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Efficacy of chemical treatments for *Acropora*-eating flatworm infestations

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Abstract

Pest management is a critical component of aquaculture operations since high stocking densities can facilitate rapid pest infestation and high stock losses. The *Acropora* eating-flatworm, *Prosthiostomum acroporae* impact the health of captive *Acropora* colonies, which are commonly grown as part of coral aquaculture for trade, research and the hobbyist sector. We investigated the efficacy of anthelmintics levamisole and praziquantel for the removal of *Acropora*-eating flatworms from *A. millepora* using one-hour chemical immersions and assessed if these treatments negatively impacted coral growth and/or caused bleaching. Coral fragments (194 total) were spread across eight treatments; levamisole infested (LI; n = 20), levamisole uninfested (LU; n = 20), praziquantel (in EtOH) infested (PI; n = 20), praziquantel (in EtOH) uninfested (PU; n = 20), handling control infested (HCI; n = 14), handling control uninfested (HCU; n = 20), EtOH control (EC; n = 40), control with no handling (NHC; n = 40). To test the efficacy of flatworm removal by short, one-hour chemical immersions, *A. millepora* fragments (54 total) were manually infested (three *P. acroporae* per fragment) and immersed separately to uninfested *A. millepora* fragments (60 total). All fragments were shaken in in a bath of seawater following immersion, then mechanically screened to recover any flatworms not removed from either immersion or shaking to determine the removal efficacy of the treatments. Furthermore, coral fragments (194 total) were photographed before treatment and four weeks following treatments to compare coral basal growth and visual signs of bleaching between infested and uninfested fragments. Levamisole and praziquantel immersions removed significantly more flatworms from *A. millepora* fragments (93% ± 3.8 and 95.0% ± 2.6 respectively; mean ± SE; $p < 0.05$) compared to the handling control (26% ± 7.5%). Chemical treatments had no significant effect on basal growth, with fragments across all treatments (including controls) increasing basal area by 73.31 ± 3.82% (mean ± SE). Furthermore, bleaching was not observed for any *A. millepora* fragments across the treatments and controls. Results from this study demonstrate that levamisole and praziquantel used in conjunction with water movement were effective at removing

>90% of *Acropora* eating-flatworms with no observable negative impacts on coral health on treated coral fragments relative to controls.

Keywords: Coral aquaculture, reef restoration, chemical treatments, coral flatworm treatment, pest management, bleaching

1. Introduction

Pest management is critical for aquaculture operations, as high stocking density and stress can facilitate the rapid spread of parasites and pathogens (Shinn et al., 2015). For example, platyhelminth (flatworms) parasites in marine environments (e.g. monogeneans infecting fish) warrant prophylactic chemical treatment of animals entering aquarium or aquaculture facilities (often during quarantine; see Hadfield & Clayton, 2011). This is necessary to prevent parasite outbreaks, which can heavily impact productivity (Liu and Bjelland, 2014; Shinn et al., 2015). While chemical treatments can be effective in managing aquaculture pests (Reed et al., 2009; Yamamoto et al., 2011, Shinn and Bron, 2012), these treatments are expensive and can be associated with reduced growth (Shinn and Bron, 2012; Paladini et al., 2017; Powell et al., 2018).

Anthelmintics are used to combat parasitic platyhelminths in agriculture, aquaculture and human medicine (Pax et al., 1996; Doenhoff et al., 2008; Park and Marchant, 2019). Anthelmintics use variable modes of action (Martin, 1997). The commonly applied anthelmintic levamisole inhibits enzymatic activity by acting as a nicotinic acetylcholine receptor agonist, causing continuous stimulation of platyhelminth muscle and subsequent paralysis (e.g. levamisole; Camacho et al., 1995; Martin, 1997; Martin et al., 1997; Ribeiro et al., 2005). Another commonly used anthelmintic, praziquantel, is thought to disrupt tegument homeostasis (Staudt et al., 1992; Pax et al., 1996; Martin, 1997; Martin et al., 1997) with an increased influx of Ca^{2+} and subsequent paralysis (Doenhoff et al., 2008). Praziquantel is used extensively to treat schistosomiasis in humans (Doenhoff et al., 2008; Park and Marchant, 2019), and has considerable potential for application in aquaculture to manage platyhelminths, but is currently not approved for non-prescription use (Shinn and Bron, 2012; Bader et al., 2018; Power et al., 2019). In aquatic organisms, praziquantel can be administered orally (Forwood et al., 2016), via the bloodstream (Justine et al., 2009), or in therapeutic bath immersions (dosage varies between 2 mg L^{-1} and 10 mg L^{-1}). The duration of treatments typically lasts a few hours to several days (Sharp et al., 2004; Reed et al., 2009; Hadfield and Clayton, 2011; Paladini et al., 2017; Bader et al., 2019).

Coral aquaculture is a burgeoning industry to support the demand of the marine ornamental trade, scientific research and reef restoration practices (Barton et al., 2017). Corals are associated with a variety of invertebrates (Stella et al., 2010), some of which can be harmful, especially in captivity (Barton et al., 2020a). The *Acropora*-eating flatworm, *Prosthiostomum acroporae* (Rawlinson, Gillis, Billings, and Bourneman 2011), is a polyclad flatworm that has been reported to be associated with corals at sites on the Great Barrier Reef (Rawlinson and Stella, 2012) and in captive aquaria (Nosratpour, 2008; Carl, 2008; Rawlinson et al., 2011; Hume et al., 2014; Barton et al., 2019; 2020). Its high fecundity and cryptic nature often result in rapid proliferation in captive environments, where it can cause colonial mortality of infested *Acropora*. Barton et al. (2019a) described the life cycle of *P. acroporae* and suggested that chemical treatment intervals of 2-3 weeks are potentially effective at breaking the life cycle between 24-30°C.

Prophylactic treatments for coral are commonly applied in the aquarium trade and come in the form of chemical immersions (commonly referred to as 'dips') of therapeutic solutions to treat a variety of ailments. Another anthelmintic, ivermectin, is used in chemical immersions (2mg L^{-1} over 5 hours) to treat the coral pest, *Waminoa* sp. (Winsor, 1990) (Leewis et al., 2009; Osinga et al., 2012). To date, levamisole HCl is the only chemical immersion suggested in the literature for the treatment of platyhelminth infestation of corals (Carl, 2008; Nosratpour, 2008). While Carl (2008) suggested a dose of 40 mg L^{-1} levamisole for one hour, no empirical evidence of the efficacy of this treatment for removal of *P. acroporae* from infested *Acropora* hosts was provided. Furthermore, Carl (2008) indicated that concentrations above 40 mg L^{-1} could cause bleaching or tissue loss in *Acropora*, and that consistent exposure can leave corals more susceptible to bleaching, however little is known about the impact on coral growth. Bleached corals are undesirable in the marine ornamental trade, and are likely to have compromised survivorship as evidenced by mortality and susceptibility to disease following bleaching events in the natural environments (Baird and Marshall, 2002; Anthony et al., 2009; Miller et al., 2009; Sakai et al., 2019).

The aim of this study was to assess the efficacy of two anthelmintics, levamisole HCl and praziquantel, for the removal of *P. acroporae* individuals from infested *Acropora* colonies. We also examined the growth and bleaching of treated corals following exposure to these chemical treatments. Identification of effective treatments that remove *P. acroporae* without compromising coral quality, is valuable to the

coral aquaculture community for pest management. Furthermore, a treatment regime can subsequently be coordinated to target specific stages of the *P. acroporae* life history.

2. Materials and methods

2.1 Coral fragment preparation

Acropora millepora colony fragments were collected in June 2019 at depths between 2-10 m from Davies Reef (18°49'21.6"S 147°39'12.5"E) located in the central Great Barrier Reef Australia (GBRMPA Permit G12/3236.1). Corals were transported to flow-through aquaria (24 °C) at the National Sea Simulator (Australian Institute of Marine Science) under natural light. Fragments were then screened using filtered seawater rinses to remove all adult *P. acroporae* and visually inspected for removal of egg clusters with tweezers to ensure subsequent infestation with a known number of flatworms (see Barton et al., 2019a). After screening, *Acropora* colonies were broken into smaller fragments (~30 x 50mm; width x height; n=194;) using coral cutters and a coral saw (Gryphon Aquasaw XL). Each fragment was then mounted on an aragonite base with cyanoacrylate glue.

Coral fragments were transferred indoors to three 250L flow-through systems and allowed to acclimate to experimental conditions for four weeks. Coral aquaria were supplied with filtered seawater (1 µm) at approximately 3 L min^{-1} and were slowly acclimated from 24 to 26°C over a period of 24 days. Fragments were illuminated by four Hydra 52 (AquaIllumination®) lights per tank to provide uniform light intensity of approximately 100 µmol m^{-2} s^{-1}. Although *A. millepora* can accommodate higher light intensity, to avoid adverse effects of excessive irradiance, light acclimation was achieved through raising irradiance to approximately 150 µmol m^{-2} s^{-1} over four weeks. A Gyre XF250 (Maxspect®) unit in each tank was used to provide internal water circulation to the coral fragments.

Fragments of *A. millepora* were screened a second time for flatworms after two weeks to remove any potential *P. acroporae* present (one individual flatworm was removed that may have hatched post the first screening). Additionally, corals were housed in a 250L flow-through aquarium with a single *Pseudocheilinus hexataenia* Bleeker, 1857, a known predator of adult *P. acroporae* (see Barton et al., 2020) providing an additional safeguard against infestation. An assortment of herbivores including *Trochus* Linneaus, 1758, *Stomatella* Lamarck, 1816 and an *Acanthurus nigrofuscus* Forsskål, 1775 were used to control undesirable algae growth on coral fragment bases during acclimation.

2.2 *Prosthiostomum acroporae* culture

Prosthiostomum acroporae were cultured *in vivo* to obtain known quantities of worms for experiments in this study. Flatworms were propagated following the methodology outlined in Barton et al. (2019a), using long term cultures of *P. acroporae* maintained on host *Acropora* colonies in three 250L flow through aquaria. In brief, various *Acropora* spp. including *A. millepora, A. spathulata*, *A. loripes*, *A. selago*, *A. latistella* and *A. muricata* were infested with *P. acroporae* via the introduction of egg capsules collected from other infested corals in culture. The temperature of the culture was adjusted from 27°C down to 26°C (the experimental temperature) over a period of three weeks prior to experiments.

Each *A. millepora* fragment for experimental infestation (54 fragments), was placed in a 2.5 L aquarium with seawater and *P. acroporae* individuals (three per fragment, 162 flatworms in total) were pipetted directly onto the coral fragments. The supply of flatworms from the culture was exhausted during the experiment so that the last treatment (control) was unable to be conducted with the 20 infested *A. millepora* (n = 14). Any flatworm that immediately moved off the coral were detectable by eye and pipetted back on once more. Flatworms that moved off the coral a second time were discarded. After five minutes each submerged coral fragment was gently shaken by hand to ensure flatworms were attached and could not be easily dislodged.

2.3 Treatment preparation and immersion

Coral fragments (n = 194) were spread across eight treatments; levamisole infested (LI), levamisole uninfested (LU), praziquantel infested (in EtOH) (PI), praziquantel uninfested (in EtOH) (PU), handling control infested (HCI), handling control uninfested (HCU), EtOH control (EC), control with no handling (NHC). Three treatments (PI, LI, and HCI) had 20 coral fragments each with the exception of HCI (n = 14; 54 total) infested with *P. acroporae* to compare the removal efficacy of the chemical immersion process, while PU, LU, and HCU had 20 uninfested fragments each (60 total). EC and NHC had 40 uninfested coral fragments each (80 total) which remained in the recovery system (see section 2.4) untouched to discern if the treatment, temporary infestation, or handling (e.g. flatworm screening method) of coral fragments had any effects on coral growth or visual bleaching.

Immersions were prepared for each treatment (excluding NHC) and added to 2 L replicate beakers. Levamisole HCl (CAS Number: 16595-80-5) is highly soluble in seawater and therefore could be prepared

directly in seawater and a 25 g L^{-1} stock was diluted to 40 mg L^{-1} in 1 µm filtered seawater in all levamisole replicates. The poor solubility of praziquantel (CAS Number 55268-74-1) in seawater required preparation of a 50 g L^{-1} stock solution in 100% ethanol added to 1 µm filtered seawater in the associated experimental replicates (PI, PU) to a final concentration of 50 mg L^{-1} praziquantel. An ethanol control treatment (EC; 0.01% Ethanol in 1 µm filtered seawater) was incorporated into the experimental design to differentiate any effects of praziquantel/ethanol versus ethanol on coral metrics (basal growth and visual bleaching signs). Handling control replicates (HC) consisted of filtered seawater (1 µm).

Once immersions were prepared, coral fragments mounted on a PVC base (to keep fragments upright), were added to their specified treatments consisting of a 2L aerated beaker (aerated with coarse bubbles through acrylic tubes). After one-hour immersion duration, fragments and the associated PVC base were removed from their respective 2 L beaker and given a vigorous five second shake in their respective rinse container containing filtered seawater, later referred to as the 'shake step'. The number of flatworms removed from the coral during the immersion and after the shake step were recorded, along with any flatworms adhering to the PVC base. Both the individual *A. millepora* fragment and the respective PVC base were rinsed in filtered seawater (26°C) to mechanically remove *P. acroporae* individuals remaining and ensure all worms were accounted for before placing corals in one of the three recovery tanks. Each tank was stocked with fish and snails in the same manner as the acclimation aquarium (*Acanthurus* nigrofuscus, *Pseudocheilinus* hexataenia, *Trochus* sp., and *Stomatella* sp.) to control algae and additionally safeguard against *P. acroporae*. The removal efficacy was calculated as the total number of flatworms removed by the treatment, including the associated shake step, divided by the total number of flatworms initially added (three per fragment). The mortality of *P. acroporae* was not measured in this study.

2.4 Monitoring coral recovery

Color change and basal growth were measured as proxies for coral recovery following chemical exposure for one month. After mechanical removal, coral fragments were placed into one of the three identical pre-conditioned 250 L aquaria in a randomly assigned position in each tank. A random number generator was used to determine the recovery tank and position within the tank before treatment. For each tank, lighting was provided by four Aqua Illumination Hydra® 52 lights (12:12 light: dark; 1 hour ramp up/down; ~150 µmol m^{-2} s^{-1}) and one Maxspect® Gyre XF250 unit. Each aquarium was fed daily

with *Artemia* nauplii at a rate of 0.35 nauplii mL^{-1} for corals. Manual handling of coral fragments was as limited as possible, with the only handling during weekly photo capture.

All corals were photographed (from the top) on their associated trays prior to chemical immersion (day 0), again the day following treatment (day 1) and weekly thereafter until day 28. For consistency, photos were taken in the dark using a computer-controlled (Adora24G, MSI®) photo cart equipped with a Nikon® DSLR D810, four Ikelite® DS161 strobes, and manually adjustable x-y stage (Figure 2). Camera settings remained consistent for all photos (shutter speed 1/8 sec, aperture f/11).

The C clade section of a CoralWatch Coral Health Chart was used to assess the color change in each coral fragment following the methods of Siebek et al. (2006). Images were first converted to greyscale in ImageJ to remove the influence of luminosity on photo color and to allow the export of mean grey values (MGV; 1-250; higher values are lighter) for each CoralWatch Coral Health category (C1 to C6) and each coral fragment in each photograph. Comparison of MGV of the coral watch color standards between time points (initial and four weeks later) indicated the consistency of photographs. Before comparison of coral fragment MGV, these raw values were corrected by 7% to reflect the uniform change in the color standards between time points. Corals that shifted two or more color categories lighter were considered bleached, while all others were not considered to have bleached.

ImageJ (FIJI ImageJ; Schneider et al., 2012) was used to measure the lateral area and circumference of each coral fragment, allowing comparison of photographs taken the day after chemical immersion and on day 28 to calculate the percentage increase in basal growth area (mm^2) (similarly to Forsman et al., 2015; Page et al., 2018) and circumference (mm) of each coral fragment. Basal growth is not only associated with overall growth in *A. millepora*, but it is required for attachment to substrate post fragmentation. This is relevant to the marine ornamental trade, where basal growth onto substrate is a sought-after feature of coral fragments examined by consumers as a qualitative indication of fragment health at the time of purchase. The implications of uncompromised basal growth are also relevant to reef restoration, where the leading cause of fragment mortality is detachment (Shafir et al., 2006; Shaish et al., 2008; Smith and Hughes, 1999).

2.5 Statistical analysis

All analyses were run using RStudio® (version 3.5.1, Rstudio PBC). Flatworm removal efficacy from the immersion step alone and efficacy after the shake step (includes removal from immersion step) were modeled separately because the shake step results are dependent on the immersion step removal. Binomial generalized linear mixed-effects models (GLMM; R package "lme4" Bates et al., 2015) were run with tray identification as a fixed effect and treatment as a random effect. This model was fitted with the "glmer" function. Normality of all the data was assessed using QQnorm and Shapiro–Wilk tests. A Tukey post-hoc test (R package "emmeans" Lenth, 2016) was used for pairwise comparison of all treatments with $P < 0.05$ as the significance threshold. For coral basal growth, lme was used to compare area data from each fragment. Bleaching response was measured as the proportional change in MGV using glm and Kruskal-Wallis, followed by a post-hoc Dunn Test. Data was visualized using (R package "ggplot2" Wickham, 2016).

3. Results and Discussion

3.1 Immersion efficacy

Treatment had a significant influence on flatworm removal for the immersion and immersion + shake step ($p < 0.05$; GLMM). Praziquantel treatments (PI) removed 90 ± 3.4% of flatworms (percent ± SE) compared to 75 ± 6.61% and 7.1 ± 3.1% removed by levamisole treatments (LI) and the handling control (HC), respectively, during chemical immersion (Figure 3). The shake step increased the efficacy of flatworm removal for LI from 75 ± 6.61% (immersion only) to 93.33% ± 3.80 (immersion and shake; Figure 3). Similarly, praziquantel removal increased from 90.0 ± 3.4% (immersion only) to 95.0 ± 2.66% (immersion and shake) while the handling control increased from 7.1 ± 3.1% to 33.33 ± 7.52% of flatworms removed due to the shake step (Figure 3). Both chemical treatments were effective at removal of *P. acroporae* individuals from infested *Acropora millepora* fragments, but there was no difference between the efficacy of levamisole and praziquantel ($p > 0.05$; Tukey post hoc). The removal observed from the 'shake step' in all treatments suggests that immersions of levamisole or praziquantel, with only aeration providing water movement, may not always remove flatworms from the coral host. These results emphasize the importance of additional water movement to improve the efficacy of chemical immersions to treat corals infested with *P. acroporae*. Similarly, praziquantel has been used to remove gill flukes from fish and enables fish recovery (e.g., 5 ppm praziquantel in seawater for 10 min) but does not remove all worms and some of the smaller worms can wedge between lamellae (Hutson et al., in press). Therefore, we suggest that a combined application of chemical treatment immersion with

a small wavemaker would increase the efficacy of flatworm removal. For commercial applications, this would be necessary to ensure water circulation while treating multiple coral fragments and/or colonies concurrently.

While there were no significant differences in flatworm removal between levamisole and praziquantel ($P > 0.05$; Tukey post hoc), we observed many flatworms in the levamisole treatment adhered to the beaker once removed from the coral, while in praziquantel, worms were clearly paralyzed and unable to adhere to treatment beakers. Furthermore, flatworms removed during the shake step after immersion in levamisole appeared to recover and adhere to the surface of shake containers, suggesting potential rapid recovery from levamisole exposure by *P. acroporae*. Praziquantel may have a more pronounced paralytic effect on *P. acroporae* because worms remained curled and unattached in their containers during immersion and after the shake step. Hirazawa et al. (2005) similarly observed immediate muscle contraction and subsequent removal of the monogenean *Heterobothrium okamotoi* Ogawa, 1991 treated with praziquantel, compared to a five minute delay using levamisole HCl in therapeutic immersions to treat the tiger puffer, *Takifugu rubripes* (Temminck & Schlegel, 1850). While future development of *in situ* treatments for *P. acroporae* are desirable, the toxicity of levamisole and the rapid degradation of praziquantel in seawater (Thomas et al., 2016) are likely to hinder these efforts. Dedicated treatment areas where corals can be immersed in praziquantel and shaken or rinsed in clean seawater baths may provide the best results with prophylactic, single preventative dips to remove flatworms before entering quarantine systems.

3.2 Coral health metrics following chemical treatment

There was no mortality observed in any coral fragments except for partial colonial mortality in a single fragment during the first week following immersion in levamisole, with the fragment later showing no further signs of tissue necrosis. Chemical treatment had no effect on the mean grey value (MGV) of all *A. millepora* fragments in the experiment, irrespective of whether corals were infested or not before being treated ($P > 0.05$; GLMM). No fragments in any treatment of the experiment were considered bleached relative to their initial MGV. These results provide evidence that the use of levamisole HCl and praziquantel did not advance observable bleaching during the four weeks following treatment. Treatment also had no significant effect ($p > 0.05$; GLMM) on the basal growth of *Acropora millepora* fragments during the experiment, with mean basal area increasing by 73.31 ± 3.82% (mean ± SE) across all treatments (Figure 4). This suggests that the prophylactic use of levamisole or praziquantel to treat

corals does not result in reduced basal growth in the short-term. This is important because growth inhibition would increase the associated cost of therapeutic treatment (Shinn et al. 2015).

3.3 Treatment cost and availability

While we validate the use of praziquantel in high concentration (50 mg L^{-1}) and low duration (i.e., immersion over one hour for the treatment of *P. acroporae* infestation on corals), availability and cost of praziquantel may vary between different countries, while levamisole is readily available globally and in use as a universal de-wormer of cattle (Varaday and Corba, 1999). Both praziquantel and levamisole are regarded as cost-effective treatments in the context of finfish aquaculture (Alves et al., 2018). Based on the average cost of 5g of each chemical from three major suppliers (Sigma-Aldrich, Tokyo Chemical Industry (TCI), and Fisher Scientific), the cost per L of treatment solution are $0.74USD L-1 for praziquantel (50 mg L-1) compared to $0.48USD L-1 for levamisole HCl (40 mg L-1), making levamisole HCl marginally more cost-effective. It should be noted that praziquantel is less toxic to human and environmental health than levamisole, which is reflected in the Australian Poisons Standard (February 2020), and examination of associated safety data sheets (Sigma-Aldriich®; L9756, P4668) in accordance with risk assessment for use in the laboratory. While praziquantel appears safe for vertebrates (Mitchell and Hobbs, 2007), further research is required to understand the toxicity of praziquantel to other organisms, and how drug resistance may be induced with increased use of praziquantel (Bader et al., 2018). Furthermore, the permitting and governance of chemical use for coral aquaculture is currently lagging as evidenced by the absence of levamisole approval for therapeutic use for corals, although it is approved for use in ornamental fish, birds, dogs and cattle in Australia (Poisons Standard February 2020). Depending on the country, the use and disposal of either of these chemicals in coral aquaculture require education, regulation, and ethics to ensure environmental responsibility.

5. Conclusion

We show that levamisole HCl and praziquantel can be used in chemical immersions in conjunction with water movement to remove >90% of flatworms from infested corals. A chemical treatment interval (time between treatments) of approximately three weeks (variable with temperature; see Barton et al. 2019), should remove the majority of flatworms from the host. Less than 100% removal efficiency of flatworms from infested corals in this study indicates the need to optimize the administration of levamisole and praziquantel treatments. Mechanical screening following chemical removal as conducted in the present study should increase flatworm removal efficacy. This protocol is suitable for treatment of

infestations in an established coral aquaculture system, or as preventative treatment of *Acropora* in quarantine.

Statement of Animal Ethics

This experiment using coral and flatworms was performed in accordance with ethical experimental procedures of James Cook University and The Australian Institute of Marine Science.

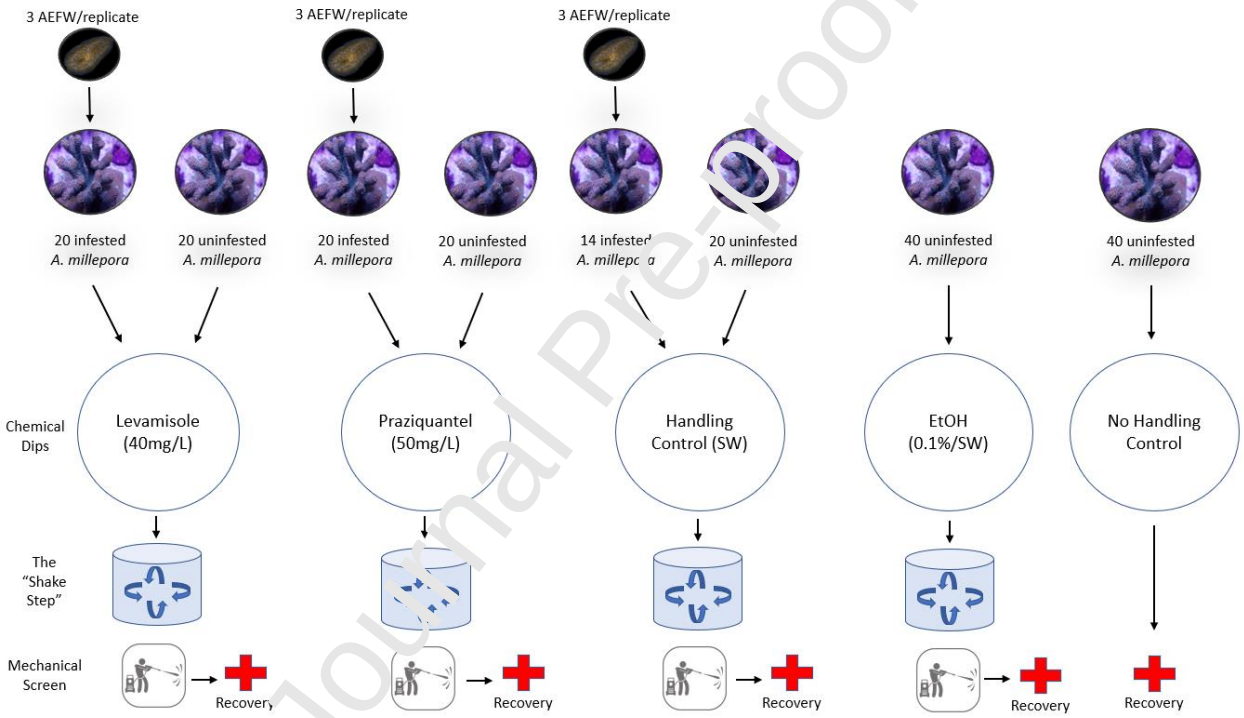

Figure 1: Schematic showing immersion procedure with all treatments (levamisole, praziquantel, handling control, EtOH control, and no handling).

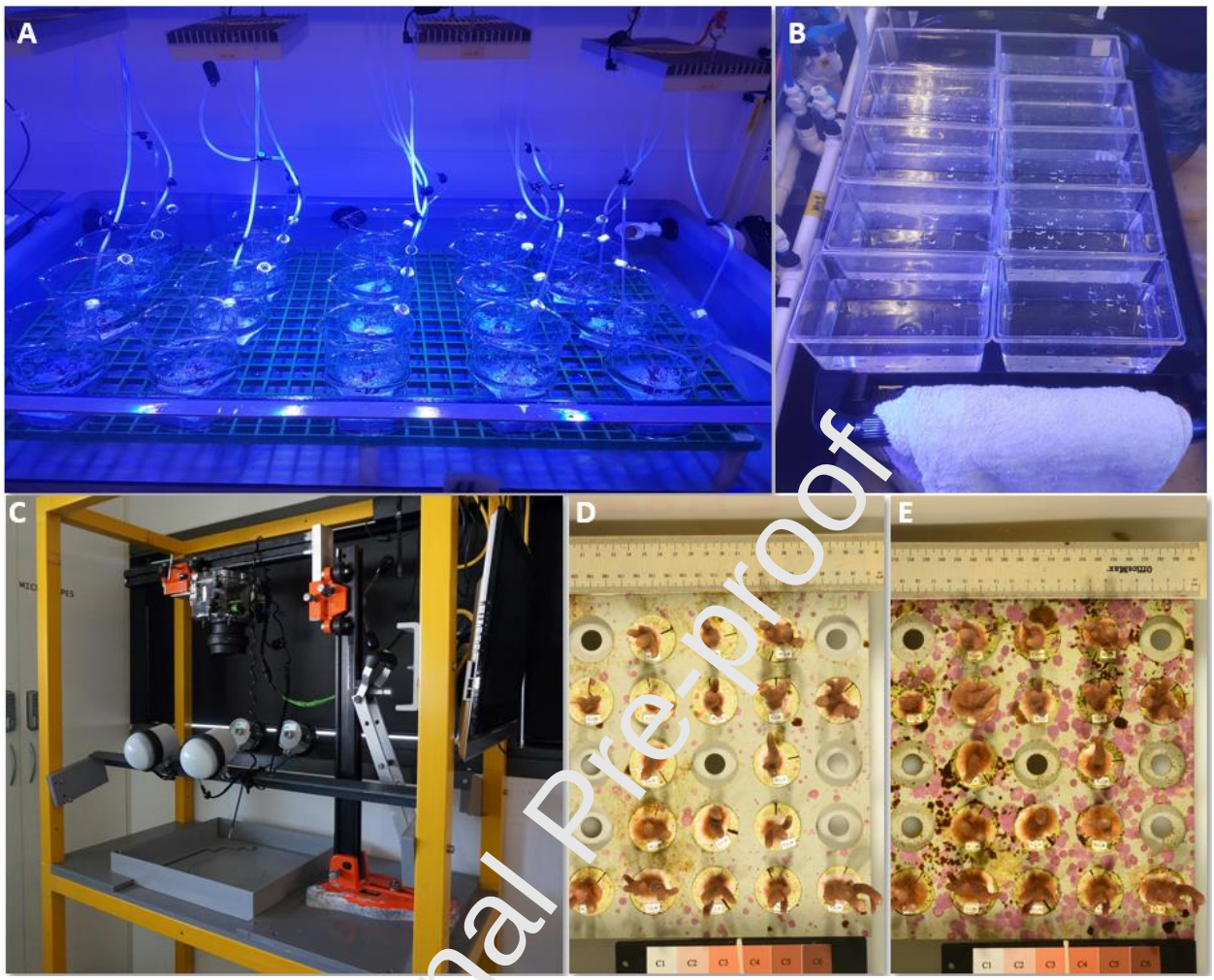

Figure 2: Photographs of experimental design: A. *Acropora millepora* fragments in their respective 2 L beakers during a one-hour chemical immersion, B. Containers with filtered seawater use for the 'shake step' after chemical immersion, C. Camera cart used for taking photos of *A. millepora* fragments, D. Initial photo (before chemical immersion) taken of *A. millepora* fragments, E. Day 28 photo taken of the same tray of *A. millepora* fragments.

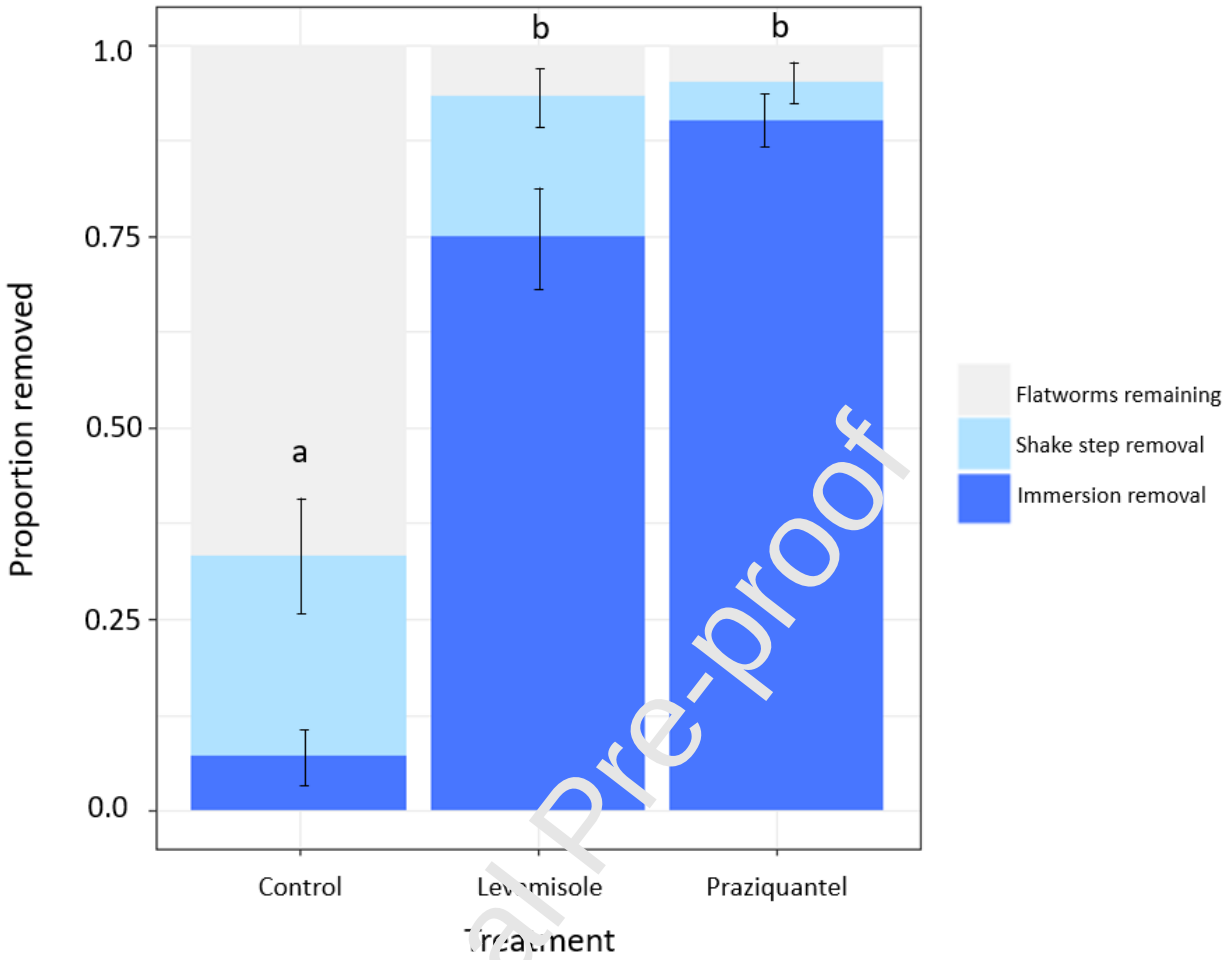

Figure 3: Stacked bar plot showing the mean percentage (± SE) of *Prosthiostomum acroporae* recovered from *A. millepora* fragments from each associated chemical treatment (Handling control, Levamisole, and Praziquantel) from each immersion, shake step, and the mechanical screening step to recover remaining flatworms. The letters (a) and (b) indicate treatments with statistical differences from each other.

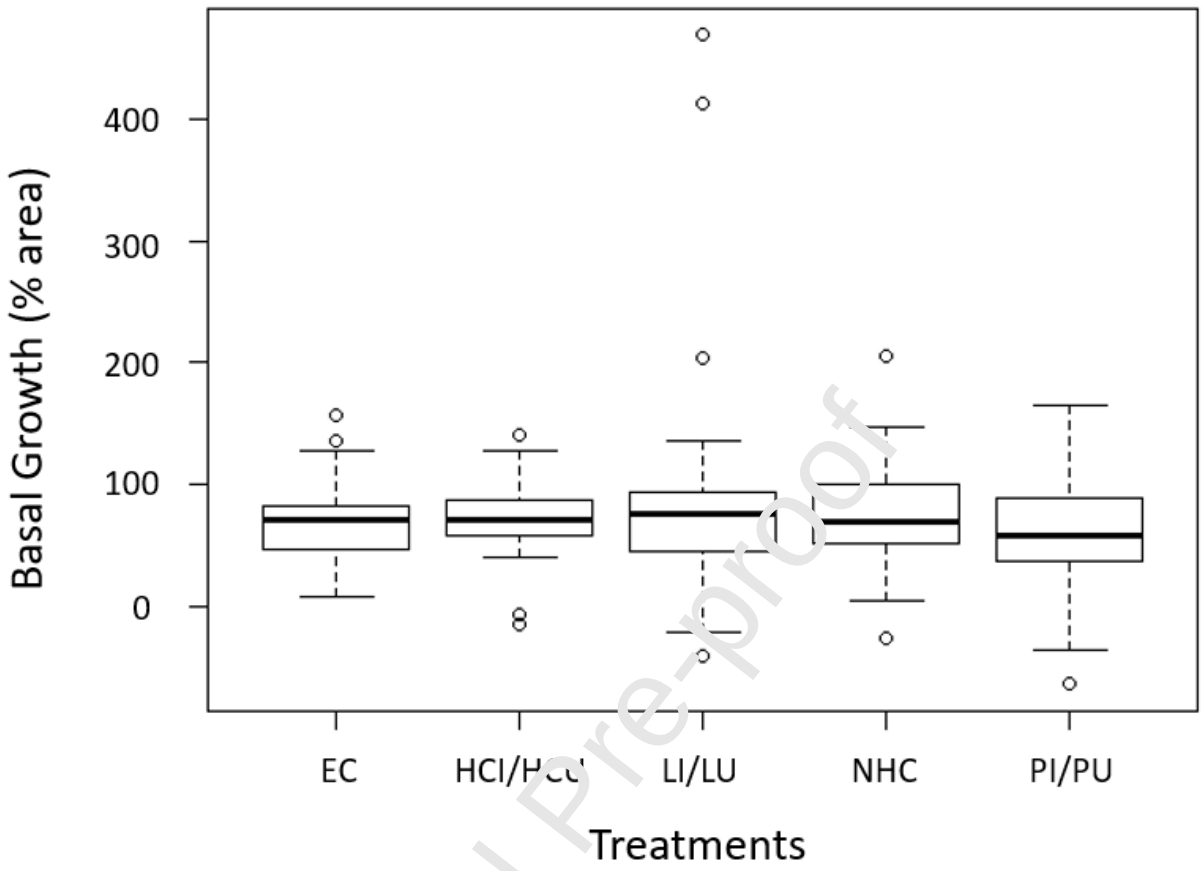

Figure 4-4: Box and whisker plot demonstrating the percentage basal growth of *Acropora millepora* in each treatment (**EC**: ethanol control, **HCI/HCU**: handling control infested and uninfested, **LI/LU**: levamisole infested and uninfested, **NHC**: no handling control, and **PI/PU**: praziquantel infested and uninfested.) after four weeks, with straight lateral lines demoting means, whiskers showing quartiles.

Contribution of authors

Jonathan Barton: Investigation, Visualization, Writing - Original draft preparation, Reviewing and Editing, Conceptualization, Methodology, Formal analysis, Data curation

Rachel Neil.: Investigation, Formal analysis, Visualization

Craig Humphrey: Supervision, Writing - Reviewing and Editing, Resources

David Bourne: Supervision, Writing - Reviewing and Editing Conceptualization

***Kate Hutson**:* Supervision, Writing - Reviewing and Editing, Project administration

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alves, C.M.G., Nogueira, J.N., Barriga, I.B., Dos Santos, J.R., Santos, G.G., & Tavares-Dias, M. 2019. Albendazole, levamisole and ivermectin are effective against monogeneans of *Colossoma macropomum* (Pisces: Serrasalmidae). Journal of Fish Diseases. 42(3), 405-412.

Anthony, K.R.N., Hoogenboom, M.O., Maynard, J.A., Grottoli, A.G., Middlebrook, R., 2009. Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. Functional Ecology. 23, 539-550. doi: 10.1111/j.1365-2435.2008.01531.x

Baird, A.H., Marshall, P.A., 2002. Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Mar. Ecol. Prog. Ser. 237, 133-141. doi: 10.3354/meps237133

Bader, C., Starling, D.E., Jones, D.E., Brewer, M.T. 2019. Use of praziquantel to control platyhelminth parasites of fish. Journal of Veterinary Pharmacology and Therapeutics. 42, 139-153. doi: 10.1111/jvp.12735

Barton, J.A., Willis, B.L., Hutson, K.S., 2017. Coral propagation: a review of techniques for ornamental trade and reef restoration. Reviews in Aquaculture. 9, 238-256. doi: 10.1111/raq.12135

Barton, J.A., Hutson, K.S., Bourne, D.G., Humphrey, C., Dybala, C., Rawlinson, K.A. (2019) The life cycle of the *Acropora* coral-eating flatworm (AEFW), *Prosthiostomum acroporae*; the influence of

temperature and management guidelines. Front. Mar. Sci. 6,524. doi: 10.3389/fmars.2019.00524

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed effects models using lme4. J. Stat. Softw. 67, 1–48. arXiv:1406.5823

Camacho, M., Alsford, S., Jones, A., Agnew, A., 1995. Nicotinic acetylcholine receptors on the surface of the blood fluke *Schistosoma*. Molecular and Biochemical Parasitology. 71, 127-134. doi: 10.1016/0166-6851(94)00039-P

Carl, M., 2008. Predators and pests of captive corals. Advances In Coral Husbandry In Public Aquariums (Public Aquarium Husbandry). 2, 31-36.

Doenhoff, M.J., Cioli, D., Utzinger, J., 2008. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Current Opinion in Infectious Diseases. 21, 659-667. doi: 10.1097/QCO.0b013e328318978f

Forsman, Z.H., Page, C.A., Toonen, R.J., Vaughan, D., 2015. Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ. 3, e1313. doi: 10.7717/peerj.1313.

Forwood, J.M., Bubner, E.J., Landos, M., D'Antignana, T., Deveney, M.R., 2016. Praziquantel treatment for yellowtail kingfish (*Seriola lalandi*): dose and duration safety study. Fish Physiology and Biochemistry. 42, 103-109. doi: 10.1007/s10695-015-0121-2

Hadfield, C.A., Clayton, L.A., 2011. Fish quarantine: current practices in public zoos and aquaria. Journal of Zoo and Wildlife Medicine. 42, 641-650. doi: 10.1638/2011-0034.1

Hirazawa, N., Ohtaka, T., & Hata, K. 2000. Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. Aquaculture, 188(1-2), 1-13.

Hutson, K.S., Mooney, A.J., and Vaughan, D.B. (in press) *Zeuxapta seriolae*. In: *Fish Parasites: A Handbook of Protocols for their Isolation, Culture and Transmission* (eds Ariadna Sitjà-Bobadilla, James Bron and Geert Wiegertjes) 5m Publishing.

Justine, J.L., Leblanc, P., Keller, F., Lester, R.J., 2009. Turbellarian black spot disease in bluespine unicornfish *Naso unicornis* in New Caledonia, caused by the parasitic turbellarian *Piscinquilinus* sp. Diseases of aquatic organisms. 85, 245-249. doi: 10.3354/dao02082

Lenth, R.V., 2016. Least-squares means: the R Package Lsmeans. J. Stat. Softw. 69:33.

Leewis, R. J., Wijgerde, T., Laterveer, M., & Osinga, R. 2009. Working With Aquarium Corals: A Book Of Protocols For The Breeding And Husbandry Of Scleractinian Corals. Rotterdam: Rotterdam Zoo.

Liu, Y., Bjelland, H.v., 2014. Estimating costs of sea lice control strategy in Norway. Preventive Veterinary Medicine. 117, 469-477. doi: 10.1016/j.prevetmed.2014.08.018

Martin, R.J., 1997. Modes of action of anthelmintic drugs. The Veterinary Journal. 154, 11-34. doi: 10.1016/s1090-0233(05)80005-x

Martin, R.J., Robertson, A., Bjorn, H., 1997. Target sites of anthelmintics. Parasitology. 114, 111-124. doi: 10.1017/S0031182097001029

Miller, J., Muller, E., Rogers, C., Waara, R., Atkinson, A., Whelan, K.R.T., Patterson, M., Witcher, B., 2009. Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs. 28, 925-937. doi: 10.1007/s00338-009-0531-7

Mitchell, A.J., & Hobbs, M.S. 2007. The acute toxicity of praziquantel to grass carp and golden shiners. North American Journal of Aquaculture, 69(3), 203-206. doi: 10.1577/A06-056.1

Nosratpour, F., 2008. Observations of a polyclad flatworm affecting acroporid corals in captivity. In: *Advances in Coral Husbandry in Public Aquariums. Public Aquarium Husbandry Series.* (eds Robert Jacob Leewis and Max Janse) Burgers' Zoo. 2, 37–46.

Osinga, R., Schutter, M., Wijgerde, T., Rinkevich, B., Shafir, S., Shpigel, M., ... & Kuecken, M. 2012. The CORALZOO project: a synopsis of four years of public aquarium science. Journal of the Marine Biological Association of the United Kingdom, 92(4), 753-768. doi: 10.1017/S0025315411001779

Page, C.A., Muller, E.M., Vaughan, D.E., 2018. Microfragmenting for the successful restoration of slow growing massive corals. Ecological Engineering. 123, 86-94. doi: 10.1016/j.ecoleng.2018.08.017

Paladini, G., Longshaw, M., Gustinelli, A., Shinn, A.P., 2017. Parasitic diseases in aquaculture: their biology, diagnosis and control. In: *Diagnosis and Control of Diseases of Fish and Shellfish*. (eds Brain Austin and Aweed Newej-Fyzul) Chichester: John Wiley & Sons, Ltd, 37-107. doi: 10.1002/9781119152125.ch4

Park, S.K., Marchant, J.S., 2019. The journey to discovering a flatworm target of praziquantel: a long TRP. Trends in Parasitology. doi: 10.1016/j.pt.2019.11.002

Pax, R.A., Day, T.A., Miller, C.L., Bennett, J.L., 1996. Neuromuscular physiology and pharmacology of parasitic flatworms. Parasitology. 113, S83-S96. doi: 10.1017/S003118200007791X

Powell, A., Treasurer, J.W., Pooley, C.L., Keay, A.J., Lloyd, R., Imsland, A.K., Garcia de Leaniz, C., 2018. Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture. 10, 683-702. doi: 10.1111/raq.12194

Power, C., Webber, C., Rough, K., Staunton, R., Nowak, B.F., Bott, N.J., 2019. The effect of different treatment strategies on *Cardicola* spp. (Trematoda: Aporocotylidae) infection in ranched southern bluefin tuna (*Thunnus maccoyii*) from Port Lincoln, South Australia. Aquaculture. 513, 734401. doi: 10.1016/j.aquaculture.2019.734401

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA

Reed, P., Francis-Floyd, R., Klinger, R., Petty, D., 2009. Monogenean parasites of fish. Fisheries and Aquatic Sciences. University of Florida IFAS Extension. FA28, USA. 4, 1-4.

Ribeiro, P., El-Shehabi, F., Patocka, N., 2005. Classical transmitters and their receptors in flatworms. Parasitology. 131, S19-S40. doi: 10.1017/S0031182005008565

Sakai, K., Singh, T., Iguchi, A., 2019. Bleaching and post-bleaching mortality of *Acropora* corals on a heat-susceptible reef in 2016. Peerj. 7. doi: 10.7717/peerj.8138

Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9(7): 671-675. PMID 22930834.

Shafir, S., Van Rijn, J., Rinkevich, B., 2006. Steps in the construction of underwater coral nursery, an essential component in reef restoration acts. Marine Biology. 149, 679-687. doi: 10.1007/s00227-005-0236-6

Shaish, L., Levy, G., Gomez, E., Rinkevich, B., 2008. Fixed and suspended coral nurseries in the Philippines: Establishing the first step in the "gardening concept" of reef restoration. Journal of Experimental Marine Biology and Ecology. 358, 86-97. doi: 10.1016/j.jembe.2008.01.024

Sharp, N.J., Diggles, B., Poortenaar, C., Willis, T.J., 2004. Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. Aquaculture. 236, 67-83. doi: 10.1016/j.aquaculture.2004.02.005

Shinn, A., Bron, J., 2012. Chapter 8: Considerations for the use of anti-parasitic drugs in aquaculture. In: *Infectious disease in aquaculture. Prevention and Control*. (ed Brain Austin) Woodhead Publishing Limited. 190-217.

Shinn, A.P., Pratoomyot, J., Bron, J.E., Paladini, G., Brooker, E.E., Brooker, A.J., 2015. Economic costs of protistan and metazoan parasites to global mariculture. Parasitology. 142, 196-270. doi: 10.1017/S0031182014001437

Siebeck, U. E., Marshall, N. J., Klüter, A., & Hoegh-Guldberg, O. 2006. Monitoring coral bleaching using a colour reference card. Coral Reefs. 25(3), 453-460.

Smith, L.D., Hughes, T.P., 1999. An experimental assessment of survival, re-attachment and fecundity of coral fragments. Journal of Experimental Marine Biology and Ecology. 235, 147-164. doi: 10.1016/S0022-0981(98)00178-6

Staudt, U., Schmahl, G., Blaschke, G., Mehlhorn, H. 1992. Light and scanning electron microscopy studies on the effects of the enantiomers of praziquantel and its main metabolite on *Schistosoma mansoni* in vitro. Parasitology Research. 78, 392-397. doi: 10.1007/BF00931694

Stella, J. S., Jones, G. P., Pratchett, M.S. 2010. Variation in the structure of epifaunal invertebrate assemblages among coral hosts. Coral reefs, 29(4), 957-973. doi: 10.1007/s00338-010-0648-8

Thomas, A., Dawson, M. R., Ellis, H., and Stamper, M. A. 2016. Praziquantel degradation in marine aquarium water. PeerJ, 4, e1857.

Várady, M., & Čorba, J. 1999. Comparison of six in vitro tests in determining benzimidazole and levamisole resistance in *Haemonchus contortus* and *Ostertagia circumcincta* of sheep. Veterinary parasitology, 80(3), 239-249.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer Verlag New York, 2016.

Yamamoto, S., Shirakashi, S., Morimoto, S., Ishimaru, K., Murata, O., 2011. Efficacy of oral praziquantel treatment against the skin fluke infection of cultured chub mackerel, *Scomber japonicus*. Aquaculture. 319, 53-57. doi: 10.1016/j.aquaculture.2011.06.045

Highlights

- Evaluated the use of chemical treatment for *Parasthiostomum acroporae* infestations in coral aquaculture
- Levamisole and praziquantel removed 93% ± 3.8 and 95.0% ± 2.6 flatworms respectively from infested *Acropora millepora* fragments
- Treatments did not compromise coral basal growth nor was there detectable bleaching following chemical treatment with levamisole or praziquantel
- Developed pest management tools for *P. acroporae* in coral aquaculture