dissected to establish microscopically the morphological state of gamete maturation (Lucero-Rincón et al 2013).

Results and Discussion. Spawning was observed in the Pianguas submerged in brackish water containing 25, 50, and 150 mM of H_2O_2 . However, they did not spawn when exposed to the low and high concentrations of H_2O_2 of 5-10 mM and 294 mM, respectively; no spawning was also observed in the control group that did not received treatment with H_2O_2 . It was also observed, that soon after the treatment, all the Pianguas died in the high-dose treatment group. No other animals died in the experiment.

Microscopic examination of the gametes collected from each of the containers where spawning was observed, showed they were eggs, indicating all the Pianguas that spawned were females. However, gross and histological examination of the animals that did not spawn, revealed that each treatment group contained both sexes, but their gametes were not fully developed. This can best be explained by the time of year when

the experiment was performed (July-August). The main reproductive season of Piangua in the Colombian Pacific coast occurs between December and March (Borda & Cruz 2004; Lucero-Rincón et al 2013).

It is well documented that H₂O₂, at a standard dose of approximately 5-7.5 mM (roughly 250 parts per million, ppm), stimulates the release of gametes in a selected number mollusc species (Morse et al 1977a; Morse et al 1978; Morse 1984). But a detailed review of the scientific literature also indicated there was great variability in the response to the chemical among the mollusc species that were studied (e.g., Helm et al 2004). Likewise, as also gathered from the literature, there appears a decrease in continuity of use of H₂O₂ to induce the spawning in captivity in other groups of molluscs (techniques summarized by Helm et al 2004).

A probable cause for the mixed results and a decline in use of the technique is the difficulty and variability there is to obtain with precision the minute concentration of H₂O₂ (250 ppm) that is required to induce the spawning. Hence the doses are too low (e.g., 60 ppm as often seen reported in the literature, e.g., Sturmer et al 2009), or too high to inhibit reproduction; and even cause death as it did in this study. The error can be compounded because of the high volatility of hydrogen peroxide in solution, and the different concentrations of the liquid available in the market (e.g., available from pharmacies from 3 to 10% by weight, and 30% in reagent-grade form).

To add to the confusion, there are a number of ways to express and calculate the relative amounts of solute and solvent in a solution (e.g., millimolar, percentages, milligrams per liter, parts per million) (Stoklosa & Ansel 1986).

Typically it depends on the background or particular branch of study (clinical, chemical and the like) of the person doing the work. Unfortunately, this frequently results in errors in the determination of the correct concentration of H₂O₂ necessary to induce the spawn. Another problem is the difficulty in getting an accurate reading on how much H₂O₂ is in solution, especially at the low concentration that may be required. The available analytical methods for routine and field determination of H₂O₂ in solution are based on titrimetric, colorimetric, or enzymatic procedures. These methods, although highly practical, have an error or deviation from the true value of 0.01 to 0.08%, equivalent to 100 and 800 ppm, respectively (eg, Hach Co., Loveland CO, USA, Taylor Technologies, Sparks MD, USA).

Conclusions. The results of this study indicated that hydrogen peroxide was able to induce spawning in *Anadara tuberculosa* kept at 25°C. This method is inexpensive and simple to apply. We recommend practical concentrations to use, from 25 to 150 mM of H₂O₂, diluted in brackish water (10 PSU). Also, the solutions should be prepared from a stock supply of 30% H₂O₂ as this product is closer to its true concentration, compared to the pharmaceutical 3-6% stock solutions that could be less accurate given the volatility of H₂O₂.

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References

- Argüello-Guevara W., Loor A., Sonnenholzner S., 2013 Broodstock conditioning, spawning, and early larval development of the tropical rock oyster *Striostrea prismatica* (Gray 1825). *Journal of Shellfish Research* 32(3):665-670.
- Borda C. A., Cruz R., 2004 [Reproduction and recruitment of the mollusc *Anadara tuberculosa* (Sowerby, 1833) in the Colombian Pacific]. *Rev Invest Mar* 25(3):185-195. [in Spanish]
- Gracia A., Díaz J. M., 2002 *Anadara tuberculosa*. In: [The red book of marine invertebrates of Colombia]. Ardila N., Navas G. R., Reyes J. (eds), INVEMAR - Ministerio del Medio Ambiente, Bogotá D. C., pp. 96-98. [in Spanish]

- Helm M. M., Bourne N., Lovatelli A., 2004 Hatchery culture of bivalves: a practical manual. FAO Fisheries Technical Paper 471, FAO, Rome, 177 pp.
- Lucero-Rincón C. H., Cantera J. R., Gil Agudelo D. L., Muñoz O., Zapata L. A., Cortes N., Gualteros W. O., Manjarres A., 2013 [Spatio-temporal analysis of the reproductive biology and recruitment of the bivalve mollusc in the Colombian Pacific coast]. *Rev Biol Mar Oceanog* 48(2): 321-334. [in Spanish]
- MAGAP - Concepto Azul, 2015 [A practical manual for the production of concha prieta "*Anadara tuberculosa*" under laboratory conditions]. Ministerio de Agricultura, Ganadería, Acuacultura y Pesca - Concepto Azul, Guayaquil, 23 pp. [in Spanish]
- Morse D. E., 1984 Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. *Aquaculture* 39(1-4):263-282.
- Morse D. E., Duncan H., Hooker N., Morse A., 1977a Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. *Science* 196(4287):298-300.
- Morse D. E., Duncan H., Hooker N., Morse A., 1977b An inexpensive chemical method for the control and synchronous induction of spawning and reproduction in molluscan species important as protein-rich food sources. In: [Symposium on progress in marine research in the Caribbean and adjacent regions-II]; papers on fisheries, aquaculture and marine biology. FAO Fisheries Report 200, Stewart H. B. (ed), FAO, Rome, pp. 291-300.
- Morse D. E., Hooker N., Morse A., 1978 Chemical control of reproduction in bivalve and gastropod molluscs, III: an inexpensive technique for mariculture of many species. *Journal of the World Aquaculture Society* 9:543-547.
- Palacios C. A., 2012 [The characterization of the women gatherers of molluscs in the river Mayorquín]. *Sabia* 1(1):102-117. [in Spanish]
- Stoklosa M. J., Ansel H. C., 1986 Pharmaceutical calculations. Lippincott Williams & Wilkins, Philadelphia, 411 pp.
- Sturmer L. N., Nuñez J. M., Creswell R. L., Baker S. M., 2009 The potential of blood ark and ponderous ark aquaculture in Florida - results of spawning, larval rearing, nursery and growout trials. Sea Grant TP-169, Sea Grant, Gainesville, 91 pp.
- Vásquez H. E., Pacheco S. P., Pérez I. M., Cornejo N. E., Córdova M. F., Kan K., 2009 [Artificial production of seed and grow-out of bivalve molluscs]. CENDEPESCA, San Salvador, 80 pp. [in Spanish]
- Velasco L. A., Barros J., 2008 [Culture of bivalves in Colombia: a utopia or bet on the future?]. In: [The present state of culture and management of bivalve molluscs and future outlook: factors that affect sustainability in Latin America]. FAO Actas de Pesca y Acuicultura 12. Lovatelli A., Farías A., Uriarte I. (eds), FAO, Rome, pp. 115-128. [in Spanish]

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