Herbivore biocontrol and manual removal successfully reduce invasive macroalgae on coral reefs

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Invasive macroalgae pose a serious threat to coral reef biodiversity by monopolizing reef habitats, competing with native species, and directly overgrowing, and smothering reef corals. Several invasive macroalgae (Eucheuma clade E, Kappaphycus clade A and B, Gracilaria salicornia, and Acanthophora spicifera) are established within Kāne‘ohe Bay (O‘ahu, Hawai‘i, USA), and reducing invasive macroalgae cover is a coral reef conservation and management priority. However, invasive macroalgae control techniques are limited and few successful large-scale applications exist. Therefore, a two-tiered invasive macroalgae control approach was designed, where first, divers manually remove invasive macroalgae (Eucheuma and Kappaphycus) aided by an underwater vacuum system (“The Super Sucker”). Second, hatchery-raised juvenile sea urchins (Tripneustes gratilla), were outplanted to graze and control invasive macroalgae regrowth. To test the effectiveness of this approach in a natural reef ecosystem, four discrete patch reefs with high invasive macroalgae cover (15 – 26 %) were selected, and macroalgae removal plus urchin biocontrol (treatment reefs, n = 2), or no treatment (control reefs, n = 2), was applied at the patch reef-scale. In applying the invasive macroalgae treatment, the control effort manually removed ~ 19,000 kg of invasive macroalgae and ~ 99,000 juvenile sea urchins were outplanted across to two patch-reefs, totaling ~ 24,000 m² of reef area. Changes in benthic cover were monitored over two years (five sampling periods) before-and-after the treatment was applied. Over the study period, removal and biocontrol reduced invasive macroalgae cover by 85 % at treatment reefs. Our results show that manual removal in combination with hatchery raised urchin biocontrol is an effective management approach for controlling invasive macroalgae at reef-wide spatial scales and temporal scales of months to years.
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ABSTRACT

Invasive macroalgae pose a serious threat to coral reef biodiversity by monopolizing reef habitats, competing with native species, and directly overgrowing, and smothering reef corals. Several invasive macroalgae (Eucheuma clade E, Kappaphycus clade A and B, Gracilaria salicornia, and Acanthophora spicifera) are established within Kāneʻohe Bay (Oʻahu, Hawaiʻi, USA), and reducing invasive macroalgae cover is a coral reef conservation and management priority. However, invasive macroalgae control techniques are limited and few successful large-scale applications exist. Therefore, a two-tiered invasive macroalgae control approach was designed, where first, divers manually remove invasive macroalgae (Eucheuma and Kappaphycus) aided by an underwater vacuum system (“The Super Sucker”). Second, hatchery-raised juvenile sea urchins (Tripneustes gratilla), were outplanted to graze and control invasive macroalgae regrowth. To test the effectiveness of this approach in a natural reef ecosystem, four discrete patch reefs with high invasive macroalgae cover (15 – 26 %) were selected, and macroalgae removal plus urchin biocontrol (treatment reefs, \( n = 2 \)), or no treatment (control reefs, \( n = 2 \)), was applied at the patch reef-scale. In applying the invasive macroalgae treatment, the control effort manually removed \( \sim 19,000 \) kg of invasive macroalgae and \( \sim 99,000 \) juvenile sea urchins were outplanted across to two patch-reefs, totaling \( \sim 24,000 \) m\(^2\) of reef area. Changes in benthic cover were monitored over two years (five sampling periods) before-and-after the treatment was applied. Over the study period, removal and biocontrol reduced invasive macroalgae cover by 85 % at treatment reefs. Our results show that manual removal in combination with hatchery raised urchin biocontrol is an effective management approach for controlling invasive macroalgae at reef-wide spatial scales and temporal scales of months to years.
INTRODUCTION

Macroalgae have become a major component to introduced marine species worldwide (Schaffelke, Smith & Hewitt, 2006) as a result of spread through vectors including biofouling, ballast water, the aquarium trade, and mariculture (Ruiz et al., 2000; Zemke-White & Smith 2006; Williams & Smith 2007). Macroalgae production has increased considerably in the last 50 years, becoming a multi-billion dollar industry in over 150 countries (FAO, 2015; Loureiro, Gachon & Rebours 2015). Macroalgae mariculture occurs throughout tropical regions cultivating primarily non-native species within genera such as Caulerpa spp., Eucheuma spp., Gracilaria spp., and Kappaphycus spp. (Zemke-White & Smith 2006; FAO, 2015; Radulovich et al., 2015).

The macroalgae industry can provide economic incentives for coastal communities and offer a lucrative alternative to struggling fisheries-based economies (Pickering & Forbes, 2002; Mate, Namudu & Lasi, 2003). However, macroalgae production can have inadvertent consequences for tropical reef biodiversity (reviews by Schaffelke & Hewett 2007; Williams & Smith 2007; Davidson et al., 2015), contributing further to the global decline of live coral (Bruno & Selig 2007; Gardner et al., 2003; Pandolfi et al., 2003; De’ath et al., 2012). An issue that’s been exacerbated by the 2015 global coral bleaching event (Hooidonk, Maynard & Planes, 2014; Eakin et al., 2017; Hughes et al., 2017) drawing further attention to the need for immediate action to protect and restore coral reefs worldwide.

Invasive macroalgae have the potential to negatively impact coral reefs by overgrowing reef building corals, outcompeting native species, and altering benthic habitat and the aquatic environment (i.e., chemistry, irradiance, sediment loading) (Russell, 1983; Woo, 2000; Conklin & Smith, 2005; Chandrasekaran et al., 2008; Rasher & Hay, 2010; Martinez, Smith &
Richmond, 2012; Davidson et al., 2015; Murphy & Richmond, 2016). Furthermore, the deleterious impacts of invasive macroalgae can be exacerbated by eutrophication, limited herbivory, or a combination of the two (Smith et al., 1981; Lapointe, 1997; Larned, 1998; Smith, Smith & Hunter, 2001; Stimson, Larned & Conklin, 2001; Thacker, Ginsburg & Paul, 2001; Vermeij et al., 2009), and can contribute to ecosystem phase shifts from coral-dominated to macroalgae-dominated reefs (Done, 1992; Stimson, Larned & McDermid, 1996; Bellwood et al., 2004). Considering the wide range of ecosystem services coral reefs provide (i.e., food security, tourism, shoreline protection, and cultural value) (Moberg & Folke, 1999), control and reduction of invasive macroalgae are a management priority for coral reef conservation.

Diverse techniques have been applied to eradicate or control marine macroalgae and include manual, chemical, and biological treatments (reviewed by Anderson, 2007). The type of techniques applied depends on the response goal (i.e., eradication or control) and is often site and species specific (Anderson, 2007). Examples include chemical treatments (i.e., bleach, salt), thermal treatments (i.e., cold shock, heating), osmotic shock (i.e., freshwater and salinity treatments) (Cheshire et al., 2002; Williams & Smith, 2004; Wotton et al., 2004; Glaspy, Cresse & Gibson, 2005; Anderson, 2007), mechanical or manual removal by hand and/or aided by vacuum or dredge pumps (Curiel et al., 2001; Miller et al., 2004; Hewitt et al., 2005; Conklin, 2007; Marks, Reed & Obaza, 2017), light attenuation, containment barriers, and even water removal or *in situ* desiccation (Anderson, 2007).

Biocontrol of invasive macroalgae is a newly emerging and promising macroalgae control technique. For instance, experimental use of sea urchin and mollusk biocontrol for controlling
invasive macroalgae species such as *Caulerpa taxifolia*, *Caulerpa racemosa*, and *Codium fragile* has been evaluated in the Mediterranean and Atlantic (Boudouresque, Lemée & Meinesz, 1996; Thibaut & Meinesz, 2000; Scheibling & Hatcher, 2007; Cebrian et al., 2009). These studies revealed successful biocontrol applications have the highest impact in areas of low infestation (Scheibling & Hatcher, 2007; Cebrian et al., 2009) and suggest invertebrate biocontrols are most effective for emerging populations of invasive macroalgae. In some cases, the effectiveness of these treatments has been limited by macroalgae toxicity to biocontrol agents (Boudouresque et al., 1996), as well as the speed and ability of biocontrol species mariculture and outplanting at adequate densities to affected areas (Thibaut, 2000). Macroalgae abatement from herbivore biocontrol has recently shown promise on Hawai`i’s reefs. The short-spined sea urchin, *Tripneustes gratilla* (Linnaeus) is a generalist herbivore native to Hawai`i and will feed on at least five species of invasive macroalgae (Stimson, Cunha & Philippoff, 2007; Westbrook et al., 2015). *T. gratilla* has the potential for application as an invasive macroalgae biocontrol agent and has been shown to reduce macroalgae biomass within cage-enclosures *in situ* (Conklin & Smith, 2005; Stimson, Cunha & Philippoff, 2007; Chon, 2014; Westbrook et al., 2015). Moreover, *T. gratilla* has low vagility, can be easily handled, and maricultured from wild urchin stock and outplanted as juveniles (~ 2.5 cm test diameter). Finally, *T. gratilla* achieves its maximum growth rate within the first two-years of life, and test size can reach 5.6 – 8.3 cm while grazing on invasive macroalgae species (Pan, 2012).

Invasive macroalgae are prominent in the Hawaiian archipelago, and as a result a number of aforementioned macroalgae control techniques have been tested in Hawai`i (Smith et al., 2004; Conklin & Smith, 2005). Nineteen documented species of macroalgae have been introduced
100 into Hawai‘i since the 1950’s, concentrated primarily on the island of O‘ahu where the main
101 shipping and military ports are located (Russell, 1992; Smith, Hunter & Smith, 2002; DLNR,
102 2003). Several Rhodophyta macroalgae species have been particularly successful at invading
103 Hawaiian reef communities, including Eucheuma clade E (N.L. Burman) F.S. Collins & Hervey,
104 and Kappaphycus clade A and clade B (Doty) Doty ex P.C. Silva (Conklin, Kurihara &
105 Shirwood, 2009), Acanthophora spicifera (Vahl) Børgesen, and Gracilaria salicornia (C.
106 Agardh) E.Y. Dawson. The introduction of these macroalgae to Hawai‘i in the mid-20th century
107 occurred through a variety of pathways including ship biofouling, ballast water discharge, and
108 mariculture experimentation and production (Doty, 1961; Russell, 1983; Russell, 1992; Smith,
109 Hunter & Smith, 2002).

110 Three Eucheumoid species of the genus Kappaphycus and Eucheuma from the Philippines, were
111 intentionally planted on reefs around Moku o Loʻe Island (Coconut Island) at the Hawai‘i
112 Institute of Marine Biology (HIMB) (Kāneʻohe Bay, Hawai‘i) for experimentation in the 1970’s
113 (Doty, 1977; Russell, 1983). Molecular techniques (Zuccarello, Smith & West, 2006; Conklin,
114 Kurihara & Shirwood, 2009) have identified these species as Kappaphycus clade A,
115 Kappaphycus clade B, and Eucheuma clade E (hereafter Eucheuma). Prior to this analysis,
116 nomenclature for these species has been inconsistent; therefore we will refer to this group
117 collectively as E/K hereafter unless referring specifically to species. E/K was left unchecked in
118 Kāneʻohe Bay for over two decades and by 1996, it had spread > 5 km from Moku o Loʻe Island
119 and were found throughout Kāneʻohe Bay (Rodgers & Cox, 1999) and continued to spread to
120 previously unaffected northern reefs adjacent to Kāneʻohe by 1999 (Conklin & Smith, 2005).
121 Eucheuma and Kappaphycus clade A are thought to spread only through vegetative propagation
and their distribution has been restricted to Kāne‘ohe Bay, whereas *Kappaphycus* clade B is able to disperse vegetatively and sexually and has been documented outside of the Bay (Conklin, Kurihara & Shirwood, 2009). *A. spicifera*, the most widely distributed non-native macroalgae in Hawai‘i (Smith et al., 2002), is thought to have been introduced and spread via ship biofouling or ballast water (Doty 1961, Russel 1983) or possibly through aquarium imports (Russel 1992). *A. spicifera* is a common fouling species on ship hulls and is able to disperse sexually and via vegetative fragmentation, which may explain its wide distribution (Smith, Hunter & Smith, 2002). The origin of *G. salicornia* are speculative, possibly arriving to Hilo Bay in the 1940’s associated with ships originating from the Philippines (Smith et al., 2004) and then later intentionally transplanted to various sites around Moloka‘i and O‘ahu, including Kāne‘ohe Bay (Russel, 1992; Smith, Hunter & Smith, 2002; Smith et al., 2004). *G. salicornia* is thought to disperse primarily via vegetative fragmentation (Smith et al., 2004).

All five species are capable of forming dense mats on the reef, overgrowing reef corals, and monopolizing reef habitats (Russel, 1983; Ask and Azanza, 2003; Conklin & Smith, 2005; Martinez, Smith & Richmond, 2012). *E/K* has been shown to be particularly damaging to corals by shading and smothering live coral and can eventually lead to mortality (Russel, 1983; Woo, 2000; Conklin & Smith, 2005; Chandrasekaran et al., 2008). *G. salicornia* can also impact reef corals by decreasing irradiance via smothering, altering water chemistry (i.e., hypoxia and hypercapnia) and increasing sedimentation surrounding reef corals (Martinez, Smith & Richmond, 2012). Although five of these invasive macroalgae species are thought to be damaging to reef biodiversity, *E/K* were deemed a management priority due to its especially...
damaging impacts to corals and its limited distribution compared to *A. spicifera* and *G. salicornia* (DLNR, 2003).

In response to the destructive impact to corals and the concern that *E/K* would continue to spread and establish on reefs outside of the bay, local managers, community members, and researchers worked to develop a control technique for invasive macroalgae with particular focus on *E/K*. Conklin & Smith (2005) tested various control methods and found that *E/K* quickly regrew after manual removal, but sea urchin biocontrol showed a sustained reduction of *E/K* in small-scale field trials. Conklin & Smith (2005) recommended combining techniques by using manual removal to remove the bulk of *E/K* biomass followed by sea urchins treatment to control regrowth. Preliminary field trials conducted by DLNR on a patch reef in Kāne‘ohe Bay supported this observation (DLNR, 2013). Based on these findings and recommendations, a large-scale invasive macroalgae control project on patch reefs in Kāne‘ohe Bay was initiated in 2008 using the combination of manual removal and sea urchin biocontrol.

The overarching goal of the project was the restoration and preservation of coral reef habitat and associated biodiversity with specific management objectives to (i) reduce invasive macroalgae on Kāne‘ohe Bay patch reefs, and (ii) stop the spread of *E/K* to unaffected reefs within and outside Kāne‘ohe Bay. Although the macroalgae control techniques applied in this study were evaluated previously in small-scale experiments, the combined use of manual removal and sea urchin biocontrol has yet to be tested as a management approach at the reef scale. In this study we evaluate the effectiveness of manual removal combined with urchin biocontrol in controlling invasive macroalgae [*E/K* (i.e., *Eucheuma*, *Kappaphycus* clade A, *Kappaphycus* clade B), *G.*]
salicornia, A. spicifera] at a reef-wide scale over two years following a Before-After Control-Impact (BACI) experimental design. We hypothesized that our proposed invasive macroalgae removal and control methods would be effective at maintaining low invasive macroalgae abundance (percent cover) over time at treatment reefs relative to untreated-control reefs. While, a factorial design testing each treatment type separately (i.e., manual removal, biocontrol, and combined treatments) might be preferred, this, was not possible due to logistic and financial challenges associated with implementing and replicating three separate treatment types at the reef-wide scale. However, previous findings of Conklin & Smith (2005) and data from the State of Hawai‘i Division of Aquatic Resources at a scale smaller than the one applied in the current study showed manual removal of invasive algae in the absence of biocontrol cannot successfully reduce invasive macroalgae cover over long term. Simply applying urchin biocontrol without manual removal was also not advised based on concerns of increased fragmentation by urchins detaching holdfasts of large E/K mats. In addition, applying urchins to a large standing crop of macroalgae would increase the amount of urchins, grazing time, and ultimate cost required to successfully treat a reef. Therefore, our goal was to use a single, most-effective treatment type (i.e., the combination of manual removal and biocontrol) and test whether this treatment was effective at reducing invasive algae cover long term among replicate patch reefs.

MATERIALS AND METHODS

Study Site

Invasive macroalgae removal and biocontrol techniques were carried out on four shallow (0.5 – 2.0 m depth) patch reefs located in central Kāne‘ohe Bay, on the windward side of O‘ahu, Hawai‘i (21°28’0”N, 157°49’0”W), which is the largest embayment in the Hawaiian Islands and
contains over 70 distinct patch reefs surrounded by a barrier reef and fringing reef system (Fig. 1). The patch reefs are island like features separated by 10 – 15 m sand bottom. Two patch reefs (Reef 26 and 27) were designated as treatment reefs, where manual removal of E/K and sea urchin biocontrol were applied, and two patch reefs (Reef 16 and 28) were designated as control reefs where no macroalgae manual removal or biocontrol were applied (Fig. 1). Study reefs were selected based on the presence of invasive macroalgae and their close proximity to each other. Designated patch reefs were approximately 11,900 m$^2$ (treatment Reef 26), 12,700 m$^2$ (treatment Reef 27), 3,100 m$^2$ (control Reef 16), and 14,500 m$^2$ (control Reef 28). Each patch reef has a distinct reef slope composed primarily of live coral and a shallower reef flat consisting of a mix of live coral, dead coral, rubble, and sand. E/K occurred on reef slopes and reef flats and ranged in size from single low growing thalli to dense mats 1 m$^2$ in area and ~ 0.3 m thick (Fig. 2a-b). Gracilaria salicornia and Acanthophora spicifera occurred primarily on the reef flats and also ranged from single thalli to mats > 1 m$^2$ and ~ 0.1 m thick (Fig. 2c-d).

**Invasive Macroalgae Control Technique**

Invasive macroalgae were controlled in two phases. First, E/K were manually removed from reefs by divers aided by an underwater vacuum system (“The Super Sucker”) that transported macroalgae from the reef to a support vessel (Fig. 3a). To a lesser extent, divers manually removed and bagged macroalgae without aid of the Super Sucker system. At the support vessel, macroalgae was bagged, weighed (wet weight to the nearest kg), and then delivered to farmers in the Kāne‘ohe Bay watershed for use as an agricultural fertilizer. Manual removal was conducted from November 2011 to March 2012 on treatment Reef 26 over 23 working days and treatment Reef 27 was cleared from March 2012 to August 2012 over 25 working days (Table 1). Divers
removed the bulk of the E/K biomass, leaving macroalgae in hard-to-reach areas (e.g., between coral fingers and within crevices), small clumps (< 400 cm³) and holdfasts to maximize the yield to effort ratio and minimize disturbance to other benthic organisms and habitats. Invasive macroalgae species *G. salicornia* and *A. spicifera* were not directly targeted by divers for manual removal.

**Sea Urchin Biocontrol**

Adult *Tripneustes gratilla* were collected from the wild and spawned at an urchin hatchery. Urchin larvae were settled and reared in tanks on land until they reached approximately 2.5 cm diameter test size (~ 4 – 6 months after spawning). A new cohort was produced every 30 – 60 days throughout the duration of the study. Following E/K manual removal, juvenile urchins were transported to the reef in trays and hand placed on the treatment reefs where *G. salicornia*, *A. spicifera*, and E/K occurred (Fig. 3b-d). A systematic approach was used to outplant urchins to achieve a relatively consistent urchin density throughout the entire reef.

On treatment Reef 26, a total of 46,913 *T. gratilla* were outplanted to affected areas, the majority of which (76 % of total) were outplanted from December 2011 to October 2012, with supplemental outplanting from July to December 2013 (19%) one additional outplanting in July of 2014 (13 % and 5 % of total, respectively) (Table 1). On treatment Reef 27, a total of 52,253 urchins were outplanted (Table 1), primarily from August 2012 to May 2013 (97 % of total) with one additional supplemental stocking (1,500 urchins) in December 2013. Stocking density of juvenile urchins was 3.9 urchins m⁻² on treatment Reef 26 and 4.2 urchins m⁻² on treatment Reef 27 (Table 1).
Invasive Macroalgae Control Costs

Control costs were calculated for field operations (i.e., manual removal and sea urchin outplanting) and sea urchin hatchery operations. Cost estimates included salaries and operating expenses (i.e., equipment, materials, supplies, fuel, and utilities).

Benthic surveys

Baseline benthic surveys were performed at all patch reefs from November 2011 to February 2012 (hereafter, Winter 2011) prior to macroalgal removal and urchin outplanting representing the “before” period of the analysis. Subsequently, benthic surveys were repeated during the treatment period at four additional times during summer and winter seasons from 2012 – 2014, representing the “after” period of the analysis. Binned sampling periods were defined as: May – June 2012 (hereafter, Summer 2012), December 2012 – February 2013 (hereafter, Winter 2012), May – June 2013 (hereafter, Summer 2013), and February 2014 (hereafter, Winter 2013). Using these five time points we analyzed changes in percent cover of invasive macroalgae (Eucheuma, Kappaphycus clade B, G. salicornia, A. spicifera), live coral, native macroalgae, crustose coralline algae (CCA), and the combined sand/rubble, bare space, turf (SBT) at treatment and control reefs.

Fixed transect locations were randomly selected using ArcGIS random point tool (ESRI, 2011) within the following strata: windward and leeward prevailing wind orientation (northeast) and habitat type (aggregate reef, mixed/unconsolidated reef, and pavement/consolidated reef situated on reef flat and reef slope areas). In addition to habitat, a windward/leeward stratification was
applied to control for the possibility of detached algae collecting disproportionately on the leeward side of reefs as a result of wind driven currents. Reef flat transects ran perpendicular to the prevailing wind direction at a bearing of ~140 degrees. Reef slope transects followed the ~1 m depth contour clockwise around the reef. The number of transects per reef varied according to reef size at an average sampling effort of one transect per ~800 m$^2$. The total fixed transects for each reef were: 6 (control Reef 16), 18 (control Reef 28), 13 (treatment Reef 26), and 14 (treatment Reef 27).

The number of transects were allocated in proportion to the total reef area first, then by primary reef habitats (aggregate and non-aggregate), then by non-aggregate sub-strata (mixed/unconsolidated reef and pavement/consolidated reef).

Mean benthic cover was estimated using a point intercept transect method (Hill & Wilkinson, 2004). Surveyors recorded the benthic cover at 0.2 m intervals along a 25 m transect ($n = 126$ points transect$^{-1}$). *T. gratilla* were surveyed at each transect location, counting all observed individuals within a 25 × 1 m belt. A correction factor of 90 % detectability (based on F. Mancini and D. Minton field trials) was used to estimate the density of urchins from transect counts.

**Data analysis**

Response variables (percent cover of invasive macroalgae, native macroalgae, CCA, coral, and SBT) were monitored over time, with baseline surveys at each patch reef (Winter 2011) designated as the “before” period and four subsequent surveys (Summer 2012, Winter 2012, Summer 2013, Winter 2013) designated as “after” periods. Treatment application (i.e., algae
removal plus urchin outplanting) was partial in Summer 2012 and complete by Winter 2012 (Table 1). Changes in community cover was assessed using a linear mixed effects model fit by restricted maximum likelihood in the lme4 package (Bates et al., 2014) in R version 3.3.0 (R Development Core Team, 2015). Treatment (E/K manual removal and biocontrol vs. no E/K removal or biocontrol) and time (before treatment applied vs. periods after treatment applied) were included as fixed effects. To account for spatial structure of the patch reef benthos habitat types within patch reefs (i.e., aggregate reef, mixed/unconsolidated reef, and pavement/consolidated reef) were included as a random effect nested within individual reefs. Reef transects were included as a repeated-measure random effect. Considering that surveys conducted over the two-year study period spanning different months and seasons, we first tested ‘season’ (i.e., summer vs. winter) separately as a fixed effect using a linear model; no effects were observed ($P \geq 0.408$) and season was not included in the final analysis. Normality of residuals and homogeneity of variance were verified using graphical inspection of standardized residuals, and data transformations were applied where assumptions of ANOVA were not met. Analysis of variance tables were generated using type-II sum of squares with Satterthwaite approximations of degrees of freedom using the package lmerTest (Kuznetsova et al., 2016). Where significant interactions were found, posthoc slice tests were performed using lsmeans (Lenth, 2016) to evaluate differences between control and treatment reefs at each time point. All data and code to reproduce figures and analyses can be found on Zenodo (xxx).

RESULTS

Initial field surveys

Mean benthic cover was comparable for all groups (i.e., invasive and native algae, coral, CCA,
bare substrate) \((\text{posthoc: } p \geq 0.721)\) at treatment and control reefs at the start of the study (Fig. 4a-e). In Winter 2011, biological benthic cover at the four study reefs was, on average, dominated by reef corals (mean ± SE) \((39 ± 13 \%)\), followed by invasive macroalgae \((21 ± 5 \%)\), CCA \((5 ± 2 \%)\), and native macroalgae \((5 ± 2 \%)\). The native macroalgae community cover was composed primarily of \textit{Dictyosphaeria versusii} \((74\%)\) and \textit{Dictyosphaeria cavernosa} \((19\%)\). Invasive macroalgae on control reefs was predominantly \textit{Gracilaria salicornia} \((11\%)\), and \textit{Eucheuma} \((7\%)\), whereas invasive macroalgae cover at treatment reefs was comparable among \textit{G. salicornia}, \textit{Acanthophora spicifera}, and \textit{Eucheuma} \((5 – 7 \%)\) (Fig. 5a-b). \textit{Kappaphycus} clade B made up the smallest component of the invasive macroalgae community \((0 – 2.5\%)\) on all study reefs (Fig. 5a-b).

**Macroalgae removal and urchin outplanting surveys**

Divers removed a total of 11,963 kg wet weight \((0.81 ± 0.14 \text{ kg m}^{-2})\) of \textit{E/K} from affected areas of treatment Reef 26 and 7,095 kg wet weight \((0.622 ± 0.05 \text{ kg m}^{-2})\) from treatment Reef 27 (Table 1). The majority of macroalgae was removed using the Super Sucker \((80\%)\) versus hand removal using bags \((20\%)\). \textit{E/K} was cleared at an average rate of \(1.48 ± 0.14 \text{ m}^2 \text{ min}^{-1}\). On treatment reefs, the mean \((± \text{ SE})\) removal effort was greater for Reef 26 \((2.36 ± 0.27 \text{ m}^2 \text{ min}^{-1})\) compared to treatment Reef 27 \((1.23 ± 0.10 \text{ m}^2 \text{ min}^{-1})\) as well as the \textit{E/K} biomass removed \(0.81 ± 0.14 \text{ kg m}^{-2}\) (Reef 26) versus \(0.62 ± 0.05 \text{ kg m}^{-2}\) (Reef 27). While stocking density of juvenile urchins was designed to be \(\sim 4 \text{ urchins m}^{-2}\) (Table 1), field surveys following urchin outplanting estimated urchin densities to be \(0.90 \text{ urchins m}^{-2}\) (treatment Reef 26) and \(0.74 \text{ urchins m}^{-2}\) (treatment Reef 27). \textit{Tripneustes gratilla} was not reported in benthic surveys on control Reefs 16 and 28.
Post-macroalgae removal and urchin outplanting surveys

E/K macroalgae manual removal and urchin biocontrol led to an 85% decline in invasive macroalgae over the study period, from 21% cover in Winter 2011 to 4% cover in Winter 2013 (Fig. 4a, 5b, 6). Invasive macroalgae was affected by the interaction between treatment and time (Table 2). On treatment reefs, percent cover of *Eucheuma*—a target of manual macroalgae removal—had declined by 59% at the first sampling time (Summer 2012), approximately 6 months after the treatment had been applied (Fig. 5b). However, total invasive macroalgae cover on treatment reefs did not significantly differ from control reefs until one year after the treatment application had begun (*posthoc*: *p* = 0.029). By Winter 2012 total invasive macroalgae cover had declined by 29% relative to Winter 2011 levels. The mean invasive macroalgae cover at control reefs fluctuated between 14 – 25% over the entire study period (Winter 2011 to Winter 2013) (Fig. 4a, 6) and comparable across all time points, (*posthoc*: *p* ≥ 0.080) except Winter 2013 where invasive algae declined relative to start of the study (*posthoc*: *p* = 0.005). *G. salicornia* and *Eucheuma* consistently dominated the invasive macroalgae community at control reefs (Fig. 5a), representing mean cover of 7 – 12% at each sampling time throughout the study period.

Mean native macroalgae percent cover ranged from 2 – 5% over the study period and decreased over time (*p* < 0.001) but not in response to treatments (*p* = 0.906) (Table 2) (Fig. 4b). The interaction of treatment × time affected coral (*p* < 0.001) and CCA cover (*p* = 0.037), and both coral and CCA increased over the study period (*p* < 0.001). However, mean coral and CCA cover did not differ among control and treatment reefs at each discrete time point (*posthoc*: *p* ≥
SBT (sand/bare/turf) was not affected by time, treatment, or their interaction ($p \geq 0.255$) (Table 2), but tended to be lower at control reefs (25 – 30 % cover) relative to treatment reefs (35 – 40 % cover) (Fig. 4e).

Invasive Macroalgae Control costs

Field portions of macroalgae removal and control operations cost an estimated $255,000 and hatchery operations cost $562,000, totaling $817,000 to treat $24,600 km$^2$ ($33 \text{ m}^2$) of affected reef.

DISCUSSION

Effectiveness of invasive macroalgae control

For invasive macroalgae control, there are few demonstrated actions available for managers when prevention and eradication attempts have failed and valuable resources and biodiversity are at risk (Anderson, 2007). Further, there are few examples of macroalgae control techniques being successfully applied beyond small-scale experiments. The present study demonstrates manual removal of invasive macroalgae, in combination with outplanting hatchery raised juvenile urchins (*Tripneustes gratilla*) for biocontrol, can be an effective approach for reducing the benthic cover of invasive macroalgae at the reef-wide scale. Invasive macroalgae was reduced by 81 %, two-years after macroalgae removal and sea urchin biocontrol was applied—a result consistent with a small-scale experiment that employed a similar control technique over a shorter time period (Conklin & Smith, 2005).
The treatments applied in this study showed promising results in controlling invasive macroalgae. Manual removal aided by the Super Sucker system was an effective means to remove E/K biomass (51% decline post manual removal) and was also an efficient means of moving thousands of kilograms of macroalgae from the reef to the support vessel at a mean removal rate of 1.48 ± 0.14 m² min⁻¹. In addition, the vacuum system captured loose macroalgae fragments created by dislodging the macroalgae; a possible risk reduction measure for macroalgae propagule dispersal. Following manual removal, invasive macroalgae continued to decline by 61% from Winter 2012 to Winter 2013 (Fig. 4a). Although individual treatment types were not tested here, we speculate that this decline was a result of T. gratilla biocontrol based on the findings of Conklin & Smith (2005), which documented steady re-growth of E/K without T. gratilla biocontrol. It should be noted that manual removal and sea urchin biocontrol manipulations deployed in this study took several months to carry-out (Table 1) and supplemental urchins were added to reefs throughout the study to account for attrition. Therefore, the first “after period” (i.e., Summer 2012) may be viewed as a transitional period in the chronology of our experiment, bridging pre-manipulation and full treatment establishment periods.

Reef-wide scale studies have strong applicability to management; however, they can also present many challenges in terms of replication and sample size. Alternatively, studies conducted at the plot-level scale may improve replication, but present their own set of challenges when extrapolating results to larger scales. In regards to this study, replication was low, but the results are highly applicable to management especially in terms of utilizing a mobile invertebrate as a biocontrol at the reef-wide scale. Our results indicate that benthic cover was comparable among
all four reefs at the onset of the study, however, it should be acknowledged that the benthic community and habitats differed between reef replicates and the sample size was low (two-treatment reefs and two control reefs). Despite these shortcomings, the treatment reefs showed clear and lasting results of invasive macroalgae decline over the course of the study indicating that this was a successful approach in controlling invasive macroalgae.

The sea urchin, *T. gratilla*, are well suited for mariculture and outplanting for the biocontrol of invasive macroalgae. *T. gratilla* are able to be propagated in a hatchery using wild stock, producing large numbers of juvenile urchins (~100,000 yr$^{-1}$) (pers. comm. D. L. Cohen, 2017) without impacting wild *T. gratilla* populations. The small size (~2.5 cm test diameter) of outplanted *T. gratilla* may also be an important factor in treating invasive macroalgae. Chon (2014) found small urchins (0.5 – 2.5 cm test diameter) were more effective at grazing invasive macroalgae than adult *T. gratilla* (~4 cm test diameter) within *in situ* enclosures. Suggesting small test-size urchins are more capable of grazing holdfasts within the small interstitial spaces of the reef.

While the primary target species for manual removal was *E/K*, other invasive macroalgae not targeted by manual removal (*Gracilaria salicornia* and *Acanthophora spicifera*) also declined over the study period (Fig. 5b). Potentially, the reductions in *G. salicornia* and *A. spicifera* cover at treatment reefs may be due to urchin herbivory also reducing the cover of these non-targeted (for manual removal) invasive macroalgae. In feeding trials, *T. gratilla* consumed all four species of invasive macroalgae found in this study, but given the choice, urchins preferred *A. spicifera*, especially among smaller test-size urchins (Westbrook et al., 2015). *T. gratilla* will
also graze *G. salicornia*, but displays the least preference for this species (Stimson, Cucha & Philippoff, 2007; Westbrook et al., 2015). Further, Westbrook *et al.*, (2015) found that *T. gratilla* were able to graze invasive macroalgae at a rate of 7.5 g d\(^{-1}\) per urchin, which they estimated to be roughly equal to the growth rate of the four species of invasive macroalgae examined.

This study demonstrates that *T. gratilla* biocontrol can be successful when applied at the patch reef-wide scale (~12,000 m\(^2\)). However, since movement of urchins were naturally confined by 10 – 15 m deep sandy habitats surrounding the patch reefs, this raises the question as to whether *T. gratilla* would be as effective in treating larger continuous reefs. Valentine & Edgar (2010) detected a significant decline of macroalgae on continuous reef habitats when *T. gratilla* are present in high densities (>4 m\(^{-2}\)) at Lord Howe Island. Stimson, Larned & Conklin (2007) found *T. gratilla* movement to be <1 m d\(^{-1}\) and suggested that this low vagility may explain its generalist diet of a wide range of macroalgae species including non-natives. The low movement rates have allowed Hawai‘i managers to utilize *T. gratilla* in spot-treating areas with high invasive macroalgae biomass and apply a manipulated urchin density in problematic locales.

It is reasonable to acknowledge the potential risk of urchin stocking in Kāne‘ohe Bay to facilitate rapid *T. gratilla* population growth. However, we believe this risk is unlikely due to a number of factors, including natural predation of *T. gratilla* by fish, decapods, and cephalopods and the close monitoring of outplanted urchin populations by managers. In addition, no natural recruitment of hatchery raised *T. gratilla* in Kāne‘ohe Bay has been observed (personal
observation). Although the urchins are reproductively viable, for reasons unknown, conditions in Kāne‘ohe Bay have not been favorable for *T. gratilla* recruitment to patch reefs.

Herbivorous reef fish grazing has also been demonstrated to have a profound impact on macroalgae cover (Williams & Poluni, 2001; Burkepile & Hay, 2006; Hughes et al., 2007; Rasher & Hay, 2013) and may have also contributed to reductions in invasive macroalgae cover across the four reefs examined in this study. Stamoulis et al., (2017) found that *G. salicornia* was the second most prevalent macroalgae species in gut contents of herbivorous reef fishes in Kāne‘ohe Bay. In addition, E/K and *A. spicifera* were identified in fishes gut contents, but were far less prevalent (Stamoulis et al., 2017). Although herbivorous fish appear to be a substantial contributor to controlling invasive macroalgae, protection of herbivorous fishes (in a small marine protected area) alone was not able to reduce invasive macroalgae levels significantly in all reef habitats (Stamoulis et al., 2017). Other Hawaiian reefs that have protection rules in place for herbivores, including *T. gratilla*, have found significant reductions in macroalgae including *A. spicifera* (Williams et al., 2016). Based on the findings of this study and others (Conklin & Smith, 2005; Stimson, Cunha & Philippoff, 2007; Westbrook et al., 2015; Chon, 2014), *T. gratilla* appears to be the most effective single biocontrol species when combined with manual removal for treating invasive macroalgae on Hawai‘i coral reefs.

*T. gratilla* are effective invasive macroalgae grazers (Conklin & Smith, 2005; Stimson, Cunha & Philippoff, 2007; Westbrook et al., 2015; Chon, 2014), however, it has been suggested that urchin herbivory may have negative effects. For instance, indiscriminate low-profile grazing on the reef substratum may reduce the survival of juvenile corals (Forsman et al., 2006), newly
settled coral recruits, or CCA (Stimson et al., 2007). CCA are an important component of reef structure and stability (Bak 1976), in addition to providing a substratum for coral recruitment and development (Morse et al., 1996, Negri et al., 2002, Harrington et al., 2004). However, we found no effect of urchin grazing treatment on coral cover or CCA. Instead, coral cover and CCA showed positive trends through time independent of treatments. Similarly, Stanley (2014) found T. gratilla had no effect on settlement or survival of six Kāne‘ohe Bay coral species and Valentine & Edgar (2010) T. gratilla outbreaks had no effect on coral cover in Lord Howe Island, Australia. Together, these results suggest T. gratilla stocked at densities for biocontrol actions do not appear detrimental to reef corals or ecologically important CCA.

The observed decrease in invasive macroalgae on control reefs over the course of the study did not result in a significant increase in any single benthic cover type as a result of the treatment (Fig. 4). However, the benthic community composition appears to have changed throughout the course of the study (Fig. 6). This shift from areas dominated by invasive macroalgae to a mix of coral, CCA, native macroalgae, and SBT (sand/bare/turf) may favor the settlement of native flora and fauna and increase the accessibility of suitable settlement substratum. Additionally, the application of manual removal plus urchin biocontrol resulted in no reductions in ecologically important benthic groups, such as corals and CCA. Approaches to control invasive macroalgae are diverse and not always benign (Anderson, 2007), and applying such treatments on ecologically sensitive habitats, such as coral reefs, demands minimal environmental impacts.
Therefore, manual removal of invasive macroalgae in combination with sea urchin outplanting proves to be a successful approach at invasive macroalgae mitigation in Hawaii. However, the effectiveness of this approach on other reef systems should be appropriately tested at small experimental scales before reef-wide treatments are applied (Conklin & Smith, 2005, DLNR, 2013). Such tests are necessary to evaluate the need for manual removal, sea urchin biocontrol or both in controlling invasive macroalgae and weighing logistic and financial constraints.

Control Costs

The control of invasive macroalgae for this study was a substantial investment by managers at a cost of $817,000 to treat 24,600 km$^2$ ($33$ m$^2$) of affected reef. This figure not only demonstrates the need to invest in invasive species prevention through strict vector management and importation rules, but also indicates the importance and value of Hawai‘i’s reefs in order to justify such a large expense. Cesar & Beukering (2003) estimated a 360 million dollar a year net benefit for Hawai‘i’s economy and a total value of 10 billion dollars. Therefore, investment in restoration and preservation of coral reef ecosystems by controlling invasive macroalgae may be a worthwhile economic investment. It should also be noted that the cost per m$^2$ of treated reef may be reduced by increased sea urchin production and further advances in sea urchin aquaculture could reduce this cost considerably.

Conclusion

Our findings show that manual removal and sea urchin biocontrol applied at a reef-wide scale are an effective approach for controlling invasive macroalgae, but should not be viewed as a
replacement for managing some of the other drivers of macroalgae phase shifts, including increased nutrients (Lapointe, 1997; Stimson, Larned & McDermid, 2001), and reduced herbivory (Hay, 1984; Hughes, 1994; Larned, 1998; Bellwood et al., 2004). The control techniques demonstrated in this study combined with watershed (Richmond et al., 2007) and herbivore (Mumby & Steneck, 2008) management are necessary to achieve broad goals of reef restoration and habitat improvement. Marine reserves and Herbivore Fisheries Management Areas have shown positive results in Hawai‘i, by increasing biomass of herbivorous reef fish and reducing cover of invasive macroalgae (Friedlander, Brown & Monaco, 2007; Williams et al., 2016). Unfortunately, native reef fish and urchin assemblages may not be capable of controlling the combined growth rates of multiple invasive macroalgae species, and therefore, a suite of management strategies may be necessary to control invasive macroalgae at a large-scale.

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Table 1. Invasive macroalgae manual removal and *Tripneustes gratilla* outplanting dates, area, and stocking density.

Table 2. Analysis of variance table for treatment and time effects on coral reef community cover.

Fig. 1. Study site location in Kāne‘ohe Bay on the windward side of the island of O‘ahu, Hawai‘i, proximate to Moku o Lo‘e (Hawai‘i Institute of Marine Biology).

Fig. 2. Invasive macroalgae species found on study reefs in Kāne‘ohe Bay: (A) *Eucheuma* clade E, (B) *Kappaphycus* clade B, (C) *Gracilaria salicornia*, (D) *Acanthophora spicifera* (photo credit: Brian Neilson).

Fig. 3. Invasive macroalgae control techniques applied in the field (A) using the Super Sucker to manually remove *Eucheuma* clade E, (B) outplanting juvenile *Tripneustes gratilla*, (C) outplanted adult *T. gratilla* surrounded by *Gracilaria salicornia* and *Acanthophora spicifera*, (D) adult *T. gratilla* surrounded by *Eucheuma* clade E, (E) before and immediately (F) after manual removal of *Eucheuma* clade E revealing crustose coralline algae (CCA) and (G) before and (H) after removal of *Eucheuma* clade E revealing live and dead coral (photo credit: (A-B) DLNR/DAR, (C-H) Brian Neilson).

Fig. 4. Mean percent cover for (A) combined invasive macroalgae (*Eucheuma* clade E / *Kappaphycus* Clade B/*Acanthophora spicifera*/*Gracilaria salicornia*), (B) native macroalgae, (C) crustose coralline algae (CCA), (D) corals, and (E) SBT (sand/bare/turf). Value are mean ± SE; *n* = 24 (control) and *n* = 26 – 27 (treatment) for each sampling time. The first time point in each figure (Winter 2011) represents the “before” time period of the study and all subsequent time points represent the “after” period. Symbols (*) represent a significant difference (*p* ≤ 0.05) between the control and treatment.

Fig. 5. Percent cover for invasive macroalgae species through time at (A) control reefs and (B) treatment reefs. Value are mean ± SE; *n* = 24 (control reefs) and *n* = 26 – 27 (treatment reefs) for each sampling time.

Fig. 6. Mean percent cover for benthic community members at control and treatment reefs before applying treatments (Winter 2011) and two years after treatment application (Winter 2013). Value are mean ± SE; *n* = 24 (control) and *n* = 26 – 27 (treatment) for each sampling time.
Table 1 (on next page)

Invasive macroalgae manual removal and *Tripneustes gratilla* outplanting dates, area, and stocking density.
Table 1. Invasive macroalgae manual removal and *Tripneustes gratilla* outplanting dates, area, and stocking density.

| Treatment | Manual Removal Dates | Manual Removal Days | E/K Removed (kg) | Urchin Outplanting Dates | Urchin Outplanting Area (m²) | Urchins Stocked | Urchin Stocking Density (urchins m⁻²) |
|-----------|----------------------|---------------------|------------------|--------------------------|-----------------------------|----------------|-------------------------------------|
| Reef 26   | Nov 2011 – Mar 2012  | 23                  | 11,963           | Dec 2011 – Dec 2013      | 11,900                      | 46,913         | 3.94                                |
|           | Mar 2012 – Aug-2012  | 25                  | 7,095            | Aug 2012 – Dec 2013      | 12,700                      | 52,835         | 4.16                                |
| Total     | Nov 2011 – Aug-2012  | 48                  | 19,058           | Dec 2011 – Dec 2013      | 24,600                      | 99,748         | 4.05                                |
Table 2 (on next page)

Analysis of variance table for treatment and time effects on coral reef community cover.

Linear mixed effect models fit by restricted maximum likelihood; analysis of variance table of Type II sum of squares and Satterthwaite approximation for degrees of freedom. Invasive macroalgae = *Eucheuma denticulatum, Kappaphycus alvarezii, Acanthophora spicifera, Gracilaria salicornia*; CCA = crustose coralline algae; SS = sum of squares; df = degrees of freedom in numerator and denominator; bold p values represent significant effects (p < 0.05).
Table 2. Analysis of variance table for treatment and time effects on coral reef community cover.

| Dependent variable | Effect          | SS     | df    | F      | p     |
|--------------------|-----------------|--------|-------|--------|-------|
| Invasive macroalgae| Treatment       | 0.031  | 1, 9  | 3.377  | 0.098 |
|                    | Time            | 1.478  | 4, 195| 40.389 | <0.001|
|                    | Treatment×Time  | 0.629  | 4, 195| 17.202 | <0.001|
| Native macroalgae  | Treatment       | 0.0005 | 1, 9  | 0.015  | 0.906 |
|                    | Time            | 0.120  | 4, 195| 8.841  | <0.001|
|                    | Treatment×Time  | 0.026  | 4, 195| 1.928  | 0.107 |
| CCA                | Treatment       | 0.0005 | 1, 9  | 0.045  | 0.837 |
|                    | Time            | 0.366  | 4, 195| 9.194  | <0.001|
|                    | Treatment×Time  | 0.104  | 4, 195| 2.606  | 0.037 |
| Coral              | Treatment       | 0.0001 | 1, 9  | 0.056  | 0.818 |
|                    | Time            | 0.181  | 4, 195| 34.783 | <0.001|
|                    | Treatment×Time  | 0.020  | 4, 195| 3.867  | 0.005 |
| Sand/bare/turf     | Treatment       | 0.007  | 1, 9  | 0.520  | 0.489 |
|                    | Time            | 0.072  | 4, 195| 1.344  | 0.255 |
|                    | Treatment×Time  | 0.048  | 4, 195| 0.893  | 0.469 |

Linear mixed effect models fit by restricted maximum likelihood; analysis of variance table of Type II sum of squares and Satterthwaite approximation for degrees of freedom. Invasive macroalgae = *Eucheuma denticulatum*, *Kappaphycus alvarezii*, *Acanthophora spicifera*, *Gracilaria salicornia*; CCA = crustose coralline algae; SS = sum of squares; df = degrees of freedom in numerator and denominator; bold p values represent significant effects (p < 0.05).
Figure 1

Study site location in Kāneʻohe Bay on the windward side of the island of Oʻahu, Hawaiʻi, proximate to Moku o Loʻe (Hawaiʻi Institute of Marine Biology).
Invasive macroalgae species found on study reefs in Kāneʻohe Bay

(A) *Eucheuma* clade E, (B) *Kappaphycus* clade B, (C) *Gracilaria salicornia*, (D) *Acanthophora spicifera* (photo credit: Brian Neilson.)
Figure 3

Invasive macroalgae control techniques applied in the field.

(A) using the Super Sucker to manually remove *Eucheuma* clade E, (B) outplanting juvenile *Tripneustes gratilla*, (C) outplanted adult *T. gratilla* surrounded by *Gracilaria salicornia* and *Acanthophora spicifera*, (D) adult *T. gratilla* surrounded by *Eucheuma* clade E, (E) before and immediately (F) after manual removal of *Eucheuma* clade E revealing crustose coralline algae (CCA) and (G) before and (H) after removal of *Eucheuma* clade E revealing live and dead coral (photo credit: (A-B) DLNR/DAR, (C-H) Brian Neilson).
**Figure 4** (on next page)

Mean percent cover of benthic cover types

(A) combined invasive macroalgae (*Eucheuma* clade E/*Kappaphycus* Clade B/*Acanthophora spicifera*/*Gracilaria salicornia*),  (B) native macroalgae,  (C) crustose coralline algae (CCA),  (D) corals,  and  (E) SBT (sand/bare/turf).  Value are mean ± SE;  $n = 24$ (control) and $n = 26 - 27$ (treatment) for each sampling time.  The first time point in each figure (Winter 2011) represents the “before” time period of the study and all subsequent time points represent the “after” period.  Symbols (*) represent a significant difference ($p ≤ 0.05$) between the control and treatment.
Invasive macroalgae (% cover)

Native macroalgae (% cover)

CCA (% cover)

Coral (% cover)

Sand/bare/turf (% cover)

Sampling Times

Winter 2011
Summer 2012
Winter 2012
Summer 2013
Winter 2013

Winter 2011
Summer 2012
Winter 2012
Summer 2013
Winter 2013

Control Reefs
Treatment Reefs

* * * * *
Figure 5 (on next page)

Percent cover for invasive macroalgae species through time

(A) control reefs and (B) treatment reefs. Value are mean ± SE; $n = 24$ (control reefs) and $n = 26 – 27$ (treatment reefs) for each sampling time.
Figure 6 (on next page)

Mean percent cover for benthic community members at control and treatment reefs before applying treatments (Winter 2011) and two years after treatment application (Winter 2013).

Value are mean ± SE; \( n = 24 \) (control) and \( n = 26 – 27 \) (treatment) for each sampling time.
