You are what you eat; The role of heterotrophic feeding in coral juvenile survival, growth, and heat tolerance.

Contents

Abstract

This scientific thesis investigates the impact of different heterotrophic feeding regimes on the survival, growth, and heat tolerance of newly settled coral juveniles' *ex-situ*. Coral reefs, facing escalating threats, especially from coral bleaching due to increasing marine heatwaves linked to climate change, necessitate innovative conservation and restoration strategies. Reef restoration practices, including *ex-situ* coral culture facilities, have become crucial in understanding coral survival mechanisms and aiding the recovery of coral ecosystems.

However, challenges in coral culture facilities, such as labour and cost efficiency, emphasize the need for optimizing early-life growth stages. This study focuses on the crucial role of heterotrophic feeding, exploring its potential to enhance coral juvenile survival, growth, and health. Heterotrophic feeding, involving the ingestion of various organisms, offers a promising avenue for supplying essential nutrients not adequately provided by photosynthesis alone.

The research aims to address gaps in understanding how nutrition influences the early life history stages of corals. The objectives include investigating the role of nutrition on newly settled coral juvenile, comparing metabolic rates, zooxanthellae, and chlorophyll densities under different diet treatments, and assessing thermal stress tolerance.

The methodology involves Acropora spawning and coral juvenile production, experimental setups with varied heterotrophic feeding treatments, and three distinct stages of investigation. Stage 1 focuses on the nutritional impact on newly settled coral juvenile, Stage 2 explores baseline metabolic rates and nutritional impacts on zooxanthellae and chlorophyll densities, and Stage 3 evaluates thermal tolerance under different heterotrophic diets.

Results indicate that heterotrophic feeding can significantly influence coral juvenile survival and growth, with potential implications for coral resilience to bleaching

events. The study contributes valuable insights into the optimization of coral culture facilities and the development of effective conservation and restoration strategies for coral reefs in the face of climate change and anthropogenic disturbances.

Introduction

Coral reefs are renowned for their biodiversity and productivity (Connell et al., 1978; Odum and Odum, 1955), but also represent a high economic value, primarily as a source of food and tourism (Bryant et al., 1998). However, these valuable ecosystems are under threat and face a myriad of challenges, with coral bleaching associated with increases in sea surface temperature being heralded as one of the most important stressors associated with the Anthropocene period (Hughes et al., 2017) The frequency, duration, and intensity of these 'marine heat waves' have increased more than 20-fold since 1981 (Laufkötter et al., 2020) and we are currently in the midst of the 4th global coral bleaching event (NOAA, 2024). Such stressors, not only alter the visual appearance of corals but critically disrupt the energy exchange between the coral host and its symbiotic algae (Hoegh-Guldberg et al., 2007).

In response to the escalating threats facing coral reefs, reef restoration practices have gained prominence as vital interventions (Rinkevich, 2014). Such practices, both in situ and ex situ, have been developed to facilitate repopulation and survival mechanisms for future generations. The process of asexual propagation (i.e. the production of genetic clonal outplants) is one of the most widely used methods to date (Boström-Einarsson et al., 2020; Ferse et al., 2021). However, despite the time and money put into these efforts, the rate of ocean warming may very well outpace the adaptive responses of corals, given the increased frequency of bleaching events and reduced time for coral recovery (Hughes et al., 2017). That said, the true adaptive capabilities of corals remain not well understood, making it difficult to accurately assess the long-term impacts of rapid climate change on these organisms.

Sexual reproduction is always a key component of population dynamics, with survival rates of juveniles being a fundamental driver of population sizes (Rapuano et al., 2017). In corals, synchronous broadcast spawning events, often characterized

by a single annual mass spawning, have long posed a number of challenges for scientists interested in understanding even the basic biology and population dynamics of corals (Craggs et al., 2017; Guest et al., 2002; Richmond and Hunter, 1990). Most notably, access to spawn occurs across a limited timeframe and there are many physical difficulties of larvae collection in the open ocean. Therefore, coral aquaculture has emerged as an important facet of reef restoration, recognized for its potential to mass-produce coral propagules for rehabilitation, experimentation, and genetic banks, as well as eliminate stressors such as predation and competition during a key life-history stage, enhancing post-settlement survival (Craggs et al., 2019; Leal et al., 2016; Petersen et al., 2008).

Furthermore, this research *ex-situ* is progressively establishing a significant role in developing an understanding of how corals may respond to environmental changes, and the underlying mechanisms of these responses (Humanes et al., 2021; Petersen et al., 2006). Gaining this knowledge will be instrumental in developing strategies for supporting conservation of coral reefs (Humanes et al., 2021; Lachs et al., 2021). Indeed, sexual coral propagation is now widely acknowledged as a scalable approach due to its facilitation of greater genotypic diversity compared to the use of coral fragmentation (Doropoulos et al., 2019; Randall et al., 2020; Vardi et al., 2021). However, the current efficiency of coral culture facilities is not as high as we would like, primarily due to low coral growth in the early life history stages (Petersen et al., 2005). In the wild, mortality can exceed 99% in the first year of life (Penin et al., 2011). In captivity there is still a considerable bottleneck during the early development stages and, together with the significant costs associated with even basic husbandry and the required facilities, this step alone has been heralded as a major hurdle that needs to be overcome (Petersen at al., 2006). As such, an integral factor in facilitating the upscaling of coral culture facilities would be the optimization of coral growth and survival in the early life history stages.

Raising newly settled spat in aquaria and keeping adult parental colonies healthy relies on maintaining high quality water parameters, correct lighting, and appropriate nutrition (Leal et al., 2016). Although their maintenance requires precision and constant monitoring and adjustment, the water and light requirements for corals are relatively well understood. In contrast, little is known about the food requirements of

young corals. However, there are now various artificial coral diets available and regularly utilized in the aquarium industry. This range of products offers diverse diets and reportedly varied benefits for optimising coral growth and survival. The diets of wild corals are diverse, and they are known to feed on bacteria (Bak et al., 1998), zooplankton (Sebens et al., 1996), microzooplankton (Ferrier-Pages and Gattuso 1998), sediment (Stafford-Smith and Ormond, 1992) and even their symbiotic algae (Wiedenmann et al., 2023). Corals also employ various feeding mechanisms to acquire their prey, including extension of tentacles, entrapment with mucus strings and/or filaments, ciliary reversal, and/or extending their mesenterial filaments (LaBarbera, 1984), all of which contribute to diet effects on levels of protein obtained, the structure of the holobiont, and metabolic processes (Anthony and Fabricius, 2000; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003). Interestingly, in an early study by Anthony (1999), assimilation efficiency of food was shown to be inversely related to suspended particulate matter (SPM) concentration. Notably, corals from turbid inshore reefs exhibit increased heterotrophic capacity on suspended sediment compared to their conspecifics on less turbid and mid-shelf reefs. This suggests the corals ability to alter its heterotrophic capacity dependent on conditions (Anthony, 2000). Such heterotrophic plasticity reveals that changes in the trophic mode of some coral species serves as a mechanism for sustaining a positive energy balance in turbid environments, broadening their physiological niche (Anthony and Fabricius, 2000).

Recent studies have even highlighted the shaping of the microbiome (important for the health and fitness of the coral) can be governed by diet (Galand et al., 2020). This range of observed impacts of diet on coral metabolism and physiology suggests that manipulation of the diet may be an efficient method to enhance fitness, health and/or survival of both adults and juvenile corals (Conlan et al., 2017a). Inorganic nutrients, including nitrogen and phosphorus, essential amino acids, and trace vitamins and minerals provided through feeding, certainly play a crucial role in augmenting coral nutrition, which is not adequately provided for by phototrophy alone (Grottoli et al., 2006). Further, when nutrient concentrations are elevated, corals shift from autotrophy to heterotrophy, stimulating calcification and growth rates, as well as increasing tissue protein content, and biomass (Ezzat et al., 2019).

Nutrition plays a pivotal role in coral health, resilience, and growth. Corals acquire nutrients through both autotrophic pathways (via photosynthesis from their symbionts) and heterotrophic feeding. However, not all nutrient sources affect corals equally. Organic nutrient sources, such as particulate organic matter and zooplankton, generally enhance coral energy reserves, tissue growth, and symbiont density (Houlbrèque and Ferrier-Pagès, 2019). In contrast, excessive enrichment with inorganic nutrients, particularly nitrate and phosphate, can destabilise coral-algal symbioses, promote algal overgrowth, and increase susceptibility to bleaching (D'Angelo and Wiedenmann, 2014; Wiedenmann et al., 2013). Recent studies have highlighted that chronic exposure to elevated inorganic nutrients impairs coral physiological performance under heat stress, resulting in reduced bleaching resistance and slower recovery (Fox et al., 2021; Hughes et al., 2020). Conversely, organic nutrient enrichment, when appropriately managed, has been shown to improve coral resilience by providing critical building blocks for proteins, lipids, and symbiont maintenance (Grottoli et al., 2021). This distinction between organic and inorganic sources underscores the importance of carefully tailoring nutritional strategies in reef restoration and ex-situ aquaculture to optimise coral health without inadvertently increasing vulnerability to environmental stressors.

Coral nutrition therefore encompasses a combination of autotrophic and heterotrophic strategies that enable survival in oligotrophic reef environments. While autotrophy through Symbiodiniaceae-derived photosynthates supplies a significant share of metabolic energy, heterotrophy offers a broader spectrum of essential nutrients (Ferrier-Pagès et al., 2011). Organic nutrients such as amino acids, fatty acids, and dissolved organic carbon are especially vital under stress conditions when photosynthetic input may be compromised. Meanwhile, inorganic nutrient availability – particularly of nitrogen and phosphorus – plays a role in supporting symbiont function and tissue growth but can be detrimental in excess (Rosset et al., 2017). This dual role of nutrition — as both a potential asset and a liability depending on the source, necessitates a nuanced approach to feeding regimes, particularly during the early life stages of corals when energy demands are high and resilience mechanisms are still developing. An understanding of these dynamics is key to informing diet formulations in ex-situ rearing and reef restoration strategies.

Even amidst the challenges posed by coral bleaching, heterotrophic feeding appears to be emerging as a critical adaptive strategy for corals to meet their nutritional requirements. For example, corals exhibit higher survival rates during bleaching events with heterotrophic feeding on lipid-enriched diets (Tagliafico et al., 2017). Further, species capable of utilizing or increasing heterotrophic feeding in the absence of symbiont-derived nutrition are better adapted to survive and recover faster after a bleaching event (Grottoli et al., 2006; Rodrigues and Grottoli, 2007; Towle et al., 2015). Additionally, stress-induced metabolic alterations to the coral, activating mechanisms for lipid reserve catabolism, have also been documented (Hillyer et al., 2017). Importantly, some of these benefits can be long-term. For example, corals appear to be able to incorporate a substantial amount of heterotrophic carbon into their tissues for almost a year following bleaching (Hughes and Grottoli., 2013). Some of these effects may also be more indirect. For example, nutrient availability, particularly the forms and ratios of nutrients such as nitrogen and phosphorus, mediate algal symbiont parasitism. In turn, stable metabolic compatibility between coral host and algal symbiont can mitigate bleaching and increase resilience to environmental stress (Morris et al., 2019).

Aim and Objectives

Coral bleaching is traditionally attributed to photo-oxidative stress under elevated temperatures and light intensity. However, recent experiments have revealed nutritional mechanisms regulating the extent of bleaching (Hughes and Grottoli, 2013; Tagliafico et al., 2017). Despite advances in coral bleaching studies (Humanes et al., 2021), a gap exists in the litreature concerning the extent and nature of heat tolerance in corals during the early life history stages. Studies have demonstrated the negative impact of increasing temperatures on coral juvenile survivorship, along with the dual effect of increasing nutrient enrichment reducing mortality (Humanes et al., 2016). Increased heterotrophic input has been identified as a mechanism which can enhance coral energy reserves, supporting greater survival and recovery from bleaching events in certain species (Grottoli et al., 2006).

This research aims of this thesis included the:

- Investigation of the role of nutrition in determining survival and growth rates of newly settled juvenile corals from 0 to 6 months old.
- Determination of the effects on baseline metabolic rates, and symbiodiniaceae and chlorophyll densities of a range of diet treatments, using dark and light respiration recordings for juvenile corals (18 months).
- Determination of the effects of diet on the thermal induced stress tolerance of juvenile corals (18 months).

This research hopes to contribute valuable insights into the impact of heterotrophic feeding regimes on the survival, growth, and heat tolerance of newly settled coral juveniles, providing essential knowledge for improving the conservation and restoration of coral reefs in the face of climate change and anthropogenic disturbances.

Materials and Methods

Experiment 1: Impact of Nutrition on Coral Juveniles (0-6 months old)

Acropora Spawning and Coral Juvenile Production

Juvenile survival increases with size, as larger corals possess a larger energy reservoir to invest into growth, immunity, and fitness, furthermore, reducing the risk of whole colony mortality by sharing resources between polyps (Raymundo and Maypa, 2014). Thus, the provision of nutrients and energy through heterotrophic feeding is likely to affect juvenile survival rates. As the *Acropora* genus, is an abundant and key component of many reefs globally, and is playing a pivotal role in reef restoration, *Acropora* corals were the focus of this study.

Forty-four colonies of *Acropora millepora*, originating from Arlington Reef, Great Barrier Reef, Australia (CITES import permit 582909/03), were transported to the Horniman Museum and Gardens in September 2019 and maintained in coral research systems programmed to replicate the environmental conditions of their *in-situ* locations. The design and parameters of these coral research systems adhered to the methodology outlined by Craggs et al. (2017). After a two-year period of

conditioning, colonies were induced to spawn *ex-situ*, employing the methods described by Craggs et al. (2019).

Spawning occurred over a four-day period (19th – 22nd November 2019). Thirty minutes before the predicted spawning time, the coral research system was isolated from the filtration sump below, and all internal water circulation was turned off. To ensure minimal exposure to air, colonies were gently transferred to white plastic 5-L buckets underwater, utilizing red light head torches. These buckets, securely lidded, were then placed on the side of the aquarium to maintain warmth and darkness. Once spawning had started, the buckets were transferred to the laboratory, where buoyant egg-sperm bundles were collected.

After the release of the egg-sperm bundles, gametes from four colonies were amalgamated, divided into 50 ml falcon tubes, and secured on a rotor at 10 rpm (Maplelab Scientific, model RM-3) until all eggs were separated. The rotation speed was then reduced to six rpm, and the tubes were left undisturbed for 45 minutes to facilitate fertilisation, following the methods of Craggs et al. (2019). The contents of all tubes were subsequently poured into a 500 ml beaker, and the sperm was gravity-siphoned, leaving the zygotes floating at the surface. Zygotes underwent three rinses with water from the research system. Following fertilization, embryos were divided into five, six-L kreisel bowls with meshed holes below the water line, facilitating water exchange. These bowls, immersed in a water bath connected to the research system, allowed for the equilibration of temperature and salinity. The initial 24 hours were designated as a static period with no water input, and at 24 hours post-fertilisation, water from the coral research system was added to the bowls via a 6 ml silicon hose at a flow rate of 200 ml min^{-1}. Planula larvae exhibited free-swimming behaviour 75 hours post-fertilization, signalling their readiness to settle. Planula larvae were then settled on 500 preconditioned Ocean Wonders Ceramic Coral Frag Plugs for 5-7 days, following the methodologies of Craggs et al. (2019).

Experimental Configuration

Four hundred ceramic plugs were randomly assigned to four treatments (100 per treatment). Each treatment involved dividing 100 plugs evenly between five replicate

12 L tanks (30 × 20 × 20 cm) (Fig. 1B), with each tank containing 20 ceramic plugs hosting coral juveniles. These 20 tanks sat in three trough-style tanks (Fig. 1A) connected to a centralized coral research system housing the broodstock coral colonies. Replicate positioning for each treatment was randomly generated to minimise confounding effects of positioning. Each tank featured a banjo-style outlet with an 800 µm mesh. Inlet water from the centralized coral system was supplied at a flow rate of 91.37 ± 6.80 Lh^{-1} into each replicate tank, following the modified methodologies of Craggs et al. (2019). The three trough-style tanks each had a central strip light (Reef Brite XHO LED, 50% white / 50% actinic blue) with a PAR of 61-79 (µmol photons m^{-2} s^{-1}).

Each aquarium tank hosted nine juvenile sea urchins (*Mespilia globulus*), spawned according to the procedures outlined by Craggs et al. (2019), to facilitate natural grazing of the algae that could harm the coral juveniles. Maintenance procedures over the six-month period involved cleaning each tank every two weeks to remove algae from the sides and outlet meshes.

Heterotrophic Feeding Regime

Four heterotrophic feeding regimes were applied (referred to as ‘treatments’ from here on), each involving five replicate 12 L tanks (30 × 20 × 20 cm) (n = 20 in total). The treatments were chosen based on knowledge that corals can consume a wide variety of prey items at varying scales. These comprised: A) non-fed control, (B) zooplankton (live rotifers, *Brachinous plicatilis*), (C) phytoplankton (*Tisochrysis* lutea - Sourced from Culture Collection of Algae & Protozoa - culture number CCAP 927/14), and (D) a novel particulate artificial coral diet. The novel diet combines a proprietary blend of Lactobacillus, Saccharomyces, and Bacillus (minimal of 1,000,000 CFU/g) to boost nutrient absorption and promote digestion. Each tank received a daily two-hour isolation with an airline for water circulation to aid prey capture, coupled with the daily allocation of the prescribed diet treatment. More specifically:

- Treatment A (Non-fed control) - No food was added during the daily isolation.

- Treatment B (Zooplankton) - Live rotifers *B. plicatilis* were cultured in 4 x 4 L glass demijohns, with a continuous culture, rotating a daily harvest, and fed according to a specified regime, and gut loaded with *Tisochrysis* before being fed to the corals. A dosage of 200 ml was provided per tank (final concentration 2.45 ± 1.33 rotifer ml^{-1}).

- Treatment C (Phytoplankton) - *Tisochrysis*, cultured in 6 x 4-litre demijohns, with a continuous culture and harvested on a daily rotation, supplied a dose of 200 ml per tank (final concentration 106,666.5 ±21,333.5 *Isochrysis* ml^{-1}.

- Treatment D (Commercial novel particulate coral diet) - A diet with a specified particle size range of 3 to 3,000 microns, fit for coral consumption (Houlbrèque and Ferrier-Pagès, 2009) administered at a dose rate in accordance with instructions (25g per 100 litres). Diet breakdown; Crude Protein - 25.5% (min), Crude Fat - 4.7% (min), Crude Fiber - 44% (max), Moisture - 6.5% (max), Phosphorus - 0.6% (min), Ash - 5% (max).

Water quality management

To maintain optimal and stable environmental conditions for coral growth, water chemistry in the coral research systems was managed using the Triton Method. This methodology allows for the precise monitoring and adjustment of macro and trace elements in reef aquaria, ensuring a chemically stable environment conducive to coral health and development.

The Triton Method is based on regular water testing using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), which provides a comprehensive analysis of water chemistry. Results from these tests inform targeted supplementation of specific elements through pre-formulated Triton dosing solutions. This approach reduces the need for routine water changes by proactively correcting imbalances and maintaining long-term chemical stability.

In accordance with the protocols established by Craggs et al. (2019), this method was employed to support the co-culturing of *Acropora millepora* and *Mespilia*

globulus. Water parameters were tested monthly using ICP-OES and supplemented accordingly using Triton Core7 Base Elements and specific element blends tailored to the system's needs.

In addition to laboratory analysis, in-house testing was performed three times per week to monitor alkalinity, calcium, magnesium, nitrate, nitrite, and ammonia, using Salifert colorimetric test kits.

The centralised coral research system also included a D&D Power Roll mechanical filter, equipped with a 5-micron filter fleece. This filtered water from the main sump before it entered the four treatment tanks, helping to maintain high water clarity and reduce particulate waste.

The following key parameters were maintained within optimal ranges throughout the experiment:

- Calcium (Ca): 400–450 mg L^{-1}
- Alkalinity (KH): 7.0–8.0 dKH
- Magnesium (Mg): 1300–1400 mg L^{-1}
- Nitrate (NO_3^-): <1.0 mg L^{-1}
- Phosphate (PO_4^{3-}): <0.03 mg L^{-1}

Influence of Heterotrophic Treatment on Coral Percentage Survival

To assess the impact of heterotrophic treatment on coral juvenile survival, settlement plugs from all replicates were imaged (Fig. 1C) bi-weekly (using a Canon 5D Mark III camera with a 100 mm macro lens) throughout the 180-day experiment duration. Coral polyps for each replicate were counted manually using the ImageJ count tool (National Institutes of Health, 2019). Percentage survival for each treatment was calculated for each replicate at each time point.

Influence of Heterotrophic Treatment on Coral Size

Images captured on day 152 for the coral survival percentage counts were also utilised to measure colony sizes and evaluate the influence of heterotrophic

treatment on total coral growth. Coral surface diameter measurements were performed using ImageJ (National Institutes of Health, 2019) (Fig. 1D), with the fixed coral settlement plug diameter of 19 mm serving as the scale.

Analysis for Experiment 1

Survival rates were investigated using Kaplan–Meier survival curves to estimate probabilities of survival over time for each treatment. To test for treatment differences between survival curves pairwise log-rank tests were conducted. Treatment effects on coral size were assessed using general linear regression. Assumptions were assessed using residual diagnostics (residuals vs fitted values and quantile –quantile plots of residuals) and Cook's distances were used to check for overly influential data points.
All analyses were conducted using the statistical programming language R (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

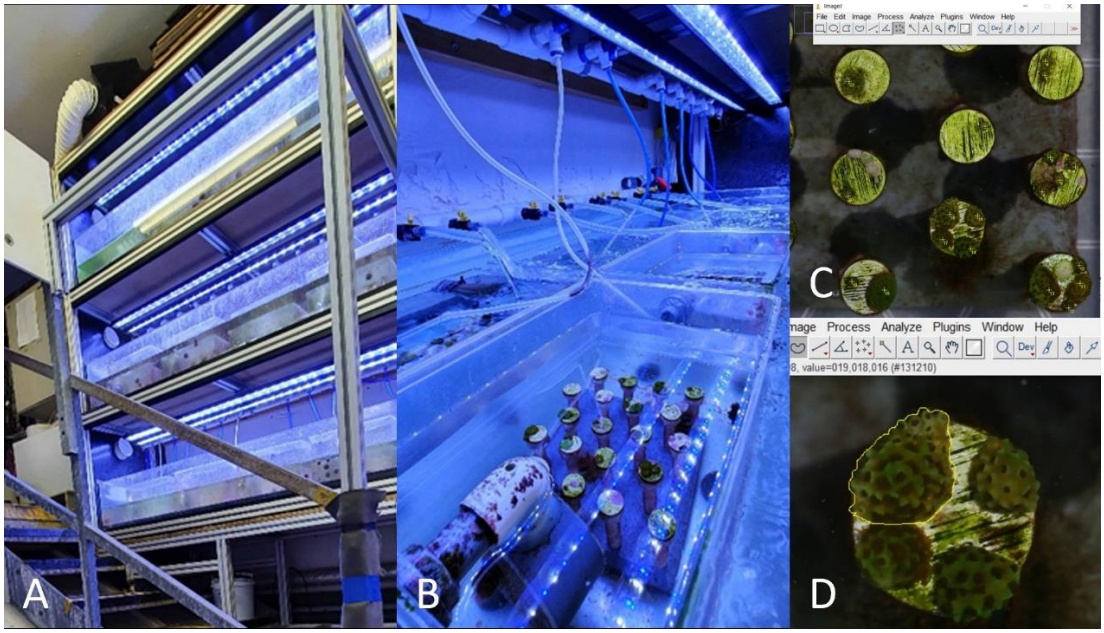

Figure 1. (A) Three trough-style tanks. **(B)** 12 L tanks containing 20 ceramic plugs hosting coral juveniles. **(C)** Image of one plug for coral juvenile survival count using ImageJ. **(D)** Coral surface diameter measurements using ImageJ.

Experiment 2a: Comparative Baseline Metabolic Rates

Physiological Assessment

Physiological differences among juvenile corals were investigated by comparing dark and light respiration rates to evaluate the impact of each heterotrophic treatment on baseline metabolic rates. At the 18-month mark, all juveniles not grafted underneath the plug were identified, and from these, eight corals were randomly chosen from each treatment group. The chosen corals were mounted on to new nylon bolt one week before the experiment to ensure the absence of calcareous or macro algae.

Experimental Configuration

The experimental configuration included a glass water bath (125.5 × 46.5 × 20 cm, L × W × H)filled with prefiltered (5 µm) water from the coral research system, maintained at a constant temperature of 27.5 °C. A central strip light (Reef Brite XHO LED, 50% white / 50% actinic blue) provided a photosynthetically active radiation (PAR) of 61–79 µmol photons m^{-2} s^{-1} at the water surface was used to provide similar light levels compared to the research system set up. This setup featured two circulation (Maxijet, 1100 L h^{-1})two 400 W heaters (Visatherm) and D&D Dual Heating and Cooling Controller. A frame secured 8 submerged magnetic stirrers Thermo Scientific micro stirrer units; Thermo Electron LED Telemodul 40c with 8-point distributor box) and10 purpose-built glass respiration chambers (400 ml) (Fig. 2B). Preliminary experiments were conducted to optimize equipment performance, determining the correct stirrer rate to maintain polyp extension and chamber duration, preventing harmful oxygen levels. A 2-point dissolved oxygen meter (Neofox GT with Foxy R Oxygen Sensor (152.4 mm) & temperature probe) ensured accurate readings, calibrated in ambient air and zero oxygen point in nitrogen gas before the experiment. The accuracy of using final O_2 mg L^{-1}readings was confirmed in a previous study by Strahl et al. (2015).

Experimental Procedure

No feed was provided to the corals 48 hours before the experiment to ensure their digestive system was empty. Each coral was acclimated for one hour to its

designated respiration chamber base using screws on the base to secure the bottom of the nylon bolt. Light incubations were performed, followed by seawater sample analysis for alkalinity (AT) and oxygen (O_2) after a two-hour incubation. Chambers were reset with new water and subjected to one-hour dark acclimation. Subsequent dark incubations were conducted and analysed for O_2 after a 2-hour incubation following modified methodologies of Strahl et al. (2015). Subsamples of seawater (0.05 L) from each incubation chamber were used for the determination of total alkalinity (AT; Xylem Analytics TitroLine 7000), and light and dark calcification rates were determined using the alkalinity anomaly technique (Chisholm and Gattuso, 1991). This process was replicated for each treatment.

Data Standardization and Calculation

Rates of net photosynthesis, dark respiration, and light and dark calcification were standardised to surface area (see below for details of measurement) and incubation time, expressed in (μmol O_2 cm^{-2} h^{-1}) and $CaCO_3$ μmol cm^{-2} h^{-1}, after subtracting values measured in the blank chambers following protocols of Strahl et al. (2019).

Experiment 2b: Nutritional Impact on Symbiodiniaceae and Chlorophyll Densities

Sample Collection and Preservation

After each experiment, corals were removed from bolts, excess water was removed by dabbing on tissue, and they were submerged in liquid nitrogen. Subsequently, corals were wrapped in two layers of aluminium foil, stored at -20 °C, the tissue was removed from the skeleton with an air gun in 10 ml of FSW (5 μm) seawater. The resulting 10 ml tissue samples were transferred to cryovials and stored at -80 °C, following protocols of Strahl et al. (2019).

Symbiodiniaceae Separation and Chlorophyll Analysis

Contents of chlorophyll a and b of the symbiodinium fraction were determined spectrophotometrically following a modified protocol of Lichtenthaler (1987), Ritchie (2008) and Strahl et al. (2019). In dark conditions, homogenate samples were thawed and separated into host and symbiodiniaceae fractions by centrifugation (3 min, 1500 × g, 4 °C) using a Hermle Z446K. Five ml of ultra filtered sea water (UFSW) was added to the symbiodiniaceae pellet, creating a supernatant, which was stored at -20 °C before determining the contents of chlorophyll a and b in the

symbiodiniaceae fraction. Subsamples of 750 µl (0.75 ml) of the supernatant were centrifuged (Labnet Prism R) for 3 minutes at 4 °C and 1500 x g. Then, 2 ml of pre-chilled 95% EtOH was added to the symbiodiniaceae pellet, followed by sonication on ice (15 s, 40% amplitude, Sonic-Vibra Cell, John Morris Scientific). The mixture was incubated on ice for 20 minutes and centrifuged (Labnet Prism R) for 5 minutes at 4 °C and 10,000 × g. The absorbencies of the supernatant containing the pigments were recorded in dark conditions using a FLUOstar Omega Multi-Mode Microplate Reader with CCD-based Spectrometer (BMG LABTECH) at 664 and 649 nm. The total chlorophyll (= chlorophyll a + b) content was calculated using the equations of Lichtenthaler (1987) and standardised to mm^2 of surface area.

500 µl (0.5 ml) subsamples of the symbiodiniaceae supernatant, were added to 100 µl (0.1 ml) of buffered formalin in a 2 ml microfuge tube for subsequent symbiodiniaceae counts.

Surface Area Determination

Coral skeletons were dried overnight in the oven at 60 °C and surface area was determined using the double wax dipping technique. Naumann et al. (2009) and Veal et al. (2010) found double dip to be the most accurate method for *Acropora*. Coral skeletons were then weighed, dipped in wax (Premium Paraffin wax pellets) at 65 °C, and the process was then repeated. A calibration curve using PVC cylinders of known surface areas was created through linear regression (R = 0.9965) to standardise weight to surface area using modified protocols of Strahl et al. (2019).

This comprehensive methodology in Experiments 2a and 2b aims to investigate baseline metabolic rates and the nutritional impact on symbiodiniaceae and chlorophyll densities in juvenile corals subjected to different diet treatments.

Experiment 3: Thermal Tolerance of Juvenile Corals Under Different Heterotrophic Diets

The third experiment aimed to evaluate the thermal tolerance of juvenile corals in response to varied heterotrophic diets, exploring its potential impact on their resilience to bleaching. To achieve this, a 90-day temperature stress experiment was conducted under controlled tank conditions.

Experimental Configuration

Seventy-two, 18-month-old *Acropora millepora* colonies were randomly chosen, with 18 colonies per treatment, from the grow-out experiment tanks where the temperature was maintained at 27.5 °C. These colonies were affixed to nylon bolts, labelled, and mounted on acrylic stands.

The 18 corals from each of four treatments were distributed across six replicate tanks, with each tank containing 12 corals representing four treatments. The positioning of replicates within tanks were randomly determined to minimise the effect of potential confounding variables due to position.

Each experimental tank setup consisted of six 10.5 L tanks (32.5 , 17.5, 18.5 cm, L x W x H), with one internal pump (Eheim compact 300, L h^{-1}) ensuring optimal water circulation. Each experimental tank was connected to a sump tank of 10.5 L (same dimensions as experimental tank) with one aquarium digital heater (Eheim, 150 watt) per sump, was connected to an aquarium controller (Neptune Systems Apex) via a programable plug socket (Neptune Systems, 6 plug Energy Bar) (Fig. 2A). Each experimental tank contained a temperature probe (Neptune Systems) connected to the aquarium controller via a programable module (Neptune Systems, PM1 module) to regulate and monitor the seawater temperature throughout the experiment. Connection between the experimental tank and the sump was obtained using a pump (Eheim universal, 600 L h^{-1}) from the sump to the experimental tank, and a one-inch return pipe from the experimental tank to the sump. A flow-through system, via a 6 mm line, fed the experimental tanks at a rate of 4.5 L h^{-1} from a broodstock research system (Craggs et al., 2017). The light regime was controlled by the aquarium controller, via to a central strip light (Reef Brite XHO LED, 50% white / 50% actinic blue) provided a PAR of 61–79 µmol photons m^{-2} s^{-1}, to provide similar light levels compared to the grow out system set up. Temperature within each experimental tank was rigorously monitored using twelve calibrated Hobo Pendant data loggers (Temp/Alarm 64k, Temcon). To ensure consistency in temperature readings, one data logger was strategically positioned at mid-depth in each tank, set to record temperature every 10 minutes following protocols of Humanes et al. (2021). A second set of loggers was used interchangeably to allow for continuous

temperature recording while data from the first set was downloaded using HOBOware, to continuously calculate DHW. This setup enabled constant monitoring of temperature variations and ensured uniform exposure to degree heating weeks (DHW) across all tanks, preventing any deviations in thermal stress conditions experienced by the coral specimens. The thermal component of the experiment commenced after a 7-day acclimation period of the corals to the experimental tanks.

Two temperatures were utilised:

1) One control tank was set to 27.5 °C (±0.23 °C), matching the temperature of the grow-out experiment tanks.

2) Heat stress conditions (five replicate tanks), with the temperature gradually increased over 90 days to ~32.5 °C (±0.22 °C). (see below for the temperature profile over time).

A sudden, rapid die-off at the end of the experiment would compromise the experimental design. However, conducting status surveys when the overall nubbin mortality is between 50-55% is considered ideal (Humanes et al., 2021). To ensure coral bleaching followed by mortality a temperature profile is needed that exhibits a faster rise in the early stages of the experiment, followed by a deceleration. Ideally, the rate of temperature rise should not accelerate, as suggested by Humanes et al. (2021).

A calculated Mean Monthly Maximum (MMM) temperature of 29.35 °C, derived from 10 years of temperature data from the Australian Institute of Marine Science (AIMS) online data for the broodstock's collection location, was used. Degree Heating Weeks (DHW) were generated for the heat stress temperature profile (see Results: Influence of heterotrophic diet on thermal tolerance Fig. 8 & 9).

The temperature stress profile included the following increments:

- Day 1: Temperature raised by 0.1 °C per hour to 29.0 °C.
- Day 4: Raised by 0.1 °C per hour to 29.5° C.

- Day 8: Raised by 0.1 °C per hour to 30.0 °C.
- Day 15: Raised by 0.1 °C per hour to 30.5 °C.
- Day 43: Raised by 0.1 °C per hour to 31 °C.
- Day 58: Raised by 0.1 °C per hour to 31.5 °C.
- Day 68: Raised by 0.1 °C per hour to 32 °C.
- Day 77: Raised by 0.1 °C per hour to 32.5 °C.

Health Status Monitoring During Temperature Stress

Each colony underwent visual inspection every other day, and daily for the last 30 days, by the same observer. Colonies were categorized as 1) healthy (no signs of discoloration), 2) first signs of bleaching, 3) bleached (fully bleached), 4) partial mortality (less than 30% of surface area dead), or 5) dead (bare skeleton without tissue) following modified methods described by Humanes et al. (2021). Simultaneously, photographs were taken. In this instance, corals were mounted on stands and transferred to a photography tank (8 L) containing water from the experimental set up, maintaining temperature and water quality to reduce stress, A standardised set up was used to ensure photographs were taken in the same conditions. This was achieved by lighting (Raleno PLV-S192 Soft Light) the corals with a set power output and kelvin temperature (5600 K). The camera (Canon 5D MKIII) kelvin setting was programmed to match to light source, to achieve true colour rendition. The camera's settings were determined by taking a light reading from an 18% standard neutral grey card (JJC GC – 1II) to ensure correct exposure. These settings were then used for all subsequent images. In addition, the camera position was fixed in relation to the photography tank, which also had a black out back and sides to prevent external light from interfering with the exposures settings (Fig. 2C and see Results: Influence of heterotrophic diet on thermal tolerance Fig. 10). This facilitated standardised photography conditions for the comparison of colour changes based on Humanes et al. (2021). The photographs served as an additional inspection step and were subsequently reviewed visually by eye to assess and categorise changes in coral colouration.

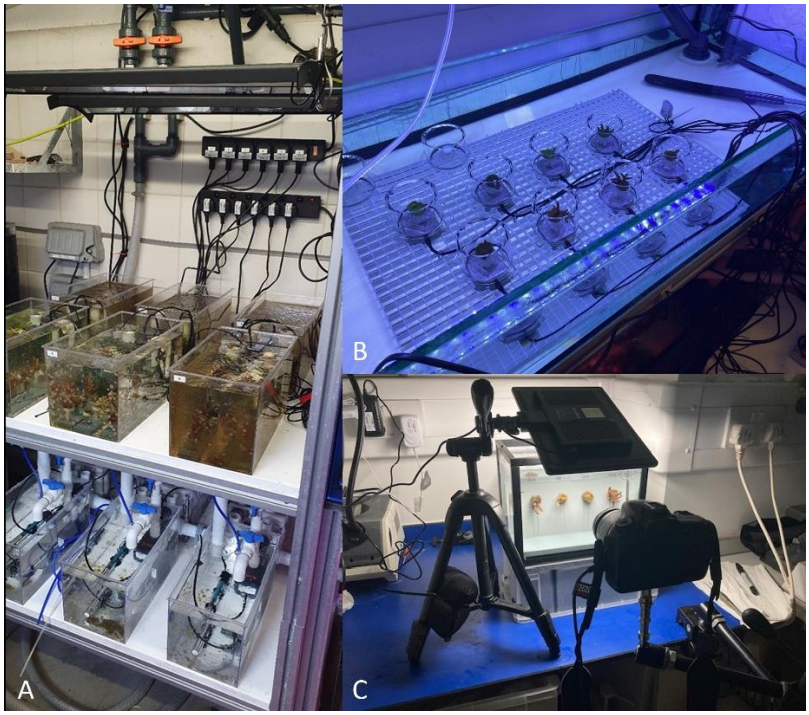

Figure 2. (A) Experimental configuration of heat tolerance experiment**. (B)** Experimental configuration of physiological assessment in respiration chambers**. (C)** Standardised photography set up for the comparison of colour changes.

Analysis of data from for Experiment 3

Kaplan–Meier survival analysis was employed to estimate and compare the probability of survival of coral juveniles over time across the different heterotrophic feeding treatments. This non-parametric method is particularly suitable for survival data containing censored observations (e.g., individuals that survived beyond the experimental period or were lost), which are common in long-term ecological studies. Kaplan–Meier estimators allowed for the visualisation of survival curves and statistical comparison of survival distributions using pairwise log-rank tests, with Bonferroni-adjusted p-values to account for multiple comparisons.

General Linear Models (GLMs) were used to examine the effects of heterotrophic feeding treatments on continuous response variables, including coral colony size, photosynthetic and respiration rates, calcification, symbiodiniaceae density, and total chlorophyll concentration. GLMs offer a flexible approach for testing the influence of

categorical predictors on normally distributed outcomes, while allowing for the assessment of key assumptions. Model assumptions, including homoscedasticity and normality, were evaluated using residual diagnostics (residuals vs. fitted values and quantile–quantile plots), and potential outliers or influential values were identified using Cook's distances.

For analyses where heteroscedasticity was detected, such as in the comparison of time to death under thermal stress (Experiment 3), a generalised least squares (GLS) extension of the linear model was applied to accommodate unequal variance across treatment groups (Zuur et al., 2009). The most appropriate variance–covariate structure was identified using a combination of Akaike Information Criterion (AIC) scores and residual plots based on restricted maximum likelihood (REML). Final model selection was conducted using backwards stepwise elimination under maximum likelihood estimation.

Specifically for Experiment 3, transition times—measured in Degree Heating Weeks (DHW)—from healthy condition to first signs of bleaching and to death (health status 5) were calculated for each coral colony. General linear regression models were initially used to assess differences in transition times and mortality between feeding treatments under heat stress conditions. Where assumptions of normality and variance homogeneity were violated, GLS models were applied as outlined above. Additionally, Kaplan–Meier survival curves were generated for each treatment group to provide a time-to-event analysis of coral mortality during the 90-day thermal stress experiment, with statistical comparisons conducted using log-rank tests consistent with the approach in Experiment 1.
Statistical significance was set at $p < 0.05$ for all tests.

Results

Influence of heterotrophic diet on coral survival.

Mortality over the 180-day period was high (Table 2), with varying responses observed across the different heterotrophic feeding treatments (non-fed control, zooplankton, phytoplankton, novel diet; see Fig. 3).

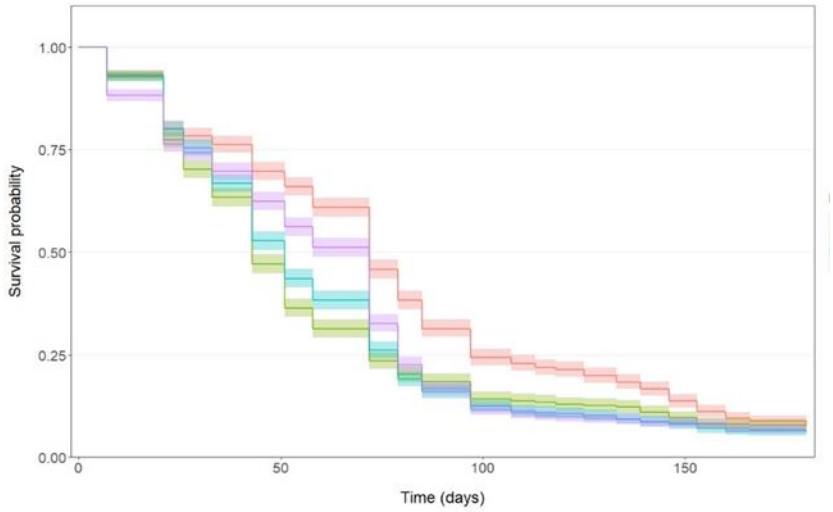

Figure 3. Kaplan-Meier survival curves of newly settled *Acropora millepora* coral juveniles exposed to differing heterotrophic treatments [non-fed control (red line), zooplankton (blue line), phytoplankton (green line), novel diet (purple line)] and grown over 180 days (mean ± s.e.m).

Pairwise log-rank tests for differences in survival rates (Table 1A) revealed significantly lower survival in all fed treatments compared to the non-fed control ($p < 0.001$ for all comparisons). No significant difference was observed between the zooplankton- and phytoplankton-fed groups ($p = 1.000$), whilst corals in the novel diet treatment had significantly higher survival compared to those fed zooplankton ($p < 0.001$) and phytoplankton ($p = 0.00359$), particularly during the middle stages of the experiment (Fig. 3).

Colony size after 152 days was significantly influenced by heterotrophic diet treatment (Fig. 4). Corals in the zooplankton (41.90 ± 31.79 mm²), phytoplankton (40.16 ± 28.59 mm²), and novel diet (25.58 ± 23.70 mm²) treatments attained significantly larger sizes than those in the non-fed control (16.48 ± 15.81 mm²) (all $p < 0.001$ or $p = 0.00189$; Table 1B). Zooplankton- and phytoplankton-fed corals were significantly larger than those fed the novel diet (both $p < 0.001$), but no difference was detected between the zooplankton and phytoplankton groups ($p = 0.609$).

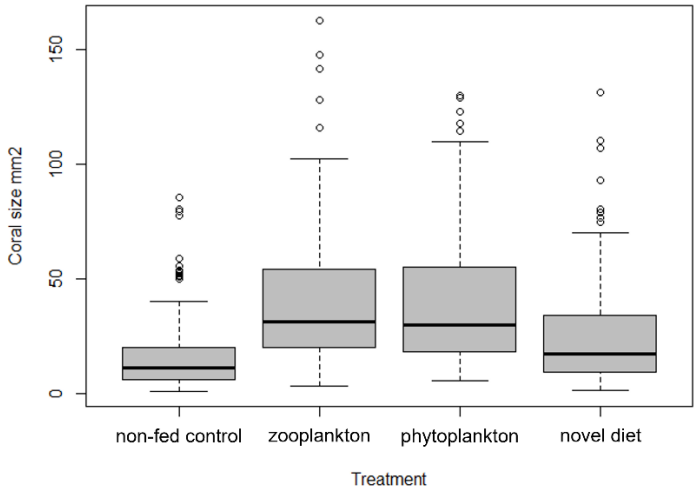

Figure 4. *Acropora millepora* mean coral diameter (mean ± s.e.m) at 152 days between treatments.

The boxplots show the median (horizontal black line), the interquartile range (grey shaded box).

Table 1. (A) Pairwise comparisons of coral survival after 180 days under different heterotrophic feeding treatments (non-fed control, zooplankton, phytoplankton, and novel diet), based on Kaplan–Meier survival curves. P-values were adjusted using the Bonferroni correction to account for multiple comparisons.
(B) Pairwise comparisons of coral colony size (mean diameter, mm²) at 152 days between treatments, based on general linear regression. An adjusted R^2 value of 0.154 was obtained for the linear regression model.
Treatments: Non-fed control, zooplankton, phytoplankton, novel diet.
Statistical significance was set at $P < 0.05$.

(A)	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p<0.001	p<0.001	p<0.001
Zooplankton	-	-	p=1.000	p=0.00359
Phytoplankton	-	-	-	p<0.001
Novel diet	-	-	-	-
(B)	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p<0.001	p<0.001	p=0.00189
Zooplankton	-	-	p=0.609	p<0.001
Phytoplankton	-	-	-	p<0.001
Novel diet	-	-	-	-

Table 2. Survival of juvenile Acropora millepora corals at 152 days post-settlement under different heterotrophic feeding treatments (non-fed control, zooplankton, phytoplankton, novel diet)

	Treatment % survival at 152 days				
	Non-fed control	Zooplankton	Phytoplankton	Novel diet	Total
Initial coral juveniles	1799	1811	1730	1878	7218
Surviving juveniles at 152 days	165	98	117	128	508
Average % survival	9.116	5.550	6.974	6.860	
Standard deviation (SD)	3.893	2.525	2.832	4.244	
Number of plugs with surviving corals	61	59	69	62	
Number of empty plugs	39	31	41	38	

Influence of heterotrophic diet on coral physiological performance.

Calcification rates ($CaCO_3$ μmol cm^{-2} h^{-1}) under light & dark conditions was significantly affected by heterotrophic diet treatment (Fig. 5A, 5B). In light conditions the treatments fed on zooplankton, phytoplankton and the novel diet all had significantly higher calcification rates than the non-fed control ($p = 0.00722$, $p = 0.02346$ & $p = 0.02398$ respectively, Table 3). No significant difference in calcification rates were seen between heterotrophic treatments.

Under dark conditions the treatments fed on zooplankton and phytoplankton had significantly higher calcification rates than the non-fed control ($p = 0.02391$ & $p = 0.00343$ respectively, Table 3). No significant difference in calcification rates was seen between the treatments fed on the novel diet and the non-fed control ($p = 0.14206$, Table 3)

In light conditions, *A. millepora* showed varied gross photosynthesis rates (μmol O_2 cm^{-2} h^{-1}) at 18 months, with the treatment fed on zooplankton displaying the highest rate of photosynthesis (Table 3, Fig. 5C). Those in the heterotrophic treatment groups fed on zooplankton and phytoplankton had a significantly higher rate of oxygen production through photosynthesis compared to the non-fed control ($p < 0.001$ & $p = 0.00567$ respectively, Table 3). No significant difference was revealed between the treatment fed on the novel diet compared to the non-fed control ($p = 0.20716$, Table 3). The treatment fed on zooplankton had significantly higher gross photosynthesis rates compared to the novel diet treatment ($p = 0.00103$, Table 3).

The respiration rates (μmol O_2 cm^{-2} h^{-1}) under dark conditions showed the treatment fed on zooplankton to have the highest rate of respiration (Table 3, Fig. 5D). Those in heterotrophic treatment group fed on zooplankton had a significantly higher rate of oxygen consumption through respiration compared to phytoplankton, the novel diet and non-fed control ($p < 0.001$, $p < 0.001$ & $p < 0.001$ respectively, Table 3). No significant difference was revealed between the other treatments.

Coral symbiodiniaceae density ($\times 10^6$ cells cm^{-2}) in the treatment fed on zooplankton was significantly higher than in all other treatment groups (non-fed control, phytoplankton and novel diet) ($p < 0.001$, Table 3, Fig. 5E). No significant difference was revealed between the other treatments.

Total chlorophyll concentrations (μg cm^{-2}) were significantly higher in heterotrophic treatments fed on zooplankton and phytoplankton compared to the novel diet treatment ($p < 0.001$, Table 3, Fig. 5F). The treatment fed on zooplankton was significantly higher than in all other treatment groups (non-fed control, phytoplankton, and novel diet) ($p = 0.00537$, $p = 0.04224$ & $p = 0.00272$ respectively, Table 3). The novel diet treatment had significantly lower total chlorophyll concentrations compared to the non-fed control ($p = 0.00272$, Table 3).

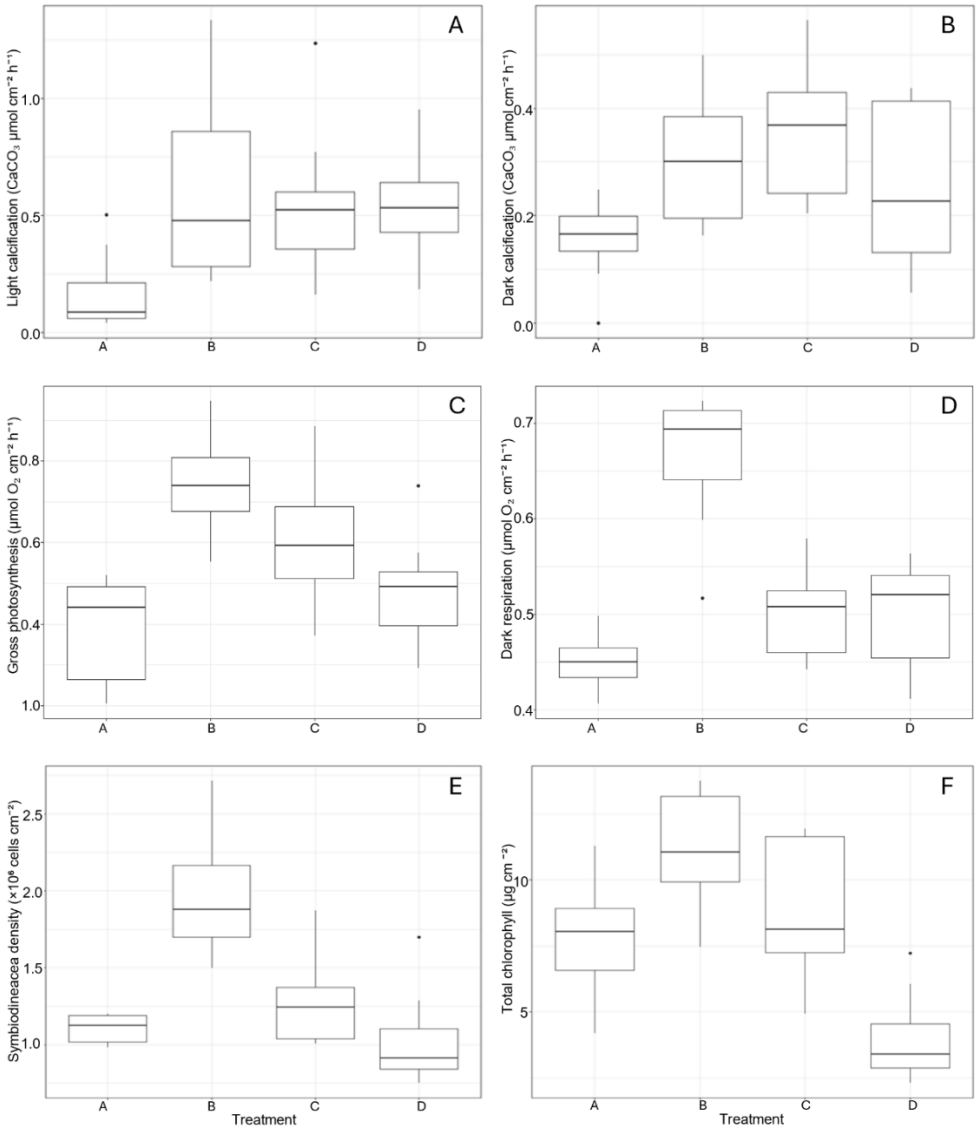

Figure 5. Physiological performance of Acropora millepora juveniles at 18 months under different heterotrophic feeding treatments **(A)** Calcification rates ($CaCO_3$ µmol cm^{-2} h^{-1}) under light conditions; **(B)** Calcification rates ($CaCO_3$ µmol cm^{-2} h^{-1}) under dark conditions; **(C)** Gross photosynthesis rates (µmol O_2 cm^{-2} h^{-1}) under light conditions; **(D)** Respiration rates (µmol O_2 cm^{-2} h^{-1}) under dark conditions; **(E)** Symbiodiniaceae densities (×10^6 cells cm^{-2}); (F) Total chlorophyll concentrations (µg cm^{-2}).

Boxplots display the median (horizontal black line) and interquartile range (white box). Treatments are coded as: A – non-fed control, B – zooplankton, C – phytoplankton, D – novel diet.

Table 3.

Pairwise comparisons of physiological parameters in *Acropora millepora* juveniles at 18 months across heterotrophic feeding treatments. Parameters assessed include calcification rates (under light and dark conditions), gross photosynthesis, respiration rates, symbiodiniaceae density, and total chlorophyll concentration. P-values were adjusted using Bonferroni correction. Statistical significance was set at $P < 0.05$.

Light Calcification	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p=0.00722	p=0.02346	p=0.02398
Zooplankton	-	-	p=0.62010	p=0.61327
Phytoplankton	-	-	-	p=0.9922
Novel diet	-	-	-	-
Dark Calcification	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p=0.02391	p=0.00343	p=0.14206
Zooplankton	-	-	p=0.4256	p=0.3876
Phytoplankton	-	-	-	p=0.10286
Novel diet	-	-	-	-
Gross Photosynthesis	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p<0.001	p=0.00567	p=0.20716
Zooplankton	-	-	p=0.06019	p=0.00103
Phytoplankton	-	-	-	p=0.09928
Novel diet	-	-	-	-
Dark Respiration	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p<0.001	p=0.0623	p=0.0843
Zooplankton	-	-	p<0.001	p<0.001
Phytoplankton	-	-	-	p=0.8804
Novel diet	-	-	-	-
Symbiodiniaceae Density	Non-fed control	Zooplankton	Phytoplankton	Novel diet

Non-fed control	-	p<0.001	p=0.227	p=0.645
Zooplankton	-	-	p<0.001	p<0.001
Phytoplankton	-	-	-	p=0.099
Novel diet	-	-	-	-
Total Chlorophyll	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p=0.00537	p=0.38099	p=0.00272
Zooplankton	-	-	p=0.04224	p<0.001
Phytoplankton	-	-	-	p<0.001
Novel diet	-	-	-	-

Influence of heterotrophic diet on thermal tolerance

The transition time (measured in degree heating weeks - DHW) to the first signs of bleaching is significantly influenced by heterotrophic diet treatment (Fig. 6A). Across the heterotrophic fed treatments, the novel diet treatment (2.46 ± 1.64 DHW) had a significantly shorter time (DHW) to first signs of bleaching compared to zooplankton (7.42 ± 2.42 DHW), phytoplankton (6.90 ± 2.77 DHW) and the non-fed control (4.78 ± 2.21 DHW) ($p < 0.001$, $p < 0.001$ & $p = 0.00985$ respectively, Table 4). The non-fed control had a significantly shorter time (DHW) to first signs of bleaching compared to zooplankton and phytoplankton treatments ($p = 0.00354$ & $p = 0.01744$, respectively, Table 4). No significant difference between zooplankton and phytoplankton ($p = 0.55388$, Table 4) was seen.

Survivorship analysis (Fig. 7) show a significantly shorter time to death in the novel diet treatment (9.36 ± 1.30) compared to non-fed control (16.11 ± 3.54), zooplankton (17.29 ± 2.64) and phytoplankton (16.33 ± 4.45) ($p = 0.00985$, $p < 0.001$, & $p < 0.001$, respectively, Table 4, Fig. 6B). Conversely, no significant difference in time to death was observed between the non-fed control compared to the zooplankton and phytoplankton treatment ($p = 0.3217$ & $p = 0.8864$ respectively, Table 4) was shown. No significant difference was seen between the zooplankton and phytoplankton treatment ($p = 0.4893$, Table 4)

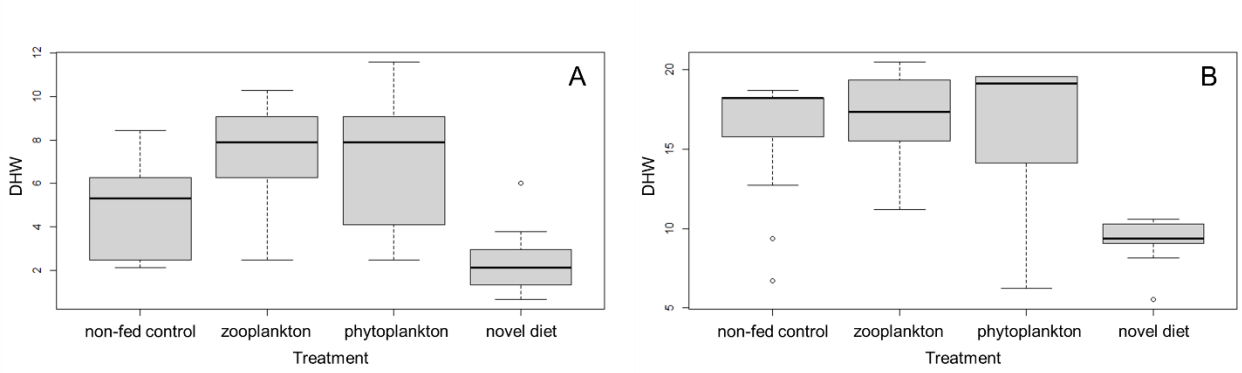

Figure 6. Transition times (degree heating weeks, DHW) for Acropora millepora juveniles under different heterotrophic diet treatments. **(A)** Time from healthy appearance to first signs of bleaching. **(B)** Time to death (health status 5) across treatments.

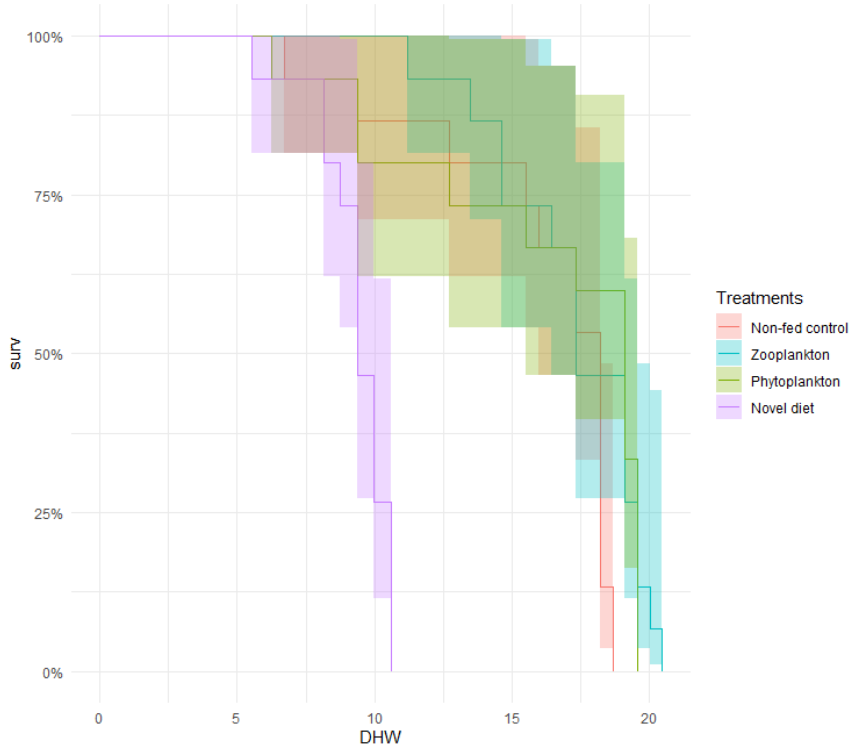

Figure 7. Kaplan–Meier survival curves of *Acropora millepora* under differing diet treatments [non-fed control (blue line), zooplankton (green line), phytoplankton (red line), novel diet (purple line)] over a 90-day heat stress experiment. SUV = percentage survival, DHW = Degree Heating Weeks).

Table 4. (A) Pairwise comparisons of transition times (degree heating weeks, DHW) from healthy appearance to first signs of bleaching between heterotrophic diet treatments (non-fed control, zooplankton, phytoplankton, novel diet), based on linear regression analysis.
(B) Pairwise comparisons of time to death (health status 5) between treatments, using generalised least squares (GLS) regression.
Adjusted $R^2 = 0.3908$ for both models. Statistical significance was set at $P < 0.05$.

(A)	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p=0.00354	p=0.01744	p=0.00985
Zooplankton	-	-	p=0.55388	p<0.001
Phytoplankton	-	-	-	p<0.001
Novel diet	-	-	-	-
Adjusted R^2 0.3908				
(B)	Non-fed control	Zooplankton	Phytoplankton	Novel diet
Non-fed control	-	p=0.3217	p=0.8864	p=0.00985
Zooplankton	-	-	p=0.4893	p<0.001
Phytoplankton	-	-	-	p<0.001
Novel diet	-	-	-	
Adjusted R2 0.3908				

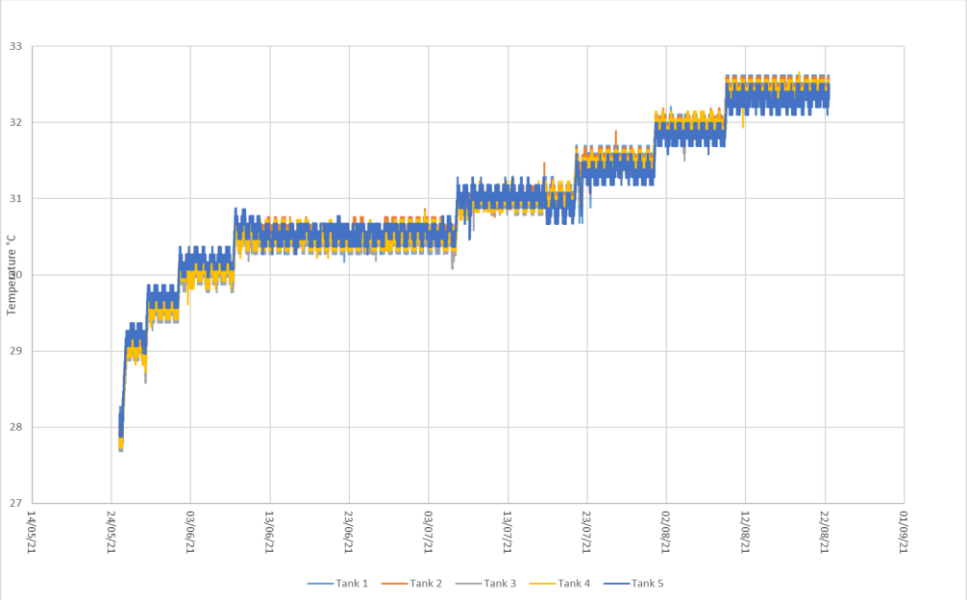

Figure 8. Temperature profile recorded for each tank (1-5).

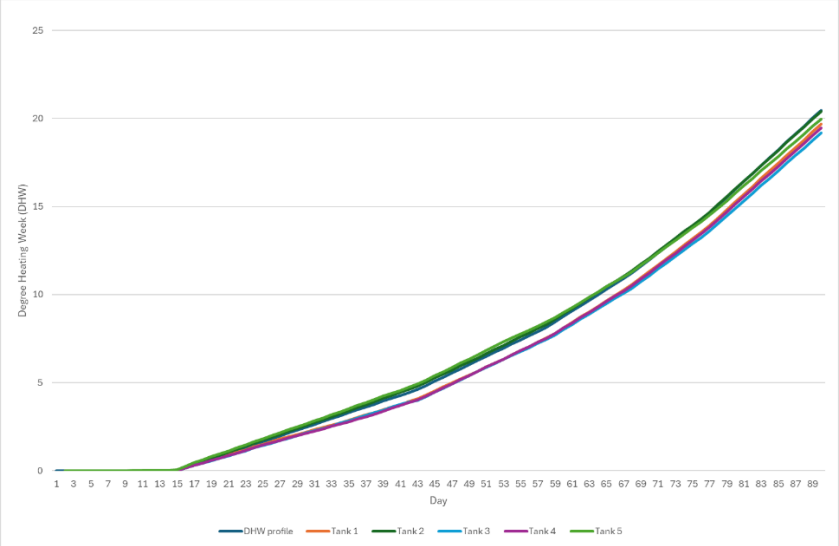

Figure 9. Temperature profile recorded in DHW for each tank (1-5) and the set profile (MMM temperature of 29.35 °C).

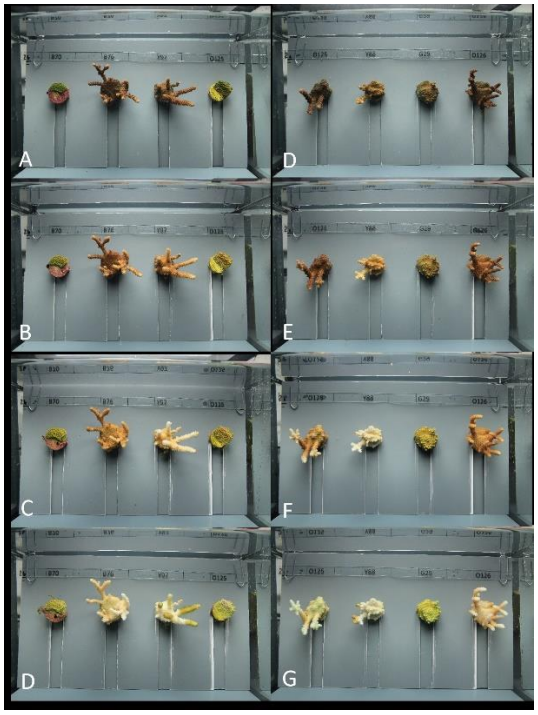

Figure 10. Standardised photographs for the comparison of colour changes (example, A,D - day 1, B,E - day 30, C,F - day 60, D,G - day 80).

Discussion

The escalating challenges posed by climate change on coral reef ecosystems underscore the need for innovative conservation and restoration strategies. Here, we examined the impact of heterotrophic feeding regimes on the survival and growth of newly settled *Acropora millepora* juveniles and the physiology and heat tolerance of 1.5-year-old *A. millepora*, highlighting the potential of nutritional interventions to enhance coral resilience.

Survival

Surprisingly, we observed a significant survival rate reduction across feed treatments compared to non-fed controls (Fig. 3). This contrasts with other work showing unfed coral juveniles having extremely poor survival rates (e.g. Petersen et al., 2008). Our observed decrease in survival in the feed treatments could result from increased competition amongst corals due to multiple coral juveniles settling on a single plug. Such close contact is known to induce allogeneic responses often leading to

mortality of both touching individuals (Rinkevich, 2004). Faster growth rates (as seen in zooplankton and phytoplankton treatments) (Fig. 4) will exacerbate such interactions. In an *ex-situ* conservation or restoration setting this suggests a necessity for a 'one-coral-per-plug' approach (Babcock and Mundy, 1996). However, allogeneic reactions may also facilitate post-settlement fusion, aiding corals in reaching a juvenile size-refuge more quickly (Petersen et al., 2008). In our study we measured survival on a per juvenile coral basis, future studies should consider recording allogeneic responses in addition to survival, to provide a more complete understanding. Another factor influencing survival results could be the high number of larvae that settled (7,218 settlers), which likely affected overall survival percentages. High settler densities can negatively impact survival, illustrating the effect of density-dependent mortality (Cameron and Harrison, 2020). Although this study did not directly quantify allogeneic responses or density-related stress mechanisms, these factors are likely contributors and should be examined in future work.

Growth rate

The initial findings indicated improved growth rates in all heterotrophic treatments compared to the non-fed controls (Fig. 4). This aligns with previous findings of enhanced growth rates resulting from heterotrophic feeding (Conlan et al., 2017a; Ferrier-Pagès et al., 2003; Grottoli et al., 2006). Heterotrophic feeding facilitates the provisioning of substantial amounts of nitrogen, phosphorus (Farrant et al., 1987; Sebens et al., 1996), and carbon (Sorokin, 1991), which are essential for coral growth. Photosynthates translocated from the zooxanthellae typically lack sufficient nitrogen, phosphorus, and amino acids (Battey & Patton, 1987; Falkowski et al., 1984; Rinkevich, 1989). Adequate nutrition during the early life stages is crucial in the highly dynamic and competitive reef environment (Colan et al., 2017a). This is particularly important in restoration projects where coral juvenile survival increases substantially with size, as larger corals possess a larger energy reservoir to invest in growth, immunity, and fitness, while also reducing vulnerability to whole colony mortality via resource sharing between polyps (Chamberland et al., 2017; Colan et al., 2017b; Raymundo and Maypa, 2014).

Significant differences between growth rates in corals under different heterotrophic feeds indicate that food composition is an important determinant of growth. Corals fed with the novel diet exhibited significantly lower growth rates compared to those receiving either zooplankton or phytoplankton, whilst there was no difference in growth between these diets, both of which yielded the highest growth rates (Fig. 4). Studies focusing on heterotrophic feeding in corals, particularly in *Acropora* species, have demonstrated highest growth and survival rates when fed with unfiltered seawater, which is rich in chlorophyll a (Conlan et al 2017a; Conlan et al., 2018), i.e. abundant phytoplankton (Ojea et al., 2004). However, using unfiltered seawater as feed presents significant implementation challenges, including infrastructure costs and increased risk of pathogen exposure. Indeed, such practice is particularly impractical for inland facilities. Further, similar to our findings, Conlan et al. (2019) demonstrated that phytoplankton feed treatments yielded the highest growth rates in *A. millepora*, surpassing even unfiltered seawater in nutritional value. *Tisochrysis* used in this study, is known for its natural abundance of DHA (Olsen et al., 1993) and richness in monounsaturated fatty acids (MUFA), highlighting its value in coral diets (Piccinetti et al., 2016). The comparable growth rates of corals fed on zooplankton and phytoplankton suggest opportunities to streamline and reduce costs in the *ex-situ* rearing of corals for restoration and conservation purposes, potentially eliminating the need to culture zooplankton.

Respiration / photosynthesis

In addition to survival and growth rates, the physiological benefits of feeding were investigated. Notably, corals fed with zooplankton showed the highest metabolic rates and symbiodiniaceae density, corresponding with significantly enhanced photosynthesis and respiration rates compared to the novel diet treatment and non-fed control (Fig. 5C & 5D). No difference was seen in net photosynthesis between the zooplankton and phytoplankton treatment and both diets had higher net photosynthesis rates compared to the non-fed control (Fig. 5C & 5D). These results are consistent with previous studies that have shown nutrient intake increases symbiodiniaceae densities, which in turn boosts coral photosynthetic capacity and energy uptake (Houlbrèque et al., 2003; Muscatine et al., 1989). Photoautotrophy in

corals has been well documented (Muscatine et al., 1981), highlighting that corals derive substantial amounts of photosynthetically fixed carbon from their symbiotic algae through translocation (Muscatine and Porter, 1977). It is suggested that while most of these photosynthetic products are utilized for respiration, only a small portion contributes to growth and reproduction (Falkowski et al., 1984). Therefore, the results showing significant increases in growth in the zooplankton feed, suggests that even if only a small portion of the benefits of increased photosynthesis go to growth and reproduction it is still enough to markedly increase growth. Additionally, the extra products generated could act as a buffer to mitigate the impacts of stress by compensating for the redirection of nutrients during stressful conditions.

Chlorophyll and Symbiodiniaceae counts

Zooplankton and phytoplankton-fed corals also showed significantly higher total chlorophyll concentrations than those fed the novel diet (Fig. 5F). The treatment fed on zooplankton showed significantly higher symbiodiniaceae densities and total chlorophyll concentrations to all other treatment groups (Fig. 5E & 5F), confirming a robust physiological state conducive to growth (Houlbrèque et al., 2003). This corresponds with the increased growth rates in these treatments (Fig. 4) and supports previous studies indicating that nutrient-rich diets increase chlorophyll levels and, subsequently, the photosynthetic capacity of corals compared to starved corals (Houlbrèque et al., 2004; Ferrier-Pagès et al., 1998). Muscatine et al. (1998) found that most corals are characterized by a predominance of host cells containing a single dinoflagellate, with continuously decreasing frequencies with those containing two or more symbionts. Houlbre`que et al (2004) demonstrated that in fed corals, the number of doublets and triplets significantly increased compared to starved corals. This indicates that feeding disproportionately enhances the number of algal components relative to animal cells. Additionally, concentrations of chlorophyll a and c2 are typically elevated in fed versus starved corals, due to either an increase in zooxanthellae density or chlorophyll content per algal cell (Dubinsky et al., 1990; Ferrier-Pagès et al., 1998; Houlbrèque et al., 2003). A simultaneous increase in the concentration of protein, lipids and chlorophyll in fed coral colonies suggests that nutrients, incorporated in particulate form are utilized by both the host

and the symbionts (Treignier et al., 2008). When feeding rates are elevated, enhanced rates of photosynthesis per unit surface area can occur, stemming from concurrent increases in symbiodiniaceae density and chlorophyll per symbiodiniaceae (Dubinsky et al., 1990). For example, Houlbrèque et al. (2003) reported that colonies of *Stylophora pistillata* fed with freshly collected zooplankton and subjected to low light levels (<200 µmoles photons m^2 s^{-1}) experienced a doubling in symbiodiniaceae concentration compared to starved corals. However, in this experiment no significant increase in symbiodiniaceae or total chlorophyll densities was observed in the treatment fed on phytoplankton compared to the non-fed control (Fig. 5E & 5F), contradicting previous findings of increased photosynthesis rates in the phytoplankton treatment. The novel diet treatment exhibited significantly lower total chlorophyll densities compared to the non-fed control and showed no difference in symbiodiniaceae densities, which is consistent with the absence of differences in photosynthesis and respiration rates relative to the non-fed control. The reasons for why these potential explanations occurred in this study and not in the previous studies discussed above is not clear.

Calcification

Increased calcification rates were observed in fed treatments against the non-fed controls in light conditions (Fig. 5A). This is aligned with the findings of Ferrier-Pagès et al. (2003), who found calcification rates to be 30% greater in fed (natural zooplankton) compared to starved corals. Indeed, Ferrier-Pagès et al. (2003) found that even moderate levels of feeding enhanced both tissue and skeletal growth. The major source of dissolved inorganic carbon for calcification is metabolic CO_2 (70% of total $CaCO_3$ deposition), whereas only 30% originates from the external seawater inorganic carbon [mostly bicarbonate (Erez, 1978; Furla et al., 2000)]. Heterotrophy may therefore provide additional CO_2 through increased metabolic rate of the coral tissue enhancing calcification (Houlbrèque, 2012). Calcification results from the delivery of both calcium and inorganic carbon to the site of calcification and the removal of protons. The calcium is acquired from seawater and delivered to the site of calcification through the calicoblastic epithelium via transcellular active transport (Marshall and Clode, 2002). Ferrier-Pagès et al. (2003), and Houlbrèque et al.

(2003) both found that feeding resulted in increased tissue growth and tissue thickening, with a proportional increase in the symbiodiniaceae density per unit surface area. Therefore, the addition of coral biomass could promote calcification rates by increasing the supply of external DIC. The increased DIC could occur via an increased number of transporting molecules or via an increase in respiration rate. Indeed, respiration rates are often higher in fed corals (Houlbrèque et al., 2003). Therefore, heterotrophy could stimulate calcification through tissue growth and the supply of metabolic inorganic carbon (Furla et al., 2000). Additionally, Allemand et al. (2004) suggested that heterotrophy was a source of aspartic acid, one of the key components of the coral matrix.

Heterotrophy can also increase tissue synthesis levels (Anthony and Fabricius, 2000; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003), increases in protein concentration with feeding have been reported for tropical corals (Al-Moghrabi et al., 1995). Such increases in protein concentration resulted in thicker tissue over each calyx (Lough and Barnes, 2000) and thus more biomass per polyp. Based on the findings of previous studies and the observation of increased respiration in corals fed zooplankton in this study, there is a strong alignment with the results indicating significantly higher calcification rates in corals nourished with zooplankton. However, the findings of Anthony et al. (2002) found that coral tissue can respond more rapidly to resource availability than the skeleton, or that the tissue's energy content might significantly contribute to coral growth.

Energy

Corals fed on zooplankton showed the highest growth and metabolic rates. Energy flow is crucial for physiological performance, influencing maintenance, growth, and reproduction, ultimately affecting survival and fitness (Maltby, 1999). Energy status in corals depends on energy intake, losses, and allocation among maintenance, growth, and reproduction (Anthony and Fabricius 2000; Ferrier-Pages et al., 1998; Grottoli et al. 2006). Feeding with zooplankton would have facilitated increased energy intake, in turn allowing greater allocation to growth and metabolism. Given that the corals in this study were juveniles then increased energy intake is likely to have been primarily allocated to growth rather than maintenance or reproduction. A

question for future research is how the balance of allocation of energy between maintenance, growth and reproduction change as corals age.

Heat Tolerance

All treatment groups exposed to the heat stress experiment experienced bleaching with increasing Degree Heating Weeks (DHW) (Fig. 6A). Stressors such as elevated sea surface temperatures can disrupt the coral-algae symbiosis, triggering the expulsion of algae and subsequent coral bleaching (Baker et al., 2018; Morris et al., 2019; Weis, 2008). Elevated temperatures disturb the photosynthetic pathways in symbiodiniaceae, producing toxic reactive oxygen species (Szabó et al., 2020; Weis, 2008) and reducing photosynthetic efficiency, thereby impairing energy transfer to the host resulting in a negative energy balance (Baker et al., 2018; Rädecker et al., 2021; Weis, 2008).

Corals fed on the zooplankton and phytoplankton treatment showed a significantly longer time to first bleaching compared to the non-fed control (Fig. 6A) which aligns with the physiological results and previous studies (Hughes & Grottoli, 2013; Rodrigues & Grottoli, 2007). Interestingly, corals on the novel diet exhibited significantly lower thermal tolerance, as they exhibited bleaching, and mortality at lower DHW levels compared all other treatments (Fig. 6A & 6B). This supports the theory that variation in diet can influence thermal resilience, potentially mitigating bleaching impacts in *ex-situ* settings (Hughes and Grottoli, 2013; Rodrigues and Grottoli, 2007). It is well known that timing of coral mass mortality correlates with bleaching severity, the duration of the bleaching event, heterotrophic activity, and pre-bleaching energy reserves (e.g., lipid stores). Therefore, the novel diet may have offered smaller energy reserves leading to lower overall survival rates in this treatment (Anthony et al., 2009; Seemann et al., 2012).

Surprisingly, there was no significant difference in the timing of mortality under thermal stress between unfed corals and those fed with zooplankton or phytoplankton (Fig. 6B).
This was despite the zooplankton-fed group showing increased metabolic rates and symbiodiniaceae and chlorophyll concentrations when fed with zooplankton or phytoplankton (Fig. 5). This outcome is intriguing, given that physiological data

suggested more substantial energy reserves in the zooplankton-fed treatments i.e. in theory these corals should have been more resistant to stress. A critical factor in assessing the impact of stressors across different resource environments is the depletion of lipid reserves in corals. Storage lipids are known to enhance heat stress resistance and improve survivorship during bleaching events (Anthony et al., 2009; Seemann et al., 2012). In corals, energy acquisition is critical and determined by photosynthetic carbon fixation, respiration, carbon transfer by symbionts, and heterotrophy by the host (Anthony and Fabricius 2000; Borell and Bischof, 2008; Ferrier-Pages et al., 1998; Grottoli et al., 2006; Sebens et al., 1996). The balance of energy availability and reserves influences coral survival during periods of resource scarcity, such as bleaching events where energy acquisition is compromised due to reduced algal symbiont and photopigment concentrations. The severity of bleaching depends on the stress duration, the symbiosis energy efficiency, availability of heterotrophic resources, and the coral species' heterotrophic feeding capacity (Grottoli et al., 2006; Rodrigues and Grottoli 2007). Host investment in digestive infrastructure, such as increasing the gastrovascular cavity's folding to enhance nutrient absorption (Stojanović et al., 2021), represents a significant energy allocation. Maintaining these structures, rich in polyunsaturated fatty acids, demands considerable energy, which impacts the buildup of lipid reserves (Burian et al., 2018). All of this makes it difficult to understand why there was no difference in the timing mortality under thermal stress between unfed corals and those fed with zooplankton or phytoplankton. However, one hypothesis to try and explain this observation is that this study focused on juveniles. It is highly likely, that at this life stage, resources from feeding were allocated primarily to growth and that the mechanisms outlined above were not instigated or prioritised.

Further, the Kaplan-Meier survival curves (Fig. 7) indicated higher survival rates in zooplankton and phytoplankton treatments, although again these were not significantly different to the unfed treatment. Despite this lack of significance, the observation offers another potential explanation of the lack of differences between the timing of mortality under thermal stress between unfed corals and those fed with zooplankton or phytoplankton The heterotrophically fed treatments might be maintaining an enhanced digestive infrastructure, thereby consuming energy reserves more rapidly compared to the non-fed treatments in stress conditions

without heterotrophic feeding. This expenditure could lead to an energy deficit, resulting in similar mortality rates at comparable DHW levels. Indeed, coral bleaching in adults is known to lead to a decrease in tissue biomass and is heavily influenced by the dynamics of lipid stores, which can make up 20–30% of the tissue biomass and serve as crucial energy reserves during stress (Porter et al., 1989). The survival of a bleached adult coral largely depends on how depleted these energy stores become and how quickly they can be replenished (Grottoli et al., 2006; Rodrigues & Grottoli 2007). In environments with harsh conditions, the physiological state of the organisms significantly predicts their survival likelihood (Gurney et al., 2013; Maltby, 1999). Feeding is generally crucial for growth and survival, with certain nuances as previously discussed. However, regarding temperature stress, all juvenile corals exhibit equal vulnerability regardless of their feeding status. The limited tissue across the entire colony during the juvenile stage leaves no room for energy reserves, which is a stark contrast to adult corals. This lack of reserves in juveniles could explain the absence of significant differences in time to mortality and why the physiological patterns observed in other studies do not apply here.

Methodology

The feeding process was indirect in that food was not delivered directly to the corals, as would be the case in the real world. However, this may have led to differences in uptake of the different feeds that are related to dispersal in the tanks. Live zooplankton and phytoplankton would remain suspended in the water column during the two-hour isolation period, whereas the novel diet's particulate nature may have accumulated in crevices or as sediment on coral tissues. This means that there would have been differences between the phytoplankton/zooplankton treatments and the novel diet in the accessibility of the feed to the corals. Additionally, if the particulates of the novel feed accumulated on the coral surfaces this may have been stress inducing, leading to growth inhibition. These effects would be more likely in the later experimental stages when increased coral growth creates low-flow areas conducive to sediment deposition (Rogers, 1990). It would be expected that such effects would be less pronounced if working with much larger adult coral colonies and/or bigger systems (volume/area) compared to the coral juveniles and smaller

tanks used in this study. This is because the surface area to volume would be significantly reduced in adult colonies, and larger tanks would have stronger and more dynamic flow systems. Further, although we attempted to keep flow rates constant throughout the experiment, individual coral juveniles would experience variation in micro-scale changes in flow patterns. Flow speeds can significantly impact on food capture efficiency, with different flow rates favouring capture of different food particle sizes (Orejas et al., 2016). Thus, future work could monitor flow dynamics, particularly at the micro-scale, as this aspect may well be important component in increase survival and growth rates in conservation practices. Potentially, any decrease in food availability due to circulation effects could be offset by increasing the density of food. Ferrier-Pagès et al. (2003) demonstrated that the number of preys ingested was proportional to prey density, and they found no saturation in feeding capability. However, any positive effects on feeding attained through increasing feed density would need to be balanced against the potential negative impact of build up of food particles on coral surfaces inducing stress.

While this study focused on the effects of heterotrophic feeding, it is important to note that water quality during the daily two-hour isolation periods may have influenced the results. Despite stable baseline parameters maintained through the Triton Method, using ICP-OES testing, targeted supplementation, and 5-micron mechanical filtration—temporary nutrient shifts during feeding may have occurred. During isolation, limited water exchange and organic inputs from feed could have led to short-term increases in dissolved or particulate organic matter. These changes may have affected coral physiology independently of direct feeding effects, particularly through altered respiration, symbiont performance, or microbial interactions. Such variability could differ between treatments depending on feed type, coral density, or flow conditions. Therefore, the observed improvements in growth, physiology, or stress tolerance may not be solely due to heterotrophy, but also influenced by shifts in water chemistry during feeding. While organic nutrients (e.g. from zooplankton or phytoplankton) are generally beneficial, distinguishing between the effects of ingestion and ambient enrichment remains a challenge (D’Angelo and Wiedenmann, 2014; Fox et al., 2021; Grottoli et al., 2021). Future studies should monitor short-term nutrient dynamics during feeding to better isolate heterotrophic effects from environmental variables. This reinforces the need to

consider water quality interactions when evaluating coral diet and performance in ex situ systems.

Conclusion

This study demonstrated that heterotrophic feeding significantly influenced the growth, physiology, and resilience of newly settled Acropora millepora juveniles. Although survival rates were lower in the fed treatments compared to the non-fed control, likely due to methodological factors such as settler density and allogeneic interactions, heterotrophic feeding markedly enhanced coral growth rates, calcification, photosynthesis, respiration, and symbiodiniaceae densities.
Corals fed with zooplankton and phytoplankton achieved significantly greater growth and improved physiological performance compared to those fed a novel diet or unfed controls, supporting the hypothesis that enhanced nutrition benefits early coral development. These treatments also delayed the onset of bleaching under thermal stress, suggesting that heterotrophic feeding can temporarily buffer corals against bleaching events. However, feeding did not extend survival under severe thermal stress, indicating that while heterotrophy enhances resilience during early bleaching stages, it does not prevent mortality once critical stress thresholds are reached.
These findings address the stated aims by showing that dietary manipulation improves early-stage coral fitness and influences thermal stress responses. However, the lack of difference in final survival rates under heat stress between fed and unfed juveniles suggests that at this life stage, energetic investments prioritise growth over reserve storage, limiting the benefits of feeding for prolonged thermal resilience. Further research is required to assess how feeding requirements shift as corals age, the long-term effects of diet on resilience, and the broader implications for reef restoration. Importantly, this work highlights the need for an integrated understanding of how heterotrophic nutrition affects not only the coral host, but also its symbiotic relationships.
Overall, the findings advocate for strategic nutritional interventions in ex-situ coral restoration protocols. Refining heterotrophic ex-situ feeding practices based physiological needs could significantly enhance coral fitness (Pandolfi et al., 2011), supporting conservation efforts in the face of escalating climate change (Sully et al., 2022).

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