AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

In vivo treatment of the gill monogenean *Pseudorhabdosynochus lantauensis* (Monogenea, Diplectanidae) on orange-spotted grouper (*Epinephelus coioides*) cultured in the Philippines

[1]Erlinda R. Cruz-Lacierda, [2]April Joy T. Pineda, and [3]Kazuya Nagasawa

[1] College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines; [2] Department of Agriculture, Miag-ao, Iloilo 5023, Philippines; [3] Graduate School of Biosphere Science, Hiroshima University, 1-4-4, Kagamiyama, Higashi-Hiroshima 739-8528, Japan. Corresponding author: E. R. Cruz-Lacierda, eclacierda@yahoo.com

Abstract. The gill monogenean *Pseudorhabdosynochus lantauensis* has been consistently observed on cultured orange spotted-grouper (*Epinephelus coioides*) fingerlings. The tolerance level of *E. coioides* to formalin (H_2CO solution) and hydrogen peroxide (H_2O_2), two of the chemicals most commonly used to treat ectoparasites, was determined. The computed 3h and 6h LC10 of formalin were 322 mg L^{-1} and 275 mg L^{-1} while the 9h LC10 and LC50 of H_2O_2 were 255 and 298 mg L^{-1}, respectively, at 28°C. The efficacy of freshwater, hypersalinity, formalin and H_2O_2 bath treatment against *P. lantauensis* was determined *in vivo*. Hydrogen peroxide at 200 mg L^{-1} for 1h was effective in eliminating *P. lantauensis* on *E. coioides* fingerlings with no apparent adverse effect on the host fish.
Key Words: grouper, formalin, hydrogen peroxide, freshwater, hypersalinity.

Introduction. In the Philippines, the orange-spotted grouper *Epinephelus coioides* (Hamilton, 1822) is the main grouper species cultured, with the brown-marbled grouper *Epinephelus fuscoguttatus* (Forsskal, 1775) coming a close second (APEC/SEAFDEC 2001). Although technology for seed production of groupers is available, there is still dependence on wild-caught seeds. The grow-out farming of groupers is conducted in floating net cages or earthen ponds.

One parasitic problem consistently observed in grouper culture is the infestation with the gill monogenean *Pseudorhabdosynochus lantauensis* (Beverley-Burton & Suriano, 1981) (Erazo-Pagador & Cruz-Lacierda 2010) (Figure 1a). In this paper, we report the efficacy of freshwater, hypersalinity, formalin and hydrogen peroxide (H_2O_2) bath treatment against this gill parasite. The tolerance level of *E. coioides* to formalin and H_2O_2 is also presented.

Materials and Methods

Fish safety tests. *E. coioides* fingerlings from a commercial farm in Roxas City, Capiz, Philippines were transported live to the laboratory of SEAFDEC Aquaculture Department. The fish were maintained under laboratory conditions for at least 3 days prior to use. Ten fish were sacrificed and measured for total length (TL, mm) and body weight (BW, g) per batch of fish prior to each experiment.

Two trials each of 24h static bioassay tests following the protocols of APHA (1980) were conducted to determine if formalin and H_2O_2 were safe for use on *E. coioides* fingerlings (BW = 1-3 g; TL = 59-76 mm). Fish were randomly assigned to glass aquaria containing 5 liters of seawater provided with aeration. Each aquarium contained ten fish. After a 1 h acclimatization period, different concentrations of formalin or H_2O_2 were

introduced to each aquarium. Five test concentrations per chemical plus a control (seawater only) were conducted per trial. All test concentrations including the control (seawater only) were done in three replicates per trial. Therefore, a total of 18 aquaria containing ten fish each or a total of 180 fish were exposed to different concentrations of either formalin or H_2O_2 per trial. Fish were observed at regular interval for behavioral changes and mortality. The median lethal concentration (LC50) values at different exposure periods were computed using the Reed-Muench (1938) method.

In vivo treatment of P. lantauensis. Apparently healthy *E. coioides* fingerlings collected from a net cage farm in Roxas City, Capiz and heavily infected with the gill monogenean *P. lantauensis* (Figure 1b) were transported live to the laboratory. The fish were fed with frozen chopped trash fish and maintained under laboratory conditions for two days. The approximate mean intensity of infection of the monogenean on gill filaments was determined a few hours prior to the experiment by sacrificing ten fish per batch of fish per treatment by dissecting the gill arches and counting the parasites from each gill arch under a stereomicroscope. The prevalence and mean intensity of infection were determined following Bush et al (1997).

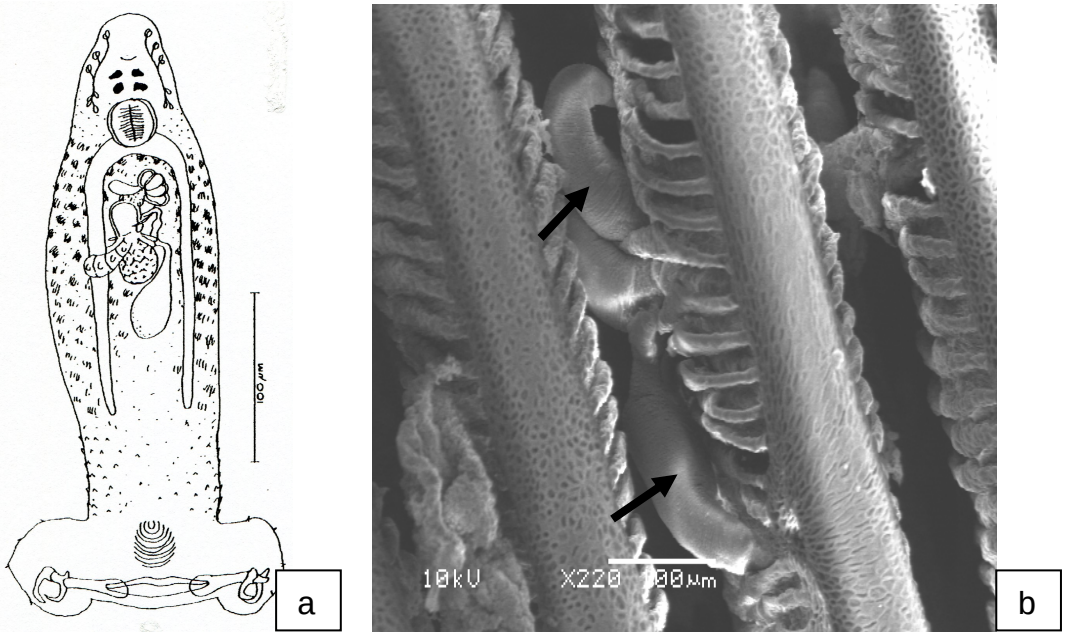

Figure 1. *P. lantauensis*: (a) entire ventral view, scale bar=100µm (source: Erazo-Pagador & Cruz-Lacierda 2010); (b) scanning electron micrograph of gills of *E. coioides* with several *P. lantauensis* (arrows) attached in between gill lamellae, scale bar=100µm.

Five fish each were randomly stocked in glass aquaria containing 5 liters of seawater provided with aeration. After a 1h acclimatization period, various treatments including freshwater, hypersalinity, formalin and H_2O_2 were introduced to each aquarium. After the desired treatment period, fish were transferred and maintained for 24h in separate labeled aquaria containing clean static seawater and provided with aeration. All fish were then sacrificed 24h after the treatment and the gills were examined microscopically to determine the effect of individual treatment on the parasites. Efficacy of the treatment was evaluated by the number of remaining attached live monogeneans on the gills of treated fish against untreated fish. Three separate trials were conducted for H_2O_2, two trials each for freshwater and formalin, and one trial for hypersaline treatments. All treatments were done with three replicates per treatment including the control (seawater only). Data were analyzed after log transformation using analysis of variance and Duncan's multiple range test (DMRT).

Results and Discussion. Diseases including parasite problems have become a major production constraint in grouper aquaculture. Aside from the actual health status of the cultured fish, they are also subjected to considerable stress during regular monitoring and handling procedures, harvest and transportation. These can be avoided through proper management of the cultured species through regular surveillance, monitoring and record keeping of health status of cultured stocks.

Although grouper culture is widespread in Asia and technology for hatchery production of grouper fry is available, seed stocks for grow-out culture are almost always wild-caught (APEC/SEAFDEC 2001). It has been previously observed that wild-caught grouper seeds are already infected with parasites prior to stocking in nursery and grow-out facilities (Leong & Wong 1988, 1995). It is also a common practice, especially in Southeast Asia, among net cage grow-out operators to stock overlapping generations of grouper, although in different net cages, but in the same farm site receiving the same water source (Leong 1997). Thus, cross-contamination among net cage-farmed groupers in the same area is most likely to happen.

A heavy monogenean population can be a potential source of stress and has been associated with outbreaks of vibriosis (Leong 1992; Chua et al 1993). In Japan, the gill monogenean *Pseudorhabdosynochus epinepheli* (Yamaguti, 1938) has caused mortalities in cultured red-spotted grouper *Epinephelus akaara* (Temminck & Schlegel, 1842) juveniles with occasional secondary infection with *Vibrio* spp. (Isshiki et al 2007).

The toxicity of chemicals to fish can vary depending on the species and treatment conditions. Thus, the tolerance of fish to chemicals should be determined prior to chemotherapy. Table 1 shows the survival rate of grouper exposed to 100-300 mg L^{-1} formalin for 3-24h. At 250 mg L^{-1} formalin, survival rate was 100% even after 9h of exposure. The computed 3h and 6h lethal concentration at 10% (LC10) were 322 and 275 mg L^{-1} formalin respectively, at an experimental water temperature and salinity of 28°C and 31 ‰. Table 2 shows the mean lethal concentrations of H_2O_2 to grouper at different exposure times. The 9h LC10 and LC50 of H_2O_2 to grouper were 255 and 298 mg L^{-1}, respectively, at 28°C and 33 ‰.

Table 1

Survival rate of orange-spotted grouper, *E. coioides*, fingerlings[a] exposed to various concentrations of formalin at different exposure periods

Concentration of formalin[b] (mg L^{-1})	*Survival (%)/Duration (h)[c]*				
	3	*6*	*9*	*12*	*24*
0 (No formalin)	100.0	100.0	100.0	100.0	83.3
100	100.0	100.0	100.0	100.0	76.6
150	100.0	100.0	100.0	96.6	70.0
200	100.0	100.0	100.0	93.3	60.0
250	100.0	100.0	100.0	86.6	30.0
300	96.6	83.3	70.0	63.3	13.3

[a] body weight = 1–3 g; total length = 59-76 mm; [b] 3 replicates/treatment; n = 10 fish/replicate/trial; 28ºC; 31 ‰ ; [c] mean of 2 trials.

Table 2

Mean lethal concentration (LC, in mg L^{-1}) of hydrogen peroxide (H_2O_2) for orange spotted grouper, *E. coioides*, fingerlings[a] at various exposure times

Exposure time (h)	*Lethal concentration (mg L^{-1})[b]*	
	LC10	*LC50*
9	255	298
12	206	262
24	150	214

[a] body weight = 1–3 g; total length = 59-76 mm; [b] mean of 2 trials with 3 replicates/treatment/trial; n=10 fish/replicate/trial; 28ºC, 33 ‰.

Acute toxicity studies on formalin and H_2O_2 have been conducted for several freshwater and marine fish (Gaikowski et al 1999; Fajer-Avila et al 2003). The reported 24h LC50 of formalin on milkfish *Chanos chanos* (Forsskal, 1775) fingerlings was 322 mg L^{-1} at 27°C and 32 ‰ (Cruz & Pitogo 1989) and the 12h LC50 of formalin on sea bass *Lates calcarifer* (Bloch, 1790) fingerlings was 273 mg L^{-1} at 27-29°C and 30 ‰ (Pascual et al 1994). Based on the reported LC50 values at different exposure periods, grouper exhibit more sensitivity to formalin and H_2O_2 than milkfish and sea bass.

Tables 3-6 show the efficacy of freshwater, hypersalinity, formalin and H_2O_2 bath treatment against *P. lantauensis* on grouper at different concentrations and exposure times. The 60-120 min freshwater bath treatment reduced the monogenean load by 85-93% (Table 3). However, these treatment durations were stressful to the fish and affected their survival. On the other hand, the 30 min freshwater treatment was not efficacious to remove the parasite, resulting in only 50% reduction of monogenean burden. Hypersaline treatment at 60 ‰ for 60 min reduced the monogenean load by up to 67% (Table 4) only, but salinities of over 45 ‰ were lethal to the fish. All the other hypersaline treatments tested were not effective. The 250 mg L^{-1} formalin 1h bath treatment reduced the monogenean load by up to 100% (Table 5) but affected survival of treated fish. Other treatments including 30 mg L^{-1} formalin for 24h (data not shown) were not effective. A 100% reduction in monogenean load was observed in fish treated for 1h with 200-300 mg L^{-1} H_2O_2 (Table 6) with no apparent adverse effect on treated fish. Similarly, 150 mg L^{-1} H_2O_2 for 1h reduced monogean burden by 96% without any apparent adverse effect on treated fish. Although 100 mg L^{-1} H_2O_2 did not result to any mortality on treated fish, it was not efficacious to remove the parasite.

The present study showed that freshwater, hypersalinity and formalin bath treatments were not efficacious against *P. lantauensis*. Although 1h freshwater and 250 mg L^{-1} formalin can significantly reduce monogenean burden, it may have an adverse effect on the treated fish. Further in this study, *P. lantauensis* on *E. coioides* can be effectively controlled using 200 mg L^{-1} H_2O_2 for 1h at a water temperature of 28°C with no apparent adverse effect on the host fish.

Table 3

Efficacy of freshwater bath treatment at various exposure periods against *P. lantauensis* infestation in orange spotted grouper, *E. coioides*, fingerlings[a]

Duration of freshwater treatment (min)	*Mean intensity*[+] *± SD*	*Efficacy of treatment (%)*	*Fish survival (%) 24h after treatment*
Control	469[a] ± 20	0.0	93.5
30	218[ab] ± 22	51.0	96.5
60	65[b] ± 5	84.5	88.5
120	50[b] ± 5	92.5	92.0

[a] mean body weight, g (range) = 4.5 (2-8); mean total length, mm (range) = 64.5 (52-80); [+] Monogenean, mean of 2 trials with 3 replicates per treatment per trial, 5 fish/replicate; means with the same superscript in a column are not significantly different (p = 0.05); SD, standard deviation; [*]28.5ºC, 30 ‰ .

Table 4

Efficacy of hypersalinity at various exposure periods against *P. lantauensis* infestation in orange-spotted grouper, *E. coioides, fingerlings*[a]

Salinity (‰)/Duration (min)	*Mean intensity*[+] *± SD*	*Efficacy of treatment (%)*	*Fish survival (%) 24h after treatment*
30 (Control)*	402[a] ± 93	0.0	90.0
45/(30)	391[a] ± 73	1.0	100.0
60/(30)	296[a] ± 26	7.0	0.0
60/(60)	107[b] ± 21	67.0	0.0

[a] mean body weight, g (range) = 3 (2-6); mean total length, mm (range) = 60 (50-80); [+] Monogenean, mean of 3 replicates per treatment, 5 fish/replicate; means with the same superscript in a column are not significantly different (p = 0.05); SD, standard deviation; [*]28ºC.

Table 5

Efficacy of 1-hour formalin bath treatment against *P. lantauensis* infestation in orange-spotted grouper, *E. coioides*, fingerlings[a]

Concentration of formalin (mg L^{-1})	*Mean Intensity*[+] *± SD*	*Efficacy of treatment (%)*	*Fish survival (%) 24h after treatment*
0 (no formalin)*	469[a] ± 20	0.0	93.5
100	400[a] ± 21	17.5	87.0
250	3[b] ± 1	99.5	90.0

[a] mean body weight, g (range) = 4.5 (2-6); mean total length, mm (range) = 65 (52-78); [+] Monogenean, mean of 2 trials with 3 replicates per treatment per trial, 5 fish/replicate; means with the same superscript in a column are not significantly different (p = 0.05); SD, standard deviation; *27.5ºC, 30‰.

Table 6

Efficacy of 1 h hydrogen peroxide (H_2O_2) bath treatment against *P. lantauensis* infestation in orange-spotted grouper, *E. coioides*[a], fingerlings

Concentration of H_2O_2 (mg L^{-1})	*Mean Intensity*[+] *± SD*	*Efficacy of treatment (%)*	*Fish survival (%) 24h after treatment*
0 (no H_2O_2)*	367[a] ± 15	0.0	95.0
100	248[a] ± 8	38.3	100.0
150	20[b] ± 2	96.0	100.0
200	0[c]	100.0	100.0
250	0[c]	100.0	100.0
300	0[c]	100.0	100.0

[a] mean body weight, g (range) = 5.3 (2-19); mean total length, mm (range) = 70.5 (52-115); [+] Monogenean, mean of 3 trials with 3 replicates per treatment per trial, 5 fish/replicate; means with the same superscript in a column are not significantly different (p = 0.05); SD, standard deviation; *28ºC, 30‰.

Liang & Leong (1992) reported that freshwater and 300 mg L^{-1} formalin for 30min was effective in reducing the intensity of the monogenean *Haliotrema* sp. on golden snapper *Lutjanus johni* (Bloch, 1792). However, they also reported that fish exhibited stress signs towards the end of the treatment period. Zafran et al (1998) reported that a 1-day bath treatment of 30 mg L^{-1} formalin was effective in the control of the gill monogeneans *Pseudorhabdosynochus* sp. and *Haliotrema* sp. on humpback grouper *Cromileptis altivelis* (Valenciennes, 1828). However in this study, 30 mg L^{-1} formalin for 24h was not effective in removing *P. lantauensis* from *E. coioides* (data not shown). Isshiki et al (2007) reported that H_2O_2 has potential for controlling the gill monogenean *P. epinepheli* infection on *E. akaara*. The recommended levels were 700 mg L^{-1} H_2O_2 for 15 min at 20-25°C or for 45 min at 10-15°C.

A relatively short exposure of 1h in 200 mg L^{-1} H_2O_2 is sufficient to effectively remove the monogenean burden without any apparent adverse effect on treated fish. The differences in toxicity between *E. coioides* and *P. lantauensis* indicate that H_2O_2 is efficacious in the control of the parasite in grouper culture.

The use of freshwater, hypersalinity, formalin and H_2O_2 to treat marine ectoparasites has been described in various reports (Yoshinaga et al 2000a, 2000b; Mansell et al 2005; Katharios et al 2006; Sitja-Bobadilla et al 2006; Isshiki et al 2007). Although formalin can effectively control parasites its negative effects on the environment as well as the health and safety risks to users are problematic (Wooster et al 2005; Sitja-Bobadilla et al 2006). Hydrogen peroxide has an environmental advantage as it breaks down to water and oxygen (Treasurer & Grant 1997). It is also considered as a Low Regulatory Priority (LRP) therapeutant by the US Food and Drug Administration and it appears to have no undesirable breakdown product. Its current application in aquaculture may be extended (Rach et al 1997).

Most parasitic diseases of cultured fish are management-related problems and can be prevented through practice of good husbandry and management techniques, including the responsible use of chemotherapeutants. It has been mentioned that in Southeast Asia fishpond and cage-culture operator practice multi-species farming at high stocking

density, with overlapping generations of fish, and no fallowing. Because of this, a practical approach to prevention and control of these diseases should be developed. This can be done by evaluation and improvements in existing management practices and determine if future disease outbreaks can be prevented by adopting changes in culture methods. The choice of an appropriate, practical and environmentally-sound treatment should go hand-in-hand with accurate disease diagnosis. On-site cage treatments for parasite should combine the use of therapeutants with net changes and cleaning to avoid fouling organisms that will enhance the proliferation of parasites.

Conclusions. Results of this study suggest that hydrogen peroxide at 200 mg L^{-1} for 1h is effective in eliminating the gill monogenean *P. lantauensis* on *E. coioides* fingerlings with no apparent adverse effect on the host fish. Freshwater and 250 formalin mg L^{-1} bath treatment for 1h can significantly reduce monogenean burden, but may affect survival of treated fish. Also, hypersalinity is not efficacious against *P. lantauensis*. The safe level of chemicals to fish should be determined prior to chemotheraphy.

Acknowledgements. This study was funded by the Government of Japan-Trust Fund under the Regional Fish Disease Project of SEAFDEC Aquaculture Department. The authors are grateful to the following: Dr. Yasuo Inui, former Fish Disease Expert of the Project, for his support and encouragement; and Helen S. Marcial and Sharon Ann G. Pedrajas for technical assistance during the early part of the study.

References

APHA, 1980 Standard Methods for the Examination of Water and Wastewater, 15th edition. American Public Health Association, Washington, D. C., 1134 pp.

APEC/SEAFDEC, 2001 Husbandry and Health Management of Grouper. APEC, Singapore and SEAFDEC, Iloilo, Philippines, 94 pp.

Bush A. O., Lafferty K. D., Lotz J. M., Shostak A. W., 1997 Parasitology meets ecology on its own terms: Margolis *et al*. revisited. J Parasitol 83:575-583.

Chua F., Loo J. J., Wee J. Y., Ng M., 1993 Findings from a disease survey: an overview of the marine fish disease situation in Singapore. Singapore J Pri Ind 21:26-37.

Cruz E. R., Pitogo C. L., 1989 Tolerance level and histopathological response of milkfish (*Chanos chanos*) fingerlings to formalin. Aquaculture 78:138-145.

Erazo-Pagador G., Cruz-Lacierda E. R., 2010 The morphology and life cycle of the gill monogenean (*Pseudorhabdosynochus lantauensis*) on orange-spotted grouper (*Epinephelus coioides*) cultured in the Philippines. Bull Eur Ass Fish Pathol 30:55-64.

Fajer-Avila E. J., de la Parra I. A., Zarate G. A., Arce R. C., Ramirez J. Z., Lozano M. B., 2003 Toxicity of formalin to bullseye puffer fish (*Sphoeroides annulatus* Jenyns, 1843) and its effectiveness to control ectoparasites. Aquaculture 23:41-50.

Gaikowski M. P., Rach J. J., Ramsey R. T., 1999 Acute toxicity of hydrogen peroxide treatments to selected lifestages of cold-, cool- and warmwater fish. Aquaculture 178:191-207.

Isshiki T., Nagano T., Milki K., 2007 Occurrence of a monogenean gill parasite *Pseudorhabdosynochus epinepheli* on red spotted grouper *Epinephelus akaara* and its experimental treatment by hydrogen peroxide bathing. Fish Pathol 42:71-74.

Katharios P., Papandroulakis N., Divanach P., 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.

Leong T. S., 1992 Diseases of brackishwater and marine fish cultured in some Asian countries. In: Diseases in Asian Aquaculture I. Shariff M., Subasinghe R. P., Arthur J. R. (eds.), Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 223-236.

Leong T. S., 1997 Control of parasites in cultured marine finfishes in Southeast Asia – an overview. Int J Parasitol 27:1177-1184.

Leong T. S., Wong S. Y., 1988 A comparative study of the parasite fauna of wild and cultured grouper (*Epinephelus malabaricus* Bloch and Schneider) in Malaysia. Aquaculture 68:203-207.
Leong T. S., Wong S. Y., 1995 Parasites of grouper, *Epinephelus suillus*, from Pulau Langkawi and Kelantan, Malaysia. J Bioscience 6:54-57.
Liang K. S., Leong T. S., 1992 Treatment of cultured golden snapper, *Lutjanus johni* Bloch, infected with monogeneans. Aquaculture 106:1-8.
Mansell B., Powell M. D., Ernst I., Nowak B., 2005 Effects of the gill monogenean *Zeuxapta seriolae* (Meserve, 1938) and treatment with hydrogen peroxide on pathophysiology of kingfish, *Seriola lalandi* Valenciennes, 1833. J Fish Dis 28:253-262.
Pascual F. C., Tayo G. T., Cruz-Lacierda E. R., 1994 Acute toxicity of formalin to seabass (*Lates calcarifer*). In: The Third Asian Fisheries Forum. Chou L. M., Munro A. D., Lam T. J., Chen T. W., Cheong L. K. K., Ding J. K., Hooi K. K., Khoo H. W., Phang V. P. E., Shim K. F., Tan C. H. (eds.), Asian Fisheries Society, Manila, Philippines, pp. 346-348.
Rach J. J., Schreier T. M., Howe G. E., Redman S. D., 1997 Effects of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. Prog Fish-Cult 59:41-46.
Reed L. J., Muench H., 1938 A simple method of estimating fifty percent endpoints. Amer J Hyg 27:493-497.
Sitja-Bobadilla A., de Felipe M. C., Pellitero P. A., 2006 *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). Aquaculture 261:856-864.
Treasurer J. W., Grant A., 1997 The efficacy of hydrogen peroxide for the treatment of farmed Atlantic salmon, *Salmo salar* L. infested with sea lice (Copepoda:Caligidae). Aquaculture 148:265-275.
Wooster G. A., Martinez C. M., Bowser P. R., 2005 Human health risks associated with formalin treatments used in aquaculture: initial study. N Am J Aquac 67:111-113.
Yoshinaga T., Kamaishi T., Segawa I., Yamamoto E., 2000a Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. Fish Pathol 35:85-88.
Yoshinaga T., Kamaishi T., Segawa I., Yamamoto E., 2000b Effects of NaCl-supplemented seawater on the monogenean, *Neoheterobothrium hirame*, infecting the Japanese flounder. Fish Pathol 35:97-98.
Zafran R. D., Koesharyani I., Johnny F., Yuasa K., 1998 Manual for fish diseases diagnosis: marine fish and crustacean diseases in Indonesia. Gondol Research Station for Coastal Fisheries, Gondol, Indonesia, 44 pp.

Received: 11 September 2012. Accepted: 14 September 2012. Published online: 25 September 2012.
Authors:
Erlinda R. Cruz-Lacierda, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miag-ao, Iloilo 5023, Philippines, e-mail: eclacierda@yahoo.com
April Joy T. Pineda, Department of Agriculture, Miag-ao, Iloilo 5023, Philippines, e-mail: joyjoy_96@yahoo.com
Kazuya Nagasawa, Graduate School of Biosphere Science, Hiroshima University, 1-4-4, Kagamiyama, Higashi-Hiroshima 739-8528, Japan, e-mail: ornatus@hiroshima-u.ac.jp

How to cite this article:
Cruz-Lacierda E. R., Pineda A. J. T., Nagasawa K., 2012 *In vivo* treatment of the gill monogenean *Pseudorhabdosynochus lantauensis* (Monogenea, Diplectanidae) on orange-spotted grouper (*Epinephelus coioides*) cultured in the Philippines. AACL Bioflux 5(5):330-336.