Vol. 12: 61–66, 2020
https://doi.org/10.3354/aei00347

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published February 13

NOTE

Biological controls to manage *Acropora*-eating flatworms in coral aquaculture

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ABSTRACT: Coral aquaculture is expanding to supply the marine ornamental trade and active coral reef restoration. A common pest of *Acropora* corals is the *Acropora*-eating flatworm *Prosthiostomum acroporae*, which can cause colonial mortality at high infestation densities on *Acropora* spp. We investigated the potential of 2 biological control organisms in marine aquaria for the control of *P. acroporae* infestations. *A. millepora* fragments infested with adult polyclad flatworms (5 flatworms fragment^{-1}) or single egg clusters laid on *Acropora* skeleton were cohabited with either sixline wrasse *Pseudocheilinus hexataenia* or the peppermint shrimp *Lysmata vittata* and compared to a control (i.e. no predator) to assess their ability to consume *P. acroporae* at different life stages over 24 h. *P. hexataenia* consumed 100% of adult flatworms from *A. millepora* fragments (n = 9; 5 flatworms fragment^{-1}), while *L. vittata* consumed 82.0 ± 26.76% of adult flatworms (mean ± SD; n = 20). *Pseudocheilinus hexataenia* did not consume any *Prosthiostomum acroporae* egg capsules, while *L. vittata* consumed 63.67 ± 43.48% (n = 20) of egg capsules on the *Acropora* skeletons. Mean handling losses in controls were 5.83% (shrimp system) and 7.50% (fish system) of flatworms and 2.39% (fish system) and 7.50% (shrimp system) of egg capsules. Encounters between *L. vittata* and *P. hexataenia* result in predation of *P. acroporae* on an *Acropora* coral host and represent viable biological controls for reducing infestations of *P. acroporae* in aquaculture systems.

KEY WORDS: *Prosthiostomum acroporae* · *Acropora*-eating flatworm · *Lysmata vittata* · *Pseudocheilinus hexataenia* · Biological control · Coral aquaculture

1. INTRODUCTION

Biological control utilizes living organisms (control agents) to suppress the population density and subsequent impact of a specific pest organism by leveraging ecological interactions through predation, parasitism, herbivory, or other natural mechanisms (Eilenberg et al. 2001). Biological controls are used extensively in agriculture, where the tactical release of parasites or predators is used to reduce insect pest species of economic importance (Smith & Basinger 1947, Simmonds et al. 1976, Greathead 1994, Eilenberg et al. 2001). In aquaculture, high stocking densities of cultured organisms can facilitate transmission of pathogens and parasites, requiring analogous approaches for disease management (Deady et al. 1995, Tully et al. 1996, Maeda et al. 1997, Powell et al. 2018). In the northern hemisphere, cleaner fishes (e.g. ballan wrasse *Labrus bergylta* Ascanius, 1767 and, more recently, lumpfish *Cyclopterus lumpus* Linnaeus, 1758) are bred in captivity and subsequently cohabited with farmed salmon (primarily *Salmo salar* Linnaeus, 1758) to remove ectoparasitic copepods (e.g. *Lepeophtheirus salmonis* [Krøyer, 1837]; Tully et al. 1996). This non-chemical

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approach to pest management is preferable to costly treatments, which stress cultured fish and reduce appetite (Skiftesvik et al. 2013, Powell et al. 2018). Within coral aquaculture and the marine ornamental trade, the peppermint shrimps *Lysmata wurdemanni* (Gibbes, 1850), *L. seticaudata* (Risso, 1816), *L. boggessi*, and *L. ankeri* Rhyne & Lin, 2005, as well as the nudibranch *Berghia* sp. are used for biological control of anemones *Aiptasia* spp. (Rhyne et al. 2004, Calado et al. 2005, Rhyne & Lin 2006). The reef fishes *Thalassoma duperrey* (Quoy & Gaimard, 1824) and *Chaetodon auriga* Forsskål, 1775 are also potential candidates to mitigate infestations of the corallivorous nudibranch *Phestilla sibogae* Begh, 1905 in captivity (Gochfeld & Aeby 1997).

Control of pests of *Acropora* spp. coral is highly desired, given that it is the most represented genus imported into many countries globally (Rhyne et al. 2014), and *Acropora* spp. are commonly used for reef restoration efforts (Barton et al. 2017). A problematic coral pest, *Prosthiostomum acroporae* (Rawlinson, Gillis, Billings, & Borneman, 2011), commonly known as the *Acropora*-eating flatworm, has plagued hobbyist aquaria for many years (Delbeek & Sprung 2005). *P. acroporae* is an obligate associate of *Acropora* spp. and actively consumes coral tissue, which results in characteristic ~1 mm circular pale feeding scars, often resulting in coral tissue necrosis. Infestations are associated with colonial mortality at high densities in captivity (Nosratpour 2008). *P. acroporae* infestations are challenging to detect because of their highly cryptic nature, which facilitates their spread into new systems undetected. Infestations impact coral health through reduction of host coral fluorescence over time and hinder the coral's ability to photoacclimate to changes in lighting conditions (Hume et al. 2014). Infestations are often not detected until compromised host health is observed through visual signs, at which point flatworm population density is high and colonial mortality of the coral may occur. There is no current empirical evidence to support effective treatment or prevention measures for *P. acroporae* infestations, although Barton et al. (2019) examined the life cycle under a range of temperature conditions and suggested timed intervention to disrupt the life cycle.

The aim of the present study was to evaluate the potential of 2 biological controls to reduce infestation by the *Acropora*-eating flatworm *P. acroporae* on coral. Biocontrol candidates included the peppermint shrimp *L. vittata* (Stimpson, 1860), which has been previously reported to remove parasites on fish and in the environment (Vaughan et al. 2017, 2018a,b), and the wrasse *Pseudocheilinus hexataenia* (Bleeker, 1857), based on anecdotal evidence that it may reduce *P. acroporae* populations in aquaria through active foraging (Delbeek & Sprung 2005). This study examined the efficacy of potential biocontrols on adults and eggs of *Prosthiostomum acroporae* in captive systems over a 24 h period *in vivo*.

2. MATERIALS AND METHODS

2.1. Species selection, husbandry, and culture

Twenty *Lysmata vittata* and 10 *Pseudocheilinus hexataenia* were purchased from Cairns Marine, Cairns, Australia, and maintained for 1 mo before any experimentation. Because of space limitations, shrimps were housed together in one 50 l flow-through aquarium system (10 turnovers d^{-1}) with approximately 5 kg of 'live' rock for hiding and protection between molts. *P. hexataenia* were housed individually in 50 l flow-through aquarium systems (10 turnovers d^{-1}) with a 60 mm PVC tee (3-way junction) each for shelter. Filtered seawater (0.04 µm nominal pore size) at 27°C was used to supply the system. Shrimps and fish were fed twice daily to satiation with a mixture of thawed Tasmanian mysid shrimp, Ocean Nutrition® Marine Fish Eggs, Ocean Nutrition® Cyclopods, and Vitalis® Platinum formulated feed. Animals were fed the morning prior to the commencement of each experimental trial but not during their trial period.

Adult *Prosthiostomum acroporae* were collected from a culture of infested captive *Acropora* spp. colonies. Flatworms were maintained in culture using established methods (see Barton et al. 2019).

2.2. Coral fragment preparation, infestation, and egg collection

To provide *A. millepora* for biological control trials, 96 *A. millepora* fragments (approximately 50 mm height; 30 mm width) were generated from donor colonies harvested from 2 colonies sourced from Davies Reef, Australia (harvested September 2017; GBRMPA Permit: G12/35236.1), and 5 captive colonies originating from Orpheus Island, Australia (harvested May 2016; G14/36802.1). A combination of bone cutters and a band saw (Gyrphon® Aquasaw XL) was used to prune *A. millepora* fragments, which were then fixed onto aragonite coral plugs (32 mm diameter) with cyanoacrylate glue.

To infest *A. millepora* fragments with *P. acroporae*, fragments were housed temporarily in individual 5 l containers. Before the start of each experimental trial, 5 *P. acroporae* individuals, approximately 3 mm in size, were directly pipetted onto each *A. millepora* fragment. After 60 s, each fragment was gently shaken to ensure *P. acroporae* had laterally appressed themselves to the host coral's tissue and were not stuck in the coral mucus (flatworms can dislodge if stuck in mucus). Any worms that detached were attempted to be reattached once and then discarded for another specimen if unsuccessful.

Egg capsules were naturally laid on *Acropora* skeleton in the *P. acroporae* culture and then harvested using bone cutters to remove the section of skeleton with these eggs. The underside of each subsequent skeletal fragment was glued onto clean aragonite disks or 'frag plugs' with cyanoacrylate glue. The number of eggs per cluster was determined by counting them under a dissecting microscope (Leica EZ4, 10–40× magnification) while immersed in seawater to prevent desiccation. Only fragments of coral skeleton bearing unhatched and undamaged egg capsules were selected for experimentation.

2.3. *L. vittata* experiments

Experiments with *L. vittata* were conducted on 4 separate trial days (i.e. 6 control and 6 treatment replicates per trial; n = 24 control; 24 treatment). On the day before each *L. vittata* trial, a random number generator was used to designate treatments and controls to aquaria. PVC blocks (80 × 80 × 25 mm; 32 mm diameter depression with central 10 × 15 mm hole to hold 32 mm diameter aragonite plugs in all replicates) were placed in each aquarium (3.5 l) before each trial. After their morning feeding, 6 *L. vittata* were haphazardly caught from their holding system using a 500 ml wide–mouth container and placed into their respective experimental tanks. *L. vittata* were given a minimum of 2 h to acclimate to their surroundings in the replicate experimental flow-through aquaria (5 l h^{-1}) maintained at 27 ± 0.1°C. *L. vittata* were considered acclimated once they settled on the bottom of each aquarium.

A. millepora fragments (1 per aquarium) infested with 5 *P. acroporae* each were introduced to each of the 3.5 l aquaria (treatment and control) for 24 h to determine if the presence of *L. vittata* (treatment) influenced the number of remaining flatworms on each coral fragment. The number of flatworms remaining was determined using a seawater screening method (Barton et al. 2019). In addition, the PVC blocks and clear tanks were inspected for flatworms with the naked eye after each trial, with any flatworms found added to the remaining total of flatworms. Experiments examining the influence of *L. vittata* on *P. acroporae* egg capsules were conducted using the same approach, with the exception of egg capsules being counted before and after the trial under a stereo microscope (Leica EZ4, 10–40× magnification). Skeletal fragments (n = 48) were divided equally across treatments and controls (i.e. n = 24 control, 24 treatment) in *L. vittata* trials with 47.27 ± 19.09 (mean ± SD) egg capsules per fragment. *L. vittata* do not forage immediately before or after molting (D. Vaughan pers. comm.), therefore any shrimps that molted during the 24 h trial were excluded (i.e. 4 replicates were removed due to molting; n = 20).

2.4. *P. hexataenia* experiments

P. hexataenia (n = 9) were acclimated for approximately 2 wk to their randomly allocated flow-through aquaria at 27 ± 0.1°C with PVC blocks in place. The 50 l aquaria (n = 9 with wrasse, 9 without) were separated by black plastic because of the acute eyesight and territorial behavior of *P. hexataenia*. After acclimation, each fish regularly accepted food and did not exhibit signs of physical or behavioral stress.

Following morning feeding of *P. hexataenia*, infested *A. millepora* fragments (5 flatworms each) were introduced to each 50 l aquarium and left for a duration of 24 h to assess if the presence of the wrasse influenced the number of flatworms remaining on each coral fragment. Flatworms were recovered using an established screening method (Barton et al. 2019). The surfaces of the aquaria and the PVC blocks holding the fragment plugs were inspected visually for any remaining worms, which were added to the total remaining flatworms if present. Experiments examining the influence of *P. hexataenia* on *P. acroporae* egg capsules were conducted similarly, but egg capsules were counted before and after inspection with a stereo microscope (Leica EZ4, 10–40× magnification). The 18 skeletal fragments used in *P. hexataenia* trials (n = 9 treatment, 9 controls) had 42.33 ± 16.95 (mean ± SD) egg capsules per skeletal fragment.

2.5. Statistical analysis

Binomial generalized linear mixed models (GLMMs) and generalized linear models (GLMs) were gener-

ated in RStudio (Version 1.0.143; R packages 'car,' Fox & Weisberg 2019, and 'lme4,' Bates et al. 2015) to assess the effect of *L. vittata* treatments on *P. acroporae* egg capsules and individual flatworms. Treatment was considered a random effect and trial identity a fixed effect in the model to ensure that there were no effects that changed the results significantly ($p < 0.05$) between *L. vittata* trials. Lacking any significant effects from trial identity in both experiments testing *L. vittata* egg and individual consumption, the GLM with pooled data denoted any significant effects ($p < 0.05$) of treatment on consumption for each experiment. Four replicates were removed from statistical analysis of the *L. vittata* vs. egg capsule experiment because these replicates molted during the experimental trial. Kruskal-Wallis tests were used to assess the results of *P. hexataenia* experiments with a significance threshold of $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

The peppermint shrimp *Lysmata vittata* consumed both settled flatworm individuals and egg capsules laid on coral skeleton. The presence of *L. vittata* significantly reduced (GLM; $p < 0.001$) *Prosthiostomum acroporae* infestations over 24 h, with 82.0 ± 26.76% of the flatworms consumed (mean ± SD; n = 20; Fig. 1). Control tanks (n = 24) showed a loss of 5.83 ± 10.77% (n = 24; Fig. 1). This indicates that approximately 94% of flatworms were recovered using the screening method, which is consistent with previous use (Barton et al. 2019). *L. vittata* also significantly reduced *P. acroporae* egg capsules (GLM; $p < 0.05$), with 63.7 ± 43.48% (n = 20) of the egg capsules removed compared to only 1.0 ± 2.99% (n = 24) in the control (Fig. 1).

Lysmata shrimps use their setae-covered antennules to detect chemical cues (via cuticular sensilla) from their environment and locate suitable prey items (Zhu et al. 2011, Caves et al. 2016). Because they do not use visual mechanisms to locate and capture prey, *L. vittata* predation on *P. acroporae* is not hindered by the camouflage of these flatworms. However, *L. vittata* must physically encounter *P. acroporae* eggs or individuals while foraging to consume them, thus potentially limiting their ability to control *P. acroporae* populations in larger aquaria (aquaria >3.5 l were not tested in this study), where the probability of a direct encounter would be limited by proximity and the availability of alternate food sources (*L. vittata* were not fed during the trials). Despite this possible limitation, *L. vittata* remain useful as a potential treatment of *P. acroporae* infestations because intimate cohabitation with *Acropora* enables shrimp to scavenge among coral branches and consume *P. acroporae* individuals and egg capsules. *L. vittata* are also an aggregating species and can be kept in high numbers when provided with sufficient food and shelter (Vaughan et al. 2018b). Future research could examine diet preferences of *L. vittata*, which may contribute to their efficacy in removing flatworms from *Acropora* colonies (e.g. Grutter & Bshary 2004).

Experimental trials with *Pseudocheilinus hexataenia* demonstrated that these fish are effective at reducing the *P. acroporae* population, with their presence having a significant effect on flatworm abundance remaining on *A. millepora* fragments (Kruskal-Wallis; $p < 0.001$). All *P. acroporae* exposed to *P. hexataenia* were removed over 24 h (100%; n = 9), compared to a loss of 7.5 ± 13.92% of flatworms (mean ± SD; n = 9) in controls. In contrast, all egg capsules were recovered intact in the experimental treatments (100%; n = 9) when cohabited with *P. hexataenia.* In

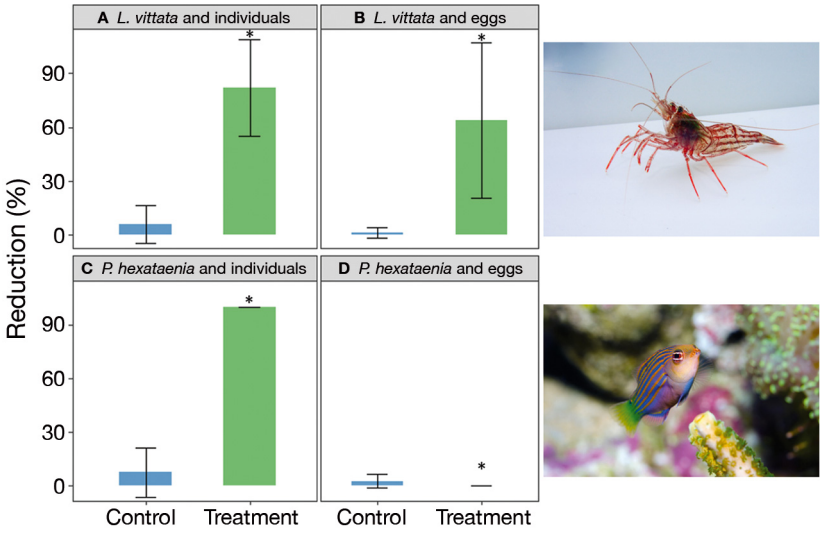

Fig. 1. Proportion of *Acropora*-eating flatworm individuals and egg capsules removed (error bars: ±SD) in the presence and absence of biocontrols. (A) *Lysmata vittata* and flatworm individuals (n = 24), (B) *L. vittata* and flatworm eggs (n = 20 egg clusters), (C) *Pseudocheilinus hexataenia* and flatworms (n = 9), and (D) *P. hexataenia* and flatworm eggs (n = 9 egg clusters). *: statistical significance between treatments and controls. Photos: = *L. vittata* and *P. hexataenia.* (*P. hexataenia* photo credit: creative commons license istockphoto.com user: marrio31 id#471448553)

the control, 2.39 ± 3.84 % egg capsules (mean ± SD; n = 9) were not recovered, resulting in significant differences between treatment and control (Kruskal-Wallis; $p < 0.05$), likely from incidental mechanical damage to egg capsules through handling.

These results indicate that *P. hexataenia* is highly efficient at eating flatworms using well-developed eyesight (Gerlach et al. 2016) but does not interact with the hard shell of flatworm egg capsules. The implementation of *P. hexataenia* as biological controls must consider their ecology and husbandry requirements. In the wild, these fish actively forage in their established territory (Geange & Stier 2009, Geange 2010), generally only coming together for mating purposes (Kuwamura 1981). While their foraging behavior appears similar in captivity, the solitary and territorial nature of *P. hexataenia* renders keeping more than 1 individual in smaller aquaria (e.g. <1000 l) problematic. More than 1 individual could be kept in aquaculture systems large enough to avoid territorial confrontation, but the 'patrol' range of this territory may remain relatively constant. It is for this reason, combined with the fact that this fish does not interact with flatworm egg capsules, that they may not be as suitable for treating acute infestations of *P. acroporae* compared to *L. vittata*. However, their performance in our trials suggests that this colorful labrid is a useful tool for consuming adult flatworms, thus mitigating the chronic impacts of a given *P. acroporae* infestation by removing or reducing the *P. acroporae* density to non-lethal levels for the *Acropora* host.

P. hexataenia and *L. vittata* identify prey items in different ways while foraging, which has implications for how they are used in the captive environment and their ecological roles in native ecosystems. Little is understood about the dynamics of wild *P. acroporae* populations, although our results may provide further understanding of the trophic relationships between *P. acroporae* and natural predators in reef ecosystems. *P. acroporae* are cryptic and there are no documented infestations causing colonial mortality of *Acropora* colonies in the wild. It does remain likely that some proportion of wild mortality of *Acropora* colonies attributed to other causes (e.g. sedimentation and algal competition) is instead experiencing negative secondary effects on coral health from *P. acroporae* infestation. However, the presence of natural predators of *P. acroporae* (e.g. *P. hexataenia* and *L. vittata*) may reduce incidences of mortality in wild *Acropora* colonies.

In captive systems, pairing both of these biological control organisms with the manual removal of *P. acroporae* egg clusters is likely to be highly effective in reducing the overall infestation within a given aquarium system. However, consideration must be given to the sustainable supply of the organisms if used as biological controls. *L. vittata* are available through the ornamental trade and can be bred in captivity. Although peppermint shrimp species from other regions (e.g. *L. wurdmenii, L. boggessi*, Rhyne & Lin 2006) were not investigated in the present study, they could also be examined for their ability to interact analogously with *P. acroporae* and could be supplied sustainably for biocontrol of flatworm infestations. Although *P. hexataenia* is categorized as Least Concern (Bertoncini 2010; IUCN Red List 2010), overharvesting for use as biological controls in the ornamental trade could impact local populations. Lessons should be taken from the Scandinavian salmonid industry, where harvesting of wrasse broodstock used for biological control of sea lice parasites has exerted considerable pressures upon wild populations (Brooker et al. 2018, Powell et al. 2018).

In summary, this study provides the first empirical evidence of potential biological control organisms for *P. acroporae* in captivity. The ability of both *L. vittata* and *P. hexataenia* to consume *P. acroporae* renders them useful preventative measures of infestation in addition to potentially being used to treat colonies infested with adult flatworms and thereby drastically reducing the impact of this pest on captive colonies. While *P. hexataenia* had no apparent interest in *P. acroporae* egg capsules, *L. vittata* displayed the added benefit of consuming egg capsules through their foraging activities, with encounters with the egg clusters likely to further control the flatworm populations in captive systems. The addition of sustainable biological control organisms adds a valuable tool for flatworm control, which is suitable for both aquarium hobbyists and large-scale coral aquaculture facilities.

Acknowledgements. We thank Brett Bolte and Rachel Neil for help in maintaining the *P. acroporae* culture. We also thank Kate Rawlinson for constructive comments on the manuscript. This project was funded through an AIMS@JCU pilot grant. Experiments were conducted in the National Sea Simulator, at the Australian Institute of Marine Science, under James Cook University Animal Ethics approval (A2466).

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Editorial responsibility: Tim Dempster, Melbourne, Victoria, Australia

Submitted: September 26, 2019; Accepted: December 9, 2019
Proofs received from author(s): February 3, 2020