Grazing of *Evechinus chloroticus* (kina) on species *Caulerpa brachypus* and *Caulerpa parvifolia*, two invasive species of seaweed in New Zealand.

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Abstract

As the two invasive species (*Caulerpa brachypus* and *C. parvifolia*) proliferate within New Zealand waters, there is a growing imperative to identify potential strategies for controlling its spread. This study investigates the prospect of leveraging the New Zealand sea urchin *Evechinus chloroticus* (kina) as a bio-control mechanism against the current Caulerpa outbreak. The research endeavours to test Kina's feeding preferences towards Caulerpa seaweed through a laboratory based investigation spanning four weeks. Three dietary treatments were employed to gauge the kina's response to Caulerpa consumption vs. native *Ecklonia radiata* seaweed.

Our results show that that kina did consume *Caulerpa* spp. when presented with only *Caulerpa* spp. and when presented with both *E. radiata* and *Caulerpa* spp.. Unfortunately, our findings must be interpreted with caution, given that this was lab based and that the kina tended to consume 100% of the seaweed presented to them (both *E. radiata* and *Caulerpa* spp.); this truncation of the data precluding detailed statistical analysis. Consuming *Caulerpa* spp.did not have any detectable deleterious impacts on the kina, in fact all animals across all treatments increased condition (using gonad growth as a condition index) with no statistically significant difference between the three treatments.

Introduction

The intentional and unintentional introduction of non-native species into terrestrial and marine environments has been a persistent phenomenon with significant ecological consequences. The study of non-native species impacts has been driven by the profound alterations they induce in natural ecosystems. While some debate exists regarding the potential positive impacts of introduced species, the prevailing body of research underscores the negative ramifications associated with such introductions. Among these, alterations to ecosystem functions and biodiversity stand out as primary consequences of non-native species introductions.

Marine ecosystems, akin to their terrestrial counterparts, grapple with the ecological challenges posed by the introduction and proliferation of invasive species. Despite the vastness and diversity of oceanic ecosystems, even coastal environments are not immune to the influx of non-native species. The seaweed genus *Caulerpa*, in particular, has garnered notoriety for its adeptness at establishing and spreading, often outcompeting native biodiversity and reshaping ecosystems.

The invasion of *Caulerpa* is not a novel occurrence. A significant event transpired in the Mediterranean following the introduction of *Caulerpa taxifolia* in 1984, identified by Meinesz et al. (2001) as one of the most threatening invasions in the region. Similarly, the invasion of *C. taxifolia* in Australian waters in April 2000 prompted efforts at eradication, with Glasby et al. (2005) experimenting with the use of sea salt as a control measure. While effective in targeting the invasive seaweed, this method also impacted native biota, underscoring the complexities inherent in invasive species management.

In addition to historical precedents, recent developments in the spread of Caulerpa within New Zealand waters further underscore the urgency of finding effective control measures. The first sightings of invasive *Caulerpa* species in New Zealand occurred in 2021 on Great Barrier Island and Mercury Island. These sightings, particularly the presence of *C. brachypus* and *C. parvifolia*, have raised grave concerns about their ecological impacts, prompting investigations into effective management strategies. The implementation of *Caulerpa* Controlled Area Notices (CAN) and ongoing surveillance efforts reflect the evolving nature of the *Caulerpa* outbreak and the need for adaptive management strategies.

Despite the challenges, the potential for mitigating *Caulerpa* outbreaks exists. Drawing from insights gained from previous invasions and ongoing efforts to control *Caulerpa* in other regions, this study endeavours to explore solutions tailored to the New Zealand context. By examining the feeding preferences of the native herbivorous sea urchin species, *Evechinus chloroticus* (referred to as kina), between native and invasive seaweeds, we aim to assess the viability of utilising kina as a bio-control tool against the *Caulerpa* invasion.

Kina consume a wide range of both drift and attached seaweeds (Schiel 1982, Barker 2013), and while there is evidence from both Europe and Australia that at least some invasive species of Caulerpa are toxic and not eaten by some invertebrates, other species such as Caulerpa racemosa and *Caulerpa lentillifera* are consumed by both humans and various herbivores. We could find no studies on herbivory on either *C. brachypus* or *C. parvifolia* .

There have been previous attempts at using kina in New Zealand as a bio-control mechanism for invasive seaweeds. In Fiordland, where significant shifts in algal communities were observed (Atalah et al., 2013) following the importation of thousands of kina to attempt to eradicate the invasive seaweed *Undaria pinnatifida*. While the method resulted in localized environmental effects, it demonstrated promise in mitigating invasive seaweed events. By elucidating kina's dietary preferences, we seek to contribute to a deeper understanding of ecological interactions in marine environments and potentially offer an effective and sustainable strategy for mitigating the Caulerpa invasion in New Zealand waters.

Furthermore, the expansion of the *Caulerpa* outbreak beyond initial containment zones highlights the challenges associated with traditional control methods and underscores the need for innovative approaches. Given the limitations of mechanical and chemical control methods and the mounting pressure posed by Caulerpa's continued spread, there is a growing interest in exploring effective and sustainable control measures. By focusing on the ecological interactions between kina and invasive seaweeds, our study aims to provide valuable insights that can inform the development of targeted management strategies for addressing the Caulerpa invasion in New Zealand waters.

Methodology

Approvals and Permits

Prior to commencing our research, we obtained approvals from Biosecurity New Zealand, obtaining authorisation for the transportation of Caulerpa to the Physical Containment 2 (PC2) laboratory at Auckland University of Technology (AUT). This laboratory enabled us to treat all discharge to ensure that any Caulerpa leaving the system had been killed.

Collection of study species

The acquisition of *Caulerpa* spp*.* and *Ecklonia radiata* species for our research was facilitated by a local environmental company, EnviroKiwi, operating on Great Barrier Island. Samples were collected on snorkel and transported to the PC2 lab at AUT. As both *C. brachypus* and *C. parvifolia* grow together in a mat-like structure, we presented the two seaweeds to the kina together, with an approximate 50:50 ratio.

A total of eighty-five kina individuals were procured by the EnviroKiwi team from Great Barrier Island, each averaging approximately 12cm in diameter. These specimens were sourced from locations along the west side of Aotea Island, where Caulerpa is known to proliferate. Special care was taken to ensure their well-being during transportation, with measures implemented to minimize desiccation stress, known to significantly impact mortality rates. The kina samples were packaged in polystyrene boxes and transported via Barrier Air, entailing a 45-minute journey from to Auckland airport. Upon arrival, the research team promptly retrieved the specimens and transported them to the AUT lab, resulting in a total travel time of approximately 3 hours for the kina individuals.

Handling and Initial Processing

Of the 85 kina individuals initially collected, an allowance was made for up to 5 individuals not surviving the journey to the lab and 20 to be sacrificed on arrival for initial assessment of condition index (see below). The remaining sixty kina individuals underwent a one-week acclimation period, allowing for potential adjustment to their new environment and recovery from any transportation-related stressors. Notably, despite the acclimation period, the kina exhibited feeding behaviors promptly and commenced feeding within two days of being placed in the laboratory setting. Throughout this period, they were provided with *E. radiata* as their food source.

System Design

Three recirculating aquaculture system were used, each with a 30-liter sump tank, which circulated water throughout the system via five 6-liter tanks dedicated to the trial individuals, propelled by a water pump. A water cooler was integrated to maintain system temperature, while an air pump facilitated aeration across the entire system, connected to bubblers. Each of the twenty bubblers per system provided aeration to the tanks. Protein skimmers were utilized within each sump tank to remove organic compounds. Mechanical filtration was achieved through the use of filter socks placed at the end of the outlet pipe, prior to water entering the biofiltration media of the sump tank.

Laboratory Conditions

Laboratory conditions were controlled, with a constant temperature of 17°C maintained using an air conditioning unit. Seawater utilised in the experiment was sourced from AUT's reservoir tank, collected in November 2022, allowing for adequate settling time and suitability for the research.

Seaweed Preparation

Seaweed utilized in the experiment was sourced weekly from Aotea Island via EnviroKiwi. Each week, seven healthy *E. radiata* plants and an equivalent amount of mixed *Caulerpa* spp. were obtained. *Caulerpa* spp. specimens were rinsed in clean seawater to remove any extraneous organisms or debris, while *E. radiata* plants underwent trimming to leave 300-600mm of frond from the stipe edge, with the stipe bisected. This preparation ensured the majority of the *E. radiata* samples consisted of the stipe with short fronds, facilitating sinking and preventing frond obstruction in the tanks. *Caulerpa* spp., lacking a stipe-like feature, was divided into equal portions based on dietary treatment parameters, with each piece affixed with a small cable tie to aid sinking and mimic natural conditions.

Experimental Design

The experiment involved sixty kina individuals distributed across three dietary treatments, with each treatment group comprising 20 kina. The three treatments were:

1. *E. radiata* only
2. *Caulerpa spp.*
3. ~50% *E. radiata* and ~50% *Caulerpa* spp.

Each week a weighed amount of seaweeds (prepared as above) was put into each of the tanks. At the end of the week any remaining seaweed was recovered and weighed. Careful attention was paid to removing any seaweed pieces ensnared in the kina's spines to ensure accurate measurements of remaining seaweed and kina weights, particularly pertinent when *Caulerpa* spp. was included in the treatments.

The kina were housed in fifteen separate 6-liter tanks distributed among three aquaculture recirculating systems (described below), with each system containing five tanks. The five tanks within each treatment were haphazardly distributed among the three recirculating systems. Within each tank, four kina (reduced to three at the end of week two to allow condition index measurement) individuals were allowed to roam freely, with individual identification not pursued, and subsequent data analysis based on averages of those individuals.

Sacrifice and Condition Index

GSI is calculated by Gonad Weight/Wet Body Weight*100 (Erickson, *et al*., 1985). We conducted condition indexes in this trial by collecting wet weight of four of the gonads from each kina, this was to allow for potential loss in one gonad when cutting open the kina. Therefore, when calculating GSI, we first found the gonad mean weight from four of the gonads and multipling this by five.

GSI was measured in 20 individuals on arrival in the laboratory to provide an initial measurement. After two weeks of the experiment, an additional 15 kina were randomly selected for sacrifice; one kina from each tank, totalling five from each treatment group. Upon conclusion of the four-week experiment, we determined the condition index of the remaining 45 kina individuals.

Statistical analysis

The statistical package "R" was used to test for differences between any affects due to the use of three separate recirculating aquaculture systems on the measured parameters and then to test for differences between treatments (ANOVA).

Results

All of the test kina survived for the entire four weeks of the experiment.

Seaweed Consumption

The amounts of seaweed fed to the kina are in Table 1. We based the amount of food given to the kina on Schiel (1982) and James (2006). Schiel used a similar laboratory set up to ours, with four kina in each tank. After five days he reported a mean seaweed removal of 22g of seaweed per tank. Added to this, James (2006), using a formulated feed, presented 1-1.5% of kina wet weight per day. The average weight of our kina was 126g, so we assumed that 2-3g of seaweed per day would be sufficient to have the kina feed *ad libitum*. As the trial progressed, it became clear that the kina were consuming virtually all of the seaweeds each week, so the amount given was increased each week, rising to 4.3% of average kina wet weight by week 4.

Table 1. Amount of seaweed presented to the kina in each week of the trial. Treatment 1=*E.radiata*, Treatment 2=*Caulerpa* spp., and Treatment 3=Mixed *Caulerpa* spp. (C) and *E. radiata* (E).

	Mean seaweed presented per kina (g)		
	Treatment 1	Treatment 2	Treatment 3
Week 1	17.95	20.8	9.8 C 8.6E
Week 2	20	22.1	11.3C 10.2E
Week 3	26.6	30.0	15.0C 13.3E
Week 4	36.5	40	20.0C 18.0E

Consumption within each treatment are shown in Figure 1. In the first week almost all of the *E. radiata* was consumed in both treatments where it occurred. *Caulerpa* spp. was consumed in both the Caulerpa only treatment and in the mixed treatment, but in both cases less than *E. radiata*. By week two, the kina were consuming 100% of both seaweeds. In week three showed a different pattern with only 84.2% (+/- 7.7SD) of E. radiata was consumed in treatment 1 and just under 100% consumed of both E. radiata (99% +/- 1.18SD) and Cualerpa spp. (97.3 +/- 3.8SSD). In week four, despite being provided with the highest amount of seaweeds across all three treatments, 100% of all seaweeds were consumed. Given the truncation of the data in many of the treatments, statistical analysis of the treatments was not possible.

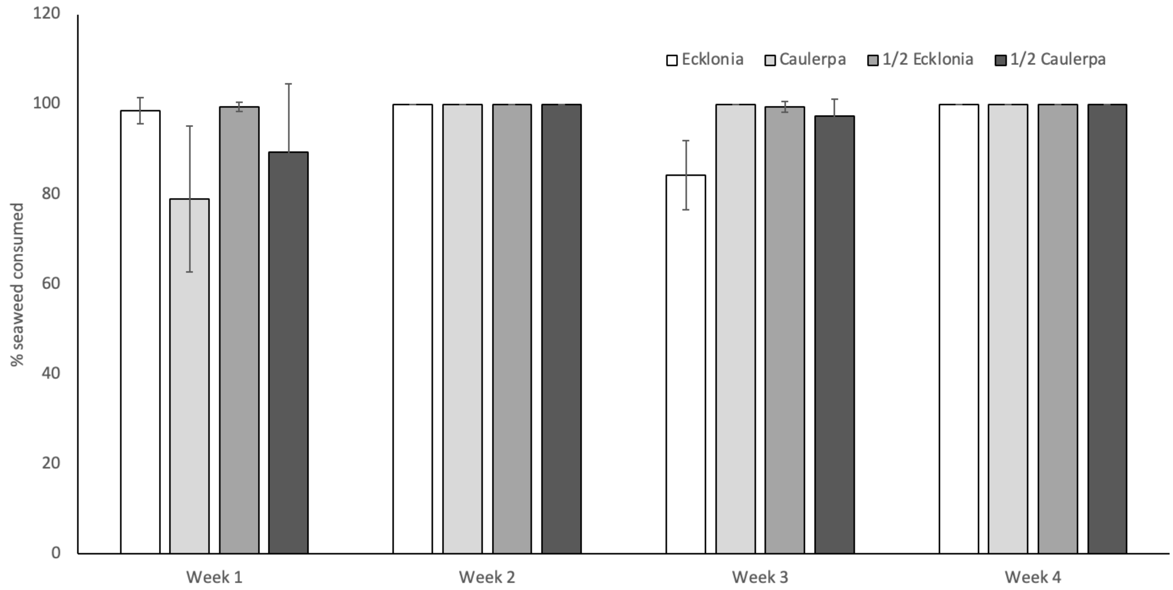

Figure 1. Mean % of seaweed consumed in each of the Treatments. Error bar = Standard Deviation.

The data gathered from the consumption of seaweed from each kina will give an understanding of feeding habits and behaviors of each Kina. By comparing each treatment, we analyzed variations in feeding behaviors based on the Kina's diet. This provided insight on Kina's preference, and that Kina's feeding behaviors is very minimally influence by the seaweed on offer.

Gonosomadic Index (GSI)

Figure 2 shows the mean kina gonosomatic indices over the course of the trial. There however, large and significant increases in the GSI over the course of the study in all of the treatments. Statistical analysis showed that there was no recirculating system effect (p-value close to 1), so a standard ANOVA was used to test for differences between treatments. The overall ANOVA F-test p-value for the null hypothesis of no treatment effect was 0.32, indicating no significant effect of treatment in terms of GSI.

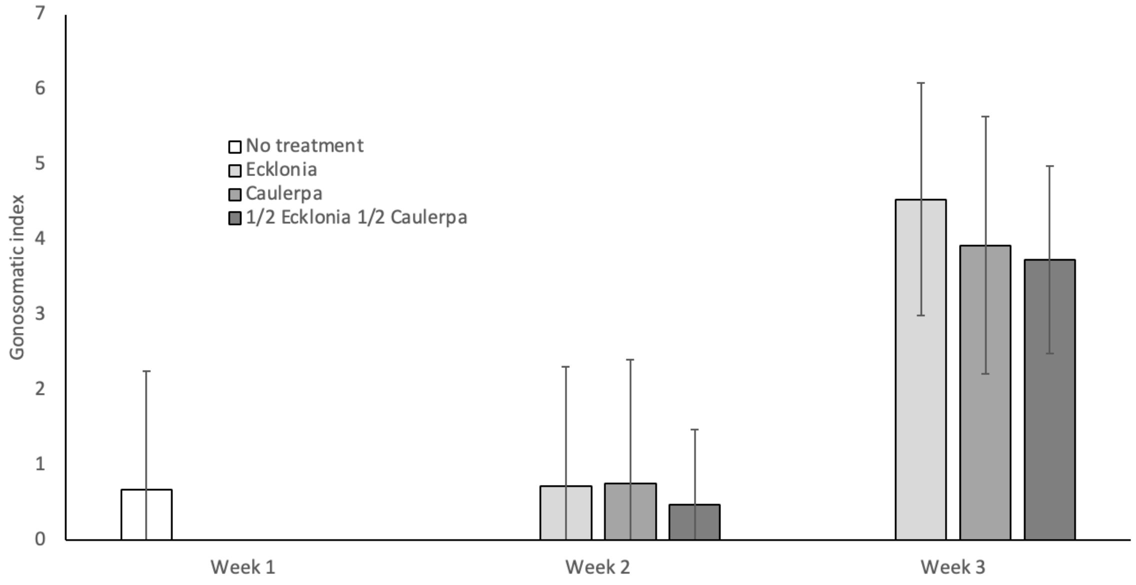

Figure 2. Gonosomatic indices of kina over the course of the trial across the three treatments. Error bars = Standard Deviation.

Discussion

This study sought to discover if *Evechinus chloroticus* (kina) would consume (*Caulerpa brachypus* and *C. parvifolia* in a laboratory setting so that they could potentially be used as bio-control agents for these species of *Caulerpa* that have found their way to New Zealand's shores and are taking over large tracts of the seafloor on islands in the Hauraki Gulf.

It should be noted at the outset that both of these invasive seaweeds will inhabit the rocky reefs that are inhabited also by kina here in New Zealand, but these seaweeds mainly extend across sandy/silty embayments, so the ability to use kina as biocontrol agents, if they will consume *Caulerpa* spp. in the wild at all, would be limited to a small part of the range of the seaweeds and other mitigation options would be needed for those non-rocky reef areas.

But the results of this study are encouraging in terms of the potential to stop or slow the spread of both *C. brachypus* and *C. parvifolia* on our rocky reefs. It is unfortunate that 100% of the seaweed was consumed in many of the treatments in this study, as we can't say for sure a) how much seaweed the kina might have consumed and b) would the kina have consumed *Caulerpa* spp. had there continued to be enough *E. radiata* present to satiate themselves.

This study does indicate that there are no detectable deleterious effects on the kina of having either mixed diet or a diet of only *Caulerpa* spp. and in fact all of the kina in all of the treatments increased gonad weight (a good indicator of overall health) throughout the four weeks of the study.

Many of the rocky reefs of the Hauraki Gulf have an over abundance of kina to the extent that "urchin barrens" are becoming more common so there may be hesitance to use these animals to clear *Caulerpa* spp. from infested rocky reefs and field trials are needed to test the findings of this study before we can be sure that kina will consume *Caulerpa brachypus* and *C. parvifolia* in the wild.

In summary – we have shown that kina will consume *Caulerpa* in a laboratory setting and that when they do so, it does not have any impact on their ability to increase their gonad weight.

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