

Growth, mortality and recruitment rates of the brown alga *Lobophora variegata* in relation to herbivory and nutrients on Bonaire



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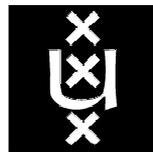
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Abstract: Declines in herbivory and increases in nutrient concentrations on tropical coral reefs are considered two of the major drivers of coral-algae phase shifts, but little is known about how growth and mortality of the dominant macroalgae in such shifts is regulated exactly. This study quantified growth and mortality rates at a small-scale and at high resolution in *Lobophora variegata* populations growing under two grazing intensities and at two different water depths on Bonaire (Southern Caribbean). Net relative growth rates were 2.2 times higher in absence of herbivores (caged treatment) compared to controls (non-caged) at 21m and 14m water depth, confirming that herbivory exerts a degree of top-down control on algae growth. Despite positive net growth rates for all treatments, high mortality rates exceeding 50% of leaves in 4 months balanced off the positive net growth rates keeping algae cover relatively stable. Only when herbivores were excluded an increase in cover of 50% was observed over 4 months. Direct evidence for mortality by grazing was only found for 6% of leaves, though there are indications that this percentage is underestimated. Recruitment of new *Lobophora* was positively influenced by the density of the already present *Lobophora* vegetation, and negatively influenced by herbivore presence. Although nutrient concentrations at the experimental sites were likely eutrophic, nutrient limitation experiments using Nutrient induced Fluorescence Transients (NIFTs) showed that *L. variegata* was limited by both phosphate and ammonium. This study shows that on Bonairean reefs herbivory still plays a strong role in controlling the local abundance and growth of macroalgae and their recruits, despite recent increases in fishing pressure. Secondly, our results demonstrate that even under eutrophic conditions *L. variegata* can be nutrient limited. This implies that a further increase in nutrient concentrations and fishing pressure could result in higher than present *Lobophora* growth rates and possibly faster degradation of coral reefs.

Nederlandse samenvatting: Een afname van herbivorie en een toename van het aantal nutriënten in het water rond tropische koraalriffen worden beschouwd als de twee belangrijkste factoren die de balans op een koraalrif kunnen laten verschuiven van koraal naar algen gedomineerd rif. Toch is er pas weinig bekend over hoe de groei en mortaliteit van de dominante macroalgen tijdens dit soort faseverschuivingen wordt gereguleerd. In dit onderzoek worden de groei en mortaliteitssnelheid gemeten van *Lobophora variegata* op kleine tijdelijke en ruimtelijke schaal, onder twee verschillende begrazingsniveaus en dieptes op Bonaire. Netto groeisnelheden waren 2.2 maal hoger bij afwezigheid van herbivoren (gesloten kooien) in vergelijking met de controlebehandeling (geen kooien) op zowel 21 als 14m diepte waarmee wordt bevestigd dat herbivorie een rol speelt bij het beteugelen van algengroei. Ondanks positieve netto algengroei zelfs bij aanwezigheid van herbivoren bleef de algenbedekking stabiel dankzij een hoge mortaliteit van de algen van meer dan 50% van de bladeren in 4 maanden. Slechts bij afwezigheid van herbivoren werd een 50% toename van de algenbedekking gemeten over 4 maanden. Bij slechts 6% van de bladeren kon met zekerheid worden gesteld dat begrazing de sterfteoorzaak was, maar er zijn aanwijzingen dat dit percentage in het echt waarschijnlijk een stuk hoger ligt. De rekrutering van nieuwe *Lobophora* kiemen werd positief beïnvloed door de dichtheid van de aanwezige *Lobophora* vegetatie, en negatief beïnvloed door de aanwezigheid van herbivoren. Hoewel er vermoedelijk eutrofe omstandigheden heersten op de experimentlocatie toonden nutriëntlimitatie experimenten aan dat *Lobophora* gelimiteerd was door de beschikbaarheid van ammonium en fosfaat. Deze studie toont aan dat herbivorie nog steeds een belangrijke rol vervult in het beheersen van de lokale algengroei op Bonairiaanse riffen, ondanks een recente intensivering van de visdruk. Ook tonen deze resultaten aan dat *Lobophora* gelimiteerd is voor bepaalde nutriënten ondanks dat deze algen onder eutrofe omstandigheden groeien. Dit houdt in dat indien nutriënt concentraties en de visdruk op Bonairiaanse riffen verder worden verhoogd, het mogelijk is dat *Lobophora* hogere groeisnelheden zou kunnen realiseren met een snellere achteruitgang van de koraalriffen als gevolg.

Resúmen na Papiamentu: Ora e piskánan ku ta kome lima riba nos refnan bira menos i ora e nutrishon pa lima por krese ta oumentá den awa rònt di nos refnan di koral (tropikal), nos tin e dos faktornan mas importante ku por kambia e balansa di un ref ku ta wordu dominá dor di koral pa un ref ku ta wordu dominá dor di lima. Te ainda ta poko konosí kon e kresementu i morto di e limanan dominante den laman ta keda regulá durante tipo di kambionan asina. Na e estudio aki nos a midi den detaya grandi kon lihé e lima *Lobophora variegata* ta krese i muri, na dos diferente nivel di kantidat di piská ku ta kome lima, i na dos diferente profundidat den laman di Boneiru. E velocidat di kresementu (nèto) tabata 2.2 bes mas haltu ora e piskánan ku ta kome lima tabata ousente (kaminda e area a wordu tapa ku kouchinan será pa tene piská pafó) en komparashon ku e situashon di kòntrol (sin kouchi, awa liber) na profundidatnan di 21 i 14 meter respèktivamente. Esaki ta konfirmá ku piskánan ku ta kome lima ta ehersé un papel importante pa evitá dominashon di kresementu di lima. No opstante e kresementu positivo rapido di e limanan i presensia di piskánan ku ta kome lima; tòg e kantidat di e limanan a keda stabil grasía na un mortalidat altu dje lima di mas ku 50% di e blachinan den un periodo di 4 luna. Solamente ora piskánan ku ta kome lima tabata ousente, nos a midi un oumento di *Lobophora* ku a tapa e ref ku 50% den 4 luna. Unikamente pa 6% di e blachinan nos por bisa ku siguridat ku piskánan a kousa nan morto, pero tin indikashonnan ku e porsentahe aki en realidat ta mucho mas altu. E densidat di e vegetashon di *Lobophora* ta pone ku e lima ta produsí mas simia. Nos a haña ku e piskánan ku ta kome lima no solamente ta kome e limanan mes, pero tambe ta pone ku e lima ta produsí mucho menos simia i konsekwentamente tin un influensia negativo mas grandi ku nos por a pensa inisialmente, i ta frena kresementu di e lima bastante. Aunke probablemente tabatin bastante nutrishon pa e lima krese den e laman na e sitionan di e investigashon, eksperimentonan den lokual e limitashon dje lima pa nutrishon a ser investigá, ta muestra ku *Lobophora variegata* ta ser limita pa ammonium i fosfat den e awa. E estudio aki ta muestra ku piskánan ku ta kome lima ainda ta ehersé un papel importante den dominashon di kresementu di limanan riba e refnan di Boneiru, a pesar di ku ultimamente tabatin un oumento den piskamentu lokal. E resultadonan tambe ta muestra ku *Lobophora variegata* ta limitá pa su nutrishon. No opstante ku e lima aki ta krese den sirkunstanshanan faborabel, tòg e lo por krese mas rapido ainda, si e awa kontené mas nutrishon. Esaki ta enserá ku si e konsentrashon di nutrishon i e piskamentu na Boneiru oumentá ainda mas, posiblemente *Lobophora variegata* por realisa un inkremento di kresementu ku por kousa un atraso rapido di e refnan di koral na Boneiru komo konsekuensia di esaki.

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1. Introduction

Historically, corals and algae have always competed for the limited amount of free space within a coral reef community. Algae and corals compete both directly and indirectly with each other (McCook *et al.*, 2001). Direct competition involves secondary metabolites, abrasion, overgrowth and shading (River & Edmunds, 2001; Jompa & McCook, 2003; Nugues *et al.*, 2004; Vermeij *et al.*, 2010; Rasher & Hay, 2010; Rasher *et al.*, 2011), whereas indirect competition goes in the form of Dissolved Organic Carbon (DOC) release by benthic macroalgae resulting in enhanced microbial abundance leading to coral mortality (Kuntz *et al.*, 2005; Kline *et al.*, 2006; Smith *et al.*, 2006).

In recent years the amount of human induced stress imposed on coral reefs in the form of amongst others overfishing and eutrophication has increased dramatically, leading to reduced coral fitness and a subsequent shift from coral to algal dominance (Hughes, 1994; McClanahan & Muthiga, 1998; Ostrander *et al.*, 2000; Gardner *et al.*, 2003; Cheal *et al.*, 2010; Lesser & Slattery, 2011; Kelly *et al.*, 2012). These shifts result in the functional degradation of the coral reef community, loss of biodiversity, reduced ecosystem complexity and coastal protection, and lost revenues for local communities (Burke *et al.*, 2011; Schep *et al.*, 2012). Coral-algae phase shifts can even lead to public health risks from increasing numbers of opportunistic pathogens (Dinsdale *et al.*, 2008).

While anthropogenic and natural disturbances could individually cause a shift, it is often the synergistic effect of multiple disturbances that ultimately lead to a phase shift (Nyström *et al.*, 2000). Healthy reefs are resilient and usually not heavily affected by disturbance (Jackson *et al.*, 2001; Pandolfi *et al.*, 2003; Mumby *et al.*, 2007), whereas multiple simultaneous disturbances can reduce the resilience of a coral reef community to a critical point after which the system collapses (Nyström *et al.*, 2000; Jackson *et al.*, 2001; Mumby *et al.*, 2007). It is also this resilience that makes the phase shifts practically irreversible (Bellwood *et al.*, 2004; Mumby *et al.*, 2007), although also coral recovery has occasionally been observed (Idjadi *et al.*, 2006; Diaz-Pulido *et al.*, 2009). However, the underlying mechanisms by which these disturbances amount to phase shifts is still poorly understood.

Insight in the processes underlying coral-algae phase shifts, like overfishing or eutrophication, is relatively scarce. These processes themselves are sometimes insufficiently studied and it is often debated how multiple processes interact leading to a phase shift. (Lapointe 1997; Hughes *et al.*, 1999; Szmant, 2002; McClanahan *et al.*, 2004; Aronson & Precht, 2006; Sotka & Hay, 2009). Nutrient concentrations and herbivory are of particular importance in regulating the outcome of coral-algae competition (Miller *et al.*, 1999; Jompa & McCook, 2002; Littler *et al.*, 2006). Macroalgae are limited bottom-up through the availability of nutrients. Corals have historically adapted to oligotrophic environments and under such circumstances outcompete macroalgae through their capacity to more efficiently use and recycle the scarce nutrients. Increased nutrient concentrations — particularly N and P — cause corals to lose their advantage under low nutrient conditions shifting the competitive outcome in favor of faster growing algae. In addition, top-down control of macroalgae occurs through the grazing of herbivores, particularly large herbivorous fish and sea urchins. These selectively graze fleshy algae reducing the amount of competition interactions with corals. Overfishing reduces grazer abundance, reducing the amount of grazing on algae thereby shifting the outcome of coral-algal competition in favor of the macroalgae.

The effects of herbivory and nutrients have been summarized in the Relative Dominance Model (RDM; Littler & Littler (1984)) (figure 1), a simple model designed to predict whether algae or corals will dominate the benthos of a coral reef under a combination of changing nutrient availability and grazing pressure. When grazing pressure is high and nutrient levels are low the system is dominated by corals and crustose coralline algae (CCA). When herbivory is reduced while nutrients are still low, reef communities become dominated by filamentous turfs, whereas high grazing and high nutrients result in domination by CCA. The most degraded state occurs when both grazing is reduced and nutrients are elevated and the system switches to a macroalgal dominated state.

Over the past 10 years, a shift from coral to macroalgal dominated reefs has occurred on the reefs of Bonaire. This phase shift is characterized by a marked increase of one specific macroalgae, the

brown frondose macroalgae *Lobophora variegata* (Phaeophyceae). Historically this alga occurred in the southern Caribbean in low abundances at greater depths, mostly below 30m (van den Hoek *et al.*, 1978; de Ruyter van Steveninck & Breeman, 1987a; Nugues & Bak, 2008). In more recent years *L. variegata* has rapidly spread from its original habitat to the shallower parts of the reef, covering most of the reef slope in some places and reaching as shallow as 3m water depth on some reefs (personal communication R. de Leon, 2012).

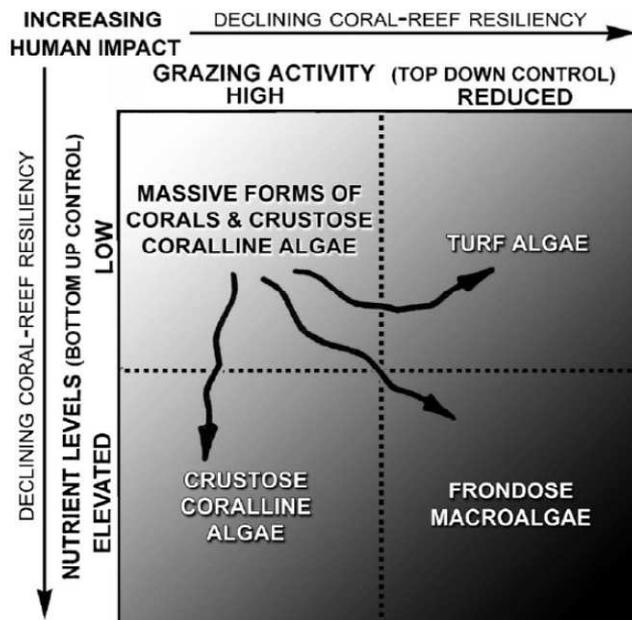


Figure 1. Coral or macroalgae domination. The RDM as it appeared in Littler *et al.* (2006): A simple model to predict likely dominant groups of benthic organisms under four environmental settings differing in grazing pressure and nutrient concentrations.

Growth and mortality rates of *Lobophora* are usually very high (de Ruyter van Steveninck & Breeman, 1987a; Mumby *et al.*, 2005). Leaf mortality rates vary from 50% of leaves in several weeks (de Ruyter van Steveninck & Breeman, 1987a) to 60% of *Lobophora*-covered patches in one year (Mumby *et al.*, 2005). Given this species' ability to grow rapidly, together with its high leaf mortality, it implies that important local factors reducing algal abundance must be present in order to explain its historically low abundances on Caribbean reefs.

While the macroalgal increase has been noted around the world, the relative importance of the essential processes that determine algae abundance, i.e. algae recruitment, growth and mortality, and the influence of herbivory and nutrients on these processes have rarely been studied. Conventional studies on *Lobophora* growth, mortality and cover over time have occasionally been done (de Ruyter van Steveninck & Breeman, 1987a; Mumby *et al.*, 2005; Ferrari *et al.*, 2012), and mostly use sampling intervals of at least one month. As *Lobophora* growth and mortality rates are usually very high, a monthly sampling interval fails to capture the smaller scale fluctuations in macroalgae growth. These studies provide little insight in the mechanisms regulating algae growth and mortality such as herbivory and nutrients, and ultimately in the possible outcome of coral-algae competition (Mumby *et al.*, 2005). Consequently, there is a need for studies on small temporal and spatial scale that determine the relative importance of herbivory and nutrients in controlling *Lobophora* dynamics, in order to explain the region-wide observed patterns of rapid macroalgae spreading.

Bonaire has the oldest Marine Park of the Caribbean (since 1979), which is often used as an example of good reef management. Bonaire has relatively healthy reefs compared to the rest of the Caribbean (Sandin *et al.*, 2008a; Sommer *et al.*, 2011) and the island's economy almost entirely

depends on tourism; especially on diving and snorkeling tourism, representing more than 57% of the total number of tourists in 2008 and generating 37-65 million USD income for the local markets (Caribbean Tourism Organization, 2011; Government evaluation TEEB Bonaire, 2012; Tourist Corporation Bonaire, 2012). Degradation of Bonaire's coral reefs would surely prove disastrous for the island's economy as reef degradation negatively impacts on the amount of visiting divers. Only 10% of visiting divers is willing to return to Bonaire if reefs are degraded, compared to 47% that would definitely not return and 43% that is not sure (Schep *et al.*, 2012).

In contrast to disturbances that threaten coral reefs on a global scale such as climate change, reduced herbivory and elevated nutrient concentrations are local stressors of coral reefs and therefore can also be treated locally, as long as the mechanisms in which they influence the reefs are understood. In order to be able to preserve coral reefs — not only in Bonaire but also elsewhere — it is extremely important that the influences of herbivory and nutrients behind the current advancement of macroalgae such as *L. variegata* are investigated and understood, so that appropriate actions can be taken to prevent further degradation of the reefs.

Research questions and hypotheses

The main research question of this research is as follows:

*Do increased nutrient concentrations and lowered grazing intensity cause an increase in *L. variegata* populations on Bonaire? If so, does such increase occur through increased growth or recruitment?*

In order to answer this question the following hypotheses will be tested:

- H1: Populations of *L. variegata* have lower growth rates and higher mortality when exposed to herbivores, and herbivory is mainly responsible for algae mortality.
- H2: Local *L. variegata* recruitment rates and dispersal increase with higher adult population densities, and decrease when exposed to herbivory.
- H3: Nutrient availability (nitrogen and phosphorus) is not limiting *L. variegata* growth.

In this study we investigate the importance of eutrophication and herbivorous fish on rates of growth, mortality and recruitment of *L. variegata* on Bonaire.

2. Material and methods

2.1. Study sites

This study was conducted on the island of Bonaire (12°9'N, 68°16'W) in the southern Caribbean with permission of the Bonaire Government and the BNMP, between February and July 2012. This period falls almost entirely in the local dry season (February-May) (Martis *et al.*, 2002). The experiments were conducted at two different sites along the leeward coast of Bonaire (figure 2). Wayaka II is the most northern site of the two, located inside the Washington Slagbaai National Park WSNP, whereas Tolo lies further to the south.

At both sites the reef slope starts at approximately 9m depth and is dominated by similar coral species, mainly *Montastraea faveolata*, *M. annularis* and *Agaricia* species. *L. variegata* abundance started at approximately 12m water depth for Tolo and increased with depth; while at Wayaka II the algae can be found as shallow as 3m water depth. For a more detailed description of the reef and reef zonation at the study sites, see Van Duyl (1985).



Figure 2. Study sites. Map showing the two different sample locations along the leeward coast of Bonaire.

2.2. Experimental setup and data collection

2.2.1. Herbivore exclusion and permanent quadrat experiment.

Experiments to assess the effects of grazing by herbivorous fish on *Lobophora* growth consisted of three different treatments: (1) closed cage treatment (fish grazing not possible), (2) open cage treatment (fish grazing possible), and (3) permanent quadrats (no cage construction; undisturbed conditions).

The herbivore exclusion experiment cages and permanent quadrats were installed at both Tolo and Wayaka II. Installation and all data collection was done using SCUBA. For the herbivore exclusion experiment open and closed cages measuring 30x30x40cm (length x width x height) were constructed using 6mm diameter rebar and 9mm mesh-size galvanized wire mesh. All cages were open at the bottom for easier fixation on the reef and equipped with a wire mesh top that could be removed for taking photographs (used to calculate growth and recruitment rates, as well as algae cover) and to account for equal light intensities in all treatments. Closed cages were completely closed on all sides, while open cages had open sides and only the corner axes to support the cage top. All cages were also equipped with wire mesh flaps at the bottom of the sides of each cage to keep the cages anchored in place once installed.

Five replicates of open and closed cages were installed on horizontal, flat patches of reef at 21 and 14m. Blocks of coral rubble ranging between 300 and 600cm² and partly overgrown with *L. variegata* were then collected and placed inside the cages. These transplants were selected to have an approximately equal cover of *L. variegata* (approximately 40% at the start of the experiment). All cages were visually inspected once a week to replace dislocated rubble and fill up holes and crevices at the installation boundary, and algae growing on the wire mesh top and sides were cleaned off using a cleaning brush.

The permanent quadrats (50x50cm) were installed at 21 and 14m water depth (n= 5 per depth) to quantify net relative growth rates of *L. variegata* under undisturbed conditions. Quadrats were installed on horizontal patches of reef with approximately equal cover of *L. variegata* (between 10 and 20% cover at 14m and between 25-40% cover at 21m) and marked with small tags in at least two of the four corners of the quadrat. In the results and discussion sections of this report the following abbreviations will be used to indicate the different treatments:

- C21: closed cage treatment at **21** m;
- O21: open cage treatment at **21** m;
- PQ21: permanent quadrat at **21** m;
- C14: closed cage treatment at **14** m;
- O14: open cage treatment at **14** m;
- PQ14: permanent quadrat at **14** m.

Sites at Tolo are indicated with a T and a W for Wayaka II. Transplants and permanent quadrats were photographed with a size reference every 14 days resulting in 7 sampling intervals for Tolo and 8 for Wayaka II between half March and June 2012 for the transplants, and 8 sampling intervals between March and end June 2012 for permanent quadrats for both Tolo and Wayaka II. All photographs were taken using a Panasonic Lumix DMC-TZ20 digital camera, after which net growth rates and algae cover were calculated on the computer using the ImageJ version 1.40 software. The program was calibrated using the size reference used in each photograph and the total transplant or quadrat area and the total area covered with *L. variegata* was measured and expressed as percentage cover.

To calculate the net growth rates, in the first photograph between 5 and 7 individual *L. variegata* leaves were randomly selected (as long as the leaf had a clear outline and was not folded or overgrown) and marked with a number and their surface area was measured using the 'polygon selections' option in ImageJ program. At each subsequent sampling interval, the same leaves from

the previous photograph were located and measured again to calculate their net Relative Growth Rate (RGR) using equation 1 (adapted from [Hunt et al., 2002](#)).

$$\text{RGR (cm}^2 \text{ cm}^{-2} \text{ day}^{-1}) = \frac{\ln(\text{cm}^2_2) - \ln(\text{cm}^2_1)}{t_2 - t_1} \quad \text{eq. 1}$$

In equation 1 cm^2_1 and cm^2_2 are leaf area (in cm^2) at interval 1 and 2 respectively and $t_2 - t_1$ is the time between these sampling intervals,. Leaves that were lost between two sampling intervals were replaced so that the number of leaves measured was always between 5 and 7 for each sampling interval.

The density of the vegetation in which the leaves monitored for the growth rate calculations were growing was recorded. Numbers 0-8 were assigned to all monitored leaves at each sampling interval, where 0 was the lowest (no sides or corners of the leaf in contact with other *L. variegata* leaves) and 8 was the highest (all sides and corners of the leaf in contact with other *L. variegata* leaves) density surrounding. Different density classes were taken as far apart as they could. One class with only the lowest density leaves (score 0 and 1) and one class with only the highest density leaves (score 7 and 8) were made and their RGR were compared to see if there was a density limitation on growth.

For all leaves used for growth rate calculations and that that disappeared between two sampling intervals the 'fate' of the leaves was monitored throughout the experiments. Monitored leaves that disappeared during the treatments were counted and categorized into the following categories: 1. *Overgrown*; the leaf is overgrown (by e.g. algae/cyanobacteria/coral etc) to such degree that its area can no longer be precisely determined. 2. *Lost*; the leaf disappeared completely and there is no evidence for grazing (i.e. bite marks, ripped leaves, parrotfish scrape marks). 3. *Grazed*; the leaf disappeared completely and there is evidence for grazing (i.e. bite marks, ripped leaves, parrotfish scrape marks). 4. *Survived*; the leaf is still present after the termination of the experiment.

2.2.2. Recruitment experiment

For this experiment similar cages as the ones used in the herbivore exclusion experiment were constructed. Ten open and 10 closed cages were installed at Wayaka II only at 14m depth using SCUBA. Five pieces of coral rubble (all derived from *M. annularis*) with high (>75%) and 5 with low (<25%) *L. variegata* cover were collected. Each piece of rubble was cut in half using hammer and chisel. All algae were scrubbed off each half using a steel brush until the limestone was clean white. One of the cleaned halves was placed inside an open cage and the other half in a closed cage. Cages were inspected and cleaned each week.

Photographs of the cleaned limestone inside the cages were at 7 day intervals (first interval was 14 days) over a period of 71 days (April - June) and the number of newly recruited *L. variegata* were quantified. In order to count new recruits each piece of rubble was visually examined and small metal rings were placed over each visible recruit before a photograph was taken (as in [Vermeij & Sandin, 2008](#)). The total number of *L. variegata* individuals on each piece of rubble was counted and the fate of each new recruit between subsequent intervals and the number of new recruits for each interval was quantified. Mortality was calculated for each interval (e.g., t_2) as the percentage of recruits that did not survive from the previous sampling interval (e.g., between t_1 and t_2).

2.2.3. Nutrient Induced Fluorescence transients experiments

NIFT experiments are useful in determining nutrient limitation in algae ([Shelly et al., 2010](#)). If a nutrient limited alga is exposed to the limiting nutrient, the alga reallocates energy from photosynthesis to nutrient uptake, resulting in a measurable shift in algae fluorescence ([Beardall et al., 2001](#); [Shelly et al., 2010](#)). A more detailed summary of the usefulness of NIFTs in determining nutrient limitation in algae is presented in [Shelly et al., 2010](#). NIFTs were measured on *L. variegata* leaves collected at Tolo and Wayaka II using the APTO device (see Appendix 1 for details) and a

DIVING-PAM underwater fluorometer (Walz GmbH, Germany). All algae used for the NIFTs were collected at 21m depth similar to the depth of the herbivore exclusion and permanent quadrats. *L. variegata* leaves were collected every morning between 07:00 and 08:00 in ziploc bags together with surrounding reef water and immediately transported in a dark cooler with seawater to the lab where they were tested for ammonium (NH_4Cl) and phosphate (KH_2PO_4) limitation. A total of 36 replicates for each nutrient were tested for each site. PAM parameters were programmed as follows;

- a. External LED light source intensity of $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ (setting 3.5)
- b. Measuring light $I = 10$
- c. Damping = 2
- d. Gain between 1 and 4 ($F_t' = 400-600$), adjust if necessary
- e. ETR-factor = 0.84
- f. Actinic light: Int = 5, width = 1, fact = 1
- g. LC-width = 0.3; LC-I depending on sample, usually 3
- h. Saturation pulse: SI = 4, SW = 0.4
- i. Light pulse = 30 seconds

Before each new measurement 15ml of the collected seawater from the ziploc bag was added to the petri dish in the APTO setup to ensure similar nutrient conditions in the petri dish as in the natural environment of the alga. One of the *Lobophora* leaves was attached between the $\frac{3}{4}$ magnets with the upper side facing the sensor. The magnets were connected to the sensor block, and subsequently lowered into the petri dish so that the sample is completely submerged. Occasional air bubbles were removed using a pipet. F_t' was checked and adjusted if not in the 400-600 range.

The PAM was covered with a black cloth and kept dark for 10 minutes prior to the measurements. This was because the earlier NIFT experiment protocols demanded the measuring of the Non-Photochemical Quenching (NPQ) which required dark adaptation of the leaves (Petrou *et al.*, 2008; Shelly *et al.*, 2010). After the NIFT experiment it was discovered that the F_m' values (maximum fluorescence in a light adapted plant) gave better results and it was decided to use this parameter to construct fluorescence curves (Personal communication J. den Haan, 2012). After the 10 minute dark period, the PAM was activated and one measurement was taken under the dark conditions before the cloth was removed. In order to maintain a constant volume during the measurements, between the 19th and 20th pulse (9:00-9:30 minutes) 1.5ml of the water was removed using a pipet, and between the 20th and 21st pulse (9:30 and 10:00 minutes) 1.5ml of the seawater was added and mixed again to control for the addition of liquid during the actual measurements. Between the 29th and 30th pulse (14:00-14:30 minutes) again 1.5ml was removed, before adding 1.5ml 1000 μM nutrient solution between the 30th and 31st (14:30-15:00 minutes) to make the nutrient concentration in the petri dish 100 μM . It is possible that the change in fluorescence does not occur immediately after the nutrient addition, therefore the algae were measured for 5 more minutes before the measurements were terminated.

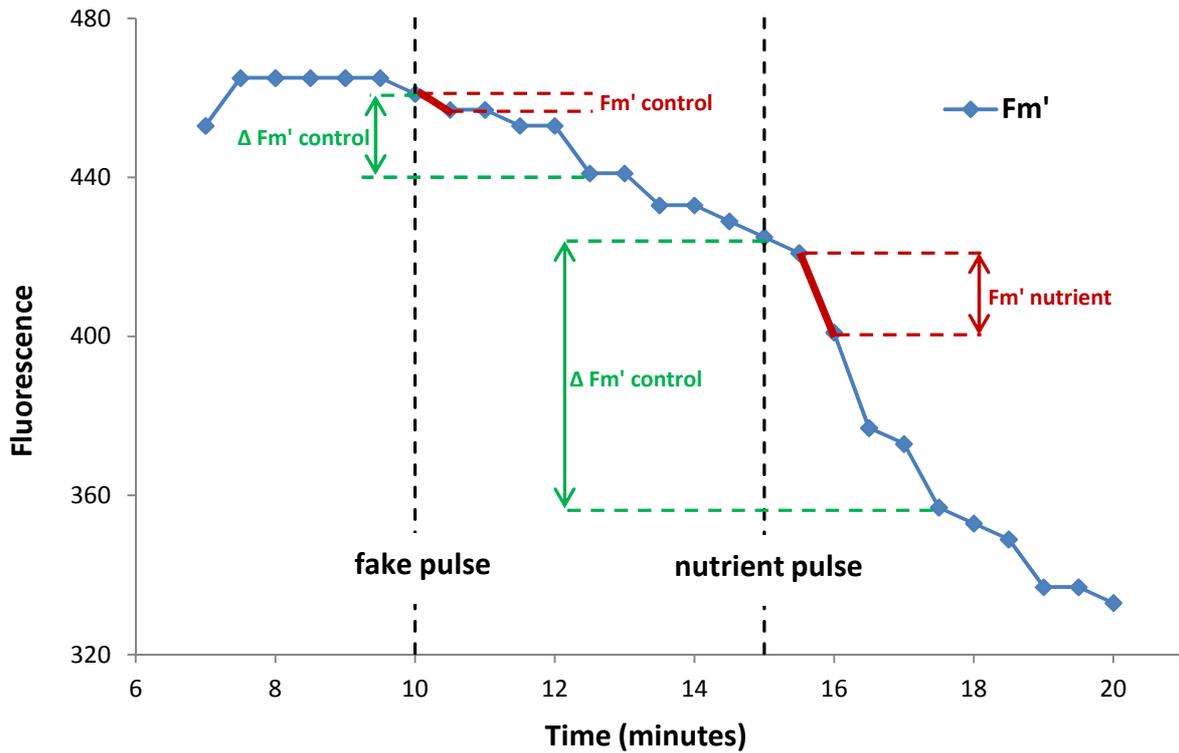


Figure 3. Example of fluorescence change over time. Fake and real nutrient pulses are added at black dashed lines at 10 and 15 minutes respectively. In red and green are the 2 different criteria (equation 2 and 3) to determine if the reaction on the reaction on the nutrient pulse is significantly stronger than on the fake pulse. In this example of a fluorescence curve the fluorescence change does not start immediately after the nutrient addition but occurs approximately 30 seconds after the addition.

In order to guarantee reliable results, first the 'healthy' algae were separated from the 'stressed' algae using the measured yield of the algae as a guideline. The threshold for 'healthy' was chosen at 0.3. If the yield drops below 0.3 the algae are stressed to a point that the possibility of a fluorescence change declines rapidly even if the algae are nutrient limited. Therefore algae with a yield lower than 0.3 were considered too stressed to give reliable results. Of the healthy algae the Fm' values (the maximum fluorescence in a light adapted plant) were measured through time. Changes in this Fm' following a nutrient pulse are an indication for limitation of that nutrient. The Fm' may also change after the addition of the fake pulse, because the increased water movement from the pulse can decrease the boundary layer on the LV leaf and thus enhancing nutrient diffusion across the boundary layer. To test whether the fluorescence reaction on the real nutrient pulse was significantly different than the fake pulse, 2 different criteria were used (Den Haan & Dekker, in preparation) (figure 3; equation 2 and 3), where $\updownarrow Fm'$ nutrient and $\updownarrow Fm'$ fake are the changes (positive or

$$\frac{\updownarrow Fm' \text{ nutrient}}{\updownarrow Fm' \text{ fake}} = \geq 2 \quad \text{eq. 2}$$

$$\frac{\Delta Fm' \text{ nutrient}}{\Delta Fm' \text{ fake}} = \geq 2 \quad \text{eq. 3}$$

negative) in fluorescence over a single time step after the nutrient and fake pulse respectively have been added, and where $\Delta Fm'$ nutrient and $\Delta Fm'$ fake are the total change (positive or negative) in fluorescence over 2.5 minutes after the nutrient and fake pulse respectively have been added. Using these two criteria, they found that they could account for at least 95% of the measured variance. If

the outcome of equation 2 and/or 3 were found to be 2 or higher, the reaction on the nutrient pulse was assumed to be significantly stronger than on the fake pulse, indicating limitation for the added nutrient.

2.2.4. Statistical analyses

Results were tested for statistical significance using the program SPSS version 20.0. All data was tested to the $\alpha=0.05$ level. Average net relative growth rates between treatments and net growth rates over time, as well as differences in recruitment and recruit mortality were compared using a one-way ANOVA test followed by Bonferroni post-hoc comparisons. A Levine's test was performed to test for homogeneity of variance (a Kruskal-Wallis nonparametric ANOVA was performed in the case of unequal variances). Density dependence in net growth rates was tested by individually comparing the growth rates of *Lobophora* leaves growing in high and low density vegetations in all different treatments using an independent Student T-test. Significance between the causes of *Lobophora* leaf mortality was tested using a two proportion Z-test (2 sided and equal variances assumed).

3. Results

3.1. Net relative growth rates

Net relative growth rates of *L. variegata* at Tolo and Wayaka II were similar for corresponding treatments (Indep. Sample T-Test $p=0.934$; $p=0.731$; $p=0.061$; $p=0.069$; $p=0.755$; $p=0.413$ respectively for the closed cage, open cage and permanent quadrat at 21 and 14m depth). Therefore data from similar treatments from the two sites was pooled (figure 4). Highest net RGRs were found for plants protected from herbivores (-herbivores; fig 4) with $0.023 \pm 0.0173 \text{ cm}^2 \text{ cm}^{-2} \text{ d}^{-1}$ (mean + 1SD; $n=314$) and $0.0217 \pm 0.0163 \text{ cm}^2 \text{ cm}^{-2} \text{ d}^{-1}$ ($n=330$) at 21m and 14m depth respectively (ANOVA $p=0.000$). The net RGRs of the open cage treatment (+herbivores; fig 4) were significantly higher than the net RGRs of the permanent quadrats (undisturbed conditions; fig 4) at 21 and 14m (ANOVA $p=0.012$ and $p=0.021$ respectively). The calculated RGRs were equivalent to a projected doubling in *Lobophora* leaf area every 31 days for the closed cage treatment and every 63 days for the permanent quadrats.

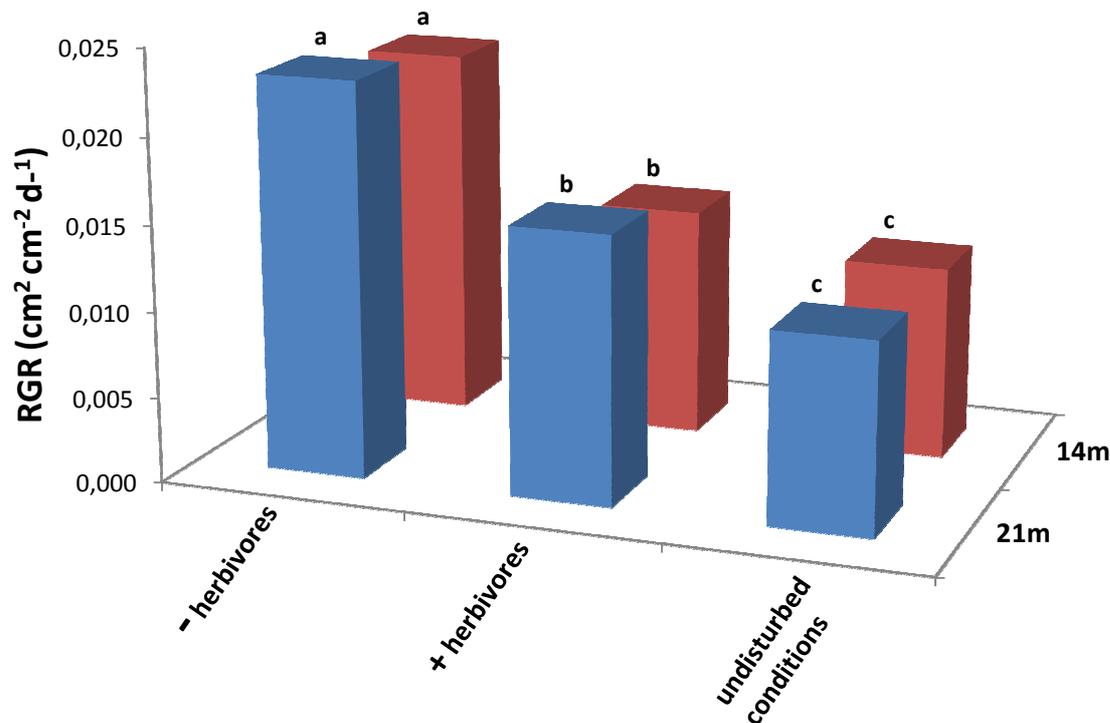


Figure 4. Net relative growth rates of *L. variegata* in the different treatments. Net growth rates of Tolo and Wayaka II are pooled together. Blue and red bars represent data from 21 and 14m respectively. -herbivores, +herbivores and undisturbed conditions are closed cage treatment, open cage treatment and permanent quadrats respectively. Letters above bars indicate significant differences in net RGR.

At Tolo at 21m depth the net RGRs fluctuated over time in the closed cage and the permanent quadrats (both ANOVA $p=0.000$) but not in the open cage treatment (ANOVA $p=0.103$) (figure 5). Highest net growth rates for all treatments were obtained around the beginning of April and May, whereas lowest net growth rates were obtained in late April. Higher or lower net growth rates were not translated in respectively increasing or decreasing *Lobophora* cover, as the pattern in *Lobophora* percentage cover change did not match the net RGRs in the corresponding treatments.

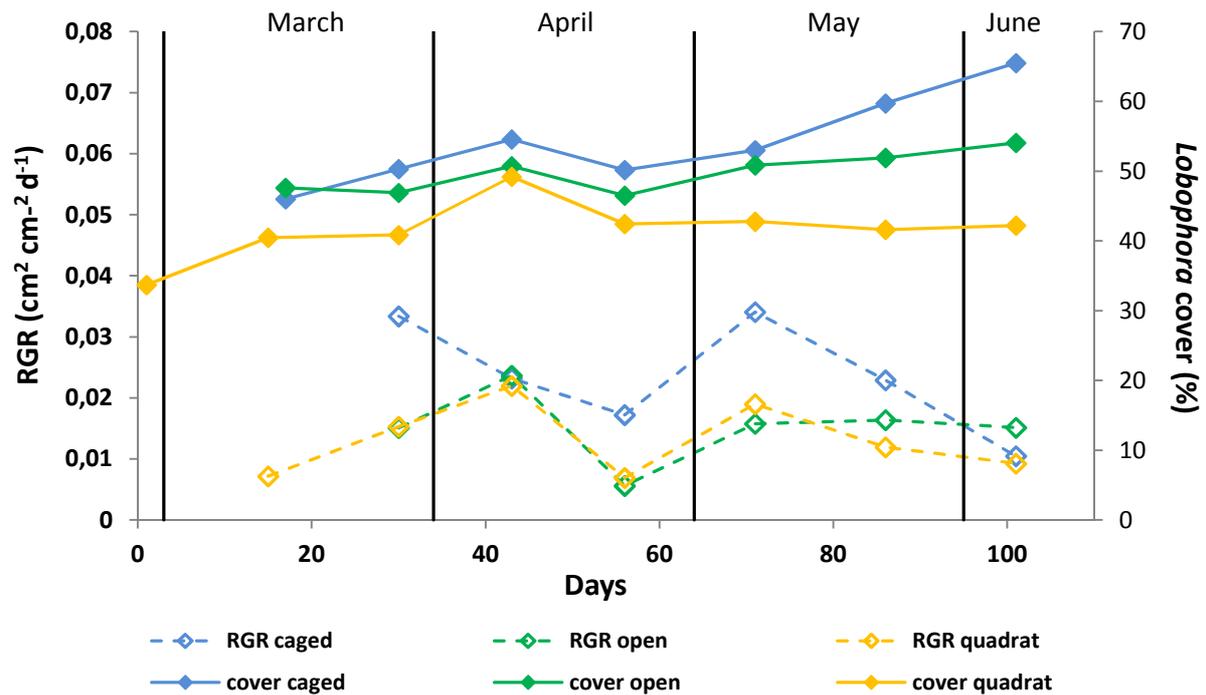


Figure 5. Net growth and cover relation. RGRs (dashed lines; primary axis) and *Lobophora* percentage cover (solid lines; secondary axis) at Tolo at 21m for the closed cages (blue), open cages (green) and permanent quadrats (yellow).

Density of the surrounding *Lobophora* vegetation did not affect net RGRs in any of the treatments (figure 6). In all treatments the net RGRs in high and low density patches was equal to the average net RGR for all leaves analyzed in the corresponding treatment (Indep Sample T-Test $p=0.114$, $p=0.477$, $p=0.944$, $p=0.076$, $p=0.902$, $p=0.6$ for the C21, O21, C14, O14, PQ21 and PQ14 treatments respectively). The data from both sites was pooled together. Net RGRs for the leaves growing in high density patches were found to be higher than the average for all leaves in the respective treatment in all cases, although not significantly different. This was also the case for the low density patches with exception of the C21 and O14 treatment.

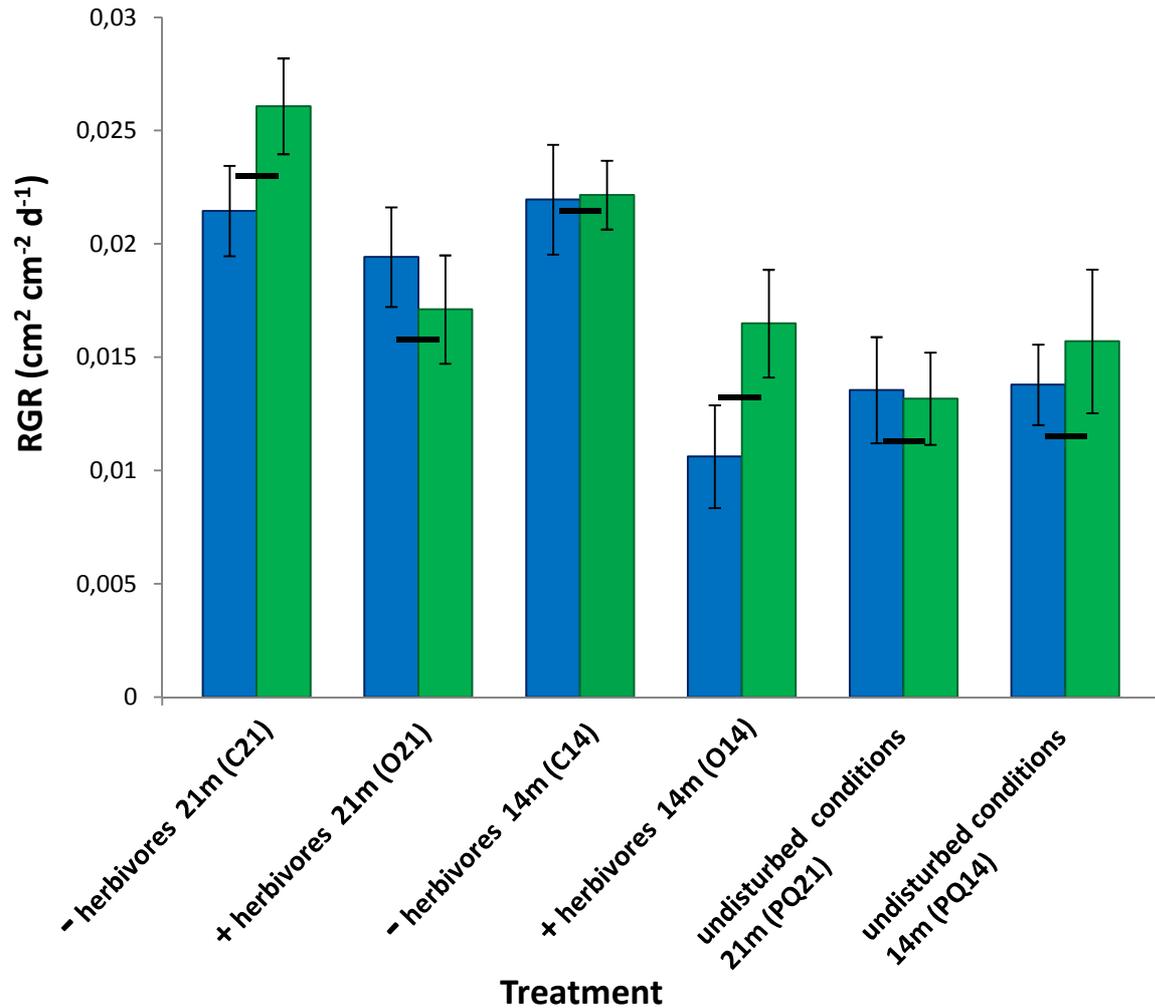


Figure 6. Density dependent growth. RGR of *L. variegata* leaves growing in low density (blue) and high density (green) patches for the different treatments. Data are pooled from Tolo and Wayaka II together. Horizontal black bars are average RGR for that treatment and error bars represent SE values.

3.2. Benthic *Lobophora* cover

Despite the observed high growth rates in, *Lobophora* cover is relatively stable through time. The projected doubling of *L. variegata* area in 31 days (as calculated from the net RGRs of the closed cage treatment in figure 4) for the closed cage treatment was not observed in the permanent quadrats (figure 7). Percentage *Lobophora* cover on the transplants in the cages and permanent quadrats only increased in the closed cage treatments, from $46.0 \pm 8.8\%$ (mean \pm 1SD) to $65.5 \pm 11.8\%$ at 21m and from $39.4 \pm 3.6\%$ to $61.7 \pm 5.1\%$ for 14m over a period of 84 days. Linear trendlines were fitted for the C21 ($R^2=0.811$; slope=0.195) and C14 ($R^2=0.995$; slope=0.274) treatment.

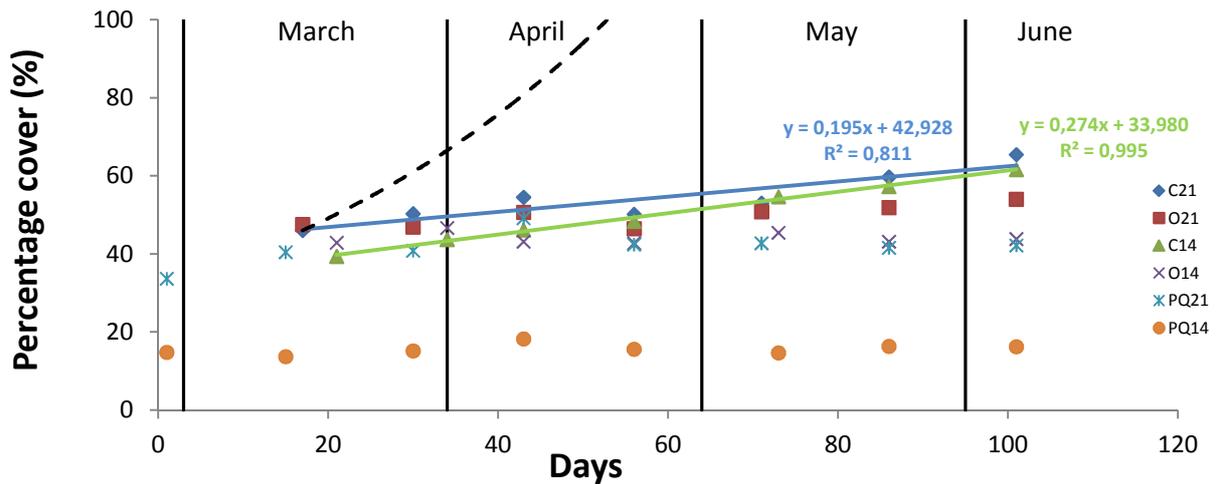


Figure 7. Percentage cover of *L. variegata* on the caged transplants and permanent quadrats at Tolo. Solid lines are the measured average increase in % cover (linear trendline) for C21 and C14 and dashed black line is the estimated % cover increase calculated for a constant RGR of $0.0229\text{cm}^2\text{cm}^{-2}\text{d}^{-1}$ as found in the closed cage treatment at 21m depth.

3.3. Algal fate

When herbivores were present overall *Lobophora* mortality did not increase, but the causes of mortality changed significantly. Between 39 and 53% of all leaves analyzed since the beginning of the experiment had survived till the end of the experiment (figure 8). This percentage was only significantly higher between the C21 (53%) and PQ21 (39%) treatment (Two-proportion Z-test $p=0.0392$; see also table 1). In the absence of herbivores the largest cause of leaf mortality was being overgrown by other *Lobophora*, i.e., 29.7% and 35.1% of the leaves at 21m and 14m depth respectively ($p=0.000$). If herbivores were present the largest cause of leaf mortality was leaves simply disappearing (lost category in figure 8) with 40.7%, 37.5%, 39.5% and 33.6% of the leaves in the open cage treatment at 21 and 14m and the permanent quadrats at 21 and 14m respectively ($p=0.000$). *Lobophora* lost by grazing represented only between 0.9% and 7.1% of all algae and was significantly lower ($p<0.049$; see table 1) in the closed cage treatments at both 21 and 14m compared to all open cage and permanent quadrat treatments with the exception of O21 (table 1).

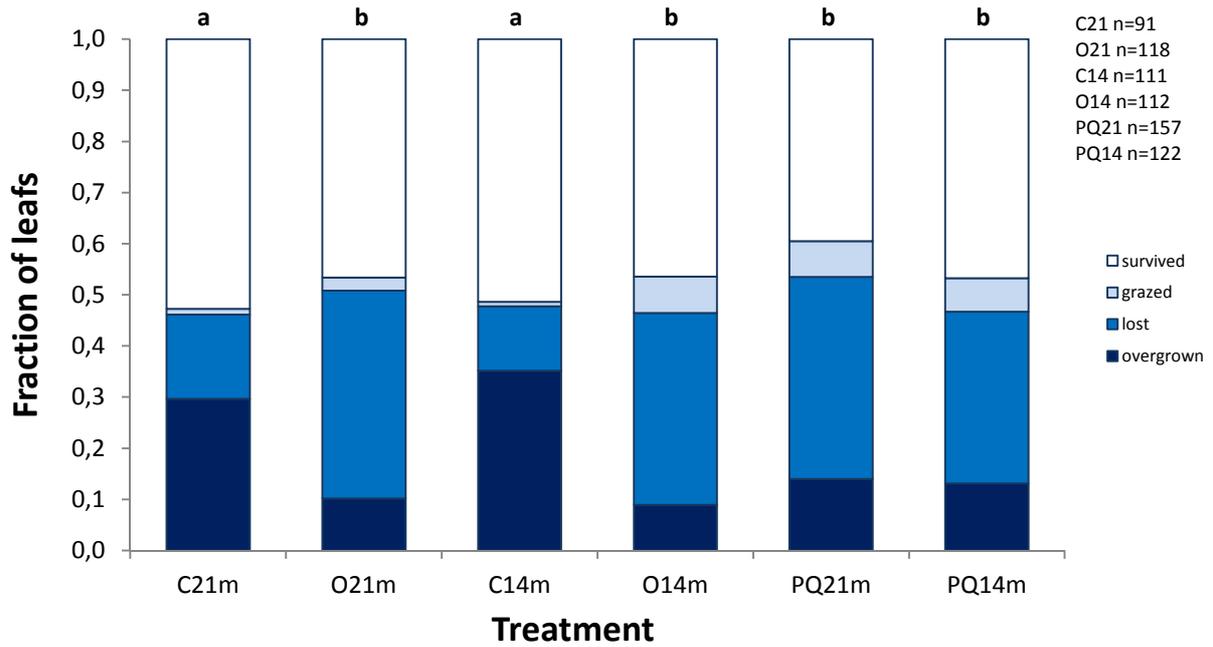


Figure 8. Fraction of *L. variegata* leaves with a particular fate per treatment. Letters above bars indicate significantly different treatments. Data from Tolo and Wayaka II is pooled together.

Table 1. *Lobophora* fate. P values for differences in algae fate between the different treatments (* significant to 0.05 level, ** significant to the 0.01 level).

	C21	O21	C14	O14	PQ21	PQ14
C21 overgrown		0.002 **	0.4512	0.0001 **	0.0023 **	0.0023 **
C21 lost		<0.0001 **	0.5451	0.0005 **	0.0002 **	0.0032 **
C21 grazed		0.4612	0.9446	0.0384 *	0.0368 *	0.049 *
C21 survived		0.3897	0.7771	0.3211	0.0392 *	0.3863
O21 overgrown			<0.0001 **	0.7961	0.3172	0.4668
O21 lost			<0.0001 **	0.6419	0.7374	0.2626
O21 grazed			0.39	0.1008	0.0922	0.1289
O21 survived			0.5451	0.8792	0.184	>0.9999
C14 overgrown				<0.0001 **	<0.0001 **	<0.0001 **
C14 lost				<0.0001 **	<0.0001 **	0.0002 **
C14 grazed				0.0211 *	0.0198 *	0.0281 *
C14 survived				0.4451	0.0512	0.5418
O14 overgrown					0.2121	0.3303
O14 lost					0.8681	0.524
O14 grazed					0.9784	0.8797
O14 survived					0.2514	0.8782
PQ21 overgrown						0.8088
PQ21 lost						0.3904
PQ21 grazed						0.8954
PQ21 survived						0.18

3.4. Recruitment

Neither herbivory nor adult *Lobophora* population density influenced the number of new *Lobophora* recruits and their mortality. The cumulative number of recruits, new recruits per sampling interval, recruit density and recruit mortality were not significantly different between treatments at the end of the experiment (figure 9a-d; ANOVA $p=0.404$, $p=0.167$, $p=0.424$ and $p=0.514$ respectively). Mortality was calculated for the last 6 sampling intervals only due to very low recruit numbers in the beginning of the experiment, and mortality percentages between sampling intervals did not drop below 10% and never exceeded 28%.

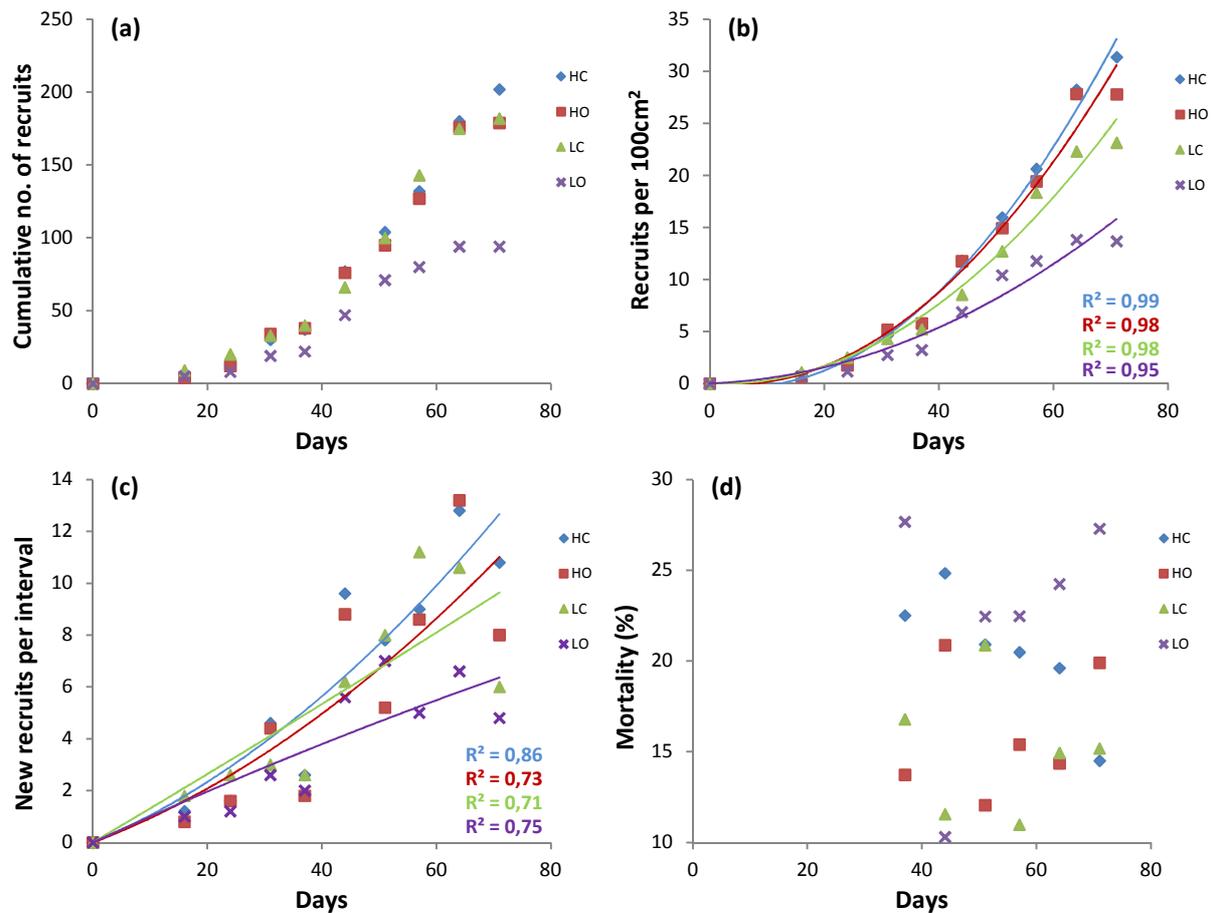


Figure 9. Recruitment patterns. Cumulative number of *L. variegata* recruits (a), recruit density (b), number of new recruits (c) and recruit mortality (d) over time. Polynomial trendlines (order 2) are fitted for (b) and (c) with corresponding R^2 values. Treatment abbreviations are HC high density - closed cage, HO high density - open cage, LC low density - closed cage and LO low density - open cage.

3.5. NIFTs

Leaves of *Lobophora* collected at both Tolo and Wayaka II showed a change in fluorescence when NH_4 and PO_4 were added. A total of 36 NIFT runs was performed for both nutrients at both sites. At Wayaka II 94.4% of the runs for NH_4 and 88.9% of the runs for PO_4 were successful with a yield of 0.3 or higher indicating healthy algae while at Tolo this percentage was 66.7% for NH_4 and 47.2% for PO_4 (table 2). Of the healthy algae at Tolo 58.3% and 52.9% showed a reaction to NH_4 and PO_4 respectively. This percentage at Wayaka II was lower with 38.2% for NH_4 and 25.0% for PO_4 , but not significantly lower compared to Tolo (Two-proportion Z-test $p=0.1307$ and $p=0.0508$ for NH_4 and PO_4 respectively).

Table 2. Nutrient Induced Fluorescence Transients. Percentage of NIFT experiments with a yield higher than 0.3 and percentage of reactions induced when tested for NH₄ and PO₄ at Tolo and Wayaka II.

	NH ₄		PO ₄	
	Tolo	Wayaka II	Tolo	Wayaka II
Total number of tests	36	36	36	36
Yield < 0.3 (non-usable NIFTs)	12	2	19	4
Yield > 0.3 (usable NIFTs)	24	34	17	32
Percentage healthy algae	66,7	94,4	47,2	88,9
Induced reaction on nutrient	14	13	9	8
No induced reaction on nutrient	10	21	8	24
Percentage induced reaction	58,3	38,2	52,9	25,0
Distinct positive reaction ↑	5	9	1	1
Distinct negative reaction ↓	4	3	7	6
Unclear reaction direction	5	1	1	1

4. Discussion

4.1. Net growth rates and herbivory

The net relative growth rates of caged *L. variegata* leaves were 2.2 times higher (0.0232 vs 0.0108 $\text{cm}^2 \text{cm}^{-2} \text{d}^{-1}$) than those of non-caged leaves, which indicates that herbivores play an important role in controlling *L. variegata* biomass and abundance on Bonaire. This coincides with results from previous studies done around the world regarding both urchin (de Ruyter van Steveninck & Breeman 1987a) and fish grazing (Miller *et al.*, 1999; Diaz-Pulido & McCook, 2003; Littler *et al.*, 2006; Sotka & Hay, 2009; Smith *et al.*, 2010). However, net growth rates were positive in all treatments and even when herbivores were present the lowest net growth rates of 0.0111 $\text{cm}^2 \text{cm}^{-2} \text{d}^{-1}$ found in the permanent quadrats indicates a doubling in *Lobophora* cover in 64 days if left undisturbed by other processes.

Despite positive net growth rates for *Lobophora* when herbivores were present, percentage cover did not increase markedly over 4 months. In the permanent quadrats at Tolo the percentage cover of *L. variegata* increased on average just 17% in 125 days, or equivalent to a RGR of 0.0056 $\text{cm}^2 \text{cm}^{-2} \text{d}^{-1}$. Even when herbivores were excluded and net growth rates increased by 2.2 times, percentage cover only increased 49% on average over 4 months. However, previous studies on long term *L. variegata* dynamics show that increases in abundance is rather a stepwise process following disturbances causing coral mortality such as hurricanes and bleaching or mass grazer mortality than a gradual smooth process (de Ruyter van Steveninck & Breeman 1987a; Mumby *et al.*, 2005; Nugues & Bak 2008; Diaz-Pulido *et al.*, 2009). The time span of the experiment was 4 months during which no disturbances occurred, possibly explaining why macroalgae cover remained relatively constant.

Another possible explanation for the relatively constant cover under high net growth rates as observed during this study are the high mortality rates of the *Lobophora* leaves. We found mortality rates of approximately 55% of the leaves in 125 days in the permanent quadrats and open cage treatments, and approximately 48% of the leaves in the closed cage treatments. The mortality rates of *L. variegata* leaves found in this study are comparable with other studies done in the Caribbean, for example those found by Mumby *et al.* (2005) of 60% of *Lobophora* patches per year, but still lower than those found by de Ruyter van Steveninck & Breeman (1987a) of on average 50% of leaves in 20 days. It is hypothesized here that the observed high mortality rates are responsible for the observation that the area covered by *Lobophora* remains relatively constant in the open cage treatments and permanent quadrats, instead of an exponential increase as predicted by the calculated RGRs.

Causes of leaf mortality differ greatly between when herbivores are present and when herbivores are excluded. When herbivores are excluded and cannot graze the algae as in the closed cage treatments, algae mortality stays high but instead of being grazed or lost the algae start to overgrow each other. It must be noted that when herbivores were present, only a small fraction of total leaf mortality could certainly be attributed to grazing by herbivorous fish (i.e. presence of bite marks or parrotfish scrape marks on the limestone). On average only 6% of the leaves analyzed are evidently grazed off by herbivorous fish in the permanent quadrats and open cage treatment over 125 days. This percentage could well be underestimated though. Of the 38% of leaves that disappeared without grazing evidence in the open cages and permanent quadrats ('lost' category, figure 8) it cannot be concluded for certain that they were not grazed, as they could have been eaten without leaving scrape marks, thereby making the percentage of algae removed by herbivores much larger.

It is not entirely clear why the net growth rates of *Lobophora* found in this study are not influenced by the density in which the algae are growing. Studies on interspecific and intraspecific competition among sessile reef organisms often show lower growth when densities increase (McCook *et al.*, 2001; River & Edmunds, 2001; Jompa & McCook, 2002; Jompa & McCook, 2003; Nugues & Bak, 2006; Chadwick & Morrow, 2011; Vermeij *et al.*, 2011). Net growth rates of macroalgae are presumably negatively influenced by density via bottom up controlled competition

for space or nutrients (Creed *et al.*, 1996; Creed *et al.*, 1998). However, we found no evidence for density dependent growth in the present study. A possible explanation for this is that leaves in open vegetations are grazed on more frequently by herbivorous fish than leaves growing in dense vegetations. The negative effects on algae growth when growing in high density vegetations is thus partly compensated by a lower grazing pressure on algae growing in dense vegetations, resulting in approximately equal growth rates. This is a result of macroalgae avoidance as well as grazing inefficiency of herbivorous fish on dense macroalgae vegetations.

Parrotfish in general do not prey specifically on *Lobophora* because of the high concentration of secondary metabolites in this alga (Littler *et al.*, 1983; Paul & Hay, 1986, Rasher & Hay, 2010; Chadwick & Morrow, 2011), quite unlike the urchin *Diadema antillarum*, who systematically scrape off benthic algae (Ogden & Lobel, 1978; Sammarco, 1982; de Ruyter van Steveninck & Breeman 1987b). Moreover, when macroalgal densities become very high, herbivorous fish can no longer efficiently graze the macroalgae (McClanahan *et al.*, 1999; McClanahan *et al.*, 2000), and instead prefer to graze on filamentous turfs (Hoey & Bellwood, 2011). Because fish are ineffective macroalgae grazers and nutritionally prefer turfs, grazing pressure on dense macroalgae vegetations will be less than on open macroalgae vegetations. As a consequence *Lobophora* growing in the more open turf vegetations are more likely to be accidentally grazed by herbivorous fish, and together with the inefficiency of fish to graze macroalgae these two factors compensate for density limited growth.

Growth and mortality rates are almost exactly the same at 21 and 14m water depth, suggesting that herbivorous fish composition and grazing intensity are approximately equal at both depths. This contrasts with historical herbivore distribution over depth, with *D. antillarum* being the dominant grazer between 0-20m water depth, and not parrotfish as is the case presently (Morrison, 1988). Before 1983 *L. variegata* was almost entirely absent between 5 and 20m water depth, exactly the range of highest *D. antillarum* densities (van den Hoek *et al.*, 1978; Bak *et al.*, 1984; Morrison 1988). After the 1983-84 *D. antillarum* mass mortality (Lessios *et al.*, 1984) herbivore composition and dominance shifted, with fish replacing the urchins (Morrison, 1988; Aronson & Precht, 2000).

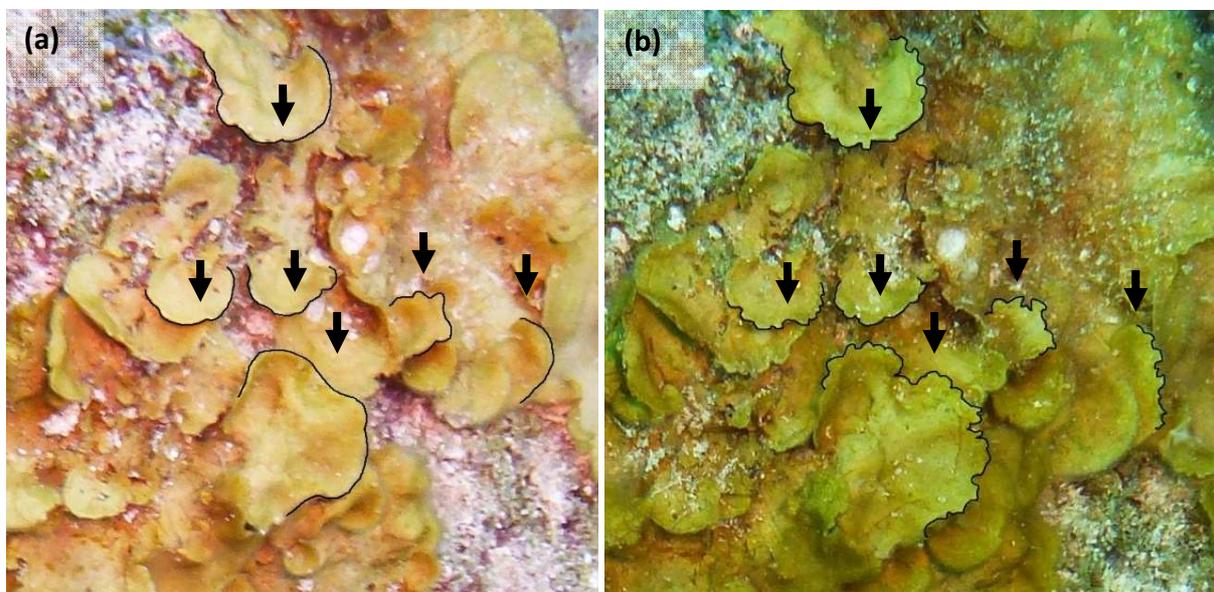


Figure 10. Microherbivore grazing. Outlines of the target algae are drawn in black and indicated by black arrows, (a) ungrazed *Lobophora* patch on 3-5-2012, (b) the same algae patch 13 days later on 16-5-2012. Note the leaf shape change from smooth to jagged.

Pristine reefs are usually characterized by extremely high fish biomass and grazing efficiency (Sandin *et al.*, 2008b). Over the last decades herbivorous fish abundance in the Caribbean has declined markedly (Paddack *et al.*, 2009). Although fish biomass is higher on Bonaire compared to most other locations in the Caribbean (Sandin *et al.*, 2008a; Sommer *et al.*, 2011), it is clear that also

here fish biomass — including herbivorous fish biomass — is much lower than it used to be. Lower herbivore density results in lower grazing pressure on algae, presumably leading to higher net algal growth rates and less algae lost through herbivory. The niche that opened up when herbivorous fish were overfished from the reefs could well have been filled up by microherbivores. During the experiments it has repeatedly been observed that small pieces were grazed off *Lobophora* leaf edges (figure 10), presumably the work of smaller reef herbivores such as shrimps, crabs or hermit crabs (Coen, 1988). It has earlier been suggested by Smith *et al.* (2001) that microherbivores may play a more important role in maintaining macroalgae than has previously been presumed. Often being overlooked, it may well be that microherbivores play a similar underrated role on Bonaire, as they are small, though very numerous. Even if microherbivores are not responsible for consuming large quantities of algae directly, they could indeed weaken *Lobophora* attachment when feeding at the base of the leaf where it is connected to the limestone substrate. This weaker attachment will likely result in the leaves being more susceptible to breaking off in water currents, thus accounting the observed high percentages of leaves disappeared without traces of grazing.

To ensure the highest chance of finding density dependence growth, only the net growth rates of *L. variegata* growing in the highest and lowest density vegetations were compared. The net growth rates of the algae growing in intermediate dense vegetations were not taken into analysis. This explains why the growth rates of the algae in high and low density vegetations in figure 6 are different than the average net growth rates for the respective treatment.

4.2. Growth rates and nutrients

At Tolo more than half of the algae tested showed a significant reaction on the addition of PO_4 and NH_4 , while at Wayaka II this percentage was lower with 25% for PO_4 and 38% for NH_4 . It is rather difficult to estimate what these percentages exactly mean. Stressed algae will less likely react on a nutrient pulse than healthy algae. We used a yield of 0.3 as baseline to separate healthy from stressed algae, but this criterion is only currently being worked out (Den Haan & Dekker, in preparation). Increasing the threshold yield for healthy algae would surely provide more accurate results but would also require the sample size to increase.

Based on the setup and choice of parameter values in the current study we argue that the percentages of reactions induced are high enough to assume that *Lobophora* is limited for both nutrients at both sites. This goes against the 'limiting nutrient concept', which says that organisms are limited by only the nutrient that is least available relative to the other nutrients. However, tropical marine macroalgae are known for their nutrient storage capacities (Larned, 1998; Fong *et al.*, 2003). Because of the capacity to store nitrogen and phosphorus, it is in this case certainly possible that *Lobophora* is limited for both nutrients simultaneously.

Ammonium and phosphate concentrations in the reef water have not been quantified in this study, but there are indications that the reefs are presently in eutrophic conditions. The reefs in Bonaire were concluded to be just above eutrophication threshold (as defined by Bell (1992) and Lapointe (1997)) for NH_4 and PO_4 in 2008 (Netherlands Antilles Coral Reef Initiative, 2012) in an extensive nutrient monitoring essay. It is expected here that the reefs are in a similar condition presently because major measures to reduce nutrient outwash (mostly sewage and from inland basins, so-called salinías) are only now being realized. The observation that *Lobophora* is limited by nutrients on a supposedly eutrophic reef shows that simply measuring nutrient concentrations is of little value when considering macroalgae growth.

Using NIFTs has a great advantage over the conventional measuring of nutrients on a reef, because it bypasses the whole discussion of exactly what nutrient levels are thresholds for eutrophication (Lapointe *et al.*, 1992; Lapointe 1997), and it targets specific species of macroalgae under specific conditions and locations. Although it appears that the reefs on Bonaire have reached eutrophication thresholds, this is certainly not the case for *Lobophora*. Since *Lobophora* is limited by both ammonium and phosphate at our sample sites, it is very likely that they have not yet reached their potential maximum growth rates. Therefore if nutrient concentrations on the reefs are

increased further in the future this could result in higher growth rates and possibly faster reef degradation. Manipulation experiments where nutrients concentrations are increased above levels found at present on Bonairean reefs are needed to further investigate this possibility.

4.3. Seasonality

We found no clear seasonal pattern in growth rates in the four month period in which the experiment was conducted. It is noted that a four month period is rather short in order to find seasonality, especially since there are only two distinct seasons on Bonaire (wet and dry) (Martis *et al.*, 2002). Our results agree with earlier studies in which seasonality of *L. variegata* was also not detected in the Caribbean (Mumby *et al.*, 2005; Ferrari *et al.*, 2012). However, clear seasonal dynamics in the growth of another brown macroalgae, *Dictyota* spp. have been shown in the Caribbean (Diaz-Pulido & Garzón-Ferreira, 2002; Mumby *et al.*, 2005; Ferrari *et al.*, 2012) as well as for *L. variegata* in Australia's Great Barrier Reef (Diaz-Pulido *et al.*, 2009). The mentioned studies have a resolution of at least one month, thereby possibly masking more subtle changes over smaller/larger time scales. Moreover, these studies focused only on percentage algae cover instead of growth rates as well, while only the study by Ferrari *et al.* (2012) takes into account herbivore fluctuation and influence.

In figure 5 it seems that there is a pattern in the fluctuations of the net RGRs at Tolo in the months April and May. When all net growth rates from all treatments and both sites are plotted together (see Appendix 2), there was no pattern in the net RGR detectable over time. It must be noted however that there should be some care taken in interpreting these results. For instance, the setup of the experiment requires that lost leaves are replaced with new leaves to keep the sample size constant. Therefore the composition of the group of algae measured changes between measuring points. As a result of this, new and different RGRs could be introduced, leading to a 'phantom pattern' that might not be there and consequently to wrong conclusions.

4.4. Recruitment

Despite that no significant differences were found in the results of the recruitment experiment, we argue that there is a trend visible in the recruitment data: recruitment rates and recruit density appear higher when herbivores were excluded and when there was a higher adult *Lobophora* population density. The small sample size (n=5) used in the recruitment experiment is likely the cause that the differences in recruitment and mortality between treatments were not significant. From figure 9 (a) and (b) it appears that re-colonization of the cleaned limestone follows logistic growth curve. An initial exponential recruitment pattern slows down after approximately 64 days into the experiment when maximum recruit densities have been reached. Not only recruitment rates, but also the maximum recruitment densities appear lower in the low versus high density rocks. This difference may be the result of the lower amount of propagules in the biofilm layer in the low density rocks.

Mortality rates of *L. variegata* appear to be largely unaffected by herbivory (figure 9d), contradicting results found by Diaz-Pulido & McCook (2003), who showed that herbivory strongly reduced *Lobophora* recruitment and growth. However, the difference between the two studies is likely due to the small sample size of the present study, or the dissimilarity in trophic reef structure between the experimental sites, as the mentioned study was done in a low nutrient, high herbivore density reef on the Great Barrier Reef in Australia, compared with the relatively high nutrient and low herbivore density reef site on Bonaire of the present study.

Little is known about *L. variegata* reproduction and it is uncertain if reproduction via spores is common in the Caribbean, and it appears that the dispersal distance of the spores is very limited (de Ruyter van Steveninck & Breeman, 1987b; Phillips *et al.*, 1994; Stiger & Payri, 1999). Moreover, no sporulating *L. variegata* individuals were observed during the entire fieldwork period. Therefore it is likely that all newly budded *Lobophora* algae were already present in the remaining biofilm on the

limestone, since if recruitment from surrounding *Lobophora* vegetations via spores would be important, there would not be such clear differences between high and low density treatments.

The experimental period of approximately 70 days was found to be too short for this experiment on this scale. After 65 days recruitment rates and density seem to stabilize, but the remaining single sampling period is too short to accurately describe the complete recruitment pattern. Also it was found that the sample size of $n=5$ per treatment was too small to give significant results. The results are very promising though, and given how little there is still known about macroalgae recruitment and dispersal, it is of great importance in understanding coral-algae phase shifts that similar but more elaborate experiments are conducted in the future.

5. Conclusions

Over the last decade the reefs on Bonaire have been showing signs of a coral-algae phase shift, partly characterized by a rapid increase in abundance and habitat range of the brown macroalgae *Lobophora variegata*. For a small island such as Bonaire the consequences of a phase shift could be catastrophic, as diving tourism is one of the main drivers of the island's economy. In the same period nutrient concentrations on the reef increased and fishing reduced herbivorous fish density, but attempts to find out what the importance of these two factors is and whether they could be responsible for the observed shift have not yet been made. As long as this remains unclear, it will be difficult for STINAPA and the BNMP to implement measures aimed to reduce or reverse this shift. The present study demonstrates that herbivory by fish is an important regulator of *Lobophora* growth on Bonaire. Even if fish abundance is much lower than some decades ago, it is shown that the remaining herbivores have a strong negative effect on net growth rates and recruitment. Our results demonstrate that in the absence of fish, net *Lobophora* growth rates are more than double compared to when fish are present. This emphasizes the importance of fishing regulations to protect remaining herbivore fish stocks. However, even in the presence of fish positive net growth rates were measured. High leaf mortality was responsible for keeping algae abundance relatively constant despite positive net growth rates, and it is demonstrated that herbivory is likely an important cause of mortality under *Lobophora* leaves. Nutrient limitation experiments show that *Lobophora* growth rates are probably sub-optimal, as there is limitation for both ammonium and phosphate. Together these findings provide detailed and high resolution information on *L. variegata* growth, mortality and recruitment rates under two different grazing intensities on Bonaire. While global threats to coral reefs are often difficult to manage, local stressors could be controlled locally with relative ease. In order to increase our knowledge on how local stressors amount to coral-algae phase shifts, further research into topics such as coral fitness, disease and recruitment, coral-algae interactions, algae physiology and light attenuation will be essential.

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Tourism Corporation Bonaire TCB

<http://www.tourismonbonaire.com/en/footer-extras/private-sector/statistieken-toerisme-bonaire>

Appendices

Appendix 1

The APTO setup is especially designed to perform NIFTs on macroalgae. Figure A1 shows a labeled representation of the APTO setup. Important components are numbered 1-7:

1. $\frac{3}{4}$ magnet clips in which the *Lobophora* leaf is secured
2. Fluorescence sensor of DIVING-PAM
3. Optic cable connecting to DIVING-PAM
4. Adjustable sensor block
5. Adjustable measuring block
6. Actinic light source
7. Petri dish

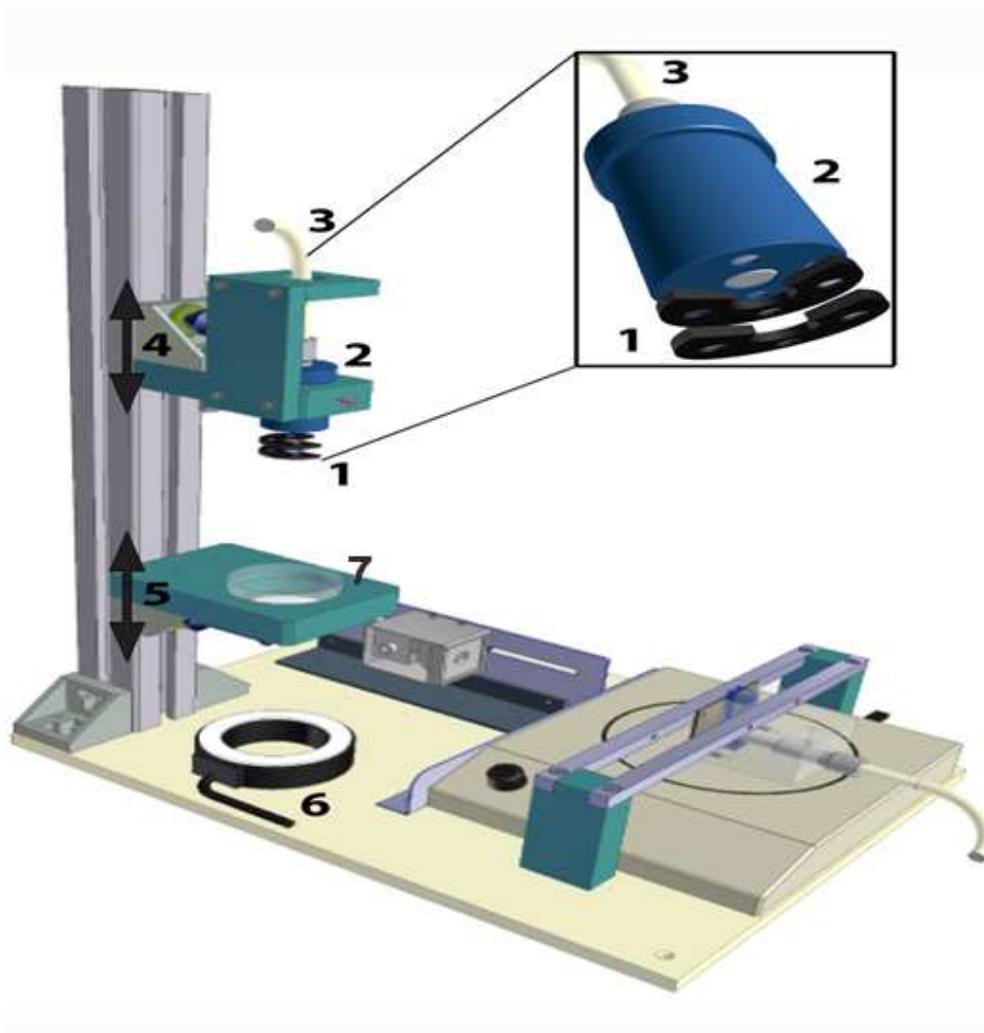


Figure A1. Schematic representation of the APTO setup.

Appendix 2

No clear pattern is observed when comparing all net growth rates over time. Net RGR was only significantly higher in early April compared to late April in 4 out of 12 treatments (Tolo C14, Tolo O14, Wayaka II O21 and Wayaka II O14, ANOVA $p < 0.05$). Some care must be taken when interpreting the data from figure A2. For example, the setup of the experiment requires that lost leaves are replaced with new leaves to keep the sample size constant. Therefore the composition of the group of leaves measured changes between sampling intervals. As a result of this, new and different RGRs are introduced, possibly leading to a 'phantom pattern' that might not be there and consequently to wrong conclusions.

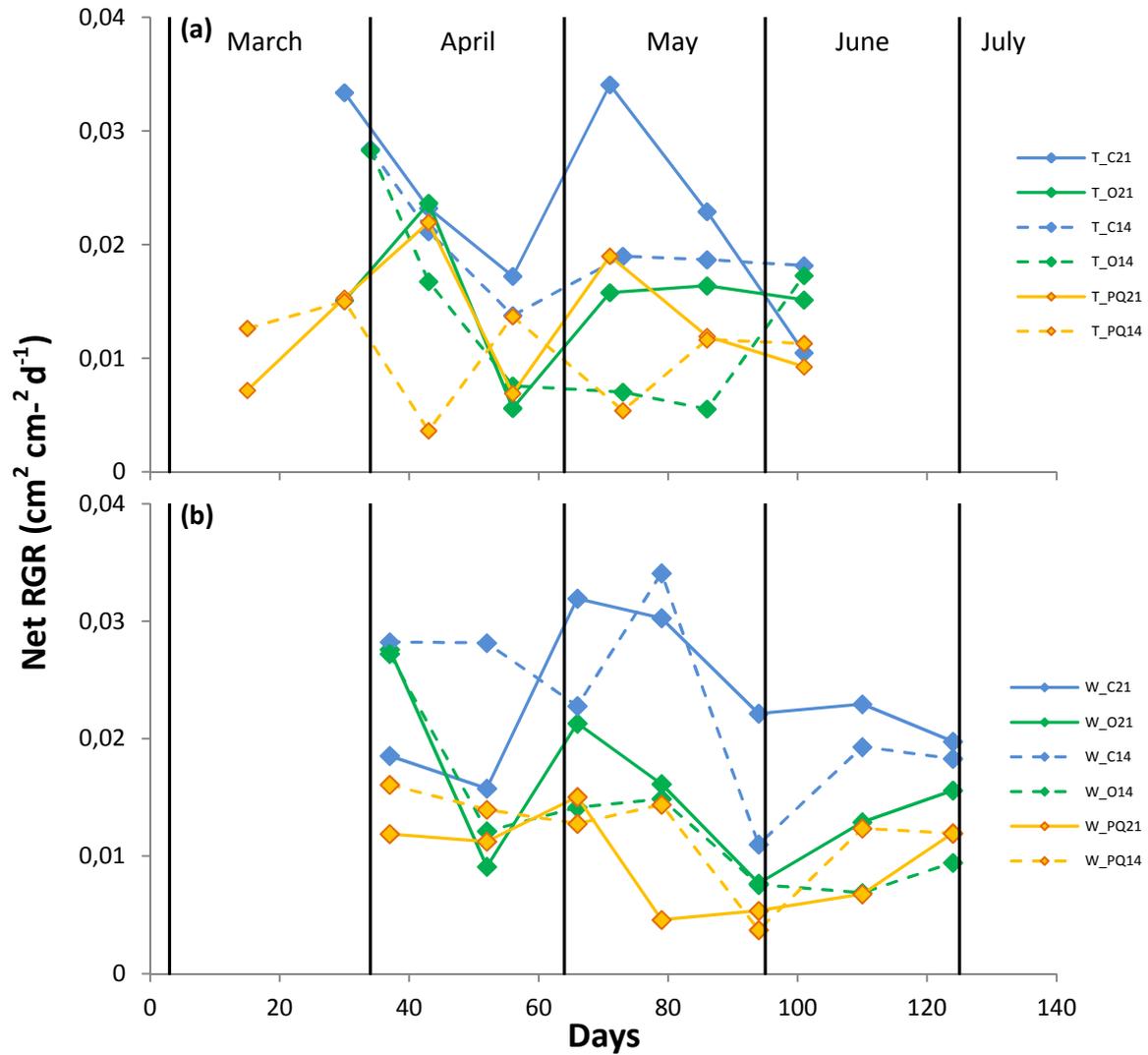


Figure A2. Net *Lobophora* growth rates over time. Timeline with the different net RGRs found for all the treatments and depths at Tolo (a) and Wayaka II (b), and different depths 21m (solid lines) and 14m (dashed lines). Abbreviations in the legend stand T for Tolo, W for Wayaka II, C for closed cage treatment, O for open cage treatment and PQ for undisturbed conditions. Vertical black lines indicate separations between months.