Control of Shell-Boring Polychaetes in *Haliotis Tuberculata Coccinea* (Reeve 1846) Aquaculture: Species Identification and Effectiveness of Mebendazole

Author(s) :Amaia Bilbao, Jorge Núñez, María Del Pino Viera, Beatriz Sosa, Hipólito Fernández-Palacios and Carmen María Hernández-Cruz

Source: Journal of Shellfish Research, 30(2):331-336. 2011.
Published By: National Shellfisheries Association
DOI: 10.2983/035.030.0219
URL: http://www.bioone.org/doi/full/10.2983/035.030.0219

Journal of Shellfish Research, Vol. 30, No. 2, 331–336, 2011.

CONTROL OF SHELL-BORING POLYCHAETES IN *HALIOTIS TUBERCULATA COCCINEA* (REEVE 1846) AQUACULTURE: SPECIES IDENTIFICATION AND EFFECTIVENESS OF MEBENDAZOLE

AMAIA BILBAO,[1,*] JORGE NÚÑEZ,[2] MARÍA DEL PINO VIERA,[1] BEATRIZ SOSA,[1] HIPÓLITO FERNÁNDEZ-PALACIOS[1] AND CARMEN MARÍA HERNÁNDEZ-CRUZ[1]

[1]*Grupo de Investigación en Acuicultura, Universidad de Las Palmas de Gran Canaria and Instituto Canario de Ciencias Marinas, PO Box 56, 35200 Telde, Canary Islands, Spain;* [2]*Departamento de Biología Animal (Zoología), Facultad de Biología, Universidad de La Laguna, 38206 La Laguna, Canary Islands, Spain*

ABSTRACT The experimental culture of *Haliotis tuberculata coccinea* is carried out at the Institute of Marine Science of Canary Islands (Gran Canaria, Spain) based on specimens captured in their natural habitat and conditioned as broodstock. In 2008, an outbreak of shell-boring polychaetes affected the culture. This study aims to identify some of the species of shell-boring polychaetes that affect *H. tuberculata coccinea* and, second, to assess the effectiveness of multiple mebendazole applications for the control of these pests. Mebendazole was applied as baths of Lomper (Esteve Laboratory, Barcelona, Spain) using the concentrations 6 mL/L, 8 mL/L, and 10 mL/L, with 0 mL/L as a control measure. Abalone were exposed to 3 mebendazole baths over a 3-day period (1×3-h bath applied for 3 consecutive days), as recommended in the drug directions for human use. After each bath, the abalone were returned to their original culture tanks until the following day. After these 3 days, the abalone were kept in their original culture tanks for a term of 1 mo. Each month, as a result of the effect of mebendazole, moribund and/or dead polychaetes became detached from the burrows and were found at the bottom of the tank during the first 15 days after the baths of mebendazole were applied. The polychaetes expelled from the shells were counted, collected, and fixed in 10% buffered formaldehyde for subsequent identification. This process was repeated month after month until no moribund and/or dead shell-boring polychaetes were found after the application of the baths of mebendazole. The shell-boring polychaete species was identified as *Polydora hoplura* (Claparède 1870). The efficacy rate of Lomper was around 99% in all the concentrations tested when it was applied during a 7-mo term (each month, 1 bath was applied for 3 consecutive days), and abalone mortality was limited to highly infested animals only. Monitoring of abalone weight gain suggested that the mebendazole treatments did not affect growth significantly during the study period.

KEY WORDS: abalone, *Haliotis tuberculata coccinea*, *Polydora* sp., mebendazole

INTRODUCTION

The abalone found in the Canary Islands is *Haliotis tuberculata coccinea* (Reeve 1846). This is a small, subtropical native species with a maximum shell length (SL) of 8 cm (Bilbao et al. 2010). Recently, the government of the Canary Islands identified *H. tuberculata coccinea* as a new species for the diversification of local commercial aquaculture. Currently, techniques for the experimental culture of this species are being developed at the Institute of Marine Science of the Canary Islands (ICCM). The culture is based on specimens captured in their natural habitat and conditioned as broodstock at the ICCM using primarily cultured algae (*Ulva lactuca* and *Gracilaria cornea*). In 2008, an outbreak of shell-boring polychaetes affected the culture, especially the oldest specimens.

Shell-boring polychaetes belong to the family Spionidae. They bore into the shells of several mollusc taxa, including oysters, mussels, scallops, abalone, and clams (Blake & Evans 1973, Lauckner 1983). The most serious shell-boring polychaete pests that affect abalone cultures worldwide are related to two spionid genera (*Polydora* and *Boccardia*) (Lleonart et al. 2003a, Simon et al. 2006) and to the sabellid species *Terebrasabella heterouncinata* (Leighton 1998, Simon et al. 2006, Moore et al. 2007). The impact of these pests depends on the severity of the infestation, the size of the host, the host species, the worm species, the environmental conditions, and the health of the host (Lleonart et al. 2003a). High levels of infestation may lead to a significant reduction in the host's flesh condition (Handley 1998, Simon et al. 2006, Sato-Okoshi et al. 2008) and may even cause mortality (Lleonart et al. 2003a).

Efforts made to treat worm infestations affecting abalone species have achieved limited success. The methods tested include exposing the worms to encapsulated toxins (e.g., liposomes) that are absorbed by the shell-boring polychaetes but not by the abalone (Shields et al. 1998), biocontrol agents using potential shell-boring polychaete predators (e.g., *Cirolana harfordi*) (Kuris & Culver 1999), air-drying of spionid-infested abalone for 2–4 h at less than 64% humidity level (Lleonart et al. 2003b), heat treatment of spionid-infested animals using seawater warmed to 28–29°C for 48 h (Leighton 1998), and freshwater immersion for more than 48 h (Moore et al. 2007). Recently, Simon et al. (2010) suggested diatom-derived polyunsaturated aldehyde 2,4-decadienal as a chemotherapeutic agent against shell-boring polychaetes. However, there are no effective treatments that, although negatively impacting the worms, do not cause any undue harm to the host abalone.

Mebendazole (methyl 5-benzoyl-1H-benzimidazol-2yl-carbamate) is a broad spectrum benzimidazole carbamate classically used in human and veterinary medicine to treat a wide range of parasitic infestations (Cózar-Bernal et al. 2010). This drug acts by holding back essential transport processes taking place within the parasites' intracellular microtubules. It works by inhibiting glucose uptake, thereby causing immobilization

*Corresponding author. E-mail: lamiak75@gmail.com
DOI: 10.2983/035.030.0219

and death of the parasite. In humans, this drug has been successfully used for the treatment of nematodes (Krishnaiah et al. 2001, Dayan 2003, De Villiers et al. 2005). In the veterinary field, the use of mebendazole has been approved for several mammalian species, including pregnant animals (Krízová-Forstová et al. 2011), and it has proved to be very active against nematodes, lungworm (*Dictyocaulus arnfieldi*), and tapeworms (*Echinococcus* and *Taenia* spp.) (cited in Cózar-Bernal et al. (2010)). In aquaculture, mebendazole has been used for controlling monopisthocotylean infestations in fish (Goven & Amend 1982, Szekely & Molnar 1990, Buchmann & Bjerregaard 1990, Mellergaard 1990, Buchmann 1993, Kim & Choi 1998). However, mebendazole showed no significant results for the control of either the monogenean *Microcotyle* sp. in the gills of cultured *Pagrus pagrus* (Katharios et al. 2006) or of the shell-boring polychaete *Boccardia knoxi* in *Haliotis rubra* (Lleonart et al. 2003b). However, these studies used a single application of mebendazole and, as in humans (Cañete et al. 2009), it is expected that multiple mebendazole applications will be more effective in controlling the parasites affecting abalone.

This study aims to identify some of the species of shell-boring polychaetes that affect *H. tuberculata coccinea* in the Canary Islands and to assess the effectiveness of multiple baths of mebendazole to control these pest species.

MATERIALS AND METHODS

Experimental Animals and Culture Conditions

The experiment was undertaken during 2008 at the ICCM, Gran Canaria Island, Spain. A total of 120 naturally infested animals (SL, 40–75 mm) were tagged, and an infestation ranking was assigned to each animal: low infested, when fewer than five boring polychaete chimneys were present on the shell; highly infested, when more than five chimneys were present and/or the external surface of the shell was partly broken. Animals were weighed to the nearest 0.1 mg for body weight, and 0.1 mm for SL, and assigned to a culture tank (an experimental unit).

The animals were reared in 12 plastic tanks of 50-L (10 abalone per replicate) containing two shelters, with 1-μm filtered and UV radiation-sterilized seawater. Water flowed through the tanks at a rate of 5 vol/day, and aeration was provided constantly. Abalone were exposed to a natural photoperiod of approximately 12 h light/12 h dark. Feeding was conducted weekly using cultured mixed algae from ICCM (*U. lactuca* and *G. cornea*). The culture tanks were cleaned, and fecal material was removed once per week. At the same time, the uneaten macroalgae were removed and replaced with fresh algae.

Treatments for Shell-Boring Polychaetes

The effectiveness of mebendazole as an anthelmintic was evaluated at three concentrations: 120 mg/L, 160 mg/L, and 200 mg/L. Mebendazole was administered as Lomper (Esteve Laboratory, Barcelona, Spain) using the following concentrations: 6 mL/L, 8 mL/L, and 10 mL/L, with 0 mL/L as a control measure.

Every month, the abalone were transferred to 24 transparent plastic aquaria with a 2-L capacity (5 abalone per aquaria), and mebendazole was added to the desired concentration. The animals were exposed to the solution as recommended in the drug directions for human use. Abalone were exposed to 3 mebendazole baths over a 3-day period (one-time 3-h bath given for 3 consecutive days). After each bath, the abalone were returned to their original culture tanks until the following day. After these 3 days, the abalone were kept in their original culture tanks for a term of 1 mo. Each month, as a result of the effect of mebendazole, moribund and/or dead shell-boring polychaetes became detached from the burrows and were found at the bottom of the tank during the first 15 days after the baths of mebendazole were applied. The shell-boring polychaetes expelled from the shells were counted, collected, and fixed in 10% buffered formaldehyde for subsequent identification. This process was repeated the following month and, for 3 consecutive days, a one-time 3-h bath was given using Lomper. Throughout the 15 days after the baths, the moribund and/or dead shell-boring polychaetes were counted, and samples were taken for subsequent identification. This process was repeated month after month until no moribund and/or dead shell-boring polychaetes were found after the application of the baths of mebendazole.

Expulsion Methods, Quantification, and Identification of Shell-Boring Polychaetes

The amount of shell-boring polychaetes not dying as a result of the overall treatment with mebendazole, and the amount of shell-boring polychaetes in the control group were determined using a chemical vermifuge to expel the shell-boring polychaetes from their burrows (Lleonart et al. 2003a). This was a solution of 500 ppm phenol in seawater (Mackenzie & Shearer 1959).

At the end of the experiment, the surviving abalone (including those in the control group) were shucked, and each shell was placed in a 200-mL container with the vermifuge solution for 5 h (Fig. 1). This allowed us to determine the abalone containing shell-boring polychaetes and the abalone that were free of the pest. After exposure, the solution was drained through a 90-μm sieve, and the shell-boring polychaetes were rinsed and transferred to Petri dishes for examination. In addition, the surfaces of the abalone shells were examined using a magnifying glass to identify any shell-boring polychaetes only partly expelled from their burrows.

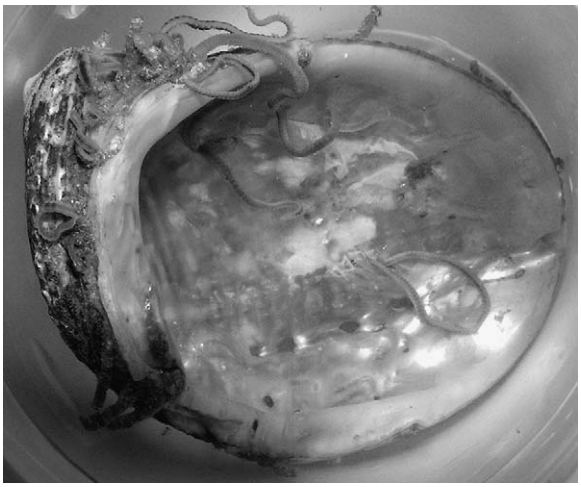

Figure 1. Shell of a highly infested abalone of the control group placed in a container of vermifuge solution. The live shell-boring polychaetes are expelled from the shell when exposed to the vermifuge.

Furthermore, during the performance of the study, whenever an abalone appeared dead in the culture tanks, its shell was immersed in the vermifuge solution to expel, count, and identify the amount of live shell-boring polychaetes found in the abalone. This was the same procedure carried out at the end of the experiment with surviving abalone.

To calculate the total amount of shell-boring polychaetes present at the start of the trial, the amount of dead shell-boring polychaetes and the number of shell-boring polychaetes resistant to the action of mebendazole must be added. The estimation of the initial amount of shell-boring polychaetes corresponded to each replication tank. The amount of shell-boring polychaetes at the start of the study equals the amount of shell-boring polychaetes expelled with the Lomper solution plus the amount of shell-boring polychaetes expelled with the vermifuge solution and found in the dead abalone plus the amount of shell-boring polychaetes expelled with the vermifuge solution at the end of the overall treatment

The efficacy rate of Lomper was defined as the percentage of shell-boring polychaetes that died after the mebendazole baths over the complete treatment, and this rate was calculated for each replication:

$$\text{Efficacy rate} = 100 - \%\ \text{of surviving shell-boring polychaetes}$$

$$\%\ \text{Surviving shell-boring polychaetes} = (\text{Amount of shell-boring polychaetes expelled with the vermifuge at the end of the complete treatment} \times 100)/\text{Amount of shell-boring polychaetes at the start of the study}$$

The shell-boring polychaete species were identified on the basis of fifth setiger chaetae, prostomium and pygidium morphology, and branchiae distribution. Descriptions of species encountered in the research are given in Pascual and Núñez (1999).

Growth

Because growth rates in abalone are age dependent, growth was estimated after separating individuals into 2 groups according to their SL: 40–59 mm and 60–79 mm. The specific growth rate for weight (SGR) and the percent weight gain (WG) were estimated as

$$\text{Specific growth rate for weight (SGR)} = 100 \times (LnW_t - LnW_0)/t$$

$$\text{Weight gain (WG)} = 100 \times (W_t - W_0)/W_0$$

where W_0 is the mean initial body weight (in grams), W_t is the mean final body weight (in grams), and t is the experimental duration (in days).

Statistical Methods

Statistical analyses were tested using an 1-factor ANOVA. Data were tested for normality prior to analysis using the Kolmogorov-Smirnov test. Bartlett's test was used to test for homogeneity of variances (Zar 1996), and Tukey's multiple comparison test was used in pairwise comparisons when significant differences ($P < 0.05$) were determined.

RESULTS

Shell-Boring Polychaetes

The shell-boring polychaete species found in the shells of abalone were identified as *Polydora hoplura* Claparède (1870). These large worms were 6.50 mm in length and 0.5–0.6 mm in width, with 56 setigers. The prostomium was fairly deeply notched, and often blackened by four small eyes. Notosetae were absent on the first setiger, and there were only ventral capillary setae. Branchiae start from setiger 7 and were absent in posterior region. Enlarged hooks (3–4) on the fifth setiger each had a lateral twisted spur, and on setiger 7 there were 2–3 bidentate sigmoid hooded hooks in the ventral position. On the last setigers, the notosetae included one enlarged, yellow, curved hook. The pygidium was open and shaped like a suction cup, with a dorsal notch, and occasionally was dark in color.

The chimneys created by *P. hoplura* on the outside surface of the abalone shell were initially located along the growing edge, although the worms were also capable of burrowing throughout the shell. When the infestation progressed, the shell-boring polychaetes tended to be evenly distributed within the shell. The chimneys were gray because of the accumulation of sediment (Fig. 2). As the infestation became increasingly heavy, the abalone formed blisters by shell material secretion (i.e., conchiolin covered with nacre) that projected into the mantle cavity to isolate the shell-boring polychaetes. The shells were occasionally broken in the area of the blisters. In these cases, the abalone were extremely vulnerable and, in most cases, died (Fig. 3). However, some of them were able to reconstruct the shell and, consequently, the shell became deformed.

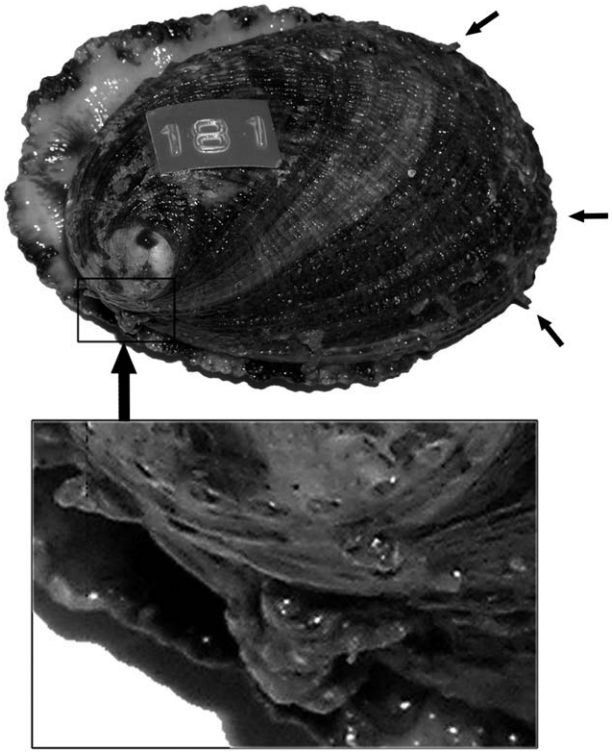

Figure 2. Typical chimneys of *P. hoplura* (arrows).

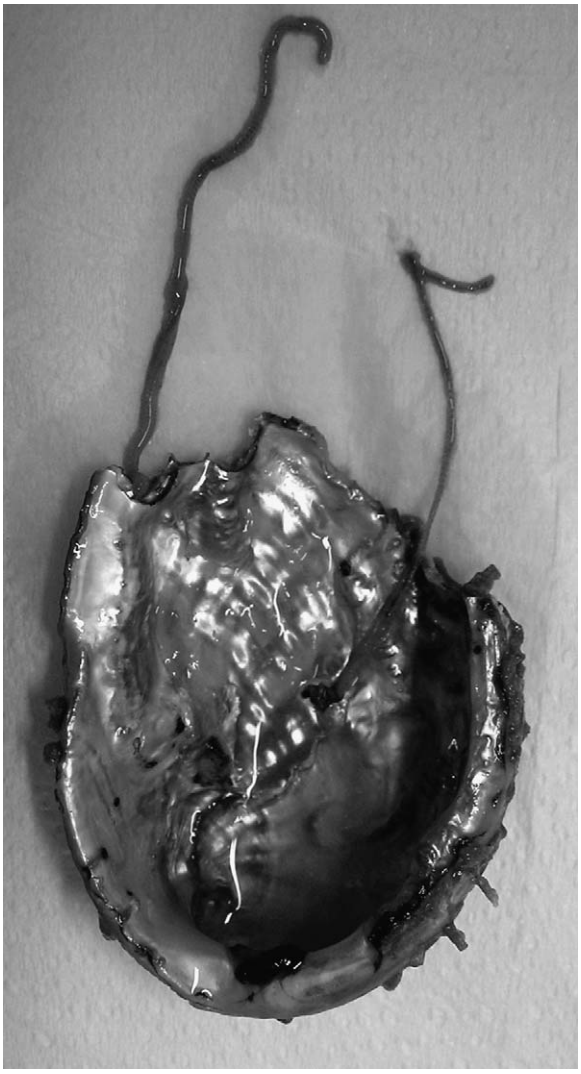

Figure 3. Typical blisters projecting into the mantle cavity as consequence of *P. hoplura* infestation in a highly infested abalone.

Treatment Against Shell-Boring Polychaetes

The initial number of shell-boring polychaetes in the abalone shells was equal ($P < 0.05$) in all replicates (Table 1). At the beginning of the trial, the results showed a positive association between the number of shell-boring polychaetes expelled and the concentration of mebendazole used (Fig. 4). Afterward, this relation disappeared and it was necessary to apply the drug 21 times over a term of 7 mo (one-time 3-h bath given for 3 consecutive days per month) to expel most shell-boring polychaetes from the abalone shells, regardless of the mebendazole concentration used.

TABLE 1.

The initial amount of *P. hoplura*, efficacy rate of *Lomper* (Esteve Laboratory, Barcelona, Spain) and abalone mortality during the course of the study.

Lomper Concentration	Initial amount of *P. hoplura*	Abalone Mortality (%)	Efficacy Rate of *Lomper* (%)
Control (0 mL/L)	185.3 ± 41.0	20.0 ± 10.0	
6 mL/L	146.3 ± 14.9	16.7 ± 5.7	99.1 ± 1.0
8 mL/L	146.7 ± 23.9	13.3 ± 15.3	98.9 ± 0.9
10 mL/L	150.0 ± 29.3	20.0 ± 0.0	98.8 ± 1.7

Values in the same column are not significantly different ($P < 0.05$) $n = 10 \times 3$.

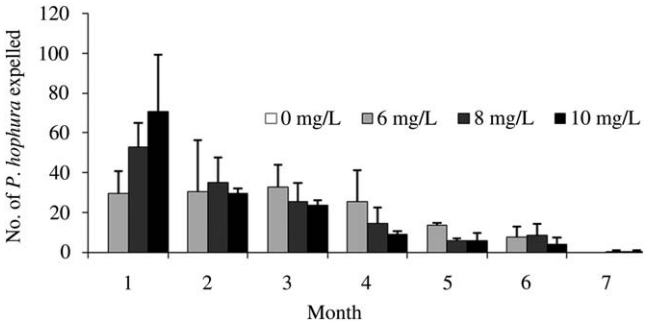

Figure 4. The number of shell-boring polychaetes expelled after 3 baths of mebendazole in each month.

The efficacy of Lomper was similar ($P < 0.05$) in all tested doses at a rate of approximately 99% (Table 1). There was no statistical difference in abalone mortality ($P < 0.05$) among the control group and the groups that were administered different concentrations of Lomper (Table 1). It is worth mentioning that mortality was only observed in highly infested animals. A similar situation was found with regard to the number of remaining shell-boring polychaetes that were expelled using the vermifuge drug. Only a few highly infested abalone still contained shell-boring polychaetes at the end of the experiment, whereas no shell-boring polychaetes were found in the abalone that originally had low-level infestations.

Growth

The size class of 40–59 mm in SL had a higher specific growth rate than the size class of 60–75 mm in SL, because these individuals were both younger and less infested (Tables 2 and 3). The SGR and WG rates did not differ statistically ($P < 0.05$) among all the mebendazole treatments and the control group (Tables 2 and 3). Thus, this result suggests that the mebendazole did not affect significantly the growth of the abalone during the period of study. However, it is worth noting that WG of the bigger abalone (SL, 60–79 mm) was positive in the control and negative in the various treatments.

DISCUSSION

The mudworm infesting *H. tuberculata coccinea* in the ICCM was identified as *P. hoplura*. This is a common pest in

TABLE 2.

Specific growth rate (SGR) and weight gain (WG) of 60–79 mm in SL of *H. tuberculata coccinea* during the experiment.

Lomper Concentration	*n*	SGR (%/day)	WG (%)
Control (0 mL/L)	10	0.01 ± 0.06	3.40 ± 14.52
6 mL/L	12	–0.02 ± 0.05	–4.82 ± 10.48
8 mL/L	11	–0.02 ± 0.04	–4.52 ± 9.96
10 mL/L	9	–0.02 ± 0.04	–4.68 ± 8.68

Values in the same column are not significantly different ($P < 0.05$).

TABLE 3.

Specific growth rate (SGR) and weight gain (WG) of 40–59 mm in SL of *H. tuberculata coccinea* during the experiment.

Lomper Concentration	*n*	SGR (%/day)	WG (%)
Control (0 ml/L)	11	0.12 ± 0.10	35.15 ± 28.54
6 mL/L	15	0.08 ± 0.11	24.00 ± 30.09
8 mL/L	11	0.03 ± 0.06	8.09 ± 16.56
10 mL/L	11	0.09 ± 0.08	25.50 ± 25.13

Values in the same column are not significantly different ($P < 0.05$).

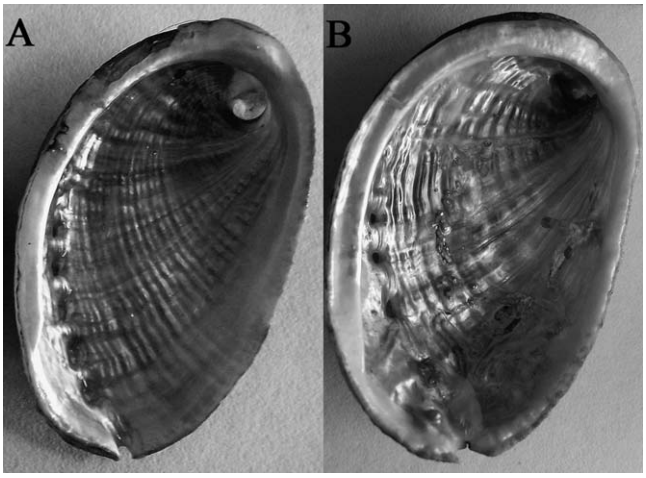

Figure 5. (A, B) Low-infested (A) and highly infested (B) abalone recovering after the treatment of mebendazole. Highly infested animals were unsightly to the consumer.

cultured abalone, as noted by several authors (Lleonart et al. 2003a, Lleonart et al. 2003b, Simon et al. 2006). This shell-boring polychaete species has been found along the coast of Europe, South Africa, New Zealand, and Australia (Ruellet 2004). In the Canary Islands, *P. hoplura* has a patchy distribution over the mesolittoral and infralittoral areas, and usually burrows into substrata containing calcium carbonate, such as stones, encrusting coralline algae, and the shells of mussels and sponges (Pascual & Núñez 1999). The current study cites, for the first time, *P. hoplura* associated with *H. tuberculata coccinea*.

The infestation develops throughout the planktonic larval stages of *P. hoplura* (Blake & Evans 1973). Because this species can reach its sexual maturity in a few months, it is capable of rapid colonization (cited in Royer et al. (2006)). Under culture conditions, as observed by other authors (Lleonart et al. 2003a, Simon et al. 2006), infestations can become sufficiently heavy to deform and even break the shell of highly infested abalone. Thus, although the meat quality is not altered by the infestation (Gray 2003), these animals lose their commercial value. No shell deformation was observed in low-infested animals according to the results obtained by Simon et al. (2004). Thus, we believe that only low-infested animals are really curable (Fig. 5).

In the current study, the efficacy rate of all the mebendazole doses tested (120 mg/L, 160 mg/L, and 200 mg/L) in multiple applications were higher (EF 98.8–99.1%) than that found by Lleonart et al. (2003b) in a single application (200 mg/L and EF 32.5%) against the shell-boring polychaete *B. knoxi* in the abalone species *H. rubra*. Therefore it can be argued that when using mebendazole, which is a broad-spectrum anthelmintic drug (Krízová-Forstová et al. 2011), multiple applications are more effective than single applications for eradicating shell-boring polychaete pests.

Although the growth rate results did not show statistical differences among the various mebendazole treatments and the control group, the bigger abalone (SL, 60–79 mm) lost weight. Taking into account that these animals were highly infested, this result confirms the observations of previous studies, which associated a high level of infestation with a loss in abalone condition (Lleonart et al. 2003a, Simon et al. 2006). This may be associated with the abalone tendency to respond to the infestation by depositing a layer of nacre over the worms to isolate them. The cost of continuously forming this layer is believed to result in a significant detriment to abalone condition (Handley 1998).

Our results suggested that the repeated application of mebendazole was more important than the concentration used. At this point, it would be interesting to test lower concentrations of the drug in multiple applications; however, it should be taken into consideration that the extended exposure to subtherapeutic dosages may result in mebendazole-resistant parasite populations (Buchmann et al. 1992). It is also noteworthy that, among the causes of parasite resistance to drugs, one of them is the presence of active anthelmintics in the natural environment of the parasite (FAO 2003, Krízová-Forstová et al. 2011). This may be a result of the disposal of drug residues directly into the environment along with the wastewater from the aquaculture facilities.

In conclusion, the current study proved that using 120 mg/L mebendazole applied 21 times for 7 mo (one-time 3-h bath given for 3 consecutive days per month) eradicated the *P. hoplura* pest in approximately 99% of highly infested abalone. However, it should be noted that the use of this type of chemical drug is a nonrenewable resource (Vial et al. 1999, Geary et al. 1999) in other words, if the parasites develop a resistance to the drug, the product will become unusable. Furthermore, total dependence on a single parasite control method has proved to be poorly sustainable and not profitable in the long term (Waller 1997, Barger 1999). Following the recommendations of the FAO (2003), it is preferable to use the anthelmintic as a timely support for a rational and sustainable control strategy and not as the exclusive tool for such purposes.

LITERATURE CITED

Barger, I. A. 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *Int. J. Parasitol.* 29:41–47.

Bilbao, A., V. Tuset, M. Viera, G. Courtois de Vicose, H. Fernández-Palacios, R. Haroun & M. Izquierdo. 2010. Reproduction, fecundity and growth of abalone (*Haliotis tuberculata coccinea*, Reeve 1846) in the Canary Islands. *J. Shellfish Res.* 29:959–967.

Blake, J. A. & J. W. Evans. 1973. Polydora and related genera as borers in mollusk shells and others calcareous substrates. *Veliger* 15:235–249.

Buchmann, K. 1993. Epidemiology and control of *Pseudodactylogyrus* infections in intensive eel culture systems: recent trends. *Bull. Fr. Pêche Piscic*. 328:66–73.

Buchmann, K. & J. Bjerregaard. 1990. Mebendazole treatment of pseudodactylogyrosis in an intensive eel-culture system. *Aquaculture* 86:139–153.

Buchmann, K., A. Roepstorff & P. J. Waller. 1992. Experimental selection of mebendazole resistant gill monogeneans from European eel *Anguilla anguilla*. *J. Fish Dis*. 15:393–400.

Cañete, R., A. A. Escobedo, P. Almirall, M. E. González, K. Brito & S. Cimerman. 2009. Mebendazole in parasitic infections other than those caused by soil-transmitted helminths. *Trans. R. Soc. Trop. Med. Hyg*. 103:437–442.

Cózar-Bernal, M. J., V. Gallardo, E. Sáez-Fernández, M. A. Holgado, J. Álvarez-Fuentes, M. Fernández-Arévalo & J. L. Arias. 2010. Role of the electrokinetic properties on the stability of mebendazole suspensions for veterinary applications. *Int. J. Pharm*. 393:161–166.

Dayan, A. D. 2003. Albendazole, mebendazole and praziquantel: review of nonclinical toxicity and pharmacokinetics. *Acta Trop*. 86:141–159.

De Villiers, M. M., R. J. Terblanche, W. Liebenberg, E. Swanepoel, T. G. Dekker & M. Song. 2005. Variable-temperature X-ray powder diffraction analysis of the crystal transformation of the pharmaceutically preferred polymorph C of mebendazole. *J. Pharm. Biomed. Anal*. 38:435–441.

FAO. 2003. Resistencia a los antiparasitarios: estado actual con énfasis en América Latina. Estudio FAO producción y sanidad animal 157. Rome: Organización de las Naciones Unidas para la agricultura y la alimentación. 51 pp.

Geary, T. G., D. P. Thompson & R. D. Klein. 1999. Mechanism-based screening: discovery of the next generation of anthelmintics depends upon more basic research. *Int. J. Parasitol*. 29:105–112.

Goven, B. A. & D. F. Amend. 1982. Mebendazole/trichlorfon combination: a new anthelmintic for removing monogenetic trematodes from fish. *J. Fish Biol*. 20:373–378.

Gray, M. 2003. Morphometrics and reproduction of *Terebrasabella heterouncinata* (Polychaeta: Sabellidae), infesting abalone (*Haliotis midae*) from different culture environments. PhD diss., Rhodes University, South Africa. 156 pp.

Handley, S. J. 1998. Power to the oyster: do spionid-induced shell blisters affect condition in subtidal oysters? *J. Shellfish Res*. 17:1093–1099.

Katharios, P., N. Papandroulakis & P. Divanach. 2006. Treatment of *Microcotyle sp*. (monogenea) on the gills of cage-cultured red porgy, *Pagrus pagrus* following baths with formalin and mebendazole. *Aquaculture* 251:167–171.

Kim, K. H. & E. S. Choi. 1998. Treatment of *Microcotyle sebastis* (monogenea) on the gills of cultured rockfish (*Sebastes schelegeli*) with oral administration of mebendazole and bithionol. *Aquaculture* 167:115–121.

Krishnaiah, Y. S. R., P. V. Raju, B. D. Kumar, P. Bhaskar & V. Satyanarayana. 2001. Development of colon targeted drug delivery systems for mebendazole. *J. Control. Release* 77:87–95.

Krízová-Forstová, V., J. Lamka, V. Cvilink, V. Hanusová & L. Skálová. 2011. Factors affecting pharmacokinetics of benzimidazole anthelmintics in food-producing animals: the consequences and potential risks. *Res. Vet. Sci*. (In press).

Kuris, A. M. & C. S. Culver. 1999. An introduced sabellid polychaete pest of cultures abalone and its potential spread to other California gastropods. *Invertebr. Biol*. 118:391–403.

Lauckner, G. 1983. Diseases of Mollusca: Bivalvia, agents: Annelida. In: O. Kinne, editor. Diseases of marine animals, vol. 2. Hamburg: Biologische Anstalt Helgoland. pp. 805–816.

Leighton, D. L. 1998. Control of sabellid infestation in green and pink abalones, *Haliotis fulgens* and *H. corrugata*, by exposure to elevated water temperatures. *J. Shellfish Res*. 17:701–705.

Lleonart, M., J. Handlinger & M. Powell. 2003a. Spionid mudworm infestation of farmed abalone (*Haliotis* spp.). *Aquaculture* 221:85–96.

Lleonart, M., J. Handlinger & M. Powell. 2003b. Treatment of spionid mud worm (*Boccardia knoxi* Rainer) infestation of cultured abalone. *Aquaculture* 217:1–10.

Mackenzie, C. L. & L. W. Shearer. 1959. Chemical control of *Polydora websteri* and other annelids inhabiting oyster shells. *Proc. Natl. Shellfish. Assoc*. 50:105–111.

Mellergaard, S. 1990. Mebendazole treatment against *Pseudodactylogyrus* infections in eel (*Anguilla anguilla*). *Aquaculture* 91:15–21.

Moore, J. D., C. I. Juhasz, T. T. Robbins & E. D. Grosholz. 2007. The introduced sabellid polychaete *Terebrasabella heterouncinata* in California: transmission, methods of control and survey for presence in native gastropod populations. *J. Shellfish Res*. 26:869–876.

Pascual, M. & J. Núñez. 1999. Poliquetos endobiontes de esponjas de la macaronesia central: Spionidae y Choetpteridae (Polychaeta: Spionida). *Rev. Acad. Canar. Cienc*. 11:121–134.

Royer, J., M. Ropert, M. Mathieu & K. Costil. 2006. Presence of spionid worms and other epibionts in Pacific oysters (*Crassostrea gigas*) cultures in Normandy, France. *Aquaculture* 253:461–474.

Ruellet, T. 2004. Infestation des coquilles d'huîtres *Crassostrea gigas* par les polydores en Basse-Normandie: recommandations et mise au point dún traitement pour réduire cette nuisance. PhD diss., Université de Caen. 536 pp.

Sato-Okoshi, W., K. Okoshi & J. Shaw. 2008. Polydorid species (Polychaeta: Spionidae) in south-western Australian waters with special reference to *Polydora uncinata* and *Boccardia knoxi*. *J. Mar. Biol. Assoc. UK* 88:491–501.

Shields, J. D., M. A. Buchal & C. S. Friedman. 1998. Microencapsulation as a potential control technique against sabellid worms in abalone culture. *J. Shellfish Res*. 17:79–83.

Simon, C. A., M. G. Bentley & G. S. Caldwell. 2010. 2,4-Decadienal: exploring a novel approach for the control of polychaete pests on cultured abalone. *Aquaculture* 310:52–60.

Simon, C. A., H. Kaiser & P. J. Britz. 2004. Infestation of the abalone, *Haliotis midae*, by the sabellid *Terebrasabella heterouncinata*, under intensive culture conditions, and the influence of infestation on abalone growth. *Aquaculture* 232:29–40.

Simon, C. A., A. Ludford & S. Wynne. 2006. Spionid polychaetes infesting cultured abalone *Haliotis midae* in South Africa. *Afr. J. Mar. Sci*. 28:167–171.

Szekely, C. & K. Molnar. 1990. Treatment of *Ancylodiscoides vistulensis* monogenean infestation of the European catfish (*Silurus glanis*). *Bull. Eur. Assoc. Fish Pathol*. 10:74–77.

Vial, H. J., M. Traore, A. H. Fairlamb & R. G. Ridley. 1999. Renewed strategies for drug development against parasitic diseases. *Parasitol. Today* 15: 393–394.

Waller, P. J. 1997. Sustainable helminth control of ruminants in developing countries. *Vet. Parasitol*. 71:195–207.

Zar, J. H. 1996. Biostatistical analysis, 3rd edition. Upper Saddle River, NJ: Prentice Hall. 662 pp.