Effectiveness of hydrochloric acid to control sea leeches (*Zeylanicobdella arugamensis*) infection on hybrid grouper fish (*Ephinephelus fuscoguttatus* x *Ephinephelus lanceolatus*)

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Abstract. Grouper fish is a mariculture commodity with a significant market value worldwide. The purpose of this study was to determine the efficacy of hydrochloric acid (HCl) in overcoming parasitic infections of sea leeches (*Zeylanycobdella arugamensis*) in vitro and in vivo, as well as the concentration and duration of safe (non-lethal) soaking on hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*). The present study used commercial HCl (32 and 36%) at concentrations of 0, 50, 100, 200, 300, 400, and 500 ppm. The sea leeches utilized were collected from naturally infected hybrid groupers, while the test fish were obtained after a month of cohabitation with leech-infected groupers. In vitro studies were performed on sea leeches (2 replications) and their eggs, which soaked in a petri dish for 15-120 minutes. Each of the 12 test specimens (in vivo) kept in plastic containers was exposed to HCl concentrations of 0, 100, 200, 300, 400, and 500 ppm. Furthermore, three fish from each treatment were taken every 15 minutes (15, 30, 45, and 60 minutes), and the condition of the fish and the sea leeches remaining attached to the fish's body was observed. In vitro and in vivo experiments revealed that soaking in 36% HCl at a concentration of 300 to 500 ppm for 15 to 60 minutes was highly effective in releasing and killing sea leeches. When compared to higher HCl concentrations (400 and 500 ppm), HCl at 300 ppm had no harmful effects on hybrid grouper. HCl released and killed sea leeches by lowering the pH of the soaking water to <4 at concentrations ≥300 ppm.

Key Words: hybrid grouper, HCl, *Zeylanycobdella arugamensis*, in vitro, in vivo.

Introduction. Grouper is a mariculture commodity with a significant market value both locally and globally (Prakasa & Perbani 2021). According to the Ministry of Maritime Affairs and Fisheries in Indonesia, grouper raising cultivation fisheries will produce 12,239.52 tons in 2022 with a total production value of 83.39 USD. Bali, a grouper farming center, recorded a production volume of 483.35 tons (3.95%) in 2022, with a production value of 1.65 USD (1.98%) (KKP 2024). The potential of marine aquaculture in North Bali, Indonesia, is constantly being increased by utilizing the unexplored potential of marine aquaculture, one of which being the grouper fish, particularly the hybrid one.

Hybrid grouper (*Epinephelus* sp.), which is the result of hybridization between tiger grouper, *Ephinephelus fuscoguttatus* (female parent), and giant grouper, *Ephinephelus lanceolatus*, is a valuable farming item (Fan et al 2020). However, significant challenges remain in the development of hybrid grouper farming, such as the infection with harmful microbes, which can result in mass mortality of grouper fish in hatcheries and floating net cages. Infections with the VNN virus (viral nervous necrosis) and iridovirus (GSDIV: grouper sleepy disease iridovirus) are diseases that frequently result in widespread mortality in grouper farming. These viral infections are typically followed by an increase in the population of *Vibrio* spp. (Mahardika et al 2020). *Vibrio* sp. infections can result in wounds or ulcers. Vibrio species that are pathogenic include *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio ordalii* and *Vibrio salmonicida* (Austin & Austin 2007). Aside from infections, parasitic diseases are also a challenge in grouper cultivation. Sea leeches (Hirudinea: *Zeylanicobdella arugamensis*) are the most abundant parasites with a frequency of 100%, followed by *Benedenia* sp., *Trichodina* sp., gill worms (*Pseudorabdosynocus* sp.), *Lepeophtheirus* sp., and *Cryptocaryon irritans* (Zafran et al 2019). The prevalence and intensity of leeches varies between species and sizes (Murwantoko et al 2018).

Sea leeches have a macroscopic size, measuring 0.5-3 cm, allowing for direct observation of massive parasitic infestations. Sea leeches typically target fish body parts such as skin, fins, operculum, and gills (Kua et al 2010; Mahardika et al 2021). Massive sea leech infections cause fish to lose their appetite, swim weakly, and thin out. Fish infected with sea leeches seem hairy, which reduces their economic value (Mahardika et al 2018). Adult leeches migrate away from their hosts and lay eggs within the cultivation facility. The attachment of sea leeches to the fish's body causes scars, which can eventually become into ulcers owing to bacterial infections such as *Vibrio* sp. (Kua et al 2010; Kua et al 2014).

Controlling marine leech infections is critical to maintaining fish healthy and increasing their economic value. The rapid propagation of parasites and the risk of transmitting parasites from one farm to another, and even to wild fish, make the parasites control challenging. Multiple types of management programs are required, which may include chemotherapy and medications as the first and best option for farmers (Buchmann 2022). Formalin, peracetic acid, potassium permanganate, and sodium chloride are common disinfectants and antiparasitic compounds used in aquaculture (Svobodova et al 2007; Valeta et al 2016; Lanikova et al 2021). The malachite green is also widely utilized in aquaculture, although the resulting residue of chemicals is toxic and may endanger the health of fish and humans (Hashimoto et al 2011). Formalin, chlorine, ivermectin, cupric sulfate, and citric acid soaking for 60 minutes have been shown to kill *Z. arugamensis* and its cocoons up to 100%, however high levels are still harmful to groupers (Mahardika et al 2019; Mahardika et al 2021; Mustikasari et al 2023).

Hydrochloric acid (HCl) is commonly utilized in the fishery industry as a disinfectant for equipment and facilities. Hydrochloric acid is a monoprotic acid in liquid form formed up of hydrogen chloride gas. HCl is utilized in a variety of industrial processes, including metal processing, steel pickling, organic compound manufacturing, pH control, and neutralization, as well as on a smaller scale in leather processing, table salt refining, and general cleaning (Isnanto 2023). In Pakistan, HCl is commonly used as an anti-coccidia drug in the poultry industry. Low-dose HCl (1,000 ppm) could be utilized as an alternative chemotherapeutic medication to treat coccidiosis (*Eimeria tenella*) (Abbas et al 2011). The stomach's hydrochloric acid helps defend the body against infections (Smith 2003). The purpose of this study was to assess the efficacy of HCl as an alternate chemical for controlling *Z. arugamensis* in vitro and in vivo.

Material and Method

General context of the experiment. This study was conducted from March to June 2023 at the Pathology Laboratory, Marine Biota Scientific Conservation Area (MBSCA), National Research and Innovation Agency (NRIA), Gondol, Penyabangan Village, Gerokgak District, Buleleng Regency, Bali, Indonesia. The *Z. arugamensis* used in this study originated from sick grouper fish stored in the Pathology Laboratory at MBSCA-NRIA. Meanwhile, the grouper for the in vivo experiment obtained from the MBSCA-NRIA hatchery.

HCl solution. The HCl solution used was technical HCl with a concentration of 32% in the in vitro test replication 1, and pure HCl with a concentration of 36% (and a molecular weight of 36.46 g mol^{-1}) (Wako, Japan) for the in vitro test replication 2, hatchability sea leech eggs and in vivo tests. The concentration of pure HCl in this study was equated to 100%. In the in vitro test, HCl was taken using a micropipette measuring 10 and 100 µL (0.01 and 0.1 mL, Dragon Lab) in amounts of 0.02, 0.04, 0.06, 0.08 and 0.1 mL, respectively. Each HCl was added to five 500 mL Erlenmeyer (Pyrex®, Iwaki) that had been filled with 200 mL seawater using a 10 mL pipette (Dragon Lab). Before adding HCL to each Erlenmeyer, the sea water is reduced by 0.02, 0.04, 0.06, 0.08, and 0.1 mL, resulting in final concentrations of 100, 200, 300, 400, and 500 ppm. Meanwhile, one Erlenmeyer was filled with 200 mL of sea water as a control (0 ppm). In the in vivo test. The HCl solution was prepared by dissolving 10, 20, 30, 40, and 50 mL of pure HCL in seawater (99.9, 99.8, 99.7, 99.6, and 99.5 L) in a plastic container volume of 120 L.

Test fish infected with sea leeches. Hybrid groupers infected with sea leeches for in vivo testing were obtained using the cohabitation method. A total of 72 healthy hybrid grouper fish were placed in four 100 L plastic containers (18 fish per container), each equipped with aeration and a stagnant water system. Subsequently 6 hybrid groupers infected with leeches were put in each container, in a 3:1 ratio. Cohabitation ran for 30 days.

In vitro test. The in vitro test was repeated twice (replications 1 and 2 with varying soaking times. Each of 18 petri dishes (diameter 8 cm) was filled directly with seawater. Sea leeches that attached to and infected the hybrid grouper fish were carefully removed with gloves on and deposited in a petri dish. Each petri dish was filled with 50 sea leeches. The petri dishes were left for 10-15 minutes until the leeches adhered closely to the bottom surface. The leeches were then rinsed three times with seawater to eliminate the fish mucus carried by the leech as well as the weak leeches that floated in the water. At the 1st replication, the sea water in each of the three petri dishes filled with sea leeches was replaced with an HCl solution according to the treatment (0 ppm-direct sea water, 50, 100, 200, 300, and 500 ppm). The 2nd replication also used HCl solutions at concentrations of 0, 100, 200, 300, 400, and 500 ppm. The ten sea leeches from each petri dishes in all treatments (0-500 ppm) were moved into a new petri dish filled with sea water using tweezers, every 30 minutes to 120 minutes in replication 1, and every 15 minutes to 60 minutes in replication 2. *Z. arugamensis*' response and survival were observed 60 minutes after being transferred to the small petri dish to allow them to recover.

In vitro assays were also performed to determine the hatchability of *Z. arugamensis* eggs after soaking in HCl for 30 and 60 minutes. A total of ten petri dishes were filled with 50-60 *Z. arugamensis*. All petri dishes were incubated for three days at 29-30°C to allow the sea leeches to lay eggs. *Z. arugamensis* were then removed from the dish with tweezers, leaving only the eggs. Sea water from each of the two petri dishes containing sea leech eggs was replaced with HCl solution at concentrations of 0, 100, 200, 300, 400, and 500 ppm. Soaking in HCl solutions of different concentrations was done for 30 and 60 minutes. After 30 and 60 minutes, the HCl solution from each petri dish was replaced with seawater. Then, all petri dishes were incubated at 29-30°C, with the seawater refilled daily. For 15 days, observations were taken on the development and number of hatched eggs. Water quality indicators such as pH, temperature, and salinity were measured in two petri dishes for each treatment.

In vivo test. A total of six 120 L plastic containers filled with seawater and an HCl solution at concentrations of 0, 100, 200, 300, 400, and 500 ppm were prepared and aerated. Each of the 12 specimens of hybrid grouper infected with sea leeches through cohabitation was placed in a plastic container. Then, after 15 minutes of soaking, three fish from each treatment were taken, at minutes 15, 30, 45, and 60. The fish were placed in a 10 L plastic container filled with seawater (5 L) and equipped with aeration.

The number of sea leeches remained attaching to each fish, as well as the fish's condition after soaking, were observed.

Data analysis. The data in this experiment was analyzed by IBM SPSS Statistics 22 version. The mortality of sea leeches after immersion in HCl solution in vitro, as well as the number of *Z. arugamensis* still attached to the body of a hybrid grouper after immersion in HCl solution (in vivo), were analyzed using normality and homogeneity tests, followed by an ANOVA variance test. The data was examined using a non-factorial randomized block design, then a Tukey's Honest Significant Difference (HSD) test was used to identify significant differences (P<0.05) among treatments. Meanwhile, descriptive analysis was performed on the number of hatched sea leech eggs.

Results. The in vitro test for replication 1 revealed that all sea leeches soaked in HCl solution at concentrations ranging from 0 to 200 ppm were alive and active for 120 minutes (P>0.05). The average mortality of *Z. arugamensis* was substantially higher (P<0.05) in HCl solution at a concentration of 300 ppm (average values 8.42±0.41) compared to 0-200 ppm (average values 0±0). The highest mortality of *Z. arugamensis* (average values 10.0±0) occurred in soaked with HCl concentration of 500 ppm which was significantly different (P<0.05) from all other concentrations. The mortality rate of sea leeches increased after 30 to 120 minutes of soaking in 300 ppm HCl, but not considerably (P>0.05) (Table 1). All *Z. arugamensis* will die when soaked in a 500 ppm HCl solution for 30 to 120 minutes. At a concentration of 500 ppm, the technical HCl solution (32%) effectively killed sea leeches, with a significantly greater number of dead leeches compared to 0-300 ppm (P<0.05).

Table 1

Mortality of *Zeylanicobdella arugamensis* after being soaked in HCl solution (32%) concentration 0 to 500 ppm for 120 minutes

HCl concentration (ppm)	*Soaking time (minutes)*				*Average*
	30	60	90	120	
0	0±0	0±0	0±0	0±0	0±0^{a}
50	0±0	0±0	0±0	0±0	0±0^{a}
100	0±0	0±0	0±0	0±0	0±0^{a}
200	0±0	0±0	0±0	0±0	0±0^{a}
300	6.67±0.58	7.33±1.15	9.67±0.58	10.0±0	8.42±0.41^{b}
500	10.0±0	10.0±0	10.0±0	10.0±0	10.0±0^{c}

Different lowercase letters within rows in the average column indicate significant differences (P<0.05).

The use of technical HCl in the in vitro replication test 1 was only able to kill *Z. arugamensis* at a concentration of 500 ppm, thus pure HCl (pro analysis) was employed in the in vitro replication test 2 to determine the ability to kill *Z. arugamensis* and their eggs at lower concentrations. The results revealed that soaking for 15 to 60 minutes in pure HCL solution with a concentration of 100-500 ppm had no effect (P>0.05) on sea leech mortality (Table 2). After soaking in pure HCl at a concentration of 200 ppm for 15-60 minutes, sea leech mortality ranged from 4.0±1.73 to 10.0±0 individuals (average values 6.0±1.04). However, mortality did not differ significantly (P>0.05) between soaking times. The mortality rate of sea leeches treated with 200 ppm HCl was substantially different (P<0.05) from those treated with soaking HCl concentrations below (100 ppm) or above (300-500 ppm). After being soaked in an HCl solution with a concentration of 300 to 500 ppm, sea leeches died at a rate of 10.0±0 (100%), which was substantially higher (P<0.05) than when soaked in a solution with a concentration of 100 and 200 ppm. All sea leeches died when soaked in a 300 ppm HCl solution for 15 minutes.

Table 2

Mortality of *Zeylanicobdella arugamensis* after being soaked in HCl solution (36%) concentration 0 to 500 ppm for 60 minutes

HCl concentration (ppm)	*Soaking time (minutes)*				*Average*
	15	30	45	60	
0	0±0	0±0	0±0	0±0	0±0[a]
100	0±0	0±0	1.0±0	1.0±0	0.5±0[a]
200	4.0±1.73	4.33±1.15	5.67±2.89	10.0±0	6.0±1.04[b]
300	10.0±0	10.0±0	10.0±0	10.0±0	10.0±0[c]
400	10.0±0	10.0±0	10.0±0	10.0±0	10.0±0[c]
500	10.0±0	10.0±0	10.0±0	10.0±0	10.0±0[c]

Different lowercase letters within rows in the average column indicate significant differences (P<0.05).

An HCl solution with a concentration of 200-500 ppm was also effective in inhibiting the development of *Z. arugamensis* egg embryogenesis, as evidenced by the eggs failing to hatch (100%) after 15 days of incubation at 29-30°C (Figure 1). A minor percentage (8-10%) of sea leech eggs in the control treatment (0 ppm) did not hatch, owing to the fact that 1-2 eggs generated by one parent failed to hatch spontaneously. However, when compared to the number of eggs that did not hatch in the soaking treatment with HCl concentrations ranging from 100 to 500 ppm, there was a significant increase (68 to 100%).

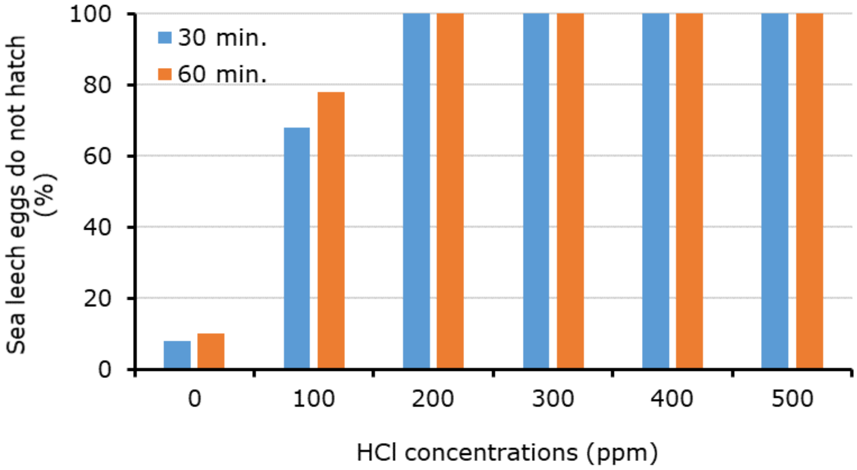

Figure 1. The percentage of *Zeylanicobdella arugamensis* eggs that do not hatch following soaking in various HCl concentration solutions.

HCl concentrations ranging from 50 to 500 ppm can impact the pH and temperature, but not the water salinity (Table 3).

Table 3

Quality of sea water used in in vitro tests

HCl treatment (ppm)	*pH*	*Temperature (°C)*	*Salinity (ppt)*
0	7.87	30.20	34
50	5.58	30.20	34
100	5.48	30.55	34
200	4.96	30.70	34
300	3.39	30.75	34
400	3.14	30.75	34
500	2.90	30.80	34

Water's pH value decreases when HCl concentration increases. Increasing the concentration of HCl can cause an increase in water temperature. The pH of the water decreased significantly (2.29) from 7.87 in the control to 5.58 at 50 ppm HCl, although the decrease was inferior (0.11-1.57) as the HCl concentration increased. When given a 100 ppm HCl solution, the water temperature increased by 0.35°C, and continued to rise slowly as the HCl concentration increased, reaching 30.80°C at 500 ppm HCl. The salinity of the seawater used in each treatment remained constant at 34 ppt.

In vivo test. In vivo test results revealed that the duration of HCl soaking had no significant effect (P>0.05) on the number of sea leeches remaining attached to and infecting hybrid grouper fish (Table 4). The concentration of HCl significantly affected the number of sea leeches infecting hybrid grouper fish (P<0.05). The concentration of 100 ppm HCl was effective than the control (0 ppm), however the concentration of 200 to 500 ppm HCl was more effective than the 0 and 100 ppm treatments. A high quantity of HCl (300-500 ppm) can eliminate all sea leeches from the body of a hybrid grouper in 15 minutes of soaking.

Table 4

The results of in vivo soaking HCl (36%) experiments on survival rate of *Zeylanicobdella arugamensis* that infect hybrid grouper fish

HCl concentration (ppm)	*Soaking time (minutes)*				*Average*
	15	30	45	60	
0	145.0±2.0	144.0±3.0	143.67±3.06	143.0±2.0	143.92±2.22[a]
100	133.33±1.53	96.0±2.0	85.0±1.0	74.33±1.15	97.17±22.27[b]
200	12.67±2.52	8.67±1.15	6.0±1.0	3.33±0.58	7.67±3.66[c]
300	0±0	0±0	0±0	0±0	0±0[c]
400	0±0	0±0	0±0	0±0	0±0[c]
500	0±0	0±0	0±0	0±0	0±0[c]

Different lowercase letters within rows in the average column indicate significant differences (P<0.05).

Observations of the hybrid grouper fish after soaking revealed that the hybrid grouper fish soaked in HCl solution at concentrations ranging from 0 to 300 ppm appeared healthy. Meanwhile, hybrid grouper fish soaked in HCl solutions at 400 and 500 ppm were observed struggling and then gasping on the surface of the rearing water from the initial phase of soaking until the 30th minute. Fish seemed weak at the bottom of the container after 45-60 minutes of soaking (Table 5). However, the fish recovered after being transferred to a tank with flowing seawater a few minutes later (20-30 minutes). The findings of this test reveal that soaking in HCl at a concentration of 300 ppm can remove all sea leeches from the bodies of hybrid grouper fish while remaining non-toxic.

Discussion. HCl is frequently used to acidify pepsin-based artificial digestive solutions in order to harvest metacercariae (trematodes) from fish hosts, amphibians and reptiles (Elsheikha & Elshazly 2008; Cho et al 2011). Kamiñski et al (2023) found that adding 1.5% HCl to cypriniform vimba bream (*Vimba vimba* L.) food improved the fish's immunity against bacterial infections. More than 700 leech species have been found to exist in freshwater, estuarine, and marine habitats, with varying morphological, physiological, and behavioral characteristics. Leeches may survive and develop in extreme environments. They can adapt to severe environmental conditions, although some species lack certain particular adaptations (Phillips et al 2020). One of them exhibits a low acidity level. He et al (2011) reported that HCl increases the acidity of seawater. Hirudinea species have been reported to live and reproduce effectively at pH 7, while some can thrive in the pH range of 4 to 10.5. *Hirudo costata*, *H. sanguisuaga*, *H. medicinalis*, and *Erpobdella octoculata* are examples of Hirudinea species that may thrive in low pH (4.40-4.58) environments. According to reports, even Hirudo and Erpobdella species can survive at extremely low pH levels. Most leeches cannot survive in pH levels below 3.0 (Tong & Jianqiu 1990; Bendell & McNicol 1991; Woke & Eze 2014; Elaltunkara et al 2022). In this study, seawater acidity had an effect on *Z. arugamensis* survival. Adding HCl to seawater at a concentration of 300-500 ppm and an acidity level of ≤3.39

can weaken *Z. arugamensis* and cause it to detach from the surface of a petri dish (in vitro) or the body of a hybrid grouper (in vivo).

Table 5
The condition of hybrid groupers after soaking in various HCl concentrations for 60 minutes

Fish condition after soaking in HCl (ppm)	*Soaking time (minutes)*			
	15	30	45	60
0	Healthy	Healthy	Healthy	Healthy
100	Healthy	Healthy	Healthy	Healthy
200	Healthy	Healthy	Healthy	Healthy
300	Healthy	Healthy	Healthy	Healthy
400	Gasping at the surface	Gasping at the surface	Gasping at the surface	Gasping at the surface
500	Gasping at the surface	Gasping at the surface	Gasping at the surface	Gasping at the surface

The survival rate test study revealed that the treatment had a substantial influence on sea leeches' survival rate. HCl soak treatment with varying lengths of time and doses had a substantial effect on the survival rate of sea leeches in hybrid grouper fish, with the duration of soaking time and dose administered being inversely proportional to sea leeches' survival rates. Sea leeches have a reduced survival rate when exposed to HCl at higher doses and/or for a longer duration of time (Table 4). This is in accordance with the findings of Suwarno & Rachmat (2019), Kua et al (2014) and Mahardika et al (2021), who determined environmental variables influencing the survival rate of sea leeches, in particular high salinity conditions reducing sea leech infestation in hybrid grouper fish.

Table 4 shows that the control concentration treatment (average values 143.92±2.22) and 100 ppm (average values 97.17±22.27) result in a high survival rate where sea leeches are still alive and attached to the hybrid grouper. However, at a treatment concentration of 200 ppm, the survival rate of sea leeches decreased drastically along with the length of soak time (average values 7.67±3.66). It can be seen that a concentration of 200 ppm HCl soaked for 60 minutes showed the lowest survival rate, even though some sea leeches survived. The treatment doses of 300 ppm, 400 ppm, and 500 ppm had a significant influence (average values 0±0) on the duration of soaking time, with a survival rate of 0%, resulting in the death of all sea leeches.

Soak HCl concentrations of 400 ppm and 500 ppm had a negative impact on hybrid grouper fish, with the fish exhibiting stress symptoms such as paleness and gasping on the water surface in the first 10 minutes before becoming weak at the bottom of the container (30-60 minutes soaking). However, at low doses, with soaks of 100, 200, and 300 ppm, there are no negative effects and soaked fish remain normal or healthy for 60 minutes, therefore such HCl concentrations are still within the safe limit for practical applications. Although HCl is efficient in killing sea leeches at high concentrations, its usage must be carefully controlled to avoid adverse effects on farmed fish. Aside from that, the duration of soaking time influences the survival rate of sea leeches and the stress level of hybrid grouper fish. The results of the analysis revealed that soaking times ranging from 15 to 30 minutes had no significant effect on the survival rate of sea leeches or the presence of stress symptoms in hybrid grouper fish, while soaking times of 45 minutes or longer had a significant impact.

Mahasri et al (2020) found that *Z. arugamensis* infection in hybrid grouper (*Ephinephelus* sp.) is a major issue in the fish farming industry. This infection can harm the fish's skin, diminish its appetite, and increase the chance of secondary infections, which can lead to death. Controlling this infection requires an effective and safe treatment. HCl has been proposed as a means of controlling sea leeches. However, HCl must be handled with attention because it is very corrosive and has the potential to harm the environment and fish health if used at unsuitable quantities. Continuous acid additions, including HCl, have been studied for practical pH regulation (Sakakibara &

Nakayama 2001; Ghafari et al 2010; Xia et al 2015). It is considered that decreasing environmental pH disrupts the metabolic processes and enzymatic functions of pathogenic microorganisms that cause diseases in fish, one of which is very efficient in reducing infections caused by sea leeches. Piscicolid leeches' osmotic control is mostly dependent on the surrounding water rather than on their hosts. Salinity is thought to be one of the most important environmental elements that affect the growth and survival of estuarine or marine species (Begon et al 1995; Bachman & Rand 2008). In tropical seas, it has been discovered that salinity and temperature influence the reproduction of *Z. arugamensis*, which may live between 10 to 40 psu in its adult and juvenile stages, but the piscicolid leech can tolerate lower salinity at 10 to 30 psu by clinging to its host fish (Kua et al 2014).

Conclusions. This study found that soaking in a 36% HCl solution at a concentration of 300 ppm for 15 minutes or more successfully releases, weakens, and kills *Z. arugamensis* while being non-toxic to hybrid groupers. More research is needed to evaluate the effects and residues of HCl when administered continuously to juvenile fish up to consumption size.

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Conflict of interest. The authors declare no conflict of interest.

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