

ORIGINAL PAPER

Homeopathy outperforms antibiotics treatment in juvenile scallop *Argopecten ventricosus*: effects on growth, survival, and immune response

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Background: Mortality from vibriosis in mollusk production is attributed to pathogenic bacteria, particularly *Vibrio alginolyticus*. Use of increasingly potent antibiotics has led to bacterial resistance and increased pathogenicity. Alternatives in sanitation, safety, and environmental sustainability are currently under analysis. To-date, homeopathy has been investigated in aquaculture of freshwater fish, but not in marine mollusks. The effect of the homeopathic complexes in the growth, survival, and immune response of the Catarina scallop *Argopecten ventricosus* were assessed.

Methods: A bioassay to assess the potential of homeopathy in improving cultivation of juvenile *A. ventricosus* was conducted for 21 days, with a final challenge of 120 h with *V. alginolyticus*. The experimental design included two homeopathic formulas The homeopathic complex Passival, consisting of *Passiflora incarnata* 30 CH, *Valeriana officinalis* 30 CH, *Ignatia amara* 30 CH and *Zincum valerianicum* 30 CH plus Phosphoricum acid 30 CH (treatment TH1) or *Silicea terra* 30 CH (TH2), two antibiotics (ampicillin = AMP, oxytetracycline = OXY), and two reference treatments (without homeopathic or antibiotic treatment = CTRL, ethanol 30° GL = ETH). Additionally, a negative control CTRL– (untreated/uninfected) is included in the challenge test. Juvenile scallops (4.14 ± 0.06 mm, 13.33 mg wet weight) were cultivated in 4 L tanks provided with aerated, filtered (1 μm), and UV-sterilized seawater that was changed every third day. They were fed a blend of the microalgae *Isochrysis galbana* and *Chaetoceros calcitrans* (150,000 cells mL⁻¹ twice a day). All treatments were directly added to the tank water and then 500 mL challenge units were inoculated with 1 × 10⁷ CFU/mL (LD₅₀) of *V. alginolyticus*.

Results: Juveniles grew significantly larger and faster in height and weight with TH2 compared to the ETH and CTRL ($P < 0.05$, ANOVA). Higher concentrations of proteins occurred in scallops exposed to TH2 (160.57 ± 7.79 mg g⁻¹), compared to other treatments and reference treatments. Higher survival rate during the challenge bioassay occurred with TH1 (85%), compared to AMP (53%), OXY (30%), and CTRL (0%), and superoxide dismutase ($P < 0.05$) was significantly higher in scallops treated with TH1, compared to other treatments and reference treatments.

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Conclusions: Homeopathic treatments improved growth and survival and enhanced survival against *V. alginolyticus* in juvenile *A. ventricosus*. This suggests that homeopathy is a viable treatment for this mollusk to reduce use of antibiotics in scallops and its progressive increase in pathogenicity in mollusk hatcheries. *Homeopathy* (2016) ■, 1–9.

Keywords: Aquacultural homeopathy; Hatchery cultivation; Bivalve health; Antibiotics; *Vibrio alginolyticus*; Immune response

Introduction

The Catarina scallop *Argopecten ventricosus* (Sowerby II, 1842) is a medium-size pectinid that ranges from the Baja California Peninsula in Mexico to Peru.¹ It is a fishing resource with aquaculture potential² and, similar to other pectinids, has increasing market demand, particularly in the Gulf of California.³ During hatchery production, some authors have addressed problems, such as susceptibility of larvae to bacterial diseases,⁴ phenol oxidase activity in larvae, juveniles, and adults,⁵ use of antibiotics in larval cultivation,⁶ and assessment of water quality during larval development to optimize cultivation protocols.⁷

Despite advances in cultivation in hatcheries to ensure continuity in juvenile (spat) production, commercial yield is still hindered by high die-offs of larvae and juveniles.^{8,9} Currently, the most relevant problems faced by growing diverse species of bivalves, including *A. ventricosus*, is the recurring presence of infectious diseases and parasites.^{10,11} Vibriosis is one of the most frequently reported diseases leading to economic losses in facilities raising bivalve mollusks.¹² Among some species, *Vibrio alginolyticus* and *V. parahaemolyticus* are reported as the most virulent strains that are naturally present in coastal systems.^{13,14} For several decades, these diseases were controlled with antibiotics, but its indiscriminate use to intensify production and obtain higher profitability favored antimicrobial resistance to treatment.¹⁵ Higher doses of new generation antibiotics, combined with chlorine and disinfectants, have generated environmental problems for floating fish farms and effluent discharges from shrimp farms.¹⁶ Homeopathic treatments are potential alternatives to control diseases in cultivated animals, aquatic and terrestrial. It is known that homeopathic treatments reduced or eliminated some antibiotics,¹⁷ improved general health of animals by strengthening their immune system,¹⁸ and even controlling the spread of endoparasites and ectoparasites.^{19–22}

Homeopathy has been successfully applied to including chickens and pigs as growth promoters,^{23,24} to control pathogenicity of *Escherichia coli* in pigs,²⁵ improve rabbit production,²⁶ and facilitate chicken vaccination.²⁷ Some homeopathic treatments (*Fator Vermes*²⁰; *Ferrum phosphoricum*, *Arsenicum album*, and *Calcarea carbonica*²¹) are used for controlling nematodes and helminths in small ruminants, mainly reducing stress that improves development and survival of the host.²² Likewise, homeopathic complexes, such as sulfur, staphysagria, calendula, and o-cillocochinum have shown a bactericidal effect that is use-

ful for treating *in vitro* plants of sugar cane infected with the bacteria *Xanthomonas albilineans* in intensive agricultural production, surpassing results using antifungal and antibacterial chemicals.²⁸ Homeopathy has not been widely explored in aquaculture, but recent results demonstrate its potential. The Nile tilapia *Oreochromis niloticus* has been treated with homeopathic complexes, such as Homeopatila™ to increase reproductive performance and success of adults,²⁹ and increase muscle quality in juvenile stock.^{30,31} Treatments applied to tilapia *O. niloticus* are able to increase morpho-functional response and the content and quality of fatty acids in muscle,^{32,33} as well as to modulate content of cortisol in muscle.³⁴ In another important edible fish, the pacu *Piaractus mesopotamicus*, physiological response to stress was successfully modulated with homeopathy.³⁵ In the ornamental fish, the molly *Poecilia* spp., homeopathic treatment induced spawning and improved the reproductive response.^{36,37} Finally, in the American bullfrog *Lithobates catesbeianus*, homeopathic treatment modified the rate of metamorphosis.³⁸

The aim of this study was to assess two homeopathic complexes and two antibiotics, the latter are regularly used in aquaculture.³⁹ We measured growth, survival, biochemical composition, and immunological response of juvenile Catarina scallops.

Materials and methods

Ethics

Care of animals met CIBNOR's institutional guidelines.

Source of juvenile stock

Juvenile *A. ventricosus* was raised in the hatchery, using routine methods described by Mazón-Suástegui.² Conditioned and sexually mature broodstock were induced to spawn by thermal shock (18–28°C) and the gametes were fertilized to obtain embryos and larvae. Veliger larvae were cultivated (15 days) in 1500 L fiberglass cylindrical tanks, filled with filtered (1 μm), UV sterilized, and gently aerated seawater, kept at 23 ± 1°C and salinity of 37 ± 0.5. Scallop larvae were fed a 1:1 mix of cultivated microalgae whose cell concentration progressively increased from 20 to 40 × 10³ cells mL⁻¹). Microalgal strains of *Isochrysis galbana* v. aff *galbana* (code UTEX LB 2307) and *Chaetoceros calcitrans* (origin from IFREMER, France) were obtained from CIBNOR's collection. These microorganisms were cultivated in glass and transparent polycarbonate tanks (2000 L), in filtered

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