Evaluation of Octocoral Responses to Global and Local Factors: Ocean Acidification, Warming and Organic Eutrophication

Susana M. Simancas Giraldo

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Susana Marcela Simancas Giraldo

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Supervisor:	Prof. Dr. Christian Wild Marine Ecology Department University of Bremen, Bremen, Germany

Examination committee:

Committee Chair:	Prof. Dr. Marko Rohlfs Population and Evolutionary Ecology University of Bremen, Bremen, Germany
1st Examiner:	Prof. Dr. Claudio Richter Bentho-Pelagic Processes, Alfred Wegener Institute, AWI Bremerhaven, Germany University of Bremen, Bremen, Germany
2nd Examiner:	Dr. Covadonga Orejas Saco del Valle Instituto Español de Oceanografía Gijón, Spain
3rd Examiner:	Ian Enochs, Ph.D. AOML Coral Program, NOAA/AOML University of Miami, RSMAS Miami, Florida, USA
1st student member:	Thomas Heran PhD Candidate, Alfred Wegener Institute, AWI Bremerhaven, Germany University of Bremen, Bremen, Germany
2nd student member:	Marie Elisabeth Kaufman PhD student, Alfred Wegener Institute, AWI Bremerhaven, Germany University of Bremen, Bremen, Germany

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A mis padres, a mi hermano y a Chrissi.
A mi familia en Colombia y a mi familia Alemana.
A mis queridos amigos.

A quienes aún caminan conmigo y
en memoria de quienes se han ido y esperan del otro lado del mar…

"Nadie habrá dejado de observar que con frecuencia el suelo se pliega de manera tal que una parte sube en ángulo recto con el plano del suelo, y luego la parte siguiente se coloca paralela a este plano, para dar paso a una nueva perpendicular, conducta que se repite en espiral o en línea quebrada hasta alturas sumamente variables. Agachándose y poniendo la mano izquierda en una de las partes verticales, y la derecha en la horizontal correspondiente, se está en posesión momentánea de un peldaño o escalón. Cada uno de estos peldaños, formados como se ve por dos elementos, se sitúa un tanto más arriba y adelante que el anterior, principio que da sentido a la escalera, ya que cualquiera otra combinación producirá formas quizá más bellas o pintorescas, pero incapaces de trasladar de una planta baja a un primer piso.

Las escaleras se suben de frente, pues hacia atrás o de costado resultan particularmente incómodas. La actitud natural consiste en mantenerse de pie, los brazos colgando sin esfuerzo, la cabeza erguida, aunque no tanto que los ojos dejen de ver los peldaños inmediatamente superiores al que se pisa, y respirando lenta y regularmente. Para subir una escalera se comienza por levantar esa parte del cuerpo situada a la derecha abajo, envuelta casi siempre en cuero o gamuza, y que salvo excepciones cabe exactamente en el escalón. Puesta en el primer peldaño dicha parte, que para abreviar llamaremos pie, se recoge la parte equivalente de la izquierda (también llamada pie, pero que no ha de confundirse con el pie antes citado), y llevándola a la altura del pie, se le hace seguir hasta colocarla en el segundo peldaño, con lo cual en éste descansará el pie, y en el primero descansará el pie. (Los primeros peldaños son siempre los más difíciles, hasta adquirir la coordinación necesaria. La coincidencia de nombre entre el pie y el pie hace difícil la explicación. Cuídese especialmente de no levantar al mismo tiempo el pie y el pie).

Llegado en esta forma al segundo peldaño, basta repetir alternadamente los movimientos hasta encontrarse con el final de la escalera. Se sale de ella fácilmente, con un ligero golpe de talón que la fija en su sitio, del que no se moverá hasta el momento del descenso."

- Instrucciones para subir una escalera -
Julio Cortázar - 1962

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Summary

Climate change, a global factor with ocean acidification and warming as its main consequences, threatens coral reefs worldwide. Concomitant local factors such as organic eutrophication occur simultaneously, but knowledge about the interaction between global and local factors is scarce. In addition, octocorals, including soft corals and gorgoniids, are highly under-investigated, although they are an essential functional group in reef ecosystems. **This thesis aimed to** evaluate the ecophysiological responses of octocorals to ocean acidification, warming and organic eutrophication simulated as dissolved organic carbon (DOC). We addressed the following **research questions: 1)** How does the physiology of different octocoral genera respond to simulated ocean acidification? **2)** How does DOC, warming, or their interaction affect the physiology of octocorals? **3)** What are the physiological responses of soft corals to prolonged warming? The approach comprised a series of aquaria experiments on octocorals spanning from 40 to 120 days. We assessed diverse metrics, including mortality, bleaching, pulsation, growth, oxygen (O_2), carbon (C) and nitrogen (N) metabolism, and N-cycling bacterial community dynamics. Findings revealed that octocorals in all treatments exhibited 100 % survival and maintained growth, no bleaching and positive photosynthesis except for *Xenia umbellata* under prolonged warming and *Pinnigorgia flava* under warming and DOC addition. In particular, *X. umbellata* showed high tolerance to local and global factors and increased N-fixation, supporting the holobiont functioning under stress. Our findings suggest that ocean acidification, warming and organic eutrophication will have lesser impacts on soft corals than most hard corals as, according to the literature, and supports soft corals as winners under future scenarios, although they are not entirely resistant to the factors assessed. This thesis contributed insights into soft corals' ecophysiological traits and mechanisms driving their tolerance, which may be key to understanding potential shifts of coral species dominance in current and future reefs. Our findings can contribute to enhancing the incorporation of soft corals within the scope of priorities for mitigating global and local factors and for devising effective reef management and improved conservation strategies.

Zusammenfassung

Der Klimawandel, ein globaler Faktor, dessen Hauptfolgen die Versauerung und Erwärmung der Ozeane ist, bedroht die Korallenriffe weltweit. Gleichzeitig mit dem Klimawandel treten lokale Faktoren wie die organische Eutrophierung auf, doch ist das Wissen über die Wechselwirkungen zwischen globalen und lokalen Faktoren noch gering. Darüber hinaus sind Oktokorallen, einschließlich Weichkorallen und Gorgoniden, noch viel zu wenig erforscht, obwohl sie eine wichtige funktionelle Gruppe in Riffökosystemen darstellen. **Ziel dieser Dissertation war es,** die ökophysiologischen Reaktionen von Oktokorallen auf die Ozeanversauerung, die Erwärmung und die organische Eutrophierung, in Form von gelöstem organischem Kohlenstoff (DOC), zu untersuchen. Aus diesem Grund haben wir uns mit den folgenden **Forschungsfragen beschäftigt: 1)** Wie reagiert die Physiologie verschiedener Gattungen der Oktokorallen auf die simulierte Versauerung der Ozeane? **2)** Wie wirken sich DOC, Erwärmung oder ihre Wechselwirkung auf die Physiologie von Oktokorallen aus? **3)** Was sind die physiologischen Reaktionen von Weichkorallen auf eine anhaltende Erwärmung? Der methodische Ansatz dieser Arbeit umfasste eine Reihe von mit Oktokorallen über einen Zeitraum von 40 bis 120 Tagen in Aquarien durchgeführten Experimenten. Wir bewerteten verschiedene Messgrößen, darunter Sterblichkeit, Bleiche, Pulsation, Wachstum, Sauerstoff- (O_2), Kohlenstoff- (C) und Stickstoff- (N) Stoffwechsel sowie die Dynamik der N-zyklischen bakteriellen Gemeinschaft. Die Ergebnisse zeigten, dass Oktokorallen in allen Behandlungen zu 100 % überlebten, ihr Wachstum beibehielten, nicht ausbleichten und eine positive Photosynthese aufwiesen, mit Ausnahme von *Xenia umbellata* bei längerer Erwärmung und *Pinnigorgia flava* bei Erwärmung und DOC-Zugabe. Insbesondere *X. umbellata* zeigte eine hohe Toleranz gegenüber lokalen und globalen Faktoren sowie erhöhte N-Fixierung, was die Funktion des Holobiont unter Stress unterstützt. Unsere Ergebnisse deuten darauf hin, dass Ozeanversauerung, Erwärmung und organische Eutrophierung geringere Auswirkungen auf Weichkorallen als auf die meisten Steinkorallen haben werden. Dies deckt sich mit dem aktuellen Forschungsstand in der Literatur. Sie sprechen dafür, dass Weichkorallen in zukünftigen Szenarien zu den Gewinnern gehören werden, auch wenn sie gegen die untersuchten

Faktoren nicht völlig resistent sind. Diese Arbeit hat Einblicke in die ökophysiologischen Eigenschaften von Weichkorallen und die Mechanismen, die für ihre Toleranz verantwortlich sind, geliefert. Dies kann für das Verständnis möglicher Verschiebungen der Dominanz in heutigen und zukünftigen Riffen entscheidend sein. Unsere Ergebnisse können dazu beitragen, Weichkorallen in die Prioritätenliste für die Abschwächung globaler und lokaler Faktoren einzubeziehen und ein effektives Riffmanagement sowie verbesserte Schutzstrategien zu entwickeln.

Resumen

El cambio climático, un factor global cuyas principales consecuencias son la acidificación y el calentamiento global, amenaza los arrecifes coralinos de todo el mundo. Adicionalmente, factores locales concomitantes como la eutrofización orgánica pueden ocurrir simultáneamente, pero el conocimiento actual sobre estos factores globales y locales y sus interacciones es escaso. Por otra parte, los octocorales, incluyendo corales blandos y gorgónidos, permanecen poco investigados, aunque son un grupo funcional esencial en los ecosistemas arrecifales. **El objetivo de esta tesis fue** evaluar las respuestas ecofisiológicas de los octocorales a la acidificación, calentamiento y a la eutrofización orgánica simulada como carbono orgánico disuelto (DOC por sus siglas en ingles). Se abordaron las siguientes **preguntas de investigación**: **1)** ¿Cómo responde la fisiología de los diferentes géneros de octocorales a la acidificación oceánica simulada? **2)** ¿Cómo afectan el DOC, el calentamiento o la interacción de ambos factores la fisiología de los octocorales? **3)** ¿Cuáles son las respuestas fisiológicas de los corales blandos frente al calentamiento prolongado? El enfoque metodológico consistió en la implementación de una serie de experimentos en acuarios y en octocorales, con duraciones entre 40 y 120 días. Se evaluaron diversos parámetros, incluyendo incidencia de mortalidad y blanqueamiento, rata de pulsación en pólipos, crecimiento, metabolismo del oxígeno (O_2), carbono (C) y del nitrógeno (N), y la dinámica de las comunidades bacterianas involucradas en el ciclo de N. Los resultados obtenidos mostraron que los octocorales en todos los tratamientos exhibieron 100 % de supervivencia y crecimiento mantenido, sin blanqueamiento y fotosíntesis positiva, excepto para *Xenia umbellata* bajo calentamiento prolongado y *Pinnigorgia flava* bajo calentamiento y adición de DOC. En particular, *X. umbellata* mostró una alta tolerancia frente a todos los factores locales y globales evaluados, y una mayor fijación de N, lo que respaldando el funcionamiento del holobionte bajo estrés. Los hallazgos de esta tesis sugieren que la acidificación oceánica, el calentamiento y la eutrofización orgánica tendrán un menor impacto en los corales blandos que en la mayoría de los corales duros, al comparar con la literatura en los últimos, y respalda a los corales blandos como futuros ganadores en escenarios futuros, aunque estos no son totalmente resistentes a

los factores evaluados. Esta tesis aporta información sobre los rasgos ecofisiológicos y los mecanismos de los corales blandos que determinan su tolerancia, lo que es clave para comprender los posibles cambios en la dominancia de las especies de coral en los arrecifes actuales y futuros. Nuestros hallazgos también pueden contribuir a mejorar la incorporación de los corales blandos en el ámbito de las prioridades para mitigar los factores globales y locales y para concebir una gestión eficaz de los arrecifes y mejores estrategias de conservación.

List of publications and manuscripts

Publications and manuscripts included as chapters within this thesis:

First author contributions:

Simancas-Giraldo SM, Martins P.P. C, Wild C, Ziegler M. Effects of ocean acidification on four different genera of tropical Octocorals. *In Prep.*

Simancas-Giraldo SM, Xiang N, Kennedy MM, Nafeh R, Zelli E, Wild C. 2021. Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic carbon eutrophication. PeerJ. DOI: 10.7717/peerj.11663

Zelli E*, **Simancas-Giraldo SM***, Xiang N, Dessì C, Katzer N, Wild C. 2023. Individual and combined effect of organic eutrophication (DOC) and ocean warming on the ecophysiology of the gorgonian *Pinnigorgia flava*. PeerJ. DOI: 10.7717/peerj.14812
* Shared first authorship.

Simancas-Giraldo SM, Storkenmaier M, Vasseur F, Ferse S, Wild, C. The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming. *In prep.*

Coauthor contributions:

Vollstedt S, Xiang N, **Simancas-Giraldo SM**, Wild C. 2020. Organic eutrophication increases resistance of the pulsating soft coral *Xenia umbellata* to warming. PeerJ. DOI: 10.7717/peerj.9182

Xiang, N., Hassenrück, C., Pogoreutz, C., Rädecker, N., **Simancas-Giraldo, SM**, Voolstra, C. R., Wild, C., & Gärdes, A. 2022. Contrasting microbiome dynamics of putative denitrifying bacteria in two octocoral species exposed to dissolved organic carbon (DOC) and warming. Applied and Environmental Microbiology. DOI: 10.1128/aem.01886-21

Other publications during the candidature - not included as thesis chapters:

P.P. Martins C, **Simancas-Giraldo SM**, Schubert P, Wall M, Wild C, Wilke T, Ziegler M. 2024. Short periods of decreased water flow may modulate long-term ocean acidification in reef-building corals. PCI Ecology. DOI: 10.1101/2024.02.23.581783

Declaration of author contribution

The detailed contributions of the PhD candidate to the multi-author journal articles and manuscripts included as chapters in the submitted doctoral thesis are presented here. The contributions are given in percentages (%) of the total work and sum up to 100 % for each category.

CHAPTER 2 | Effects of ocean acidification on four different genera of tropical Octocorals

Simancas-Giraldo SM, Martins P.P. C, Wild C, Ziegler M.

Experimental concept and design	80 %
Experimental work and/or data acquisition	80 %
Data analysis and interpretation	90 %
Preparation of figures and tables	100 %
Drafting of the manuscript	100 %

CHAPTER 3 | Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic carbon eutrophication

Simancas-Giraldo SM, Xiang N, Kennedy MM, Nafeh R, Zelli E, Wild C.

Experimental concept and design	20 %
Experimental work and/or data acquisition	80 %
Data analysis and interpretation	80 %
Preparation of figures and tables	100 %
Drafting of the manuscript	90 %

CHAPTER 4 | Organic eutrophication increases resistance of the pulsating soft coral *Xenia umbellata* to warming

Vollstedt S, Xiang N, **Simancas-Giraldo SM**, Wild C.

Experimental concept and design	10 %

Experimental work and/or data acquisition	40 %
Data analysis and interpretation	30 %
Preparation of figures and tables	20 %
Drafting of the manuscript	0 %

CHAPTER 5 | Individual and combined effect of organic eutrophication (DOC) and ocean warming on the ecophysiology of the gorgonian *Pinnigorgia flava*

Zelli E*, **Simancas-Giraldo SM***, Xiang N, Dessì C, Katzer N, Wild C.

*** Shared first authorship.**

Experimental concept and design	20 %
Experimental work and/or data acquisition	60 %
Data analysis and interpretation	50 %
Preparation of figures and tables	70 %
Drafting of the manuscript	30 %

CHAPTER 6 | Contrasting microbiome dynamics of putative denitrifying bacteria in two octocoral species exposed to dissolved organic carbon (DOC) and warming

Xiang, N, Hassenrück, C, Pogoreutz, C., Rädecker, N, **Simancas-Giraldo SM,** Voolstra, C. R., Wild, C., & Gärdes, A.

Experimental concept and design	10 %
Experimental work and/or data acquisition	30 %
Data analysis and interpretation	10 %
Preparation of figures and tables	0 %
Drafting of the manuscript	10 %

CHAPTER 7 | The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming

Simancas-Giraldo SM, Storkenmaier M, Vasseur F, Ferse S, Wild, C.

Experimental concept and design	20 %
Experimental work and/or data acquisition	60 %
Data analysis and interpretation	80 %
Preparation of figures and tables	90 %
Drafting of the manuscript	100 %

Date: 29.04.2024

Signature:

Chapter 1

General Introduction

1.1 Climate change: the greatest challenge

Climate change as a byproduct of anthropogenic activities and human development is the primary concern of our century and the major threat to reef ecosystems worldwide (IPCC, 2021). Since the beginning of the industrial era, human development has dramatically increased the atmospheric concentrations of greenhouse gases, leading to rapid increases of such gases in the earth's atmosphere and the oceans (Riebesell et al., 2011). In particular, the current increase rate of carbon dioxide (CO_2) is two to three orders of magnitude faster than previously observed shifts, e.g., during the past glacial and interglacial periods (Zachos et al., 2005; Hönisch et al., 2012). Consequently, these changes have driven substantial shifts in the physicochemical characteristics of the ocean environment, representing a paradigm for the earth's climate geological history and jeopardising many coral reef ecosystems (IPCC, 2021).

By recognising the current situation and its intricacy, many studies have provided evidence of the ecological responses of coral reefs to recent climate change (as reviewed in Parmesan 2006). These effects vary from a higher incidence of mass coral bleaching, mortality, and reef biodiversity decline (Hughes et al., 2017; Eddy et al., 2021) to a wide range of subtle yet fundamentally important changes in physiological and ecological processes within these ecosystems (Hoegh-Guldberg, 2011). Beyond the consensus that severe coral mortality can result in the collapse of the coral reef ecosystems, it has also been acknowledged that shifts to alternative states in which reef-building corals are not the primary dominant benthic organisms are also currently taking place across many regions worldwide (Moritz et al., 2018; Hughes et al., 2018).

In detail, current projections suggest that coral reefs are losing up to 70–90 % of their coral species, with little evidence of reef-building corals and other benthic organisms being able to adapt to the incoming changes (Hoegh-Guldberg, Jacob & Taylor, 2018). With reefs undergoing severe declines, an estimated 30 % are already severely damaged, and up to 60 % may be lost by 2030 (Wilkinson, 2002; Hughes et al., 2003). These expected coral losses have also been forecasted to be inevitable (Hoegh-Guldberg, 1999; Knowlton, 2001) and, suppose dire scenarios, heavily impacting marine

life dependent on them (Hughes et al., 2018; Gao et al., 2020; IPCC, 2021). Moreover, shifting patterns and increasing tempos of coral mortality have created widespread agreement that substantial global losses of coral reefs have already occurred (Knowlton, 2001), while most recent studies suggest that climate change impacts are to be considered irreversible within the next decades (Rapp, 2014; IPCC, 2021). Altogether, current findings conclude that reef ecosystems will either undergo major changes or become rare globally by the middle of the century (Bellwood et al., 2004; Hughes et al., 2010; Edmunds & Riegl, 2020).

1.2 Coral reefs and their relevance as marine ecosystems

Coral reefs are rich marine ecosystems mainly constituting biological constructions that host an extensive biological diversity, occupying a total estimated area close to 0.1 % of the ocean surface (Allemand & Osborn, 2019). These ecosystems are not only hotspots and central storehouses of incredible biodiversity, being an oasis in an oceanic desert (Wilkinson, 2002; Allemand & Osborn, 2019), but they are also among the most endangered ecosystems due to climate change. Despite their limited area coverage, coral reefs are home to about 30 % of the world's marine species described to date out of the 274,000 known (Porter & Tougas, 2001). Within coral reefs, at least 32 out of the 34 recognised animal Phyla are represented, making them nearly 400 times richer in species diversity than other oceanic ecosystems. Further, their biodiversity per square kilometre remains comparable to that of tropical rainforest ecosystems, although, in the latter, only nine animal Phyla are generally represented (Reaka-Kudla, 1997; Wilkinson, 2002).

Moreover, the relevance of coral reef ecosystems lies in the goods and services that they provide as well, as they create the necessary conditions for human settlement and potential development in nearby coastal areas (Moberg & Folke, 1999; Wild et al., 2011); although it is by no means limited to their potential utility to our kind. In this context, coral reefs are found within the waters of over 100 countries, many of which correspond to lower to middle-income nations (Spalding et al., 2017). Among these ecosystems, tropical reefs especially provide habitat to many marine organisms, which in turn supply

us with goods and services worth trillions of US dollars per year (Costanza et al., 2014; Hoegh-Guldberg, 2015; Knowlton, 2021). Thus, the lower to middle-income nations where these coral reefs are present strongly depend on them to sustain their well-being and development (Knowlton, 2021).

In detail, tropical coral reefs provide coastal communities with food, employment, recreational and touristic opportunities, besides other socio-economic services (Kittinger et al., 2012; Spalding et al., 2017) and non-material and cultural values (Hein et al., 2019). The touristic value of coral reefs generates global estimates of US$36 billion per year, and at least a quarter of all small-scale fishermen worldwide depend directly on them (Teh, Teh & Sumaila, 2013; Spalding et al., 2017; Knowlton, 2021). Further, while producing a wide range of biochemical compounds of high interest for bioprospection and medical applications (Molinski et al., 2009), coral reefs also provide coastal and shoreline protection for people living near them (Harris et al., 2018). Since they are efficient wave breakers, they significantly buffer wave energy, reducing storm damage and flooding consequences and ultimately mitigating risks posed by natural hazards (Beck et al., 2028; Harris et al., 2018). Despite increasing awareness of coral reefs ecosystem socio-economic and intrinsic value and their provided ecosystem services to the world's population (Darling et al., 2019), coral reefs remain threatened (Anthony et al., 2017; Hoegh-Guldberg, Jacob & Taylor, 2018; IPCC, 2018, 2021; Burke et al., 2023). From a broad perspective, the factors posing major strains on tropical coral reefs are of two types: global and local, while the biological responses from benthic communities to these factors are multifaceted and do not present simple correlations (Ban, Graham & Connolly, 2014; Boyd et al., 2018; Cornwall et al., 2023). Therefore, the present and future of these marine ecosystems will be shaped mainly by these critical global and local influences (Pandolfi et al., 2011; Edmunds et al., 2014; Bell, Micaroni & Strano, 2022).

1.3 Global factors: Ocean acidification and warming

Global factors such as ocean acidification due to increased seawater acidity and ocean warming are, for instance, the most concerning outcomes of climate change and as mentioned, both pose serious threats and challenging scenarios to every tropical coral reef system and their benthic species (Riebesell et al., 2010, 2011; Hughes et al., 2018; Gao et al., 2020; Cornwall et al., 2023; Thirukanthan et al., 2023). For instance, the phenomenon known as 'ocean acidification' depicted in Figure 1.1 occurs naturally and results from the dissolution of CO_2 in the oceanic waters, causing a subsequent decrease in the seawater pH (Riebesell et al., 2011). The decrease occurs according to the following reaction described in Riebesell et al. (2011) and Allemand and Osborn (2019):

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \qquad (1)$$

Where, HCO_3^- represents the bicarbonate ion and H^+ the acidic hydrogen. As a consequence of Reaction 1, when atmospheric carbon dioxide reacts with the oceanic waters, it causes a decrease in the concentration of carbonates after a complex series of reactions that increase the concentrations of carbonic acid and bicarbonate in the seawater (Kleypas et al., 1999). In detail, when the carbonate ion, which constitutes the fundamental building block for the formation of hard coral skeletons by reacting with calcium (Reaction 2) to form calcium carbonate ($CaCO_3$), becomes less available when increasing the acidity of the seawater due to protons (H^+) produced in excess, it becomes available to react with the carbonate ion as shown in the Reaction 3 (Allemand & Osborn, 2019), ultimately affecting the concentration of carbonate ions in the seawater (Riebesell et al., 2011; Allemand & Osborn, 2019; IPCC, 2021).

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \qquad (2)$$

$$H^+ + CO_3^{2-} \rightarrow HCO_3^- \qquad (3)$$

Besides, Reactions 1 and 3 represent consecutive acid dissociation steps of dissolved carbon dioxide, further increasing proton production and thus reducing the sea water pH

even more (Riebesell et al., 2011). Specifically, ocean acidification resulting mainly from the high introduction of anthropogenic CO_2 into the oceans has caused the rapid changes observed in the seawater chemistry, including alarmingly accelerated rates in pH decrease during the last decades (Kleypas et al., 1999; Orr et al., 2005; Caldeira et al., 2007), when compared to an ocean where the pH has remained stable during the last 300 million years (Allemand & Osborn, 2019). This phenomenon has led to an increase of 30 % in the acidity of the oceans since the beginning of the industrial era, with a pH decrease of at least one unit (from 8.2 to 8.1), projected to reach 7.8 by the year 2100, at the end of this century (Orr et al., 2005; Lerman et al., 2011; IPCC, 2021).

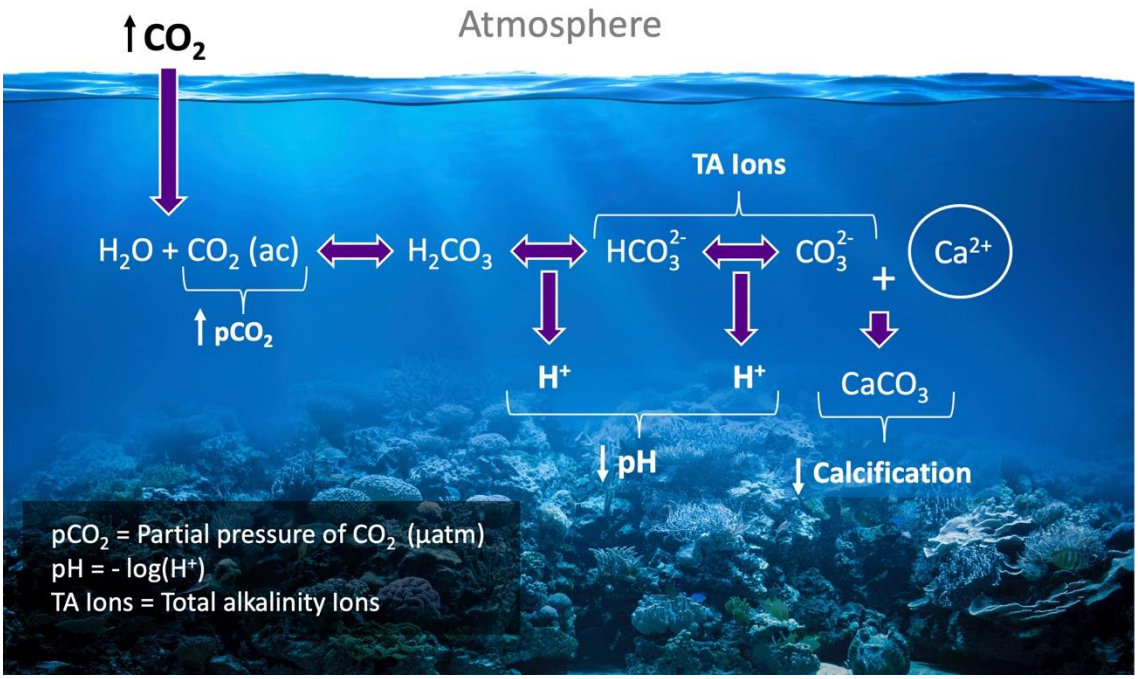

Figure 1.1 | Ocean acidification modifies seawater chemistry. As carbon dioxide (CO_2) levels increase due to anthropogenic activities, ocean waters absorb more CO_2. In turn, this leads to enhanced seawater acidity via an increase of carbon dioxide partial pressure (pCO_2), which results in decreased pH and less availability of the required ions for the formation of calcareous structures. Heightened acidity impedes coral calcification, the process by which corals build their calcium carbonate ($CaCO_3$) skeletons, resulting in weakened and bleached reefs in many cases. Additionally, ocean acidification can disrupt crucial processes affecting other aspects of the formation of coral structures, further jeopardising the health and resilience of coral reef ecosystems.

Furthermore, beyond changing the quantities of carbonate required for skeletal formation in many organisms (Kleypas et al., 1999; Comeau, Cornwall & McCulloch, 2017), modifying a key parameter for animal physiology, such as the pH, has profound biological consequences (Comeau, Cornwall & McCulloch, 2017). The complex chemical system shaping seawater composition and its saturation states play a significant role in the pH equilibrium of biological fluids (Pörtner, Langenbuch & Reipschläger, 2004). The pH can thus regulate a large portion of the cellular processes taking place, e.g. the optimal activity of many proteins and the calcifying fluids responsible for controlling the formation of sedimentary structures, as it occurs in hard corals (Tambutté et al., 1995; Allemand & Osborn, 2019), and that results in the structural framework of coral reefs. Coral reefs, thus, are one of the most susceptible marine ecosystems to ocean acidification since mainly hard corals and other calcareous organisms are projected to face intense pressures under these conditions, especially for precipitating calcium carbonate into their skeletons (Gattuso, Allemand & Frankignoulle, 1999; Kleypas et al., 1999; Hoegh-Guldberg et al., 2007; Doney et al., 2009; Kleypas, 2011). Nevertheless, despite all the research efforts conveyed to date, further studies are still needed before gaining a complete understanding of the impacts of ocean acidification on coral reefs and especially on the coral species inhabiting them (Hilmi et al., 2013; Hoegh-Guldberg et al., 2017).

Although it remains a major endeavour to understand how marine ecosystems will respond to changes in seawater chemistry and lowered pH, for many instances within ocean acidification research (Kroeker et al., 2013), it is challenging to definitively attribute biological impacts to acidification alone, as pH shifts often occur in concert with other environmental factors such as, e.g., ocean warming (Zachos et al., 2005; Kline et al., 2015). Ocean warming is the most immediate and pressing global threat to coral reefs (Hughes et al., 2017, 2018). Higher surface water temperatures occur in the oceans due to increased greenhouse gas emissions into the atmosphere (Cheng et al., 2019), imposing tremendous strain on the coral reef ecosystems, which are already withstanding high stress due to multifactor impacts.

Described changes in the ocean temperature include even rapid increases down to at least 700 m depths (IPCC, 2021), but especially for tropical shallow reefs, the warmer temperatures have proven to cause mass coral bleaching, catastrophic coral mortality rates and outstanding increases in coral disease prevalence (Anthony, Connolly & Hoegh-Guldberg, 2007; Hughes et al., 2018; Knowlton, 2021). Warmer than usual temperatures can thus effectively impact coral benthic communities, as also observed, e.g., in 1997-1998, during El Niño and La Niña phenomena of those years (Wilkinson, 2004). For instance, for many coral species being sensitive to thermal stress, the symbiosis between coral and its associated zooxanthellae and microbiota can be hampered by increases as small as 1 °C above the average summer sea surface temperature, leading to overall symbiosis breakdown (Ziegler et al., 2017, 2019; Allemand & Osborn, 2019). Regarding bleaching due to thermal stress, as it occurs for ocean acidification (Anthony et al., 2008), high variability in the coral response also occurs even at intraspecific levels (Dobson et al., 2021; Humanes et al., 2022). Thus, the cellular mechanisms behind the symbiosis breakdown occurring in bleaching are still very much debated (Lesser, 2007; Weis, 2008; Baird et al., 2009; Tolleter et al., 2013), as well as the respective roles of the host and symbionts (Baird et al., 2009; Dove & Hoegh-Guldberg, 2011; Voolstra & Ziegler, 2020).

Moreover, both IPCC (2018) and (2021) reports confirmed potential losses of 90 % of reef-building corals given a rise in global temperature of 1.5 °C and an almost complete loss of coral populations worldwide (>99 %) if there is a rise in temperature of 2 °C. Current warming projections foresee scenarios where temperature increases can reach and overcome these 1.5 °C to 2 °C thresholds within the next 50 years, and multiple lines of evidence present, with very high confidence that several tropical coral reefs that exist today will disappear even if global warming is limited to 2 °C (Frieler et al., 2013; IPCC, 2021). Furthermore, changes in the response to climate change rarely operate in isolation from regional and localised factors (Cornwall et al., 2021). Therefore, the effect of global warming of 1.5°C versus 2°C should also be considered in the light of multiple factors, such as the so-called local factors (Wild et al., 2009; Ateweberhan et al., 2013), which may accumulate and interact with thermal stress byproduct of global ocean

warming, to produce complex risks, hazards and impacts on coral reefs systems (Wild et al., 2009; Ateweberhan et al., 2013; Pogoreutz et al.,2017; IPCC, 2021).

1.4 Local factors: Eutrophication as dissolved organic matter

With growing human populations and improved storage and transport systems, the scale of human impacts on reefs has increased exponentially, as have local factors (Baum et al., 2015; Baum et al., 2016; Guest et al., 2016). At this local level, the main threats to coral reefs include overfishing, unsustainable tourism and the development of coastal infrastructures, diverse forms of pollution, sedimentation, and eutrophication (Burke et al., 2011; Wiedenmann et al., 2013). These threats, which can be addressed via regional and local management, have been reported not only to weaken many coral species (Fabricius, 2005; Kuntz et al., 2005; Wiedenmann et al., 2013), thus reducing their resilience to global factors (Ateweberhan et al., 2013), but also to act in synergy with those same factors, amplifying in many cases their effects due to complex interactions (Carilli et al., 2010; Ateweberhan et al., 2013; Pendleton et al., 2016).

Organic eutrophication as dissolved organic carbon (DOC) represents a local factor that may occur as a consequence of sewage pollution or sedimentation (Kline et al., 2006; Haas, Al-Zibdah & Wild, 2009; Pogoreutz et al., 2017) and can play a fundamental role as well in driving coral physiological responses, the stability of algal and microbiome-host associations, and thus their associated health status (Kline et al., 2006; Pogoreutz et al., 2017). In tropical reefs where corals exhibit a high component of symbiotic interactions and are strongly dependent on these relationships (e.g., autotrophic corals highly reliant on their photosynthetic symbionts), the effects of organic eutrophication and specifically its effects as DOC are still highly underinvestigated (Ateweberhan et al., 2013; Pogoreutz et al., 2017) although most findings suggest that they may cause an overall adverse impact on hard corals and thus deserve further attention (Kuntz et al., 2005; Kline et al., 2006; Smith et al., 2006; Haas, Al-Zibdah & Wild, 2009). In detail, DOC in elevated concentrations can cause an imbalance of the coral holobiont symbiotic associations, affecting aggregate formations and influencing bacterial metabolism (and other microbes) in hard corals (Pogoreutz et al., 2017; McDevitt-Irwin et al., 2017, 2019),

ultimately causing bleaching (Pogoreutz et al., 2017) and signifying a serious risk. Moreover, some studies have suggested that the interactions of this factor (Ateweberhan et al., 2013), in particular with ocean warming, may pose a threat to many hard coral species (Rodrigues & Grottoli, 2007; Edmunds, 2007; Pörtner, 2008; Bourne et al., 2009), but for others, it may result in negligible or beneficial effects (Fabricius et al., 2013; McCauley & Goulet, 2019), alleviating the impact of other factors via an increase of heterotrophic intake, energy resource management and overall trophic strategy adaptability (Anthony & Fabricius, 2000; Grottoli, Rodrigues & Palardy, 2006; Fabricius et al., 2013). However, few studies have engaged in research mainly focused on organic eutrophication and, in particular, DOC and its interaction with other global factors, such as warming beyond hard corals.

1.5 Octocorals: The neglected reef component

Coral species that compose the reefs are also architects of their functional services and form close bonds with organisms living there (Wilkinson, 2004). When coral reefs have disappeared or transformed, portraying very different landscapes with modified ecosystem services and functionality (Glynn, 1993; Wild et al., 2011; Frieler et al., 2013), the coral reefs as we know them will change, leading to phase shifts from hard corals to other invertebrates such as soft corals, sponges, and benthic algae (Bellwood et al., 2006; Maliao, Turingan & Lin, 2008; Inoue et al., 2013; Enochs et al., 2015; Lasker et al., 2020), thus undergoing overall negative impacts. In general, hard corals have always been studied intensively in terms of their performance relative to other functional groups, such as macroalgae (Mumby, Hastings & Edwards, 2007; Edmunds et al., 2014), given they are the primary engineers and building components of coral reefs, and their situation is critical under climate change circumstances (Wild et al., 2011; Hughes et al., 2018; Cornwall et al., 2021). It has been observed that many hard coral species suffer reductions in their reproductive rates and growth (Baird & Marshall, 2002; Edmunds, 2012), and in cases of extreme temperatures, many of them bleach and subsequently die out of the stress imposed (Anthony, Connolly & Hoegh-Guldberg, 2007; DeCarlo et al., 2017; Hughes et al., 2018). Thus, it is understandable that hard corals have been under the main spotlight to this date. However, another essential component of coral

reefs is the octocorals, illustrated in Figure 1.2 and which, despite their high relevance, have been strongly neglected compared to hard corals regarding the study of their responses to climate change (Sánchez, 2016; Lasker et al., 2020; Schubert, Brown & Rossi, 2020).

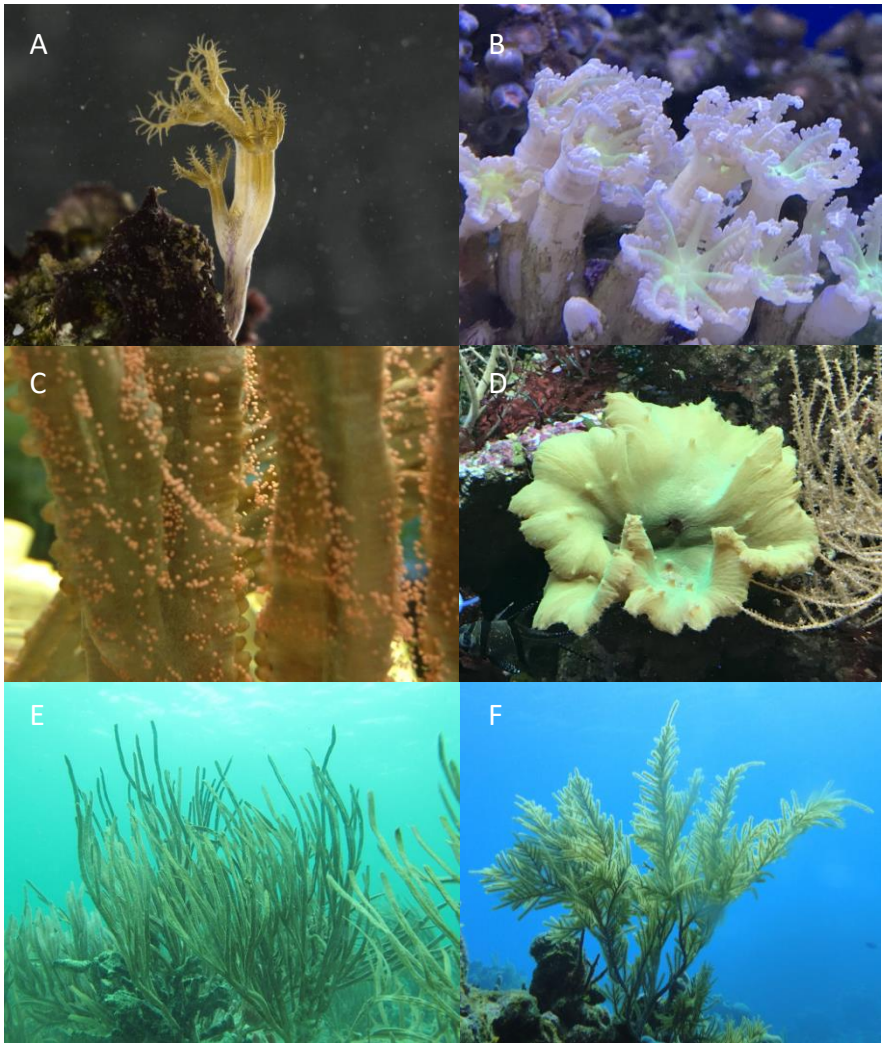

Figure 1.2 | Octocorals: a highly biodiverse and vital component of tropical coral reefs. Soft corals belonging to the class Octocorallia (A-F) are outstandingly biodiverse, generally well-represented across coral reef ecosystems worldwide, and exhibit astonishing adaptability to various environments. Corals within this group showcase a remarkable amount of variation in their biological traits, architectures, and growth modes, together with an important degree of phenotypical plasticity. Some octocorals may be soft-bodied and highly flexible, with fast growth rates, while others portray more rigid structures and slow growth rates. Octocorals with encrusting growth modes form extensive layers over surfaces, maximising space utilisation, while others, having branching architecture, extend intricate tree-like structures which provide habitat complexity for diverse marine organisms.

Octocorals, also commonly addressed as soft corals (as we will interchangeably address them during this thesis work after Fabricius (2011) for simplicity), are a crucial component of the benthic communities of the tropical coral reefs. They dominate many tropical reefs worldwide (Edmunds et al., 2015; Lasker et al., 2020) and have a ubiquitous distribution (Fabricius & De'ath, 2008; Sánchez, 2016). Although soft corals are not true reef-building corals, they are essential calcium carbonate contributors to the sediments (Velimirov & Böhm, 1976). In addition, they provide substratum and habitat for several reef organisms (Sánchez, Zea & Díaz, 1998) and enhance habitat complexity and reef's three-dimensional structure ((Fabricius, 2011; Sánchez et al., 2014; Newman et al., 2015; Sánchez, 2016). In the tropical shallow waters, this group shows an exceptionally high diversity where it is possible to find over ten species within sympatric distributions in small areas of only a few square meters (Edmunds et al., 2016; McFadden et al., 2017; Conti-Jerpe, Pawlik & Finelli, 2022). These corals provide other marine organisms, such as reef fishes and many other closely associated invertebrates, places to reproduce and shelter from predators, comprising interesting model cases of masquerade camouflage (Sánchez, 2016; Schubert, Brown & Rossi, 2020). Moreover, soft corals provide habitat and feeding substrates. Therefore, they are considered to be genuine foundation species (Sánchez, 2017), while at the same time, they show fascinating evolutionary trends (Sánchez, 2016; McFadden et al., 2017), where their varied ecological strategies and divergence potential are certainly not only promising in contributing to the future maintenance of their ecological roles in reefs (Tsuonis & Edmunds, 2017) but also to their potential capacity to outcompete calcifying species (Norström et al., 2009; Tsounis & Edmunds, 2017; Tkachenko, Dung & Ha, 2022; Coffroth et al., 2023), making them particularly exceptional candidates to become winners under selected phase shifts scenarios.

1.6 Thesis premise: Knowledge gaps and aim

Knowledge gaps: soft corals under global and local factors

With more than 3500 species composing the soft corals group, this taxon remains highly underinvestigated in the context of global and local factors (Lasker et al., 2020). Their

incredibly high biodiversity has made them challenging to study, as it is exceptionally complicated to identify their species and to differentiate clear boundaries across taxa, which appear to be morphologically inexistent and, in many cases, require further molecular assessments (Prada, Schizas & Yoshioka, 2008; McFadden et al., 2017). Precisely, given this outstanding amount of diversity, there is little resolution and a wide range of physiological responses from many soft coral species in the face of climate change environmental pressures that remain unexplored (Guinotte & Fabry, 2008; Fabricius et al., 2011; Hale et al., 2011). Contrasting the widely documented adverse effects that global and local factors (ocean acidification, warming and eutrophication) have on hard corals, such as compromising their metabolic, physiological and reproductive processes and growth and calcification rates (Hoegh-Guldberg et al., 2007; Kurihara, 2008; Guinotte & Fabry, 2008; Kleypas, 2011; Albright, 2011; Kroeker et al., 2013; DeCarlo et al., 2015), there is still comparatively limited information on how soft corals behave under these factors (Lasker et al., 2020; Coffroth et al., 2023). However, the studies performed to date report distinct responses to acidification and warming (Gabay, Benayahu & Fine, 2013; Gabay et al., 2014; Gómez et al., 2014; Enochs et al., 2016; Parrin et al., 2016) and eutrophication (Baum et al., 2016; McCauley & Goulet, 2019) across several soft coral species, suggesting soft corals may have higher tolerance and resistance towards these factors. Yet, this hypothesis still requires further experimental evidence (Lasker et al., 2020). With coral communities worldwide indeed threatened and rapidly changing through shifts in their species composition (Alvarez-Filip et al., 2013; Tkachenko, Dung & Ha, 2022), better-fitted species to the changing chemistry and temperatures of the seawater (e.g. robust soft coral species) may become dominant (Tkachenko, Dung & Ha, 2022; Coffroth et al., 2023). Thus, the overall dominance of coral communities in reefs may shift from reef-building hard corals to soft corals (Norström et al., 2009; Tkachenko, Dung & Ha, 2022; Coffroth et al., 2023), implying significant consequences for functional and structural shaping of the reefs as well as their complexity and ecosystem integrity (Alvarez-Filip et al., 2013; Wilkinson, 2002). Given this situation, double efforts are urgently needed to better understand soft corals as a highly relevant component of coral reefs in transition, also highlighting the compelling need to gain better comprehension and insights on their response to global

and local factors, which will ultimately shape the future landscapes of coral reefs worldwide.

Thesis Aim

This thesis work aimed to comprehensively evaluate the ecophysiological responses of soft corals to ocean acidification and warming as global factors and organic eutrophication or DOC as a selected local factor, considering these corals' responses towards DOC and warming as likely interacting factors. Thus, this thesis sought to investigate further the contrasting response patterns associated with the previously mentioned factors found for selected soft coral species and their potential for resistance and tolerance.

1.7 Research questions and working hypothesis

To improve our understanding of the effects of global and local factors on the ecophysiological performance of soft corals, this thesis addressed the following research questions and hypotheses:

1. How do different soft coral genera respond comparatively to simulated ocean acidification scenarios, and to what extent do their physiological responses suggest potential for tolerance or resistance?

Hypothesis 1: *Soft corals will be able to withstand ocean acidification. However, their overall capacity for tolerance and resistance will vary as a function of their intrinsic biological and physiological features.*

The studies performed to date on the effects of ocean acidification on soft corals have shown contrasting findings and even species-specific responses across closely related taxa (Sprung & Delbeek, 1997; Albright, 2011; Gabay, Benayahu & Fine, 2013; Gabay et al., 2014; Hoegh-Guldberg et al., 2017), with some of them suggesting that soft corals may be less affected by ocean acidification than hard corals (Kurihara, 2008; Fabricius,

2011; Lasker et al., 2020). Nevertheless, further experimental evidence addressing this specific coral group is still pending (Lasker et al., 2020). In **Chapter 2** of this thesis, we employed aquaria experiments to evaluate further the physiological responses of four different genera of soft corals towards ocean acidification (pH 7.78, pCO_2 > 1000 ppm), comparing them across a broad phylogenetic range.

2. Does organic eutrophication as dissolved organic carbon (DOC) affect soft corals, or are they able to cope with this factor? Does warming and its potential interaction with DOC influence soft corals' responses?

Hypothesis 2: We hypothesised that the individual and combined effects of DOC enrichment and high temperatures would impact soft corals, influencing their health status by altering their ecophysiological parameters and promoting diazotrophs' growth and activity.

Increased DOC can cause bleaching in hard corals with negative responses at the level of the host, their microbiota, and its associated zooxanthella (Haas, Al-Zibdah & Wild, 2009; Pogoreutz et al., 2017). In addition, this local factor is expected to impact coral responses in varied ways, given concomitant warming (Fabricius et al., 2013; Morris et al., 2019). In the context of soft corals, few studies have specifically approached organic eutrophication or its interaction with other factors (McCauley & Goulet, 2019). Thus, in **Chapters 3 to 6,** we assessed the effects of DOC enrichment, warming, and the combination of both factors on the physiological responses of the octocorals *Xenia umbellata* **(Chapters 3 and 4)** and *Pinnigorgia flava* **(Chapter 5)**, together with the effects of both factors on their microbiota associated with denitrification processes **(Chapter 6)**.

3. What are the physiological responses of soft corals to prolonged warming as a single factor under the context of climate change and distinct warming scenarios primarily focusing on the soft coral *X. umbellata* as a model species?

Hypothesis 3: The soft coral *X. umbellata* will be able to overcome the adverse effects of warming as a single factor; however, when prolonged, exposure to thermal stress will cause adverse effects on this soft coral.

Although current evidence suggests that soft corals may be resistant to warming, this remains inconclusive, especially for soft corals belonging to the group xeniids, as only a few studies have evaluated their responses beyond short-term experiments (Strychar et al., 2005; Sammarco & Strychar, 2013; Parrin et al., 2016). In **Chapter 7** of this thesis, we investigated further the physiological responses of the soft coral *X. umbellata* as a study model and its sensitivity and potential tolerance under the context of prolonged or mid-term warming exposures.

1.8 Thesis structure and manuscript outline

This thesis work consists of eight chapters, where the first and last chapters are dedicated to the general introduction **(Chapter 1)** and a final discussion **(Chapter 8)**, respectively. The remaining six chapters in between consist of four published manuscripts **(Chapters 3 to 6)** and two additional manuscripts in preparation for publication in international peer-reviewed journals **(Chapters 2 and 7)**. All manuscripts included in this thesis contribute to understanding ocean acidification and warming effects as global factors and organic eutrophication or DOC as a local factor on soft corals. The thesis chapters are outlined as follows:

Chapter 2: Effects of ocean acidification in four different genera of tropical Octocorals.

Simancas-Giraldo SM, Martins P.P. C, Wild C, Ziegler M.

Manuscript in prep.

Author contributions:

• **Susana M. Simancas-Giraldo** conceived and designed the experiments, performed the experiments, analysed and interpreted the data, prepared figures and/or tables, and authored the manuscript draft.

• Catarina Padilha Pires Martins conceived and designed the experiments, performed the experiments and contributed to data interpretation.

• Cristian Wild contributed to the experiment design and project development.

• Maren Ziegler conceived and designed the experiments, guided the aquaria project and contributed to data interpretation and project development.

Chapter 3: Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic carbon eutrophication.

Simancas-Giraldo SM, Xiang N, Kennedy MM, Nafeh R, Zelli E, Wild C.

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Author contributions:

• **Susana M. Simancas-Giraldo** conceived and designed the experiments, performed the experiments, analysed and interpreted the data, prepared figures and/or tables, authored or reviewed manuscript drafts, and approved the final draft.

• Nan Xiang conceived and designed the experiments, authored or reviewed manuscript drafts, contributed to data interpretation, and approved the final draft.

• Meghan Moger Kennedy and Rassil Nafeh performed the experiments, authored or reviewed manuscript drafts, contributed to data interpretation, and approved the final draft.

• Edoardo Zelli performed the experiments, authored or reviewed manuscript drafts, contributed to experimental design, and approved the final draft.

• Christian Wild conceived and designed the experiments, authored or reviewed manuscript drafts, and approved the final draft.

Chapter 4: Organic eutrophication increases resistance of the pulsating soft coral *Xenia umbellata* to warming.

Vollstedt S, Xiang N, **Simancas-Giraldo SM**, Wild C.

Manuscript published in PeerJ. DOI: 10.7717/peerj.11663

Author contributions:

• Svea Vollstedt conceived and designed the experiments, performed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed manuscript drafts, and approved the final draft.

• Nan Xiang performed the experiments, analysed the data, prepared figures and/or tables, contributed to experimental design, authored or reviewed manuscript drafts, and approved the final draft.

• **Susana M. Simancas-Giraldo** performed the experiments, analysed the data, contributed to experimental design, authored or reviewed manuscript drafts, and approved the final draft.

• Christian Wild conceived and designed the experiments, authored or reviewed manuscript drafts, and approved the final draft.

Chapter 5: Individual and combined effect of organic eutrophication (DOC) and ocean warming on the ecophysiology of the gorgonian *Pinnigorgia flava*.

Zelli E*, **Simancas-Giraldo SM***, Xiang N, Dessì C, Katzer N, Wild C.

*** Shared first authorship.**

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Author contributions:

• Edoardo Zelli conceived and designed the experiments, performed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed manuscript drafts, and approved the final draft.

• **Susana M. Simancas-Giraldo** conceived and designed the experiments, performed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed manuscript drafts, and approved the final draft.

• Nan Xiang conceived and designed the experiments, performed the experiments, authored or reviewed manuscript drafts, and approved the final draft.

• Claudia Dessì performed the experiments, analysed the data, authored or reviewed manuscript drafts, and approved the final draft.

• Nadim Daniel Katzer performed the experiments, analysed the data, authored or reviewed manuscript drafts, and approved the final draft.

• Arjen Tilstra analysed the data, authored or reviewed manuscript drafts, and approved the final draft.

• Christian Wild conceived and designed the experiments, authored or reviewed manuscript drafts, and approved the final draft.

Chapter 6: Contrasting microbiome dynamics of putative denitrifying bacteria in two octocoral species exposed to dissolved organic carbon (DOC) and warming.

Xiang, N, Hassenrück, C, Pogoreutz, C., Rädecker, N, **Simancas-Giraldo SM,** Voolstra, C. R., Wild, C., & Gärdes, A.

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Author contributions:

• Nan Xiang designed and conceived the research, performed the aquaria project, analysed sequencing data, conducted the statistical analysis, data visualisation and interpretation, wrote the manuscript and approved the final draft.

• Christiane Hassenrück analysed sequencing data, conducted statistical analysis, reviewed drafts of the manuscript, provided improvement suggestions, and approved the final draft.

• Claudia Pogoreutz conducted data visualisation and interpretation, wrote the manuscript, reviewed drafts, provided improvement suggestions, and approved the final draft.

• Nils Rädecker conducted data visualisation and interpretation, wrote the manuscript, reviewed drafts, provided improvement suggestions, and approved the final draft.

• **Susana M. Simancas-Giraldo** performed the aquaria project, reviewed manuscript drafts, provided improvement suggestions, and approved the final draft.

• Christian R. Voolstra reviewed drafts of the manuscript, provided improvement suggestions, and approved the final draft.

• Christian Wild designed and conceived the research, contributed coral samples and reagents, reviewed drafts of the manuscript, provided improvement suggestions, and approved the final draft.

• Astrid Gärdes designed and conceived the research, contributed coral samples and reagents, reviewed manuscript drafts, provided improvement suggestions, and approved the final draft.

Chapter 7: The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming

Simancas-Giraldo SM, Storkenmaier M, Vasseur F, Ferse S, Wild, C.

Manuscript in prep.

Author contributions:

• **Susana M. Simancas-Giraldo** conceived and designed the experiments, performed the experiments, analysed and interpreted the data, prepared figures and/or tables, and authored or reviewed manuscript drafts.

• Madline Storkenmaier conceived and designed the experiments, performed the experiments, contributed to data analysis and figure preparation, and reviewed manuscript drafts.

• Francine Vasseur contributed to the experiment design, performed the experiments, and reviewed manuscript drafts.

• Sebastian Ferse contributed to data analysis and interpretation and reviewed manuscript drafts.

• Christian Wild conceived and designed the experiments, contributed improvement suggestions and reviewed manuscript drafts.

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Chapter 2

Effects of ocean acidification on four different genera of tropical Octocorals

First author contribution

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Ecophysiological Responses of Four Octocoral Genera to Ocean Acidification

Susana M. Simancas-Giraldo[1], Catarina P. P. Martins [2], Christian Wild[1], Maren Ziegler[2]

[1] Marine Ecology Department, Universität Bremen, 28359 Bremen, Germany
[2] Department of Animal Ecology and Systematics, Justus Liebig Universität, 35392 Giessen, Germany

Corresponding Author:
Susana Marcela Simancas-Giraldo[1]
Email address: sussim@uni-bremen.de

In prep.

2.1 Abstract

Climate change, and in particular ocean acidification (OA), have differing effects on marine organisms. Interestingly, selected octocorals have shown to be more resistant to OA when compared to reef-building corals in terms of their survival, growth, and calcification. However, our current knowledge of the effects of OA on the octocorals group is, in general, still limited. Existing studies on octocorals suggest high species-specificity in their ecophysiological response to OA, but more comprehensive work covering several taxa is needed. In this study, corals belonging to the octocoral genera *Pinnigorgia*, *Plexaurella*, *Sinularia* and *Xenia*, selected to cover a broad phylogenetic range, were exposed for three months to OA conditions expected under the RCP 8.5 scenario for the year 2100 (pH 7.78, pCO_2 > 1000 ppm). Our results on the physiological parameters assessed, including P:R ratios, maximum photochemical (F_v/F_m) and effective photochemical efficiency ($\Delta F/F_m'$), polyps pulsation frequencies and coral growth, showed that the acidification treatment had no significant adverse effects for any of the soft coral genera assessed. In addition, no mortality or bleaching was observed, indicating tolerance to OA conditions in all genera. This general tolerance to OA may give soft corals an additional advantage over the more susceptible hard corals under climate change. Based on these results, soft corals may become an increasingly dominant ecological functional group in global reef ecosystems of the future.

2.2 Introduction

Atmospheric carbon dioxide (CO_2) concentrations have surged in recent decades at alarming rates, leading to sea surface pH anomalies over the last 40 years (Lopes et al., 2018; IPCC, 2021). This phenomenon, known as ocean acidification (OA), results from the dissolution of CO_2 in oceanic waters, causing a decrease in their pH levels (Allemand & Osborn, 2019). To date, few studies have evaluated octocorals responses to environmental changes and specifically to OA (e.g. Bramanti et al., 2013; Gabay, Benayahu & Fine, 2013; Gómez et al., 2014; Enochs et al., 2016; Lopes et al., 2018), while considerable research has been focused on the effects of OA on reef-building hard

corals. Consequently, there is scarce knowledge on how octocorals will respond to this global factor, especially considering the vast biodiversity present within this group and their distinct taxa (Lasker et al., 2020).

OA poses a significant threat to coral reef ecosystems, affecting calcification rates and various biological processes of hard coral species, the main reef framework builders (Anthony et al., 2008; Doney et al., 2009; Edmunds, 2012). In addition, acidification is likely to affect a varied range of biological aspects of the coral holobiont, including the relationship between the host and its symbiotic associations (Anthony et al., 2008; Grottoli et al., 2018) and crucial physiological processes such as coral nutrition (Grottoli, Rodrigues & Palardy, 2006), reproduction (Albright, 2011) and photosynthetic productivity (Crawley et al., 2010; Comeau, Carpenter & Edmunds, 2017; Schoepf et al., 2017; Albright, 2018). However, numerous studies highlight a significant disparity in hard corals sensitivities to OA (Kroeker et al., 2010; Kline et al., 2015; Allemand & Osborn, 2019). Similarly, studies on octocorals under OA conditions have shown contrasting findings, with species-specific responses observed across various taxa (Hoegh-Guldberg et al., 2007; Kurihara, 2008; Albright, 2011; Gabay, Benayahu & Fine, 2013; Gabay et al., 2014). Despite this variability, experimental evidence suggests that it may be reasonable to hypothesize that octocorals will be less affected by OA than hard corals (Lasker et al., 2020). For instance, research has shown that certain octocoral species, e.g., *Eunicea fusca* and *Eunicea flexuosa,* can maintain their linear extension and sclerite calcification even when exposed to elevated CO_2 concentrations (Gómez et al., 2014; Enochs et al., 2016). In detail, the studies by Gómez et al. (2014) found that the octocoral *Eunicea fusca* can maintain its linear extension and sclerite calcification, although reduced when exposed to a range of CO_2 concentrations from 285 to 4.57 ppm (pH range 8.1 - 7.1), throughout 30 d. On the other hand, Enochs et al. (2016) found no negative impacts on *Eunicea flexuosa* after assessing similar metrics on this octocoral, undergoing 49 d of exposure to reduced pH 7.75 and 8.01 as near-present day conditions. Moreover, previous studies on soft-bodied octocorals like *Heteroxenia fuscescens*, *Ovabunda macrospiculata*, and *Xenia umbellata* have indicated minimal effects of OA on selected physiological metrics under acidic conditions (Gabay, Benayahu, & Fine, 2013; Gabay et al., 2014; Tilstra et al., 2023). In these studies, OA did

not seem to affect the sclerite formation or photosynthetic metrics of *H. fuscescens* and *O. macrospiculata* (Gabay, Benayahu & Fine, 2013; Gabay et al., 2014; Tilstra et al., 2023), while in the study by Tilstra et al. (2023), *X. umbellata* photosynthetic metrics remained unaffected, although pulsation and growth decreased.

With over 3500 described species in modern tropical reefs (Fabricius, 2011; McFadden, Ofwegen & Quattrini, 2022), Octocorals represent an essential component exhibiting diverse morphologies, growth modes, biological architectures, and high phenotypical plasticity at many levels (Sánchez, 2016; Calixto-Botía & Sánchez, 2017; Schubert, Brown & Rossi, 2020). Despite their importance as a functional group in reefs, only a few octocoral species have been explored under the context of OA, with previous work focusing on the immediate responses of single or closely related coral species (Dupont & Pörtner, 2013a; Lasker et al., 2020). Thus, our understanding of octocoral responses in the face of OA is still in its infancy. Moreover, although the notion of some octocorals being more resistant to increased acidity than other corals persists, additional experimental evidence is still pending (Lasker et al., 2020), where understanding how ecosystems as a whole will respond to OA remains the ultimate challenge (Dupont & Pörtner, 2013b). In these regards, comprehensive approaches covering a wider range of taxa are still needed to comprehend better how octocorals are affected by OA. Thus, a comparative assessment across selected representative members of major octocoral groups comprising a broader phylogenetic range may be valuable to derive an overview of this coral group's sensitivities.

The present study aimed to answer the effects of OA on different octocoral genera, how they respond comparatively to simulated OA scenarios, and to what extent their physiological responses suggest a potential for tolerance or resistance. To address these questions, we implemented a 158 d aquaria experiment (from October 15th, 2019, to March 12th, 2020), simulating OA conditions for 3.3 months (i.e. 100 d). Octocoral colonies of the genera *Pinnigorgia*, *Plexaurella*, *Sinularia* and *Xenia* were selected to cover a broad phylogenetic range and exposed to OA conditions expected under the IPCC (2014) RCP 8.5 scenario for the year 2100, pH 7.78, pCO_2 > 1000 ppm (Pachauri et al., 2014; IPCC, 2021). We hypothesized that octocorals would be able to withstand

ocean acidification. Still, their overall capacity for tolerance and resistance will vary as a function of their intrinsic biological and physiological features.

2.3 Materials and Methods

Study specimens

Our experiments were performed at the 'Ocean2100' facility within the Department of Animal Ecology & Systematics of the Justus-Liebig University (JLU) in Giessen, Germany. All coral colonies were acquired and bred under artificial conditions in aquaria more than two years prior to our experiments. Mother colonies of the octocoral genera *Xenia* and *Pinnigorgia* were initially bred at the research facilities of the Marine Ecology group in the Center for Environmental Research and Sustainable Technology (UFT) at the University of Bremen, Germany. These were transported to the Giessen facilities on August 25th, 2019, before the start of the experiments. On the other hand, the mother colonies belonging to the genera *Plexaurella* and *Sinularia* were always bred and maintained at the research facilities in Giessen. The mother colonies were propagated into smaller fragments of *Xenia* (n = 80), *Sinularia* (n = 72), *Plexaurella* (n = 80) and *Pinnigorgia* (n = 80) and given a minimum of 10 d and a maximum of 32 d to heal. We procured initial small sizes around 1 to 1.5 cm^2 for *Sinularia* and *Xenia* (i.e. an average size of 6 to 16 polyps per fragment) and sizes ranging between 1.5 to 2 cm^2 for the branching genera *Pinnigorgia* and *Plexaurella*. During the healing period, the octocoral fragments were first kept at the facilities in 256 L culturing aquaria and under an 11:13 light:dark photoperiod, light intensity of 230 µmol m^{-2} s^{-1}, water temperature of 26.0 ± 0.5 °C and salinity of 35.

Experimental setup and OA treatments

The experimental setup, the OA treatments, the seawater chemistry, and the chemical parameters of our experiments have already been described in our additional study by Martins et al. (2024), together with further details in its supplementary materials. Briefly, the aquaria experimental setup consisted of eight 120 L tanks arranged in two

vertical sets of four tanks (see Figure 2.1), all together in circulation with an aquarium acting as a sump and holding a return pump. The experimental aquaria system was kept in circulation with a bigger main closed recirculating system composed of multiple aquaria (8000 L in total). This arrangement was preferred to maintain higher stability within the experimental system during the whole term of the experiment. The water inflow rate into the experimental system corresponded to 20–40 L h^{-1} (i.e. 100 % tank volume turnover every 3–6 h), and water exchanges of approximately 10 % of the water volume were performed for the bigger main system weekly.

Figure 2.1 | Experimental setup and treatment distribution. Panel A shows the eight-aquaria experimental system, together with an exemplary view in panel B of the soft and hard corals in each aquarium. The hard coral fragments were part of different assessments corresponding to our additional publication dedicated to the effects of OA and water flow on these corals (Martins et al., 2024), while the octocorals fragments correspond to the individuals evaluated in this study. The semi-randomized OA treatment distribution within our experimental system is depicted as well, with 4 tanks assigned to the control condition (light blue) and 4 tanks to the high pCO_2 treatment (dark blue).

Moreover, every aquarium in the eight-tank experimental system was equally equipped with a pH sensor (GHL, Germany) calibrated biweekly using NBS buffers, a thermostat (300 W; 548, Schego, Germany), two pumps at each side of each aquarium to ensure evenly distributed water flow (ES-28, Aqualight, Germany), and a wave generator (6208, Tunze, Germany). The pump arrangement and wave generator provided a flow velocity of 6 cm s^{-1} measured at the coral's fragments position. Further, every tank had two white light lamps (54 W, Aqua-Science, Germany). Salinity was monitored daily using a conductivity sensor (TetraCon 925, WTW, Germany).

The seawater pH in each aquarium was controlled individually using a Profilux 3 computer (GHL, Germany), and the temperature and pH data were monitored and recorded continuously. The aquaria seawater conditions were adjusted accordingly via the computerized system, and CO_2 was bubbled independently into each aquarium on demand, given the feedback of the pH and temperature sensors. The OA treatments were generated via CO_2 bubbling into the corresponding tanks using custom-installed solenoid valves, and CO_2 dissolution and dispersion was achieved via one of the pumps in each of the tanks. The pH values in this study are always expressed here on a total scale. In addition, total alkalinity (TA) was assessed during the experiment, every 2–4 days the first two weeks and every 1–2 weeks thereafter. TA was measured via open-cell potentiometric titrations using a titrator (TitroLine 7000, SI Analytics, Germany) together with a glass pH electrode (A 162 2M-DIN-ID, SI Analytics, Germany), after the protocols by Riebesell et al. (2011) and the SOP3b protocol by Dickson et al. (2007). Subsequently, TA was calculated using the modified Gran approach (Millero, 2013). Alkalinity was monitored daily and controlled by an alkalinity controller (Alkatronic, Focustronic, Hong Kong) coupled with two automatic in-house constructed calcium reactors (pH 6.2–6.4, coral rubble) and dosing of $NaHCO_3$. The seawater carbonate chemistry was calculated using the program CO2SYS (v25; Pelletier, Lewis & Wallace 2007), with carbonic acid dissociation constants from Mehrbach et al. (1973) and refit by Dickson and Millero (1987). A summary of the seawater chemistry parameters in our experiments can be found in Table 2.1.

Table 2.1 | Seawater chemistry parameters during the OA experiment. Summary of the sweater chemistry parameters over the acidification exposure period. Values are shown as mean ± SD with their corresponding measurement replication (n). The listed abbreviations for some parameters indicate: pH_T, pH on the total scale; TA, total alkalinity; pCO_2, partial pressure of CO_2; DIC, dissolved inorganic carbon; Ω_{ca}, calcite saturation; Ω_{ar}, aragonite saturation.

	Control	Ocean Acidification
Salinity	34.6 ± 0.4 (10)	34.7 ± 0.4 (10)
Temperature (ºC)	25.9 ± 0.3 (1,736)	25.9 ± 0.2 (1,503)
pH_T	7.98 ± 0.13 (1,736)	7.78 ± 0.13 (1,503)
Daily Minimum pH_T	7.79 ± 0.12 (10)	7.60 ± 0.12 (10)
Daily Maximum pH_T	8.19 ± 0.04 (10)	8.01 ± 0.06 (10)
TA (µmol kg^{-1})	2,155 ± 52 (47)	2,155 ± 60 (44)
pCO_2 (µatm)	480 ± 171 (1,736)	813 ± 286 (1,503)
Daily Minimum pCO_2 (µatm)	244 ± 31 (10)	413 ± 67 (10)
Daily Maximum pCO_2 (µatm)	769 ± 195 (10)	1,262 ± 330 (10)
DIC (µmol kg^{-1})	1,906 ± 76 (1,736)	2,009 ± 74 (1,503)
CO_2 (µmol kg^{-1})	13 ± 5 (1,736)	23 ± 8 (1,503)
HCO_3^- (µmol kg^{-1})	1,709 ± 109 (1,736)	1,857 ± 94 (1,503)
CO_3^{2-} (µmol kg^{-1})	184 ± 43 (1,736)	129 ± 33 (1,503)
Ω_{ca}	4.45 ± 1.05 (1,736)	3.13 ± 0.81 (1,503)
Ω_{ar}	2.94 ± 0.69 (1,736)	2.07 ± 0.53 (1,503)

Note: This table has been modified from its available version online in Martins et al. (2024).

Two conditions were implemented in the experimental system, one corresponding to the control and the other to the OA treatment. The OA treatments and controls were arranged within the experimental system using a semi-randomized design, with four aquaria acting as control and four as the high pCO_2 condition, respectively. The OA treatment (also referred to through the text as high pCO_2 treatment) was implemented according to the expected RCP 8.5 scenario for the year 2100 and following a diel diurnal-nocturnal oscillation pattern as it occurs in natural environments, with a diel range of 0.4 pH units (see exemplary Figure 2.2). On the other hand, the control condition was stablished to simulate the non-acidified present scenario. The high pCO_2 treatment corresponded to 7.78 ± 0.13 (mean ± SD; daily range: 7.60–8.01) and the control pH condition to 7.98 ± 0.13 (mean ± SD; daily range: 7.79–8.19). The control pH

condition remained significantly higher than the high pCO_2 treatment during the 3.3 months of OA exposure (LMM-ANOVA, F = 298, p < 0.001).

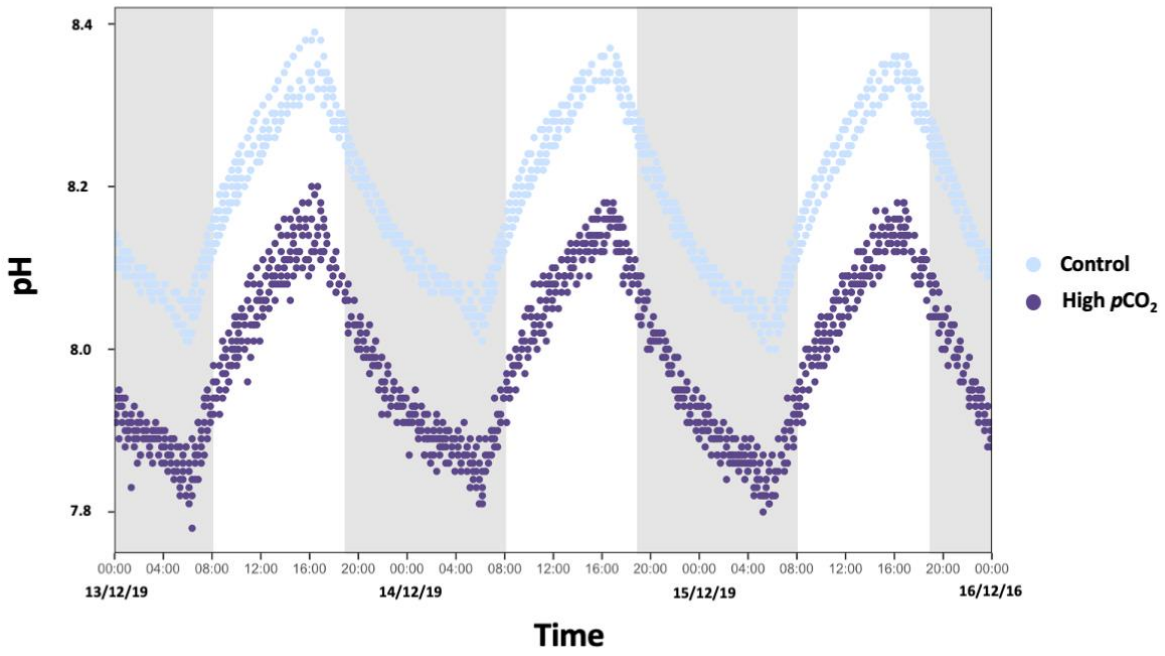

Figure 2.2 | Exemplary pH behavior in the control and OA treatments depicting diurnal-nocturnal oscillation. The data illustrates the pH treatment and control tank trends (n=8, with 4 tanks per condition), which remained significantly different across the whole extent of the experiment (LMM, p < 0.001). Shaded areas indicate night-time and the control condition is shown in light blue, while the high pCO_2 treatment is in dark blue.

Experimental timeline and metrics evaluated

After the healing period, the fragments were transferred to the experimental system on October 15th, 2019, where they remained under 11:13 light:dark photoperiod, light intensity of 176 ± 31 µmol m^{-2} s^{-1}, water temperature of 26 °C and salinity 35. The fragments underwent acclimation for 37 d before decreasing the system pH to simulate the acidification scenarios. The pH was gradually decreased by 0.01 – 0.02 units daily for 15 d until reaching the decreased pH target by December 4th, 2019. After that, the coral fragments remained exposed to the OA for 100 d before their assessment using PAM on March 12th, 2020, and the final photographs were taken on March 21st for final growth assessments. After concluding the measurements, the corals in the system were reserved or frozen in case of additional measurements before ending the experiment

entirely not long after. Moreover, the metrics evaluated through our experiments included mortality and bleaching incidence, the P:R ratios as a proxy of metabolic balance between photosynthesis and respiration (and thus as an assessment of physiological performance), photosynthetic efficiency, which was measured as a proxy of coral health and productivity, and coral growth. The experimental timeline in Figure 2.3 shows data acquisition time points and their frequency per metric.

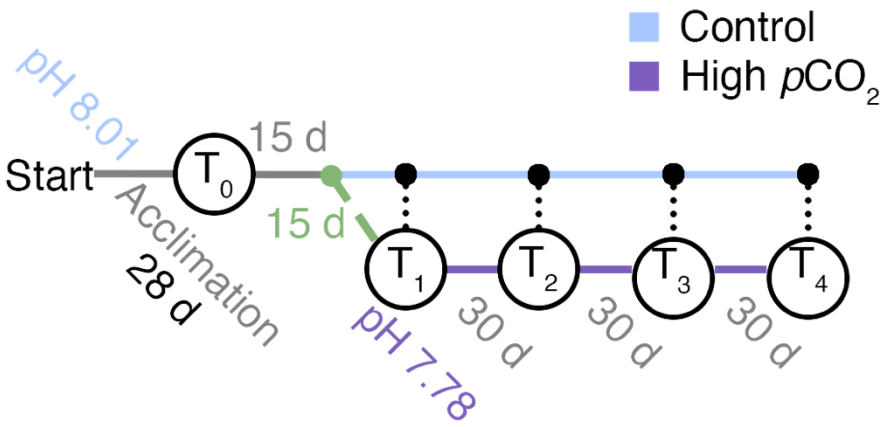

Figure 2.3 | OA Experimental timeline. The schematic overview of the complete experiment shows the most relevant time points since the "start" of the experiment, when the coral fragments were first transferred into the experimental system by October 15th, 2019, after completing their healing period. After that, the octocorals underwent an acclimation period and the first data acquisition for the P:R ratio, *Xenia* pulsation and growth metrics was performed (T_0). Upon completing the first data acquisition, the system pH was decreased in the high pCO_2 treatment tanks over 15 days, starting November 21st, 2019. The treatment target pH was reached by December 4th, followed by the first data acquisition time point under OA conditions (T_1). Data acquisition was performed again after reaching the time points corresponding to T_2 and T_3 for the mentioned metrics, except for *Xenia* pulsation, which was assessed with a higher frequency over time. Finally, a PAM data set and photographs were acquired for the metrics corresponding to photosynthetic efficiency and growth during T_4 on March 11th and 12th, 2020 and March 21st, 2020, respectively.

Mortality, bleaching and P:R ratios over time

All coral fragments within the experiment were visually inspected, and their health status was recorded daily to assess fragment mortality and bleaching. Tissue integrity and behavioural cues were also followed over time, and photographs of the individuals in the experiments were taken monthly after each incubation session, starting from the

acclimation period. These methods guaranteed a non-destructive approach, allowing us to follow all individual fragments throughout the experiments. For this study, coral death was defined as the cessation of respiratory activity together with the occurrence of tissue dissolution and coral bleaching was defined as the complete whitening of the coral fragments accompanied by impaired photosynthetic activity.

The P:R ratio of the coral fragments in our experiments was calculated after measuring the photosynthetic and respiration rates of three coral fragments per aquaria of each genus over time through light and dark incubations, following the protocols by Herndl & Velimirov (1986) adapted for octocorals in Bednarz et al. (2012) and Simancas-Giraldo et al. (2021). The incubations were performed monthly, starting during the acclimation period and were completed over four days, one coral genus at a time. On each day of incubation, three selected fragments were chosen from each aquarium and placed into 151 mL jars sealed airtight with seawater from the same aquaria. The incubated fragments were always the same ones throughout the whole experiment. Moreover, a control jar containing a coral holder without fragments was always included in each aquarium to monitor planktonic background metabolism. At the beginning and end of every incubation, the dissolved oxygen concentration in these jars was measured using an optical oxygen sensor (FDO 925, WTW, Germany) and recorded with temperature measurements. Additional oxygen concentration, temperature, and salinity measurements within each of the experimental aquaria were performed before each incubation session. The light and dark incubations took place within a room adapted for this purpose and immersed in water baths equipped with temperature controllers (STC-1000 digital) connected to thermostats and pumps (1W RESUN SP500), providing continuous water flow to maintain the incubation jars at a constant temperature of 26 °C. The water baths had their own illumination source, consisting of white lamps that provided relatively even illumination during the light incubations, comparable to that of the experimental system.

Furthermore, magnets were arranged directly under each of the water baths so that they drove motion for stirrers added inside the jars during the incubation period, ensuring constant water motion during the measurements. The Light incubations were

performed at least 2 h after turning on the lights in the system. In contrast, the dark incubations were performed on the same day, in the afternoon, after the light incubations, and under complete darkness. The incubation times were adjusted depending on genus, fragment size and oxygen consumption rates, always avoiding oxygen saturation and within a 0.5 to 2.5 h time frame. We calculated the photosynthesis and respiration rates using the differences between the end and start oxygen concentrations of the dark and light incubations to calculate the P: R ratios. These values were further corrected by jar volume, incubation time, and planktonic background by subtracting the oxygen concentration of the control jars without fragments from those containing fragments per tank. The P:R ratios were ultimately obtained by dividing the measured photosynthesis over the respiration rates, performing the calculations after Krueger (2019).

Photosynthetic efficiency

We assessed the photosynthetic performance of five representative fragments of each genus per aquaria. These measurements were executed through 2 d during March 11th and 12^{th,} 2020, after approximately 100 d of exposure to the OA treatment (i.e. from December 4th to 12^{th,} 2020). For these specific measurements and practical reasons, the coral fragments of each genus were briefly brought (five fragments at a time) into a provisional small aquarium (20 x 30 cm) filled with approximately 8 L of seawater taken from one of the control aquaria. The provisional aquarium was equipped with a small submersible pump (1W RESUN SP500) to ensure water circulation and a heater connected to an STC-1000 digital temperature controller and set to 26 °C. Additional white, fluorescent lamps installed over the aquarium provided light intensity at the measurement spot of ~160 µmol m^{-2} s^{-1}. Each fragment was measured with a PAM-2500 chlorophyll fluorometer (Walz, Germany) and using the following settings: measuring light Intensity = 6, saturation pulse intensity = 8, saturation pulse wide = 80, gain = 2 and damping = 2. All measurements were procured at the same measurement spot on the fragments and on comparable regions between fragments of each genus. For every measurement, a constant distance between the fragment and the optical fibre was ensured via coupling a small plastic tube cut diagonally to create a 45° angle with respect

to the optic fibre. Thus, the minimum and maximum distance from the PAM optical fibre to the tips were 2.8 cm and 3.4 cm, respectively. Before every measurement session, we performed a correction for background fluorescence noise, pointing the probe at nothing while immersed within the seawater contained in the provisional aquaria. For each genus, maximum photochemical efficiency Fv/Fm and the effective photosynthetic efficiency YII were assessed 1 h after lights were turned off in the system to ensure the fragments were in the dark-adapted state before being measured. The measurement strategy for each coral genus differed. For *Xenia*, the coral fragments were measured ensuring complete tissue coverage within the measurement area of the optical fiber and always including polyps within the said area.

On the other hand, for *Sinularia* corals, the optical fibre generally covered almost the whole area of the fragments, given their small sizes. Regarding the branching octocorals, *Plexaurella* and *Pinnigorgia* were always measured approximately 1 cm under the tip of a selected branch, and particularly for *Pinnigorgia*, given its thin branch surface, the branch was always laid on top of a black surface card providing a non-reflective background during the measurement. All the fragments were returned immediately after being measured and placed in their corresponding aquarium.

Pulsation

Several xeniids present a conspicuous pulsation behaviour reserved for several species within this group. The *Xenia* fragments in our experiments presented this specific behaviour, which we evaluated over time as a non-invasive response metric. Thus, *Xenia* fragment pulsation was visually recorded for five of the 10 colonies available within each of our aquaria in the experimental system and followed over time for the same individuals and during the whole experiment. Pulsations were visually counted on individual polyps of these fragments for 60 s each, and the number of pulsations was recorded regularly, and always by mid-day, to ensure they were at peak activity during the assessment. The fragments remained inside their corresponding aquaria and were unperturbed during the counting sessions.

Coral growth

Coral growth was evaluated using the photographic records three fragments per genus and aquarium. We used the photographs taken at the start of the experiments during the acclimation period in November 2019 and the last set of photographs taken in March 2020, after 109 d of exposure to the OA treatment. The fragments were photographed using a fixed professional setup with even illumination from a lateral view for all genera and an additional 90° top view for *Xenia* and *Sinularia,* given their growth morphologies. The photographs were taken using a Nikon D7000 camera, and the setup and acquisition parameters were always the same. We also ensured the same constant distance from the camera to the coral fragments, which were immersed briefly in another provisional small aquarium for each photograph. The fragments were placed within the provisional aquaria using seawater from one of the acidified aquaria for the fragments belonging to the high pCO_2 treatment and from one of the control aquaria for those fragments belonging to the control treatments. The photographs were taken, ensuring an immersion period as short as possible, and water was replaced accordingly to avoid any sharp water chemistry changes or temperature loss during the photographic sessions. Moreover, for every photograph, we included an accompanying size and colour reference to be used during the subsequent image analysis, and the photographs corresponding to the selected time points were processed using the open-source software FIJI (Schindelin et al., 2012).

To compare across the different genera, we calculated a geometrical approximation of the surface area for the fragments of each genus, adjusting the most suitable geometric form according to the genus growth morphology and architecture. The geometric forms used for modelling the surface area of each genus were proposed using our own modified version of the geometric approach by Naumann et al. (2009) for hard coral surface area quantifications. The numerical values for the geometric parameters composing the surface models were directly obtained from the measured photographs. We measured fragment height and ratio for the genera *Xenia* and *Sinularia,* fragment height and width for the genera *Pinnigorgia* and *Plexaurella*, given the radial growth of the first and the branching morphologies of the last. Finally, we evaluated coral growth

for each genus in terms of the delta in surface area between the two-time points assessed and compared the growth of the fragments between the control condition and the high pCO_2 treatment per each genus and between them.

Statistical data analysis

To evaluate significant differences in the metrics assessed through our experiments, we implemented Linear Mixed-effects Models (LMM). These models were fit per each metric employing the package Lme4 from Bates et al. (2015) and were built considering different fixed factors in accordance with each metric. For instance, for evaluating the P:R ratios, the LMM models considered as fixed factors: treatment (2-level factor, control and high pCO_2), genus (4-level factor, *Xenia*, *Sinularia*, *Pinnigorgia* and *Plexaurella*), growth morphology (2-levels factor, branching and encrusting), time (i.e. corresponding to the data acquisition time points) and the interaction between the factors treatment and time. Regarding the LMM model generated for the PAM data, only the factors treatment, genus, and growth morphology were considered, while for *Xenia* pulsation, the factors considered were treatment, time, and the interaction between both factors. Finally, for the statistical model corresponding to the growth assessment, we considered the factors of treatment, genus, the interaction between both, and the factor growth morphology. For all the models described, the factor aquaria ID was defined as random, together with the factor individual for the model built for coral growth. Model diagnostics and fit were evaluated using residual plots, while model sensitivity was tested through data leverage. Model selection was made via direct model comparisons, loglikelihood and AIC selection criteria assessment. Factors not contributing to the models' explained variance were removed after the model comparison steps. Statistical significance was determined using ANOVA types II and III accordingly (Zuur et al., 2009) and defined for every analysis as $p < 0.05$. Whenever significant differences were found, Post hoc tests were implemented (see our supplementary information). All statistical analyses and graphs within this study were carried out using the computational software R version 3.5.2 (R Development Core Team, 2013).

2.4 Results

During the whole extent of our experiments, no mortality or bleaching occurred due to the acidification treatments for any of the octocoral genera evaluated. In addition, all corals seemed visually healthy without any conspicuous changes on their pigmentation, or any compromised structures. Their overall behavior also suggested no particularly negative response towards the treatments from the direct visual assessments. Regarding the P:R ratios (Figure 2.4), despite during the base line time period corresponding to T_0 *Xenia* and *Plexaurella* showed differences between the control and high pCO_2 treatments, both were comparable between the OA treatment and control through T_1 to T_3 (LMM; $P > 0.05$). Remaining as net autotrophs by T_3. These results were also supported by consistent photosynthetic efficiency above 0.5 indicating healthy and net photosynthetic coral fragments for these genera by T_4 together with no statistically significant differences between control and high pCO_2 treatment for this parameter (LMM; $P > 0.05$; Figure 2.5). Both genera also showed copious and consistent positive growth overtime (see our supplementary information), especially conspicuous for *Xenia*. In addition, although there were significant differences between control and the high pCO_2 treatment for *Xenia*'s pulsation rates overtime with the high pCO_2 treatment becoming progressively lower (LMM; $P \leq 0.05$; Figure 2.6), their order remained within the 40 and 30 pulsations per minute, which is considered within the range of healthy Xeniid pulsation behavior.

Furthermore, the octocoral genera Pinnigorgia showed no significant differences between the control and the high pCO_2 treatment for either its P:R ratios or the photosynthetic efficiency assessments (LMM; $P > 0.05$; Figure 2.4 and 2.5), although the P:R ratios for the high pCO_2 treatment suggested the fragments were under compensation, the PAM assessments suggested otherwise, while growth remained positive and did not suggest outstanding differences between control and the OA treatment. Although more profuse branching and bigger colonies seemed to occur more often in the control condition by the end of the experiment.

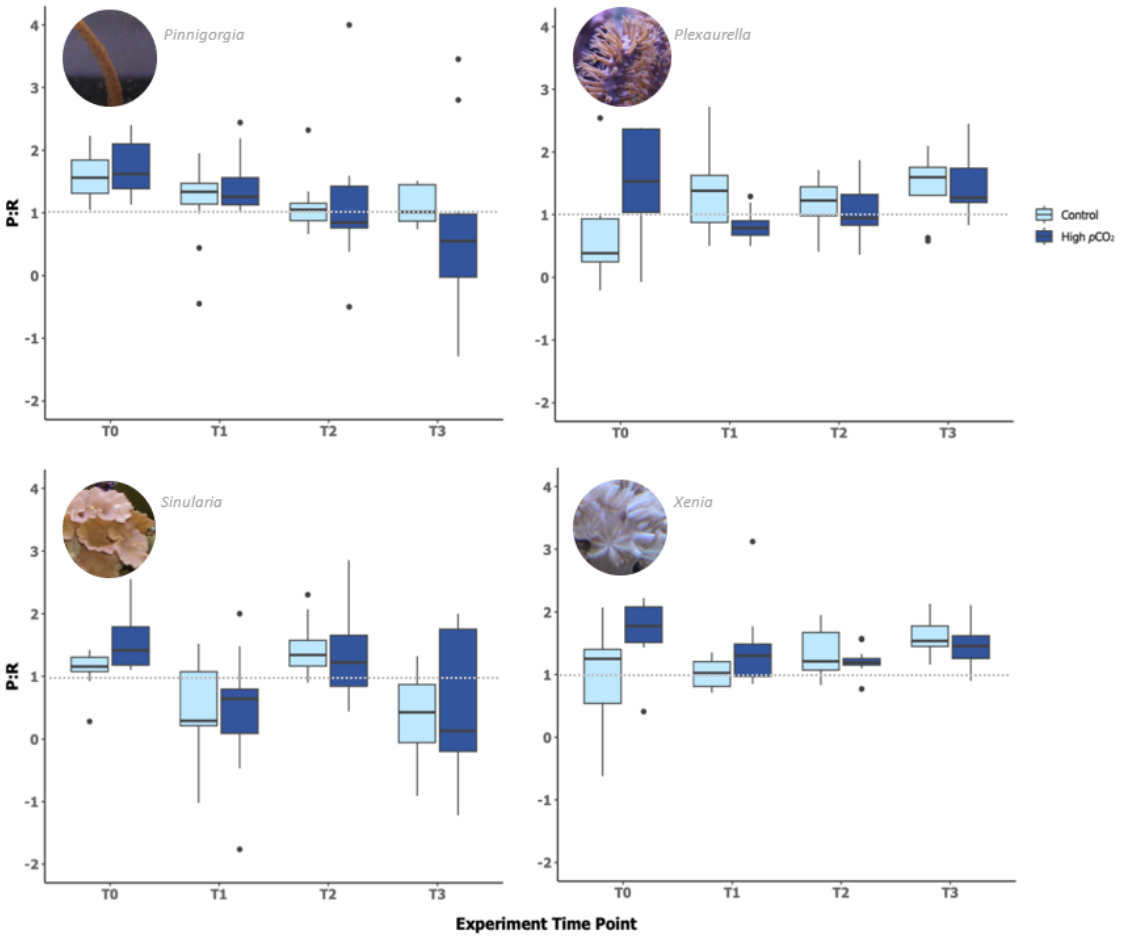

Figure 2.4 | Daily integrated P:R ratios under high pCO_2 for the four octocoral genera *Pinnigorgia*, *Plexaurella*, *Sinularia* and *Xenia*. The P:R behavior over time is shown for the genera assessed. The dotted line highlights the threshold for autotrophy (P:R = 1). The boxes show the control condition (light blue) and OA condition (dark blue). The data depicts n = 4 replicates per treatment, with 3 coral fragments measured per tank in each treatment.

Finally, the genera *Sinularia* which showed the slowest growth and relative smaller sizes through the experiment (and at its end) when compared to the other genera, did not present a consistent pattern in its P:R ratios over time, which may explain the significant differences observed for this genus in the observed P:R response. The fragments of this genus seemed to alternate between compensation and net autotrophy showing a P:R inconclusive pattern which explained the significant differences observed over time (LMM; $P \leq 0.05$; Figure 2.5). On the other hand, although the photosynthetic efficiency assessment showed these fragments were net photosynthetic by T_4 (Figure 2.5), their growth remained modest.

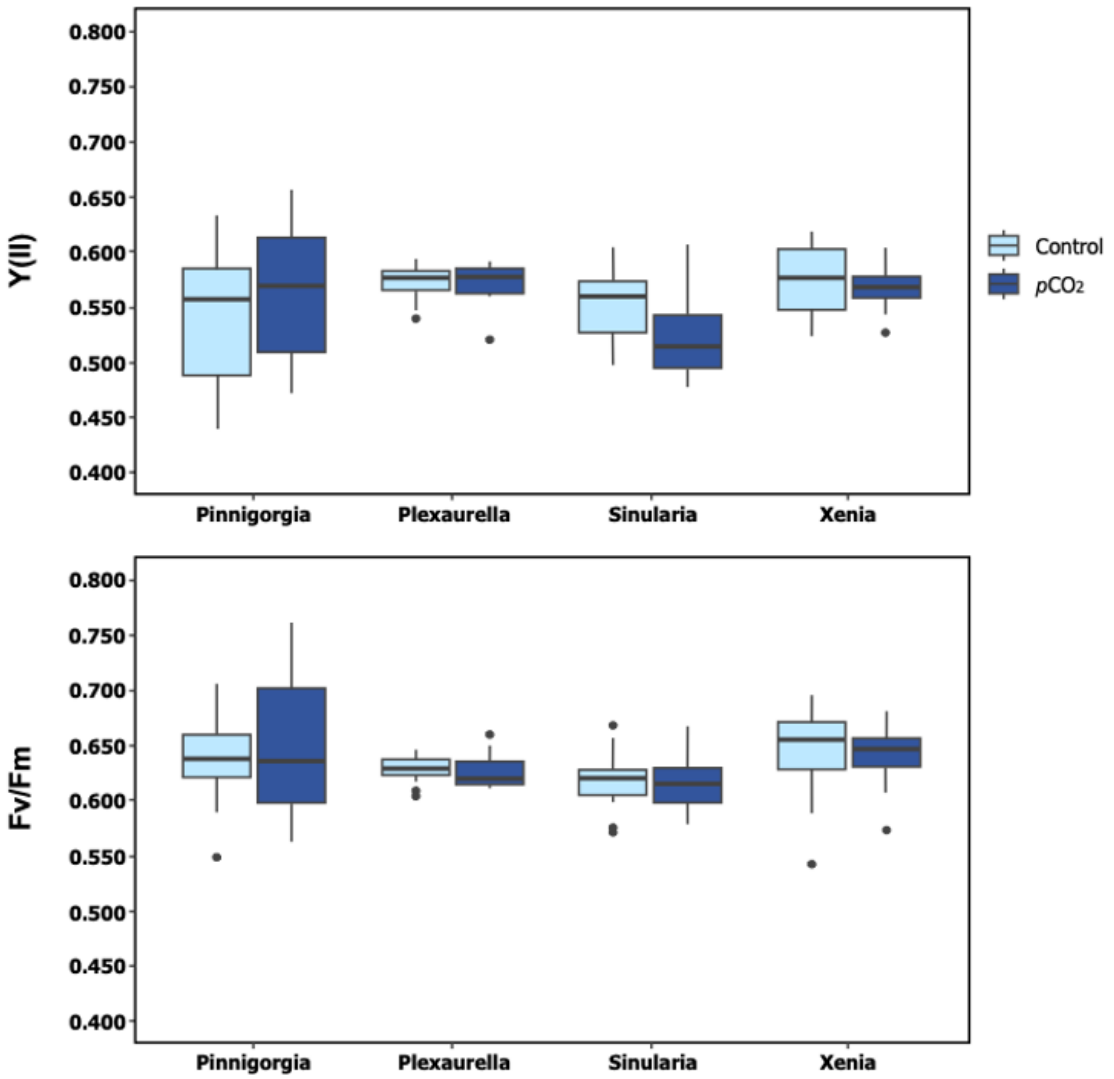

Figure 2.5 | Photosynthetic efficiency for the octocoral genera assessed. Fv/Fm and Y(II) after 3-month OA exposures are shown for the octocorals Pinnigorgia, Plexaurella, Sinularia and Xenia. 5 coral fragments were assessed per each genus (treatments n = 4). F_v/F_m values above 0.5 can be considered as indicator of good coral health.

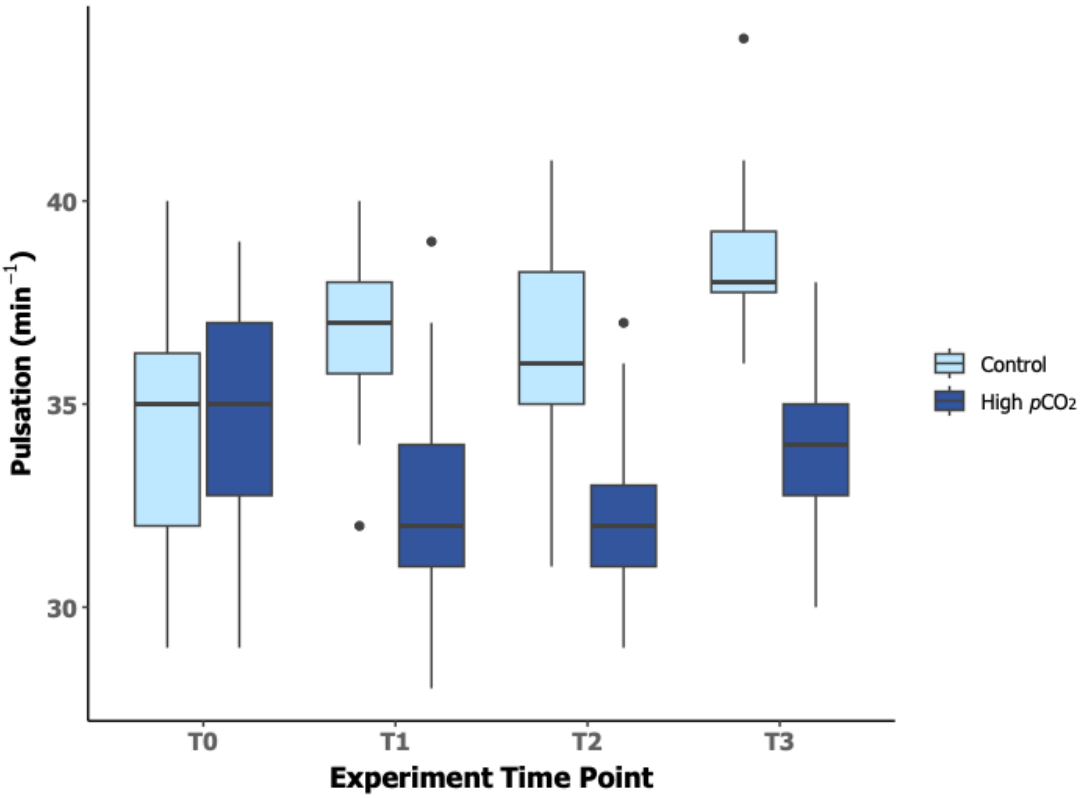

Figure 2.6 | *X. umbellata* pulsation rates under OA conditions. Pulsation rates over the span of a minute were evaluated periodically through the experiment. The assessment was performed for a total of 3 coral fragments per tank. The color codes indicate the control condition in light blue, and the high pCO_2 treatment in dark blue.

2.5 Discussion

The findings in our study confirmed not only octocorals potential tolerance towards OA and a degree of variation in their response being specific to their different growth morphologies and biological traits , but also a contrast to what has been reportedly found for hard corals in general under OA scenarios, which being strong calcifiers exhibit mostly large and negative effects of OA across varied metrics, particularly, calcification, growth, and photosynthesis (Kroeker et al., 2010; Comeau, Carpenter & Edmunds, 2017), despite the fact of some hard coral species remain particularly resistant towards this factor (Barkley et al., 2017; Krueger et al., 2017).

In addition, in this study we showed that over the extend of our experiment, octocorals photophysiology and growth remained ultimately resilient to the effects of OA across different genera, and this may be transferable to several groups within the broad phylogenetic spectrum available within octocorallia. The potential implications of this research could offer new insights into the resilience of coral reefs in the face of increasing ocean acidity.

Effects of ocean acidification treatments on Octocorals over time

Overall, the OA treatments did not have any particularly detrimental impact on the octocoral genera evaluated, in terms of their health overtime, and results of our study align with the general notion that octocorals seems particularly resistant to this stressor (Gabay et al., 2014; Lasker et al. 2020). In general, there are diverse views regarding potential resistance of octocorals towards global stressors, where there is a significant variability in their responses for instance to stressors such as ocean warming, where octocoral can vary drastically across regions in their sensitivity towards stress (Fabricius et al., 2011; Osman et al., 2018). However, regarding ocean acidification, reported octocoral responses seem to converge to higher tolerance of these organisms towards this stressor (Lasker et al., 2020;), and our findings of the present study contributes as well to this notion. For instance, field studies have shown that octocorals can be highly resistant to ocean acidification, even below the calcite saturation point (Sánchez et al., 2019). This resistance is further supported by other findings, suggesting that octocorals can acclimate and withstand rising levels of ocean acidification, even under conditions that exceed what is expected to occur by the end of the present century (Inoue et al., 2013; Gabay, Benayahu & Fine, 2013).

As suggested, there was no mortality or bleaching for any of the octocoral genera evaluated contrasting with many of their tropical coral-reef relatives (Lopes et al., 2018) and hard corals (Kroeker et al., 2010). In hard corals, high pCO_2 may induce coral bleaching although the mechanisms through which this occurs remains still understudied (Albright, 2018). However, coral mortalities and their frequencies give a clear message regarding OA as coral reefs threatening factor (Doney et al., 2009). Our

findings are in agreement with other studies showing that octocorals, such as those in the Caribbean, may exhibit a lower susceptibility to bleaching compared to scleractinian corals, and when bleaching does occur (Coffroth et al., 2023), however the mechanisms of bleaching in this groups of organisms is seldom observed in field or recorded and also remains relatively obscure (Prada, Weil & Yoshioka, 2010; Lasker et al., 2020), since it can be observed as well in field that sensitivities may vary even among corals in sympatry, and that in some species particularly pigmented, ongoing bleaching processes may be masked by their own host pigments (Coffroth et al., 2023). In the corals from our study, the various photosynthetic activity assessments we performed confirmed that bleaching did not occur in our experiments, and that all genera that we exposed to the OA treatment remained relatively insensitive to it especially in terms of their photophysiology and growth.

Comparative responses to ocean acidification across genera

In this study, we hypothesized that octocorals possess inherent resilience to OA, but their capacity for tolerance and resistance would vary based on their biological and physiological features. Here, we found they were above all resistant to this factor, nevertheless, differences intrinsic of each genera suggested that under OA scenarios each group may count with a diverse set of advantages under these scenarios. For instance, our results aligned to those of Enochs et al. (2016) where we did not find particular detrimental effects of OA on the growth of the branching genera *Pinnigorgia* and *Plexaurella*, respectively, while our findings also support the findings by Gómez et al. (2014) where coral growth is maintained under OA conditions. Both species from those studies constitute relatively fast growers among branching species as well as *Plexaurella* does. Within this study we found that the relatively slower growth of species like *Pinnigorgia* may represent a disadvantage when competing with other fast-growing species. Nevertheless, both will have potential to outcompete most hard corals being slow and generally sensitive to OA (Kroeker et al., 2010; Lopes et al., 2018). In addition, all these species have the potential to colonize space three dimensionally and conquer spaces in the water column that hard corals may not be able to reach depending on their growth morphologies (Edmunds et al., 2014; Lasker et al., 2020). All this suggests

additional advantages for these corals that will play a role as contenders in the acidified reefs of the coming future. Moreover, the high photosynthetic metabolism that *Xenia* portraits, aided by its pulsing behavior and summed to its high growth rates suggest that this species will represent the actual strongest contender among the octocorals assessed, given its photosynthetic metabolism and growth being impervious to the effects of OA. On the other hand, *Sinularia* corals, although also resistant to the negative effects of OA, do not portray particular advantages that allow it to specially outcompete other species.

Furthermore, it is important to highlight that intraspecific variability within groups is also highly relevant in the context of OA. In general, observed responses to OA stress has been also illustrated as well when comparing studies on the effects of OA on e.g. the coral *H. fuscescens* which showed discrepancies in its pulsing behavior between studies (Sprung & Delbeek, 1997; Gabay, Benayahu & Fine, 2013). On one hand, Sprung & Delbeek (1997) reported complete cessation of its pulsing behavior when this octocoral was subjected to acidified pH values, while Gabay et al. (2013) described that in contraposition to these findings, no changes in pulsing behavior occurred when the corals were exposed to acidified conditions. Pulsing behavior physiology has been uncovered in detail and found to benefit this octocoral through enhancing its photo-physiological processes (Kremien et al., 2013), where this behavior can aid coral health maintenance under stress (Vollstedt et al., 2020). Our study contrasts some of these findings by supporting no OA effects on for instance *X. umbellata*. However, our study highlights that, variations in physiological response metrics at intra- and inter-specific levels, and in particular variation in sensitivities to OA depending on biological traits specific to taxonomical groups (e.g. pulsation, growth morphologies, branching patterns) deserve further attention as they may underpin important implications at the level of ecosystem responses (Kroeker et al., 2010). Finally, conducting more comparative evaluations across diverse octocoral genera to OA, may further support us in identifying which other groups are better suited to withstand its effects, potentially making them better competitors and thus, a potential layer of complexity that may persist in the face of the present increasing ocean acidity and in the long term, in the future reefs (Dupont & Pörtner, 2013a; Lasker et al., 2020).

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Chapter 3

Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic carbon eutrophication

First author contribution

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Photosynthesis and respiration of the soft coral *Xenia umbellata* respond to warming but not to organic eutrophication

Susana Marcela Simancas-Giraldo[1], Nan Xiang[1,2], Meghan Moger Kennedy[1], Rassil Nafeh[1], Edoardo Zelli[1], Christian Wild[1]

[1] Marine Ecology Department, Universität Bremen, Bremen, Germany
[2] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research for Polar and Marine Research, 27570 Bremerhaven, Germany

Corresponding Author:
Susana Marcela Simancas-Giraldo[1]
Email address: sussim@uni-bremen.de

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3.1 Abstract

Eutrophication with dissolved organic carbon (DOC) as a far under-investigated stressor, and ocean warming, can strongly affect coral reefs and hard corals as major reefs ecosystem engineers. However, no previous studies have investigated the metabolic responses of soft corals to DOC eutrophication or its interaction with ocean warming. Thus, we investigated respiration and photosynthesis response of *Xenia umbellata*, a common mixotrophic soft coral from the Indo-pacific, to (1) three levels of DOC eutrophication simulated by glucose addition over the first 21 days of the experiment and (2) ocean warming scenarios where the temperature was gradually increased from 26 °C (control condition) to 32 °C over another 24 days in an aquarium experiment. We found no significant difference in response to DOC treatments and all corals survived regardless of the DOC concentrations, whilst subsequent exposure to simulated ocean warming significantly decreased gross photosynthesis by approximately 50 % at 30 °C, and 65 % at 32 °C, net photosynthesis by 75 % at 30 °C and 79 % at 32 °C, and respiration by a maximum of 75 % at 30 °C; with a slight increase at 32 °C of 25 %. The ratio between gross photosynthesis and respiration decreased by the end of the warming period but remained similar between controls and colonies previously exposed to DOC. Our findings suggest that soft corals may be more resistant than hard corals to DOC eutrophication and in consequence, may potentially experiment in less magnitude the negative effects of increased temperature or subsequently both stressors. The results of this study may contribute to explaining the successful role of soft corals in phase shifts, as reported from many coral reefs where predicted declines in reef ecosystems' health due to increased eutrophication levels can be exacerbated by future warming.

3.2 Introduction

Coral reefs are among the most important ecosystems on earth, providing a wide variety of ecological services of high importance for human development and survival (Connell, 1978;

Hughes et al., 2003). However, these ecosystems face multiple global (Doney et al., 2009; Hughes et al., 2017) and local stressors (Jackson et al., 2001; Dubinsky & Stambler, 2011; Mora et al., 2011; Lamb et al., 2018) that threaten their functionality, biodiversity, and survival. Evidence of the negative impacts of organic eutrophication and warming as local and global stressors has been widely reported for coral reef benthic communities worldwide (Walther et al., 2002; Hughes et al., 2003; Walther, 2010; Fabricius et al., 2013). In particular, eutrophication as dissolved organic carbon (DOC) has been shown to cause a direct effect on the coral holobiont through destabilization of the relationships between symbiotic algae, microbial community, and the coral host (Szmant, 2002; Kuntz et al., 2005; Kline et al., 2006; Pogoreutz et al., 2017); while DOC can also indirectly threat coral reefs by the alteration of trophic relationships in reef communities (Haas et al., 2016). Besides, thermal stress due to global warming poses a growing threat to global reefs, causing hard coral bleaching and mortality (D'Angelo and Wiedenmann, 2014; Hughes et al., 2018), as sea surface temperature has risen rapidly in the past 50 years and is expected to continue rising (Poloczanska et al., 2016; Chaidez et al., 2017; Hughes et al., 2017). Under the current business-as-usual trend, warming is likely to reach an increase of 1.5 °C between 2030 and 2052 (de Coninck et al., 2018). Thermal stress already causes depressed coral colony growth, lower reproduction rates (Baird & Marshall, 2002), and high coral mortality (Hughes et al., 2003; Walther, 2010), where these events have become more frequent and widespread during the past few decades (Baker, Glynn & Riegl, 2008).

Previous reports have shown that there is a worldwide trend of decline in coral reefs, with the possibility of losing 60 % of global reefs by 2030 (Hughes et al., 2003; Bellwood et al., 2004). However, the available evidence indicates that, at a global scale, reefs may undergo significant changes in response to climate change rather than disappear entirely (Hughes et al., 2003). Coral reef dominance will most probably change, favouring the species that are more resistant to local and global stressors in future scenarios (Alvarez-Filip et al., 2013). Moreover, under ocean warming predicted for the end of the century and water pollution, phase-shifts favouring soft corals dominance could deeply transform these ecosystems

(Alvarez-Filip et al., 2013; Inoue et al., 2013; Baum et al., 2016), given the differential functionality and environmental complexity that soft corals provide (Sánchez, 2016). The current evidence shows that soft coral abundance in most regions worldwide either increased or maintained, while in contrast, hard coral cover has declined over the last decades (Stobart et al., 2005; Lenz et al., 2015). Such phase shifts, associated with changes in bottom-up dynamics, can become stable through positive feedback mechanisms (Norström et al., 2009). In consequence, soft corals already represent the most abundant and species-rich order of octocorals on Indo-Pacific coral reefs (Fabricius, 2011), and in some areas, their density can equal or exceed that of hard corals (Fabricius et al., 2011; Lenz et al., 2015).

Soft corals' ability to thrive with relatively high abundances and diversity under different and harsh environmental conditions (Fabricius & De'athDe'ath, 2008) can be explained by their particular ecophysiological characteristics. In specific, soft corals portray mixotrophic species with high nutritional plasticity (Fabricius & Klumpp, 1995; Baker et al., 2015; Schubert, Brown & Rossi, 2016; Rossi et al., 2018) where mixotrophic corals can acquire nutrients via their symbionts' autotrophic activity and host heterotrophy (Fabricius & Klumpp, 1995).

While there are cases where mixotrophy in hard and soft corals can be comparable (Conti-Jerpe et al., 2020), it is generally accepted that soft corals show lower dependency on autotrophy compared to hard corals in both shallow and deep water (Fabricius & Klumpp, 1995; Schubert, Brown & Rossi, 2016; Pupier et al., 2019), together with high variability in the contribution of heterotrophic versus autotrophic nutrition (Schubert, Brown & Rossi, 2016). This high plasticity concerning trophic strategy results in critical physiological differences in carbon acquisition mechanisms (Fabricius & Klumpp, 1995; Wild et al., 2004; Pupier et al., 2019) and ultimate energy allocation. Driving requirements for processes such as calcification (Allemand et al., 2011), growth (Lasker et al. 2003; Sánchez et al. 2004), and

reproduction, which significantly differ between both groups in terms of modes and frequencies (Kahng, 2011).

Despite their high relevance as part of the benthic communities on reefs and their high potential for resistance towards climate change stressors (Schubert, Brown & Rossi, 2016; Sánchez, 2016; Rossi et al., 2020), soft corals have been strongly neglected in comparison to hard corals, which has resulted in soft corals and their responses towards stress being little or poorly understood. For hard corals, studies suggest that elevated DOC can stimulate coral-associated microbes' proliferation, increasing coral hosts' vulnerability (Kuntz et al., 2005; Kline et al., 2006). Whilst exposure to organically enriched treatments can potentially reduce the resistance and resilience of hard corals to heat stress via impacts on coral metabolism due to changes in photosynthetic activity or respiration rates, causing bleaching and ultimately lower survival (Fabricius et al., 2013; Pogoreutz et al., 2017; Morris et al., 2019).

From the existing literature, there is little to no evidence of soft coral responses to such stressors, and it is surprisingly unclear if soft corals physiology may exhibit similar negative responses as observed for hard corals: towards increased DOC levels (Haas, Al-Zibdah & Wild, 2009; Pogoreutz et al., 2017), or warming (Al-Sofyani & Floos, 2013; Lyndby et al., 2018); or if they portray elevated or reduced thermal tolerance when simultaneously exposed to combined stressors (Fabricius et al., 2013; Pogoreutz et al., 2017). In our most recent study on this topic by Vollstedt et al. (2020), we found evidence suggesting that DOC addition had a positive effect on the heat tolerance of the soft coral *Xenia umbellata*. However, our assessment concentrated exclusively on functional and ecological variables such as pulsation rates and growth, while there is still limited knowledge about the main physiological processes taking place in these soft corals under both stressors.

It has been thoroughly reported that external nutrient availability, together with internal nutrient metabolism, can underpin the thermal tolerance of hard corals (Morris et al.,

2019). Therefore, this study addresses the question of how DOC addition, warming, or both stressors combined affect photosynthesis and respiration as proxies for the metabolic status of soft corals under stress while complementing the ecological findings from our previous study (Vollstedt et al., 2020). Here, we aimed to resolve (1) if DOC as simulated organic eutrophication may have a negative effect on soft corals (Kuntz et al., 2005; Kline et al., 2006; Smith et al., 2006) or if soft corals can cope with this stressor (Baum et al., 2016; McCauley & Goulet, 2019), and, (2) if simulated warming causes a negative impact on these corals, while under DOC exposure that may potentially enhance or diminish coral resistance (Pogoreutz et al., 2017).

Here, we investigated the effects of single DOC treatments and the subsequent addition of warming scenarios as a further stressor in a two-stage aquaria experiment, with a total duration of 45 days. We focused on a regression-based experimental design by testing multiple exposure levels, as previously suggested by Fabricius et al. (2013). We assessed the physiological responses of the soft coral *X. umbellata* as a model, a widespread species found in the Indo-Pacific and the Red Sea (Al-Sofyani & Niaz, 2007; Janes, 2013), in terms of its dark respiration rates, photosynthesis, and the P:R ratio.

3.3 Materials and Methods

Study Species and Sample Preparation

Species identification was performed according to the criteria by Reinicke (1997) and following the methodologies by McFadden et al. (2019). Thus, we carried out Sanger sequencing of two mitochondrial (mtMutS, COI) and one nuclear ribosomal (28S) gene PCR amplicons. The species identity was concluded to be *Xenia umbellata* (Xiang et al., 2020 unpublished data) and further confirmed based on Halász et al. (2019) and its original source, the Red Sea. *Xenia umbellata* (Lamarck, 1816) is a soft coral that belongs to the order Malacalcyonacea in the family Xeniidae. These pumping soft corals have been

previously described as mainly autotrophic, portraying a reduced gastrovascular cavity and enhanced photosynthetic activity (Schlichter, Svoboda & Kremer, 1983; Kremien et al., 2013).

Fragments of this coral were propagated from mother colonies previously kept in captivity for more than two years in advance to the start of our experiments. For this study, we included 160 propagated colonies with sizes ranging between 1 and 2 cm^2. Additional colony fragments of the same species (140 colonies) were present in the experimental system but reserved for a different study. Furthermore, each colony was attached to a coral frag plug (Aqua Perfect plug/ Round 1cm (AP-7004-0)) employing rubber bands and allowed to subsequently heal for at least 10 days before the start of the acclimation period. These colony fragments were initially maintained under controlled conditions at a main tank in the aquaria facilities, filled with artificial seawater at a salinity of 35 ± 0.2 ppt, pH of 8.2 ± 0.01, temperature of 25.6 ± 0.6 °C (mean ± SE), and exposed to a 12:12 photoperiod at constant light intensity. Once healed, the 160 colonies were subject to 11 days of acclimation and, subsequently, randomly distributed into the tanks of our experimental system, allocating ten colonies per tank.

During the experiment's total extent, the colonies were continuously monitored to assess and record their evolution over time and control for the occurrence of bleaching. For instance, we performed a swift check on the coral colonies' survival percentages, starting with the 10 initial colonies in each tank. From there on, the colonies were continuously tracked daily through visual and direct inspection of tissue firmness, colony aspect and colouration, together with photographs taken over time at the end of each incubation day. We accompanied these inspections with additional confirmation from our respiration rate records over time. On the other hand, we used the information collected on colony colouration and photographic confirmation as a qualitative approximation to bleaching. Observations were documented, for initial ease, as presence-absence of conspicuous signs of bleaching, understood as complete and prominent whitening of the coral colonies. We

collected additional notes on paler colourations on sight and further contrasted our visual inspections to photosynthetic activity records, aiming to evaluate in detail the colonies upon the occurrence of bleaching.

Experimental Setup

Our study took place at the research facilities of the Marine Ecology group in the Center for Environmental Research and Sustainable Technology (UFT) at the University of Bremen, Germany.

The experiment consisted of two phases: a first phase that included single DOC additions and a second phase that consisted of additional thermal stress treatments. The experimental assays were carried out on a close aquaria system, including 16 individual glass tanks, each with a total volume of 60 L. In further detail, each of these tanks was divided into two technical parts: a 50 L front part, housing the experimental colonies, and a 10 L back part functioning as a filtration tank or sump, holding a pump (EHEIM CompactOn 300 pump; EHEIM GmbH and Co. KG, Germany), a heater to manipulate and control temperature (3613 aquarium heater. 75 W 220-240 V~; EHEIM GmbH and CO. KG, Germany), a skimmer (EHEIM SkimMarine 100; EHEIM GmbH and Co. KG, Germany), and an outflow providing constant water exchange between the front and the back part of the tank. Illumination was provided by LED lights (Royal Blue – matrix module and Ultra Blue White 1:1 – matrix module, WALTRON daytime® LED Light, Germany) in an array of blue and white combination arrangements equally adjusted for each tank and at a constant mean intensity of 120.8 ± 10.2 µmol quanta m^{-2} s^{-1} and exposed to a 12:12 photoperiod. Moreover, the experimental tanks were filled with artificial seawater prepared with sea salt (Tropic Marin® ZooMix Sea Salt) free from synthetic additives, nitrates and phosphates and containing all trace elements found in natural seawater concentrations. The aquaria were maintained at equivalent conditions to those in the main tank with a salinity of 35.4 ± 0.4 ppt, pH of 8.2 ± 0.01, and temperature of 26 ± 0.4 °C (mean ± SE) during the first 21 days

before the increased temperature treatments. Salinity, temperature, and evaporation rates were monitored continuously three times per day and adjusted if required. Whilst additional chemical water parameters, such as the pH, KH, Ammonium (NH_4^+), Nitrite (NO_2^-), Nitrate (NO_3^-) and Phosphate contents (PO_4^{3-}), were measured and adjusted manually twice per week. Dissolved O_2 concentrations in the tanks were unaffected by the enrichment, remaining always higher than 6 mg/L. Further details on chemical parameters and their recorded values can be found summarized in Vollstedt et al. (2020) and Zelli et al. (2023). Furthermore, 10 % water exchanges were performed in the experimental tanks daily during the mornings, ensuring near-to-natural seawater parameters and maintaining algae growth under control.

Organic Enrichment and Temperature Treatments

In order to assess the effects of organic eutrophication on *X. umbellata*, 12 of the experimental aquaria were randomly arranged into three DOC treatments and a control condition. Thus, the coral colonies arranged in the experimental tanks were kept under three DOC concentration treatments: a low concentration of 10 mg L^{-1}, a medium concentration of 20 mg L^{-1} and a high concentration of 40 mg L^{-1}. Organic enrichment was performed daily through glucose (D-Glucose) additions, while the control condition was represented by untreated aquaria (2 to 3 mg L^{-1}). The colonies remained incubated in the tanks under the previously mentioned conditions during the whole execution of the experiment. To keep DOC concentrations constant in our treatments, the DOC contents of each tank were measured twice a day with a TOC Analyzer (TOC-L CPH/CPN PC-Controlled Model, Shimadzu, Japan). Measurements were always performed every morning and in the afternoon (10:00 and 17:00). Using the results from the morning measurements, the required amount of glucose to be added after consumption was calculated and subsequently added in the afternoon from a mother solution freshly prepared every day, before the second monitoring measurement. This first phase of the experiment had a total duration of 21 days, with 10 incubation time data points collected at higher frequencies

during the initial week of the experiment and including a baseline incubation before the start of the treatments.

During the second phase of the experiment, i.e. from day 21 and until day 45, warming effects on *X. umbellata* corals were assessed through three temperature treatments. Four additional tanks (procuring the same conditions as the DOC untreated controls) were prepared and included as controls for the temperature treatment. These controls remained untreated with DOC and at a constant temperature of 26 °C until the end of the experiment. Thus, the experimental tanks were arranged as 16 aquaria, with four of them as the aforementioned controls and 12 of them representing the low, medium and high DOC concentrations plus a DOC untreated control. The later 12 tanks experienced the temperature treatments achieved through stepwise increases over time: 28, 30, and 32 °C. In further detail, on day 21 of the experiment, a slow temperature increase of 2 °C in a matter of 5 days was performed. It was then kept stable for three days after reaching the target temperature, and physiological measurements were performed before increasing the temperature again by 2 °C more until reaching the maximum of 32 °C. Temperature treatments were selected and conducted in consideration of the IPCC report 2018, to which natural temperature changes would apply (de Coninck et al., 2018). A total of nine incubation data points in time, plus a baseline, were collected during this second phase.

Photosynthesis and respiration rates

Physiological measurements were carried out following the established beaker incubation technique by Herndl & Velimirov (1986) and procedures described by Bednarz et al. (2012) for the same xeniid species. Net photosynthesis and respiration were measured for three organisms per treatment (i.e. 3 coral colonies per 4 experimental conditions during the first experimental phase (n = 12), and 3 coral colonies per 5 experimental conditions distributed in the 16 tanks for the second experimental phase, thus (n = 16). Our incubations were always performed in the mornings (~2 h after LED lamps switched on in the system). As

higher respiration rates in light-adapted corals relative to dark-adapted corals (Anthony and Hoegh-Guldberg, 2003; Porter et al., 1984) provide a better estimate for calculating gross photosynthesis (Bednarz et al., 2012). At the start of each measurement day, one coral colony was randomly selected from the ten organisms available in each tank and picked from it. The selected colonies were collected underwater in 151 mL glass jars to avoid air exposure. Observing they did not show signs of stress due to manipulation and remained pumping during the measurements. The initial O_2 concentrations were measured in each jar using a salinity-corrected oxygen optode (HACH HQ40d multimeter, HACH Lange GmbH and Co., Germany). To measure planktonic background metabolism, a single plug containing no coral colony was recovered from each aquarium and introduced into a glass jar following the same procedure as the collected colonies. These plugs served as controls undergoing the same treatments as each of the colonies chosen. Once measured, the jars were carefully closed airtight, avoiding the presence of air bubbles and taken for incubation procedures.

Dark incubations took place in a darkened room, inside water baths conditioned in order to ensure no light penetration and constant temperature. After 2-3 hours of incubation, the jars were re-opened, directly measured and end time and O_2 concentration values were recorded per each sample. While photosynthesis may exhibit a lag response during the first minutes of dark incubations, O_2 evolution can be considered negligible under the context of 2 to 3 hours incubation time (Bednarz et al., 2012). To obtain net photosynthetic activity, we performed 2-3 hours of light incubations. After measuring the start O_2 concentration for every sample, we closed airtight each glass jar and placed it back into the experimental system. We allocated each jar inside the original aquarium from which its colony (or single plug) was picked. All the jars remained in the experimental tanks to ensure constant irradiance and temperature during the incubation procedure. When the incubation time was finally over, each jar was re-opened and measured once more to record the end time and O_2 concentrations of each sample.

Furthermore, O_2 fluxes obtained from the dark and light incubations were normalized by incubation time (hours; mg O_2 L^{-1} h^{-1}), corrected for incubation volume and normalized to coral surface area (mg O_2 m^{-2} h^{-1}), therefore obtaining gross, net photosynthesis and respiration rates as a final result from the calculations. The surface area of the colonies was calculated using the approach by Bednarz et al. (2012), where the area approximation of a polyp (which we obtained through assessment of taken photographs of the individual colonies) can be multiplied by the number of polyps in each colony, and counted for each colony after finishing each incubation.

Data Analysis

All statistical analyses were performed in the computing software R version 3.0.2 (R Development Core Team, 2013) using the Lme4 package (Bates et al., 2015). To test for differences in the treatments, a Linear Mixed-effects model (LMM) analysis was used for obtaining fitting models to the data per each predictor assessed. We evaluated data normality for each physiological response using a Shapiro–Wilk test and Q-Q plots as an additional aid for visual inspection. We carried out model diagnostics as the models were being constructed, and Pearson's residual variance was evaluated through residual plots to confirm that fitted model assumptions were met. Models were calculated separately for each experimental phase, including the complete data for all the incubations performed in the first 10 data time points plus a baseline for the first phase of the experiment and 9 data time points plus day 21 as a baseline for the second experimental phase with simulated warming. To assess model sensitivity, we evaluated further Cook's distances and data leverage. Two data points identified as strong outliers (magnitude way larger than >1.5 times the interquartile range of the data) were removed from the analysis after outlier treatment since they prevented model convergence. Furthermore, for the LMM analysis, the factors evaluated as fixed variables included: 1. DOC concentration: four levels for the first part of the experiment and five levels for the subsequent part, including warming conditions as a stressor. 2. Temperature: three levels factor, only tested for the second part

of the experiment, where warming temperatures were included as a treatment. The variable time was also included to evaluate the factors in a longitudinal assessment. The interaction between the factors DOC x time and DOC x temperature was evaluated as well. The significance of fixed effects was determined using an ANOVA type III (Zuur et al., 2009), with Satterthwaite's method for degrees of freedom approximation (Kuznetsova, Brockhoff & Christensen, 2014). Models were considered statistically significant when found to be supported by *p-values* of $P < 0.05$. Where significant differences were observed, we performed additional Tukey tests (Supplementary Information S1 and S2), using the ''glht'' function from the multicomp package (Hothorn et al., 2008) and the package emmeans (Lenth, 2019) for results confirmation and visual inspection.

3.4 Results

During the first phase of the experiment, i.e. throughout the first 21 days, all the coral colonies survived regardless of their exposition to the DOC concentration treatments. In contrast, by the end of the subsequent warming period, i.e. from day 21 to 45, mortality and visible stress signs were observed only for the heat-stressed controls in the absence of DOC (Vollstedt et al., 2020). For these colonies, mean survival decreased to 80.9 %, while 38 % of the surviving ones in this condition showed size reductions, shrunk polyps, thinner stalks and depressed tentacles with compromised pinnate structures. Moreover, the colonies under warming and in the different DOC concentrations retained a less stressed and relatively healthy appearance, while their survival was 100 % until the end of the experimental term. Subtle lessening in colouration occurred in 41 % of the colonies in the high DOC concentration treatment and for 26 % of the colonies in the medium DOC concentration treatment. However, no conspicuous sign of bleaching, understood as complete whitening of the colonies accompanied by null photosynthetic activity, was observed for any of the treatments during the extent of the experiment.

DOC Organic Enrichment

For *X. umbellata* colonies exposed to different DOC concentrations during the first 21 days of the experiment, no impact of the DOC treatments was observed for any metric (Table 3.1).

Table 3.1 | Linear mixed-effects model results for gross and net photosynthesis, respiration rates (mg O2 m-2 h-1), and the P:R ratio of *X. umbellata* corals under single DOC addition. Type III Analysis of Variance with Satterthwaite's approximation method for degrees of freedom.

Factor		Gross photosynthesis		Respiration		Net photosynthesis		P:R ratio	
Fixed Effects	***df***	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
DOC	3	1.206	0.386	1.06	0.421	1.394	0.338	0.589	0.639
Time	9	6.712	**$9.20e^{-07}$ *****	6.41	**$1.464e^{-06}$ *****	3.371	**0.00186 ****	2.370	**0.0213***
DOC x Time	27	1.215	0.257	1.09	0.379	0.955	0.538	0.939	0.558

Note: *P*-values defined as significant at a threshold of $P < 0.05$ are highlighted in bold.

Thus, the *p*-values for DOC concentrations treatments were not significant for gross photosynthesis response (LMM; F = 1.21, P =0.39; Figure 3.1A), respiration (LMM; F = 1.06, P =0.42; Figure 3.1B), net photosynthesis (LMM; F = 1.39, P =0.34; Figure 3.1C), or the P:R ratio (LMM; F = 2.37, P =0.02; Figure 3.1D). The interaction between DOC treatments and time was not significant either for any of the metrics assessed (LMM; $P > 0.05$; Table 3.1). Only the variable time was significant (LMM; $P < 0.05$) for every metric. Net photosynthesis and respiration, and thus gross photosynthesis, showed initial higher values, which reached stability after four days of treatment exposition and remained stable until the end of the experiment's first phase. In particular, gross photosynthesis rates reached similar mean values among treatments, rounding 22 ± 7.00 mg O_2 m^{-2} h^{-1} on average, while the P:R ratio response rounded 2.5 ± 0.3 by day 21 of the experiment, well above 1.5, a conservative threshold for net autotrophy (Wilkinson, 1983).

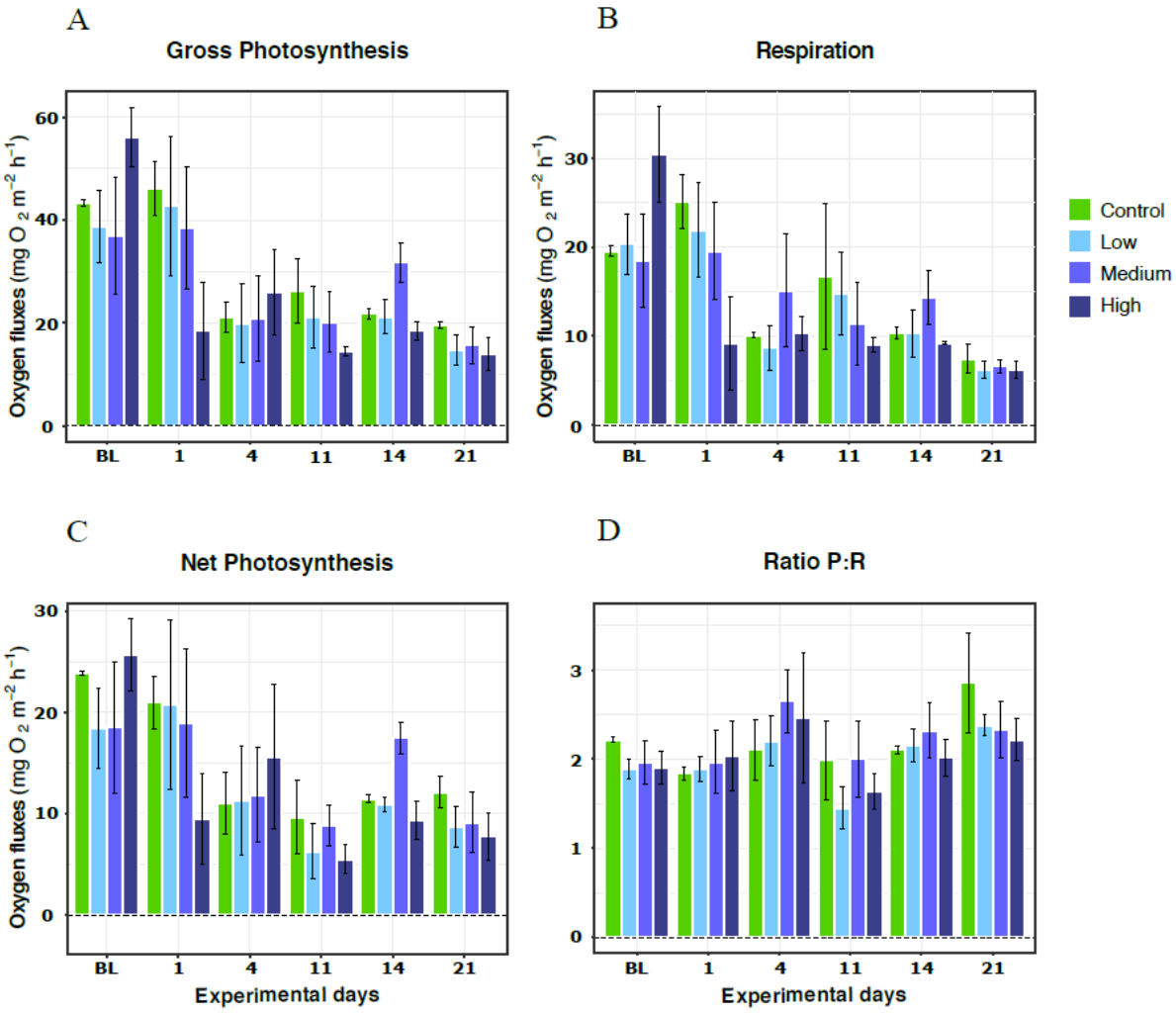

Figure 3.1 | Respiration rates, net photosynthesis, gross photosynthesis (mg O_2 m^{-2} h^{-1}), and P:R ratio of *X. umbellata* under single DOC simulated organic eutrophication over time. Control condition: 2-3 mg/L (green), Low: 10 mg/L (light blue), Medium: 20 mg/L (medium blue), and High: 40 mg/L (dark blue). Bars values indicate mean ± s.e.m. for n=12 (3 samples x 4 conditions).

Warming Temperature Treatments and DOC addition

When exposed to simulated warming scenarios, there was a significant effect of the temperature factor for every metric (LMM; $P < 0.05$; Table 3.2). A general decline was observed in response to the increased temperatures, together with significant differences between most pairwise temperature comparisons (Supplementary Information S1 and S2).

In contrast, no significant effect was found for the DOC treatments or the interactions between DOC and Temperature, or DOC and time for any metric (LMM; $P \geq 0.05$; Table 3.2).

Table 3.2 | Linear mixed-effects model results for gross and net photosynthesis, respiration rates (mg O2 m-2 h-1), and the P:R ratio of *X. umbellata* corals under simulated warming and continuous DOC addition. Type III Analysis of Variance with Satterthwaite's approximation method for degrees of freedom.

Factor		Gross photosynthesis		Respiration		Net photosynthesis		P:R ratio	
Fixed Effects	***df***	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
DOC	4	0.855	0.515	0.772	0.563	1.505	0.206	2.440	0.0512.
Temperature	3	33.13	**$6.3e^{-15}$ *****	17.90	**$2.35e^{-09}$ *****	36.87	**$<2e^{-16}$ *****	11.06	**$2.16e^{-06}$ *****
Time	7	1.902	0.0774.	4.701	**0.00014 *****	1.562	0.155	1.585	0.147
DOC x Temp.	12	0.551	0.876	0.947	0.504	0.705	0.743	0.924	0.526
DOC x Time	24	0.553	0.951	0.760	0.776	0.622	0.909	1.487	0.087.

Note: *P*-values defined as significant at a threshold of $P < 0.05$ are highlighted in bold.

Furthermore, significant effects observed were primarily due to the factor temperature and not to the factor time, which was not significant for most metrics (LMM; $P > 0.05$; Table 3.2), except respiration (LMM; $F = 4.70$, $P < 0.05$; Table 3.2).

In further detail, gross photosynthesis (Figure 3.2A) showed approximately a 50 % decrease from the 26 °C to the 28 °C condition and from 28 °C to 30 °C. At 32 °C, an overall 65 % reduction was observed compared to the 26 °C condition for all DOC treatments. Besides, respiration response at 28 °C portrayed mean values being 50 % less than the recorded ones at the start of the temperature stress phase and a further 75 % reduction at 30 °C for the heat-stressed control together with the DOC treated conditions, followed by a 25% positive increase from 30 to 32 °C (Figure 3.2B). Moreover, net photosynthesis (Figure 3.2C) showed a substantial reduction of approximately 79 % at 28 °C for the heat-stressed controls, while the DOC treatments and the temperature treatment control decreased together, in lesser magnitude. After the temperature increased to 30 °C and subsequently to 32 °C, all the DOC treatments and controls alike decreased even further by approximately 75 % at 30 °C and

79 % at 32 °C, with respect to 26 °C. On the other hand, the P:R ratio (Figure 3.2D) showed statistically comparable mean values across all the DOC treatment conditions and both controls, together with a steady decrease from 26 °C through 28 °C, 30 °C and at 32 °C.

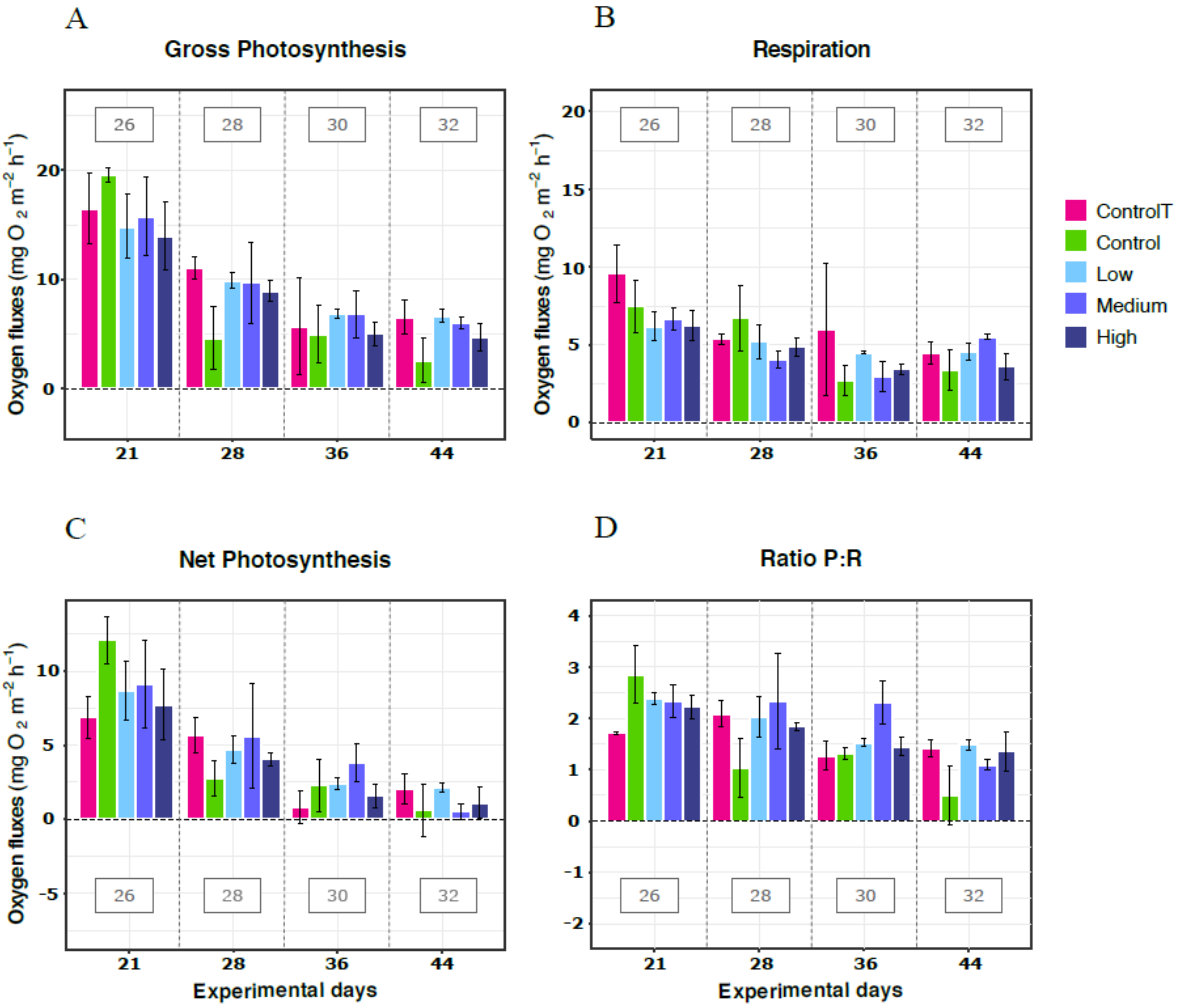

Figure 3.2 | *X. umbellata* response in terms of respiration rates, net photosynthesis, gross photosynthesis (mg O2 m-2 h-1), and P:R ratio to warming stress and prolonged DOC addition over time. Control condition for the temperature treatment (ControlT): 2-3 mg/L DOC at constant 26°C over time (pink), heat-stressed control (Control): 2-3 mg/L (green), Low: 10 mg/L (light blue), Medium: 20 mg/L (medium blue), and High: 40 mg/L (dark blue). Bars values indicate mean ± s.e.m. for n=16 (3 samples x 4 heat-stressed conditions plus 4 samples for the non-heat stressed control).

When contrasting temperature responses (Figure 3.3), all pair comparisons were significant for the P:R ratio (Table S1.4, Figure S2.4; $p<0.05$), except 28 °C to 30 °C and 30 °C to 32 °C.

At 28 °C, coral colonies had average P:R values rounding 1.5, while at 30 °C and 32 °C, most P:R values remained approximately between 1.5 and 1.0, where P:R = 1.0 defines compensation.

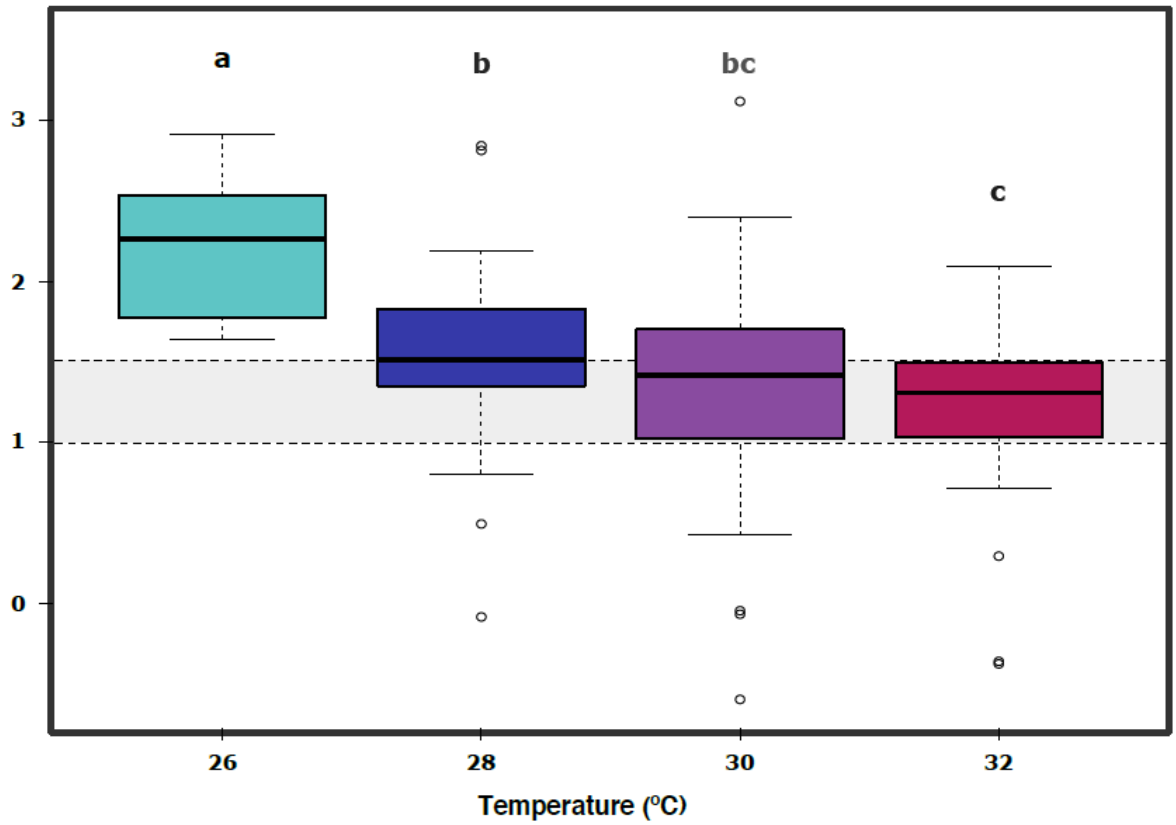

Figure 3.3 | *X. umbellata* P:R ratio trend as a function of increased temperature over time. The grey space between dashed lines represents the zone between compensation where P:R = 1.0 and the conservative threshold for net autotrophy P:R ≥ 1.5 (Baker et al., 2015). Letters, together with colours, indicate significant differences between groups as determined by Tukey's test ($P < 0.05$) for the factor temperature on the P:R metric.

3.5 Discussion

Our study of the effects of DOC eutrophication as glucose addition and simulated warming on the soft coral of *X. umbellata* shows that net and gross photosynthesis, respiration and the P:R ratio are completely insensitive to DOC organic enrichment, even under exceptionally high concentrations at both, 26 °C and increased temperatures. This contrasts

with hard corals, which are negatively impacted by DOC at non-stressful temperatures and therefore, potentially impacted by DOC under heat stress. Moreover, the detrimental response of *X. umbellata* to temperature stress, however milder for these corals when compared to many other hard coral responses to thermal stress (Prada et al., 2010; DeCarlo et al., 2017; Osman et al., 2018; Monroe et al., 2018; Ziegler et al., 2019), demonstrates that *X. umbellata* shows considerable resistance to this stressor, even though higher temperatures may negatively drive the photosynthesis and respiration response in this species. These results agree with the suggested outcome from the ecological response for *X. umbellata,* where pulsation rates did not suggest disturbances caused by DOC addition but rather under simulated warming at 30 °C and 32 °C (Vollstedt et al., 2020). However, no statistical significance was found for the interaction term between temperature and DOC for any of the response variables at any temperature step. From the physiological perspective in our present study, we found no beneficial effect of DOC on *X. umbellata* response, despite the suggested benefit of DOC on the ecological variables assessed in our previous study. Thus, there seem to be differences between the ecological and physiological metric responses of *X. umbellata* to DOC and heat stress, and the ecological benefits of DOC did not appear to translate into physiological benefits. Furthermore, DOC did not increase sensitivity to warmer temperatures in this soft coral, in contrast to hypothesized expectations where warming scenarios may pose a greater threat given the ubiquitous presence of diazotrophs in most coral holobionts (Pogoreutz et al., 2017). Our results support the likelihood that the mixotrophic *X. umbellata* soft coral may be potentially able to endure challenging conditions imposed by high eutrophication better than their counterpart, the calcifying hard corals. DOC resistance may convey a considerable advantage for soft coral dominance, potentially even under future changing climate scenarios.

Effects of single DOC addition on Respiration and Photosynthesis

There was no effect of single DOC addition on *X. umbellata* photosynthesis or its respiration rates, regardless of the concentrations assessed. Previous studies on the effects of organic enrichment on hard corals have extensively reported its adverse effects on many species. For instance, decreased photosynthetic activity and additional negative impacts in photosynthesis-related metrics have been described by, e.g. Pogoreutz et al. (2017), for the hard coral *Pocillopora verrucosa*, by Haas et al. (2009) for *Acropora* sp. by Fabricius et al. (2013) for *Acropora millepora* and *Montipora tuberculosa* and, by Weber et al. (2012) for *Montipora peltiformis*. In particular, Pogoreutz et al. (2017 and 2018) reported already strong damaging effects for concentrations such as 10 mg L^{-1}, while the study of Kline et al. (2006) showed that DOC levels of 25 mg L^{-1}, compared to our medium-level concentrations (i.e. 20 mg L^{-1}), caused disruptions of the balance between the hard corals and their associated microbiota. In contrast, after additional acclimatization within the first days of the experiment, photosynthesis and respiration rates of *X. umbellata* in our study were comparable to values expected for healthy untreated colonies, in agreement with previous references by Bednarz et al. (2012). Both parameters remained relatively constant until the end of the single DOC addition exposure phase, i.e. the first 21 days. Besides, no detrimental effects seemed to have occurred due to the over-stimulated growth of coral mucus-associated microbes expected from organic carbon loading with glucose (Kuntz et al., 2005).

Furthermore, the absence of a DOC effect on photosynthesis in our study may indicate that the requirements for this physiological process were already being met through the available energy byproduct of regular metabolism (Comeau, Carpenter & Edmunds, 2017). Whilst a lack of an increase in photosynthesis could suggest that the *Symbiodinium* of *X. umbellata* did not make use of the readily available carbon source. Stark increases in net and gross photosynthesis activity under high DOC concentrations have been reported to happen via heightened CO_2 availability in *hospite* in another pumping cnidarian (Rädecker et al., 2017). However, this did not seem to be the case in our study. As an additional

observation from the physiological point of view, coral calcification can be enhanced through different mechanisms. Indirectly, via increasing the photosynthetic activity of the symbionts (Goreau, 1959; Falkowski et al., 1984; Muscatine 1990), where calcification and photosynthesis have been traditionally accepted as tightly coupled (Goreau, 1959; Falkowski et al., 1984; Muscatine 1990; Burriesci et al., 2012; Kopp et al., 2015); and also directly, via light absorption by the coral host (Cohen et al., 2016, Eyal et al., 2019) and heterotrophic feeding (Houlbrèque and Ferrier-Pagès 2009). Calcification processes in soft corals such as xeniids may not be as energetically costly as calcification in hard corals; this might enable the coral to have a larger energetic budget as surplus, also from photosynthesis, that may be available for resistance and other processes, e.g. pumping, growth or mucus production. Furthermore, differences in mineralized structure components, intracellular or extracellular calcification, and calcite or aragonite incorporation can influence the photosynthesis response to specific stressors (Hofmann & Bischof, 2014). Under environments in which energetic demands may exceed metabolic capacity, individuals with maintenance costs less sensitive to environmental stressors may be more likely to survive by changing the allocation of their available energy (Pan, Applebaum & Manahan, 2015).

On the other hand, the null effect of DOC on respiration could reflect multiple pathways of metabolic responses, ranging from potentially no effect to more complex scenarios involving energetic trade-offs among critical processes that change the allocation of energy to individual functions while conserving the overall metabolic costs (Pan, Applebaum & Manahan, 2015; Comeau, Carpenter & Edmunds, 2017). Moreover, a negligible effect of sugar enrichment on respiration (and net and gross photosynthesis) due to only DOC addition contrasts with previous findings from works on other soft-bodied cnidarians and hard corals where glucose enrichment has been shown to stimulate respiration in the cnidarian mixotrophic model organism, *Cassiopeia* sp., (Rädecker et al., 2017), and to increase respiration rates while decreasing gross photosynthesis in the hard coral *P. verrucosa* as in Pogoreutz et al. (2017).

Regarding the P:R ratios, there was no statistical evidence of physiological dose-response relationships occurring by using multiple DOC levels of exposure. Therefore, the colonies remained healthy and autotrophic, regardless of DOC as a stressor, suggesting that until 40 g L^{-1} of DOC concentration, DOC levels were still inside the range of *X. umbellata* response optima with P:R ratios by the end of the single DOC addition period comparable to non-treated *X. umbellata* corals as reported by Bednarz et al. (2012). These findings are consistent with non-affected pulsation rates described by Vollstedt et al. (2020), regardless of DOC concentrations.

Warming Temperatures Effects and its Synergy with DOC

During the warming phase of our study, the temperature had a significant effect on net and gross photosynthetic activity, which showed a negative trend proportional to temperature increases. For both metrics, all pair temperature contrasts were significant (Tables S1.1 and S.1.3, Figures S2.1 and S2.3; $p<0.05$), except 30 °C to 32 °C. Besides, the DOC treatments and the control remaining at 26 °C did vary together with the temperature treatments. Substantial decreases in photosynthetic O_2 evolution and maximum quantum yield are known to be early responses of Symbiodinium to heat stress, following the overwhelming of photoprotective mechanisms (Jones et al., 1998). However, it is remarkable that for *X. umbellata*, positive net photosynthesis was still observed at 32 °C, although harshly reduced. These results suggest that the expected impact of elevated temperatures on *X. umbellata* may be much lesser than previously suspected (Strychar et al., 2005).

Regarding respiration, a decrease was observed between 28 °C and 30 °C, with a slight increase again from 30 °C to 32 °C (Table S1.2, Figure S2.2; $p<0.05$). A decrease in respiration under stress may be indicative of a reduction in metabolic costs and perhaps explicit metabolic depression (Pörtner, Langenbuch & Reipschläger, 2004). While in contrast, elevated respiration as a function of thermal stress could result from higher-performing

costs, as it occurs in the case of increased respiration rates under elevated pCO_2, caused, for example, by perturbed active cellular transport or an increasing cost of other metabolic activities (Comeau, Carpenter & Edmunds, 2017).

Furthermore, the ratio P:R trend showed a steady decrease from 26 °C through 28 °C, 30 °C and finally 32 °C, all significant when compared to 26 °C (Table S1.4, Figure S2.4; $p<0.05$); together with longitudinal significance of the temperature. A significant difference in the 26 °C to 28 °C steps suggests already thermal stress responses to a 2.0 °C increase in temperature. Nevertheless, the P:R ratio remained above the compensation threshold (P:R = 1.0) at 28 °C, 30 °C and 32 °C for all the temperature treatments but not for the heat-stressed control at 32 °C, which also showed a decrease in survival. The coral colonies remained mostly autotrophic until 32°C with P:R ratios above compensation (P:R > 1.0) but below or rounding the conservative net autotrophic threshold (P:R > 1.5) for 30 °C and 32 °C. Thus, *X. umbellata* ratios described here suggest strong net autotrophic behaviour at 26 °C, moving within the heterotrophic – autotrophic continuum towards a more heterotrophic character at 30 °C and 32 °C (Wilkinson, 1983; Baker et al., 2015). The autotrophic P:R ratio observed at 26 °C can be considered higher than those of other soft coral species. However, it is still in the lower range compared to observations for hard corals, rounding from 2.0 to 4.0 (Fabricius & Klumpp, 1995; Bednarz et al., 2012). It is also particular that the *X. umbellata* P:R ratio is far lower than the 8.3 ratio recorded for the equally pulsating xeniid *Heteroxenia fuscescens* (Kremien et al., 2013). Nevertheless, its ability to pulsate may allow it to reach higher energetic yields than other non-pulsating corals (Kremien et al., 2013). Altogether, the diversity of P:R ratios among taxa may reflect the differential potential for species-specific tolerances towards stressors, where modulation strategies and coping mechanisms may highly vary and be dependent on the particular nature of the stressor and the available energetic budget of the coral holobiont (Pan, Applebaum & Manahan, 2015) in complement to its nutritional plasticity (Schubert, Brown & Rossi, 2016; Rossi et al., 2020; Conti-Jerpe et al., 2020), associated microbial community dynamics (Neave et al., 2019), and surrounding seawater conditions.

Besides *X. umbellata*'s potential ability to enhance its autotrophic energy input, these soft corals have as well a non-negligible mixotrophic component. The P:R trend observed through temperature increases suggests that corals would go in the direction of shifting to heterotrophy by reaching values below compensation (Baker et al., 2015), given enough time. Traditionally, soft corals have been described to rely more on heterotrophic feeding due to their lower photosynthetic productivity when compared to hard corals, as supported by lower P:R ratios rounding 1.0 to 1.3 (Fabricius & Klumpp, 1995). Moreover, heterotrophic feeding has been shown to aid various hard coral species in sustaining a positive energy balance under thermal stress or eutrophication (Anthony & Fabricius, 2000; Borell & Bischof, 2008; Conti-Jerpe et al., 2020). However, in our study, particular heterotrophic uptake of DOC as glucose, as a mechanism to endure thermal stress, could not be confirmed from the physiological point of view, given the lack of statistical significance for the interaction term between DOC and temperature together with lack of evidence favouring utilization of already available carbon (no significance for the DOC factor in any metric). These findings contrast with earlier studies where *X. umbellata* has been suggested to balance carbon deficiency under inorganic nutrient enrichment by increasing DOC heterotrophic uptake (Bednarz et al., 2012). While other soft corals are known to be able to uptake dissolved organic matter directly as an energy source, especially when available in excess (Schlichter, 1983). In our case, we cannot discard that *X. umbellata* could eventually use this or other resources to sustain its metabolism if its behaviour eventually shifts towards a sustained heterotrophic regime. Nevertheless, this suggestion remains to be further studied for confirmation.

As an additional remark, DOC eutrophication has been shown to induce bleaching in hard corals but did not in *X. umbellata*. For instance, sugar enrichment, without temperature and light stress, can initiate a bleaching response in the hard coral *Pocillopora verrucosa,* together with a decrease in gross photosynthesis (Pogoreutz et al., 2017). On the other hand, thermal stress has been thoroughly reported to cause bleaching in hard corals (Weis, 2008; Santos et al., 2014; Cardini et al., 2016; Hughes, Kerry & Simpson, 2018; Ziegler et al.,

2019) and soft corals as observed in, e.g. *Xenia* sp. by Strychar et al. (2005) and other octocoral species in diverse scenarios (Prada et al., 2010; Rossi et al., 2018; Slattery et al., 2019). However, bleaching was not observed during our experiments despite the decreased photosynthetic activity in *X. umbellata* under warmer temperatures. These observations align with the idea that autotrophy reliance does not appear to necessarily explain bleaching sensitivity in octocorals (Lasker et al., 2020). Moreover, the notion that soft corals might be less responsive to warm water bleaching than hard corals has been persistent (Prada et al., 2010; Schubert, Brown & Rossi, 2016; Goulet et al., 2017; Lasker et al., 2020). Even though this is still uncertain, given the lack of studies testing this specific hypothesis, it is clear that there is a degree of species-specific resistance concerning climate change impacts that will underpin the setup for potential winners and losers in future scenarios.

Tolerance to environmental pressures in the long term should depend on the sustainability of energy allocation under stress, the long-term impact on organismal performance, and the ability to support other essential physiological processes (Pan, Applebaum & Manahan, 2015). Time significance in our study may support the idea that the longer the colonies remain under stress, the stronger its effects may become. This factor significance was supported for our single DOC addition experimental phase during the first 21 days. However, this could not be addressed for the second phase of our experiments (i.e. days 21 to 45), where we increased temperature together with continuous DOC addition. Further work is necessary to elucidate the effects of longer terms of stress in these corals and pathways of energy acquisition and allocation that may underpin tolerance mechanisms in these species when incubated under organic enriched conditions. However, the long-standing question in metabolic regulation remains at what point sublethal stress would become lethal due to energy limitations (Pan, Applebaum & Manahan, 2015).

3.6 Conclusions

Overall, the present study shows that the photosynthesis and respiration of the pulsating xeniid *X. umbellata* is completely insensitive to high organic eutrophication concentrations between 10 and 40 mg L^{-1} at 26 °C and under heat stress over time. These findings provide evidence that the effects of the DOC stress factor alone are potentially trivial for this group of corals. In contrast, *X. umbellata* portrays negative sensitivity to temperature in terms of its photophysiology, which remained mild at intermediate thermal stress (i.e. 28 °C) and increased at higher temperatures, as observed at 30 °C and 32 °C. Nevertheless, bleaching was not observed, and at 32 °C, the colonies remained above compensation (P:R = 1.0) and below the net autotrophy threshold (P:R = 1.5), indicating mixotrophic behaviour, with displacement towards heterotrophy as temperature increased. These results may suggest that the effects of elevated temperatures on *X. umbellata* can be potentially modulated. Such modulation could occur via modifications of the total supply and demand for metabolic energy whenever possible (Comeau, Carpenter & Edmunds, 2017). However, it remains to be further investigated if they can be effectively overcome.

Interestingly, there was no statistical evidence for a synergistic interaction between DOC and temperature in the metrics assessed in our study. DOC organic eutrophication, expected to worsen the effects of thermal stress in hard corals (Pogoreutz et al., 2017), did not show, from a physiological point of view, any statistical effects supporting DOC sensitivity enhancements towards increased temperature stress in *X. umbellata*; neither statistical effects supporting DOC enhanced thermal tolerance via utilization of DOC as an additional energetic resource (Bednarz et al., 2012, Vollstedt et al., 2020). Nevertheless, there is likely a potential additive or synergistic effect of DOC and heat stress through different mechanisms, which have yet to be confirmed.

Altogether, our findings show that soft corals can be remarkably resistant to DOC effects, in contrast to hard corals that are susceptible to DOC. Under nutrient enrichment and highly

eutrophicated environments, hard corals have shown pronounced negative responses such as (but not limited to) e.g. bleaching, increased mortality and affected photophysiology (Kuntz et al., 2005; Kline et al., 2006; Haas et al., 2016; Pogoreutz et al., 2017; Morris et al., 2019). Our findings show that *X. umbellata* soft corals can better withstand highly increased DOC conditions. Thus portraying higher resistance to this stressor than hard corals. As a final remark, it would be valuable to visit further the conceptual framework within which organic eutrophication effects, together with thermal stress, are understood in soft corals (and for most octocoral species) as many particularities and the mechanics of how resistance may be achieved in soft corals remains unknown.

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Chapter 4

Organic eutrophication increases resistance of the pulsating soft coral *Xenia umbellata* to warming

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Organic eutrophication increases resistance of the pulsating soft coral *Xenia umbellata* to warming

Svea Vollstedt[1], Nan Xiang[1,2], Susana Simancas[1], Christian Wild[1]

[1] University of Bremen, Faculty of Biology and Chemistry, Department Marine Ecology, Leobener Str. 6, 28359 Bremen, Germany
[2] ZMT, Tropical Marine Microbiology Group, 28359 Bremen, Germany

Corresponding Author:
Svea Vollstedt[1]
Email address: sv_vo@uni-bremen.de

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4.1 Abstract

Recent research indicates that hard corals in a process that is termed phase shift are often replaced by soft corals in reefs. The simultaneous occurrence of local (i.e. organic eutrophication as highly under-investigated parameter) and global (i.e. ocean warming) factors may facilitate these phase shifts as hard corals are negatively affected by both ocean warming and organic eutrophication. Knowledge about soft coral responses to environmental change remains incomplete, although these organisms are becoming important players in reefs. The present study thus investigated the individual and combined effects of organic eutrophication (as glucose addition) and warming on the ecological data of the pulsating soft coral *Xenia umbellata*. We assessed health status, growth and pulsation rates of soft corals in a 45-day aquarium experiment, with first manipulation of organic eutrophication (no, low, medium and high glucose addition) over 21 days followed by step-wise increases in water temperature from 26 to 32 °C over 24 days. Findings revealed that glucose addition did not affect health status, growth and pulsation rates of the investigated soft corals. Under simulated ocean warming, soft corals that had experienced organic eutrophication before, maintained significantly higher pulsation rates (averaging 22 beats per minute—bpm) and no mortality compared to the controls that showed a decrease of 56 % (averaging 15 bpm) in pulsation rates and mortality of 30 % at water temperatures of 32 °C compared to 26 °C. This apparently positive effect of organic eutrophication on the ecological data of soft corals under an ocean warming scenario decreased with increasing water temperature. This study thus indicates that (a) organic eutrophication as additional energy source up to a certain threshold may increase the resistance of soft corals to ocean warming and (b) pulsation rates of soft corals may be used as inexpensive, easily detectable, and non-invasive early warning indicator for ocean warming effects on benthic reef communities. When comparing findings of this study for soft corals with previous results for hard corals, it can be assumed that soft corals under the predicted increases of organic eutrophication and warming gain more and more competitive advantages. This may further facilitate phase shifts from hard to soft corals in warming reefs.

4.2 Introduction

Coral reefs are biodiversity hotspots and among the most productive ecosystems in the world (Connell, 1978). Although they cover only less than 1 % of the ocean floor, coral reefs are of high economic value (Moberg & Folke, 1999). Besides their ecological and economic importance, coral reefs are extremely vulnerable ecosystems (Connell, 1978; Hoegh-Guldberg, 1999; Moberg & Folke, 1999). During the last decades, an alarming change with a sharp decline in species diversity and habitats has been described for coral reefs (Sebens, 1994). Approximately 20 % of hard corals worldwide are already irreversibly destroyed and an additional 30 % are strongly damaged (Bange et al., 2017). There is hardly any unaffected reef left (Jackson et al., 2001; Hughes et al., 2003).

Hard corals produce a rigid aragonite endoskeleton and create a unique and stable inorganic carbonate substrate and 3D framework system (Scheffers et al., 2003). Therefore, reef-building corals are essential ecosystem engineer organisms and highly important for reef-associated biodiversity (Wild et al., 2011; Wild & Naumann, 2013). Recently, several studies suggest hard corals respond negatively to ocean warming and/or elevated nutrient concentrations (Wooldridge & Done, 2009; Fabricius et al., 2013). At many locations in the Indo-Pacific phase shifts from hard to soft corals have been observed (Norström et al., 2009). Soft corals therefore may become essential to reef ecosystems in the future (Hoegh-Guldberg, 1999).

Coral reefs worldwide are susceptible to the effects of global warming. Over the last 50 years, the sea surface temperature has raised by 0.11 °C per decade and it is estimated to continue warming by 0.6–2.0 °C before 2100 (Chaidez et al., 2017). Another global threat is the rising atmospheric carbon dioxide CO_2 it reduces the ocean pH and causes ocean acidification (Doney et al., 2009; Rodolfo-Metalpa et al., 2010; Inoue et al., 2013).

Organic eutrophication is a highly under-investigated factor that may contribute to coral reef decline (Rabalais et al., 2009). Terrestrial runoff washes nutrients, sediments and pollutants from urbanized or fertilized seaside into coastal waters and thereby represents a major source of organic eutrophication (Fabricius et al., 2013). Furthermore, domestic and industrial contaminants increase the degree of organic contamination (Braga et al., 2000). The excess input of dissolved organic carbon (DOC) is a typical organic eutrophication in tropical waters. Nevertheless, DOC is also released naturally by algae, seagrass and corals (Smith et al., 2006; Haas et al., 2010a; Haas et al., 2010b). Kline et al. (2006) reported that DOC increased coral mortality due to a disruption of the symbiosis between the hard coral *Orbicella annularis* (Ellis & Solander, 1786) and its associated microbiota. Coral reef ecosystems depend on the symbiosis between coral hosts and their intracellular photosynthetic dinoflagellates family *Symbiodiniaceae* (see LaJeunesse et al., 2018). Nevertheless, the associated bacteria importantly contribute to the functioning of the entire coral. Nitrogen (N) cycling microbes are commonly associated with corals and provide N to both, the *zooxanthellae* and the coral host (Rädecker et al., 2015; Pogoreutz et al., 2017). Since organic eutrophication provides fast and directly digestible sugar, microbes enhance their growth (Kuntz et al., 2005; Pogoreutz et al., 2017). The proliferation and thereby higher microbial activity is detrimental because of the enhanced O_2 depletion which could have severe consequences for ecosystem functioning and the coral metabolism (Wild et al., 2010). Although temporal hypoxia is common in reef ecosystems, severe hypoxic events can cause widely coral mortality (Simpson, Cary & Masini, 1993). Different studies suggest that high temperature stimulates microbial proliferation and in addition increase the nitrogenase activity (Santos et al., 2014; Cardini et al., 2016). This excess (microbial) fixed nitrogen may lead to a disruption of the N limitation of hard coral associated zooxanthellae, subsequently leading to coral bleaching via a cascade of processes described by Pogoreutz et al. (2017). Such bleaching susceptibility of corals may be species-specific (Hoegh-Guldberg, 1999), and knowledge about soft coral bleaching responses is scarce.

Soft like hard corals depend on the metabolic communication between coral host, endosymbiotic zooxanthellae and a diverse microbial community. *Xenia umbellata* (Lamarck, 1816) is a common soft coral mainly distributed in the Indo-Pacific and the Red Sea and occurs there in high abundances (Al-Sofyani & Niaz, 2007; Janes, 2014). This soft coral colonizes both sand slopes and hard substrates in a water depth of 3–25 m (Janes, 2014). The distinctive, rhythmic pulsation of their tentacles, first recorded by Lamarck about 200 years ago, makes them remarkable (Kremien et al., 2013). This mechanism may enhance the photosynthetic efficiency and prevent refiltration as Kremien et al. (2013) detected for the closely related species *Heteroxenia fuscescens*. The enhancement of photosynthesis may be maintained by fast removal of excess oxygen at the coral surface and may account for the reduced gastrovascular capability of pulsating xeniid corals (Kremien et al., 2013). In addition, the tentacles support the ability to be a heterotrophic suspension feeder (Al-Sofyani & Niaz, 2007). However, *X. umbellata* is able to live autotrophic based on photosynthesis products (Schlichter, Svoboda & Kremer, 1983) from the zooxanthellae.

There are pronounced gaps of knowledge in relation to the response of soft corals to a combination of local and global stressors since most previous research concentrated on hard corals and the effect of either a local or a global stressor. In addition, organic eutrophication is a highly under-investigated stress factor, although it commonly occurs in coastal areas. This study thus aims to answer the following research questions:

1) How does organic eutrophication (as glucose addition) affect pulsation and growth rates along with the health of the soft coral *X. umbellata*?
2) Does organic eutrophication (as glucose addition) influence the resistance of the soft coral *X. umbellata* to ocean warming?

We aimed to answer these research questions by implementing a laboratory manipulation experiment over 45 days. With the resulting experimental data and the available literature, we compare the responses of soft in comparison to hard corals to organic eutrophication

and warming in order to assess potential winners and losers under the predicted future scenarios.

4.3 Materials and Methods

Experimental setup

The soft corals used in the experiments are originally from the northern Red Sea but kept in the maintenance aquarium of Marine Ecology Department since more than 2 years prior to the experiments. The soft coral genus *Xenia* is very common in the Indo-Pacific reef ecosystems (Al-Sofyani & Niaz, 2007; Janes, 2014). We identified the soft coral according to Reinicke (1997), as species *X. umbellata* Lamarck (1816), which is a common pulsating *Xenia* species from the Red Sea. Five big colonies (5 x 7 x 12 cm) were fragmented into 160 small colonies (1–2 cm in width) using a scalpel. Subsequently, these colonies were attached to cubical-shaped calcium carbonate coral holders (1 x 1 cm) with rubber bands. The colonies could heal and grow on the holders for 7 days. Thereafter, the rubber bands were cut off and the colonies were evenly distributed among 12 experimental aquaria tanks (volume: 60 L; details, please see below) and left there for acclimation for 10 more days. The tanks were still connected with the main tank and a stable water flow was maintained. After acclimatization, the first ecological data were recorded as a baseline (day 0) for the experiment.

The experiment was conducted using an aquarium tower which was made out of 12 individual tanks. The tanks were arranged in three levels with four tanks in one level. Each tank had a volume of 60 L, consisting of technical and experimental parts. The volume of water in the tanks was 50 L. Each tank was equipped with 20 *X. umbellata* colonies. In the technical part of the tanks a thermostat (3613 aquarium heater. 75 W 220–240 V; EHEIM GmbH and Co. KG, Germany) and a pump (EHEIM CompactOn 300 pump; EHEIM GmbH and Co. KG, Germany) were installed. To maintain a stable temperature and prevent the water

from cooling, styrofoam boards were placed on top of the aquaria when needed. The LED light (Royal Blue—matrix module and Ultra Blue White 1:1—matrix module, WALTRON daytime® LED light, Germany) was a source of heat, so it was placed on top of the aquaria and adjusted in height. Light intensity of 120.8 ± 10.2 µmol quanta m^{-2} s^{-1} and a day-night rhythm of 12–12 h.

Further, 10 % of the water was replaced on a daily basis, assuring close to natural sea water parameters with a high renewal rate of seawater in the tanks. Maintenance conditions were kept constant, and the algae growth was kept within limits by repeated cleaning. Water parameters were monitored twice a week. Temperature and salinity were measured every day and adjusted manually. In the first part of the experiment the temperature was kept stable around 26 °C, while in the second part an increase of temperature was performed. There were no significant differences between treatments in salinity (average: 35.4 ± 0.4). Chemical parameters for all tanks are summarized in Table 4.1.

Table 4.1 | Mean values (± SD) of water parameters maintained in all tanks.

Parameter	Mean values (± SD)
pH	8,1 ± 0,1
KH	7,2 ± 1,0
Ca^{2+}	447,8 ± 66,4 ppm
Mg	1493,1 ± 293,5 ppm
NO^{2-}	<0,01 ppm
NO_3	< 0,5 ppm
NH_4	< 0,05 ppm
PO_4	< 0.02 ppm

Manipulation of organic eutrophication and temperature

In nine aquaria, organic eutrophication was manipulated by daily additions of D-Glucose anhydrous (purity: 99 %, Fisher Scientific U.K. Limited, Loughborough, UK). To maintain three different treatments, we measured TOC as a proxy for glucose addition and calculated the

addition to reach: 10 mg/L as low concentration, 20 mg/L as medium concentration and 40 mg/L as high concentration. After the first addition, we monitored the glucose addition regularly with a Total Organic Carbon Analyzer TOC-L (Shimadzu Corporation, Kyoto, Japan) in order to adjust glucose addition as response to consumption. Every afternoon after sampling, the missing amount of glucose was added in form of a stock solution (D-Glucose, concentration 40 mg/mL) to achieve the desired final concentrations. This procedure was continued throughout the experiment, while the stock solution was prepared daily. The other three tanks were maintained at ambient glucose levels (control 2–3 mg/L). The glucose concentrations were based on previous studies that manipulated organic eutrophication (Kline et al., 2006; Pogoreutz et al., 2017).

The temperature was kept stable at 26 °C for the first three weeks of the experiment. After day 21, the temperature was increased by 2 to 28 °C within 5 days and then kept stable for 3 days. This was repeated two more times until the maximum temperature of 32 °C was reached. The light intensity was measured with a LI-1400 Data Logger (LI-COR, Inc., Lincoln, NE, USA) once a week over the entire duration of the experiment. This resulted in a mean light intensity of 120.8 ± 10.2 µmol quanta m^{-2} s^{-1}. The temperature treatment was chosen to imitate the possible natural temperature in the Red Sea, a distribution area of *X. umbellata*. In the southern Red Sea, a temperature of 33 °C is reached between late July and mid-August. Here the organisms may be yet close to their thermal limits (Chaidez et al., 2017). Thereby, Strychar et al. (2005) used 32 °C as a maximum temperature as well and found that *Xenia* spp. is susceptible to relatively small temperature changes.

Ecological assessments

We used the pulsation and growth rates to assess the sublethal effects. Pulsation rates were always quantified by the same observer and the same methodology. Samples were taken 13 times in the first 3 weeks having a higher resolution in the first 2 weeks and fewer counting in the last. During the second part of the experiment pulsation rates were counted

before and after each temperature increase. Three random colonies from each tank were selected about noon every sampling day to avoid the corals being disturbed via glucose addition or other measurements. We decided to use pulsation per minute as a comparable unit. Pulsation rates that occurred in 1 min were then counted for a single polyp by using a stopwatch. A complete pulsation was defined as the time that a polyp needed to open and close all its tentacles. After 7 days the pulsation was counted 30 s to save time but was calculated to pulsation rates per minute afterwards.

To calculate the growth rate (new polyps per day), always the polyps of the same three colonies per tank were counted over time. Therefore, the colony was taken out of the tank without air exposure, and the number of polyps of every colony was counted using tweezers in order to avoid double-counting. With the data of polyp numbers, the growth rates of new polyps per day were determined.

Data analyses

Statistical analyses were performed using GraphPad Prism 8 and SPSS 17.0. All data presented in the text, figures and tables are expressed as means ± SD. The significant differences in variables between treatments were analyzed by one-way analysis of variance (ANOVA) with Duncan's test. For each timepoint and temperature an individual ANOVA was performed. The assumptions for an ANOVA were checked a priori. The effect $p < 0.05$ was regarded as significant and $p < 0.01$ was considered as highly significant.

4.4 Results

Glucose tank concentration monitoring

The mean TOC concentrations were measured over the first and last week of the experiment 18 h after the prior glucose addition. Our mean values were 2.95 ± 0.36 mg L^{-1} (control), 5.84 ± 0.99 mg L^{-1} (low), 11.72 ± 2.09 mg L^{-1} (medium), 26.57 ± 4.56 mg L^{-1}, respectively.

Mortality

After 3 weeks exposure to elevated organic nutrient concentrations, no mortality was detected. The mortality of corals was observed during the last days of the temperature experiment. 30 % of the control corals with increased temperature died. Remaining colonies were shrunken and showed visible signs of stress. In comparison, no coral of the glucose treatments died until the end of the experiment and remaining colonies looked less damaged.

Pulsation rates

On baseline day 0, all pulsation rates ranged from 35 to 44 beats min^{-1}. After glucose addition for 21 days, pulsation rates showed no significant change among all groups (p = 0.198) (Figure 4.1A). With the first temperature increase to 28 °C, there was no significant difference between any treatment (p = 0.153; Figure 4.1B) and no difference compared to control colonies at 26 °C. However, the increased temperature control showed a significant decrease in pulsation rate compared to 26 °C and a significant decrease ($p < 0.05$) by 52 and 33 % compared to all glucose addition treatments at 30 and 32 °C, respectively. Soft corals in the glucose addition treatments decreased their pulsation by around 30 % at 32 °C compared to 26 °C, but they exhibited over two times higher pulsation rates than the control in 32 °C.

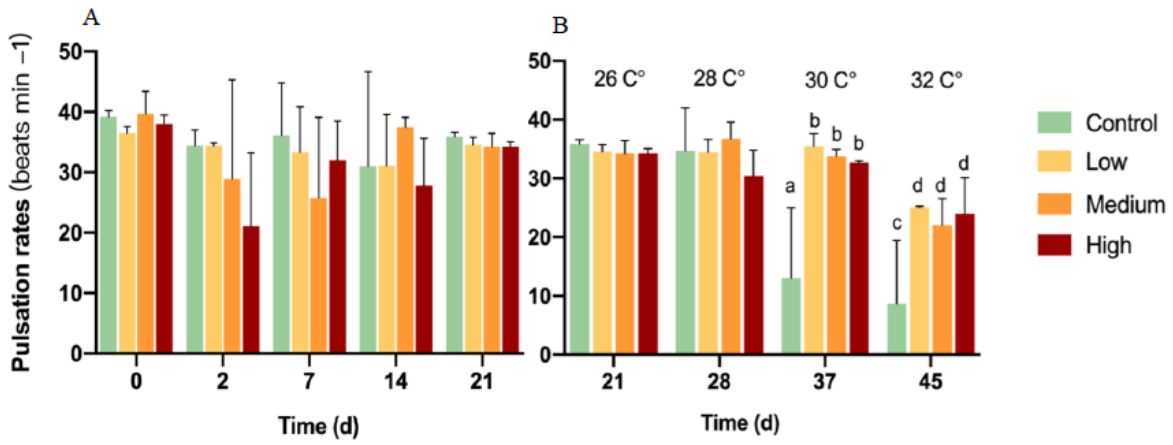

Figure 4.1 | Pulsation rates of corals at different glucose concentrations during the experiment. Columns indicate mean values of three replicates with error bars providing the respective SD. **(A)** Exclusive glucose experiment over time. (B) Glucose treatments with step-wise increased temperature over time. **(B)** starts with day 21 at 26 °C as a starting point for the temperature increase. Letters indicate statistically significant differences ($p < 0.05$).

Growth rates

While the control colonies exhibited a growth rate of 0.59 new polyps d^{-1}, the enriched treatments caused growth rates of 0.96 (low), 0.86 (medium) and 0.68 (high) new polyps d^{-1} (Figure 4.2). Thereby, glucose enrichment maintained the soft coral growth rate by 62 % (low), 45 % (medium) and 13 % (high) compared to the controls. During the first week of temperature increase to 28 °C, a positive growth rate was detected in all treatments. With increased temperature to 30 °C, medium and high glucose treatments increased in polyp numbers, while the other treatments showed negative growth rates. With increased temperature to 32 °C, only the high glucose and ambient glucose group showed positive growth rates. It is worthy to note, that in the high glucose treatment, corals continued to slowly grow even until the end of the experiment. There were no significant differences ($p = 0.09–0.70$) between treatments at particular temperatures.

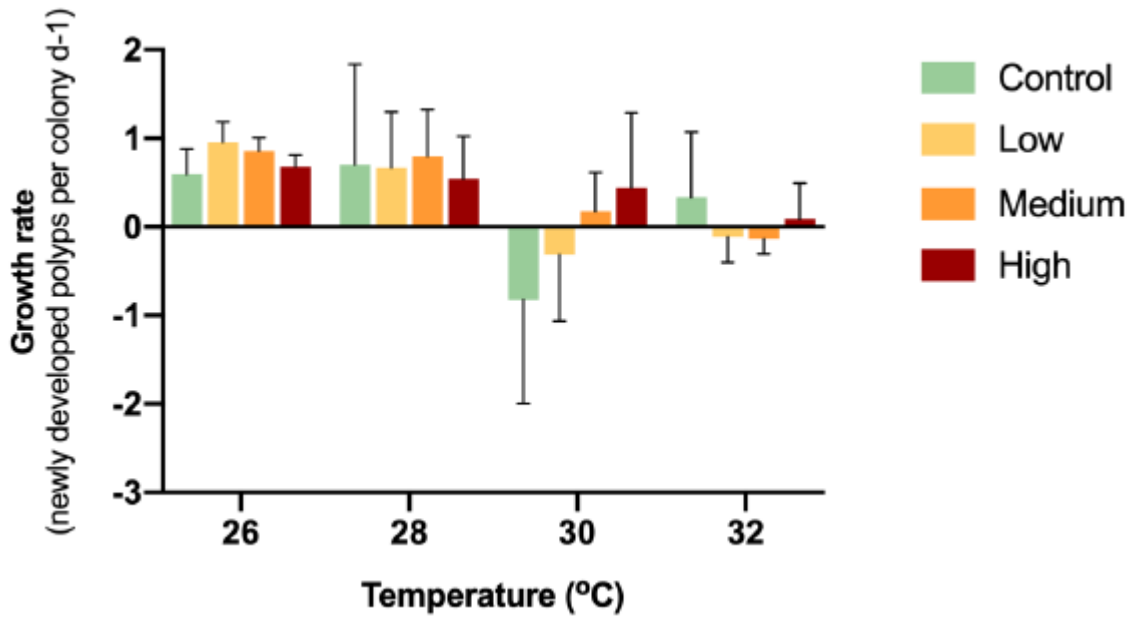

Figure 4.2 | Growth rates of corals with different glucose concentrations and temperatures during the experiment. Columns indicate mean values of three replicates with error bars providing the respective SD. The growth rates did not show any statistically significant differences ($p > 0.05$).

4.5 Discussion

How does organic eutrophication (as glucose addition) affect pulsation and growth rates along with the health of the soft coral *X. umbellata*?

Findings revealed that glucose addition did not affect polyp growth and pulsation rates. The pulsation rates of *X. umbellata* showed a slight decrease in all treatments after 21 days of glucose exposure, although pulsation rates ranged within the limit of in situ pulsation rates of 34 to 42 beats min^{-1} (Kremien et al., 2013). Our findings do thus not suggest a disturbance of the soft coral caused by glucose addition. The comparison of all treatments at ambient temperature showed positive and similar growth rates of *X. umbellata*. Other experiments showed hard coral mortality with comparable glucose loading of 25 mg/L over a period of 30 days (Kuntz et al., 2005; Kline et al., 2006). Kline et al. (2006) proposed a species-specific response to glucose loadings likely mediated by dynamics of the symbiont community. In *X.*

umbellata, the positive response could point towards a symbiont community that can benefit from moderate glucose addition as energy source. Furthermore, soft corals are known to have antimicrobial properties in their coral mucus (Ritchie, 2006) and may have lower growth of potentially pathogenic bacteria compared to hard corals. In general, the disruption of the functioning of the symbiosis can lead to lower or stagnating growth rates up to mortality (Hoegh-Guldberg, 1999). Our results show less detrimental effects on soft corals under these specific experimental conditions. The findings are contrary to previous findings when sugar- stressed hard corals exhibited negative changes in photosynthetic and nutrient cycling properties which are very similar to bleaching responses (summarized in Table 4.2) (Pogoreutz et al., 2017).

Also, the heterotrophic feeding ability may be of potential importance for *X. umbellata* when coping with eutrophication. It is an additional energy source, most likely enhanced by polyp pulsation, and it may maintain the host functions even with autotrophic disruption (Wild & Naumann, 2013; Wooldridge, 2013). Switching from photo-autotrophy to mixotrophy, could have enhanced the growth rates of *X. umbellata* in treatments with elevated glucose levels. It needs to be investigated to what extent a switch to heterotrophic feeding is possible, because previous findings are contradicting. While Gohar (1940) and Schlichter, Svoboda & Kremer (1983) classified *Xenia* as completely autotrophic, Fabricius & Klumpp (1995) proposed, that *Xenia* is dependent on both feeding strategies. Soft corals have poor predatory abilities compared to hard corals (Fabricius & Klumpp, 1995) but this does not mean they cannot feed on particulate organic matter such as phytoplankton, labile detritus, microzooplankton and bacteria (Fabricius & Dommisse, 2000). Furthermore, glucose addition can have direct effects being an energy source for reef associated microbes or indirect effects as increased turbidity. Especially within closed systems, indirect effects have a considerable influence. In our experiment, the indirect effect of increased turbidity was particularly strong to observe (no measurements were performed) in the high glucose treatment during the last week of the experiment. This could have reduced the ability of soft corals to utilize energy via photosynthesis. High turbidity may have decreased the

possible combined effect of photo-autotrophically and heterotrophically fixed carbon energy sources.

Table 4.2 | Enrichment experiments with different organic carbon concentrations and coral species. All previous studies were conducted with hard corals in ambient reef temperature and showed negative impacts of elevated glucose compared to control treatments with ambient glucose levels. The present study showed neutral effects of glucose addition in combination with ambient temperature and positive effects of glucose on pulsation rates and mortality of soft corals compared to control treatments in elevated temperature. Negative (–), positive (+) and neutral (o) implications for corals are indicated.

Study	Species	Glucose concentration (mg/L)	Temperature (°C)	Time (days)	Results (compared to control)	Implications
Pogoreutz et al., 2017	*Pocillopora verrucosa*	10	27	28	Pronounced paling	–
Kline et al., 2006	*Monstrea annularis*	12,5 25	Ambient reef temperature	30	5-fold higher, significant mortality in both levels	–
Kuntz et al., 2005	*M. annularis, Agaricia tenuifolia, Porites furcata*	5 25 (lactose addition)	Ambient reef temperature	30	Species-specific higher mortality rate	–
Haas et al., 2009	*Acropora*	110	21.5 – 23.2	90	Reduced chlorophyll a tissue concen-tration, colour decrease	–
This study	*Xenia umbellata*	10 20 40	26	21	No significant effect	o
This study	*Xenia umbellata*	10 20 40	32	45	Significantly higher pulsation rate, reduced mortality in all treatments	+

Does organic eutrophication (as glucose addition) influence the resistance of the soft coral *X. umbellata* to ocean warming?

Under simulated ocean warming, soft corals that had experienced organic eutrophication before, maintained significantly higher pulsation rates and showed no mortality compared to the controls. The reduction of pulsation could be the first step of *X. umbellata* to cope with temperature stress as energetic investment is minimised. Therefore, pulsation rates may be used as inexpensive, easily detectable and non-invasive early warning indicator for ocean warming effects on benthic communities. Already 64 years ago, Horridge (1956) published work about "the responses of *Heteroxenia* to stimulation and to some inorganic ions". The only other related studies focused on the toxicity and sublethal effects of crude oil (Cohen, Nissenbaum & Eisler, 1977; Studivan, Hatch & Mitchelmore, 2015). Cohen, Nissenbaum & Eisler (1977) pulsation with a decline to less than 50 % when exposed to crude oil. Studivan, Hatch & Mitchelmore (2015) suggested *Xenia elongata* as bioindicator for coral species in other locations than the Indo-Pacific reefs because of its high sensitivity to changes in water quality.

Nevertheless, pulsation provides great benefits for the coral colony. The enhancement of photosynthesis during the day and the prevention of refiltration during both day and night (Kremien et al., 2013). Thus, a maintained pulsation may protect *X. umbellata* exposed to warming. In treatments with elevated glucose levels, the switches from primarily photo-autotrophically to combining heterotrophically fixed carbon and photosynthesis, could have supported energetic investment required for pulsation. Organic eutrophication as additional energy source may thus increase the resistance of soft corals to ocean warming.

In general, heterotrophic feeding can support corals when photosynthetic activity is reduced by photodamage (Borell & Bischof, 2008; Wooldridge, 2013). In limited amounts, glucose addition may enhance the availability of photosynthetically fixed carbon to the coral host. This excess fixed carbon may be stored in tissues as lipids and create an important energy reserve or directly be channeled into growth and reproduction (Wild & Naumann,

2013; Wooldridge, 2013). With low and moderate levels of autotrophic disruptions, the coral host may also be able to consume the existing energy reserves (Grottoli, Rodrigues & Palardy, 2006) that likely have been fueled by previous glucose addition.

Throughout the temperature experiment, little mortality was noted among soft coral colonies, but they did not maintain their normal appearance and showed visible signs of stress. While the increased temperature up to 28 °C showed a positive growth rate in all treatments (not notably different from growth rates calculated at 26 °C), in general the growth rates decreased with increasing temperature.

However, an increase up to 28 °C did not negatively affect the soft corals. In the Red Sea, an among others distribution area of *X. umbellata*, 28 °C are common water temperatures (Chaidez et al., 2017). Therefore, the colonies may potentially be resistant to a short exposure time. 30 °C showed the first evidence of negative influence of elevated temperature on *X. umbellata* colonies. Other studies concluded the same and showed higher rates of mortality with temperatures >30.5 °C (Strychar et al., 2005; Cantin et al., 2010). Some studies even predict xeniids to be more susceptible to temperature stress than other Octocorallia (Strychar et al., 2005; Sammarco & Strychar, 2013). In the present study, no significant difference in growth rates could be detected between corals at different glucose treatments. In addition, the high glucose treatment continued to slightly grow even until the end of the experiment, on the contrary to the lowest glucose concentration. These findings may suggest that glucose addition supports *X. umbellata* to maintain growth and increase the ability to withstand the effect of elevated temperature.

In contrast to our findings, high levels of glucose caused strong negative effects on two hard corals, showing that coastal eutrophication produces an additional stress factor that even outweighed nutritional benefits (Fabricius et al., 2013). These results highlight the fact that coral mortality patterns may depend on each type of stressor, the species of coral, and the duration of exposure time as Kuntz et al. (2005) mentioned.

Ecological perspective

Human activity will likely further increase organic eutrophication as a local stressor of marine ecosystems, and climate change will further increase ocean warming. Even achieving the ambitious goal of 1.5 °C of global warming under Paris Agreement, will lead to a loss of 70–90 % of reef building corals versus today (Hoegh-Guldberg et al., 2018). Given this severity of coral loss, understanding the ecophysiology of fast-growing xeniids is an important aspect for future predictions (Wild & Naumann, 2013). Besides, Norström et al. (2009) reported benthic reef community shifts which are often related with changes in the dynamics of bottom-up factors (e.g. nutrient enrichment). *Xenia* was observed to overgrow large fields of rubble in the Komodo National Park in Indonesia where blast fishing destroys living coral at an extensive rate (Fox et al., 2003).

Different recovery and reproduction strategies (r- and k-strategies) are also involved in the abundance of coral types after severe mortality or bleaching events with increased frequency (Hoegh-Guldberg, 1999). Soft corals often have high fecundity and several dispersal modes to rapidly colonize damaged reef areas (Fox et al., 2003), while hard corals may not be able to get mature or reproductive before the next period of intensive environmental stress. Soft corals have a competitive advantage against hard corals because of higher resilience to temperature induced bleaching and ocean acidification (Inoue et al., 2013; Wild & Naumann, 2013). Findings of a study on *Ovabunda macrospiculata* suggest the octocoral tissue to have a possible protective role against sclerite loss under acidic conditions (Gabay et al., 2014). In addition, powerful chemical defense mechanisms benefit soft corals (Fox et al., 2003).

The results of the present study suggest that *X. umbellata* has a high resistance to a combination of organic eutrophication and warming. These findings show contradictory results to previous studies (summarized in Table 4.2) and speak for the fact, that soft corals respond differently than hard corals when exposed to organic eutrophication. When comparing findings of this study for soft corals with previous results for hard corals (Haas,

Al-Zibdah & Wild, 2009; Pogoreutz et al., 2017), it can be assumed that soft corals under the predicted increases of organic eutrophication and warming gain more and more competitive advantages. This may further facilitate phase shifts from hard to soft corals in warming reefs.

Reef-associated biodiversity is under threat (Sebens, 1994). With a loss of diversity and a dominance of soft corals, a decline of important ecosystem functions is predicted. A loss of reef-building corals will lead to a decrease in 3D framework systems and lessened release of mucus (Wild & Naumann, 2013). Still, the overall productivity of the coral reef may not necessarily decrease because of the higher photosynthesis to respiration ratio that Kremien et al. (2013) discovered for the pulsating *H. fuscescens* compared with non- pulsating hard and soft corals. But xeniid soft corals do not release extensive amounts of organic matter into the reef environment and, therefore, cannot function as an energy carrier and particle trap (Bednarz et al., 2012; Wild & Naumann, 2013).

However, in this way, the high economic and social capacity of coral reefs will not be preserved (Hoegh-Guldberg et al., 2018). Although climate change is a global issue, reducing local stressors can help in maintaining reefs and enhancing reef resilience including limiting the long-term damage (Hughes et al., 2003). Reducing organic eutrophication is one promising management strategy to avoid phase shifts from hard to soft corals.

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Chapter 5

Individual and combined effect of organic eutrophication (DOC) and ocean warming on the ecophysiology of the gorgonian *Pinnigorgia flava*

First author contribution

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Individual and combined effect of organic eutrophication (DOC) and ocean warming on the ecophysiology of the Octocoral *Pinnigorgia flava*

Edoardo Zelli*[,1,2], Susana M. Simancas-Giraldo*[,1], Nan Xiang[1,3], Claudia Dessì[1,4], Nadim Katzer[1], Arjen Tilstra[1], Christian Wild[1]

* These authors contributed equally to the study.
[1] Marine Ecology Department, Faculty of Biology & Chemistry (FB 2), University of Bremen, 28359, Bremen, Germany
[2] School of Science, University of Waikato, Tauranga, New Zealand
[3] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany
[4] Università degli Studi di Cagliari, Dipartimento di Scienze della Vita e dell'Ambiente, Via Tommaso Fiorelli 1, 09126, Cagliari, Italy

Corresponding Author:
Edoardo Zelli[1,2]
Email address: ez14@students.waikato.ac.nz

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5.1 Abstract

Dissolved organic carbon (DOC) enrichment and ocean warming both negatively affect hard corals, but studies on their combined effects on other reef organisms are scarce. Octocorals are likely to become key players in future reef communities, but they are still highly under-investigated with regard to their responses to global and local environmental changes. Thus, we evaluated the individual and combined effects of DOC enrichment (10, 20 and 40 mg L^{-1} DOC, added as glucose) and warming (stepwise from 26 to 32 °C) on the widespread Indo-Pacific gorgonian *Pinnigorgia flava* in a 45-day laboratory experiment. Oxygen fluxes (net photosynthesis and respiration), as well as Symbiodiniaceae cell density and coral growth were assessed. Our results highlight a differential ecophysiological response to DOC enrichment and warming as well as their combination. Individual DOC addition did not significantly affect oxygen fluxes nor Symbiodiniaceae cell density and growth, while warming significantly decreased photosynthesis rates and Symbiodiniaceae cell density. When DOC enrichment and warming were combined, no effect on *P. flava* oxygen fluxes was observed while growth responded to certain DOC conditions depending on the temperature. Our findings indicate that *P. flava* is insensitive to the individual effect of DOC enrichment, but not to warming and the two stressors combined. This suggests that, if temperature remains below certain thresholds, this gorgonian species may gain a competitive advantage over coral species that are reportedly more affected by DOC eutrophication. However, under the expected increasing temperature scenarios, it is also likely that this octocoral species will be negatively affected, with potential consequences on community structure. This study contributes to our understanding of the conditions that drive phase shift dynamics in coastal coral reef ecosystems.

5.2 Introduction

Gorgonians (Octocorallia; Malacalcyonacea [= Gorgonians]) have, in general, a flexible internal gorgonin skeleton which in some species take on tree- or bush-like forms that can

reach considerable sizes (up to >1 m in height). This non monophyletic group differs greatly from the most studied Scleractinian corals (Hexacorallia), also known as hard or reef-building corals, because the latter have a hard, calcium-based skeleton which, in most cases, constitutes the very foundation of the reef ecosystem (Roberts & Hirshfield, 2004). Nevertheless, both scleractinian and gorgonians are generally considered ecosystem engineers due to the key ecological role they carry out in ecosystem functioning (Jones et al., 1994, Rossi et al., 2017). In particular, gorgonians typically have an arborescent shape that forms three-dimensional structures which increase environmental complexity. While gorgonians are not a recognized taxonomically valid group, this term is useful for referring to soft corals that have a skeletal axis composed of gorgonin (McFadden et al., 2010), and a more extensive three-dimensional structure as a result of their axial skeleton. These organisms often create underwater forests that foster biodiversity either as a substrate for epifaunal communities or by acting as nursery areas for a large number of species (Sánchez et al., 2016; Rossi et al., 2017). In tropical coral reef ecosystems, the ecological functioning of many gorgonian species is strongly dependent on the relationship between the coral host, its associated microbes and associated Symbiodiniaceae algae (i.e., collectively the coral holobiont), which provide the foundation for their ecological success, as they do for many other tropical coral species. In this mutualistic association, the coral host provides inorganic nutrients in exchange for photosynthetically fixed carbon (photosynthates) and amino acids translocated from the Symbiodiniaceae (Muscatine & Porter, 1977), which fuels gorgonian growth (Ramsby, 2014).

Anthropogenic impacts on a local and a global scale can threaten the coral-Symbiodiniaceae symbiosis (Pogoreutz et al., 2017; Rädecker et al., 2021; Tilstra et al., 2017). Increased water temperatures can induce malfunctioning of the Symbiodiniaceae photosynthetic apparatus leading to a reduction in Symbiodiniaceae cell density and subsequently photosynthetic activity (Weis, 2008, Fitt et al., 2001; Hughes et al., 2017, 2018). Moreover, extended periods of high temperatures generally lead to coral mortality due to physiological damage and impaired metabolism (Glynn, 1984; Berkelmans et al., 2004). These negative impacts of

increased temperatures are described in both hard corals (Santos et al., 2014; Cardini et al., 2016; Hughes, et al., 2018; Ziegler et al., 2019) and octocorals, such as gorgonians (Lasker et al., 2003; Harvell et al., 2001; Prada et al., 2010). Gorgonians often respond similarly compared to the more extensively studied hard corals – i.e., display bleaching processes whereby large extents of the coral rapidly pale through loss of their algal endosymbionts via destabilization of the coral–algal symbiosis. Coral bleaching may lead to diminished photosynthetic activity and eventually to mortality when prolonged over long periods (Sugget and Smith 2020 and references therein).

Yet, coral resilience towards temperature-induced impacts can be simultaneously affected by other stressors (Hughes et al., 2003; Brodie et al., 2011), including complex combinations of stressors arising from global climate change and local degraded water quality. The presence of humans in the proximity of coral reefs can result in an elevated input of nutrients into reef waters. Nutrients associated with human activities, i.e., particulate and dissolved inorganic and organic matter, can enter reef ecosystems via riverine influx, diffuse discharge, or as aeolian dust (Cuet et al., 1988; Fabricius and De'ath 2004; Brodie et al., 2009, 2012; Weber et al., 2012). Some corals may benefit from particulate organic matter enrichment because it enhances feeding rates and growth, providing even higher competitive advantages, especially for species more dependent on heterotrophic filter feeding. A consequence of this is a potential community shift from corals that can grow at extremely low food concentrations to more heterotrophic and less diverse coral communities (Fabricius, 2005). However, anthropogenic eutrophication of coastal waters has also been linked to a decline in coral cover (Bednarz et al., 2012; Wiedenmann et al., 2013; Pogoreutz et al., 2017).

Land sourced runoff containing elevated nutrient concentrations may result in a wide range of impacts on hard coral communities (Grigg 1995; Ward and Harrison 2000; Koop et al., 2001; Loya et al., 2004; Fabricius and De'ath 2004; Fabricius et al., 2004, 2007); including reduced recruitment (Loya et al., 2004; Fabricius, 2005), modified trophic structures

(Fabricius and De'ath 2004), altered biodiversity (van Woesik et al., 1999), and increased mortality (Ward and Harrison 1997; Harrison and Ward 2001; Kline et al., 2006). Under extreme situations, such impacts can result in the collapse of the coral community (Smith et al., 1981). Furthermore, experiments on hard corals indicate that increased nutrient levels can reduce tolerance to heat stress (Cardini et al., 2015), which assigns critical importance to local management of water quality to mitigate the pressure induced by global climate change (Webb et al., 1975; Hoogenboom et al., 2012). Thus far, studies assessing the impacts of nutrient enrichment on coral reefs have primarily focused on hard corals (e.g., Wiedenmann et al., 2013; Vega Thurber et al., 2014; Cardini et al., 2015) with very few studies on octocorals. Gorgonians may have a capacity to cope with inorganic nutrient enrichment (Fleury et al., 2004; McCauley et al., 2019), while organic matter fluxes and metabolic activity in other octocorals may be negatively affected (Bednarz et al., 2012; Baum et al., 2016; McCauley et al., 2019).

Nevertheless, water quality has many components which have not been as widely studied and deserve more attention, such as dissolved organic carbon (DOC). In hard corals, DOC enrichment can cause a breakdown of the coral-Symbiodiniaceae symbiosis (Pogoreutz et al., 2017), similar to thermally stressed corals (Rädecker et al., 2021). This process is intimately linked with nitrogen (N) availability (Wiedenmann et al., 2013; Wooldridge, 2013; Vega Thurber et al., 2014; Rädecker et al., 2021). DOC enrichment may stimulate the fixation of atmospheric N_2 by increased microbial -i.e., diazotrophic- activity, increasing N concentration and triggering the rapid uptake of N by Symbiodiniaceae (Pogoreutz et al., 2017). This may eventually lead to phosphorus (P) starvation and a stoichiometric shift in the N:P ratio, causing the photosynthetic apparatus to malfunction resulting in the onset of bleaching (Tchernov et al., 2004; Wiedenmann et al., 2013; Wooldridge, 2013). Other studies showed hard coral mortality under DOC enrichment treatments and similar experimental duration (Kline et al., 2006; Kuntz et al., 2005). Recent studies assessing the individual and combined effects of DOC enrichment and increased water temperatures on the fleshy, pulsating octocoral *Xenia umbellata* reported that DOC enrichment had a

positive effect on its heat tolerance when functional and ecological variables, i.e., pulsation, were considered (Vollstedt et al., 2020). DOC enrichment also did not have a significant effect on the ecophysiology (oxygen production, consumption, and growth) of *X. umbellata* suggesting that certain octocorals may be more resistant to individual DOC enrichment than hard corals (Simancas et al., 2021). In line, *X. umbellata* showed decreased gross photosynthetic activity at 28°C and 30 °C but still positive net photosynthesis at 32 °C displaying certain degree of resistance to elevated temperatures.

Despite empirical evidence showing that octocorals are becoming more abundant, displaying more significant functional roles than in the past (Lenz et al., 2015; Ruzicka et al., 2013), and potentially representing a "new normal" for some coral reefs, they remain largely under-investigated (Lasker et al., 2020). In particular, how the combined effects of local (e.g., organic eutrophication) and global factors (e.g., warming) influence the ecophysiological responses in gorgonians is still largely overlooked. Thus, in this study, we investigated how the individual and combined effects of DOC enrichment and increased temperatures affected the ecophysiology of the Symbiodiniaceae-associated gorgonian *Pinnigorgia flava* (Nutting, 1910). Following previous studies on nutrient addition and thermal stress (Fabricius et al., 2013; McCauley et al 2019), we assessed the effects of individual DOC concentrations and a subsequent stepwise increase in temperature on fragments of the gorgonian coral *P. flava* in a 45-day manipulative experiment. We hypothesized that the individual and combined effects of DOC enrichment and high temperatures would negatively impact this gorgonian's ecophysiology. Specifically, we expected coral photosynthesis, respiration activity and growth rates to significantly decrease under both individual and combined effects of DOC enrichment and increased temperature (with stronger negative effects under multiple stressors). As such, the present study assessed the effects of 1) organic eutrophication, i.e., DOC enrichment, 2) increased water temperatures, and 3) a combination of both factors on the ecophysiology of the gorgonian *P. flava* by measuring photosynthesis and respiration activity via oxygen fluxes, Symbiodiniaceae cell densities and coral growth (i.e., changes in surface area), over 45 days.

5.3 Materials and Methods

The experiment was carried out at the Marine Ecology laboratory of the Centre for Environmental Research and Sustainable Technology (UFT), University of Bremen, Germany.

Experimental tank setup

Experimental design, methodologies, and the seawater parameters are described by Vollstedt et al. (2020) and Simancas-Giraldo et al. (2021). Summarizing, our experiment was divided into two temporal stages consisting of 21 and 24 days, respectively. In the first experimental stage, 12 tanks (3 tanks per treatment including 3 controls) were used to test the individual effects of three different DOC concentrations (low: 10 mg L^{-1}, medium: 20 mg L^{-1} and high: 40 mg L^{-1}), while the control tanks were kept without DOC additions at environmental conditions (2 to 3 mg L^{-1}). During the second experimental stage, warming scenarios were implemented and four additional tanks were added as controls for the increased temperature treatments. In this stage, we tested the individual and simultaneous effects of warming by raising the water temperature following a stepwise increase from 26 °C to 32 °C (2 °C per week) in every tank except for the four tanks assigned as temperature controls. In further detail, 16 tanks were prepared in total for the experiments comprising this second experimental stage and ensured to have starting comparable conditions. Then, 12 of these tanks were used to accommodate the individual DOC treatments and the corresponding controls (i.e., DOC controls) during the first stage, while the additional four tanks remained in wait for the start of the second stage of experiments. These additional tanks were installed and held with identical initial conditions to the ones in the DOC control tanks of the first stage and were employed as temperature controls during the second stage of the experiments, when all DOC treatments, including the DOC controls, were exposed to warming conditions.

Each experimental tank had a total volume of 60 L and consisted of two parts: a back part acting as a technical tank separated by a glass division from a front part which contained the experimental fragments. Both parts were connected by a pump and an outflow situated in the glass division between the frontal and back part that allowed water exchange between these two sections. Thus, each 60 L tank represented an independent closed system with its own circulation. Above each tank, a LED light simulated daylight conditions. All tanks were filled with artificial seawater (Tropic Marin ® ZooMix Sea Salt) and kept at the same conditions as the tank from which parent colonies originated. One month prior to the experiments, water was cycled altogether with the parental colonies tank (i.e., the maintenance tank) for a minimum period of two weeks, before closing connections between tanks making each of them independent. Seawater salinity was kept at 35 ± 0.6 ppt, pH of 8.2 ± 0.1, and temperature at 26.0 ± 0.3 C (mean ± SE) and exposed to a 12:12 light:dark period at constant light intensity (120.8 ± 10.2 µmol m^{-2} s^{-1}), while additional chemical water parameters, such as the pH, KH, Ammonium (NH_4^+), Nitrite (NO_2^-), Nitrate (NO_3^-) and Phosphate contents (PO_4^{3-}) were measured and adjusted manually twice per week. A general summary on the chemical parameters through the experiment can be found in Vollstedt et al. (2020) while other relevant parameters such as salinity and pH can be found summarized by treatment in Xiang et al. (2021). We additionally present details on the recorded mean values measured per tank through the experiment in our supplementary information S1.

A parallel study on the soft coral *X. umbellata* was performed in conjunction with the current experiment. In particular, each experimental tank contained four frames, two holding n=10 *P. flava* and two holding n=10 *X. umbellata* fragments. The additional frames with *X. umbellata* fragments were used for different studies besides this one (i.e., Vollstedt et al., 2020; Simancas et al., 2021 and Xiang et al., 2021). Our selection of DOC treatment concentrations for this experiment was based on previous studies that manipulated glucose loading (Kline et al., 2006; Pogoreutz et al., 2017) alongside previous findings by e.g., Baum

et al. (2016), where some octocoral species were shown to be able to inhabit highly eutrophicated zones in particular reefs. Untreated DOC tanks (2 to 3 mg L^{-1}) were employed as DOC control condition. The temperature treatments (28 °C, 30 °C, 32 °C) were selected to simulate the predicted rising ocean temperatures based on the 2018 IPCC report (De Coninck et al., 2018). DOC concentrations were measured using a Total Organic Carbon (TOC) analyzer (TOC-L CPH/CPN PC-Controlled Model, Shimadzu, Japan) twice a day, and adjusted by adding a standard solution containing D-Glucose anhydrous (purity: 99%, Fisher Scientific U.K. Limited, Loughborough, UK). The water temperature was measured daily and kept under stable conditions using a heater for each tank and salinity was kept steady by adding demineralized water to the system to compensate for evaporation.

Experimental implementation

P. flava was molecularly identified by Xiang et al. (2021) and selected for this study based on its widespread occurrence in the Indo-Pacific (Vargas et al., 2020) and its relatively simple breeding and maintenance in experimental tanks (Conci et al., 2019, Vargas et al., 2020; Vargas et al., 2022). The identity of the *P. flava* associated Symbiodiniaceae of our fragments was not confirmed through molecular means. However, since *P. flava* has been consistently reported to be associated with *Cladocopium sp.* (Goulet et al., 2008a), we inferred our *P. flava* fragments to be associated with a Symbiodiniaceae species within this genus. (see Goulet et al., 2008b and the Symbiodiniaceae Style Guide" by Parkinson et al. Lab, 2022 -for further details). We therefore did not expect the specific species of Symbiodiniaceae to play a significant role in the interpretation of our results.

During this study, approximately 280 fragments (2 ± 0.5 cm in length) of the gorgonian *P. flava* were propagated randomly from three clonal mother colonies (similar sized). The mother colonies initially originated from the Caribbean and were kept in a 420 L aquarium in the facility for more than one year prior to the start of our experiment. Except for water flow rates, we kept the same conditions of this aquarium for all the control tanks used for

this experiment (Supplementary Information S1). Each fragment was subsequently attached to calcium carbonate plugs (Aqua Perfect frag plug for light grid / Round 1cm (AP-7004-0) using coral glue (D-D AquaScape Construction Epoxy). Two plastic grids were used to fit a total of ten gorgonian fragments per grid. The fragments were then randomly assigned to their corresponding experimental tanks and allowed to acclimatize before the start of the experiment. A total of 240 *P. flava* fragments (20 frags per tank x 12 tanks) were employed during the first stage of the experiment (individual DOC addition). These fragments were acclimated in the experimental system for five days before the start of the first stage. The remaining 40 fragments were kept at ambient conditions in the maintenance tank for the first stage of the experiment. These fragments were then distributed evenly in the temperature control tanks and given five days to acclimate before the second stage of the experiment started.

Quantification of oxygen fluxes

The oxygen (O_2) fluxes were calculated according to Bednarz et al., (2012). Three fragments from each treatment were transferred to individual incubation glass chambers for measurements for oxygen quantification. In addition, one glass chamber per tank was filled solely with seawater to serve as a control to account for planktonic background metabolism (i.e., control glass chamber). The starting O_2 concentration in each chamber was measured using a salinity-corrected O_2 optode sensor (FDO®925 Optical Dissolved Oxygen Sensor, range:0.00 to 20.00 mg O2 L-1, accuracy: ± 0.5% of the value, MultiLine® IDS 3430, WTW). All chambers were sealed airtight (without any air bubbles inside) and incubated twice per day, once for measuring O_2 production, i.e., net photosynthesis, and once for O_2 consumption, i.e., respiration. O_2 production was measured through incubations performed under light conditions, putting back the sampled glass chambers into the experimental tanks to keep the water temperature steady. Each glass chamber was opened, and end O_2 concentrations were measured as soon as the first incubation was concluded. The glass chambers were then closed immediately and subsequently incubated under complete

darkness to measure O_2 consumption. During this measurement, the glass chamber was placed in darkened water baths which were completely clad with a handmade black coating and located inside a dark room. The temperature in the water baths was kept constant via thermostats, mirroring the temperatures in the corresponding experimental tanks. Thus, both water temperature and dark conditions were ensured during the dark incubations. The incubations lasted for approximately two hours each. O_2 fluxes were subsequently calculated from these dark and light incubations, where O_2 initial concentrations were subtracted from the final concentrations and the results were normalized to the incubation time. The O_2 fluxes measured were further corrected by the background seawater control signal, subtracting the O_2 flux measured in the control glass chamber from the O_2 flux in the glass chamber containing the coral fragment. These corrections were further standardized by the incubation water volume and the calculated O_2 fluxes were finally normalized to the corresponding coral fragment surface area.

Symbiodiniaceae density

Symbiodiniaceae cell counting was performed at the end of the experiment on day 45 by randomly selecting *P. flava* fragments (n = 3) from each aquarium treatment and cutting a ~1.5 cm tip. The branch tips were weighed in a four-digit analytical balance, to measure wet weight. Host tissue was then separated from its central gorgonin axis by mechanical removal of the tissue using a scalpel. Subsequently, simple mechanical movements were employed to separate the tissue from the axis. The resulting tissue slurry was collected in 2 mL Eppendorf tubes and homogenized mechanically with 1 mL of demineralized water using Eppendorf micropestles. Symbiodiniaceae cell density was subsequently quantified microscopically immediately after extraction using an improved Neubauer hemocytometer. Final counts were normalized to the corresponding coral fragment wet weight (Forcioli et al., 2011; McCowan et al., 2011; Cardini et al., 2015).

Growth measurements

Growth of *P. flava* fragments was measured by image analysis using photographs taken continuously throughout the experiment (n = 3). The same fragments that were chosen for O_2 fluxes were photographed over time. Photographs of the fragments were taken once per week, always from a lateral view. The small fragments were glued as single unbranched fragments at the start of the experiment, and photographs were taken, always procuring to capture the full extension plane of each photographed fragment. A camera (Canon EOS 650 D, Canon Inc., Japan) with an unchanged camera setting and the objective (EF-S 18-55 IS II Objective, Canon Inc., Japan) were used to keep identical conditions such as the height of the lens above the floor (85 cm), height of tank above the floor (75 cm) and distance tank-camera (31 cm). The same photographic setup, with a constant distance to the specimens and a known size measurement reference were used every time the fragments were photographed. Subsequently, photographs were edited and processed using ImageJ software (version 1.44). Growth was derived by assuming the fragments to have the shape of a cylinder. Both length and the width (diameter) of the fragment were measured. As the fragment's diameter was slightly different throughout its length, the top, middle and bottom of the fragment were measured to calculate an average. Finally, changes in the coral fragments' surface area were calculated over time.

Data Analysis

Data analyses were carried out using the computing software R version 3.5.2 (R Core Team, 2018) and Rstudio version 1.1.456 (R Core team, 2018) and the R package "Lme4" from Bates et al. (2015). In order to check whether there were any significant differences among the treatments, a Linear Mixed-Effects Model (LMM) was used for O_2 fluxes and growth, whilst a simple Linear Model (LM) was used to evaluate the Symbiodiniaceae cell densities. After an outlier treatment was performed, LMM models that suited the data were

calculated and verified using model diagnostics: i.e., model fit quality was carefully assessed using Pearson's residuals variance plots for each parameter together with linearity checks of the factors tested during the model's construction. The best model was chosen according to results obtained from direct model comparisons via ANOVA type II, alongside AIC criterion for additional comparison and best model selection. To estimate the significance of the fixed factors, we implemented an ANOVA type III for O_2 fluxes and Symbiodiniaceae cell densities, and an ANOVA type II for growth (Zuur et al., 2009). Corresponding approaches for degrees of freedom approximation were used accordingly (Kuznetsova et al., 2014). When p-values were determined to be $p < 0.05$, fixed factors were considered statistically significant. Whenever significant differences were found, a corresponding post hoc Tukey test was executed using the R package "emmeans" by Lenth (2019). In further detail, the analyses were performed independently for each stage, ensuring consistency with the experimental design, i.e., we created a LMM dedicated to the first stage: individual DOC addition in 12 tanks, with DOC (four levels), time and the interaction thereof as fixed factors. Additionally, a second model was created for the second stage: with DOC addition and increased temperature in 16 experimental tanks, DOC (five levels), temperature, and the interaction of DOC and temperature as fixed factors. The aquaria tank's information (i.e., the corresponding tank identity) was included as a random factor in all our LMM models to account for additional sources of noise or unwanted variation related to differences among tanks. Furthermore, the donor colony identity information was included as random factor in the growth assessment models but not in the O_2 fluxes or the Symbiodiniaceae cell density models, always favoring model fit and statistical power. Hence, caution is advised when interpreting these results, as the models excluding this factor do not consider colony slope variations. As the four temperature control tanks were solely utilized for the second stage of the experiment, they were only included in the statistical analysis thereof. In addition, except for flow rate related parameters, the measured system characteristics of these four tanks did not differ statistically when compared to those of the DOC control tanks nor to the maintenance tanks (see Supplementary Information S2).

5.4 Results

DOC enrichment

For *P. flava* fragments exposed to different DOC concentrations, neither DOC, time nor the interaction between DOC and time showed any significant effect on the O_2 fluxes (LMM; $p > 0.05$; Table 5.1).

Table 5.1 | Linear mixed-effects model for O_2 production and consumption rates (mg O_2 m^{-2} h^{-1}) of *P. flava* corals under individual DOC addition. Type III analysis of variance with Satterthwaite's approximation method for degrees of freedom.

Factor	O_2 consumption			O_2 production		
Fixed Effects	***df***	**F**	**p**	***df***	**F**	**p**
DOC	3	0. 6482	0. 6058	3	0. 9971	0. 4422
Time	4	0. 6109	0. 6578	4	1. 3105	0. 2871
DOC x Time	12	0. 6796	0. 7579	12	1. 5900	0. 1445

The O_2 production rates showed mean values that varied from a minimum of 1.06 ± 0.37 mmol O_2 cm^{-2} h^{-1} to a maximum of 1.32 ± 0.41 mmol O_2 cm^{-2} h^{-1} by the end of the first stage of the experiment (Figure 5.1A). The O_2 consumption rates showed a stable trend over time where mean values recorded at the end of the first stage were within a minimum of 0.65 ± 1.17 mmol O_2 cm^{-2} h^{-1} and a maximum of 1.61 ± 1.22 mmol O_2 cm^{-2} h^{-1} (Figure 5.1B). Cell density was measured only at the end of the experiment when all treatments had already experienced the same temperature increase. Furthermore, growth showed no significant differences when exposed to individual DOC effects (LMM; $p = 0.19$; Table 5.2).

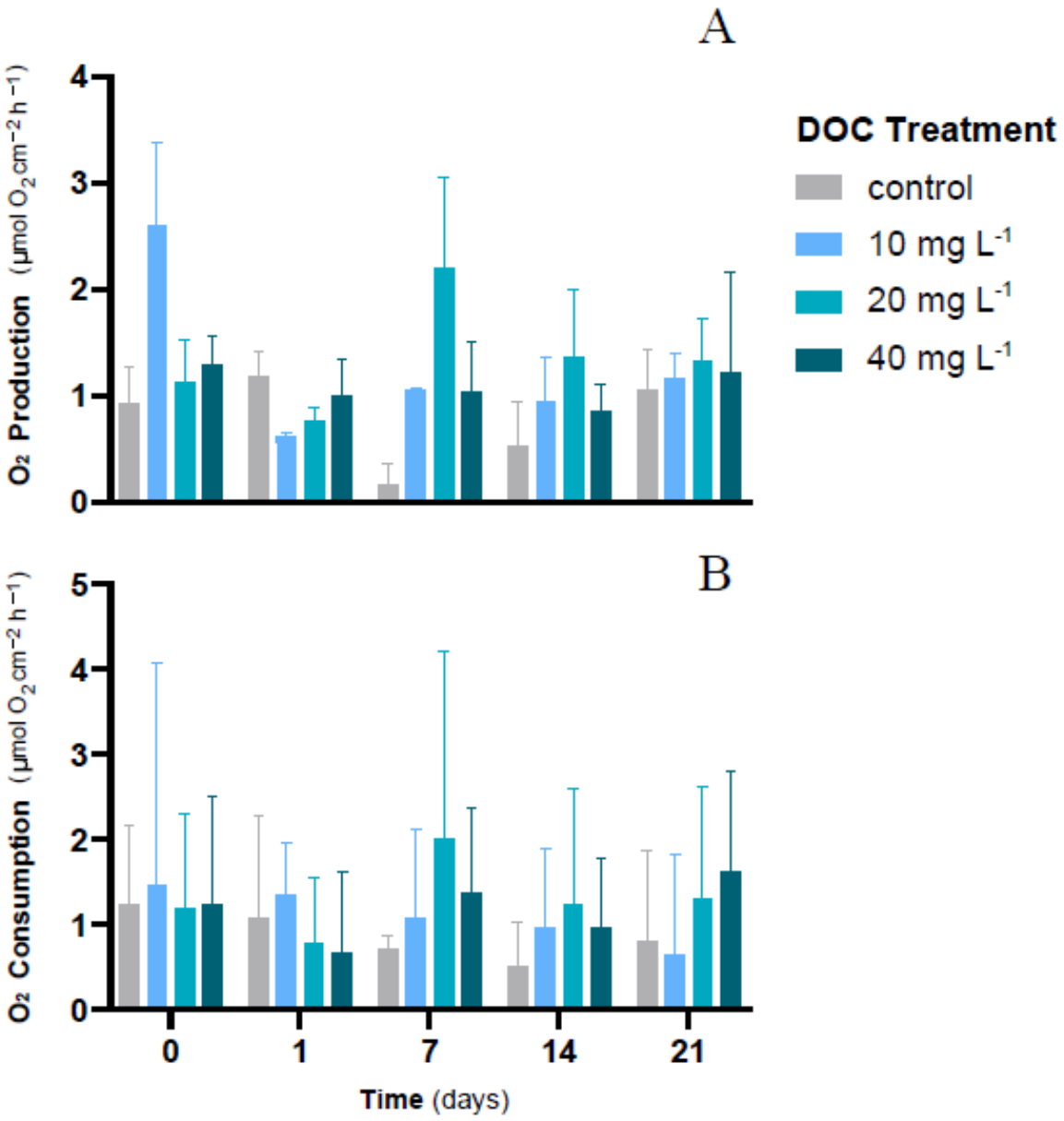

Figure 5.1 | *P. flava* O_2 fluxes response to individual DOC addition. O_2 production **(A)** and consumption **(B)** rates (mg O2 m -2 h -1) of *P. flava* under simulated DOC organic eutrophication over time. The control: 2-3 mg L^{-1} (grey), and the treatment conditions low: 10 mg L^{-1} (light blue), medium: 20 mg (teal) and high: 10 mg L^{-1} (dark blue) are represented accordingly. Individual DOC enrichment did not alter O_2 fluxes of the *P. flava* fragments at any of the DOC treatment concentrations assessed. Bars values indicate mean ± s.e.m. for n =3 corals per treatment.

Table 5.2 | Linear mixed-effects model for *P. flava* corals growth as change in surface area. Type II analysis of variance with Satterthwaite's approximation method for degrees of freedom.

		Growth		
Fixed Effects	**Experimental Stage**	***df***	**χ2sq**	**p**
DOC	1	3	4.490	0.1967
DOC	2	4	8.758	0.05701
Temperature	2	3	2.431	0.564
DOC x Temperature	2	12	48.949	**6.1e-06 *** **

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

Increased temperature

When *P. flava* fragments were exposed to simulated warming scenarios, O_2 production rates showed a decreasing trend towards higher temperatures (Figure 5.2A). In contrast, O_2 consumption rates were not significantly affected by the increased temperatures (Figure 5.2B; p = 0.81; Table 5.3). In particular, O_2 production was strongly reduced under increased temperature when compared to the temperature control. The factor temperature had a significant effect on O_2 production (LMM; F = 6.95, p = 0.001; Table 5.3), and subsequent pairwise temperature comparisons were significant only for the contrast between 26°C and 32°C and 28°C and 32°C (p = 0.0024 and p = 0.0021 respectively (Supplementary Information S3.1).

Table 5.3 | Linear mixed-effects model for O_2 production and consumption rates (mg O_2 m^{-2} h^{-1}) of *P. flava* corals under DOC enrichment and warming. Type II analysis of variance with Satterthwaite's approximation method for degrees of freedom.

Factor		**O_2 consumption**			**O_2 production**	
Fixed effects	***df***	**F**	**p**	***df***	**F**	**p**
DOC	4	0. 7785	0. 56	4	0. 8370	0. 5310
Temperature	3	0. 3254	0. 8069	3	6. 9528	**0. 0010****
DOC x Temperature	12	1. 9910	0. 0592	12	1. 2085	0. 3199

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

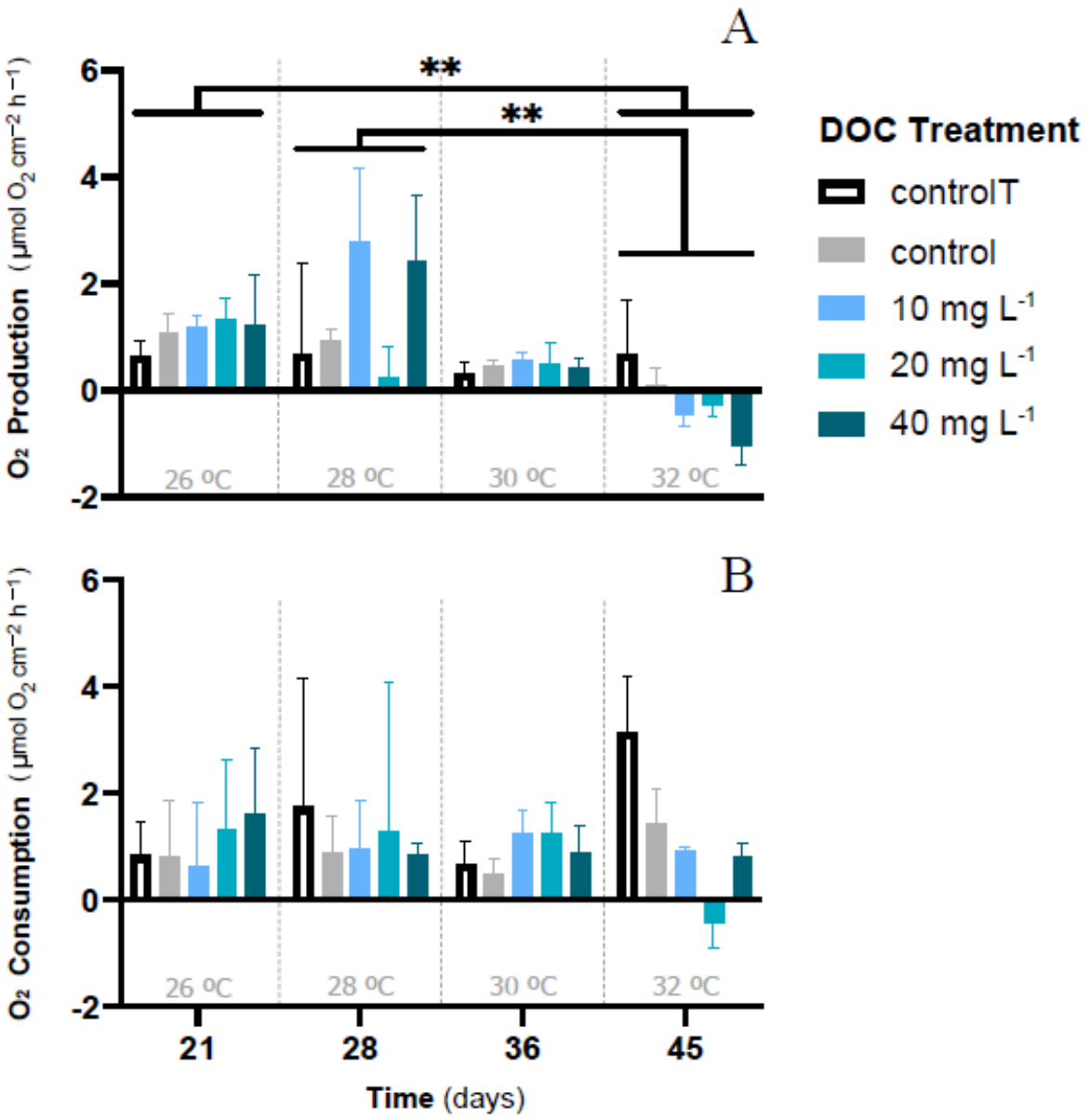

Figure 5.2 | *P. flava* O_2 fluxes response to DOC enrichment and warming. *P. flava* response in terms of **(A)** O_2 production and **(B)** consumption rates (mg O2 m -2 h -1) to increased temperature and prolonged DOC addition over time. The temperature control: 2-3 mg L^{-1} at 26 ºC (white), the combined increased temperature treatments including the DOC control: 2-3 mg L^{-1} (grey), and the DOC treatments, low: 10 mg L^{-1} (light blue), medium: 20 mg (teal) and high: 10 mg L^{-1} (dark blue) are represented accordingly. Under increased temperatures O_2 production rates were negatively affected at 32 °C, and significantly different for the contrasts between 26-32 ºC and 28-32 ºC. However, no significant effects were found on *P. flava* O_2 consumption rates, regardless of temperature. Asterisks mark statistically significant differences ($P < 0.05$; LMM), and the quantity of asterisks displayed indicate the corresponding p-value significance codes. The bars values indicate mean ± s.e.m. for n =3 corals per treatment.

Moreover, the temperature factor alone did not have a significant effect on *P. flava* growth ($p = 0.56$; Table 5.2). However, a significant reduction of Symbiodiniaceae cell density was observed for the fragments exposed to heat stress at 32 °C at the end of the experiment (LM; $p = 5.35e\text{-}10$; Figure 5.3).

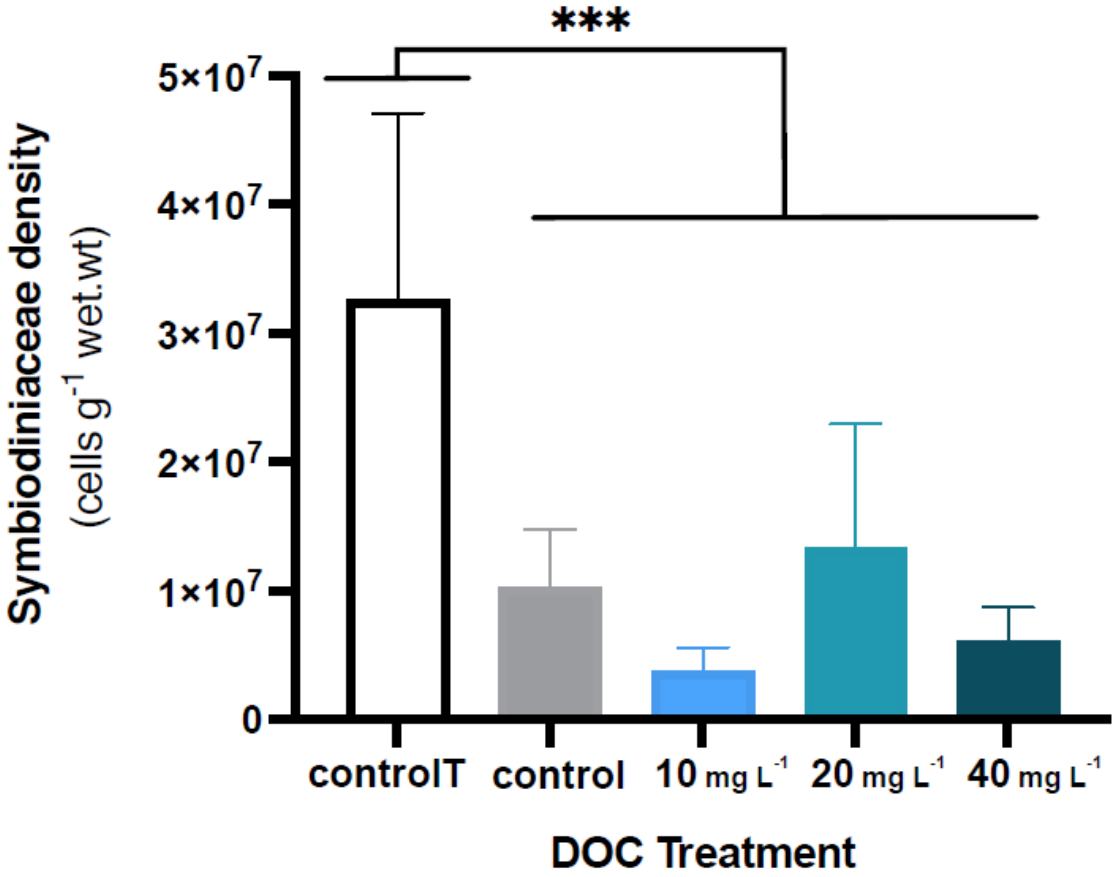

Figure 5.3 | *P. flava* Symbiodiniaceae density in response to DOC enrichment and warming. *P. flava* Symbiodiniaceae cell densities (cells g^{-1} wet.wt) by the end of the experiment (day 45), corresponding to increased temperature of 32 ºC and prolonged DOC addition. The graph presents the temperature control: 2-3 mg L^{-1} at 26 ºC (white), the combined increased temperature treatments including the DOC control: 2-3 mg L^{-1} (grey), and the DOC treatments, low: 10 mg L^{-1} (light blue), medium: 20 mg (teal) and high: 10 mg L^{-1} (dark blue). Significant reduction of Symbiodiniaceae cell densities was observed by the end of the experimental term when increased temperature reached 32 °C, for all the treatments where the fragments had been exposed to increased temperatures. Asterisks mark statistically significant differences ($P < 0.05$; LMM), and the quantity of asterisks displayed is proportional to the corresponding p-value significance code. The bars values indicate mean ± s.e.m. for n =3 corals per treatment.

DOC enrichment and warming

For the second stage of the experiment, i.e., where the gorgonian fragments were exposed to DOC enrichment and warming, neither the DOC treatment nor the interactions between DOC and temperature had a significant effect on the *P. flava* fragments' O_2 production or consumption (Table 5.3). However, there was a significant effect in the interaction between DOC and temperature on growth (LMM; p = 6.1e-06; Table 5.2; Figure 5.4).

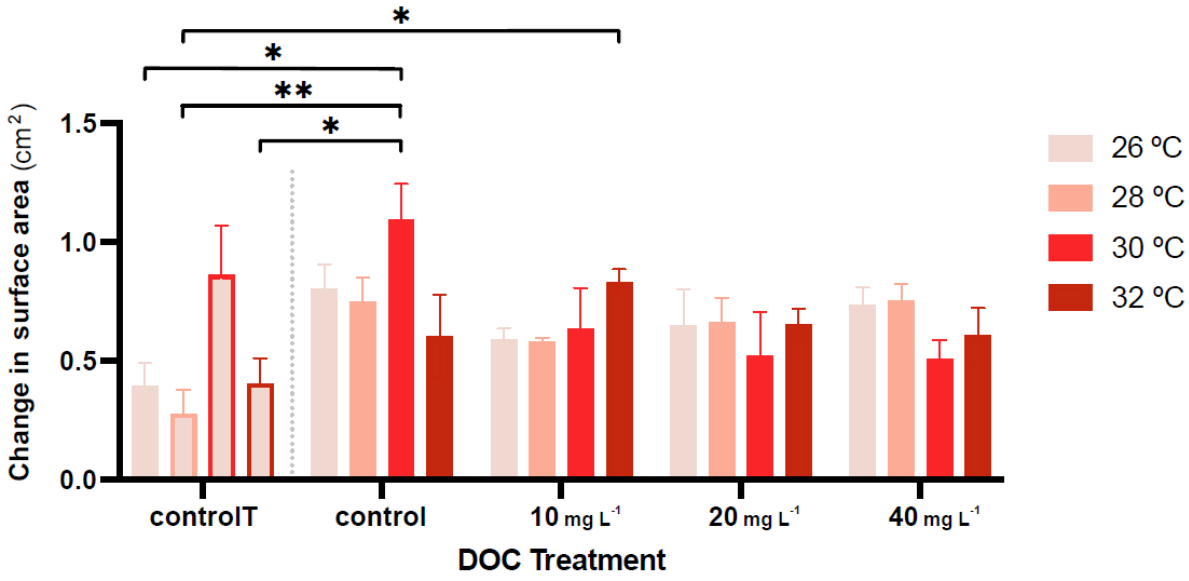

Figure 5.4 | *P. flava* growth as change in surface area in response to DOC enrichment and warming. Coral surface area changes (cm^2) corresponding to every temperature condition at each DOC treatment. The bar graph shows growth under individual DOC addition and warming as a coupled stressor (increasing red intensity scale). Temperatures per each DOC treatment are shown as 26 ºC (light pink), 28 ºC (salmon), 29 ºC (red) and 32 ºC (dark red). The first four bars correspond to the temperature control condition (controlT) with no DOC addition, constantly at 26 ºC, but at each temporal step in which the rest of the system reached the corresponding temperature treatment targets. Thus, these four bars' outlines display increasing red intensities accordingly. While neither DOC nor temperature had any effect on growth, their interaction had a significant effect with the effect of DOC on growth varying depending on the temperature value. Asterisks mark statistically significant differences ($P < 0.05$; LMM) while the bars values indicate mean ± s.e.m. for n =3 corals per treatment. The number of asterisks displayed on top of the lines indicate the corresponding p-value significance code.

The post hoc test with fixed DOC factor intercept varying across temperatures highlighted significant differences across the low (10 mg L^{-1}) DOC treatment ($p = 0.0046$) and the temperature control condition ($p = 0.0091$) at 26 ºC (see Supplementary Information S3.2). In detail, the significantly different contrasts included some of the pair combinations between the temperature control condition and the DOC control at 30 ºC and, the temperature control condition and the low (10 mg L^{-1}) DOC treatment at 32 ºC (see Figure 5.4 and Supplementary Information S3.3, S3.4 and S4 for further details).

5.5 Discussion

Effects of DOC concentration enrichment

Our results showed that individual DOC enrichment did not alter O_2 fluxes, or growth in *P. flava* fragments at any DOC concentration. Our findings contrast from previous scientific evidence conducted on hard coral species such as e.g., *Orbicella annularis* and *Pocillopora verrucosa*, which showed negative responses toward DOC enrichment since it likely triggers microbial activity, a consequent stoichiometric shift in the N:P ratio, and potential bleaching response. For instance, DOC concentration enrichment can initiate bleaching responses in hard corals (Kuntz et al., 2005; Kline et al., 2006; Haas et al., 2016; Pogoreutz et al., 2017; Morris et al., 2019). Nevertheless, our results match with recent studies conducted on the pulsating octocoral *X. umbellata* which showed no negative responses to individual DOC treatments regardless of their concentrations at the ecophysiological level (Simancas et al., 2021). Soft corals may display higher tolerance to enriched DOC concentration in the water than hard corals, by either up taking the available DOC as an additional source of energy via the host (Fabricius., 2011), or by regulating internal nutrient availability of the holobiont via the role that the host-associated bacterial communities (e.g., denitrifying bacteria) might play under changing environments (Xiang et al., 2021).

Effect of temperature

Regarding temperature increases, the findings of this study on *P. flava* resemble the results observed in our previous works conducted on *X. umbellata*. The latter coral species is not sensitive to DOC enrichment, but shows a negative response to warming, though milder when compared to the present study (Vollstedt et al., 2020; Simancas-Giraldo et al., 2021, Xiang et al., 2021). Specifically, we found no effect of warming on *P. flava*'s O_2 consumption rates, regardless of the temperatures reached during the warming stage of our study. However, we observed significant differences in O_2 production rates when subjected to warming. In particular, the rising water temperatures during the second stage of the experiment negatively affected O_2 production rates in *P. flava* at 32 °C. Such results contrast with studies performed on other gorgonian species from the Caribbean region such as e.g., *Eunicea fleaxuosa* or *Eunicea tourneforti* (Goulet et al., 2017), but align with the well-known negative trends observed on several hard coral species in response to thermal stress (Monroe et al., 2018; Ziegler et al., 2019). Both decreased O_2 production and maximum quantum yield are among the first reactions of hard corals to thermal stress. This may lead to the dysfunction of photoprotective mechanisms and impair CO_2 fixation of the coral-associated Symbiodiniaceae; thus, causing bleaching responses (Jones et al., 1998). In general, thermal stress is a widely reported factor to cause bleaching in both hard corals (Cardini et al., 2016; Hughes et al., 2018; Ziegler et al., 2019) and octocorals (Strychar et al., 2005; Slattery et al., 2019), including gorgonians (Lasker et al., 2003; Harvell et al., 2001; Prada et al., 2010; Rossi et al., 2018). Moreover, the observed reduction of O_2 production under high-temperature conditions aligned with the decreased Symbiodiniaceae cell densities observed at 32°C may have been signaling the onset of an early bleaching response in our *P. flava* coral colonies. The loss of Symbiodiniaceae cells due to environmental stressors such as elevated seawater temperatures, may lead to symbiosis breakdown which in turn causes coral bleaching (Fitt et al., 2001; Lesser et al., 2011; Karim et al., 2015). However, despite the significant drop in Symbiodiniaceae cell density during the warming phase, the fragments appeared only moderately bleached, and no mortality

was observed. This suggests a given potential for thermal tolerance until a certain threshold, that might be higher than that of many hard coral species reportedly sensitive towards warming (Hughes et al., 2018; Ziegler et al., 2019). Explanations for this relative tolerance could be related to many aspects of the coral holobiont including (but not limited to), species specific traits (e.g., resistant bacterial or microbial communities), capability to modify holobiont parameters (Goulet et al., 2017; Xiang et al., 2021), differences in colony morphology (Conti-Jerpe et al., 2022) or potential adjustments of nutritional behaviors (Grottoli et al., 2006). Further, as highlighted by Wooldridge (2014), many aspects of coral bleaching cannot be explained solely by the loss or persistence of algal symbionts amongst coral species but also by other host coral traits (e.g., metabolic rates, heterotrophic feedings capacity) which are also believed to influence the thermal tolerance. Thus, all these aspects should deserve further exploration in future studies in octocorals, including data on Symbiodiniaceae identity which likely would be needed to improve conclusions on the physiological responses of the octocoral holobiont.

Despite the experiments by Wooldrige (2014) being conducted on hard coral species, the occurrence of certain host traits in octocoral species may justify their potential resistance to thermal stress. In addition, despite the contribution of the Symbiodiniaceae to the energy budget of octocorals being species-specific (Sorokin 1991), overall, it may be lower in some octocoral species compared to hard corals (Baker et al., 2015; Ferrier-Pagès et al., 2015). Furthermore, environmental parameters such as e.g., water flow regimes or flow speed have also been shown to affect bleaching resilience, growth, mortality and to enhance coral feeding (Nakamura et al., 2003). In this study, we did not measure water flow speeds directly and water flow regimes significantly varied when comparing the maintenance tank to the rest of the experimental tanks in our system. Despite we controlled additional variation for this factor through our experimental manipulations and during our statistical analyses, flow rates discrepancy may have impacted our observed results, especially those concerning growth rates during the second stage of our experiments. Thus, the interpretation of our findings should consider these additional potential effects on coral

tolerance related responses. Moreover, some octocoral species display higher trophic plasticity concerning nutrition compared to hard coral species (Schubert et al., 2017). This has been related to a higher capacity to cope with stress conditions as Symbiodiniaceae loss may not necessarily lead to a significant change in the coral energy input, preventing some octocorals from starving and dying (Goulet et al., 2017; Schubert et al., 2017).

On the other hand, some hard corals show notable resilience capacity after bleaching by switching from acquiring fixed carbon via primarily photoautotrophic means to primarily heterotrophic means (that is, feeding) (Grottoli et al., 2006), a response that may occur also among octocoral species (Schubert et al., 2017; Lasker et al., 2020). Although we did not assess this through our study, we speculate that *P. flava* could potentially compensate the decreasing Symbiodiniaceae density and lower photosynthetic activity by either modifying or regulating additional holobiont-related parameters such as e.g., bacterial community structure, photosynthetic pigment activity or heterotrophic capacity (Goulet et al., 2017; Xiang et al., 2021; Schubert et al., 2017). This would allow *P. flava* to effectively cope with increasing water temperatures under a diminished Symbiodiniaceae community and adverse environmental conditions eventually gaining advantages over other coral species such as hard corals until a certain threshold is met.

Moreover, these hypotheses may also contribute to explain the evidences of community phase shifts from hard coral-dominated towards octocoral-dominated reefs, as reported by recent studies in the Indo-Pacific as well as Caribbean regions (Hoegh-Guldberg et al., 2009; Enochs et al., 2015; Lasker et al., 2020). However, as shown in this study, the elevated temperature still implies negative consequences for the ecophysiology of *P. flava* (i.e., decreased photosynthetic activity, moderate bleaching response) which will likely aggravate if conditions persist in the future, as seen in previous works on octocorals (Lasker et al., 2003; Harvell et al., 2001; Prada et al., 2010; Rossi et al., 2018).

Effects of DOC concentration enrichment and warming

The present study is the first to investigate the individual and combined ecophysiological effects of DOC enrichment and warming on the tropical gorgonian species *P. flava*. Our findings show that the interaction between DOC concentrations and temperature did not affect *P. flava* O_2 fluxes, while a significant effect was observed for growth. Growth was subsequently shown to respond differentially to determined DOC concentrations depending on the temperature, a feature that may confer an advantage or disadvantage to the coral under stress scenarios depending on the DOC concentration-temperature combination experienced. Furthermore, DOC concentration enrichment did not increase sensitivity to warmer temperatures in *P. flava* which contrasts with our hypothesis but aligns with our previous findings for *X. umbellata* corals (Vollstedt et al., 2020; Simancas et al., 2021). When compared to hard corals, these results suggest that *P. flava* may behave differentially towards the individual effects of DOC enrichment and warming, especially when bleaching response and growth are considered. Moreover, there was no overall effect of either temperature or the DOC concentration factor on growth. However, there was a significant effect in their interaction, in which there was an increase at 32 °C under low (10 mgl-1) DOC concentrations and the effect of DOC concentrations on growth varied depending on the temperature. It is likely that *P. flava* responds to these factors through mechanisms that actuate via diverse photochemical adjustments or physiological pathways, which should be further investigated in the future.

5.6 Conclusions

Our findings suggest that the gorgonian species *P. flava* is not affected by individual DOC enrichment, in contrast to many other hard coral species. We also observed a significant decrease in *P. flava* O_2 fluxes and Symbiodiniaceae cell densities under higher temperatures, together with significant decreases in growth when subjected to elevated DOC concentrations and warming simultaneously. Thus, we advocate that the gorgonian

octocoral in our study can still be negatively affected by increased water temperatures, despite showing substantial resistance to individual DOC enrichment. Nevertheless, DOC effects can vary depending on the temperature as observed in *P. flava*'s growth. The negative effects of expected climate change scenarios on this and potentially other octocoral species may lead to further structural simplification in coral reefs communities and ecological shifts towards alternative benthic assemblages which might gain competitive advantages (e.g., macroalgae) under altered environmental conditions (McManus et al., 2004; deYoung et al, 2008; Sguotti et al., 2018; Adam et al., 2021). For these reasons, we suggest future studies to further explore the effects of combined local and global stressors on octocoral species e.g., gorgonians, accounting for consequences of potential ecological transformation of coral reef communities' structure and productivity.

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5.8 References

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Chapter 6

Contrasting microbiome dynamics of putative denitrifying bacteria in two octocoral species exposed to dissolved organic carbon (DOC) and warming

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Contrasting microbiome dynamics of putative denitrifying bacteria in two octocoral species exposed to dissolved organic carbon (DOC) and warming

Nan Xiang[1,2,3], Christiane Hassenrück[3,4], Claudia Pogoreutz[5], Nils Rädecker[5,6], Susana Marcela Simancas Giraldo[2], Christian R. Voolstra[5], Christian Wild[2], Astrid Gärdes[1,3,7]

[1] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany
[2] Marine Ecology Department, Faculty of Biology and Chemistry, University of Bremen, 28359 Bremen, Germany
[3] Leibniz Centre for Tropical Marine Research, Fahrenheitstraße 6, 28359 Bremen, Germany
[4] MARUM - Center for Marine Environmental Sciences, University of Bremen, 28359 Bremen, Germany
[5] Department of Biology, University of Konstanz, 78457 Konstanz, Germany
[6] Laboratory for Biological Geochemistry, School of Architecture, Civil and Environmental Engineering, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
[7] Hochschule Bremerhaven, An der Karlstadt 8, 27568 Bremerhaven, Germany

Corresponding Author:
Nan Xiang[1,2,3]
Email address: nanxiang@uni-bremen.de

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6.1 Abstract

Mutualistic nutrient cycling in the coral-algae symbiosis depends on limited nitrogen (N) availability for algal symbionts. Denitrifying prokaryotes capable of reducing nitrate or nitrite to dinitrogen could thus support coral holobiont functioning by limiting N availability. Octocorals show some of the highest denitrification rates among reef organisms, however little is known about the community structures of associated denitrifiers and their response to environmental fluctuations. Combining 16S rRNA gene amplicon sequencing with *nirS* in-silico PCR and quantitative PCR, we found differences in bacterial community dynamics between two octocorals exposed to excess dissolved organic carbon (DOC) and concomitant warming. While bacterial communities of the gorgonian *Pinnigorgia flava* remained largely unaffected by DOC and warming, the soft coral *Xenia umbellata* exhibited a pronounced shift towards *Alphaproteobacteria* dominance under excess DOC. Likewise, the relative abundance of denitrifiers was not altered in *P. flava*, but decreased by one order of magnitude in *X. umbellata* under excess DOC likely due to decreased proportions of *Ruegeria* spp. Given that holobiont C:N ratios remained stable in *P. flava* but showed a pronounced increase with excess DOC in *X. umbellata* host, our results suggest that microbial community dynamics may reflect the nutritional status of the holobiont. Hence, denitrifier abundance may be directly linked to N availability. This suggests a passive regulation of N cycling microbes, which could help stabilize nutrient limitation in the coral-algal symbiosis and thereby support holobiont functioning in a changing environment.

6.2 Importance

Octocorals are important members of reef-associated benthic communities that can rapidly replace scleractinian corals as the dominant ecosystem engineers on degraded reefs. Considering the substantial change in the (a)biotic environment that is commonly driving reef degradation, maintaining a dynamic and metabolically diverse microbial community might contribute to octocoral acclimatization and ecological adaptation. Nitrogen (N)

cycling microbes, in particular denitrifying prokaryotes, may support holobiont functioning by limiting internal N availability, but little is known about the identity and (a)biotic drivers of octocoral-associated denitrifiers. Here, we show contrasting dynamics of bacterial communities associated with two common octocoral species, the soft coral *Xenia umbellata* and the gorgonian *Pinnigorgia flava* after a six-week exposure to excess dissolved organic carbon (DOC) under concomitant warming conditions. The specific responses of denitrifier communities associated with the two octocoral species aligned with the nutritional status of holobiont members. This suggests a passive regulation of this microbial trait based on N availability in the coral holobiont.

6.3 Introduction

Coral reefs are hotspots of marine biodiversity and primary productivity in oligotrophic tropical oceans (Muscatine & Kaplan, 1994). Corals, the ecosystem engineers of these reefs, are key to supporting these ecosystems (Wild et al., 2004). The symbiosis with intracellular dinoflagellate algae of the family Symbiodiniaceae is central to this ecological success as it enables corals access to heterotrophic as well as autotrophic nutrient sources to support growth and productivity (Muscatine & Porter, 1977; Muscatine, 1990). In particular, the translocation of organic carbon (C) in the form of photosynthates by symbiotic algae is a major energy source for the coral host, which provides inorganic nutrients and carbon dioxide from its catabolism to support algal photosynthesis (Falkowski et al., 1993). However, the efficient symbiotic trade of C in the coral-algae symbiosis depends heavily on limited nitrogen (N) availability of algae. Constant N limitation of algal symbionts is required to limit their populations and ensure the accumulation of excess photosynthates available for translocation (Falkowski et al., 1993; Dubinsky & Jokiel, 1994). Given the pronounced environmental fluctuations and seasonality on coral reefs, the functioning of symbiosis thus depends on active and/or passive regulation of nutrient availability for algal symbionts, summarized in (Rädecker et al., 2015). Importantly, the nutrient availability in the symbiosis does not depend on interactions of the host and its symbiotic algae alone (Benavides et al.,

2016; Bednarz et al., 2017). Corals also associate with a diverse prokaryotic microbiome with varying degrees of taxonomic flexibility depending on host species and environmental conditions (Voolstra & Ziegler, 2020). Many members of the prokaryotic microbiome are or may be actively involved in the provision and recycling of limiting nutrients such as N, Phosphorus (P), thereby altering nutrient availability for the holobiont (Lesser et al., 2004; Raina et al., 2009; Pogoreutz et al., 2017a; Pogoreutz et al., 2017b). As such, the ecological success of corals likely depends on an intricate functional interplay of all its microbial associates. This diverse multi-species assemblages termed the coral holobiont extends the metabolic properties of its members and may help their rapid adaptation to changing environmental conditions (Rohwer et al., 2002).

For millions of years, the functional interplay between holobiont members has formed the basis of the ecological success of corals and the reefs they support (Rosenberg et al., 2007; Bourne & Webster, 2013; Pogoreutz et al., 2020). Yet in recent decades, anthropogenic activities have led to widespread coral mortality and reef degradations (Hughes et al., 2017; Hoegh-Guldberg, 1999). Global and local stressors such as ocean warming and labile DOC loading are known to disrupt holobiont functioning, resulting in coral bleaching, i.e., the collapse of the coral-algal symbiosis, or coral diseases (Kuntz et al., 2005; Kline et al., 2006; Bourne et al., 2008). This symbiotic breakdown is not exclusively restricted to interactions between the coral host and their algal symbionts, but involve other members of the holobiont as well, e.g., prokaryotes (Ziegler et al., 2017; Voolstra & Ziegler, 2020; Kushmaro et al., 2001). Given that the stability of coral-algal symbiosis is dependent on the N-limited state of algal symbionts, microbial N cycling may stabilize or destabilize holobiont functioning depending on the environmental condition (Cardini et al., 2015; Rädecker et al., 2015; Pogoreutz et al., 2017b).

In particular, denitrifiers, i.e., prokaryotes that encompass the reduction of nitrate or nitrite to dinitrogen gas (Braker et al., 1998), might help alleviate excess N stress of holobionts (Rädecker et al., 2015). Denitrifiers appear to be widely associated with many reef

organisms and their activity was recently confirmed in corals from the Red Sea (Tilstra et al., 2021). Specifically, it has been observed that the activity of microbial denitrification in coral holobionts increased with environmental N levels (El-Khaled et al., 2020). As such, changes in community structure, abundance and activity of denitrifiers might directly affect coral holobiont functioning by altering N availability for other holobiont members. While it remains unexplored that how environmental changes such as ocean warming and excess DOC influence coral-associated denitrifying bacterial communities.

To date, most studies have focused on the bacterial community dynamics of scleractinian corals given their importance as ecosystem engineers (Ziegler et al., 2016; Ziegler et al., 2017). In contrast, even though octocorals constitute highly abundant members of benthic communities of coral reefs, their bacterial community dynamics under environmental stress remain largely unexplored (Van de Water et al., 2018a). Octocorals were recently shown to exhibit some of the highest denitrification rates among Red Sea reef organisms and substrates (El-Khaled et al., 2021), indicating a potential importance of this microbial functional trait to octocoral health and holobiont functioning. However, the (a)biotic drivers of denitrifier abundance and community composition in octocoral holobionts are unknown. Here, we set out to explore the effects of excess DOC and concomitant warming on bacterial community structure with a focus on diversity and abundance of putative denitrifiers associated with the pulsating soft coral *Xenia umbellata* (Lamarck, 1816) and the gorgonian *Pinnigorgia flava* (Nutting, 1910). The experiment consisted of two consecutive phases. In phase one, excess DOC enrichment was performed over the course of 21 days at varying concentrations via administration of daily glucose dosing. In phase two, the continued DOC enrichment was combined with step-wise warming for 24 days (Figure 6.1). By doing so, we aimed to: first, provide a comprehensive overview of the bacterial symbionts associated with two common and taxonomically distinct octocoral species and their response to excess DOC and ocean warming; and second, to provide a comparative assessment on the abundance, diversity, and community dynamics of associated putative denitrifiers in these octocoral holobionts.

6.4 Results

Overview of 16S rRNA gene sequencing data.

Over the duration of 45 days of the aquarium experiment with two octocoral species, using 4 levels of DOC concentrations and 2 different temperatures, a total of 78 coral fragments were collected for 16S rRNA gene amplicon sequencing on the Illumina MiSeq platform. Of these, 76 samples passed quality control, resulting in 8,145 amplicon sequence variants (ASVs) based on 1,150,262 sequences. Of total ASVs, 5,703 ASVs were present in both coral species. 1,847 ASVs were exclusively associated with the soft coral *X. umbellata* and 595 ASVs with the gorgonian *P. flava* only. Under undisturbed condition, the total number of bacterial ASVs associated with *X. umbellata* (6,344 ASVs at day 0) was higher than in *P. flava* (4,260 ASVs at day 0). However, over the course of our experiment, *X. umbellata* showed a decline in total ASV diversity regardless of DOC treatments (1,618 and 1,058 ASVs for day 21 and 45, respectively), as well as significantly reduced Inverse Simpson Index (Two-way ANOVA, Time-effect: $F_{2, 24} = 14.97$, $P < 0.001$, DOC-effect: $F_{4, 24} = 5.76$, $P = 0.002$). Specifically, compared to day 0, the Inverse Simpson Index experienced an 80% reduction at day 21 (Tukey's HSD, $P = 0.003$) and an 84% reduction at day 45 (Tukey's HSD, $P < 0.001$) (Figure S1; Figure S2). In contrast, ASV numbers and Inverse Simpson Index remained stable in the *P. flava* bacterial microbiome (Two-way ANOVA, Time-effect: $F_{2, 26} = 1.57$, $P = 0.227$, DOC-effect: $F_{4, 26} = 0.20$, $P = 0.936$; Figure S1; Figure S2) throughout the experiment.

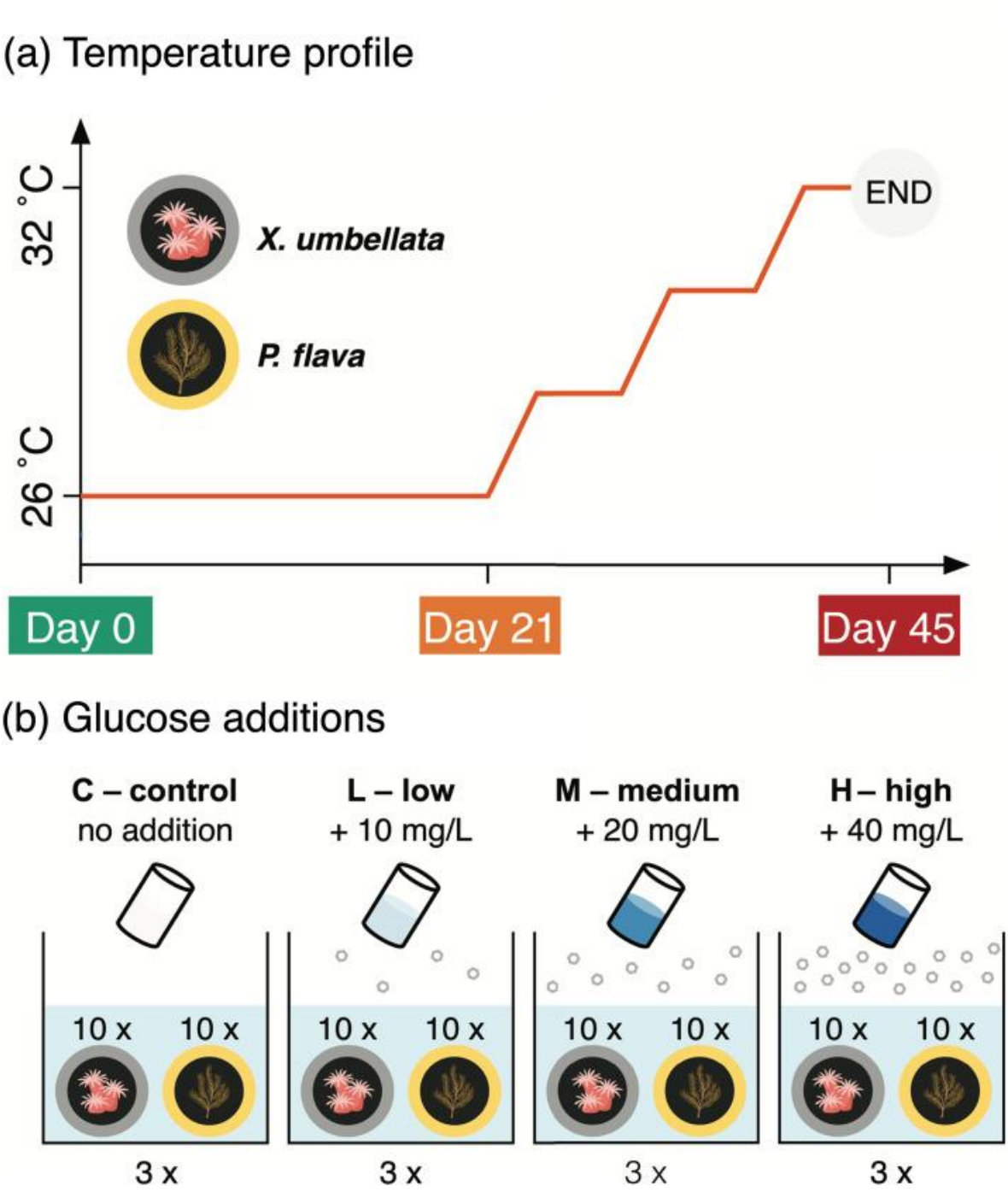

Figure 6.1 | Design of the conducted manipulative aquarium experiment. (a) Step-wise increases in temperature started from day 21 to day 45 with an increase in 2°C every 8 days. **(b)** Dissolved organic carbon (i.e., glucose) additions were applied daily throughout the experiment.

Distinct bacterial communities and bacterial community responses of *X. umbellata and P. flava*.

At day 0, i.e. just prior to the start of the experiment, both octocoral species were associated with distinct dominant bacterial taxa (ANOSIM, R = 0.68, *P* = 0.001; Figure 6.2b; Figure 6.2c). In *X. umbellata*-associated bacterial communities, *Alphaproteobacteria* (25-30% proportion; ca. 70% of sequences affiliated to *Rhodobacteraceae*), *Bacteroidia* (20-25% proportion; ca. 75% of sequences affiliated to *Kordia*) and *Gammaproteobacteria* (15-25% proportion; ca. 70% of sequences affiliated to *Alteromonadaceae*) were identified as dominant classes. In contrast, *P. flava* was dominated by *Alphaproteobacteria* (20-40% proportion; ca. 65% of sequences affiliated to *Rhodobacteraceae*), *Gammaproteobacteria* (10-40% proportion; ca. 16% of sequences affiliated to *Vibrionaceae*) and *Campylobacteria* (15-80% proportion; ca. 70% of sequences affiliated to *Arcobacteraceae*) (Figure 6.2c).

X. umbellata bacterial communities significantly varied across timepoints (PERMANOVA, $F_{2, 22}$ = 5.07, *P* = 0.001), and were significantly affected by excess DOC (PERMANOVA, $F_{3, 22}$ = 1.92, *P* = 0.003) (Figure 6.2c). At day 21, bacterial community structure of *X. umbellata* at control did not markedly change from day 0 (ANOSIM, R = 0.52, *P* = 0.132; Figure 6.2c). Yet, bacterial communities under excess DOC separated well from the control (ANOSIM, R = 1.00, *P* = 0.132; Figure 6.2c) by showing a two-fold increase in the proportion of *Alphaproteobacteria*, primarily ASVs affiliated to *Rhodobacteraceae* (63%-75% proportion of *Alphaproteobacteria*) and *Hyphomonadaceae* (25-28%). Until day 45, following continuous excess DOC paired with a temperature increase to 32°C, *X. umbellata* bacterial communities were dominated by *Alphaproteobacteria* (mainly *Rhodobacteraceae*; 75-80% of all sequences in this class), a higher proportion than in corals with excess DOC at day 21 (ANOSIM, R = 1.00, *P* = 0.132; Figure 6.2c). *P. flava*-associated bacterial communities differed significantly over time (PERMANOVA, $F_{2, 24}$ = 2.60, *P* = 0.001). This was first and foremost driven by the 8-fold increased proportion of ASV5, which was affiliated with *Paraspirulinaceae*, in corals without excess DOC. In contrast, the bacterial community

structure of *P. flava* did not significantly respond to excess DOC throughout the experiment (PERMANOVA, $F_{3, 24} = 0.99$, $P = 0.500$; Figure 6.2c).

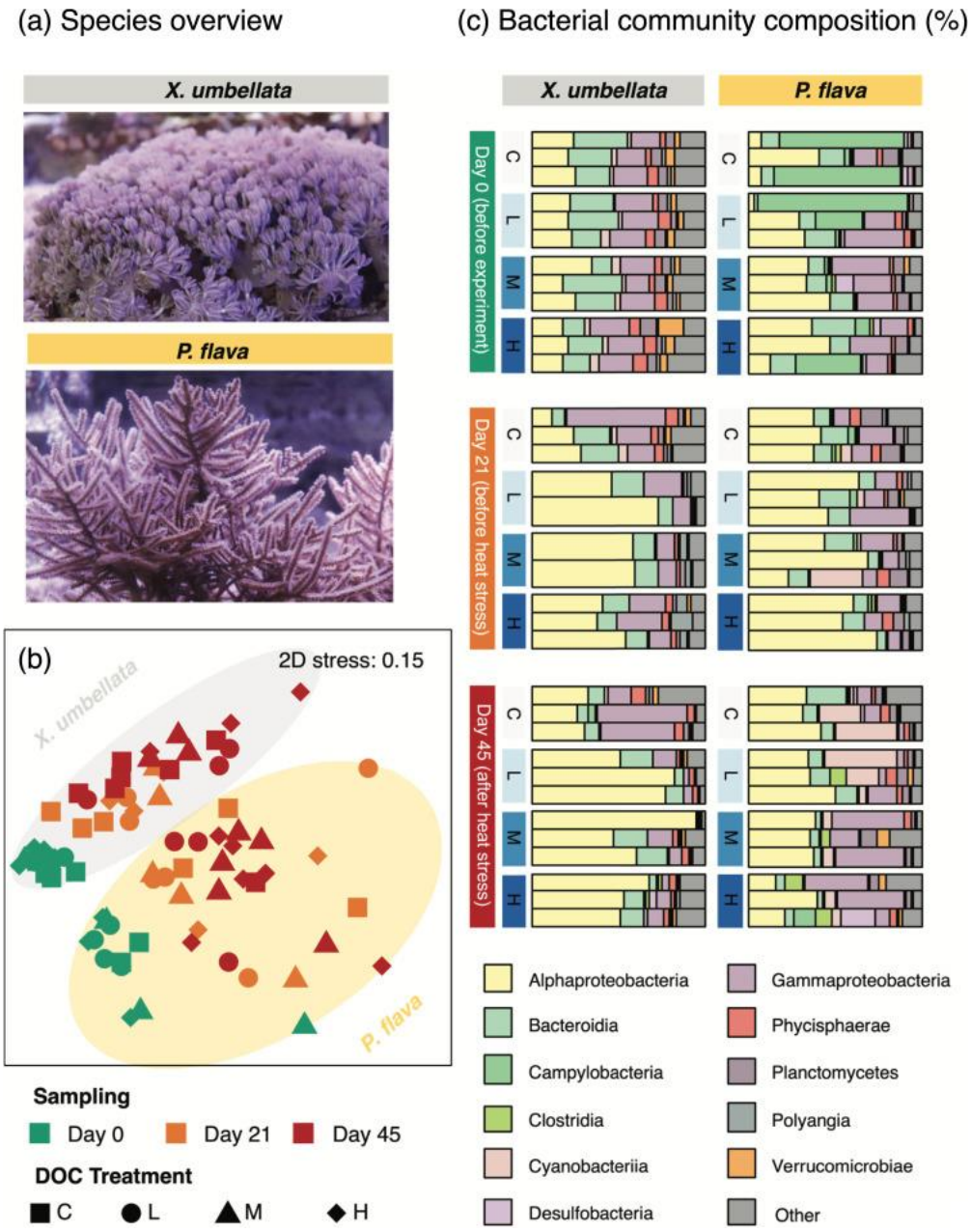

Figure 6.2 | Bacterial community compositions of corals *X. umbellata* and *P. flava* over the course of the experiment. (a) Representative photographs of corals. **(b)** Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarity matrix of bacterial community compositions associated with coral samples at day 0, day 21, and day 45. **(c)** Stacked bar plots of bacterial community compositions of corals across different timepoints and DOC treatments. Stacked bar plots display the 10 most abundant bacterial classes (>1%, determined for each coral species separately).

Pronounced effects of excess DOC on composition and abundance of putative denitrifiers associated with *X. umbellata*.

The *nirS* in-silico PCR and 16S rRNA gene sequencing retrieved a total of 97 ASVs distributed over 14 putative denitrifying genera from 76 octocoral samples. Putative denitrifying taxa were represented by 12 genera and 75 ASVs and accounted for approximately 10% of the *X. umbellata* bacterial community. The putative denitrifying community in *P. flava* varied more across samples than in *X. umbellata*, with denitrifiers being represented by 14 genera from 79 ASVs, accounting for up to 23% of the overall bacterial community. *Ruegeria* (ca. 90% of denitrifier-affiliated sequences, across 23 ASVs dominated by 60-65% ASV8 and 26-35% ASV11) showed its dominance in both octocoral denitrifying communities at day 0. *Labrenzia* (incl. ASV43, ASV126 and ASV179) was the second most dominant genus and occupied a higher proportion in *P. flava* (Figure 6.3a). The denitrifying community of *X. umbellata* revealed a ca. 80% reduction in sequence proportion related to *Ruegeria* spp. and a ca. 30% increase in the proportion of sequences affiliated with *Dinoroseobacter* spp. after 21 days of excess DOC (Figure 6.3a). However, this shift did not affect the cumulative relative abundance of putative denitrifiers, as estimated by *nirS* gene relative abundance (ANOVA, $F_{3, 24} = 2.280$, $P = 0.105$; Figure 6.3b). Denitrifying community structure in *P. flava* showed no significant response to DOC, as well as a non-significant change in the *nirS* relative abundance based on qPCR (ANOVA, $F_{3, 24} = 0.961$, $P = 0.427$; Figure 6.3b).

The denitrifiers of *X. umbellata* revealed significant changes in community structure across treatments at day 45. This was primarily due to the reduced proportion of *Ruegeria* spp. under excess DOC and concomitant warming (Figure 6.3a; Figure 6.4). This shift in the community was further reflected in a significant decrease in *nirS* relative abundance in corals under excess DOC (ANOVA, $F_{6, 24} = 2.45$, $P = 0.05$; Figure 6.3b), especially when comparing the high DOC treatment (at a concentration of 40 mg L^{-1}) to the control (Tukey's HSD, $P = 0.03$). Conversely, denitrifiers in *P. flava* showed no significant change in

community structure or cumulative relative abundance (*nirS* qPCR, ANOVA, $F_{6, 24} = 0.93$, $P = 0.49$; Figure 6.3b) in the presence of excess DOC and warming.

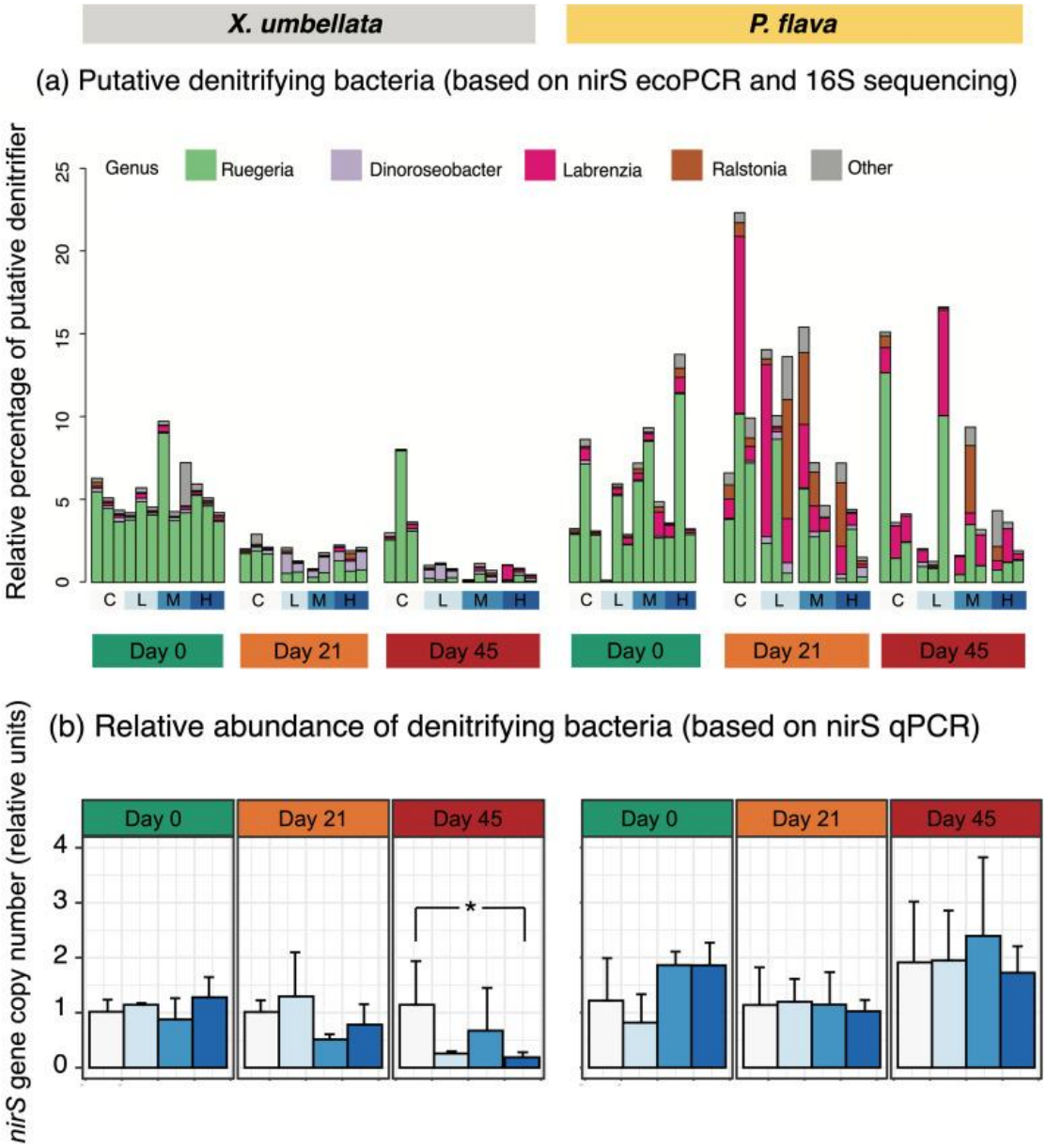

Figure 6.3 | Community compositions and relative abundances of putative denitrifiers in corals *X. umbellata* and *P. flava* over the course of the experiment. (a) Relative proportions of denitrifier genera of corals *X. umbellata* and *P. flava* inferred by *nirS* in-silico PCR in relation to the total bacterial community from 16S rRNA gene sequencing. **(b)** Relative fold changes in copy numbers of *nirS* gene referenced to 16S rRNA gene and in relation to the day 0 control samples (n = 3) of corals *X. umbellata* and *P. flava*. Values are means ± SD, and the asterisk indicates statistically significant differences (* $p < 0.05$).

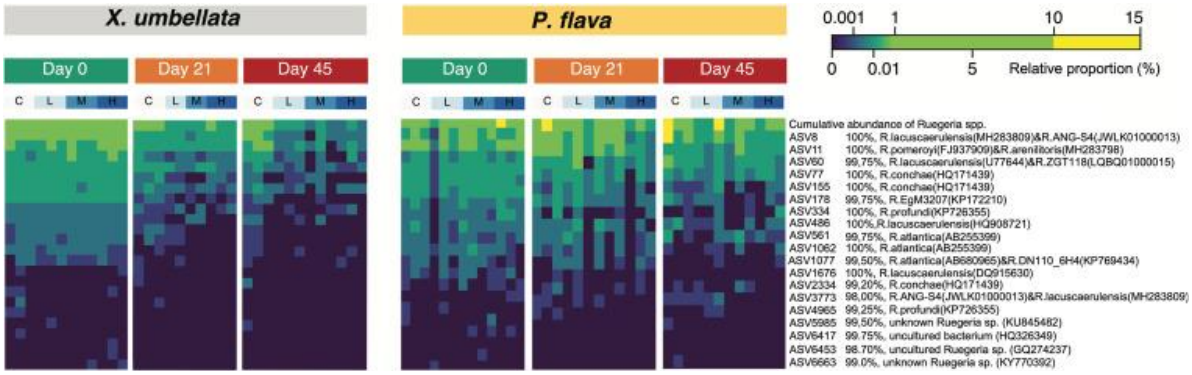

Figure 6.4 | Relative proportions of putative denitrifying *Ruegeria* spp. ASVs in total bacterial communities associated with corals *X. umbellata* and *P. flava* across different timepoints and DOC treatments.

Physiological changes in *X. umbellata* subjected to excess DOC and warming.

No change in phenotype was observed for the two octocoral species with excess DOC for 21 days. Even at the end of the experiment after combined excess DOC and warming conditions, *X. umbellata* still maintained a healthy appearance across excess DOC groups (Figure 6.5a). In contrast, moderate bleaching was observed in *P. flava* under higher DOC concentrations (i.e., 20 mg L^{-1}, 40 mg L^{-1}; Figure 6.5a). At day 45, *X. umbellata* with excess DOC showed 30% higher C:N ratios in host tissues compared to their control counterparts (ANOVA, $F_{3, 8} = 12.59$, $P = 0.002$; Figure 6.5c), while no significant difference was observed in C:N ratios of Symbiodiniaceae (ANOVA, $F_{3, 8} = 1.002$, $P = 0.44$; Figure 6.5c). This was accompanied by a moderate albeit not significant increase in host $\delta^{15}N$ signatures (ANOVA, $F_{3, 8} = 2.7$, $P = 0.116$), as well as a 25% increase in $\delta^{15}N$ signatures of Symbiodiniaceae under excess DOC (ANOVA, $F_{3, 8} = 19.44$, $P < 0.001$; Figure 6.5c).

There was no statistically significant difference across treatments in C:N ratios of *P. flava* host (ANOVA, $F_{3, 8} = 2.435$, $P = 0.14$) and Symbiodiniaceae (ANOVA, $F_{3, 8} = 2.508$, $P = 0.133$), nor in $\delta^{15}N‰$ signatures of host (ANOVA, $F_{3, 8} = 1.396$, $P = 0.313$) and Symbiodiniaceae (ANOVA, $F_{3, 8} = 0.234$, $P = 0.87$) (Figure 6.5c).

At the end of the experiment, seawater nutrient levels did not significantly differ among treatments with regard to NH_4^+ (ANOVA, $F_{3, 8} = 0.069$, $P = 0.975$) and PO_4^{3-} (ANOVA: $F_{3, 8} = 10.08$, $P = 0.969$) levels (Figure 6.5b). Nevertheless, NO_2^- (0.22 ± 0.19 µM) and NO_3^- (1.60 ± 0.57 µM) concentrations from the control were two to four-fold higher (NO_2^-, ANOVA, $F_{3, 8} = 1.502$, $P = 0.286$; NO_3^-, ANOVA, $F_{3, 8} = 0.741$, $P = 0.557$; Figure 6.5b) than those of aquaria with excess DOC (NO_2^-: 0.05 ± 0.04 µM; NO_3^-: 0.84 ± 0.66 µM).

6.5 Discussion

N cycling microbes, including denitrifiers, are considered key players in the coral holobiont, as they might help in maintaining a N-limited state critical for the functioning of the coral-algae symbiosis (Rädecker et al., 2015; Ngugi et al., 2020; Rädecker et al., 2021). In current study, we provide the first assessment of the overall bacterial and denitrifying community structures associated with two octocoral species as well as their abundances and dynamics under excess DOC and warming in a six-week aquarium experiment. We found pronounced octocoral species-specific responses to excess DOC in bacterial community structure in general, and in denitrifier community composition and abundance, which aligned with patterns in C:N ratios of host tissues. Taken together, our results suggest a link between octocoral-associated denitrifiers and nutritional status, i.e. N availability of the holobiont, as well as a passive regulation of denitrifier communities as discussed below.

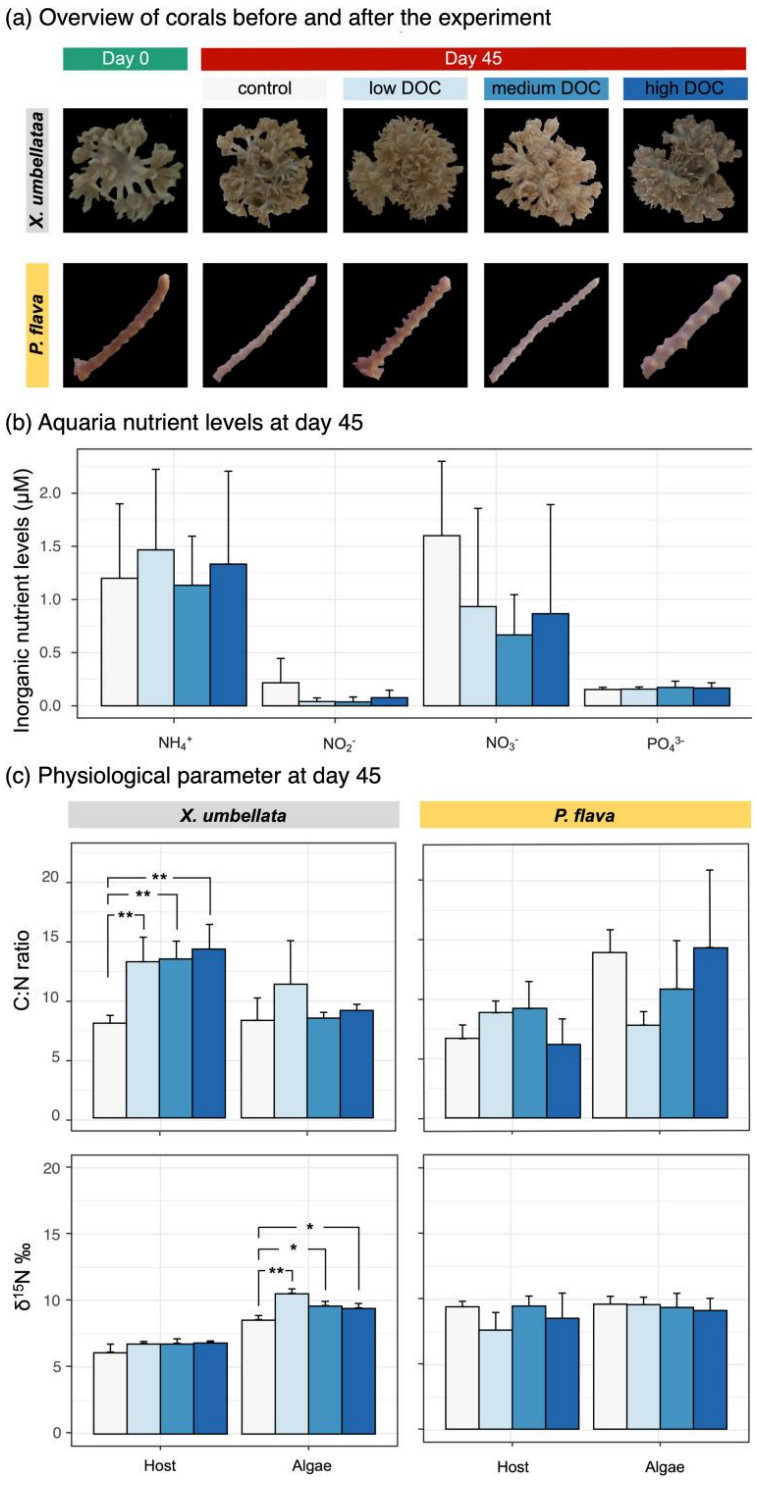

Figure 6.5 | Physiological changes in corals *X. umbellata* and *P. flava* at the end of the experiment. **(a)** Phenotypes of corals *X. umbellata* and *P. flava* before and after the experiment. **(b)** Seawater inorganic nutrient levels in all aquaria at the end of the experiment. **(c)** Elemental (carbon and nitrogen) changes in both coral holobionts. Values are means ± SD, and the asterisk indicates statistically significant differences (* $p < 0.05$, ** $p < 0.01$).

Distinct bacterial community structures between octocoral species

Bacterial community structure and diversity were distinct between two octocoral species *X. umbellata* and *P. flava* at the beginning of the experiment, even though these corals were maintained in the same aquaria for years. At the bacterial class level, *P. flava* was initially dominated by *Alpha-* and *Gammaproteobacteria* as well as *Campylobacteria*; *X. umbellata* was dominated by *Alphaproteobebacteria*, *Bacteroidia*, and *Gammaproteobacteria*. Our results are in line with previous findings that octocoral-associated bacterial communities are species-specific (Van de Water et al., 2017; Van de Water et al., 2018b; McCauley et al., 2020; Osman et al., 2020). This also concurs with the abundantly reported host-specific microbial assemblages for scleractinian corals, as well as a structural similarity to scleractinian coral bacterial symbionts at the class level (Rohwer et al., 2002; Littman et al., 2009; Ziegler et al., 2016; Ziegler et al., 2017; Ziegler et al., 2019). The gorgonian *P. flava* showed a lower bacterial diversity, which is consistent with previous reports (Van de Water et al., 2017; Van de Water et al., 2018b). Due to the broad leaf-like morphology and comparatively small polyps, algal symbionts may maximize photosynthetic activity to sustain a high autotrophic capacity in gorgonians (Baker et al., 2015). The invariable C:N ratio in the *P. flava* holobiont in our study suggests a relatively poor heterotrophic capacity, lending support to previous observations by Baker et al., 2015. The low heterotrophic capacity of *P. flava* may restrict bacterial diversity (Meistertzheim et al., 2016). Limited niche space for bacterial associates due to morphological and physiological features of *P. flava* may be another reason for the observed low bacterial diversity. Being an octocoral, *P. flava* does not build a porous calcium carbonate skeleton that may offer niche space for a number of specialized endolithic microbes, as known from scleractinian corals (Yang et al., 2016; Pernice et al., 2020), nor does it secrete a thick surface mucus layer like *X. umbellata* (Bednarz et al., 2012) that may provide favorable habitat for prokaryotic growth, including that of anaerobes (Kellogg, 2004; Sharon & Rosenberg, 2008).

Species-specific responses of octocoral-associated bacterial communities to excess DOC

Little information on drivers of octocoral microbial dynamics is available. Here we showed for the first time that octocorals exhibit species-specific responses to excess DOC and warming with regard to their bacterial communities. Over time, excess DOC led to conspicuous changes in the bacterial community structure of *X. umbellata*, as characterized by a marked continuous increase in *Rhodobacteraceae,* making up to 80 % of the overall bacterial community at the end of the experiment. We cannot disentangle the direct and indirect effects of DOC on associated microbiomes. While based on qPCR data of 16S rRNA gene, we found an overall increase of bacterial abundance in *X. umbellata* under excess DOC. In contrast, no such change in response to excess DOC was observed in the bacterial community structure or abundance of *P. flava*. The absence of change in bacterial community structure in *P. flava* aligns with prior reports that gorgonian corals host a group of extremely robust bacterial communities over large geographic scales (Correa et al., 2013), seasonal variations (Van de Water et al., 2018b) and even in the presence of environmental stressors such as increased temperatures and ultraviolet radiation (McCauley et al., 2020). These observations suggest a differential 'flexibility' or plasticity in bacterial community responses, as recently reported for a number of scleractinian corals (Pogoreutz et al., 2018).

This apparent interspecific plasticity of octocoral microbiomes may be explained by the initial differences in bacterial diversity and community structure between these two octocoral species. In general, coral bacterial microbiomes with greater diversity likely show a higher degree of functional redundancy and may exhibit more flexible associations in response to environmental change, which align with the ecological resilience of the holobiont (Reshef et al., 2006; Torda et al., 2017; Ziegler et al., 2017; Louca et al., 2018; Voolstra & Ziegler, 2020). In contrast, the lower diversity of the bacterial community associated with *P. flava* might suggest bacteria are highly selected and likely inhabit host-constructed niches, as previously reported (Ainsworth et al., 2015). These may be

dominated by a few specialized species of high abundance (Pogoreutz et al., 2018) and vary less under environmental perturbation (Gardner et al., 2019).

Observations of such a species-specificity may pertain to functional traits of the animal host and/or algal symbionts, such as heterotrophic capacity, nutritional status, or ecological resilience, including but not limited to heat tolerance as well as (a)biotic environmental drivers (Ziegler et al., 2017; Bednarz et al., 2019; Gardner et al., 2019; Camp et al., 2020). Differences in trophic strategies between the two octocoral species in particular may be of potential importance for their contrasting responses of the overall bacterial community under excess DOC. *Xenia* sp. is thought to be entirely autotrophic (Schlichter et al., 1983). However, Fabricius and colleague argued that *X. umbellata* may exhibit a mixotrophic lifestyle, i.e., relying on autotrophic and heterotrophic food sources (Fabricius & Klumpp, 1995). Our findings of increased C:N ratios in *X. umbellata* under excess DOC lend support to the latter. Indeed, the capacity to feed heterotrophically may allow *X. umbellata* to benefit from the surrounding DOC, thereby also creating a favorable environment for heterotrophic bacterial propagation, which may subsequently alter the bacterial community structure.

X. umbellata is known for its distinctive pulsation behavior, which may contribute to its ecological resilience by mixing of the surface boundary layer (Kremien et al., 2013; Wild & Naumann, 2013). Of note, excess DOC remarkably enhanced their pulsation rates (Vollstedt et al., 2020) and net primary productions (Simancas-Giraldo et al., 2021), but whether this increase in pulsation may have an effect on the microbial plasticity of *X. umbellata* remains to be determined. Some endosymbiotic Symbiodiniaceae are known to feed heterotrophically (Jeong et al., 2012), suggesting that excess DOC in *X. umbellata* may also promote the heterotrophic growth of Symbiodiniaceae. Given Symbiodiniaceae-associated bacteria often form a major component of the coral microbiome (Lawson et al., 2018; Matthews et al., 2020), changes in Symbiodiniaceae physiology or even community

composition likely contribute to microbial community dynamics observed in the present study.

Distinct patterns of microbial plasticity between two octocoral species appeared to be primarily driven by the increase of *Rhodobacteraceae* (ASV1: Roseobacter clade CHAB-I-5; ASV9 and ASV13: unclassified Rhodobacteraceae) exclusively in *X. umbellata*. This observation contrasts the findings of (Pogoreutz et al., 2018) reporting on the structural inflexibility in the bacterial microbiome of the scleractinian coral *Pocillopora verrucosa* under excess DOC. The pronounced increase in sequences proportions affiliated to multiple *Rhodobacteraceae* ASVs however align well with genomic evidence and ecological observations (Jahreis et al., 2008; Cárdenas et al., 2018). *Rhodobacteraceae* are known for their considerable versatility with regard to C utilization, which allows them to thrive and rapidly proliferate in high-DOC environments (Jahreis et al., 2008; Cárdenas et al., 2018).

A passive regulation of denitrifiers in octocoral holobiont functioning

Coral-associated diazotrophs (N-fixing prokaryotes) have previously been suggested to play a central role in supporting holobiont fitness and functioning when the surrounding environmental N availability is low (Cardini et al., 2015; Rädecker et al., 2015). In contrast, denitrifiers have been proposed to be important in maintaining the coral-algae symbiosis in an N-limited state (Rädecker et al., 2015), yet our understanding on whether their abundance in the coral holobiont is of physiological or ecological relevance remains poor. On average, the proportion of putative denitrifiers in the microbiome was higher in *P. flava* than in *X. umbellata*. This difference between host species may be attributed to their distinct trophic strategies. Microbial community compositions in coral holobionts are highly selective due to different host functional or life history traits pertaining to e.g. development, physiology, and metabolism (Bourne & Webster, 2013; Webster & Reusch, 2017; Ziegler et al., 2019). Due to a relatively low capacity to obtain (in)organic N sources from the surrounding environment, *P. flava* is likely to rely more on symbiotic N cycling

microbes to acquire or remove N to fulfill its metabolic requirements (Pogoreutz et al., 2017a; Tilstra et al., 2019; Tilstra et al., 2021).

In the presence of N, bioavailable C sources including glucose usually favor denitrification and hence the growth of denitrifiers (Beauchamp et al., 1989). However, excess glucose caused no stimulating effects on the relative abundance of denitrifiers in our study. This apparent contradiction might be explained by the following two considerations: first, denitrifier populations might be regulated by N availability; consequently, denitrifier abundance may not necessarily increase in the presence of high DOC loads in a N-limited environment. Second, if denitrifiers were not limited by environmental N availability, different denitrifying taxa may exhibit differential preferences for C sources. For instance, some *Rhodobacteraceae* taxa dominate microbial glucose uptake in coastal North Sea waters (Alonso & Pernthaler, 2006), others while may be suppressed by allochthonous glucose input, but follow fluctuations in population dynamics of primary producers (Allers et al., 2007).

Importantly, a reduction in the cumulative relative abundance of denitrifiers (as reflected in *nirS* relative gene copies quantified by qPCR) during DOC enrichment was exclusively observed in *X. umbellata*. In contrast to *P. flava*, this soft coral species showed an increase in C:N ratios in the animal host under excess DOC at the end of the experiment. As increased C:N ratios imply a relative decrease of N availability, associated changes in the nutritional status of the host may directly impact its interaction with associated denitrifiers. The notion of reduced N availability for holobiont members is further corroborated by the increase in $\delta^{15}N$ signatures, potentially indicating a reduced uptake of inorganic N from the seawater and an increased retention of N within the holobiont.

A passive regulation of N cyclers could thereby directly support overall holobiont functioning under fluctuating environmental conditions. In periods of low N availability, reduced denitrifier abundance (and therefore overall denitrification activity) might reduce

the competition for N source in the coral holobiont and favor N uptake by the algae to support their growth. Likewise, rapid growth of denitrifiers during periods of excess N availability could increase the competition for N source between holobiont members, thereby alleviating excess N stress, and ultimately stabilizing the coral-algal symbiosis.

Can denitrifiers provide new insights into Symbiodiniaceae-bacteria interactions?

By inference, all four dominant denitrifiers identified in our study appear to form close associations with Symbiodiniaceae instead of coral hosts. The predominant putative denitrifier *Ruegeria* spp., formerly named *Silicibacter* spp., are known as dinoflagellate-associated bacteria. They are attracted to and capable of catabolizing degrading DMSP produced by the dinoflagellate host (Miller et al., 2004). *Labrenzia* spp. were previously reported to be 'core' microbiome members of Symbiodiniaceae in the coral holobiont (Lawson et al., 2018; Matthews et al., 2020). Given their ability to produce DMSP (an osmolyte and powerful scavenger of reactive oxygen species), the consistent association of *Labrenzia* spp. might potentially assist in reducing oxidative stress of Symbiodiniaceae (Lawson et al., 2018). Likewise, *Dinoroseobacter* spp. were previously shown to supply their dinoflagellate host with essential nutrients, specifically the essential vitamins B_1 and B_{12}, to support their growth in particular under the nutrient-limitation state (Wagner-Döbler et al., 2010). *Ralstonia* spp. were identified as intracellular, i.e. occurring within the endodermal coral host cells in close proximity to the Symbiodiniaceae and were proposed to be implicated in the functioning of the coral-algae symbiosis (Ainsworth et al., 2015).

Notably, we observed differential responses of different putative denitrifying taxa to experimental treatments. Specifically, an increasing proportion of *Dinoroseobacter* spp. concurs with a decreasing proportion of *Ruegeria* spp. in *X. umbellata* with excess DOC. At this point, the causes and consequences of altered denitrifier community structure and abundance for coral holobiont functioning remain to be determined. Functional studies will be required to link these microbiome dynamics with related denitrifying activities and

nutritional states of holobionts to disentangle the potential role of denitrifiers in octocoral holobiont fitness and functioning. Further localization of denitrifiers in the intact symbiosis could help us better understand the interactions between octocoral-associated N cycling microbes with other members of the holobiont: the animal host, Symbiodiniaceae, and other microbes in a changing environment.

6.6 Conclusions

Phase shifts on coral reef ecosystems have been linked to a number of environmental stressors. Among these, the detrimental effects of excess DOC (typically associated with sewage and reduced water quality) on scleractinian corals have received considerable attentions in the past (Kuntz et al., 2005; Kline et al., 2006; Haas et al., 2016). Changes in the activity, abundance and community structure of N cycling microbes have been discussed as critical components to corals' response to environmental DOC loading. While an imbalance in N cycling significantly affected the structure and functions of bacterial microbiomes associated with scleractinian corals, very little information is available for octocorals, which are globally abundant particularly on reefs undergoing phase shifts (Norström et al., 2009). Here, we have provided a first insight into the dynamics of octocoral-associated bacterial communities with an emphasis on putative denitrifying communities under excess DOC and warming. The dynamics of denitrifiers aligned with the nutritional status of the soft coral host, which implies their critical role in regulating internal nutrient availability of the holobiont in a changing environment. In order to obtain a better understanding on the interactions between octocoral holobiont members, future studies should expand to a comparative taxonomic framework of octocoral host species and link their functional and life history traits to their microbial communities, in particular potentially critical functional groups such as N cyclers. Integrated holistic approaches combining '-omics' approaches and cultivation-dependent methods may aid such a challenging endeavor.

6.7 Materials and Methods

Coral preparation and maintenance.

The soft coral *X. umbellata* and the gorgonian *P. flava* were cultivated (temperature: 26 ± 0.5 °C; pH 7.8 ± 0.2; salinity: 35 ± 3 ‰;) for more than 2 years at the Marine Ecology Department of the University of Bremen. Coral species were identified by barcoding gene (*COI*, *mutS* and 28S rRNA gene) amplification and sanger sequencing. Small *X. umbellata* fragments (1-2 cm in side length) were cut from 5 mother colonies (5×7×12 cm) and fixed on cubical-shaped calcium carbonate coral holders (1×1 cm) with rubber bands. After fragments recovered from fragmentation for 7 days and attached to the holders, rubber bands were removed. Simultaneously, branches of 3-4 cm in height were cut from 4 mother colonies (18×1×24 cm) of *P. flava,* and subsequently attached to coral holders using aquarium moss coral fix glue. Thereafter, 120 fragments of *X. umbellata* and 120 fragments of *P. flava* were distributed among 12 experimental aquaria tanks (water volume 50 L) and acclimated for 10 days prior to the experiment. Each aquarium was equipped with a thermostat (3613 aquarium heater, 75 W 220-240 V), a pump (EHEIM Compact On 300 pump) and a protein skimmer that were all purchased from EHEIM GmbH and Co. KG in Germany to maintain stable aquarium conditions. Additionally, LED lights (Royal Blue-matrix module and Ultra Blue White 1:1-matrix module, WALTRAt day time® LED light, Germany) were used to simulate day - night rhythm of 12 h-12 h at the intensity of 120.8 ± 10.2 µmol quanta m^{-2} s^{-1}. No additional feeding was provided and 10% of the artificial seawater was exchanged daily to maintain stable water parameters. To verify stable environmental conditions, salinity (C: 35.39 ± 0.05; L: 35.38 ± 0.08; M: 35.50 ± 0.08; H: 35.11 ± 0.09) and pH (C: 8.15 ± 0.01; L: 8.05 ± 0.02; M: 8.05 ± 0.02; H: 8.03 ± 0.02) were monitored throughout the experiment (Figure S4).

Experimental design, sampling and DNA extraction.

In the first phase of experiment, temperature was kept constant at 26°C for 21 days (Figure 6.1). Daily additions of glucose to the aquaria based on a stock solution (D-Glucose, 40 mg mL-1) were used to simulate four different glucose enrichment levels: control (no addition), low (equivalent to 10 mg L-1), medium (equivalent to 20 mg L-1) and high (equivalent to 40 mg L-1). Daily measurements of total organic carbon (TOC) using a TOC-L analyzer (Shimadzu, Japan) were used to approximate glucose levels in the aquaria and the daily dosing of glucose was adjusted accordingly to achieve the desired enrichment levels. For the second phase, temperature was increased gradually in all aquaria, ultimately adding 2°C every 8 days to a final temperature of 32°C. The range of 26oC to 32oC represents a latitudinal gradient of mean maximum temperature from north to south across the Red Sea, where *X. umbellata* is widely abundant (Benayahu & Loya, 1984). The temperature maximum of 32oC is also close to the thermal physiological limit of this species (Chaidez et al., 2017). During this gradual ramping phase, all DOC treatments were continued as described above (Figure 6.1). The experiment was terminated at day 45.

Corals for molecular analysis were collected at day 0 (before DOC additions, as baseline data), after the first phase of the experiment (day 21, DOC treatments at 26oC), and after the second phase (day 45, DOC treatments at 32^{o}C). For this, fresh coral samples were collected and immediately frozen in liquid nitrogen and stored at -80^{o}C until further processing. Additionally, samples for physiological measurements and aquarium seawater inorganic nutrients were collected at day 45 as outlined below. Frozen coral samples were ground into powder over liquid nitrogen using mortar and pestle. Genomic DNA was extracted according to the instruction of Quick-DNA Universal Kit Quick Protocol for Solid Tissue (ZYMO RESEARCH, USA). Afterwards, DNA was quantified by spectrophotometry at 260 nm and 280 nm (Tecan Infinite 200 PRO, Austria) and quality-checked by 1% (wt/vol) agarose gel electrophoresis (Biometra Horizon 58, Germany).

Illumina MiSeq 16S rRNA gene sequencing and sequence analysis.

The 16S rRNA gene amplicon sequencing was conducted at LGC genomics (Berlin, Germany). The hypervariable regions V3-V4 of the bacterial 16S rRNA gene were amplified and sequenced using the primer pair S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). The PCRs included 1-10 ng of DNA extract (total volume 1µl), 15 pmol of each forward primer and reverse primer in 20 µL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline GmbH, Germany) and 2 µl of BioStabII PCR Enhancer (Sigma-Aldrich Co., Germany). For each sample, the forward and reverse primers had the same 10-nt barcode sequence for multiplexing. PCRs were carried out for 35 cycles using the following parameters: 1 min 96°C pre-denaturation; 96°C denaturation for 15s, 55°C annealing for 30s, 70°C extension for 90s, hold at 8°C. The Illumina library was pooled and size selected by preparative gel electrophoresis, and the sequencing was conducted on the Illumina MiSeq platform in a 2×300 bp paired-end run using V3 Chemistry.

After demultiplexing and removal of primer sequences from the raw paired-end reads by LGC genomics, further sequence processing was performed according to the DADA2 (1.14.1) pipeline for the generation of exact amplicon sequence variants (ASVs) (Callahan et al., 2016). Specifically, sequences were filtered and quality trimmed to 225 bp (forward) and 235 bp (reverse) at a maximum expected error rate of 5. Trimmed sequences were pooled and used for error learning and denoising. In total, 77 samples were pooled with 3,858,215 reads in 1,264,025 unique sequences. Denoised sequences were merged, followed by Chimeras removal according to default parameters. A total of 26,902 chimeras were identified out of 40,839 ASVs, singletons generated during the merging step were removed. ASVs between 400 to 430 bp were retained and taxonomically classified by "assignTaxonomy" based on the SILVA database release 138 (Quast et al., 2012). ASVs that matched chloroplast and mitochondrial sequences were removed prior to further analysis.

An *in silico* PCR for the nitrite reductase *nirS* primer pair, nirS-1F (5'-CCTAYTGGCCGCCRCART-3') and nirS-qR (5'-TCCMAGCCRCCRTCRTGCAG-3') (Mosier & Francis, 2010) was used to characterize the putative denitrifier community. The program ecoPCR (Ficetola et al., 2010) from OBI tools 1.01.22 was launched against the Ensembl Bacteria release 42 with a maximum 3 mismatches and a zero mismatch zone of 2 bp at the 3' end of each primer, retaining fragments between 50 bp and 500 bp. Resultant sequences from ecoPCR with the fragment size between 224 - 227 bp were blasted against the GenBank Nucleotide database (NCBI nucleotide BLAST, date accessed 2020/04/05), and sequences that were not identified as originating from denitrifying *nirS* were removed. The genus affiliation of the remaining *nirS* fragments was used as potential denitrifying taxa to recover denitrifier communities based on our 16S rRNA gene sequencing results. In addition, ASV sequences affiliated to the predominant genus were aligned to SILVA 138, and the accession numbers of nearest relatives from SILVA Incremental Aligner (SINA) output were used to obtain a higher resolved taxonomic path.

Quantification real-time PCR (qPCR) of denitrifying *nirS* gene.

We assessed the denitrification potential in the coral holobiont via the relative quantification of *nirS* gene, which catalyzes the conversion of nitrite to nitric oxide in the denitrification cascade (Zumft, 1997) and has been previously used to determine denitrifier abundance and diversity (Braker et al., 1998; Mosier & Francis, 2010; Lee & Francis, 2017). The *nirS* gene was amplified using the same primer pair *nirS*-1F, qR (Mosier & Francis, 2010) previously used for *in silico* PCR as outlined above, and validated with Sanger sequencing (StarSEQ, Mainz, Germany). The relative quantification of *nirS* gene abundance was done by qPCR using the CFX96^{TM} Touch Real-Time PCR Detection System (BIO-RAD, USA) by SensiFAST™ SYBR® No-ROX Kit (Bioline, USA). C_T values of *nirS* gene amplicons (as a proxy of denitrifier abundance) were referenced against C_T values of 16S rRNA gene amplicons (as a reference for total bacterial abundance) using the primer-pair Bact-16S_784F: 5'-AGGATTAGATACCCTGGTA-3' and Bact-16S_1061R: 5'-CRRCACGAGCTGACGAC-3'

(Andersson et al., 2008), according to the delta-delta Ct method ($2^{-\Delta\Delta Ct}$) (Livak & Schmittgen, 2001). 10-20 ng of DNA extract was used for 16S rRNA gene and *nirS* qPCRs. Final cycling conditions consisted of a hot-start activation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 65°C (*nirS* gene in *X. umbellata*) or 60°C (*nirS* gene in *P. flava* and 16S rRNA gene for both species) for 20 s, and extension at 72°C for 30 s. Final extension was carried out at 72°C for 10 s followed by a melting curve from 65 to 95°C with increase of 0.5°C steps every 5 s. The qPCR efficiency was validated by calibration curves of genomic DNA from *E. coli* (ATCC 25922) targeting 16S rRNA gene and DSM 428 *Alcaligenes eutropus H16* targeting *nirS* gene separately.

Seawater inorganic nutrient and coral elemental analysis.

Nutrient samples were collected in triplicates at the end of the experiment (i.e., day 45). 50 mL of aquaria seawater was collected through 0.45 µm filters in 50 mL sterilized centrifuge tubes, and immediately frozen at -20°C until further analysis. Nutrient levels were measured spectrophotometrically using the Infinite 200 PRO (Tecan Infinite 200 PRO, Austria) according to (Grasshoff et al., 2009). Coral samples for elemental and isotope analysis were collected at day 45. Fresh coral fragments were immediately frozen in liquid nitrogen and stored at -80°C until further processing. Corals were thawed at room temperature and homogenized for 30 s at 3,500 rpm with an Ultra Turrax (IKA, Germany). The resulting homogenized coral slurry was separated into coral tissue and algal symbiont fractions by centrifugation at 3,000 g for 5 min (Eppendorf, Germany). The host fraction, i.e. the resulting supernatant was carefully removed by pipetting without disturbing the algal symbiont pellet. Algal symbiont pellets were resuspended in 0.22 µm filtered seawater (FSW) in sterilize 2 mL Eppendorf tubes, and host and algal samples were dried at 40°C for one week. After the dried matter was pulverized using clean mini pestles; 1.0 mg of coral tissue or algal symbiont sample was used for measuring total nitrogen content (TN). Further, 1.0 mg of coral tissue or algal symbiont sample mixed with 200 µL 1 mol L^{-1} HCL was used for analyzing total organic carbon content (TCorg). TN and TCorg were analyzed

in an elemental analyzer (Euro EA, Germany), Isotopic $\delta^{15}N$ and $\delta^{13}C$ signatures were measured using an Isotope Ratio MS (Thermo Fisher, USA), following (Moncada et al., 2019).

Statistical analysis.

All statistical analyses were performed in R studio 3.6.1, specifically *vegan* for multivariate statistics (Oksanen, 2015) and *ggplot2* for visualization (Warnes et al., 2016). Alpha diversity indices were calculated based on repeated (n=100) random subsampling of ASVs to the minimum library size at sequencing depth 3,000. Data normality was determined by the Shapiro–Wilk test, and statistical differences between different timepoints and DOC treatments for each species was tested using two-way analysis of variance (ANOVA) with Tukey's HSD as a post-hoc comparison. Beta dispersion of samples was conducted for each coral species using function "betadisper". Beta diversity was evaluated between species, timepoints and DOC treatments by non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities of relative ASV proportions. Analysis of similarities (ANOSIM) was used to illustrate the dissimilarity of bacterial community structures between two coral species at day 0. To illustrate the significant difference in bacterial variations at ASV level from different timepoints and DOC treatments, permutational multivariate analysis of variance (PERMANOVA, 'adonis' function, 999 permutations) based on Bray-Curtis dissimilarity was applied along with ANOSIM as post-hoc comparisons. An ANOSIM R value close to 1 suggests a strong separation between groups, while values close to 0 indicates an overlap between groups (Ramette, 2007). After log transformation to meet data normality, homogeneity of variance and independence, the qPCR data was analyzed by two-way ANOVA and Tukey's HSD. Seawater inorganic nutrient levels in aquaria and elemental changes in animal host and algal symbionts respectively were analyzed by one-way ANOVA and Tukey's HSD.

Data Accessibility.

The Sanger sequencing data derived from *COI*, *mutS*, and 28S rRNA gene PCR amplicons for octocoral identification have been deposited in the European Nucleotide Archive (ENA) under the project accession number PRJEB43824. Primer-clipped DNA sequences generated by Miseq 16S rRNA amplicon sequencing were deposited on NCBI under BioProject accession number PRJNA718022.

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Chapter 7

The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming

First author contribution

Simancas-Giraldo SM, Storkenmaier M, Vasseur F, Ferse S, Wild, C. The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming. *In prep.*

The soft coral *Xenia umbellata* displays comparably high resilience against prolonged ocean warming

Susana Marcela Simancas-Giraldo[1], Maren Storkenmaier[1], Francine Vasseur[1], Sebastian Ferse[1,2], Christian Wild[1]

[1] Marine Ecology Department, Universität Bremen, Bremen, Germany
[2] Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany

Corresponding Author:
Susana Marcela Simancas-Giraldo[1]
Email address: sussim@uni-bremen.de

In prep.

7.1 Abstract

Ocean warming, one of the main consequences of climate change, poses a significant challenge for coral reefs worldwide. Although evidence suggests that soft corals may resist warming, this remains inconclusive, as only a few studies have evaluated their responses beyond short-term experiments (primarily envisioned to assess acute heat stress). This study thus evaluated the soft coral *Xenia umbellata*'s response to prolonged elevated temperatures (i.e., > 20 days of prolonged warming) in terms of mortality, bleaching, pulsation, growth, as well as oxygen (O_2), carbon (C) and nitrogen (N) metabolism. Using a series of aquaria experiments, we evaluated three temperature scenarios: 26 °C, 29 °C, and 32 °C. Under 29 °C (11-degree heating weeks, DHW), no mortality was observed despite bleaching and reduced growth occurred for 61.2 % of the coral population. In addition, pulsation rates and the O_2, C and N metabolism of the unbleached corals remained comparable to the control at 26 °C. On the other hand, prolonged 32 °C (22 DHW) caused up to 80 % mortality and 93% bleaching, decreased pulsation, growth and O_2 metabolism, together with shifted C-fluxes and trophic status. The shifts in trophic status were associated with concomitant bleaching. *X. umbellata* showed 100 % and 25 % probability to survive 11 and 22 DHW beyond 25 days, with 38.8 and 6.7 % of the coral population remaining unaffected, although increased bleaching likelihood of 67 % and 94 %. Our results suggest that *X. umbellata* shows high intraspecific variability to warming, and despite prolonged exposure exacerbated its effects, it has a high potential for tolerance at intermediate temperatures, e.g., 11 DHW and to a certain degree at 22 DHW. This contrasts with the 8 DHW severe bleaching and mortality threshold usually linked to most of the sensitive hard and soft coral species recorded. *X. umbellata*'s intraspecific variability may increase its competitive advantage, enabling it to remain as winner over more sensitive species, which are projected to be lost by the middle of this century. However, in the long term, its persistence may be eventually threatened by future climate change scenarios.

7.2 Introduction

Global warming is among the most prominent threats, posing challenging scenarios for benthic reef communities worldwide (Heron et al., 2016; Hughes et al., 2018; Pandolfi et al., 2011). Rapid increases in temperatures and changes in the earth's climate are manifesting in every region and across the whole climate system, according to the latest Intergovernmental Panel on Climate Change (IPCC) Reports (Arias et al., 2021; de Coninck et al., 2018; Leung et al., 2019). Over the next 20 years, global temperatures are expected to increase by at least 1.5 °C, with most recent models projecting steady increases of 0.2 °C per decade (Masson-Delmotte et al., 2018), implying higher chances of crossing the warming threshold of 2.0 °C within the next 50 years (Arias et al., 2021). Further, the mean annual sea surface temperature over subtropical and tropical regions has increased by 0.4 to about 1.0 °C per year over the past 40 years (Masson-Delmotte et al., 2018), with locations such as the central Red Sea experiencing notably higher temperatures (Chaidez et al., 2017). These observed changes are unprecedented, and some are irreversible over scales of hundreds of years (Arias et al., 2021; de Coninck et al., 2018; Leung et al., 2019). Under these scenarios, consequences are expected to be catastrophic for coral reefs (de Coninck et al., 2018; Leung et al., 2019), threatening the ecological integrity and functionality of these ecosystems (Bozec & Mumby, 2015; Eddy et al., 2021; Hughes et al., 2018). Due to their unique ecological demands, corals are prone to experience declines of up to 70 – 90 % at a 1.5 °C increase compared to total losses at 2.0 °C ocean warming (Frieler et al., 2012; Hoegh-Guldberg et al., 2018; Masson-Delmotte et al., 2018). Moreover, thermal susceptibility is considerably variable across coral taxa, even at the species level (Furby et al., 2013; Marshall & Baird, 2000; Ziegler et al., 2019), where many key reef-building corals have varied thermal susceptibilities (Chaidez et al., 2017; Roik et al., 2016), from pronounced vulnerability in a considerable number of cases (Hughes et al., 2018; Monroe et al., 2018; Ziegler et al., 2019) to high resistance in some others (Bay & Palumbi, 2014; Krueger et al., 2017). Most studies have concentrated on assessing the thermal tolerance of hard corals (Hughes et al., 2018), especially for species highly sensitive to

narrow temperature regimes. Temperature increases can impair coral ecophysiology even below bleaching thresholds (Rädecker et al., 2021), affect reproduction, growth, and calcification rates (Carlon et al., 1996; Schneider & Erez, 2006), and cause severe coral mortality events (Baird & Marshall, 2002; Baker et al., 2008; Leggat et al., 2019), while in addition, increased length of exposure to warming can exacerbate the adverse effects of temperature on these corals (Rädecker et al., 2021). Whilst hard corals have justifiably attracted most of the attention, field monitoring has suggested negligible mortality and high resilience in octocoral taxa (Lasker et al., 2020; Prada et al., 2010; Sánchez, 2017), together with differential responses to warming (Lasker et al., 2020; 2021; Sánchez, 2016). Although heat-induced bleaching has also been reported for this group (Lasker et al., 2020; Prada et al., 2010), there are still deep knowledge gaps regarding these corals, whose responses to warming remain highly underinvestigated (Lasker et al., 2020).

The genus *Xenia* is among the few soft coral groups for which its metabolism and physiological responses to heat stress have been studied to date. This important tropical genus is widespread across numerous coral reefs, such as those in the Indo-Pacific region and the Red Sea (Halász et al., 2019; McFadden et al., 2019). Xeniidae corals show fast growth rates, substantial vegetative reproduction, and high potential as effective colonisers (Benayahu, 1985, 1991). As a result, this genus has successfully spread, even invading new regions in Venezuela and Brazil in the South American Caribbean (de Carvalho-Junior et al., 2023; Ruiz Allais et al., 2014). Moreover, xeniids have proven to be excellent competitors against hard coral species residing in these reefs (Ruiz Allais et al., 2014), showing strong potential to quickly overtake newly available niches that might arise due to climate change pressures. Despite these facts, only a limited number of past studies have been carried out on xeniids in the context of thermal stress (Parrin et al., 2016; Sammarco & Strychar, 2013; Strychar et al., 2005). However, these studies were conducted throughout 12 to 48 h and mainly focused on assessing heat-shock-induced bleaching and responses to acute thermal stress. They showed that *Xenia* sp. coral hosts could become exapted under warmer temperatures and are susceptible to bleaching, releasing their zooxanthellae symbionts at

maximally 30 C (Sammarco & Strychar, 2013). However, they lost their zooxanthellae considerably less than other soft and hard coral species subjected to similar scenarios (Strychar et al., 2005). In contrast, other xeniids have displayed a relevant degree of tolerance to warming, retaining positive photosynthesis in additional short-term experiments (Netherton et al., 2014; Parrin et al., 2016) and in most recent studies on the soft coral *Xenia umbellata* (Lamarck, 1816) featuring stepwise temperature increases with upheld short periods of warming (i.e., five days). These studies applied additional inorganic (Klinke et al., 2022; Mezger et al., 2022; Thobor et al., 2022) and organic eutrophication levels (Simancas-Giraldo et al., 2021; Vollstedt et al., 2020; Xiang et al., 2021) and their findings suggest that *X. umbellata* may be able to either attenuate or withstand physiological thermal stress. Nevertheless, the sensitivity and potential tolerance of *X. umbellata* still require further testing, e.g., under the context of longer-term warming exposures.

Hence, we aimed to study the response of the soft coral *Xenia umbellata* to prolonged warming, i.e., constantly elevated temperatures beyond 20 days of exposure. Thus, we evaluated the metabolic and ecophysiological responses of *X. umbellata* in a moderate-duration thermal stress experiment with 29 °C and 32 °C as simulated warming scenarios and 26 °C as control. We sought to resolve if adverse effects would be observed and exacerbated due to increased length of exposure to warming. In addition, we used a survival analysis and a Bayesian approach to assess *X. umbellata* survival probabilities beyond the term of our experiments and to provide a probabilistic framework to evaluate its bleaching susceptibility. With this research, we intend to contribute new insights and update the knowledge on soft corals' responses to thermal stress, especially from the Xeniidae family.

7.3 Materials and Methods

Experimental setup

We propagated 240 coral fragments (1.0 to 1.2 cm) from five *X. umbellata* parental colonies. The mother colonies were held in our facilities in preparation for this study for over a year at the Marine Ecology Department of the University of Bremen. Our experiments were carried out in two consecutive runs or trials, where new fragments were freshly propagated for each (n = 120 fragments per trial). After propagation, the fragments were allowed 14 days to attach to carbonate stones, and once healed, they were allocated into the experimental system consisting of 12 independent tanks with a capacity of 60 L each and distributed in a tower-like arrangement. Ten fragments were assigned to each tank and acclimatised for at least 15 days before introducing the warming treatments. These tanks were kept at initial control conditions with a salinity of 35.40 ± 0.40, pH of 8.20 ± 0.01, temperature of 26.00 ± 0.03 °C (mean ± SE), and light intensity of 109.20 ± 0.20 µmol m^{-2} s^{-1}, with LED lights (Royal Blue and white–matrix module, Waltron, Germany) providing even illumination in a 12:12 photoperiod for each tank. The tanks were distributed in a tower-like arrangement with three levels from top to bottom and four tanks per level. Each tank was separated into two technical parts: a 50 L front section that housed the coral fragments and a 10 L back section that served as a sump. This sump hosted technical equipment, pumps, and thermostats (± 0.50 °C accuracy, Eheim, Germany), allowing individualised tank temperature monitoring and control. Temperature and salinity were measured twice daily using a multimeter probe (HQ40d multimeter, HACH, Germany) and additional data loggers (Onset Hobo Pendant Temp/Light). Evaporation rates were monitored and adjusted constantly, and 20 % of the water volume was exchanged weekly. All tanks were maintained with additive-free artificial seawater (Tropic Marin ZooMix Sea Salt) to ensure nutrient conditions similar to those of natural reef environments. The water chemical parameters, including pH, Potassium hydride (KH), Ammonium (NH_4^+), Nitrite

(NO_2^-), Nitrate (NO_3^-), and Phosphate (PO_4^{3-}), were monitored and adjusted twice per week, while all the aquaria were cleaned weekly.

Further, although Xeniidae corals have been classified as mainly autotrophic (Gohar, 1940; Schlichter et al., 1983), other studies support mixotrophic (Fabricius & Klumpp, 1995) and plausible dominant heterotrophic behaviours (Al-Sofyani & Niaz, 2007). Thus, we provided all tanks with 20 mL Reef-Roids (PolyLab, USA) as coral food daily to ensure sufficient nutrient supply. Moreover, the coral species used in this study was identified molecularly as *Xenia umbellata* (Xiang et al., 2021). This zooxanthellate soft coral belongs to the family Xeniidae (order Malacalcyonacea) commonly distributed in warm, light-flooded waters of the Red Sea, where it is among the most abundant soft corals (Halász et al., 2019). The species' identity was validated based on its source, the Red Sea, and criteria established by Reinicke (1997) and Halász et al. (2019).

Temperature treatments

We implemented two warming scenarios: 29 °C and 32 °C. The selection of treatments considered the temperature increases expected according to the 2018 IPCC report (de Coninck et al., 2018), the range of temperatures expected for the Red Sea (Chaidez et al., 2017), and those evaluated for other Xeniids in previous studies by Parrin *et al.* (2016), Sammarco & Strychar (2013) and Strychar et al. (2005). The control condition was chosen as 26 °C, accounting for the mean temperature recorded for the Red Sea across seasons (Chaidez et al., 2017). The temperature treatments were allocated using four aquaria per treatment, and each aquaria row (i.e., a level) corresponded to a specific treatment (n = 80 coral fragments per treatment for both trials). After the acclimation period, the temperature was ramped up gradually over five days until reaching the target temperature. These treatments represented prolonged elevated temperatures of a moderate-duration thermal stress experiment, as defined by Grottoli et al. (2021), with more than 20 d of constant exposure, corresponding to 11 and 22 DHW, respectively, by the end of both trials

(i.e., by 25 d). A trial was carried out as long as a minimum of three tanks per treatment had enough individuals available for the metrics assessments. All procedures described were repeated as closely as possible in both trials, with additional treatment randomisation during the second trial. For the first trial, level I represented 32 °C, level II 29 °C, and level III 26 °C, while for the second trial, level I corresponded to 26 °C, level II to 32 °C and level III to 29 °C. Temperature treatment evolution over time is shown for the merged data corresponding to both trials in Figure 7.1 and detailed per each trial in Figure S1.

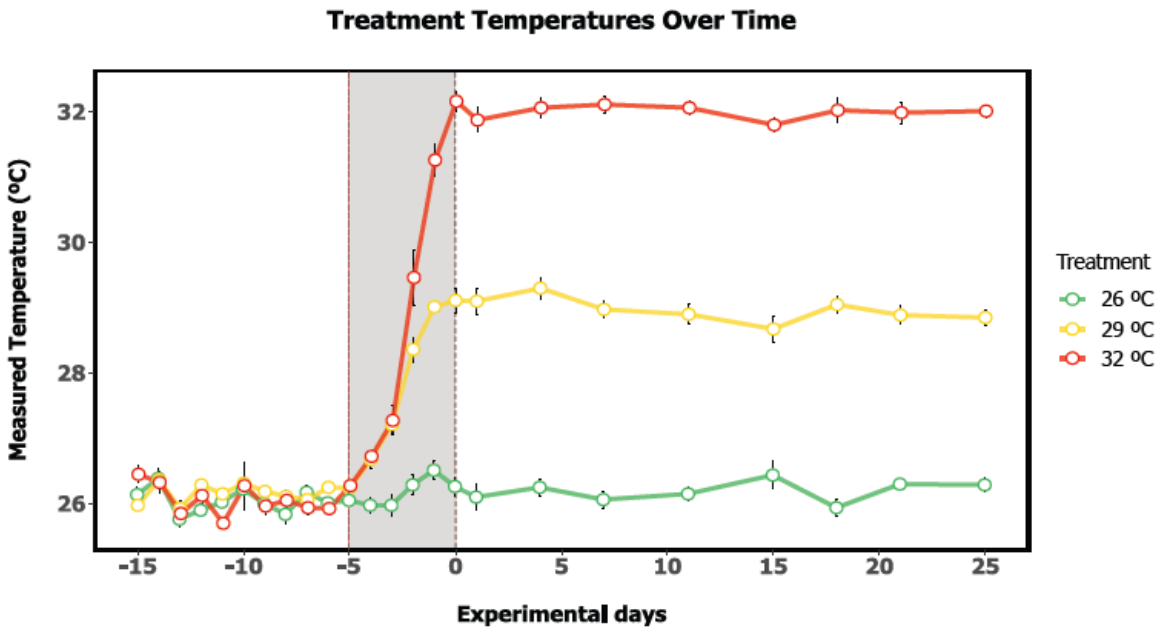


Figure 7.1 | Treatment temperatures through the experiment. The temperature behaviour over time (mean, ± s.e.m.) is illustrated for the control condition at 26°C (green) and heat-stressed conditions at 29°C (yellow) and 32°C (red). The data depicts n = 8 aquaria as replicates per treatment, comprising the combined data for the experimental trials 1 and 2. The grey region represents the temperature ramp-up phase until reaching manipulation targets, while the regions before and after the grey zone represent the acclimation and the heat-stress treatment phase, respectively.

Mortality and bleaching prevalence

We assessed coral mortality and bleaching for all 240 fragments in both experimental trials through daily visual observations and recordings of coral health status. Coral mortality was

assessed visually and using a small pipette to test tissue firmness of each fragment. A coral was considered dead when its respiration ceased and its tissue dissolved completely. Moreover, Additional weekly photographs accompanied the visual inspections and were used to assess bleaching prevalence qualitatively. The coral fragments were scored as bleached only upon complete whitening and impaired photosynthetic activity.

Pulsation and growth rates

X. umbellata pulsation was recorded for the 120 fragments in both trials with n = 24 per treatment. The fragments were followed over time, counting pulsations during 60 s for the same individuals. For this assessment, we used videos of coral fragments, which were always taken by midday, to ensure they were at peak activity. On the other hand, *X. umbellata* growth rates were calculated using photographic records of every fragment in the experiment. Every fragment was photographed in a fixed underwater setup and from a 90° top view, including a size and colour reference. Subsequently, each picture was processed on the open-source software FIJI (Schindelin et al., 2012), and the coral area was contoured. Finally, the differences in fragment area at the experiments' start and end were calculated and then related to daily change, thus obtaining the fragments' growth rate.

Oxygen fluxes

We measured the photosynthetic and respiration rates over time through light and dark incubations (n = 24 for both trials), following the Herndl & Velimirov (1986) and Bednarz et al. (2012) protocols and procedures detailed in Simancas-Giraldo et al. (2021). Briefly, three *X. umbellata* fragments were randomly chosen from each tank, placed in 151 mL jars filled with seawater and sealed airtight. An additional jar per tank without fragments was used as a control for planktonic background metabolism. The O_2 fluxes were measured in triplicate in these jars using a salinity-corrected optode (HQ40d multimeter, ± 0.05 % accuracy, HACH, Germany) at the start and the end of each incubation. The Light

incubations were performed by submerging the sealed jars directly within each treatment-specific aquaria, while the dark incubations took place within temperature-controlled water baths under complete darkness. The incubations always started 1 h after turning on the lights in the system and were carried out for 2 h to avoid O_2 saturation. To obtain net and gross photosynthesis (P_{net} and P_{gross}) and respiration rates (R), we averaged the three measurements per incubated fragments of the same tank and calculated the differences between the end and start O_2 concentrations (Δ_{dark} or Δ_{light}, respectively). These values were corrected by jar volume (V), coral fragment surface area (a), incubation time (t), and planktonic background (subtracting the control jars' O_2 concentrations from those with fragments). The respiration rate was calculated as:

$$R = \left|\frac{\Delta_{dark} \cdot V}{a \cdot t}\right| \quad (1)$$

net photosynthesis as:

$$P_{net} = \frac{\Delta_{light} \cdot V}{a \cdot t} \quad (2)$$

and gross photosynthesis as the sum of respiration and net photosynthesis:

$$P_{gross} = P_{net} + R \quad (3)$$

The P:R ratio was obtained by dividing gross photosynthesis over the respiration rates. In addition, we calculated the light R:P ratio, which reflects the percentage of produced O_2 directly used for respiration by the holobiont during the light phase (Krueger, 2019).

$$R{:}P_{light} = \frac{R\ h^{-1}}{P_{gross}\ h^{-1}} \times 100 \quad (4)$$

Further, the surface area of the fragments was determined according to Bednarz et al. (2012) via geometrical approximation. This approach allowed us to assess coral development over time without sacrificing individuals and to retain comparability with other studies.

TOC fluxes, CHARTOC and CZAR

Total organic carbon (TOC) was measured by calculating the organic matter fluxes mediated by the coral fragments. We assessed three tanks per temperature condition (n = 18 for both

trials). During the O_2 fluxes measurements, 25 mL water samples were collected directly from the incubation jars at the start and the end of each dark incubation in glass vials combusted at 550 °C. The samples were measured using a TOC-L analyser with an autosampler function (Shimadzu, Japan). The TOC contents were measured using the direct non-purgeable organic carbon (NPOC) method, and the TOC-L machine was calibrated by conducting a three-point calibration using 10 mL of a 1000 mg/L standard solution of 2.12 g potassium hydrogen phthalate diluted in 1 L ultrapure water. The TOC concentration differences between the end and start of the dark incubation period were used to calculate the TOC fluxes. The resulting values were corrected by planktonic background metabolism, fragment surface area, incubation time, and jar volume. The TOC fluxes were used to compute the proportionate contribution of heterotrophically derived organic C ($CHAR_{TOC}$) to the coral daytime C budget relative to respiration after Grottoli et al. (2006) and Levas et al. (2015). The percentage contribution of zooxanthellae-acquired C to the daily animal respiration (CZAR) was calculated according to Muscatine et al. (1981), converting the daily integrated P:R ratio to C equivalents. We assumed a mole-to-mole relationship of consumed CO_2 to produced O_2 during photosynthesis and respiration, respectively (Grottoli et al., 2006; Muscatine, 1981), and used the photosynthetic (PQ) and respiratory quotients (RQ) as in Rossi *et al.*, (2020):

$$GP_C = mg\ O_2\ Produced \cdot 0.375\ PQ^{-1} \quad (5)$$

$$R_C = mg\ O_2\ Consumed \cdot 0.375\ RQ \quad (6)$$

Finally, PQ and RQ were assumed to be 1.1 and 0.8, respectively (Muscatine, 1981; Rossi et al., 2020).

Stable isotopes analysis

At the end of the experiments, n = 12 fragments were randomly collected, ensuring four samples per treatment. The fragments were rinsed thoroughly with distilled water to eliminate excess salt contents and oven-dried at 60 °C for 72 h or until the measured weight stabilised. The subsequent analyses followed the protocols by Karcher *et al*. (2020) for Red

Sea Xeniidae samples. Briefly, we homogenised the dried samples through maceration without separating the zooxanthellae and host fractions and weighed one to two milligrams of each sample in thin aluminium capsules. The stable isotope abundances were measured to determine the elemental and stable isotopic compositions of C and N via combustion in a Thermo Electron Isotope Ratio Mass Spectrometer. We excluded two samples given technical issues during measurements for the first and insufficient dry material for the second. Thus, we analysed n = 10 samples with a minimum of three samples per treatment. Further, international laboratory standards were employed to calibrate the system: Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 for C and N, respectively. Isotopic values were expressed in δ notation as parts per thousand (‰) differences from the international standards:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000 \quad (7)$$

Here, X equals ^{15}N or ^{13}C, and R the heavier-to-lighter isotopic ratio ^{13}C:^{12}C or ^{15}N:^{14}N.

Statistical data analysis

We used the computational software R version 3.5.2 (R Development Core Team, 2013) for all graphs and statistical analyses, which were conducted over the merged data for both trials. To test for significant differences in the metrics assessed, we used Linear Mixed-effects Models (LMM) together with 1) a survival analysis to estimate survivorship probabilities and their differences across treatments and 2) a Bayesian approach to evaluate bleaching likelihood and its prevalence. For the LMM models, a model fit to the data was implemented per each predictor using the package Lme4 (Bates et al., 2015). We diagnosed model fit using residual plots, while model sensitivity was tested through data leverage. Model assumptions were confirmed, and strong outliers (far larger than 1.5 times the interquartile data range) were located using the Bonferroni outlier test function from the package 'car' (Fox et al., 2012) and removed if appropriate. The LMM models included temperature, time, and the interaction of both as fixed factors. Aquaria, coral individual,

and experimental trials were assigned as random factors for every metric assessed, except for the bleaching prevalence and stable isotope metrics. We excluded time as a fixed factor for the bleaching analyses and for the stable isotope LMM models, we included temperature, bleaching status and their interaction as fixed factors, and only experimental trial as a random factor. Best model selection was performed via model comparisons, AIC criteria, and loglikelihood assessments. For some of the isotopic metrics, LM was preferred over LMM models when the first provided a better fit to the data according to robustness and the selection output. Statistical significance was determined using ANOVA types II and III and defined for every analysis as $p \leq 0.05$ (Zuur et al., 2009). When significant differences were found, Post hoc tests were implemented (see our Supplementary Information). To evaluate *X. umbellata* survivorship probabilities, we modelled the shape of the survivorship curve per treatment and calculated the survival rates by estimating its conditional probabilities at each time point where mortality events occurred. We tested whether survival probabilities differed across treatments using a Log-rank test considering right-censored data (Kassambara et al., 2017; Therneau, 2015). Finally, to estimate *X. umbellata* bleaching likelihood, we fitted a Bayesian multilevel model using a predictive framework and the probabilistic programming language 'Stan', version 2.19.2 (Carpenter et al., 2017).

7.4 Results

Mortality and bleaching prevalence

Mortality occurred only for the coral fragments exposed to 32 °C (Figure 7.2) and showed intraspecific variability, with its onset beginning after four to eight days of treatment exposure (approx. 6 DHW). Cumulative mortality at 32 °C and 22 DHW reached up to 80 % for the first and 30 % for the second trials. From this coral population, 73 % showed tissue shading and died without experiencing intermediate bleaching.

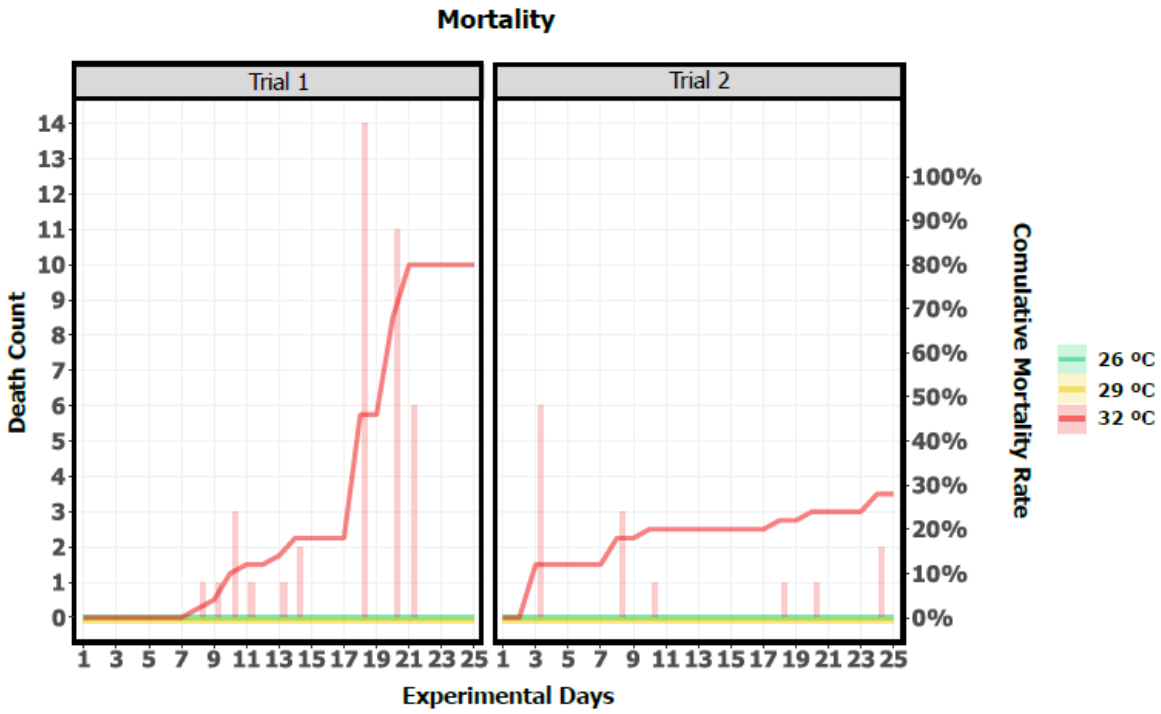


Figure 7.2 | Coral cumulative mortality (%) over time after exposure to increased temperature treatments. The control condition (26°C), intermediate heat-stressed conditions (29°C), and acute heat-stressed condition (32°C) are all represented by the green, yellow, and red lines, respectively. The data depicts the mortality recorded during the two experimental trials executed. Both trials included a total of n = 80 individuals per treatment at the start of each manipulation with 8 independent tanks per treatment and 20 corals per each.

In contrast, 93 % of the corals that survived the 32 °C treatment bleached at 22 DHW (Figure 7.3), while 6.7 % retained their healthy behaviour and appearance. Moreover, 61.2 % of the corals exposed to prolonged 29 °C and 11 DHW bleached, while 38.8 % remained healthy. The temperature treatments explained the proportions of bleaching observed (LMM; $P < 0.05$; $F = 34.4$; $P = 1.5e^{-6}$), and all temperature pair comparisons were significantly different.

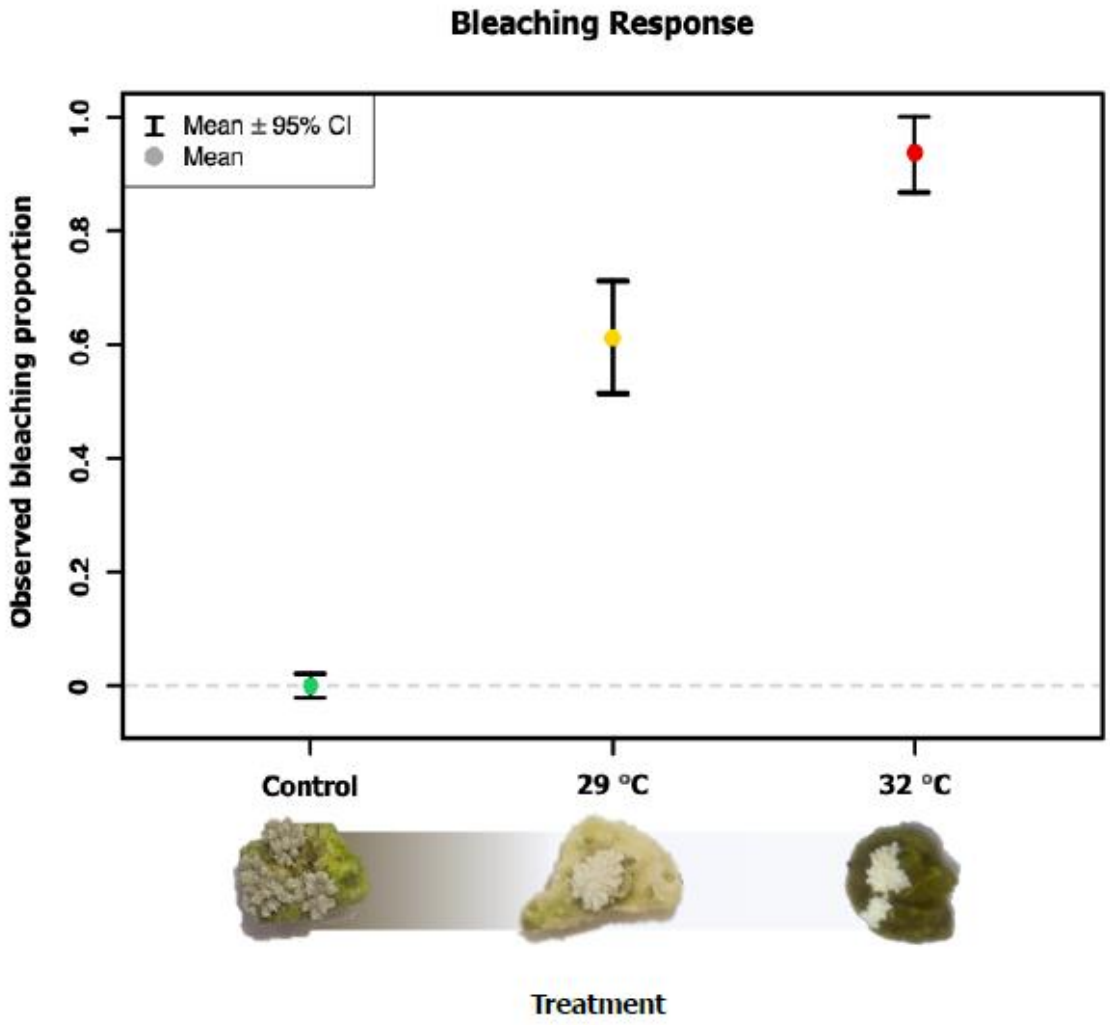


Figure 7.3 | *X. umbellata* bleaching response towards increased temperature treatments. The data depicts the mean coral bleaching response ± 95% confidence intervals. The coral colonies at 26°C control condition (green) showed no bleaching, whereas the intermediate heat-stressed condition at 29°C (yellow) and the treatment at 32°C (red) both caused coral colonies to bleach. Each treatment included n = 80 colonies, with 10 healthy individuals per tank by the start of the experimental manipulations.

Pulsation and growth rates

X. umbellata pulsation response over time and growth rates were significantly affected by prolonged warming (LMM; $P < 0.05$; Table 7.1; Figure 7.4 and S3.1). For pulsation, every temperature pair comparison was significantly different, while for the growth rate, every control-treatment pair was significant. At 29 °C and 11 DHW, almost 40 % of the fragments remained pulsating at rates comparable to those of the controls. In contrast, the remaining corals were negatively affected by temperature and progressively reduced their pulsation, correlating with transitions to bleached appearance and shrunken sizes. On the other hand, in the 32 °C treatment, a substantial reduction of pulsation rates and sizes was observed already during the first week, together with complete cessation of pulsing behaviour for most corals after 12 d (approx. 10 DHW). By 25 d and at 22 DHW, most of the surviving corals had either experienced tissue shading or bleaching, completely stopped their pulsation and displayed hampered growth rates.

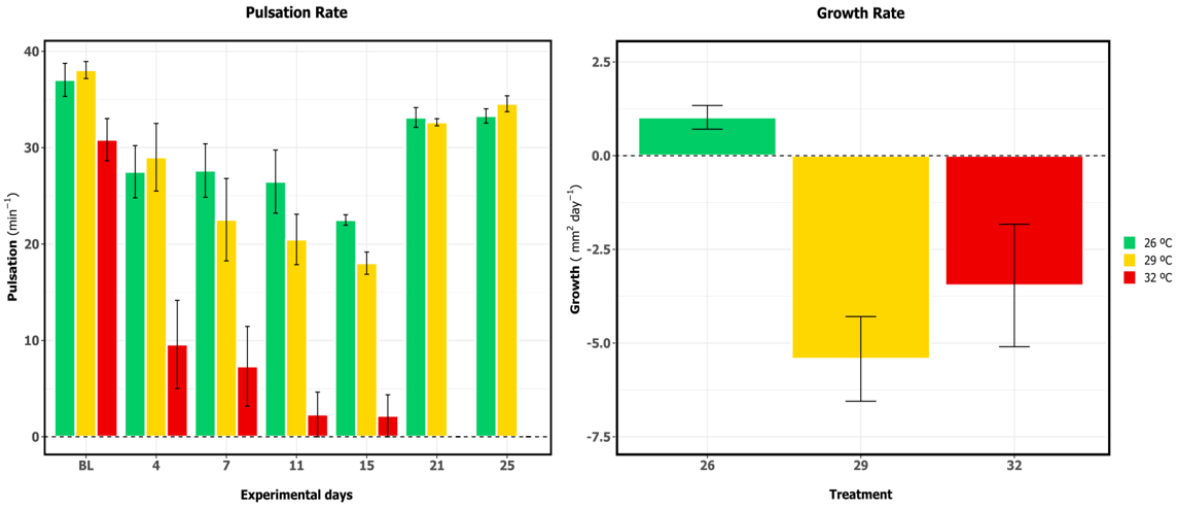


Figure 7.4 | *X. umbellata* pulsation and growth rates. Colour codes show the temperature conditions where green represents the control at 26°C, and yellow and red indicate the heat-stressed conditions at 29°C and 32°C, respectively. Pulsation rates assessment was performed for a total of 120 coral fragments over time, with n = 8 replicates per treatment and 5 coral fragments measured per tank, while on the other hand, growth rates were quantified starting with a total of 80 coral fragments with n = 8 replicates per treatment and 10 coral fragments monitored per tank.

Table 7.1 | Linear mixed model results for *X. umbellata* pulsation rate (min^{-1}) over time and growth rate ($mm^2\ day^{-1}$). Type II Analysis of Variance with Wald Chi-square tests. Significant *P*-values are highlighted in bold ($p \leq 0.05$).

Factor	Fixed Effects	*df*	Chisq	p
Pulsation Rates	Temperature	2	2993.7	**$< 2.2e^{-16}$ *****
	Time	14	4141.5	**$< 2.2e^{-16}$ *****
	Temperature x Time	28	2831.0	**$< 2.2e^{-16}$ *****
Growth Rate	Temperature	2	91.375	**$< 2.2e^{-16}$ *****

Note: *P*-values defined as significant at a threshold of $P < 0.05$ are highlighted in bold.

Oxygen fluxes

All the fixed factors evaluated (i.e., temperature, time, and their interaction) were significant for respiration and every photosynthetic metric (LMM; $P < 0.05$; Table 7.2; Figure 7.5 and S6.1). In addition, both temperature treatments were significantly different compared to the control condition for gross and net photosynthesis and the P:R ratio. Respiration and the light R:P ratio showed significant differences for the control and 29 °C, and 32 °C and 29 °C pairs. By the end of the experiments, when 29 °C equated to 11 DHW and 32 °C to 22 DHW, respectively, gross photosynthesis and respiration rates decreased by 25 % and 50 % at 29 °C and 75 % for both metrics at 32 °C. Net photosynthesis and the P:R ratio showed increases of approximately 50 % under 29 °C and reached comparable values to the control when the experiment concluded. Yet, both exhibited 50 and 71 % decreases, respectively, at 32 °C and 22 DHW. On the other hand, corals under 26 °C and 29 °C showed mean P:R values exceeding 1.5, the conservative threshold for autotrophy (Wilkinson, 1983), in contrast to corals under 32 °C which displayed values between 1.5 and 1.0, corresponding to the compensation range (Baker et al., 2015; Wilkinson, 1983) or below. Conversely, the light R:P ratio for the 32 °C treatment showed the highest percentages of O_2 directly consumed for respiration at 22 DHW, which decreased by 40 %.

Table 7.2 | Linear mixed-effects model results for gross and net photosynthesis, respiration rates (mg O_2 m^{-2} h^{-1}) and the P:R and light R:P ratio of *X. umbellata* corals under sustained warming over time. Type II Analysis of Variance with Wald Chi-square tests. Significant *P*-values ($p \leq 0.05$) are highlighted in bold.

Factor	Fixed Effects	*df*	Chisq	p
Gross photosynthesis	Temperature	2	21.62	**< 2.2e^{-16} *****
	Time	8	66.33	**< 2.2e^{-16} *****
	Temperature x Time	16	124.42	**< 2.2e^{-16} *****
Respiration	Temperature	2	34.64	**3.013e^{-10} *****
	Time	8	300.35	**< 2.2e^{-16} *****
	Temperature x Time	16	54.45	**1.099e^{-05} *****
Net photosynthesis	Temperature	2	18.45	**< 2.2e^{-16} *****
	Time	8	78.44	**< 2.2e^{-16} *****
	Temperature x Time	16	194.568	**< 2.2e^{-16} *****
P:R	Temperature	2	21.75	**1.89e^{-05} *****
	Time	8	189.68	**< 2.2e^{-16} *****
	Temperature x Time	16	197.36	**< 2.2e^{-16} *****
Light R:P	Temperature	2	108.01	**< 2.2e^{-16} *****
	Time	6	131.71	**< 2.2e^{-16} *****
	Temperature x Time	12	86.635	**< 2.205e^{-13} *****

Note: *P*-values defined as significant at a threshold of $P < 0.05$ are highlighted in bold.

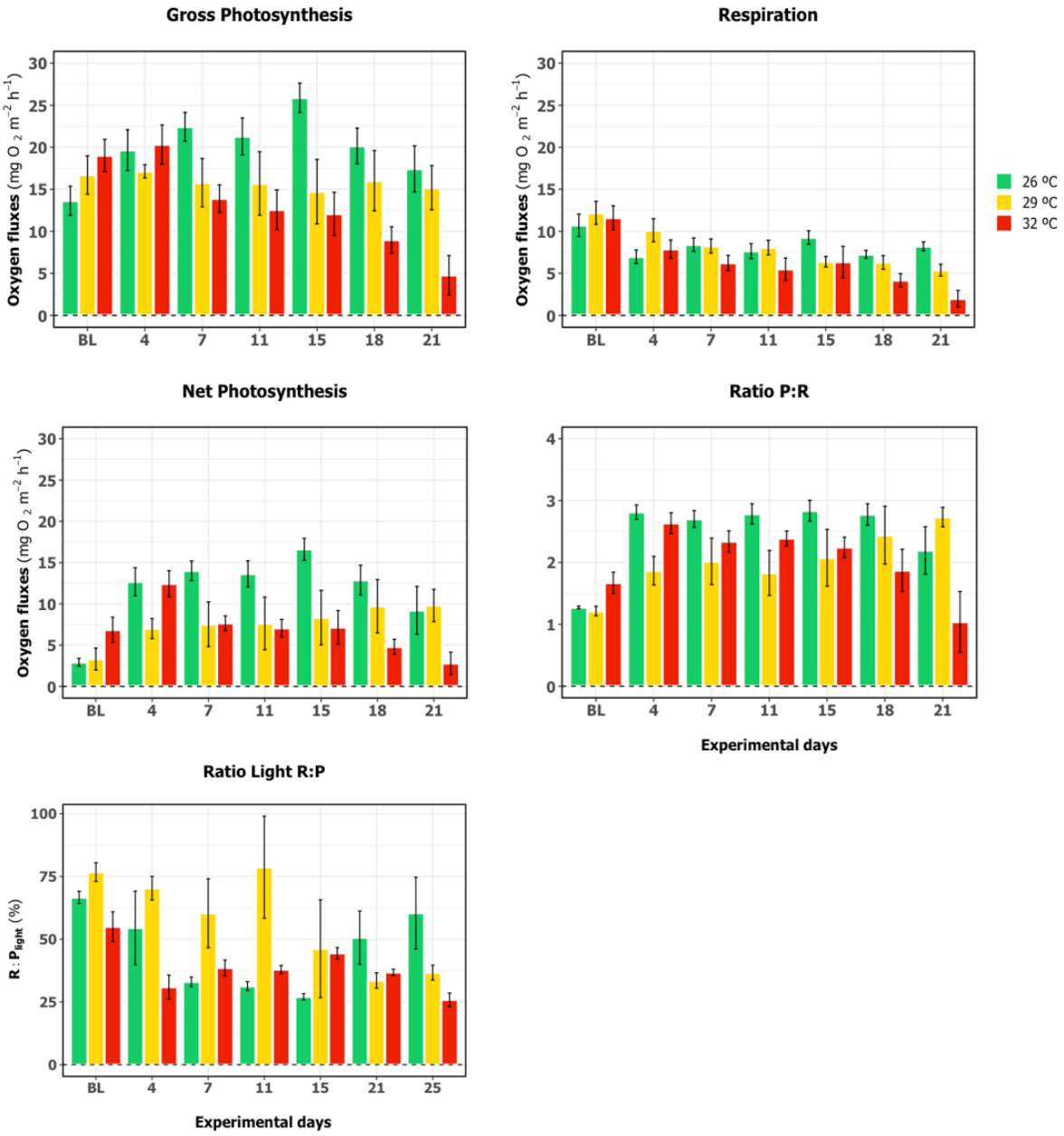


Figure 7.5 | *X. umbellata* gross photosynthesis, respiration, net photosynthesis rates, (mg O_2 m^{-2} h^{-1}), P:R and light R:P ratios under sustained warming scenarios over time. Behaviour over time is shown for the temperature control condition at 26°C (green) and heat-stressed conditions at 29°C (yellow) and 32°C (red). Bars values indicate mean ± s.e.m. The data depicts n = 8 replicates per treatment, with 3 coral fragments measured per tank in each treatment.

TOC fluxes, CHARTOC and CZAR

Net TOC fluxes were significantly affected by temperature and time but not by their interaction (LMM; $P > 0.05$; Table 7.3; Figure 7.6). However, all factors and their interaction affected $CHAR_{TOC}$ and CZAR (LMM; $P < 0.05$; Table 7.3; Figure 7.6). Further inspection of the TOC, $CHAR_{TOC}$ and CZAR metrics showed significant differences between the control and 32 °C, and 32 °C and 29 °C treatments. The corals' TOC fluxes for the control and 29 °C treatments remained positive until the end of the experiment. However, with magnitudes close to zero across the experimental time (See Supplementary Information S9.1-2 and S11.1-2). On the other hand, the net TOC fluxes at 32 °C indicated a shift in the OC metabolism after 15 d of exposure (12 DHW) and high variability towards the end of the experiment at 22 DHW, while $CHAR_{TOC}$ showed similar behaviour to that of the net TOC fluxes. Further, every temperature condition initially showed CZAR values between 80 and 100 % after a first decrease in the 29 °C condition compared to the control and overshoots in the 32 °C condition by experimental days 4 and 25. From day 7 to 11, corals at 29 °C showed highly variable responses that returned by day 15 to be comparable to the control condition. After this point, mean CZAR values remained above 100 % for every condition.

Table 7.3 | Linear mixed-effects model results for *X. umbellata* TOC fluxes, $CHAR_{TOC}$ and CZAR. Type II Analysis of Variance tests. Significant *P*-values ($p \leq 0.05$) are highlighted in bold.

Factor	Fixed Effects	*df*	Chisq	p
TOC flux	Temperature	2	7.261	**0.025 ***
	Time	6	36.945	**$4.318e^{-05}$ *****
	Temperature x Time	12	19.037	0.085
$CHAR_{TOC}$	Temperature	2	31.129	**0.005 ****
	Time	6	51.152	**$5.044e^{-05}$ *****
	Temperature x Time	12	21.445	**0.044 ***
CZAR	Temperature	2	115.62	**$< 2.2e^{-16}$ *****
	Time	6	183.22	**$< 2.2e^{-16}$ *****
	Temperature x Time	12	105.65	**$< 2.2e^{-16}$ *****

Note: *P*-values defined as significant at a threshold of $P < 0.05$ are highlighted in bold.

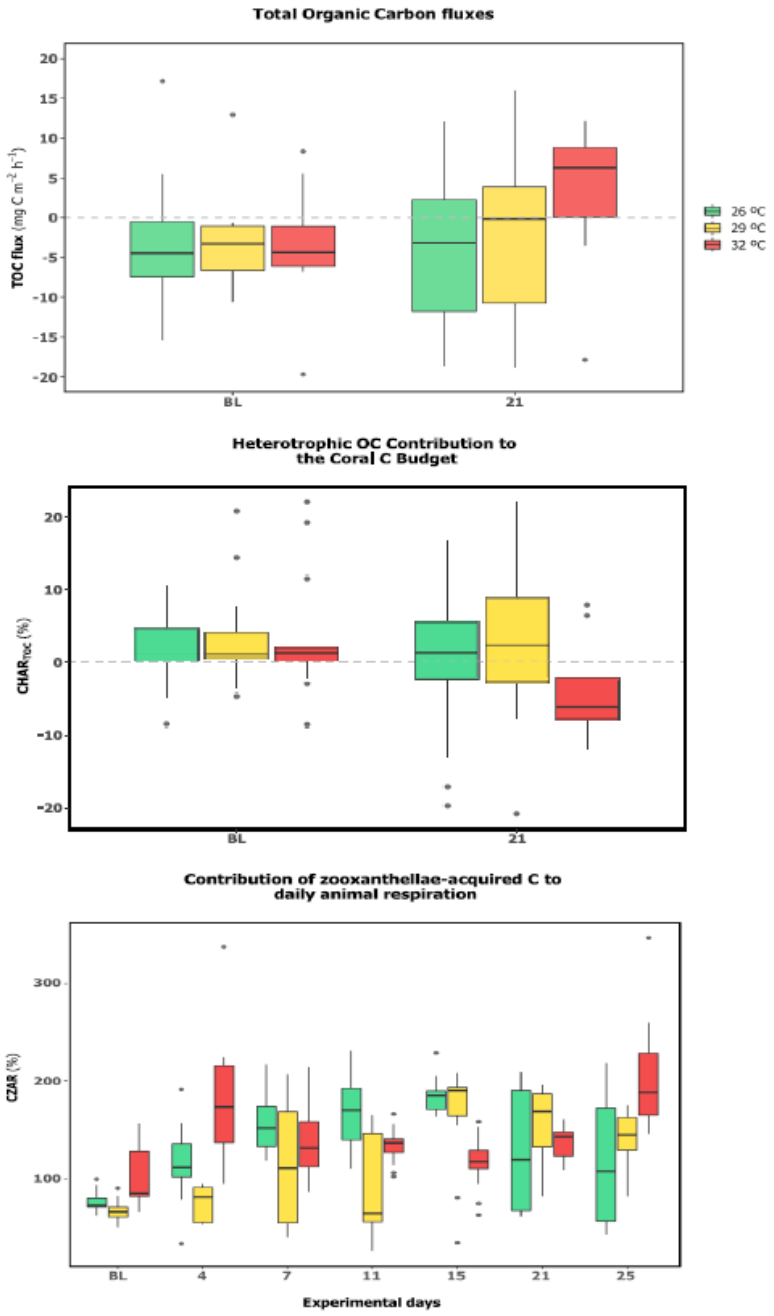


Figure 7.6 | Zoom-in into TOC and $CHAR_{TOC}$ fluxes and CZAR behaviour over time for *X. umbellata* corals under increased temperatures. The figure shows TOC and $CHAR_{TOC}$ fluxes before increasing temperatures at baseline BL (day -5) and on day 21. For the TOC fluxes, negative rates indicate organic matter uptake, while positive rates indicate loss or release. On the other hand, negative $CHAR_{TOC}$ values indicate carbon losses, whilst positive ones indicate uptake. In addition, CZAR indicates the percent contribution of autotrophically acquired carbon available to the coral energy budget over time. 26°C (green) represents the control condition, whereas 29°C (yellow) and 32°C (red) indicate heat-stressed treatments. Each treatment included n = 6 replicates, with 3 corals measured per tank.

Isotopes δ13C, δ15N, C and N contents, and C:N ratios

By the end of the experiment, means for *X. umbellata* $\delta^{13}C$ and $\delta^{15}N$ isotopes showed high variability for the 29 and 32 °C treatments (Figure 7.7), with no significant differences across temperature conditions (LMM; $P > 0.05$; Table 7.4).

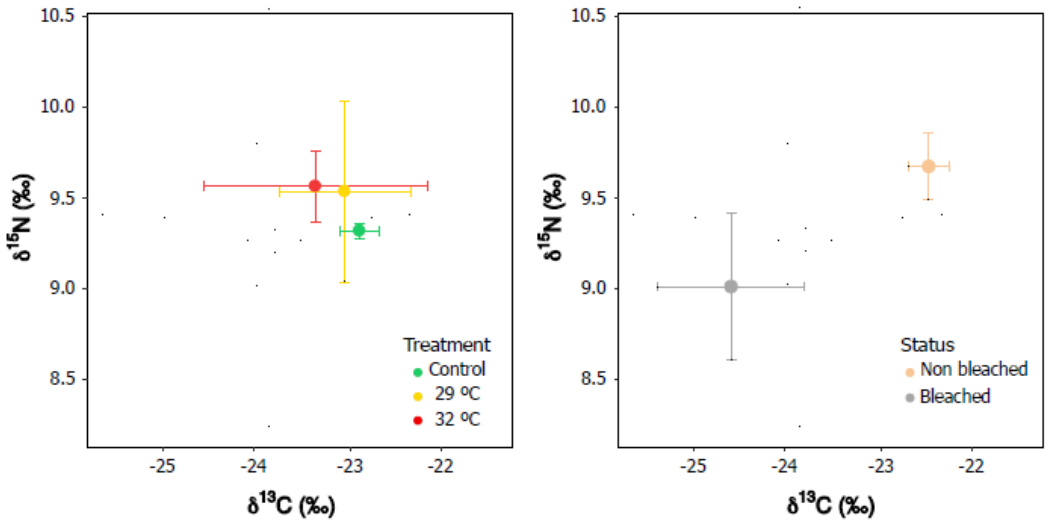


Figure 7.7 | Biplots for δ13C and δ15N values for *X. umbellata* corals after exposition to increased temperatures. The plot portrays δ13C and δ15N putative values (mean ± SE) per treatment and per bleaching status after 25 days of experiment. In the first plot showing the values per treatment, 26°C (green) represents the control condition, whereas 29°C (yellow) and 32°C (red) indicate heat-stressed treatments. The bleached condition (grey) and the non-bleached condition (light brown) are presented in the second plot. Each treatment included at least n = 3 replicates per temperature treatment.

In line with this, the C:N ratio showed no significant differences when contrasting temperature treatments (LMM; $P > 0.05$; Table 7.4; Figure 7.8). However, elevated temperatures significantly affected the *X. umbellata* %C and %N contents (LMM; $P < 0.05$; Table 7.5; Figure 7.8), where every pair comparison for the factor temperature was significant. The bleaching status explained the observed responses for the $\delta^{13}C$ and $\delta^{15}N$ isotopes, %C and %N contents and C:N ratios (LM; $P < 0.05$; Table 7.4 and Table 7.5), despite the high variability in the $\delta^{13}C$ and $\delta^{15}N$ isotopes and the %C and %N contents. The temperature and bleaching status interaction resulted significant only for the %C and %N contents (LMM; $P < 0.05$; Table 7.5). Moreover, the $\delta^{13}C$ and $\delta^{15}N$ values of *X. umbellata*

ranged from -24.5 ‰ to -22.0 ‰ and from 9.0 ‰ to 10.0 ‰, respectively, across temperature treatments. Regarding bleaching status, $\delta^{13}C$ and $\delta^{15}N$ showed values from -25.6 ‰ to -23.1 ‰ and from 8.5 ‰ to 9.4 ‰, for the bleached corals and from -22.7 ‰ to -22.1 ‰ and 9.5 ‰ to 9.8 ‰, respectively, for the non-bleached ones. Further, the %C and %N mean contents remained higher for bleached corals, approximately 48 % and 6 %, when compared to 26 % and 3 % for the non-bleached ones. This resulted in a lower C:N ratio for the bleached corals compared to the non-bleached ones, of 9.4 and 8.0, accordingly.

Table 7.4 | Linear models summary for $\delta^{13}C$ and $\delta^{15}N$ values for *X. umbellata* corals after exposition to increased temperatures. Significant differences were determined using a Type II Analysis of Variance and considering $p \leq 0.05$.

Factor	Fixed Effects	*df*	F	p
$\delta^{15}N$ (‰)	Temperature	2	3.309	0.121
	Bleaching status	1	11.790	**0.0186 ***
	Temperature x Bleach	1	3.493	0.121
$\delta^{13}C$ (‰)	Temperature	2	1.363	0.3368
	Bleaching status	1	15.064	**0.0116 ***
	Temperature x Bleach	1	0.002	0.9694
C:N	Temperature	2	0.937	0.451
	Bleaching status	1	8.212	**0.0352 ***
	Temperature x Bleach	1	0.0300	0.8695

Table 7.5 | Summary for linear mixed-effects models of *X. umbellata* carbon and nitrogen stable isotope contents. Significant differences were assessed using a Type II Analysis of Variance test. *P*-values $p \leq 0.05$ were considered as significantly different.

Factor	Fixed Effects	*df*	Chisq	p
% C	Temperature	2	82.261	**$< 2.2e^{-16}$ *** **
	Bleaching status	1	4.306	**0.03799 ***
	Temperature x Bleach	1	156.343	**$< 2.2e^{-16}$ *** **
% N	Temperature	2	201.24	**$< 2.2e^{-16}$ *** **
	Bleaching status	1	11.73	**0.00061 *** **
	Temperature x Bleach	1	338.17	**$< 2.2e^{-16}$ *** **

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

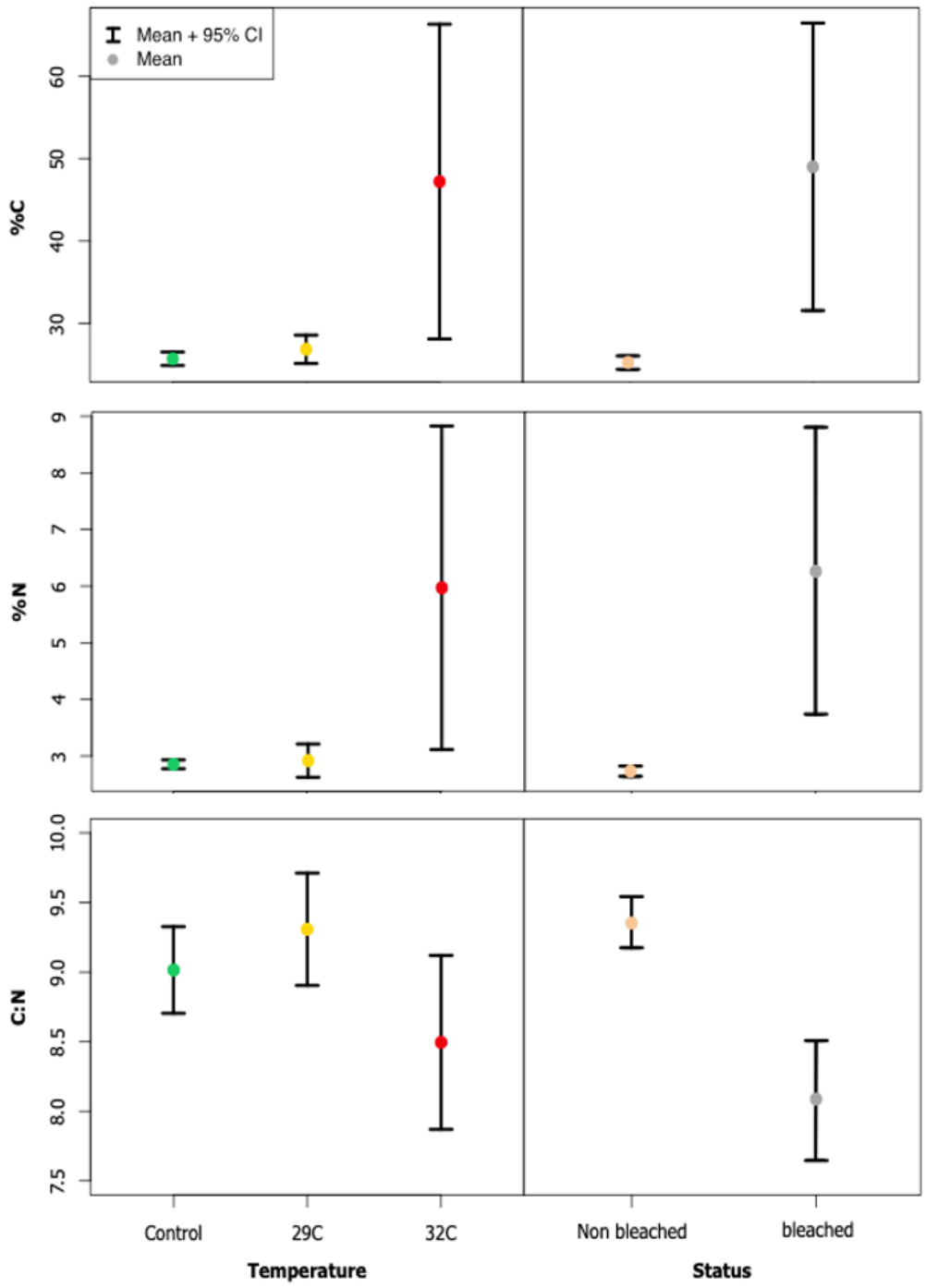


Figure 7.8 | *X. umbellata* %C, %N, and C:N isotopic ratio by the end of the increased temperature treatment. The plots depict 95% confidence intervals (mean ± SE) for the coral fragment's response per treatment (left) and per bleaching status (right) at the end of the experimental manipulations. Here, 29°C (yellow) and 32°C (red) denote treatments that have been exposed to heat stress, while 26°C (green) represents the control condition. The bleached condition and the unbleached condition are represented here in grey and light brown, respectively. Each treatment included at least n = 3 replicates per temperature treatment.

Survival and predicted bleaching prevalence

Observed survivorship for the control at 26 °C and the temperature treatment at 29 °C and 11 DHW corresponded to 100 %. In comparison, 28 % (22 out of 80) of the fragments survived at 32 °C and 22 DHW. Specifically, *X. umbellata* showed an 88 % probability of surviving 6 DHW (i.e., 5 days into 32 °C exposure) and 75 % and 50 % when heat stress equated 12 DHW and 17 DHW (i.e., 15 and 20 d at 32 °C), respectively. The median survival time corresponded then to 20 days at 32 °C (i.e., 17 DHW). The survival rates for the fragments under the 32 °C treatment were significantly different from the 29 °C treatment and the control (log-rank test, $p < 0.001$; Figure 7.9).

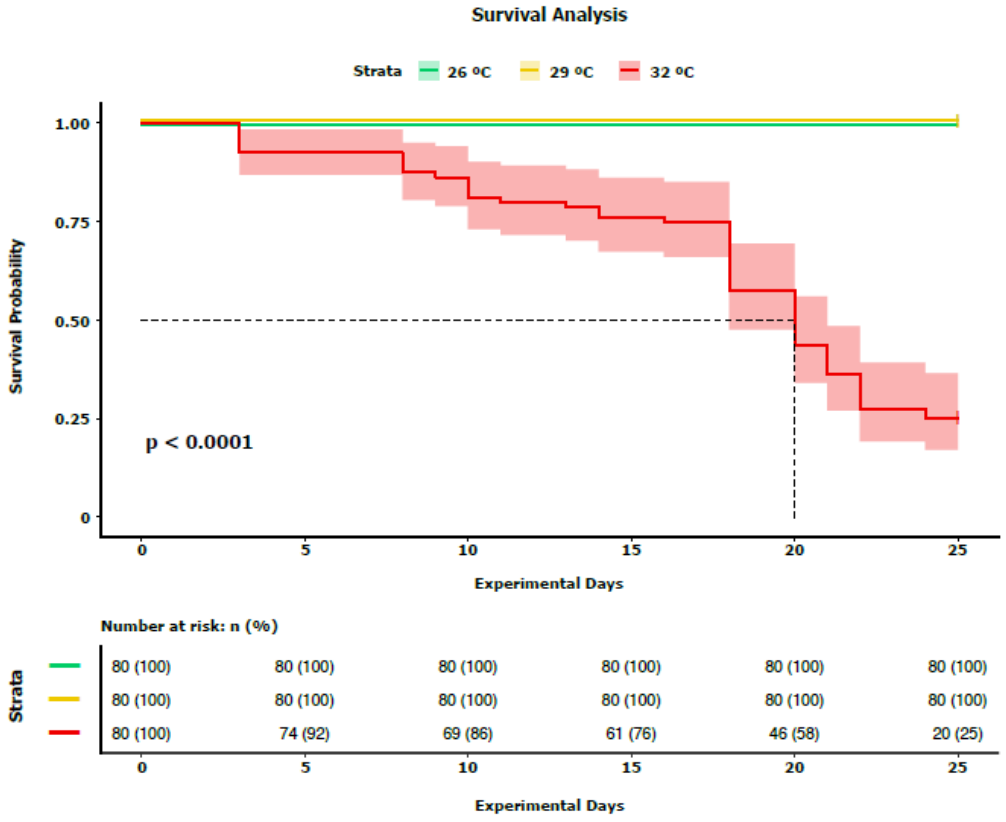


Figure 7.9 | Survival probability functions of *X. umbellata* corals exposed to temperature treatments over time. The 26°C control condition for the temperature treatments (green) and the intermediate 29°C heat-stressed condition (yellow) showed 100% survival during the whole extent of the experiment, whereas mortality occurred at 32°C (red). The curves depict the coral survival probabilities calculated based on the combined data for the two experimental trials conducted. Thus, each treatment included n = 80 fragments by the start of the experimental manipulation with 10 initial fragments per tank.

By 17 DHW, the proportion of at-risk observed corals corresponded to 58 % of the total treatment population (46 out of 80 corals), compared to 25 % (20 out of 80 corals) by 22 DHW. Moreover, the survival curves at 32 °C for both experimental trials crossed, indicating nonproportional hazard rates (Figure S15.1). Thus, there were no detectable differences in the survival probabilities between trials. On the other hand, the likelihood of observing bleached corals under the elevated temperature treatments corresponded to 67 % for 29 °C and 11 DHW and 94 % for the 32 °C treatment at 22 DHW, implying a 33 % and 6 % probability of not observing bleaching. These predicted bleaching probabilities per treatment are shown in Figure 7.10. Furthermore, the probability that prolonged elevated temperature had a negative effect on the coral fragments was above 90 % for both temperature treatments (Figure S16.1), with significant negative effects for the 29 °C ($P = 0.012$; $P < 0.05$) and the 32 °C ($P = 0.00067$; $P < 0.05$) treatments.

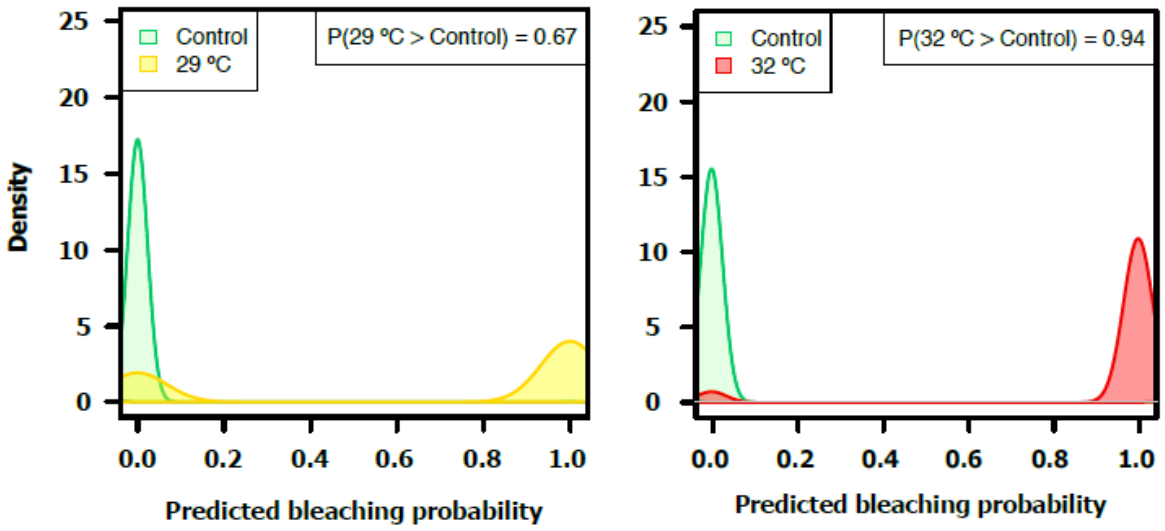


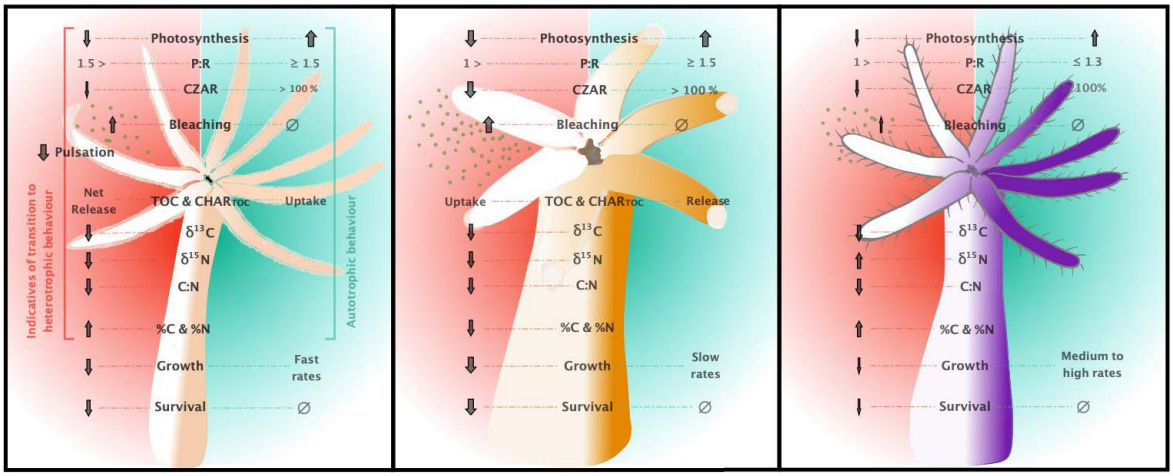
Figure 7.10 | Predicted bleaching probabilities for *X. umbellata* colonies from each experimental condition. Density plots showing the predicted bleaching probability of a randomly chosen coral from the heat-stressed groups 29°C (yellow) and 32°C (red), respectively. The heat-stressed treatment density plots are compared to the calculated distribution for the control groups at 26°C (green), where no bleaching occurs.

7.5 Discussion

This study explored the sensitivity, potential survival and bleaching prevalence of *X. umbellata* under prolonged warming scenarios of 29 °C and 32 °C. Our findings show that the degree of sensitivity of this coral can differ significantly and show important intraspecific variability. Although it ultimately becomes negatively affected by prolonged exposure to elevated temperatures. For instance, the most adverse effects of prolonged warming were observed at 32 °C, representing an initial increase of 6 °C and 22 DHW by the end of the experiment, while a prolonged temperature of 29 °C (i.e., initially a 3 °C change that reached 11 DHW) already impaired *X. umbellata* ecophysiology and its metabolism, yet to a lesser extent. Interestingly, under 11 DHW, no mortality was observed, the survivorship probabilities of *X. umbellata* did not decrease regardless of co-occurring bleaching, and under 22 DHW, the probability of surviving beyond 25 days remained at 25 %, with a portion of the total population still healthy under both warming scenarios. These findings particularly contrast with the 8 DHW threshold, which corresponds to the maximum level of heat stress bleaching alert (Heron et al., 2016) that is generally associated with severe bleaching and up to 100 % mortality for most hard and soft coral species being thermally sensitive. Our results also suggest that constantly elevated temperatures eventually triggered the onset of bleaching and that prolonged exposure exacerbated the adverse effects of warming over time. In line with this, while our survival analyses confirmed *X. umbellata* survivorship potential to constant warming exposure, our Bayesian approach indicated that both temperature treatments increased the likelihood of observing bleaching. Thus, our study revealed that prolonged exposure to warming had cumulative negative impacts on *X. umbellata* health and survival, especially at the highest temperature. However, differing physiological sensitivity may contribute to this species' relative tolerance, enhancing its potential competitive advantage when compared to other thermally sensitive corals under the selected warming scenarios and especially at intermediate temperatures.

X. umbellata showed intraspecific variability at prolonged intermediate temperature

Under 29 °C and until reaching 11 DHW, *X. umbellata* corals exhibited milder heat-stress responses and assorted usage of the available metabolic energy. Intraspecific sensitivity to temperatures in terms of bleaching and physiological responses has been observed in other xeniids such as *Phenganax parrin*i, *Sarcothelia* sp., and *Sympodium* sp. with significant variations even across closely related taxa (Netherton et al., 2014; Parrin et al., 2016; Steinberg et al., 2022). In general, some soft corals such as *Sarcophyton ehrenbergi*, *Sinularia* sp., and in special xeniids can be insensitive to 29 °C exposures, especially when subjected to short-term or heat-shock stress experiments (Sammarco & Strychar, 2013; Strychar et al., 2005). For many soft and hard corals, 29 °C remains within the scope of their thermal limits (Heron et al., 2016). However, through prolonged exposure experiments (which better simulate heat stress accumulation), it is possible to identify species thermal maxima and the conditions for which increased temperature can trigger bleaching (Marzonie et al., 2022; Humanes et al., 2022). For instance, in Figure 7.11, we present a general comparison between *X. umbellata* and diverse hard and soft coral taxa. For most hard coral taxa, low levels of bleaching can be observed starting at 2-3 DHW, while typically at 4 DHW and 8 DHW, 30-40 % and 70 to 90 % of corals show mild and severe bleaching and mortality, respectively (Osman et al., 2018). On the other hand, although bleaching in soft corals seems less severe and the notion of this coral group being more resistant than hard corals has persisted (Lasker et al., 2020; Goulet et al., 2017), soft coral bleaching (which can reach up to >50%) has also been reported for some taxa at comparable temperature scenarios (Prada et al., 2010). Regarding *X. umbellata*, this coral displayed no mortality and retention of photosynthetic activity and pulsation at intermediate temperature and at 11 DHW, although roughly more than half of the population bleached. However, a net autotrophic character indicated positive symbiont productivity (Netherton et al., 2014), although impacted by prolonged warming. Maintained photosynthetic efficiency is a key energy source for upholding regular metabolic demands in pumping xeniids (Gohar, 1940; Kremien et al., 2013; Schlichter et al., 1983).



	P:R	trophic strategy	bleaching threshold	susceptibility rank	Physiological effects: compromised photosynthesi	Physiological effects: reduced growth	Physiological effects: mortality	References
Sarcophyton spp	1.8	Autotroph	< 8 DHW	M	●	○	○	Fabricius & De'ath 2008 Travesso et al. 2023 Porter et al. 2021
Sinularia sp	1.3	Heterotroph	< 8 DHW	M	●	○	●	Slattery et al. 2023 Ferrier-Pagés et al. 2021 Goulet et al. 2008
Scleronephthya spp	0.3	Heterotroph	>8 DHW	L	○	○	○	Fabricius & Klumpp 1995
Dendronephthya spp	0.8	Heterotroph	>8 DHW	L	○	○	○	Fabricius & Klumpp 1995
Xenia umbellata	2.5 to 3	Autotroph	> 11 DHW	L	○	●	○	This Study Thobor et al. 2022
Pocillopora darmicornis	2.21	Autotroph	< 4 DHW	H	●	●	●	Zaneveld et al. 2016 Sahin et al. 2023
Pocillopora verrucosa	1.6	Autotroph	< 4 DHW	H	●	○	●	Sawall et al. 2015 Sahin et al. 2023 Evensen et al. 2022
Seriatopora hystrix	2.8	Autotroph	< 4 DHW	H	●	●	●	Frieler et al. 2013 Hoegh-Guldberg & Smith 1989
Stylophora pistillata	3.0	Autotroph	< 4 DHW	H	●	●	●	Porter et al. 1984 Hoegh-Guldberg & Smith 1989
Acropora spp	1.5	Autotroph	< 4 DHW	H	●	●	●	Monroe et al. 2018 Frieler et al. 2013 P.P. Martins et al. 2024
Anacropora matthaii	2.0	Autotroph	< 4 DHW	H	●	○	○	Monroe et al. 2018 Heron et al. 2016
Porites lobata	1.6	Autotroph	< 4 DHW	H	●	●	●	Monroe et al. 2018
Pavona spp	1.5	Autotroph	< 4 DHW	H	●	●	●	Monroe et al. 2018
Siderastrea radians	1.5	Autotroph	< 4 DHW	H	●	●	●	Fournie et al. 2012 Frieler et al. 2013

Figure 7.11 | Physiological response comparison between *X. umbellata* and diverse hard and soft coral taxa sensitive to thermal stress. Schematic comparison between *X. umbellata* (Light peach) and soft (Purple) and hard (Orange) corals is shown here, together with selected species thermal stress responses reported in the literature. The cladogram gathers both octocorals (purple clade) and hexacorals (orange clade). *X. umbellata* seems particularly tolerant to thermal stress when compared to its hard coral counterparts.

Despite the underlying mechanism facilitating photosynthetic activity for *X. umbellata* remaining unclear, our findings align with Netherton et al. (2014) and Parrin (2015), who showed that xeniids can continue to photosynthesise despite acute stress and bleaching via symbiont migration within the coral coenenchyma. Under 29 °C, *X. umbellata* potentially utilised autotrophically acquired energy effectively, explaining the healthier coral population observed. Conversely, prolonged thermal stress and concomitant bleaching (when observed) impaired this coral's energy balance and tissue biomass (Anthony et al., 2007; Fitt et al., 2000; Zelli et al., 2023). Overall, negative growth rates and complete interruption of pulsation were observed for every coral experiencing bleaching, indicating energy reserve depletion and compromised resources (Anthony et al., 2009). However, at intermediate temperatures, an alternative interpretation suggests that gradual pulsation decreases may have served as an initial energy-saving mechanism to alleviate energy consumption (Schubert et al., 2020). In line with this, the respiration, R:P ratio, and CZAR metrics also illustrated how consumption differences between the warming treatments support a better performance for the 29 °C corals. While withstanding a 3 °C increase required less energy investment than a 6 °C change, prolonged warming resulted in negative responses, supporting the notion of adverse effects due to heat accumulation reported for other coral taxa (Conti-Jerpe et al., 2020; Rädecker et al., 2021). Nonetheless, a better energy balance and no mortality compared to the highest temperature suggests intermediate sensitivity at 29 °C, with better tolerance and potential resilience chances.

Furthermore, the bleached *X. umbellata* isotopic signatures associated with the C and N metabolism revealed a disparity in this coral's trophic and metabolic behaviour. For every isotopic metric, these differences were primarily explained by bleaching status rather than temperature. However, prolonged warming impacted the mean %C and %N contents while its synergy with bleaching amplified its effects. The N isotopic metrics suggested carbohydrate losses for the bleached corals in line with reduced sizes, indicating affected biochemical energy reserves (McCauley & Goulet, 2019), while $\delta^{15}N$ depletion suggested N

fixated products assimilation into the coral holobiont (Muscatine et al., 1989; Pernice et al., 2012). Although soft and hard corals' behaviour may differ, our observations align with those of hard corals under heat stress, where the symbionts can act as a sink for fixed N ('selfish symbiont'), while the host may not take up much N, if at all, and is rather expected to show net releases of N from its tissues (Pogoreutz et al., 2017; Rädecker et al., 2015). Nevertheless, in this study, the symbionts and host were not separated during sample processing. Thus, it is difficult to discern if remaining symbionts in the tissues could have driven the $\delta^{15}N$ signature or whether the coral host assimilated this isotope via translocation or a different mechanism such as, e.g. direct symbiont assimilation (Wiedenmann et al. 2023). Moreover, the bleached corals also displayed an overall shift towards heterotrophy supported by $\delta^{13}C$ and $\delta^{15}N$ metrics, higher mean %C and %N contents and lower C:N ratios (C Ferrier-Pagès, 2019; Conti-Jerpe et al., 2022). This indicates a higher reliance on external C and N sources differing from those of the non-bleached corals (Rodrigues & Grottoli, 2007; Rossi et al., 2020; Schoepf et al., 2013), which effectively relied more on photosynthesis. The relevance of autotrophy and heterotrophy to the coral energy budget has been scarcely studied in octocorals (Pupier et al., 2019, 2021; Rossi et al., 2020), while there is an ongoing debate on the trophic classification of xeniids. Some authors classify them as mainly autotrophic (Gohar, 1940; Schlichter et al., 1983), and others suggest a more heterotrophic character (Al-Sofyani & Niaz, 2007; Lewis, 1982). Here, *X. umbellata* displayed trophic plasticity, potentially enabling it to benefit of previously unavailable resources (Ferrier-Pagès, 2019; Sturaro et al., 2021), in which case, may confer a level of competitive advantage to this coral in the context of better coping with this stressor and outcompeting more sensitive hard coral species at least at intermediate temperatures, and under scenarios where selected forms of pollution and eutrophication may represent additional sources of available organic carbon (Fabricius et al., 2013; Baum et al., 2016). For instance, although we found no significant differences among treatments for $\delta^{13}C$ and $\delta^{15}N$, their range for the heat-treated corals indicated trophic status disruptions with enhanced coverage of their potential trophic niche (Layman et al., 2007). Thus, energy reserve sustenance via OC uptakes might have been possible for

the bleached coral population (Fabricius, 2011; Schoepf et al., 2013). However, its benefits were limited.

X. umbellata is sensitive to prolonged warming

Overall, prolonged warming impacted almost every metric assessed. Constantly elevated temperatures had notably adverse effects at the highest temperature (i.e., 32 °C), with observed coral mortality exclusively within this treatment and a significant number of individuals sustaining heavy tissue shading without bleaching. The most adverse effects included significantly reduced O_2 metabolism and pulsation rates, the highest bleaching prevalence among surviving colonies, and marked shifts in the coral C fluxes from uptake to net releases. In addition, *X. umbellata* photobiology, reflected by its net and gross photosynthetic performance and its P:R and light R:P ratios, was remarkably impacted where most corals were either within or under the compensation range (Baker et al., 2015; Wilkinson, 1983), with low respiration rates indicating metabolic depression (Comeau et al., 2017; Pan et al., 2015). Our findings align with classic studies for *Xenia* sp., such as those by, e.g., Sammarco & Strychar (2013) and Strychar et al. (2005), and with our previous *X. umbellata* studies (Bednarz et al., 2012; Simancas-Giraldo et al., 2021; Vollstedt et al., 2020; Xiang et al., 2021), but contrast with studies that did not find temperature effects in some of their assessed metrics (Klinke et al., 2022; Mezger et al., 2022; Thobor et al., 2022). Nonetheless, the prolonged temperature treatments explained the coral responses observed in our study due to enhanced heat accumulation, given longer exposure to warming. Reductions in the coral energetic budget can typically affect a wide range of its life and maintenance functions, including, e.g., reproduction (Enochs & Glynn, 2017; Ziegler et al., 2019), growth (Anthony & Fabricius, 2000; Anthony et al., 2009), and pulsation behaviour (Vollstedt et al., 2020). Here, polyp pulsation demonstrated its potential as a sublethal indicator of coral health status over time, where a prolonged temperature of 32 °C decreased it steadily before hampering it completely. Despite being energetically costly (Kremien et al., 2013), pulsation enhances photosynthesis via remotion of boundary layer

O_2 accumulation and thus increases RuBisCo enzymatic activity and effective photosynthetic surface (Kremien et al., 2013; Mass et al., 2010; Wild & Naumann, 2013). Halted pulsation can reduce photosynthetic efficiency and cause negative feedback on the coral's energetic budget. On the other hand, prolonged exposure to the highest temperature also affected *X. umbellata* C and N metabolism. Shifts in the TOC and $CHAR_{TOC}$ fluxes, which aligned with the changes in %C and %N contents, indicated net C losses relative to the respiratory demand and shifts in the heterotrophic organic C contribution to the energy budget (Levas et al., 2015; Rossi et al., 2020). As a note of caution, we suggest carefully interpreting these results, given the high variances observed for the OC fluxes. Nevertheless, the overall TOC and $CHAR_{TOC}$ metrics indicated minor heterotrophic contributions to the host (Grottoli et al., 2006; Levas et al., 2015, 2016), with the heterotrophic intake being insufficient to counter harmful effects of warming (Grottoli et al., 2006). Other studies have also reported high variability in TOC flux responses to temperature (Levas et al., 2015; Mezger et al., 2022). However, an additional interpretation may indicate health-status-specific responses (Dobson et al., 2021) and substantial intraspecific variability. Further, CZAR remained consistently high for the corals in this treatment, besides the increase in %C. Yet, most corals failed to use this available energy even if in a surplus (Grottoli et al., 2006; Muscatine, 1981), and thus, we presume them likely starved due to reduced photoassimilate translocation (Fine et al., 2002), and therefore unable to meet their metabolic demands.

Exposure length exacerbated warming effects

Physiological impacts of temperature amplified by exposure have been abundantly recorded for hard corals (Hughes et al., 2018; Lough et al., 2018; Ziegler et al., 2019) and to a lesser extent for soft coral species (Lasker et al., 2020). For *X. umbellata*, the period under heat stress determined coral response, where its extent and synergies intensified the negative impacts of warming (Rogers et al., 2009). Specifically, temperature effects evolved faster under 32 °C, and the longitudinal effect of time and its interaction with temperature

explained almost every metric assessed, supporting coral health status worsening and sensitivity thresholds decreasing progressively (Rädecker et al., 2021; Schoepf et al., 2015). In addition, bleaching incidence occurred beyond the week of exposure, underlining that bleaching can take time, as also described for other hard coral species (Rädecker et al., 2021). These findings were consistent with cumulative heat stress effects, which can also determine individual coral bleaching thresholds (Berkelmans, 2002). Moreover, prolonged warming of an already weakened coral due to bleaching worsened its health status. However, corals under 29 °C coped better, a feature that might allow a chance for potential acclimation since exposed corals may adapt to higher water temperatures over time through, e.g., genetic or ecophysiological mechanisms (Bay & Palumbi, 2014; Voolstra et al., 2021; Weis, 2010).

Bleaching susceptibility and survival: Projecting the future via predictive approaches

To study *X. umbellata* survivorship, we evaluated to which extent prolonged warming impacted its survival probabilities and mortality risk. On a mechanistic level, survival can be described by the functional link between the coral's physiological condition and observed cumulative mortality (Anthony et al., 2009). However, caution should be exercised when interpreting the cumulative mortality observed here at 32 °C, as it displayed a faster progression during the first trial, potentially influenced by improved handling experience. Survival analysis can comprehensively explore projected survivorship, considering event timings, censoring, and covariates (Yap, 2004). Conversely, Bayesian statics constitute a powerful approach to further study bleaching prevalence, allowing inferences about realisable values not yet observed, or in other words, prediction of future observations given the actual bleaching observed (Billheimer, 2019). Regarding survivorship, 29 °C temperature prolonged over 25 days (11 DHW) did not challenge *X. umbellata* survival probabilities, while at 32 °C survival rate decreased. Nevertheless, this coral showed a median lifespan of 20 days at 32 °C, with a 25 % probability of surviving past 22 DHW before experiencing increased mortality risk. Overall survival is determined by

temperature exceeding the organism's thermal capacity, which can depend on the maximum temperatures it has previously experienced, among other factors (Arroyo et al., 2022; Hughes et al., 2019). This coral species displayed notable intraspecific variability concerning survivorship and bleaching prevalence. Considering solely survival, it remained within acceptable margins (≥ 75 %) at 18 DHW. Although the significant reduction in projected survivorship observed at 22 DHW underlines the sensitivity of *X. umbellata* to such warming scenarios, it also unveiled the threshold to which physiological acclimation may still have an acting window (Weis, 2010). Yet, our Bayesian predictive approach revealed that both prolonged warming scenarios, 11 DHW and 22 DHW, had an above 95 % chance of impacting these corals negatively, increasing the likelihood of bleaching in the long run, albeit to varying degrees. Within the time frame of our experiments, *X. umbellata* thermal limits related closely to the maximum temperatures that these species can experience in their original habitat, the Red Sea, and to the proposed threshold (> 8 DHW) for bleaching and mortality occurrence for hard coral species residing within this and other habitats (Eladawy et al., 2022; Kayanne 2017; Osman et al., 2018; Heron et al., 2016). For instance, the Red Sea represents a latitudinal temperature gradient transitioning from moderately warm in the north to very warm (28 to 32 °C) in the south, where heat anomalies are consistently encountered over varied timeframes (Chaidez et al., 2017; Osman et al., 2018). These conditions may favour high phenotypic plasticity and heat tolerance through selection mechanisms (Schoepf et al., 2015; Sturaro et al., 2021; Voolstra et al., 2021). Whether thermal response differences observed for this species are genetically fixed or a consequence of acclimation (Bay & Palumbi, 2014; Cunning et al., 2016) remains to be investigated. However, our findings for survival and projected bleaching yield additional evidence of disparate heat stress responses even among corals within the same populations, ultimately suggesting the coexistence of distinct response mechanisms (Guest et al., 2012; Núñez-Pons et al., 2023; Voolstra et al., 2021), especially for the thermal-tolerant portion of the population, wherein a number of individuals may remain completely unaffected. Such coral populations displaying variable intraspecific

susceptibilities may hold a remarkable potential for further thermal adaptation and higher resilience (Guest et al., 2012; Humanes et al., 2022).

7.6 Outlook

Under current climate change scenarios and in an ever-changing ocean, a holistic understanding of warming effects on soft corals is essential, given our scarce knowledge about this critical functional group in coral reefs worldwide. Here, *X. umbellata* displayed intraspecific variability in its physiological response, especially at intermediate temperatures, together with adverse responses to prolonged warming over time. These findings not only explain why this species demonstrates higher resistance compared to more sensitive soft and hard coral species in natural environments (Lasker et al., 2020; Steinberg et al., 2022; Ziegler et al., 2019) but also highlight that it is not entirely impervious to rising temperatures. Assorted susceptibility and intraspecific variability in *X. umbellata* may be key to understanding its exceptional adaptative abilities and potential resilience. Notably, xeniids are highly diverse and exhibit considerable physiological and trophic plasticity, along with a range of interesting mechanisms to withstand stress. Recent reports suggest that *X. umbellata* may take advantage of simultaneous local eutrophication, such as dissolved organic carbon (DOC), NO_3^-, or PO_4^{3-}, to withstand thermal stress (Klinke et al., 2022; Mezger et al., 2022; Thobor et al., 2022; Vollstedt et al., 2020; Xiang et al., 2021). With increasing temperatures and more frequent and intense thermal anomalies, effective coral reef management and conservation efforts should prioritise mitigating ocean warming. Nevertheless, this stressor may facilitate the selection of ecophysiological traits, ultimately promoting the competitive advantage of *X. umbellata*, an already formidable competitor within tropical coral reefs, that may pose a significant threat to sensitive coral species in the face of warming oceans.

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Chapter 8

General Discussion

8 General Discussion

This thesis deepens our insights on the ecophysiological responses of soft corals as potential winners of climate change, to ocean acidification and warming as global factors, organic eutrophication, or DOC as a selected local factor, and warming and DOC as likely interacting factors. Together these factors sum up to the current coral reef's crisis and cause the contrasting response patterns observed in soft corals that motivated this research. Thus, within this thesis work, we investigated in further detail if the selected soft corals may have the ability to withstand the mentioned factors, the mechanisms and traits that may allow them to do so, how these factors may affect their prospective resistance and tolerance when present, and how they may ultimately drive their potential to thrive in the oceans of the future.

8.1 Key Findings

Under simulated ocean acidification scenarios expected for the year 2100:

1. The soft corals genera *Xenia*, *Pinnigorgia*, *Plexaurella*, and *Sinularia* are all remarkably tolerant to ocean acidification. Their coping capacity varies as a function of growth morphology and genus-specific physiological features **(Chapter 2).**

When exposed to simulated eutrophication as DOC and stepwise increased warming:

2. The bleaching susceptibility, oxygen metabolism and photosynthetic metrics of the soft coral *Xenia umbellata* are insensitive to DOC enrichment and while this coral is considerably resistant to warming, thermal stress may eventually affect its photosynthesis and respiration response **(Chapter 3)**.

3. The soft coral *X. umbellata* is highly resistant to the combination of DOC and warming in terms of its pulsation and growth, while DOC may aid to increase its resistance to warming **(Chapter 4)**.

4. The gorgonacean *Pinnigorgia flava* is insensitive to the individual effect of DOC enrichment, but not to warming and the combination of both factors in terms of its photophysiological responses and growth **(Chapter 5)**.

5. Under high DOC and concomitant warming, *X. umbellata* and *P. flava* show differing bacterial community dynamics suggesting passive regulation of the N cycling microbiota which overall supports the coral holobiont functioning **(Chapter 6)**.

Under prolonged exposure to constant warming:

6. *X. umbellata*, shows high intraspecific variability and potential for tolerance specially at intermediate temperatures. Although mid-term exposure to high temperatures exacerbates its negative effect due to cumulative stress **(Chapter 7)**.

8.2 Overview

With coral communities worldwide certainly threatened and rapidly changing through shifts in their species composition (Alvarez-Filip et al., 2013; Tkachenko, Dung & Ha, 2022), better fitted species to the changing chemistry and temperatures of the seawater (e.g. robust soft coral species), may become dominant, implying important consequences for functional and structural shaping of the reefs as well as their present and future complexity, and ecosystem integrity (Wilkinson, 2002; Alvarez-Filip et al., 2013). Current projections suggest that the dominance of coral communities in tropical reefs may shift from reef-building hard corals to soft corals, particularly under ocean acidification and warming conditions predicted for the end of the century (Alvarez-Filip et al., 2013; Flato et al., 2013; Frieler et al., 2013; Inoue et al., 2013; Lasker et al., 2020). Furthermore, besides ocean acidification impacts on water chemistry and warmer temperatures, water quality diminishing can also control the relative abundance and physiology of dominant soft corals, thus also contributing to phase shifts from hard to soft coral dominance (Baum et al., 2016). Soft corals are one of the several taxa that have been overlooked for decades in what concerns analyses of coral reef community

dynamics or their physiological responses to stress (Pupier et al., 2019; Lasker et al., 2020). This has only resulted in a strong lack of resolution in the soft corals group at many levels, rendering our comprehension of how present and future reefs will look like rather diffuse. Thus, the effects of the mentioned global and local factors that are ultimately threatening coral reefs, on this specific group, are a pressing matter for our understanding of the ecological services and landscapes that coral reefs will provide in the incoming decades.

In line with these antecedents, this thesis and its manuscripts contributed to enhance our comprehension of the effects of ocean acidification, warming and DOC as global and local factors, respectively. In detail, our research focused on warming and acidification single exposures provided mechanistic understanding of how these factors drive soft coral physiological responses **(Chapters 2 and 7)**, while our studies combining warming and DOC provided further insights on the synergistic effects of these combined factors on soft corals, addressing every component of the holobiont, including the host, its associated Symbiodiniaceae and denitrifying microbiota **(Chapters 3 to 6)**. In addition, the approach implemented in the studies included in this thesis comprised a series of prolonged to mid-term manipulative aquaria experiments performed under controlled conditions at the aquaria research facilities of the department of Marine Ecology at the University of Bremen, at the UFT, and at the Ocean 2100 facility in the Justus Liebig University, within the department of Animal Ecology and Systematics. This specific approach enabled us to assess the triggers and a representative range of soft coral responses to these factors in isolation from unwanted sources of variation, increasing our resolution to estimate these factors effects in the diverse metrics evaluated within our studies.

Further, this thesis work also contributed to increase the literature corpus on soft corals ecophysiology and their microbiome responses in the context of climate change and its byproduct factors, which is otherwise scarce in comparison to the more abundant corpus of studies for hard corals. Moreover, the outputs of this thesis refined our understanding of the performance of the selected soft coral species studied under stress covering a diverse collection of factors and a broad phylogenetic range particularly in

the specific case of our acidification experiments **(Chapter 2)**. This thesis also addressed how the imposed DOC treatments affected soft corals and their tolerance and resistance at the organism level, with detailed insights into their ability and mechanisms to cope with both DOC and thermal stress **(Chapter 3 to 6)** and warming alone **(Chapter 7)**. Overall, the research presented hereby confirmed that in most case scenarios soft corals, and in particular *X. umbellata* showed an outstanding potential to withstand both local and global factors, supporting their claim as potential winners under climate change. This claim may remain true specially concerning acidification conditions and organic eutrophication combined with warming scenarios **(Chapter 2 to 6)**, and unless factors such as global warming are sustained over specifically prolonged periods, as observed during our study of constant exposures to increased thermal stress **(Chapter 7)**. The findings of this thesis can be extrapolated to the ecological context where they aligned to the already mentioned findings suggesting that under ocean acidification (Alvarez-Filip et al., 2013; Flato et al., 2013; Inoue et al., 2013), warming and eutrophication conditions (Fabricius et al., 2013; Baum et al., 2016; Pogoreutz et al., 2017; Sánchez, 2017) phase-shifts may eventually favor soft corals dominance over hard coral communities (Frieler et al., 2013; Edmunds et al., 2014; Lasker et al., 2020), transforming deeply these ecosystems.

8.3 Octocorals responses to ocean acidification

In the face of climate change, considerable efforts are being dedicated to elucidating the processes and the biological and ecological traits of resistant corals which may influence shifts in the present and future coral community structures (Edmunds et al., 2014). Under ocean acidification scenarios and the specific context of soft corals more comprehensive studies covering varied taxa are still missing. Thus, in **Chapter 2** of this thesis we explored a varied collection of octocoral genera including *Pinnigorgia*, *Plexaurella*, *Sinularia* and *Xenia*. Overall, we found that these soft corals were not jeopardized if at all by this factor in terms of the diverse physiological metrics we evaluated through our experiments and after being exposed to three months of ocean acidification treatment (pH 7.78). Within the framework of ocean acidification, most studies, mainly focused on calcifying coral species, have revealed detrimental impacts

on their growth, calcification rates, and survival (e.g., Smith & Roth, 1979; Smith & Buddemeier, 1992; Marubini & Atkinson, 1999) and numerous studies conducted over the past decade using a range of approaches both in the laboratory and in natural environments at different scales and durations have mostly confirmed these findings (Kline et al., 2015), although they have also highlighted that coral responses are more nuanced and variable than indicated by early ocean acidification experiments (e.g., Ries, Cohen & McCorkle, 2009; McCulloch et al., 2012).

Our findings from **Chapter 2** showed that the evaluated octocorals are remarkably tolerant when subjected to this global factor and when compared to most hard coral groups under acidified scenarios (as it also the case when comparing to hard coral responses in the context of warming in **Chapter 7** and warming and concomitant DOC as global and local factors in **Chapters 3 to 6**). Nevertheless, ocean acidification impacted the growth of the branching gorgonaceans under our treatments as evidenced for *P. flava* and as also observed for other hard coral branching species, although the gorgonaceans in our experiments still withstood better the negative effects of ocean acidification. For instance, research carried out on coral reefs naturally located within reach of CO_2 vents or natural sources of carbon dioxide occurring in locations such as Papua New Guinea (Fabricius et al., 2011) or Iwotorishima Island in Japan (Inoue et al., 2013) showed in the first case, that massive hard coral species might be able to resist up to a maximum of pH 7.7, while in contrast, branched coral species were prone to be particularly sensitive (Fabricius et al., 2011). On the other hand, for the second case, soft coral species present along an acidification gradient did tolerate well all conditions over the transects that reached their maximum close to the acidification source, while hard corals didn't (Inoue et al., 2013). In addition, where calcifying species such as hard corals may be considerably negatively affected as already stated (Kleypas et al., 1999; Venn et al., 2011; DeCarlo et al., 2015), soft coral species, such as *Eunicea fusca* and *E. flexuosa*, can withstand acidified conditions, in terms of maintaining continuous calcification and even linear extension, nevertheless at slower rates (Gómez et al., 2014; Enochs et al., 2016), which again aligned to our results for the gorgonacean corals evaluated in **Chapter 2**. On the other hand, another case of study is the soft coral *Heteroxenia fuscescens*, which pulsing behaviour has been studied recently and found by Kremien et

al. (2013) to benefit this octocoral by enhancing its physiological processes (as also confirmed for *X. umbellata* in our study combining DOC and warming in **Chapter 4**). Decreased pH conditions on chosen biological features of *H. fuscescens* had shown marked discrepancies between different studies (Sprung & Delbeek, 1997; Gabay, Benayahu & Fine, 2013), where Sprung & Delbeek (1997) described halted pulsation under acidified conditions contrasting to the findings by Gabay et al. (2013), who reported no changes in pulsing behaviour or other features, including, e.g. the number of zooxanthellae per tissue protein when these corals were exposed to acidified conditions. Our study of the soft coral *X. umbellata* showed reduced pulsation under ocean acidification, however the pulsation behaviour remained within a healthy range together with its photosynthetical parameters **(Chapter 2)**, implying that although this coral is not impervious to this factor, it can still be considered as a robust soft coral. Moreover, under ocean acidification, soft corals coping performance varied depending on genus-specific physiological features **(Chapter 2)**. A finding that we also evidenced when comparing the physiological responses towards DOC and concomitant warming observed between *X. umbellata* in **Chapters 3 and 4** to those observed for *P. flava*, specifically in **Chapter 5**. The effects of all these factors on the evaluated soft corals and their coping mechanisms seemed thus to be associated to an extend to the corals' morphology and growth mode, as also noted when comparing our findings from **Chapters 2 to 4**, with those of **Chapter 5**, and to specific features such as e.g., *X. umbellata*'s pulsation behavior, where all these traits taken together influenced soft corals tolerance and resistance. Overall, the findings of **Chapter 2** dedicated to the context of ocean acidification support soft corals as winners and align to what has been suggested by other authors such as Fabricius et al. (2011) and Lasker et al. (2020), where it appears that if there are winners and losers in a more acidic world, the reef composition, species dominance and ecology will profoundly change towards a decrease in coral biodiversity and an eventual increase in soft corals and macroalgal cover. Further, our findings for soft corals' resistance under ocean acidification **(Chapter 2)** are not limited to this global factor, as already mentioned, but also to the additional factors studied during this thesis work, i.e., DOC and warming whenever acting individually or in combination **(Chapter 3 to 7)**.

8.4 Effects of single and combined DOC and warming on soft corals

Eutrophication has strongly affected many coral reefs worldwide, with some of the best examples of reef degradation found in tropical systems in the Caribbean and temperate regions (Hughes, 1994; Edmunds, 2002) or in other reefs e.g. from in the Red Sea to those found off Jakarta, the Polynesian islands, or the biosphere reserves in the Philippines due to poor land management on highly populated neighbouring islands (Wilkinson, 2002; Jessen et al., 2013; Baum et al., 2015). Nevertheless, this factor's effects can also extend to relatively well-managed areas such as the reefs in eastern Australia, where, despite comparatively well-run locations being the standard, nutrient run-off still represents a significant challenge (Leon & Warnken, 2008). Soft corals have portrait higher tolerance to eutrophication in field as reported in studies such as those performed by Baum et al. (2015) and (2016), however no previous studies to date had approached the effects of organic eutrophication or DOC as a single factor or its combination with thermal stress in soft corals. Within this thesis work, we addressed the combined and single effect of these local and global factors on soft corals for the first time, with **Chapters 3 to 6** mainly dedicated to both, while in **Chapter 7** we provided further insights and details in the context of soft coral responses to single mid-term warming. Thus, within this collection of studies, we examined the impact of individual DOC treatments and the subsequent introduction of warming scenarios in a two-phase experimental design, over 45 days of exposure for the case of DOC and warming as combined factors **(Chapters 3 to 6)**, while for the case of mid-term warming single exposures, we performed two iterations of aquaria experiments over 25 days each and under constant increased temperatures **(Chapter 7)**. Our findings showed that the soft coral *X. umbellata* **(Chapters 3, 4, 6 and 7)** demonstrated high tolerance to both factors while *P. flava* was relatively more affected by warming alone and the synergistic interaction between DOC and warming **(Chapters 5 and 6)**. In general, regarding warming, the results from **Chapters 3 to 7** aligned to other findings suggesting that octocorals seem to have higher temperature tolerances together with less bleaching susceptibility and mortality (Prada, Weil & Yoshioka, 2010; McCauley & Goulet, 2019; Coffroth et al., 2023), despite they can bleach specially under increased thermal stress **(Chapters 5, 6 and 7)** and be even more affected whenever this factor is particularly

sustained overtime **(Chapter 7)**. Moreover, we found that high intraspecific variability **(Chapter 7)** together with other mechanisms that contribute to lessen the negative effects of stress such as e.g., enhanced mixotrophy or transition to heterotrophy **(Chapters 3 and 5)**, or pulsation **(Chapter 4)** are key to support these corals endurance when compared to more sensitive tropical corals (see comparative **Figure 7.11,** in **Chapter 7**). Likewise, our results from **Chapters 3 to 7** are compatible with the outstanding tolerance that octocorals can show as well when exposed to thermal stress in field scenarios as illustrated in the study by Prada et al. (2010), where surveys in Costa Rica revealed that even though some octocoral groups were undergoing bleaching, these were significantly less affected than a percentage of the hard coral cover component within the surveyed reefs.

Further, in hard corals, DOC eutrophication can cause bleaching and impair photosynthetic performance as seen for the coral *Pocillopora verrucosa,* suggesting that under both factors' corals may be negatively affected due to synergistic effects of both factors (Pogoreutz et al., 2017). When comparing our findings for the soft corals *X. umbellata* **(Chapters 3, 4 and 6)** and *P. flava* **(Chapters 5 and 6)** with previous results for hard corals, our studies confirmed that both soft corals may gain competitive advantages over hard corals particularly under the projected thermal stress increases, given future ocean warming, and organic eutrophication if management is not implemented to reduce these factors impacts at regional and global scales (Shaver, Burkepile & Silliman, 2018; Knowlton, 2021). On the other hand, given soft corals have been generally overlooked during regular monitoring and surveys and there is still much data resolution and targeted experiments missing before confirming that the effects of these factors on this coral group are not being underestimated (Lasker et al., 2020; Coffroth et al., 2023), our findings in **Chapters 3 to 6** specifically contribute to the available literature corpus on soft corals on this context and supports the notion that certain groups of soft corals (specially *X. umbellata* as shown in **Chapters 3, 4 and 6**) can endure and even benefit from eutrophicated conditions, as also shown by Baum et al. (2016) in their study in Jakarta Bay, where they found higher dominance in soft coral cover even under highly eutrophicated conditions, aligning with other suggestions by Fabricius et al. (2011) and (2013), who proposed potential beneficial effects for this

group of corals under such environments. Nevertheless, in a detailed overview, our studies on *P. flava* also revealed potential negative effects on this coral supported by diminished physiological status and observed mild bleaching **(Chapter 5 and 6)**, together with the less flexible behaviour of its denitrifying bacterial community when compared to *X. umbellata* **(Chapter 6)**, indicating species specific responses in terms of their bacterial communities dominance and behaviour ultimately translated to differentiated coral health status **(Chapter 6)**. Thus, although this gorgonacean still results more robust than thermally sensitive hard corals, it seems to show a more intermediate competitive capacity when contrasted with *X. umbellata*.

Furthermore, under the framework of DOC and overall, for ocean warming, coral bleaching and mortality represent overarching topics of discussion. Although *X. umbellata* **(Chapter 7)** and *P. flava* can bleach (as observed in **Chapters 5 and 6**), *X. umbellata* represents a highly resilient coral regarding this aspect (as actually shown for every global and local factor evaluated in **Chapters 2 to 4, 6 and 7**); while *P. flava* may be less resistant, although still holding certain competitive advantage **(Chapters 5 and 6)**. In general, bleaching can affect a reef of several square kilometres within a few hours (Allemand & Osborn, 2019), making this phenomenon evident to the naked eye and intrinsically dramatic when unfolded to its fullest. Hard coral colonies of hundreds of years old may die within months or days (Yu et al., 2006; Hughes et al., 2018a; Thompson, 2022), showing the exceptional character of this phenomenon as well. For octocorals, bleaching may remain cryptic, or lead to mortality and complete coral dissolution as observed in **Chapter 7**, making this phenomenon relatively difficult to pinpoint and trace overtime in field conditions without implementing focused online monitoring. On this regard, our experimental approaches provided us a particular advantage to overcome this challenge and to gain insights on octocoral bleaching responses for every factor tested from **Chapter 2 to 7**, where we found the collection of octocoral genera assessed to be remarkably resistant to bleaching. Further, even though the first reports of coral bleaching date to the turn of the 20th century, it wasn't until the eighties that we started to see this phenomenon become recurrent (Allemand & Osborn, 2019) and by the decade of the nineties, during 1997 and 1998, mass bleaching events affected coral reefs in almost every part of the world, causing mortalities of 16 %

of reef-building hard corals (Wilkinson, 2004; Frieler et al., 2013). Additional key examples include mortality occurrences as a result of extreme bleaching events, as observed e.g. in Kiribati, where death rates of at least 80 % were observed (Ezzat & Courtial, 2016), or some parts of the Great Barrier Reef, which though far from any direct human impact, bleached by 99 %, with resulting mortality rounding 30 % and up to 90 % in some specific areas such as the Lizard Island, where an estimated 29 % of shallow-water coral cover was lost during this event (Hughes, Kerry & Simpson, 2018; Hughes et al., 2018b). Through our research work, bleaching incidence and mortality remained low or inexistent **(Chapters 2 to 6)**, again, suggesting dire scenarios for hard coral future and present persistence when compared to octocorals subjected to stress. However, under mid-term prolonged warming, projected survival for *X. umbellata* resulted reduced (particularly at highest temperatures) and bleaching likelihood increased **(Chapter 7)**, showing that soft corals are not completely invulnerable. A dramatic example in alignment with our findings are recent observations after the latest warmer temperatures affecting coral reefs in Florida (USA), during 2023, which resulted to be the highest recorded in decades according to the latest National Oceanic and Atmospheric Administration (NOAA) report from 2023. During this warming event, bleaching and high rates of octocoral mortality were observed for diverse groups present within various of these reefs, with many of them exhibiting states beyond possible recovery (*Person. Comm.* Ian Enochs). Taken together, the findings of this thesis work and the evidence to date suggests that the full story of coral future persistence is not completely settled, and winners may turn into losers or remain as winners mainly depending on the perfect confluence of specific scenarios and their intrinsic capabilities to face stress, as we are already witnessing in many tropical reefs (Grottoli et al., 2014; Slattery, Pankey & Lesser, 2019). Thus, this thesis work also highlights the importance of interpretating our findings in the context of coral long-term persistence, where even though corals can overall recover following stress periods and large-scale bleaching events, the ecosystem may take up to two decades before reaching their pre-bleaching form or at least a new favourable stable state under certain circumstances (Baker, Glynn & Riegl, 2008), ultimately favouring corals which portray higher tolerances and resilience.

8.5 Synoptic answers to the proposed research questions

Summarizing our research findings, we provide below the synoptic answers to the research questions that we solved through this thesis work, together with the answers to their corresponding proposed hypothesis and the overview of our key findings depicted in **Figure 8.1**.

1. How do different soft coral genera respond comparatively to simulated ocean acidification scenarios, and to what extent do their physiological responses suggest potential for tolerance or resistance?

Hypothesis 1: *Soft corals will be able to withstand ocean acidification. However, their overall capacity for tolerance and resistance will vary as a function of their intrinsic biological and physiological features.*

> ***Yes, soft corals are highly tolerant to ocean acidification although their responses varied across genera.***

In **Chapter 2** of this thesis, we further evaluated the physiological responses of four different genera of soft corals towards ocean acidification. Overall, the four genera assessed including the Gorgonacean corals *Pinnigorgia* and *Plexaurella*, and the Malacalcyonaceans *Xenia* and *Sinularia* were remarkably tolerant when exposed to the three-month ocean acidification treatments simulating the RCP8.5 scenario (pH 7.78 pCO_2 > 1000 ppm), expected for the year 2100. Our experimental manipulation provided insights into these soft corals' taxa comparative response towards acidification where we found that their potential tolerance may give them an additional advantage over more susceptible hard and soft corals under climate change. In addition, the coping capacity of these four genera was shown to be species-specific, and varied as a function of their morphology, growth mode and physiological features, where the evaluated malacalcyonaceans seemed less sensitive than gorgonaceans, although showing mild affectation of metrics such as pulsation for *X. umbellata*, which seemed prone to recovery overtime after an initial negative response. Moreover, the gorgonian branching corals *Pinnigorgia* and *Plexaurella* seemed negative effected by ocean acidification in

terms of linear growth and diminished branching patterns, despite still retaining growth, that resulted particularly marked for the genus *Pinnigorgia*, aligning with previous findings for the gorgonian *E. fusca* which also retained positive linear extension despite being affected by ocean acidification (Gómez et al., 2014). The current trends showing increasing abundance of soft corals worldwide may reflect the appearance of communities composed of new combinations of taxa reflecting the prevailing physical and chemical conditions (Lasker et al., 2020). Since soft corals do not produce massive calcareous skeletons and may be able to better exploit additional available niches and space given their three-dimensional growth modes (specially gorgonians), it is reasonable to postulate that they will be less affected by ocean acidification than hard corals (Fabricius et al., 2011; Tsounis & Edmunds, 2017; Lasker et al., 2020; Coffroth et al., 2023). Nevertheless, their morphological and physiological features vary significantly across taxa given the high biodiversity that this coral group comprises (Bayer, 1961; McFadden, Sánchez & France, 2010; Fabricius, 2011), and thus, so too, should their sensitivity and susceptibility (Bramanti et al., 2013; Gómez et al., 2014; Enochs et al., 2016; Tsounis & Edmunds, 2017).

2. Does organic eutrophication as dissolved organic carbon (DOC) affect soft corals, or are they able to cope with this factor? Does warming and its potential interaction with DOC influence soft corals' responses?

Hypothesis 2: We hypothesised that the individual and combined effects of DOC enrichment and high temperatures would impact soft corals, influencing their health status by altering their ecophysiological parameters and promoting diazotrophs' growth and activity.

> ***Yes, however the observed effects were species specific and varied depending on a species-factor combination basis.***

Organic eutrophication as DOC is a highly under-investigated stress factor, although commonly occurring in coastal areas. From the existing literature, there was little to no evidence of soft coral responses to such factors, and it was unclear if soft corals' physiology will exhibit negative responses as observed for hard corals towards increased

DOC (Haas, Al-Zibdah & Wild, 2009; Pogoreutz et al., 2017), or warming (Al-Sofyani & Floos, 2013; Lyndby et al., 2018); or if they show elevated or reduced thermal tolerance when simultaneously exposed to combined factors (Fabricius et al., 2013; Pogoreutz et al., 2017). In this thesis work, the effects of organic eutrophication as DOC enrichment, warming, and the combination of both factors on the physiological responses of the Malacalcyonacean *X. umbellata* **(Chapters 3 and 4)** and the Gorgonian *P. flava* **(Chapter 5)**, together with their effects on their bacterial communities associated with denitrification processes **(Chapter 6)** where comprehensively explored, implementing both factors as single and combined treatments and following a regression-based experimental design testing multiple exposure levels. Under simulated eutrophication as DOC and stepwise increased warming, the bleaching susceptibility, net and gross photosynthesis, respiration, and the P:R ratio of the soft coral *X. umbellata* were completely insensitive to DOC organic enrichment, even at extremely high DOC concentrations. Moreover, the detrimental response of *X. umbellata* to temperature stress, however mild, demonstrated that this coral is considerably resistant to this factor. Although higher temperatures may negatively impact its photosynthesis and respiration response **(Chapter 3).** In addition, the soft coral *X. umbellata* showed high resistance regarding the combination of organic eutrophication and warming in terms of its pulsation and growth, while also demonstrating the ability to employ organic eutrophication at specific concentrations and up to a certain threshold, as additional energy source in order to potentially increase its resistance to ocean warming **(Chapter 4)**. On the other hand, the gorgonacean coral *P. flava* also evaluated under the same treatment settings resulted to be insensitive towards the individual effect of DOC enrichment, but not to warming or the combination of both factors. Hinting that if temperature remains below certain thresholds, this gorgonian species may gain a competitive advantage over coral species reportedly more sensitive to DOC eutrophication. However, under heavily increased warming this octocoral will be most probably negatively affected **(Chapter 5).** Finally, although soft corals can exhibit some of the highest denitrification rates among reef organisms and substrates, the drivers of their denitrifiers' abundance and community composition are unknown, even though these symbionts may be essential in determining coral health status. When exposed to organic carbon eutrophication as DOC and concomitant warming, the soft corals *X.*

umbellata and *P. flava* showed significant differences between their denitrifying bacterial community in terms of their dominance and dynamics. These were directly linked to Nitrogen availability and reflected the nutritional status of the coral holobiont, also suggesting a passive regulation of Nitrogen cycling microbes, which could help stabilize nutrient limitation in the coral-algal symbiosis thereby supporting holobiont functioning under climate change scenarios **(Chapter 6)**. In the literature, it has been thoroughly reported that external nutrient availability, together with internal nutrient metabolism, can underpin the thermal tolerance of hard corals (Morris et al., 2019), with increased DOC causing similar bleaching to the one observed in thermally stressed corals, and stimulated Nitrogen fixation via increased microbial (i.e., diazotroph) activity (Pogoreutz et al., 2017, Rädecker et al., 2021). Besides, malacalcyonaceans and gorgonians may show dissimilar responses, with some species negatively affected in terms of their organic matter fluxes and metabolic activity (Bednarz et al., 2012; Baum et al., 2016; McCauley & Goulet, 2019). Altogether, our findings confirmed that the octocorals *X. umbellata* and *P. flava* in our experiments may withstand future scenarios comprising DOC and warming better than many other hard coral species.

3. What are the physiological responses of soft corals to prolonged warming as a single factor under the context of climate change and distinct warming scenarios primarily focusing on the soft coral *X. umbellata* as a model species?

Hypothesis 3: The soft coral *X. umbellata* will be able to overcome the adverse effects of warming as a single factor; however, when prolonged, exposure to thermal stress will cause adverse effects on this soft coral.

> ***Yes, although this soft coral is overall highly resilient to warming in comparison to other soft corals and most hard corals.***

Chapter 7 of this thesis work was dedicated to gaining detailed understanding of the ecophysiological responses of the soft coral *X. umbellata* as a study model to increase our understanding of soft corals and specifically Xeniids' responses to warming. After implementing moderate-duration thermal stress experiments, i.e., at constantly increased temperatures beyond 20 days of exposure, with two simulated warming

scenarios we found that *X. umbellata* corals showed an outstanding potential to endure thermal stress, specially at intermediate temperatures, portraying various features that explain why this coral could be an excellent competitors against hard coral species residing in tropical coral reefs, as it has been observed for related Xeniids with similar biological and physiological features (Ruiz Allais et al., 2014; de Carvalho-Junior et al., 2023). Moreover, only a limited number of past studies have been carried out on xeniids in the context of thermal stress, in comparison to the many focusing on hard corals, or in specific genus such as e.g., Acropora or Pocillopora (Hughes, Kerry & Simpson, 2018; Ziegler et al., 2019). In further detail, the group of Xeniids has shown during assessments by, e.g. Strychar et al. (2004), Netherton et al. (2014) and Parrin et al. (2016), contrasting responses together with very distinctive response mechanisms (Parrin et al., 2016). For instance, Some Xeniid species show high sensibility and bleaching susceptibility (Strychar et al., 2005; Sammarco & Strychar, 2013), while others show a high degree of tolerance to warming, retaining positive photosynthesis despite concomitant bleaching and specialised mechanisms to mitigate warming effects such as e.g. symbiont migration (Netherton et al., 2014; Parrin et al., 2016). Our findings in this chapter allowed us to confirm that *X. umbellata* belongs to the xeniids group that can be considered as particularly resilient, and allowed us to characterize the biological features and physiological responses that can be associated to its resistance and high tolerance, including higher temperatures required to trigger enhanced mortality and bleaching in comparison with most hard corals, and an interesting component of intraspecific variability that may enable *X. umbellata* to further adapt to increased thermal stress in the future, although this global factor has a cumulative effect over time on these corals. Overall, this soft coral and its associated responses to warming, yielded underappreciated insights into the capacities of soft corals to adapt or acclimatise to this global factor.

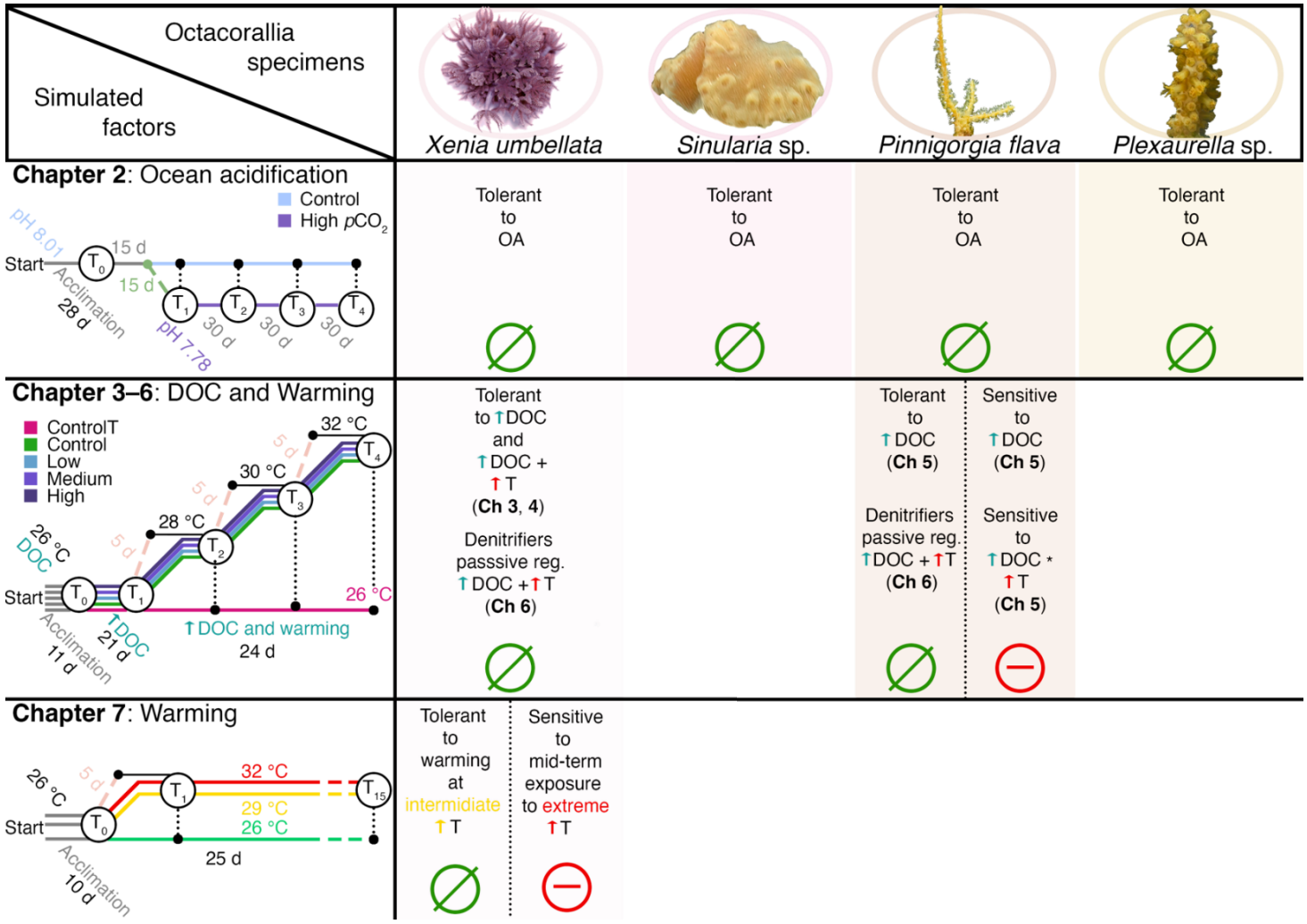

Figure 8.1 | Overview on the physiological response and tolerances of the octocorals assessed through this thesis. The Experimental timelines and designs are shown for both global and local factors evaluated together with octocoral response. The key findings for **Chapters 2 to 7** are thus summarized accordingly.

8.6 Concluding remarks

Overall, coral reefs represent an essential resource not only to virtually every maritime tropical and subtropical country but also to every nation as a collective (Wilkinson, 2002; Knowlton, 2021). Nevertheless, and despite the wide variety of socio-economic and ecological services that these ecosystems provide (Wild et al., 2011; Hein et al., 2019), local efforts at protecting regional coral reefs have failed to reverse their declines over the past 30 years, while global management has also been jeopardised due to a lack of consensus on implementation priorities and their emphases (Hughes et al., 2017). Under the projected climate change scenarios, many coral reefs will change as they respond to the previously mentioned factors rather than disappear completely (Hughes et al., 2003). Thus, given the current climate change situation, double efforts are urgently required to better understand soft corals as a highly pertinent component of coral reefs in transition, emphasizing the relevance that their responses to ocean acidification and other likely interacting global and local factors, such as ocean warming, and organic eutrophication have in shaping the future landscapes of coral reefs worldwide. Moreover, the effects of ocean acidification and ocean warming, and consequences of lower pH values, elevated pCO_2 and increased sea surface temperatures (SSTs) will have diverse implications for future reefs (Riebesell et al., 2011; Allemand & Osborn, 2019; Leung, Russell & Connell, 2019; IPCC, 2021). In the past, most ocean acidification and warming events have occurred over much longer time scales and at slower rates than the current anthropogenic ocean acidification and warming (Hönisch et al., 2012; de Coninck et al., 2018; IPCC, 2018, 2021), which suggests that present events may have more severe consequences than past perturbations according to the available geological record (Hoegh-Guldberg et al., 2007; Pelejero, Calvo & Hoegh-Guldberg, 2010; IPCC, 2018, 2021). Furthermore, the collection of findings from this thesis work contributes to the notion that ocean acidification, warming and organic eutrophication will have a lesser impact on soft corals while heavily impacting the vast majority of the reef-building coral species (Wilkinson, 2004; Prada, Weil & Yoshioka, 2010; Gómez et al., 2014; Pogoreutz et al., 2017; IPCC, 2018, 2021; Allemand & Osborn, 2019; Morris et al., 2019; Ziegler et al., 2019; Lasker et al., 2020). Although, soft corals are not completely impervious to these factors alone or acting synergistically in selected combinations.

Despite direct experiments comparing hard and soft corals under these scenarios are still missing and some reef-building-coral species appear resistant to acidification and warming, e.g., their growth in length doesn't appear reduced when exposed to ocean acidification, most hard corals are nevertheless affected by greater skeletal porosity and are prone to form more fragile branches and show reduced calcification rates (Tambutté et al., 2015; Rippe et al., 2018; Ziegler et al., 2019). Thus, our findings and most hard coral studies support that the later may remain as less competitive given they are highly sensitive specially to eutrophication and ocean warming (Morris et al., 2019; Ziegler et al., 2019), while ocean acidification will as well continue to affect them (Allemand & Osborn, 2019). In the future, tropical coral reefs will change probably favoring the species that may show higher resistance under both, global and local factors, and thus soft corals may remain as winners since they seem to show higher adaptative potential to outcompete more sensitive hard coral species (Alvarez-Filip et al., 2013; Lasker et al., 2020). In addition, thermal anomalies and, thus, coral bleaching events are projected to become more intense and frequent (IPCC, 2021), with increasing odds of bleaching episodes becoming annual under specific scenarios (Frieler et al., 2013). For instance, regarding the incidence of warming temperatures, Hughes et al. (2017) found that the duration in which the SSTs exceed the summer average increased as the temperature rose from 1998 to 2016 (Allemand & Osborn, 2019), while projections for specific global regions, such as the Mediterranean, the Caribbean, or the Red Sea, also suggest trends hinting at SST increases that may lead to important coral reef affectations (Pandolfi et al., 2011; Cornwall et al., 2021; Eladawy et al., 2022). Everything suggests not only a dark future for coral reefs but also that a holistic understanding of these effects, their mechanistic pathways and their potential biological consequences is still pending, in fact for both, hard and soft corals. This recommendation should specially consider hard and soft corals under the context of competition, given the wide inter and intraspecific variability that their responses show towards stress as reportedly found for many coral reef species (Kroeker et al., 2013), together with broader combinations of global and local factors that are yet to be considered. Moreover, this
thesis contributed insights into soft corals' ecophysiological traits and mechanisms driving their tolerance. Advancements in this knowledge area may be key to broaden

our understanding of the potential shifts of coral species dominance that may take place in current and future coral reefs. The findings of this thesis may also contribute to improve the incorporation of soft corals within the scope of priorities for mitigating global and local factors, that may in turn contribute to devising more effective reef management and improved conservation strategies. In the long term, the arms race between those coral species less affected by bleaching and less prone to exacerbated mortality and the more sensitive species inhabiting tropical reefs will be determinant in the establishment and persistence of the diverse coral species that will dominate the future reefs.

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Appendix

Supplementary Information

Supplementary Information | Chapter 2

Supplementary Information S1.

Table S1.1 | Anova *Xenia* pulsation results

Factor		Pulsation rates	
Fixed effects	***df***	**χ^2sq**	***p***
High pCO2	1	36.962	6.8e-15***
Time	3	24.520	1.9e-14***
High pCO2 * Time	3	61.538	2.9e-12***

Table S1.2 | Anova results LMM P:R Ratio analysis for the octocorals Xenia, Sinularia and Plexaurella, Pinnigorgia

Factor		P:R	
Fixed effects	***df***	**F value**	***p***
High pCO_2	1	36.962	6.8e-15***
Genus	3	24.520	1.9e-14***
Time	3	61.538	2.9e-12***

Supplementary Information S2.

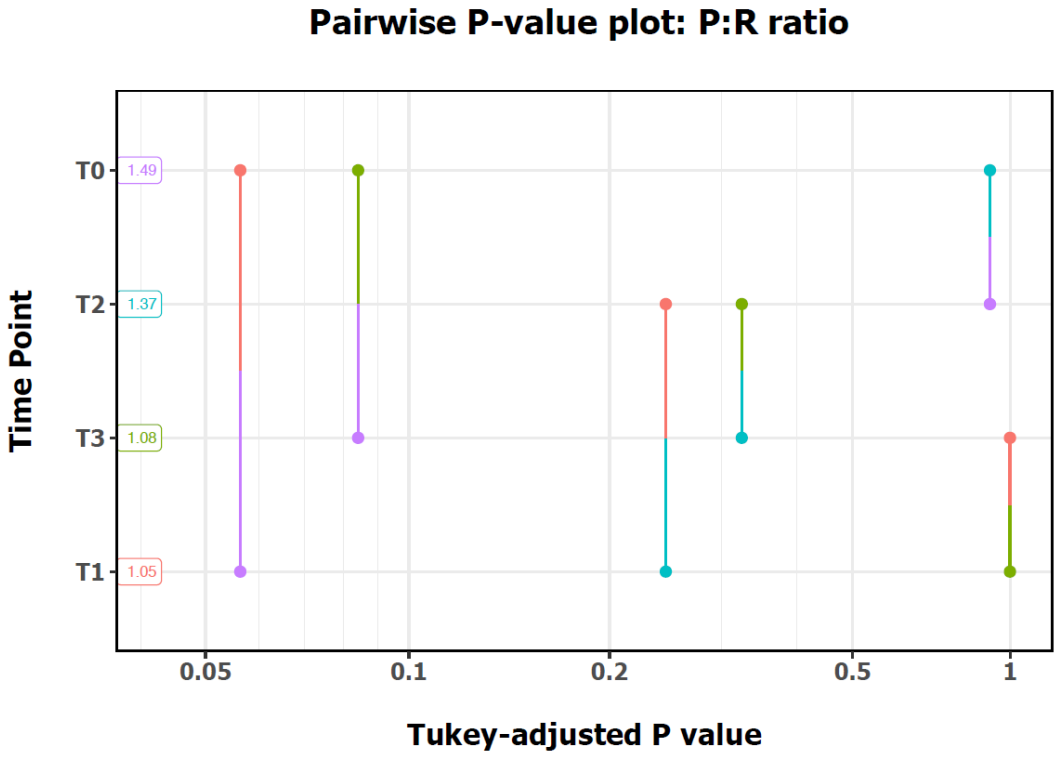


Figure S2.1 | Time factor pairwise comparisons for P:R ratio – Time Points. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

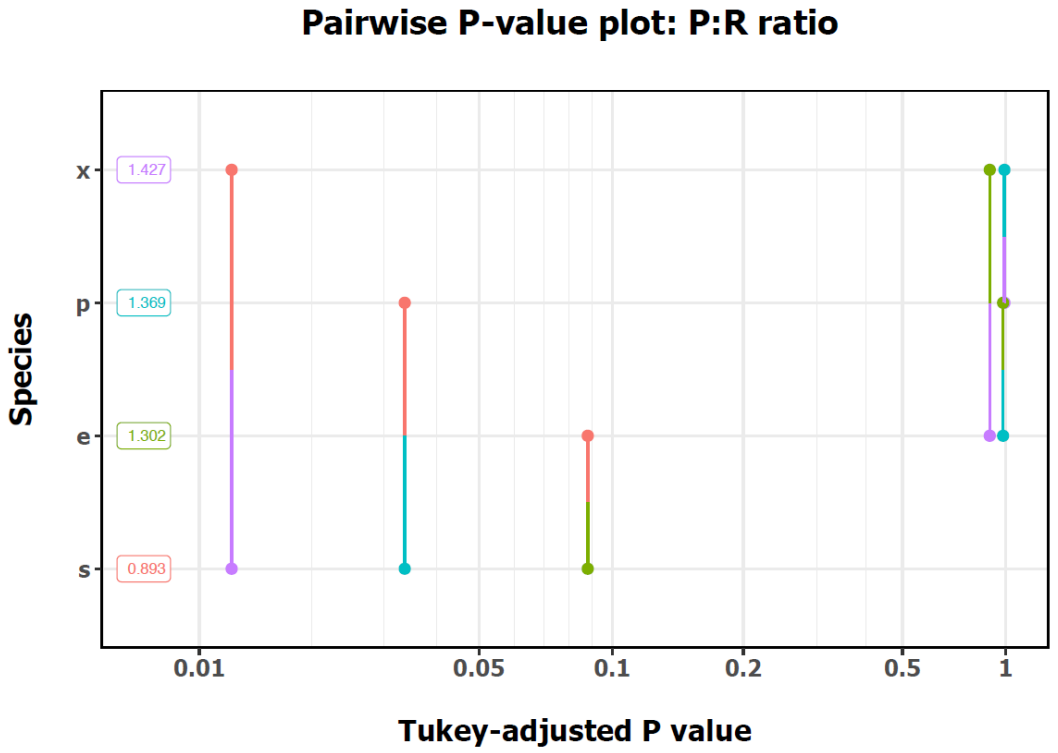


Figure S2.2 | Time factor pairwise comparisons for P:R ratio – Species. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

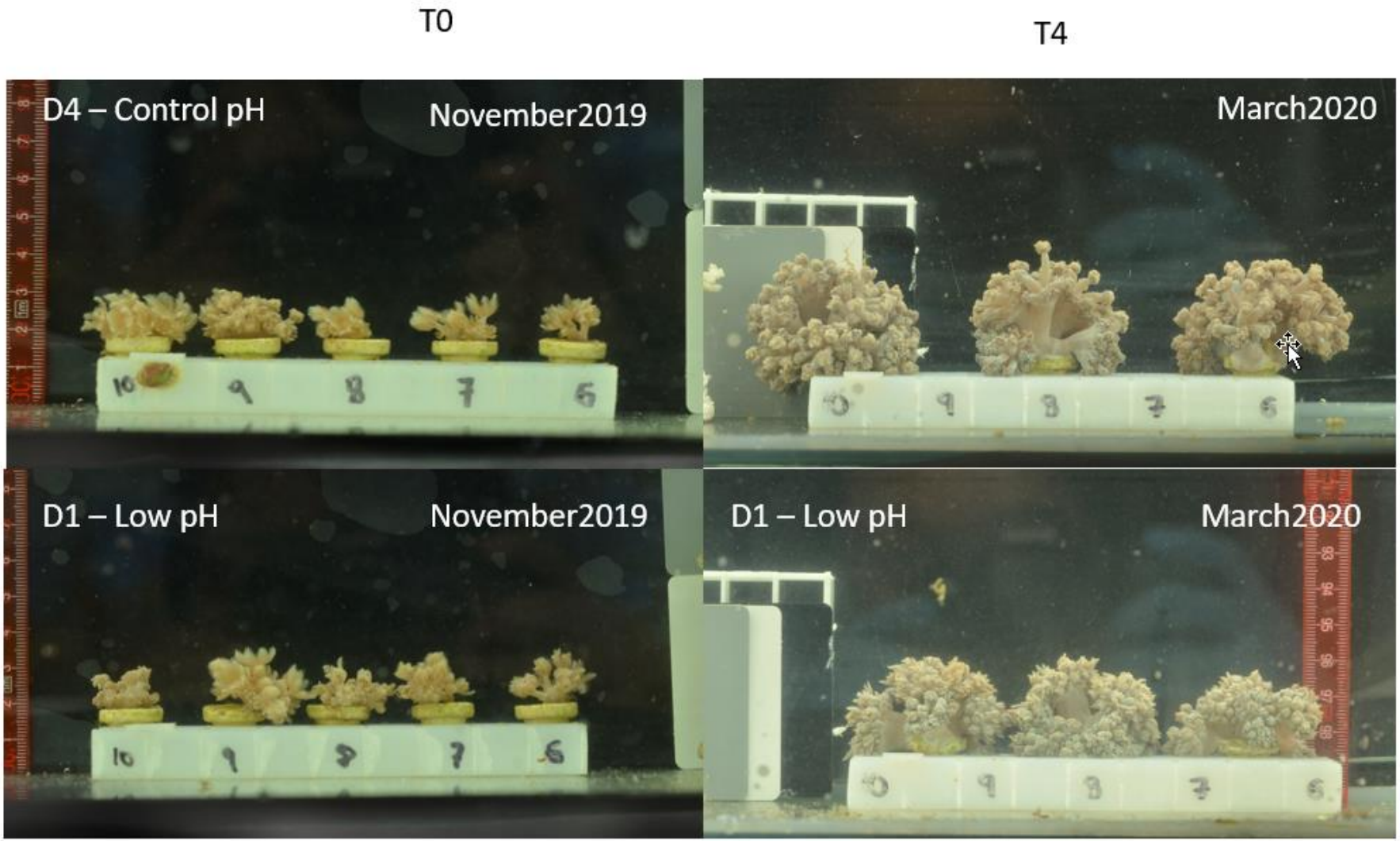
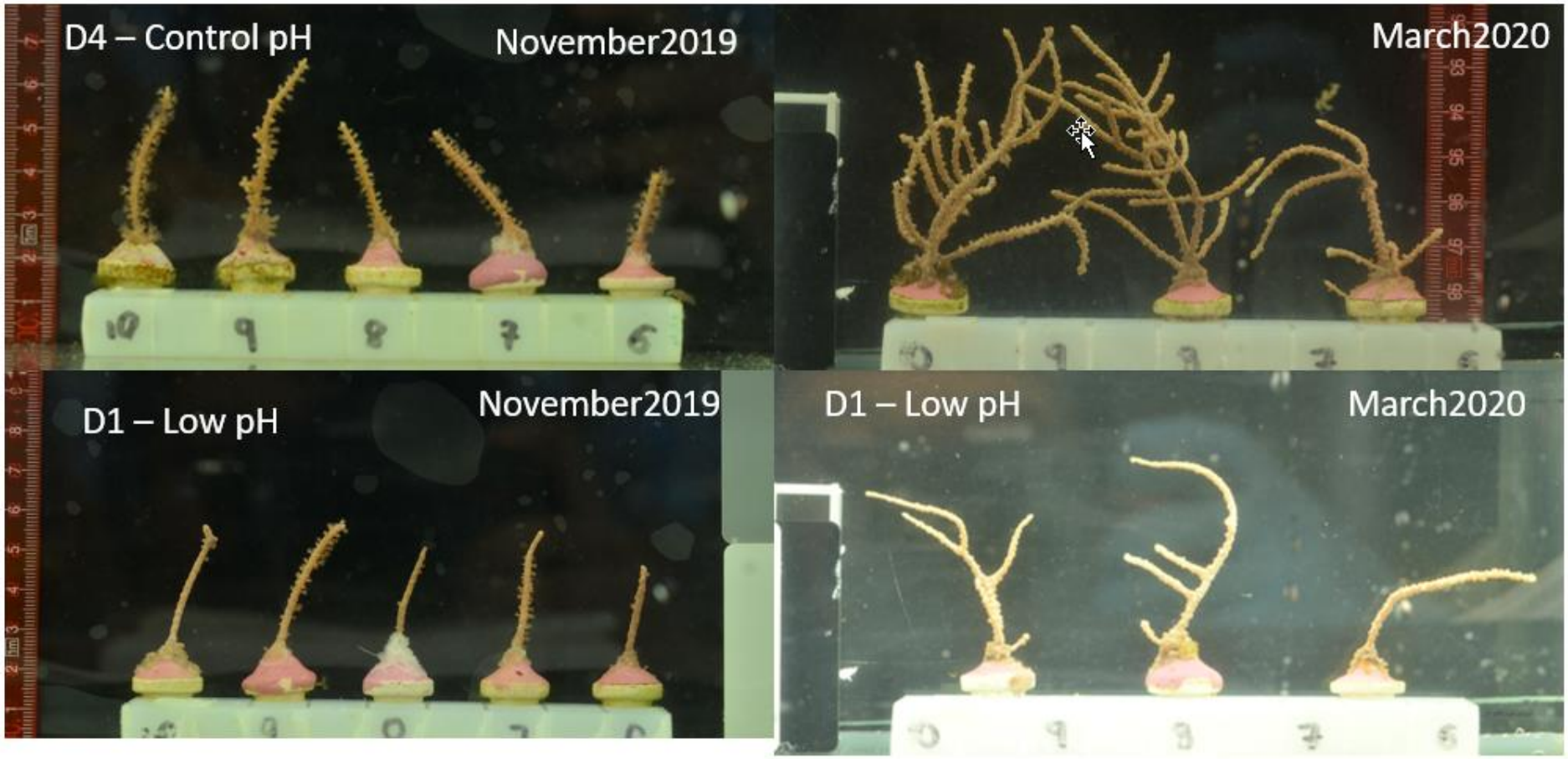
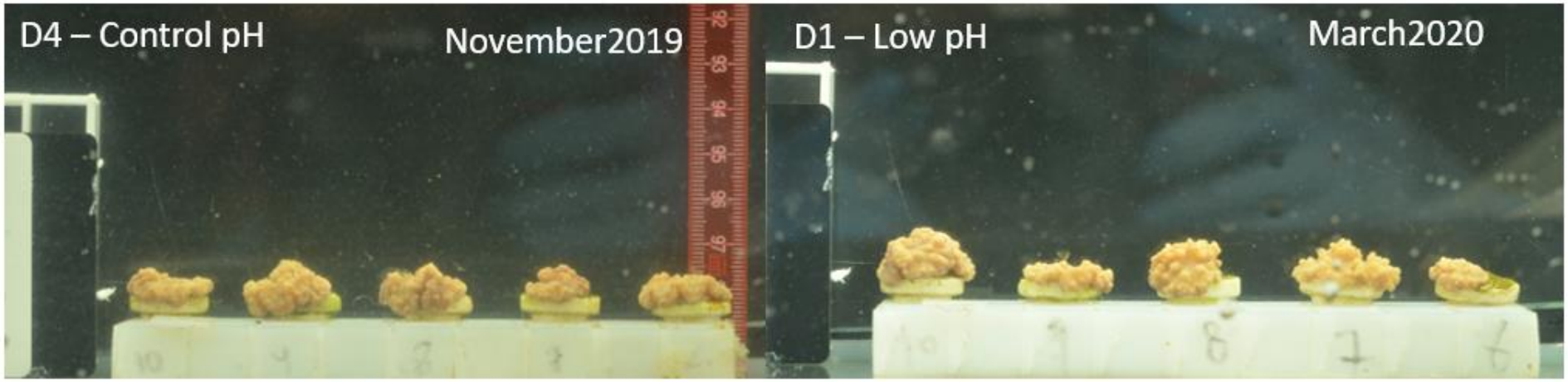


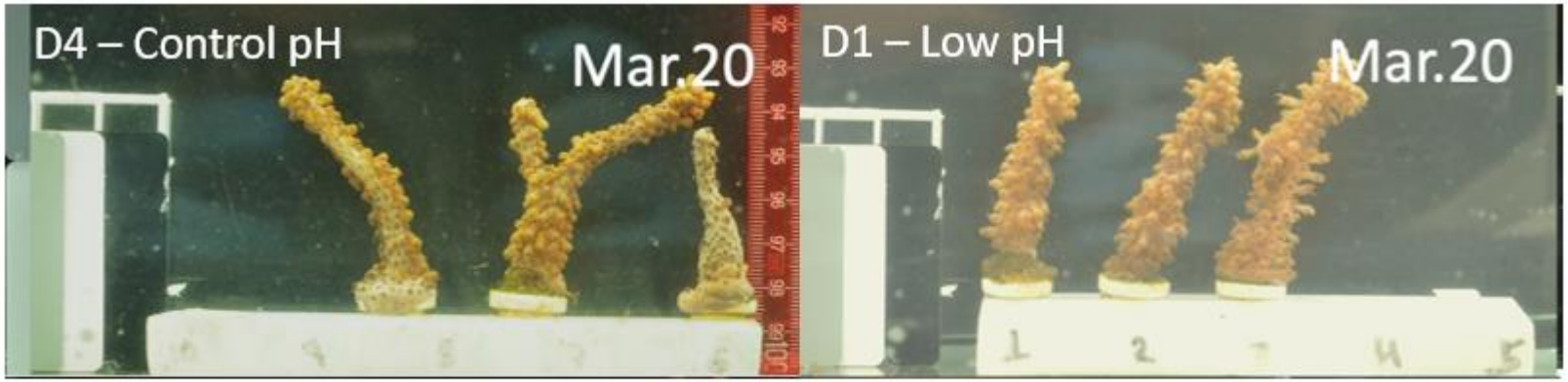
Figure S2.3 | Octocoral growth over time: Xenia, Pinnigorgia, Plexaurella and Sinularia, T0 to T4

T0

T4


D4 – Control pH
November2019
March2020
D1 – Low pH
November2019
D1 – Low pH
March2020





Control

Low pH

Supplementary Information | Chapter 3

Supplementary Information S1. Summary tables: Tukey pairwise comparisons of means for the temperature factor. Tukey contrasts for gross and net photosynthesis, respiration rates and the P:R ratio of *X. umbellata* corals, under simulated warming and DOC additions.

Table S1.1 | Temperature pairwise mean comparisons for *X. umbellata* gross photosynthesis. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
26 - 28	-7.5817	1.1165	-6.790	**4.47e-11 *****
26 - 30	-11.9203	1.1193	-10.650	**< 2e-16 *****
26 - 32	-11.0462	1.1193	-9.869	**< 2e-16 *****
28 - 30	-4.3385	0.7745	-5.602	**6.36e-08 *****
28 - 32	-3.4645	0.7742	-4.475	**1.53e-05 *****
30 - 32	0.8740	0.7782	1.123	0.261

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

Table S1.2 | Temperature pairwise mean comparisons for *X. umbellata* respiration. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
26 - 28	-1.7566	0.6157	-2.853	**0.00433 ****
26 - 30	-4.7236	0.6189	-7.632	**1.39e-13 *****
26 - 32	-3.2084	0.6157	-5.211	**7.50e-07 *****
28 - 30	-2.9670	0.4295	-6.908	**2.45e-11 *****
28 - 32	-1.4518	0.4247	-3.418	**0.00126 ****
30 - 32	1.5152	0.4293	3.529	**0.00125 ****

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

Table S1.3 | Temperature pairwise mean comparisons for *X. umbellata* net photosynthesis. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
26 - 28	-5.4096	0.7017	-7.709	**5.06e-14 *** **
26 - 30	-7.5156	0.7035	-10.683	**< 2e-16 *** **
26 - 32	-7.8073	0.7035	-11.098	**< 2e-16 *** **
28 - 30	-2.1060	0.4868	-4.326	**3.04e-05 *** **
28 - 32	-2.3977	0.4868	-4.925	**2.53e-06 *** **
30 - 32	-0.2917	0.4894	-0.596	0.551

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

Table S1.4 | Temperature pairwise mean comparisons for *X. umbellata* P:R ratio. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
26 - 28	-0.6651	0.1734	-3.837	**0.000499 *** **
26 - 30	-0.9063	0.1742	-5.202	**9.83e-07 *** **
26 - 32	-1.0305	0.1738	-5.930	**1.82e-08 *** **
28 - 30	-0.2412	0.1199	-2.011	0.088577.
28 - 32	-0.3654	0.1193	-3.063	**0.006577 ** **
30 - 32	-0.1242	0.1206	-1.030	0.303036

Note: *P*-values defined as significant at a threshold of P < 0.05 are highlighted in bold.

Supplementary Information S2. Temperature factor pairwise comparisons. Tukey adjusted P-value plots of estimated marginal means for gross and net photosynthesis, respiration rates and the P:R ratio of *X. umbellata* corals under simulated warming and DOC additions.

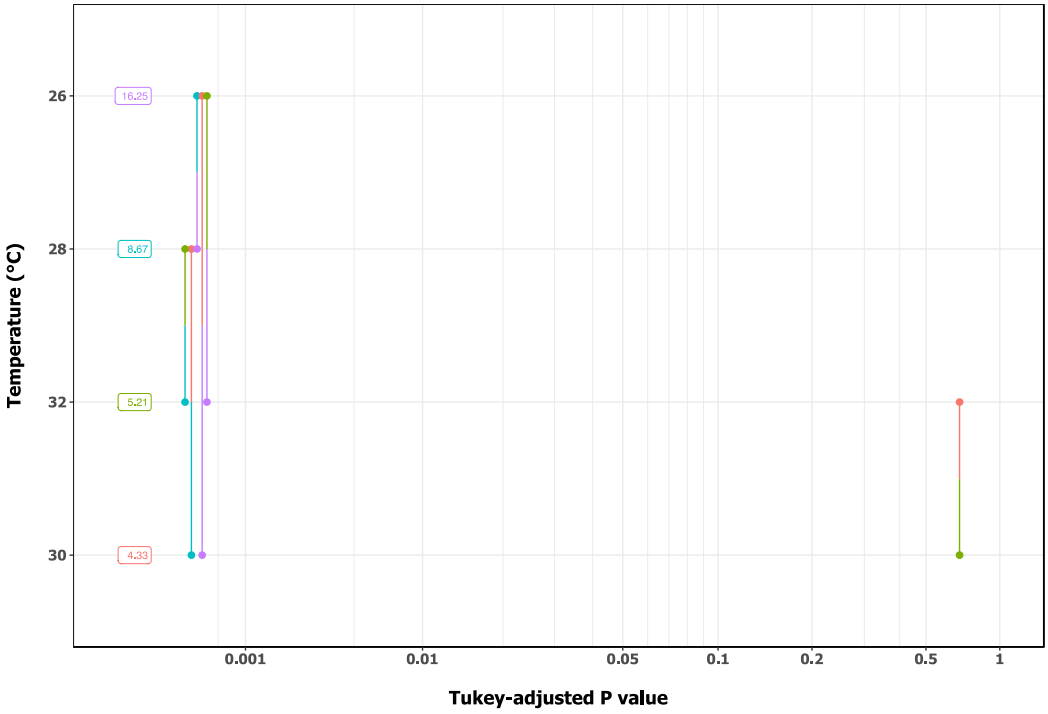


Figure S2.1 | Temperature pairwise comparison for *X. umbellata* gross photosynthesis. Tukey adjusted P-value plot.

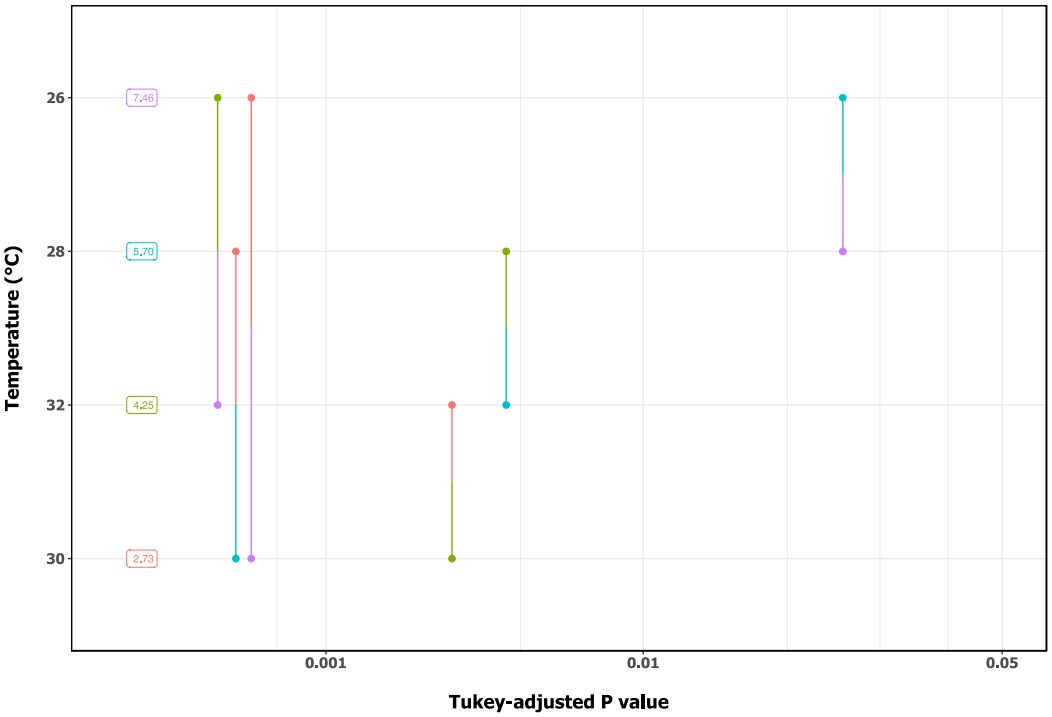


Figure S2.2 | Temperature pairwise comparison for *X. umbellata* respiration. Tukey adjusted P-value plot.

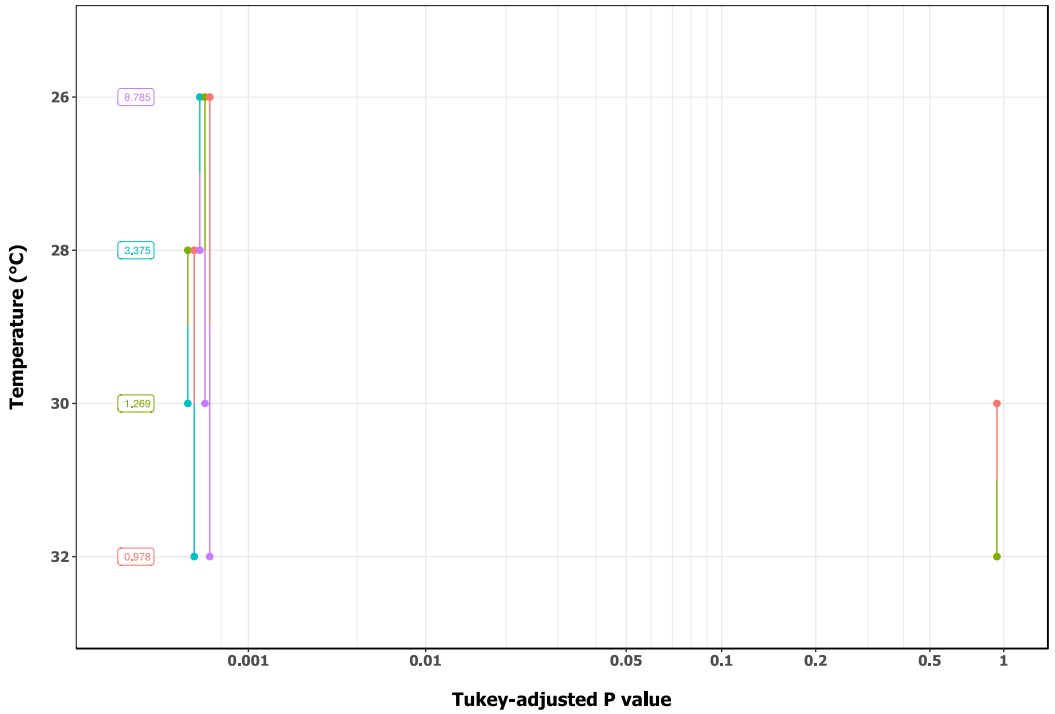


Figure S2.3 | Temperature pairwise comparison for *X. umbellata* net photosynthesis. Tukey adjusted P-value plot.

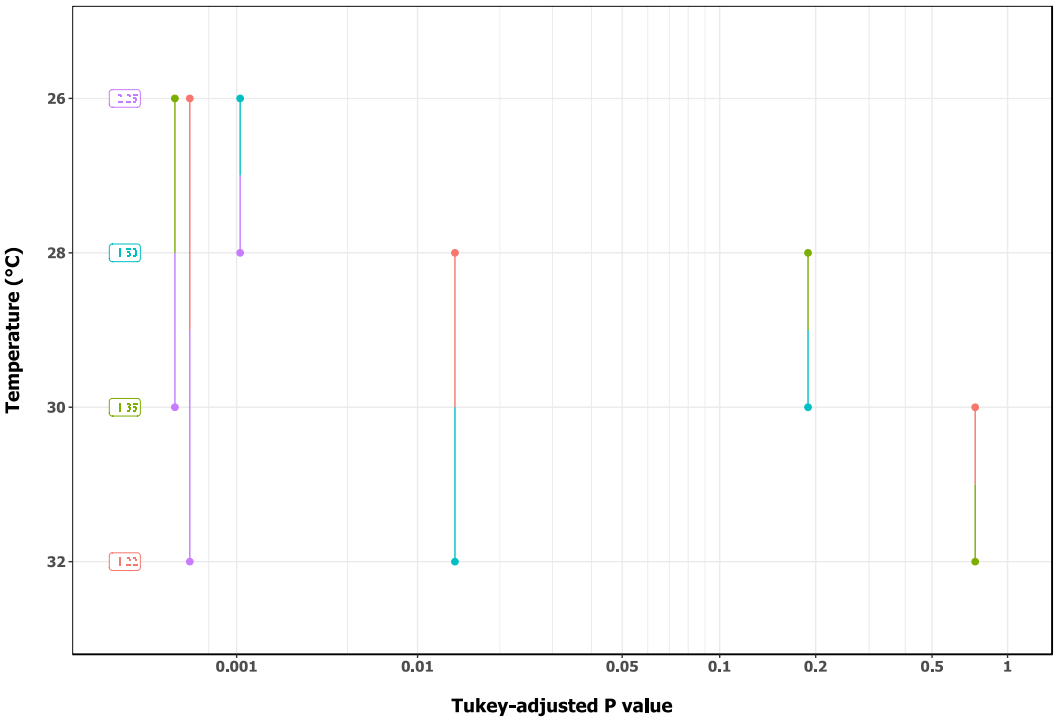


Figure S2.4 | Temperature pairwise comparison for *X. umbellata* P:R ratio. Tukey adjusted P-value plot.

Supplementary Information | Chapter 4

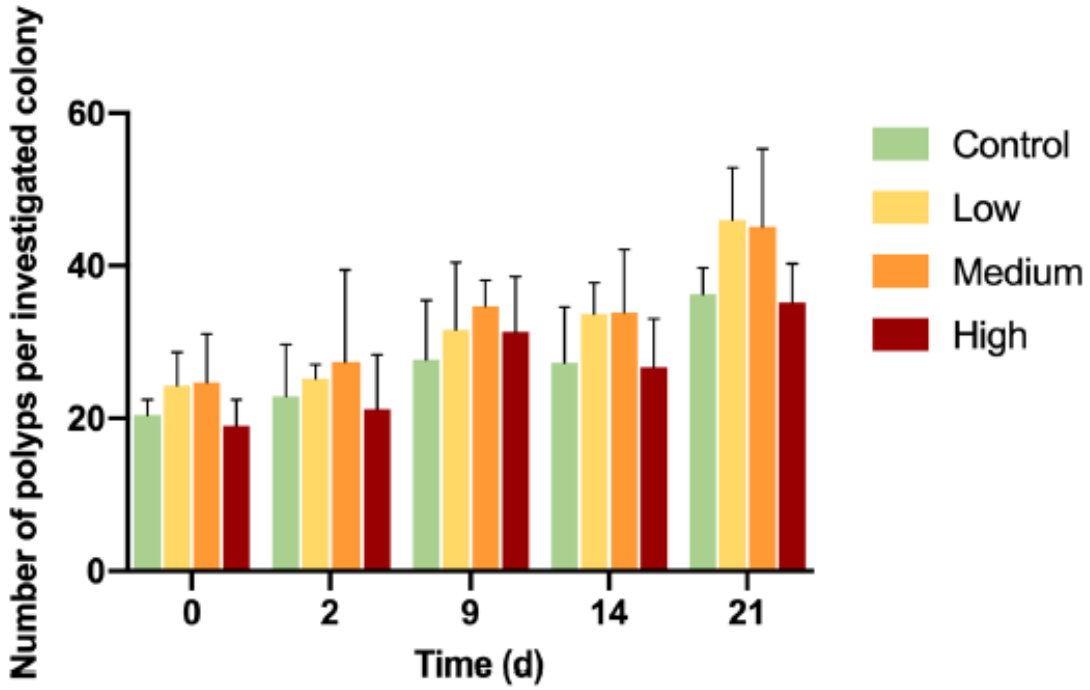


Figure S1.1 | Polyp numbers of different treatments over time. Columns indicate mean values of three replicates with error bars providing the respective SD.

Supplementary Information | Chapter 5

Supplementary Information S1. Controlled experimental background parameters measured through the study.

Table S1.1 | Summary of background parameters measured through the complete experiment depicting mean values ± SD for every tank across all treatments.

	Tank ID	**Light** (µmol m^{-2} s^{-1})	**Salinity** (ppt)	**pH**	**KH**	**Ca** (ppm)	**Mg** (ppm)	**NO_2** (ppm)	**NO_3** (ppm)	**NH_4** (ppm)	**PO_4** (ppm)	**Flow rate** (L s^{-1})	**Turnover time** (s)
Mantainance Tank	Main	121.86 ± 5.48	35.40 ± 0.25	8.20 ± 0.01	7.33 ± 0.58	446.67 ± 70.24	1445.00 ± 218.10	<0.01	<0.5	<0.05	<0.02	1.39	302.16
ControlT	13	119.90 ± 0.36	35.10 ± 0.74	8.17 ± 0.08	7.13 ± 0.83	450.00 ± 87.51	1450.00 ± 415.55	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	14	120.38 ± 0.36	35.34 ± 0.56	8.20 ± 0.01	7.25 ± 0.89	447.50 ± 86.81	1430.00 ± 341.43	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	15	120.34 ± 0.22	35.09 ± 0.55	8.17 ± 0.08	7.13 ± 0.64	442.50 ± 83.11	1407.50 ± 346.23	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	16	119.95 ± 0.37	35.52 ± 0.48	8.20 ± 0.01	7.00 ± 0.71	447.50 ± 93.16	1437.50 ± 279.48	<0.01	<0.5	<0.05	<0.02	0.083	722.89
Control	1	121.46 ± 0.15	35.25 ± 0.55	8.18 ± 0.06	7.25 ± 0.75	450.00 ± 57.52	1413.33 ± 134.66	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	8	122.14 ± 0.31	35.36 ± 0.54	8.20 ± 0.02	7.33 ± 0.78	448.33 ± 53.57	1519.17 ± 338.83	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	9	120.92 ± 0.68	35.46 ± 0.66	8.19 ± 0.03	7.25 ± 1.06	450.00 ± 69.02	1550.00 ± 355.17	<0.01	<0.5	<0.05	<0.02	0.083	722.89
DOC 10 mg/l	4	121.96 ± 1.38	35.18 ± 0.76	8.16 ± 0.08	7.25 ± 0.97	435.00 ± 68.82	1506.67 ± 290.37	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	7	121.74 ± 0.59	35.38 ± 0.61	8.15 ± 0.09	7.17 ± 0.58	451.67 ± 79.75	1496.67 ± 227.05	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	12	118.82 ± 0.97	35.73 ± 0.75	8.20 ± 0.02	7.17 ± 1.03	448.33 ± 74.57	1430.00 ± 363.77	<0.01	<0.5	<0.05	<0.02	0.083	722.89
DOC 20 mg/l	2	120.44 ± 0.44	34.99 ± 0.54	8.18 ± 0.06	7.17 ± 0.94	440.00 ± 71.35	1533.33 ± 314.77	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	6	122.68 ± 2.60	35.26 ± 0.60	8.20 ± 0.01	7.25 ± 0.97	446.67 ± 46.19	1500.83 ± 275.70	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	10	120.22 ± 0.084	35.53 ± 0.61	8.18 ± 0.06	7.25 ± 0.75	435.00 ± 57.92	1476.67 ± 281.40	<0.01	<0.5	<0.05	<0.02	0.083	722.89
DOC 40 mg/l	3	120.40 ± 1.02	35.21 ± 0.57	8.16 ± 0.08	7.17 ± 1.03	468.33 ± 54.24	1509.17 ± 187.98	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	5	120.82 ± 0.41	35.22 ± 0.67	8.05 ± 0.13	7.25 ± 0.87	448.33 ± 67.40	1543.33 ± 310.64	<0.01	<0.5	<0.05	<0.02	0.083	722.89
	11	121.98 ± 0.28	35.55 ± 0.67	8.16 ± 0.08	7.33 ± 1.23	448.33 ± 61.18	1428.33 ± 239.61	<0.01	<0.5	<0.05	<0.02	0.083	722.89

Note: the values presented here for flow rates (L s^{-1}) and turnover time (s) were obtained via calculating their corresponding theoretical values for each tank according to Grottoli et.al. (2021). Here, flow rate was calculated in each tank as a volumetric water flow rate per unit time, which in a closed system tank would correspond to the fluid output from the exhaust of the pump. On the other hand, the water turnover time would correspond to the time required to replace the entire volume of water in the tank, assuming the tank is continuously well mixed. This value was calculated here for each tank, by dividing the tank volume by it corresponding flow rate.

Table S1.2 | Summary of background parameters prior to the start of the first experimental stage of the study corresponding to individual DOC additions. The table shows mean values ± SD for every tank across all treatments.

	Tank ID	**Light** (μmol m^{-2} s^{-1})	**Temperature** (°C)	**DOC** (mg/l)	**Salinity** (ppt)	**pH**	**KH**	**Ca** (ppm)	**Mg** (ppm)	**NO_2** (ppm)	**NO_3** (ppm)	**NH_4** (ppm)	**PO_4** (ppm)
Mantainance Tank	Main	119.4	25.93 ± 0.25	2.77	35.33 ± 0.06	8.20	7.0	440.0	1720.0	<0.01	<0.5	<0.05	<0.02
Control	1	121.5	26.37 ± 0.25	2.51	35.30 ± 0.20	8.20	7.0	440.0	1740.0	<0.01	<0.5	<0.05	<0.02
	8	122.4	26.20 ± 0.26	2.96	35.33 ± 0.15	8.20	7.0	440.0	1720.0	<0.01	<0.5	<0.05	<0.02
	9	119.8	26.47 ± 0.31	2.71	35.20 ± 0.82	8.20	7.0	420.0	1720.0	<0.01	<0.5	<0.05	<0.02
DOC 10 mg/l	4	121.3	26.67 ± 0.25	2.65	35.27 ± 0.31	8.20	7.0	400.0	1760.0	<0.01	<0.5	<0.05	<0.02
	7	121.4	26.50 ± 0.17	2.76	35.60 ± 0.40	8.20	7.0	440.0	1600.0	<0.01	<0.5	<0.05	<0.02
	12	119.1	26.23 ± 0.12	2.58	35.43 ± 0.21	8.20	7.0	400.0	1680.0	<0.01	<0.5	<0.05	<0.02
DOC 20 mg/l	2	120.4	26.13 ± 0.32	2.55	35.47 ± 0.29	8.20	7.0	380.0	1600.0	<0.01	<0.5	<0.05	<0.02
	6	118.2	26.47 ± 0.12	2.80	35.47 ± 0.25	8.20	7.0	420.0	1620.0	<0.01	<0.5	<0.05	<0.02
	10	120.3	26.20 ± 0.26	2.62	35.57 ± 0.15	8.20	7.0	380.0	1660.0	<0.01	<0.5	<0.05	<0.02
DOC 40 mg/l	3	120.1	26.50 ± 0.17	2.55	35.30 ± 0.17	8.00	7.0	420.0	1620.0	<0.01	<0.5	<0.05	<0.02
	5	120.7	26.37 ± 0.35	2.70	35.30 ± 0.20	8.00	7.0	440.0	1600.0	<0.01	<0.5	<0.05	<0.02
	11	121.9	26.40 ± 0.35	2.56	35.40 ± 0.10	8.20	7.0	380.0	1780.0	<0.01	<0.5	<0.05	<0.02

Table S1.3 | Summary of background parameters prior to the start of the second experimental stage of the study, right after individual DOC addition and before implementing increased temperature treatments. The table shows mean values ± SD for every tank across all treatments.

	Tank ID	**Light** (µmol m^{-2} s^{-1})	**Temperature** (°C)	**DOC** (mg/l)	**Salinity** (ppt)	**pH**	**KH**	**Ca** (ppm)	**Mg** (ppm)	**NO_2** (ppm)	**NO_3** (ppm)	**NH_4** (ppm)	**PO_4** (ppm)
Mantainance tank	Main	120.7	25.97 ± 0.23	2.49	35.43 ± 0.12	8.20	7.0	380.0	1520.0	<0.01	<0.5	<0.05	<0.02
ControlT	13	120.2	25.90 ± 0.52	2.52	35.47 ± 0.06	8.20	7.0	440.0	1560.0	<0.01	<0.5	<0.05	<0.02
	14	120.3	25.93 ± 0.32	2.89	35.20 ± 0.46	8.20	7.0	380.0	1500.0	<0.01	<0.5	<0.05	<0.02
	15	120.1	26.00 ± 0.10	2.57	35.13 ± 0.15	8.00	7.0	380.0	1480.0	<0.01	<0.5	<0.05	<0.02
	16	120.5	25.90 ± 0.20	2.61	35.43 ± 0.31	8.20	7.0	400	1520.0	<0.01	<0.5	<0.05	<0.02
Control	1	121.3	26.00 ± 0.60	2.66	35.63 ± 0.42	8.20	7.0	400.0	1520.0	<0.01	<0.5	<0.05	<0.02
	8	121.6	26.03 ± 0.42	2.57	35.33 ± 0.12	8.20	7.0	380.0	1400.0	<0.01	<0.5	<0.05	<0.02
	9	121.1	26.20 ± 0.10	2.51	35.57 ± 0.25	8.20	7.0	380.0	1580.0	<0.01	<0.5	<0.05	<0.02
DOC 10 mg/l	4	121.6	25.83 ± 0.49	10.0	34.67 ± 0.47	8.20	7.0	380.0	1580.0	<0.01	<0.5	<0.05	<0.02
	7	121.3	25.87 ± 0.21	10.0	35.37 ± 0.15	8.20	7.0	380.0	1480.0	<0.01	<0.5	<0.05	<0.02
	12	119.4	26.06 ± 0.41	10.0	35.37 ± 0.38	8.20	7.0	400.0	1400.0	<0.01	<0.5	<0.05	<0.02
DOC 20 mg/l	2	120.1	26.07 ± 0.40	20.0	35.17 ± 0.15	8.20	7.0	440.0	1520.0	<0.01	<0.5	<0.05	<0.02
	6	122.7	26.13 ± 0.25	20.0	35.30 ± 0.70	8.20	7.0	480.0	1480.0	<0.01	<0.5	<0.05	<0.02
	10	120.2	26.07 ± 0.32	20.0	35.30 ± 0.30	8.20	8.0	400.0	1520.0	<0.01	<0.5	<0.05	<0.02
DOC 40 mg/l	3	120.5	26.10 ± 0.52	40.0	34.80 ± 0.70	8.00	7.0	480.0	1520.0	<0.01	<0.5	<0.05	<0.02
	5	120.7	25.77 ± 0.46	40.0	34.77 ± 0.64	8.20	7.0	480.0	1400.0	<0.01	<0.5	<0.05	<0.02
	11	121.7	26.10 ± 0.20	40.0	35.30 ± 0.36	8.20	7.0	400.0	1520.0	<0.01	<0.5	<0.05	<0.02

Supplementary Information S2. Statistical background parameters comparisons

Table S2.1 | Statistical results for background parameters comparison through the complete experiment. The statistical analysis was performed via a simple LM where contrasts were done across all experimental treatments including: the DOC control tanks (Control), the temperature control tanks (ControlT), the initial maintenance tank where the colonies were breed, and the tanks corresponding to the DOC manipulated conditions 10, 20 and 40 mg/l. There were no significant differences between tanks regarding the background parameters presented in the table, and all tanks showed comparable conditions except for waterflow related parameters in the maintenance tank. These water flow parameters were not included here but were significantly different (LM; $p < 0.05$) as expected according to the values observed in supplementary information S1.

Parameter	*df*	F	p
Light (µmol m^{-2} s^{-1})	5	0.8202	0.5601
Salinity (ppt)	5	0.2922	0.9074
pH	5	2.0622	0.1473
KH	5	2.3253	0.1131
Ca (ppm)	5	1.4526	0.2808
Mg (ppm)	5	1.2441	0.3529

Table S2.2 | Statistical results for background parameters prior to the start of the first and second experimental stages, respectively. The parameters were statistically analysed via simple LM contrasting across experimental treatments. For the analysis corresponding to the parameters prior to the start of the first stage, we compared: the DOC control tanks (Control), the initial maintenance tank where colonies were breed, and the corresponding tanks to the DOC manipulated conditions 10, 20 and 40 mg/l. In addition, for the analysis corresponding to the second experimental stage, we included the temperature control tanks (ControlT) and compared the DOC parameter only among the tanks representing control conditions and the maintenance tank. No significant differences were found in any of the background parameters shown in the table, except for the waterflow related parameters in the maintenance tank (not included here, LM; $p < 0.05$; please see supplementary information S1 for further reference on raw data values).

	Stage 1			Stage 2		
Parameter	***df***	**F**	**p**	***df***	**F**	**p**
Light (µmol m^{-2} s^{-1})	4	0.9605	0.4786	5	0.5784	0.7162
Temperature (°C)	4	2.4185	0.1339	5	1.3067	0.3294
DOC (mg/l)	4	0.4064	0.7994	2	0.5932	0.5873
Salinity (ppt)	4	2.4697	0.1287	5	1.8451	0.1845
pH	4	3.0769	0.08247	5	0.5405	0.7424
KH	4	1.4615	0.2997	5	0.5176	0.7582
Ca (ppm)	4	1.4327	0.3076	5	2.7276	0.07687
Mg (ppm)	4	0.9982	0.4617	5	0.1475	0.9766

Supplementary Information S3. Post hoc model analyses: Tukey test results summary tables

Table S3.1 | Post-hoc multiple comparisons test results. *P. flava* O_2 production assessment during the experiments' second stage, including warming and DOC addition treatments.

Contrast	*df*	p
26-28	44.6	0.9995
26-30	44.0	0.3003
26-32	44.0	**0.0024** **
28-30	44.6	0.2640
28-32	44.6	**0.0021** **
30-32	44.0	0.1939

Table S3.2 | Post-hoc comparison test results for *P. flava* fragments change in surface area. Simplified initial assessment of the significant interaction term between temperature and DOC, fixing the DOC treatment factor intercept and varying it across temperatures.

	Estimates for Temperature			Contrast by DOC Treatment	
Fixed Factors	**28 °C**	**30 °C**	**32 °C**	***df***	**p**
ControlT	0.0305	-0.0826	0.4408	3	**0.0091** **
Control	0.2468	0.2041	0.4400	3	0.0875
High	0.1398	0.1338	-0.1128	3	0.2877
Low	-0.3875	-0.3573	-0.4305	3	**0.0046** **
Medium	-0.1600	-0.0021	-0.2609	3	0.1071

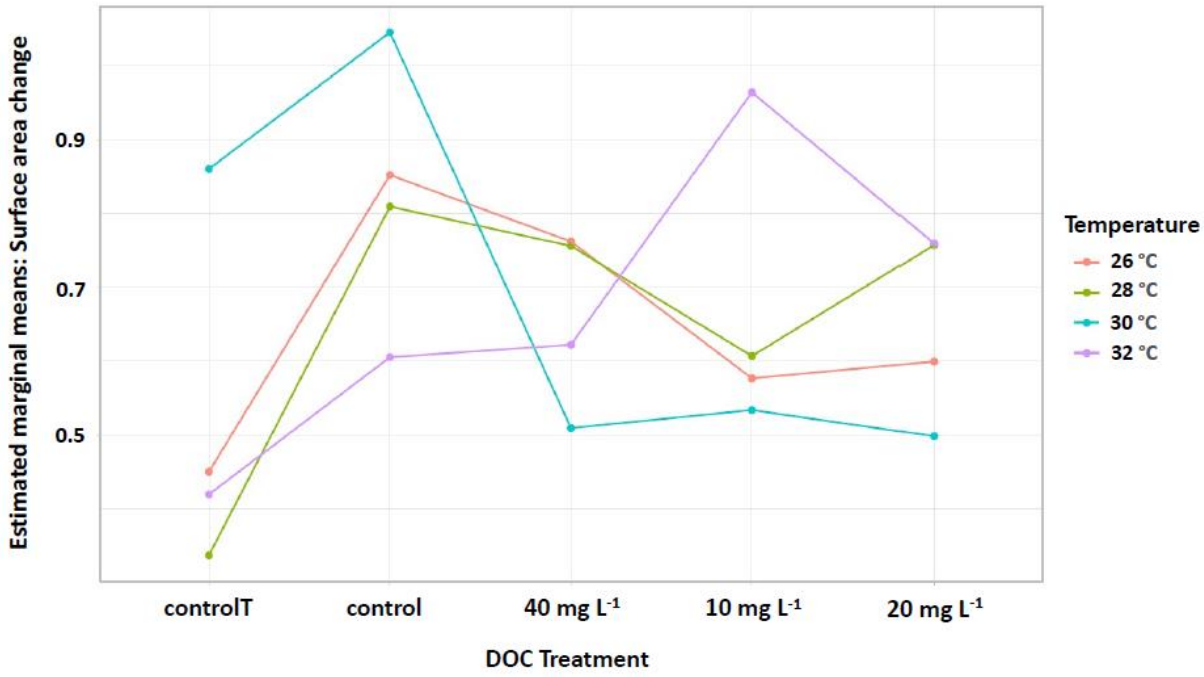


Figure S3.3 | Model marginal means for the *P. flava* fragments change in surface area. Graph depicting the contrasts across DOC treatments and at each temperature treatment.

Table S3.4 | Detailed results of Post-hoc pair comparisons test for *P. flava* fragments change in surface area. Complete summary of the significant interaction term assessment between temperature and DOC treatment pairs. Please see previous figure S3.3 for graphical reference of these results. Here, ControlT represents the control that remained always at 26 degrees during the second phase experiments while the increased temperature treatments correspond to the DOC control tanks (Control) and the DOC manipulated conditions 10 (Low), 20 (Medium) and 40 (High) mg/l. The contrasts are shown in the table as "DOC treatment.temperature - DOC treatment.temperature". With this notation we indicate pair comparisons performed per each DOC condition at each increased temperature point. Here, e.g., "Low.30 – High.32" would represent the comparison between Low DOC treatment when temperature had been increased at 30 C contrasted to High DOC treatment when the temperature had been increased at 32 C. For the special case of ControlT, e.g., "ControlT.28 – Medium.32" would represent the comparison between ControlT at 26 C temperature, but at the time point where the rest of the experimental tanks were exposed to 28 C; contrasted to the Medium DOC treatment when the tanks were exposed to 32 C, and so on. Significant differences are highlighted in bold.

Contrast	Estimate	SE	*df*	t.ratio	p
ControlT.26 - Control.26	-0.4021	0.1488	30.1314	-2.7030	0.4607
ControlT.26 - Low.26	-0.1267	0.1481	33.8046	-0.8554	1.0000
ControlT.26 - Medium.26	-0.1493	0.1481	33.8046	-1.0080	0.9999
ControlT.26 - High.26	-0.3120	0.1481	33.8046	-2.1065	0.8317
ControlT.26 - ControlT.28	0.1131	0.0931	3.7525	1.2146	0.9887
ControlT.26 - Control.28	-0.3594	0.1413	29.7706	-2.5427	0.5643
ControlT.26 - Low.28	-0.1569	0.1481	33.8046	-1.0592	0.9999
ControlT.26 - Medium.28	-0.3071	0.1481	33.8046	-2.0737	0.8478
ControlT.26 - High.28	-0.3060	0.1555	43.8959	-1.9683	0.8979
ControlT.26 - ControlT.30	-0.4103	0.1425	43.3399	-2.8786	0.3427
ControlT.26 - Control.30	**-0.5954**	**0.1488**	**30.1314**	**-4.0022**	**0.0367 ***
ControlT.26 - Low.30	-0.0837	0.1481	33.8046	-0.5651	1.0000
ControlT.26 - Medium.30	-0.0484	0.1481	33.8046	-0.3267	1.0000
ControlT.26 - High.30	-0.0594	0.1555	43.8959	-0.3818	1.0000
ControlT.26 - ControlT.32	0.0305	0.1168	5.6304	0.2610	1.0000
ControlT.26 - Control.32	-0.1553	0.1555	43.8959	-0.9990	1.0000
ControlT.26 - Low.32	-0.5142	0.1481	33.8046	-3.4715	0.1143
ControlT.26 - Medium.32	-0.3093	0.1481	33.8046	-2.0881	0.8408
ControlT.26 - High.32	-0.1722	0.1481	33.8046	-1.1625	0.9996
Control.26 - Low.26	0.2754	0.1629	35.2032	1.6905	0.9697
Control.26 - Medium.26	0.2528	0.1629	35.2032	1.5517	0.9868
Control.26 - High.26	0.0901	0.1629	35.2032	0.5530	1.0000

Control.26 - ControlT.28	0.5151	0.1488	30.1314	3.4631	0.1223
Control.26 - Control.28	0.0427	0.0915	3.2398	0.4663	1.0000
Control.26 - Low.28	0.2452	0.1629	35.2032	1.5052	0.9904
Control.26 - Medium.28	0.0950	0.1629	35.2032	0.5829	1.0000
Control.26 - High.28	0.0961	0.1696	43.7439	0.5665	1.0000
Control.26 - ControlT.30	-0.0082	0.1579	43.1130	-0.0521	1.0000
Control.26 - Control.30	-0.1933	0.1505	6.5945	-1.2842	0.9913
Control.26 - Low.30	0.3184	0.1629	35.2032	1.9544	0.8995
Control.26 - Medium.30	0.3537	0.1629	35.2032	2.1712	0.7983
Control.26 - High.30	0.3427	0.1696	43.7439	2.0206	0.8765
Control.26 - ControlT.32	0.4326	0.1515	30.5134	2.8558	0.3688
Control.26 - Control.32	0.2468	0.1696	43.7439	1.4549	0.9940
Control.26 - Low.32	-0.1121	0.1629	35.2032	-0.6880	1.0000
Control.26 - Medium.32	0.0928	0.1629	35.2032	0.5698	1.0000
Control.26 - High.32	0.2299	0.1629	35.2032	1.4113	0.9953
Low.26 - Medium.26	-0.0226	0.1623	38.0337	-0.1393	1.0000
Low.26 - High.26	-0.1853	0.1623	38.0337	-1.1416	0.9997
Low.26 - ControlT.28	0.2397	0.1481	33.8046	1.6187	0.9796
Low.26 - Control.28	-0.2327	0.1562	35.1747	-1.4901	0.9914
Low.26 - Low.28	-0.0302	0.1279	4.9981	-0.2362	1.0000
Low.26 - Medium.28	-0.1804	0.1623	38.0337	-1.1117	0.9998
Low.26 - High.28	-0.1793	0.1691	43.9963	-1.0607	0.9999
Low.26 - ControlT.30	-0.2836	0.1573	43.9196	-1.8036	0.9494
Low.26 - Control.30	-0.4687	0.1629	35.2032	-2.8768	0.3512
Low.26 - Low.30	0.0430	0.1623	38.0337	0.2648	1.0000
Low.26 - Medium.30	0.0783	0.1623	38.0337	0.4824	1.0000
Low.26 - High.30	0.0673	0.1691	43.9963	0.3983	1.0000
Low.26 - ControlT.32	0.1572	0.1508	34.1036	1.0420	0.9999
Low.26 - Control.32	-0.0286	0.1691	43.9963	-0.1693	1.0000
Low.26 - Low.32	-0.3875	0.1623	38.0337	-2.3871	0.6667
Low.26 - Medium.32	-0.1826	0.1623	38.0337	-1.1248	0.9997
Low.26 - High.32	-0.0455	0.1623	38.0337	-0.2803	1.0000
Medium.26 - High.26	-0.1627	0.1623	38.0337	-1.0023	0.9999
Medium.26 - ControlT.28	0.2624	0.1481	33.8046	1.7714	0.9535

Medium.26 - Control.28	-0.2101	0.1562	35.1747	-1.3453	0.9973
Medium.26 - Low.28	-0.0076	0.1623	38.0337	-0.0467	1.0000
Medium.26 - Medium.28	-0.1578	0.1279	4.9981	-1.2345	0.9912
Medium.26 - High.28	-0.1567	0.1691	43.9963	-0.9269	1.0000
Medium.26 - ControlT.30	-0.2610	0.1573	43.9196	-1.6598	0.9764
Medium.26 - Control.30	-0.4461	0.1629	35.2032	-2.7380	0.4344
Medium.26 - Low.30	0.0656	0.1623	38.0337	0.4041	1.0000
Medium.26 - Medium.30	0.1009	0.1623	38.0337	0.6217	1.0000
Medium.26 - High.30	0.0899	0.1691	43.9963	0.5321	1.0000
Medium.26 - ControlT.32	0.1798	0.1508	34.1036	1.1919	0.9994
Medium.26 - Control.32	-0.0060	0.1691	43.9963	-0.0355	1.0000
Medium.26 - Low.32	-0.3649	0.1623	38.0337	-2.2478	0.7552
Medium.26 - Medium.32	-0.1600	0.1623	38.0337	-0.9855	1.0000
Medium.26 - High.32	-0.0229	0.1623	38.0337	-0.1410	1.0000
High.26 - ControlT.28	0.4251	0.1481	33.8046	2.8698	0.3568
High.26 - Control.28	-0.0474	0.1562	35.1747	-0.3036	1.0000
High.26 - Low.28	0.1551	0.1623	38.0337	0.9556	1.0000
High.26 - Medium.28	0.0049	0.1623	38.0337	0.0300	1.0000
High.26 - High.28	0.0060	0.1691	43.9963	0.0354	1.0000
High.26 - ControlT.30	-0.0983	0.1573	43.9196	-0.6252	1.0000
High.26 - Control.30	-0.2834	0.1629	35.2032	-1.7394	0.9608
High.26 - Low.30	0.2283	0.1623	38.0337	1.4064	0.9956
High.26 - Medium.30	0.2636	0.1623	38.0337	1.6240	0.9799
High.26 - High.30	0.2526	0.1691	43.9963	1.4944	0.9920
High.26 - ControlT.32	0.3425	0.1508	34.1036	2.2705	0.7402
High.26 - Control.32	0.1567	0.1691	43.9963	0.9268	1.0000
High.26 - Low.32	-0.2022	0.1623	38.0337	-1.2455	0.9990
High.26 - Medium.32	0.0027	0.1623	38.0337	0.0168	1.0000
High.26 - High.32	0.1398	0.1279	4.9981	1.0936	0.9970
ControlT.28 - Control.28	-0.4725	0.1413	29.7706	-3.3425	0.1568
ControlT.28 - Low.28	-0.2699	0.1481	33.8046	-1.8226	0.9410
ControlT.28 - Medium.28	-0.4202	0.1481	33.8046	-2.8370	0.3756
ControlT.28 - High.28	-0.4191	0.1555	43.8959	-2.6955	0.4564
ControlT.28 - ControlT.30	-0.5234	0.1425	43.3399	-3.6717	0.0639

ControlT.28 - Control.30	**-0.7084**	**0.1488**	**30.1314**	**-4.7623**	**0.0055 ****
ControlT.28 - Low.30	-0.1968	0.1481	33.8046	-1.3284	0.9976
ControlT.28 - Medium.30	-0.1614	0.1481	33.8046	-1.0900	0.9998
ControlT.28 - High.30	-0.1724	0.1555	43.8959	-1.1090	0.9998
ControlT.28 - ControlT.32	-0.0826	0.1168	5.6304	-0.7070	1.0000
ControlT.28 - Control.32	-0.2684	0.1555	43.8959	-1.7262	0.9658
ControlT.28 - Low.32	**-0.6272**	**0.1481**	**33.8046**	**-4.2348**	**0.0184 ***
ControlT.28 - Medium.32	-0.4223	0.1481	33.8046	-2.8514	0.3673
ControlT.28 - High.32	-0.2852	0.1481	33.8046	-1.9259	0.9092
Control.28 - Low.28	0.2025	0.1562	35.1747	1.2967	0.9983
Control.28 - Medium.28	0.0523	0.1562	35.1747	0.3347	1.0000
Control.28 - High.28	0.0534	0.1632	43.8539	0.3273	1.0000
Control.28 - ControlT.30	-0.0509	0.1509	43.3229	-0.3374	1.0000
Control.28 - Control.30	-0.2359	0.1249	5.0981	-1.8897	0.8624
Control.28 - Low.30	0.2757	0.1562	35.1747	1.7653	0.9554
Control.28 - Medium.30	0.3110	0.1562	35.1747	1.9915	0.8849
Control.28 - High.30	0.3001	0.1632	43.8539	1.8389	0.9403
Control.28 - ControlT.32	0.3899	0.1442	30.1838	2.7037	0.4602
Control.28 - Control.32	0.2041	0.1632	43.8539	1.2509	0.9990
Control.28 - Low.32	-0.1548	0.1562	35.1747	-0.9909	1.0000
Control.28 - Medium.32	0.0501	0.1562	35.1747	0.3211	1.0000
Control.28 - High.32	0.1872	0.1562	35.1747	1.1988	0.9994
Low.28 - Medium.28	-0.1502	0.1623	38.0337	-0.9256	1.0000
Low.28 - High.28	-0.1491	0.1691	43.9963	-0.8821	1.0000
Low.28 - ControlT.30	-0.2534	0.1573	43.9196	-1.6116	0.9823
Low.28 - Control.30	-0.4385	0.1629	35.2032	-2.6915	0.4640
Low.28 - Low.30	0.0732	0.1623	38.0337	0.4509	1.0000
Low.28 - Medium.30	0.1085	0.1623	38.0337	0.6684	1.0000
Low.28 - High.30	0.0975	0.1691	43.9963	0.5769	1.0000
Low.28 - ControlT.32	0.1874	0.1508	34.1036	1.2422	0.9990
Low.28 - Control.32	0.0016	0.1691	43.9963	0.0093	1.0000
Low.28 - Low.32	-0.3573	0.1623	38.0337	-2.2011	0.7827
Low.28 - Medium.32	-0.1524	0.1623	38.0337	-0.9388	1.0000
Low.28 - High.32	-0.0153	0.1623	38.0337	-0.0943	1.0000

Medium.28 - High.28	0.0011	0.1691	43.9963	0.0067	1.0000
Medium.28 - ControlT.30	-0.1032	0.1573	43.9196	-0.6561	1.0000
Medium.28 - Control.30	-0.2882	0.1629	35.2032	-1.7692	0.9546
Medium.28 - Low.30	0.2234	0.1623	38.0337	1.3765	0.9966
Medium.28 - Medium.30	0.2588	0.1623	38.0337	1.5941	0.9833
Medium.28 - High.30	0.2478	0.1691	43.9963	1.4657	0.9936
Medium.28 - ControlT.32	0.3376	0.1508	34.1036	2.2383	0.7595
Medium.28 - Control.32	0.1518	0.1691	43.9963	0.8981	1.0000
Medium.28 - Low.32	-0.2070	0.1623	38.0337	-1.2754	0.9987
Medium.28 - Medium.32	-0.0021	0.1623	38.0337	-0.0131	1.0000
Medium.28 - High.32	0.1349	0.1623	38.0337	0.8314	1.0000
High.28 - ControlT.30	-0.1043	0.1642	39.7100	-0.6353	1.0000
High.28 - Control.30	-0.2893	0.1696	43.7439	-1.7058	0.9693
High.28 - Low.30	0.2223	0.1691	43.9963	1.3150	0.9982
High.28 - Medium.30	0.2576	0.1691	43.9963	1.5239	0.9901
High.28 - High.30	0.2467	0.1755	39.7100	1.4051	0.9958
High.28 - ControlT.32	0.3365	0.1581	43.8936	2.1288	0.8246
High.28 - Control.32	0.1507	0.1755	39.7100	0.8585	1.0000
High.28 - Low.32	-0.2082	0.1691	43.9963	-1.2313	0.9992
High.28 - Medium.32	-0.0033	0.1691	43.9963	-0.0193	1.0000
High.28 - High.32	0.1338	0.1691	43.9963	0.7916	1.0000
ControlT.30 - Control.30	-0.1850	0.1579	43.1130	-1.1722	0.9996
ControlT.30 - Low.30	0.3266	0.1573	43.9196	2.0770	0.8507
ControlT.30 - Medium.30	0.3619	0.1573	43.9196	2.3016	0.7233
ControlT.30 - High.30	0.3510	0.1642	39.7100	2.1374	0.8185
ControlT.30 - ControlT.32	0.4408	0.1454	43.3512	3.0322	0.2607
ControlT.30 - Control.32	0.2550	0.1642	39.7100	1.5530	0.9874
ControlT.30 - Low.32	-0.1039	0.1573	43.9196	-0.6604	1.0000
ControlT.30 - Medium.32	0.1010	0.1573	43.9196	0.6426	1.0000
ControlT.30 - High.32	0.2381	0.1573	43.9196	1.5143	0.9908
Control.30 - Low.30	0.5117	0.1629	35.2032	3.1407	0.2209
Control.30 - Medium.30	0.5470	0.1629	35.2032	3.3575	0.1433
Control.30 - High.30	0.5360	0.1696	43.7439	3.1600	0.2029
Control.30 - ControlT.32	**0.6258**	**0.1515**	**30.5134**	**4.1317**	**0.0265 ***

Control.30 - Control.32	0.4400	0.1696	43.7439	2.5943	0.5248
Control.30 - Low.32	0.0812	0.1629	35.2032	0.4984	1.0000
Control.30 - Medium.32	0.2861	0.1629	35.2032	1.7561	0.9574
Control.30 - High.32	0.4232	0.1629	35.2032	2.5976	0.5257
Low.30 - Medium.30	0.0353	0.1623	38.0337	0.2176	1.0000
Low.30 - High.30	0.0243	0.1691	43.9963	0.1440	1.0000
Low.30 - ControlT.32	0.1142	0.1508	34.1036	0.7570	1.0000
Low.30 - Control.32	-0.0716	0.1691	43.9963	-0.4235	1.0000
Low.30 - Low.32	-0.4305	0.1279	4.9981	-3.3670	0.3246
Low.30 - Medium.32	-0.2256	0.1623	38.0337	-1.3896	0.9962
Low.30 - High.32	-0.0885	0.1623	38.0337	-0.5451	1.0000
Medium.30 - High.30	-0.0110	0.1691	43.9963	-0.0649	1.0000
Medium.30 - ControlT.32	0.0789	0.1508	34.1036	0.5228	1.0000
Medium.30 - Control.32	-0.1069	0.1691	43.9963	-0.6325	1.0000
Medium.30 - Low.32	-0.4658	0.1623	38.0337	-2.8695	0.3525
Medium.30 - Medium.32	-0.2609	0.1279	4.9981	-2.0405	0.8071
Medium.30 - High.32	-0.1238	0.1623	38.0337	-0.7627	1.0000
High.30 - ControlT.32	0.0898	0.1581	43.8936	0.5683	1.0000
High.30 - Control.32	-0.0960	0.1755	39.7100	-0.5466	1.0000
High.30 - Low.32	-0.4548	0.1691	43.9963	-2.6903	0.4598
High.30 - Medium.32	-0.2499	0.1691	43.9963	-1.4783	0.9929
High.30 - High.32	-0.1128	0.1691	43.9963	-0.6674	1.0000
ControlT.32 - Control.32	-0.1858	0.1581	43.8936	-1.1754	0.9996
ControlT.32 - Low.32	-0.5446	0.1508	34.1036	-3.6108	0.0836
ControlT.32 - Medium.32	-0.3397	0.1508	34.1036	-2.2524	0.7511
ControlT.32 - High.32	-0.2027	0.1508	34.1036	-1.3436	0.9973
Control.32 - Low.32	-0.3589	0.1691	43.9963	-2.1227	0.8278
Control.32 - Medium.32	-0.1540	0.1691	43.9963	-0.9107	1.0000
Control.32 - High.32	-0.0169	0.1691	43.9963	-0.0998	1.0000
Low.32 - Medium.32	0.2049	0.1623	38.0337	1.2623	0.9988
Low.32 - High.32	0.3420	0.1623	38.0337	2.1068	0.8336
Medium.32 - High.32	0.1371	0.1623	38.0337	0.8445	1.0000

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Supplementary Information S4. Complementary graphs: *P. flava* fragment changes in surface area as a function of time

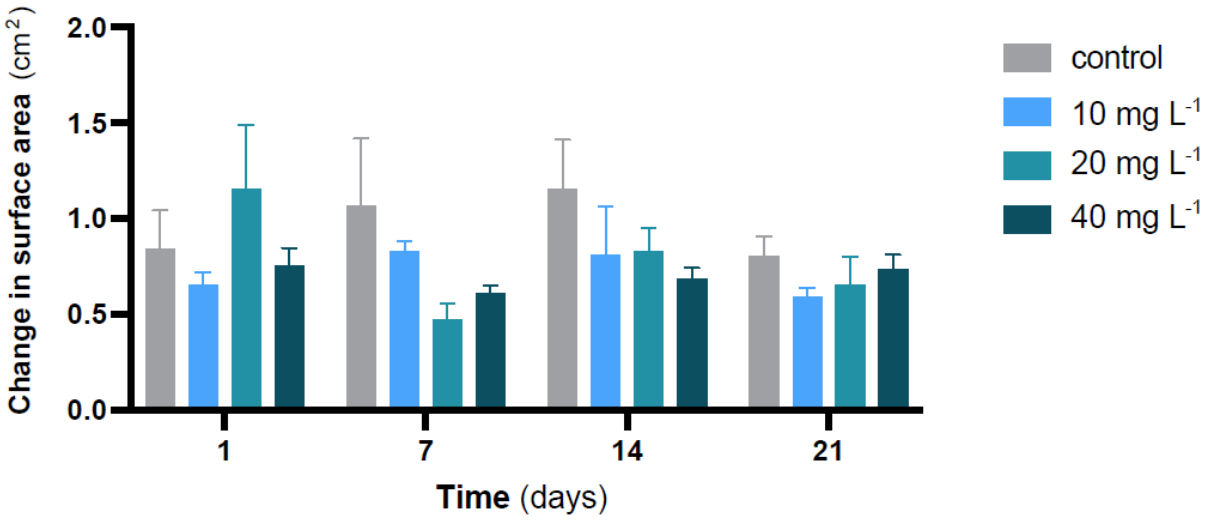


Figure S4.1 | Changes in *P. flava* fragments surface area as a function of time for the first experimental stage. Including Individual DOC additions as treatment.

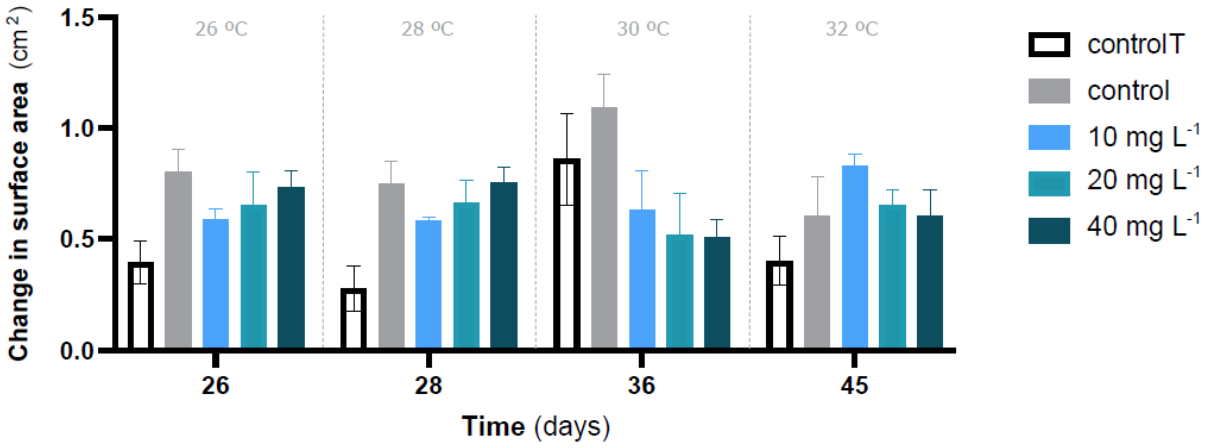


Figure S4.2 | Changes in *P. flava* fragments surface area as a function of time for the second experimental stage. Including simultaneous warming and DOC addition as treatments.

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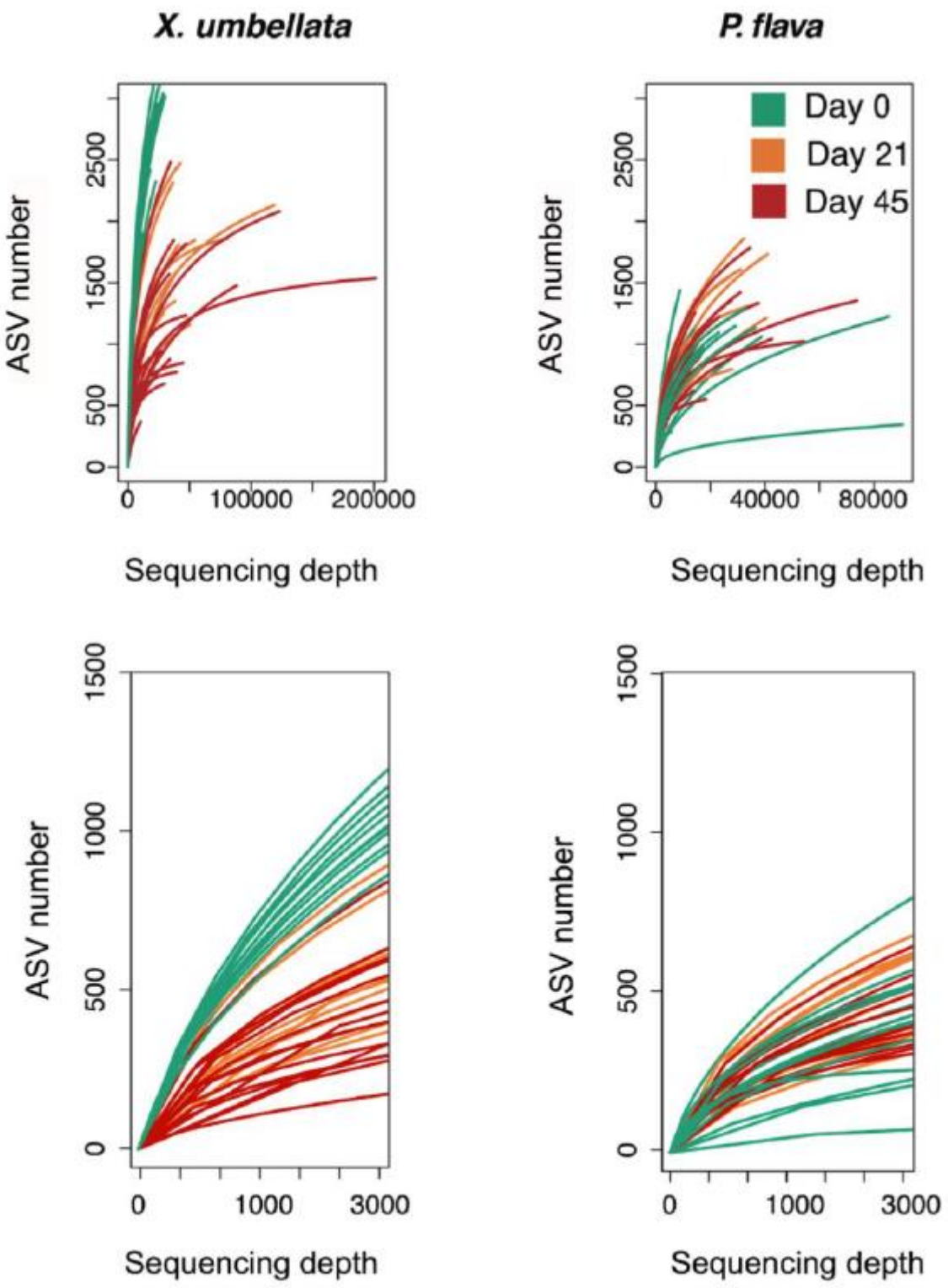

Figure S1.1 | Rarefaction curves of bacterial communities in corals *X. umbellata* and *P. flava*. (a) ASV numbers with sequencing depth of 200,000 in *X. umbellata* and ASV numbers with sequencing depth of 80,000 in *P. flava*. **(b)** ASV numbers at the minimum library size with sequencing depth of 3,000 in corals *X. umbellata* and *P. flava*.

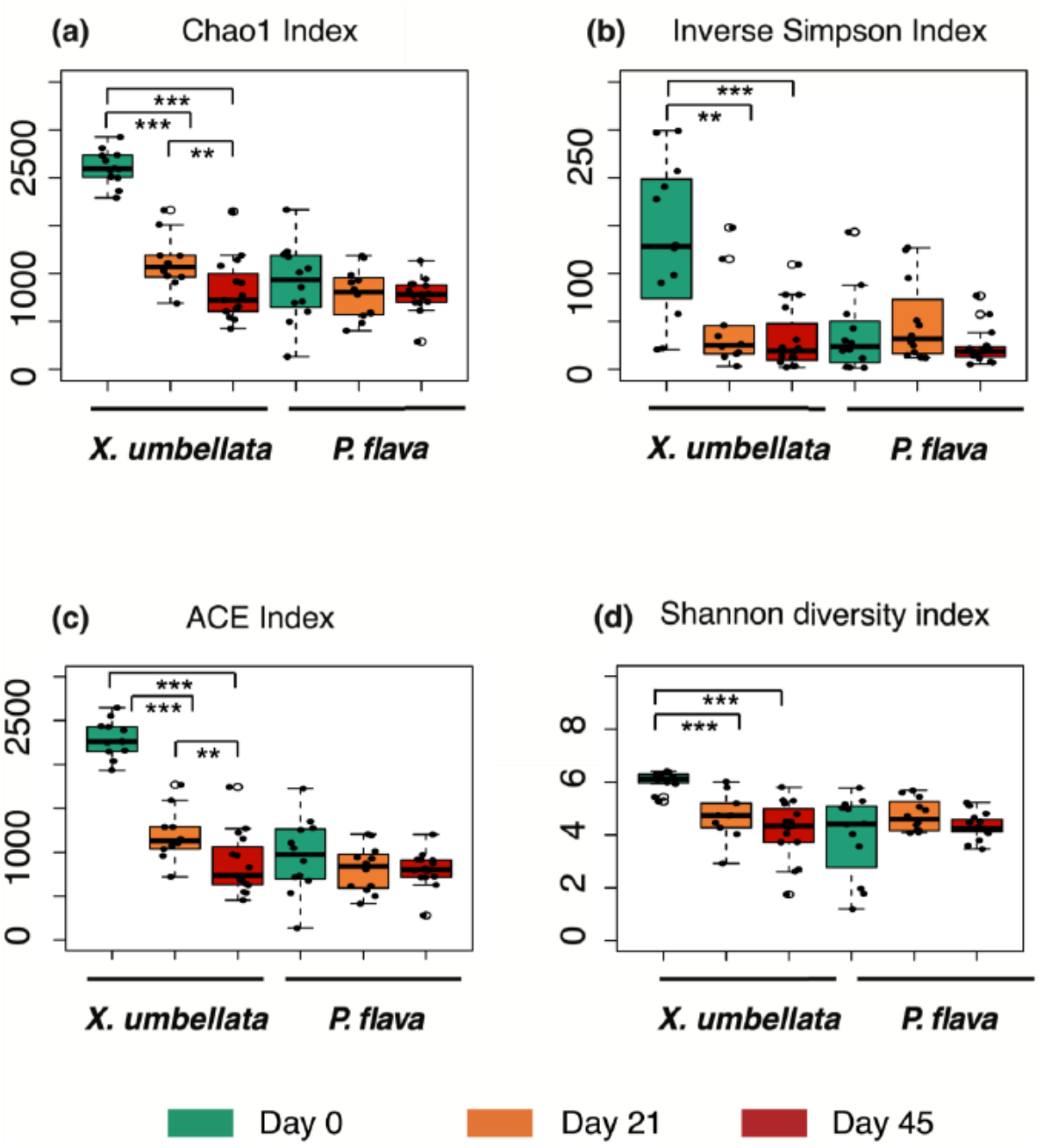

Figure S1.2 | Alpha diversity of bacterial communities at the minimum library size with sequencing depth of 3,000 in corals *X. umbellata* and *P. flava*. The asterisks indicate statistically significant differences compared to day 0 for each species separately (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). **(a)** Chao1 index. **(b)** Inverse Simpson Index. **(c)** ACE index. **(d)** Shannon diversity index.

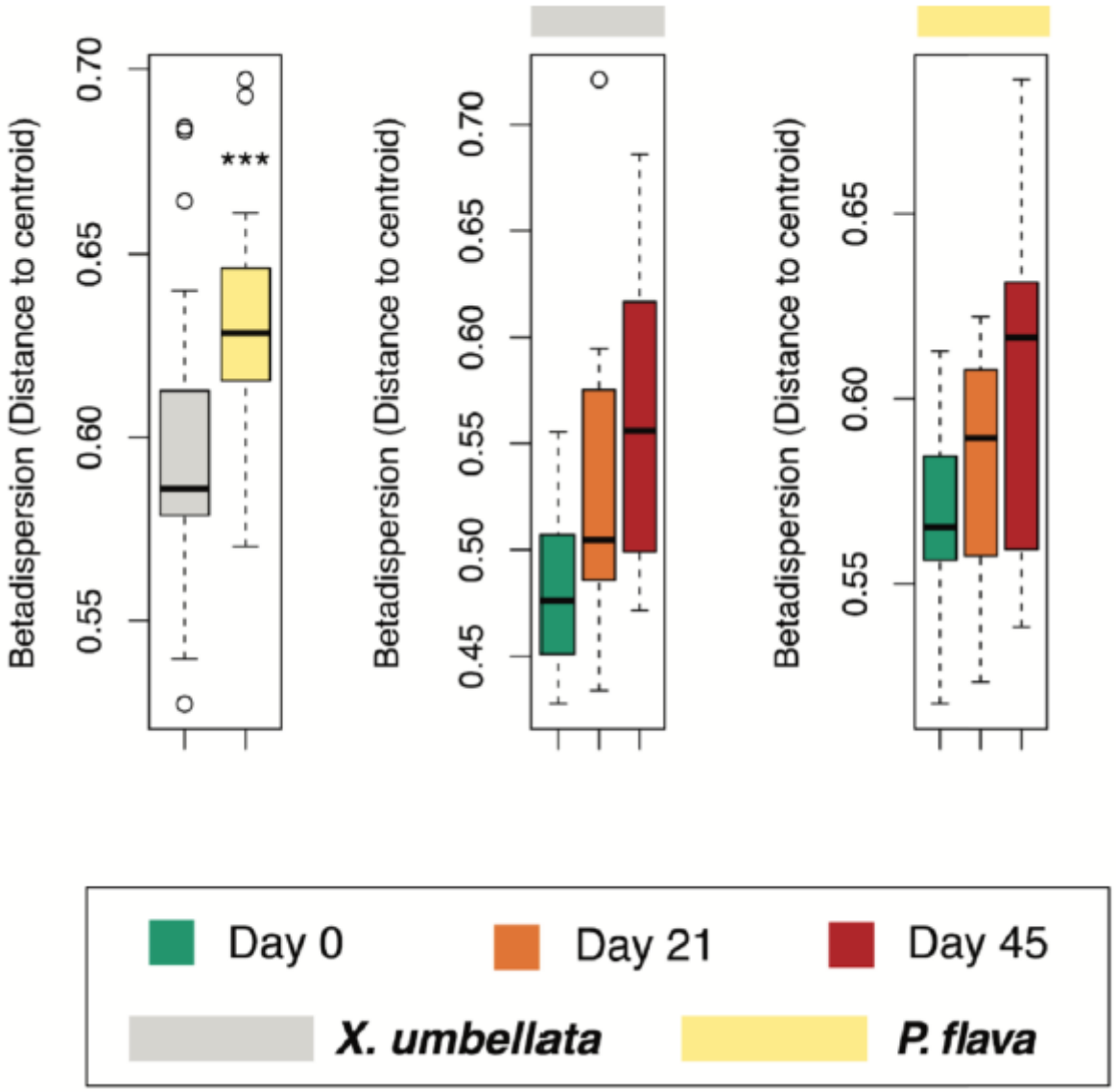

Figure S1.3 | Multivariate dispersion of bacterial communities in corals *X. umbellata* and *P. flava*. The asterisks indicate statistically significant differences between groups (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

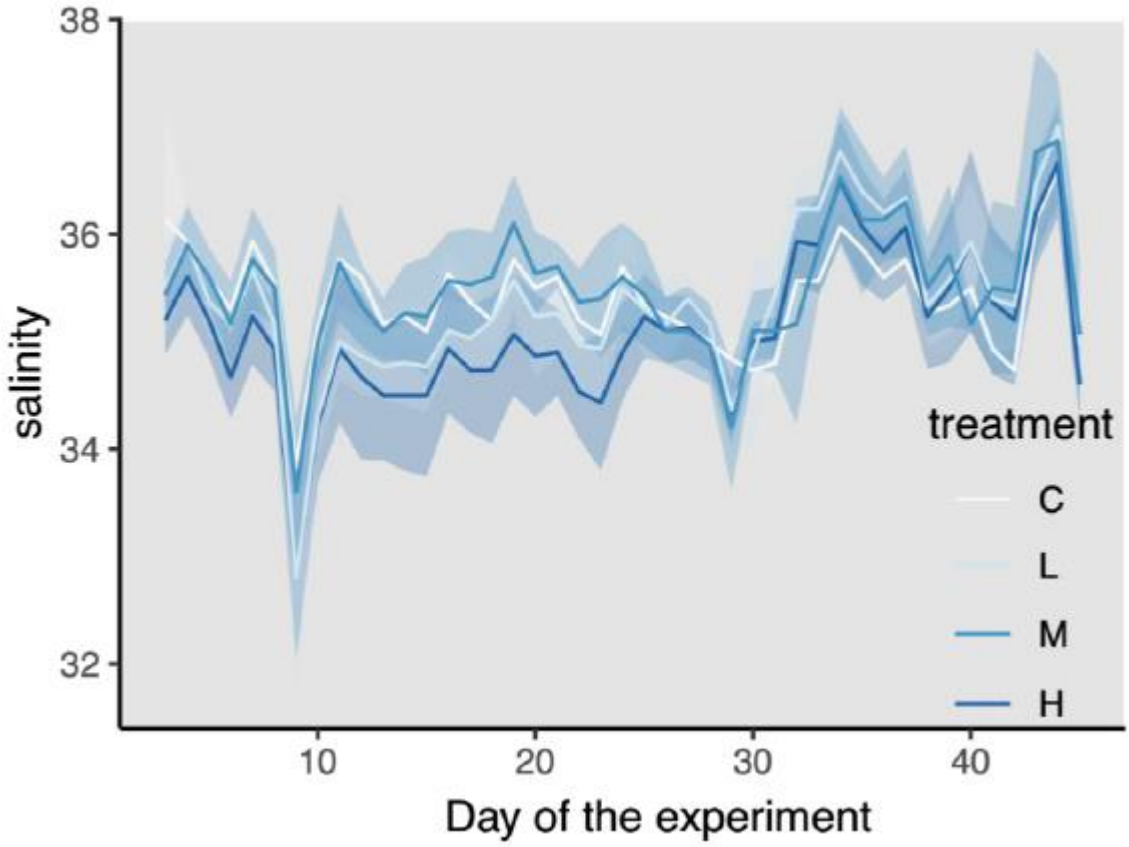

Figure S1.4 | The fluctuations in salinity (daily measurement) throughout the experiment. Values are shown by means ± SE.

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Supplementary Information S1.

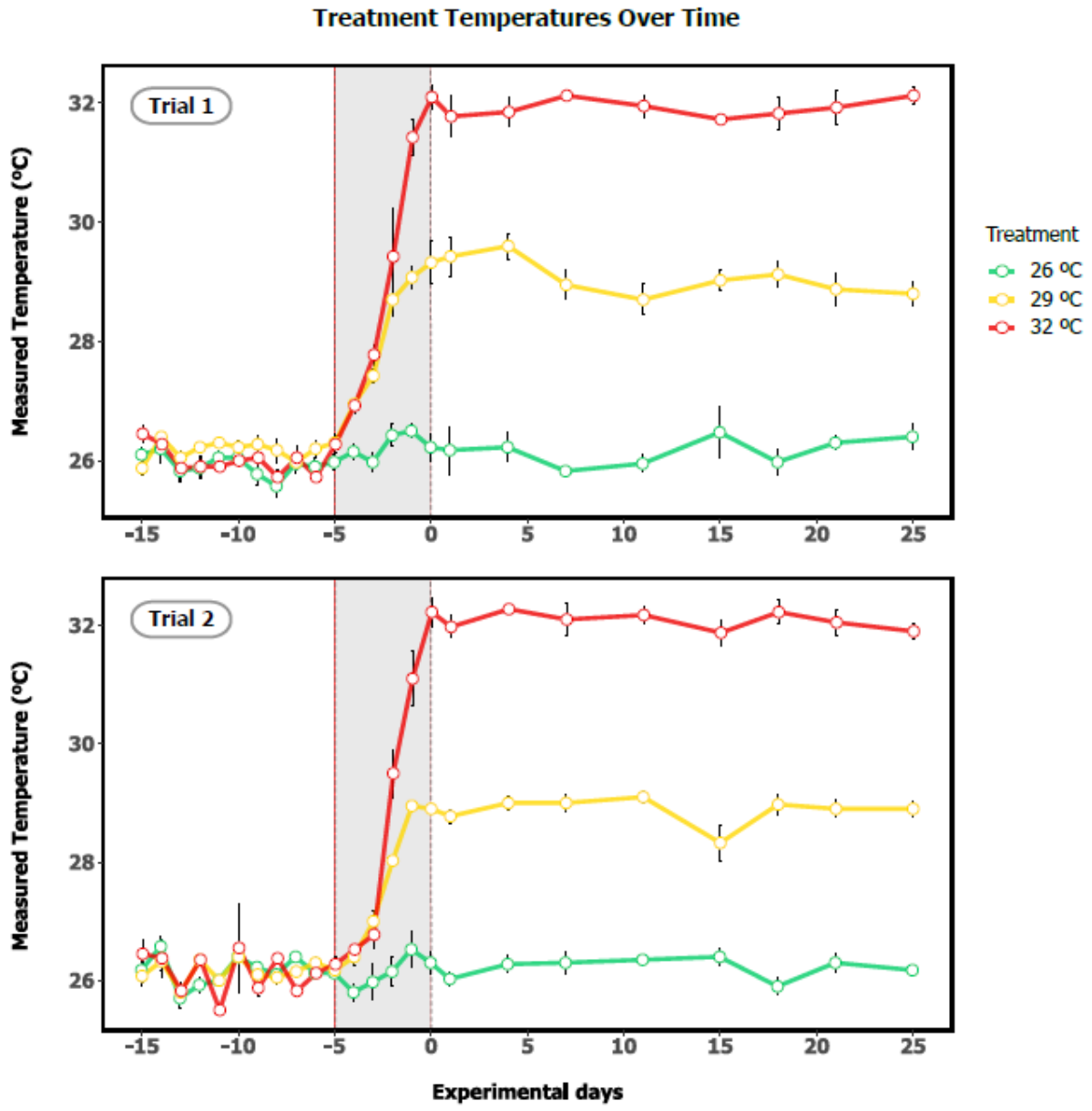

Figure S1.1 | Experimental system temperatures over time. Aquaria temperatures (mean ± s.e.m.) recorded for each implemented treatment throughout the study. 26°C represents the control condition (green), 29°C the intermediate heat-stressed conditions (yellow) and 32°C the acute heat-stressed condition (red). The data depicts the three experimental phases undergone during each experimental trial: acclimation, temperature increase phase (grey delimitated region), and finally the sustained warming phase. Each data point shows the mean temperature value recorded per treatment over time, where n = 4 independent tanks per treatment for each trial.

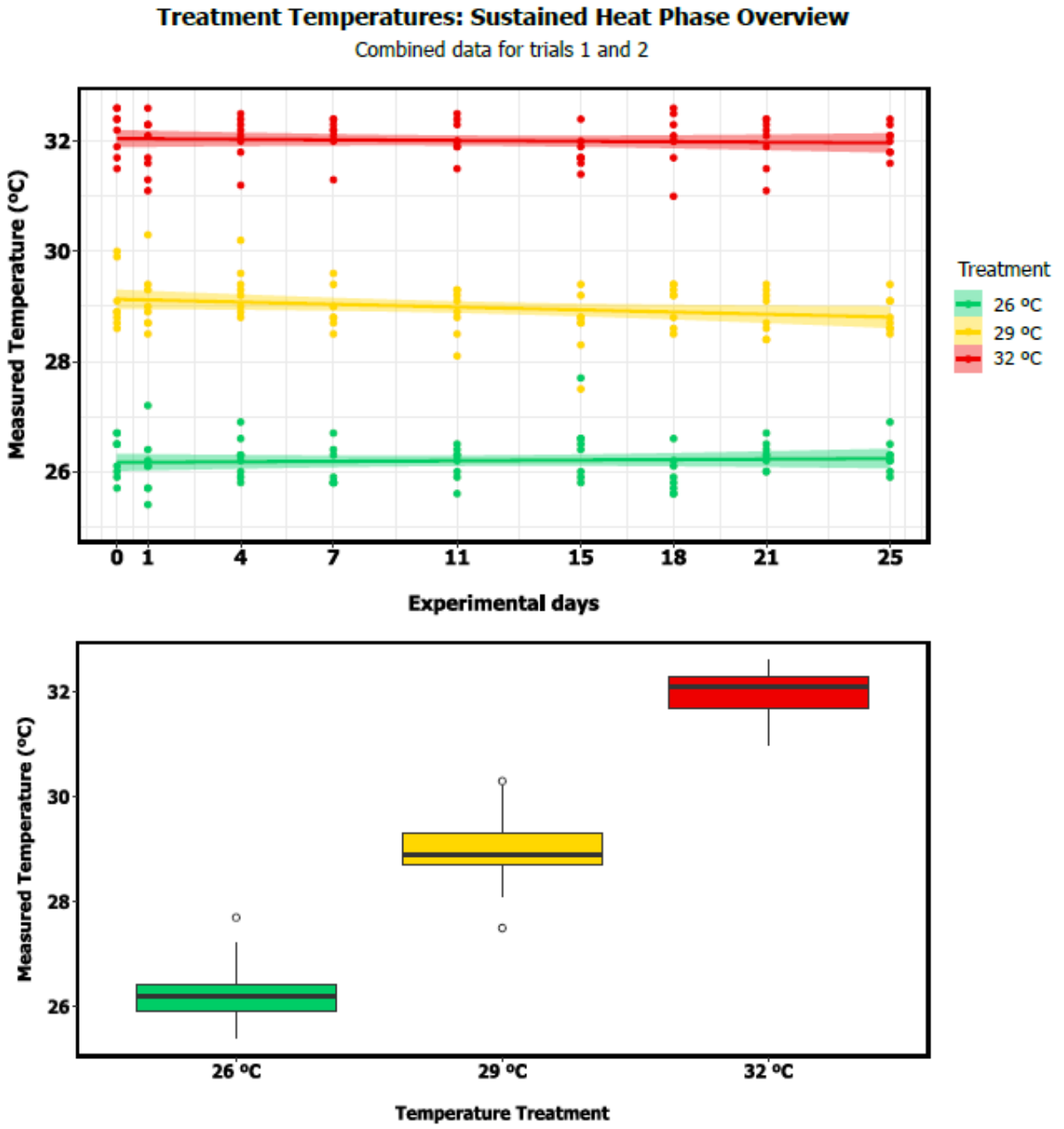

Figure S1.2 | Detailed overview on the temperature treatment Behaviour during the sustained warming phase the of experimental manipulation. The summary graphs portray the Behaviour for the combined trials 1 and 2. There were no significant differences between both trials when assessing the temperature treatments imposed (LMM; $p > 0.05$).

Supplementary Information S2. Observed bleaching pairwise comparisons for the temperature factor. Tukey plots representing adjusted P-values of the calculated marginal means for the observed bleaching response of *X. umbellata* under sustained warming scenarios.

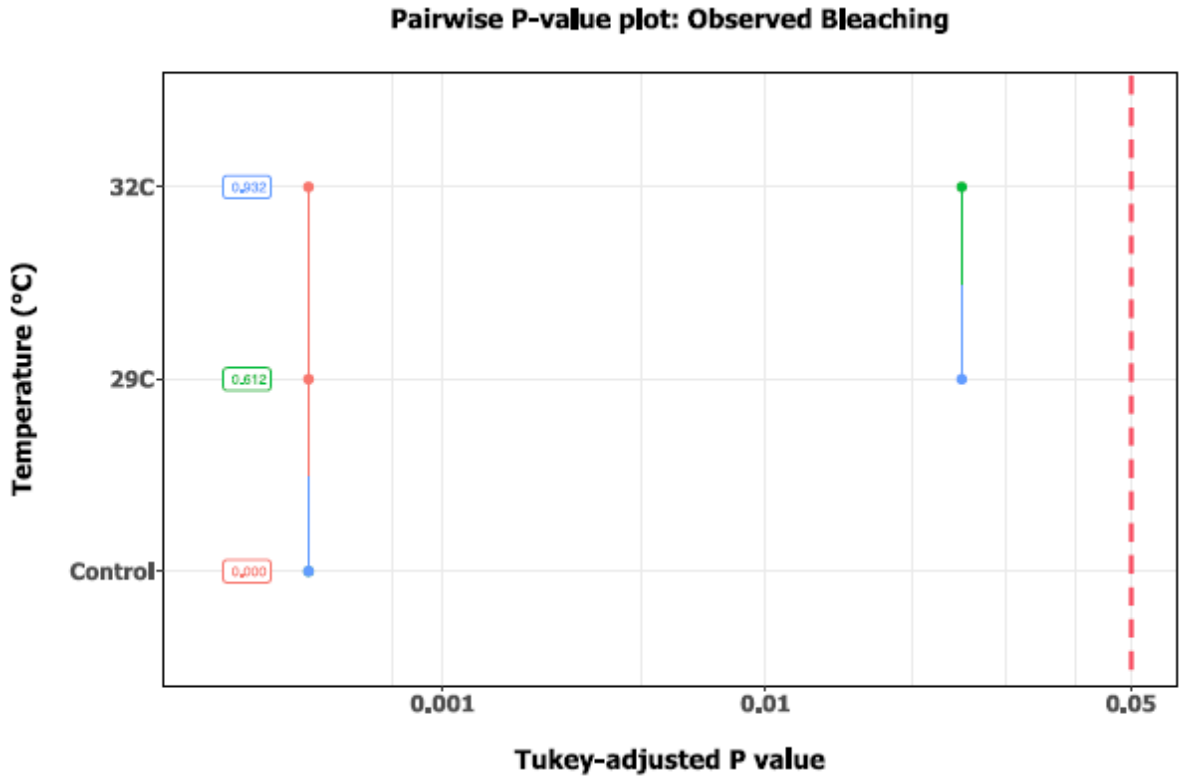

Figure S2.1 | Temperature pairwise comparison for *X. umbellata* observed bleaching response through the experiment. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S3.

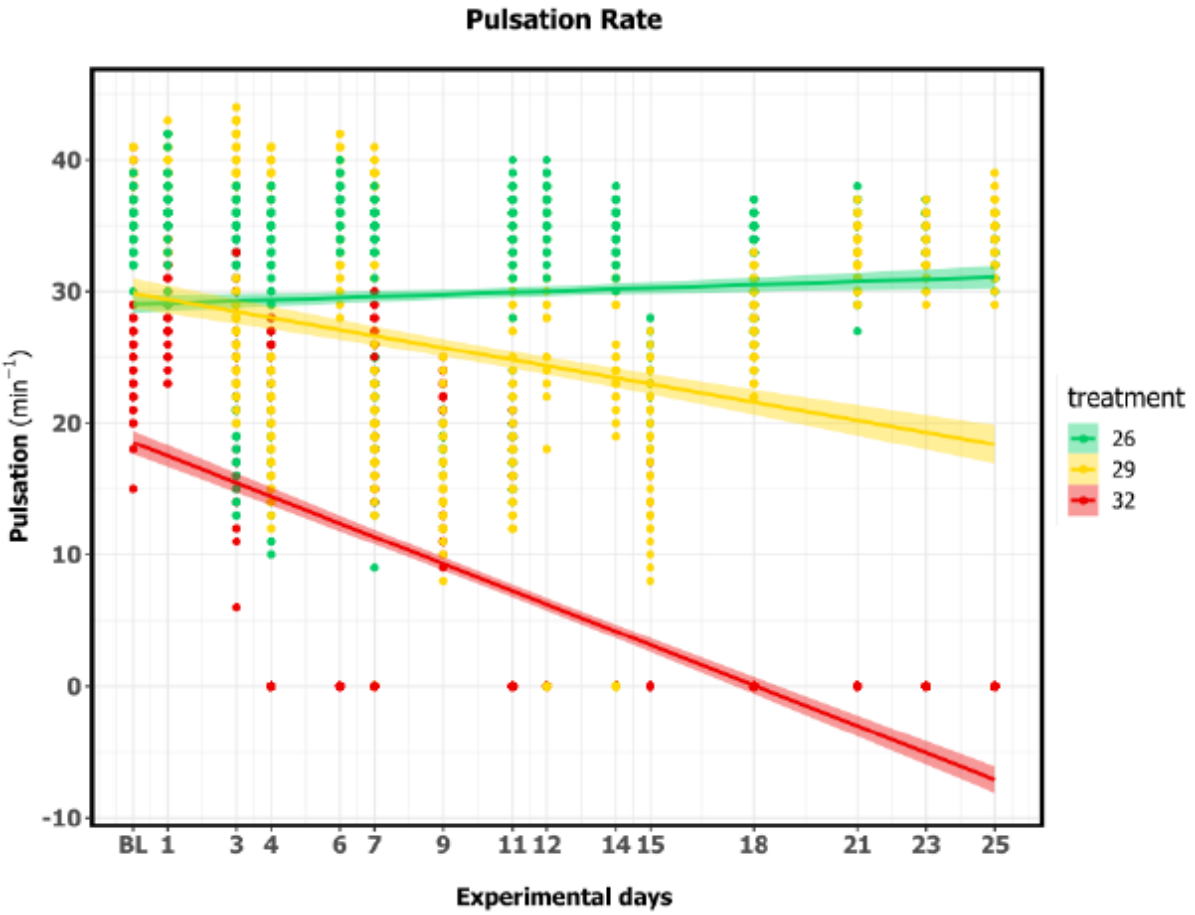

Figure S3.1 | *X. umbellata* pulsation rates under sustained warming scenarios over time. Linear fit showing the coral polyps pulsation behavior over time as a function of temperature. The color code indicates warming treatments where 26°C represents the control condition (green), and 29°C and 32°C heat-stressed conditions (yellow and red respectively). Data depicts n = 8 replicates per treatment, with pulsation counted for 4 polyps per coral fragment, and 5 fragments measured per tank overtime.

Supplementary Information S4. Pulsation rates pairwise comparisons for the temperature and time factors. Tukey plots representing adjusted P-values of the calculated marginal means for polyp pulsation rates (min^{-1}) of *X. umbellata* corals under sustained warming.

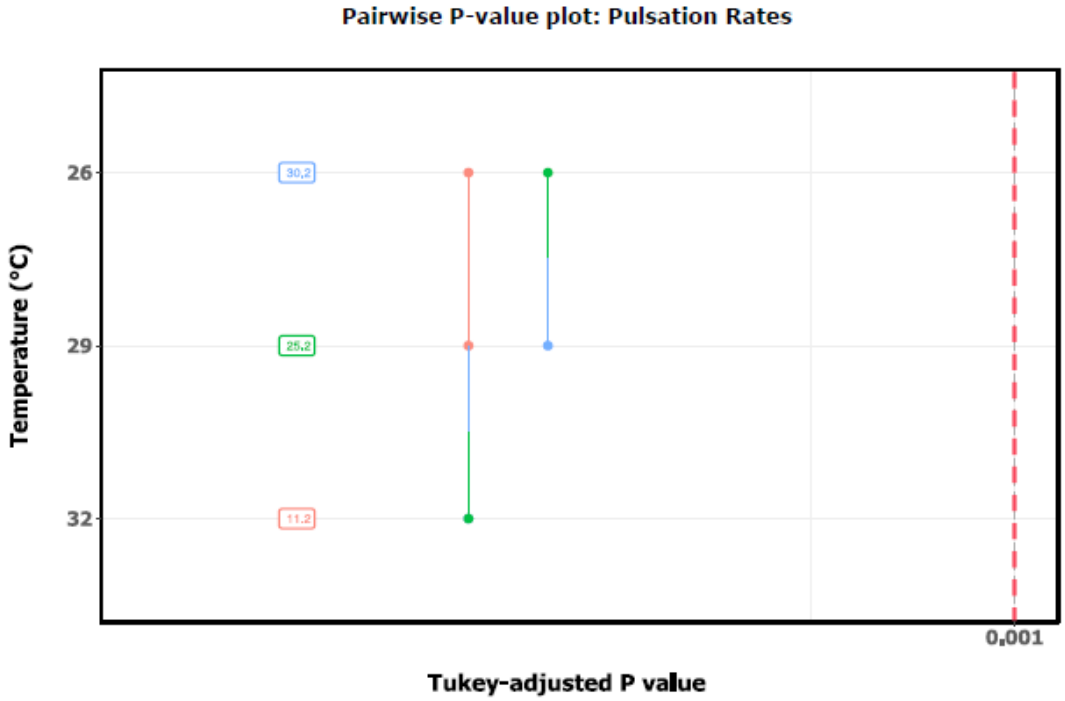

Figure S4.1 | Temperature factor pairwise comparisons for *X. umbellata* pulsation rates. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

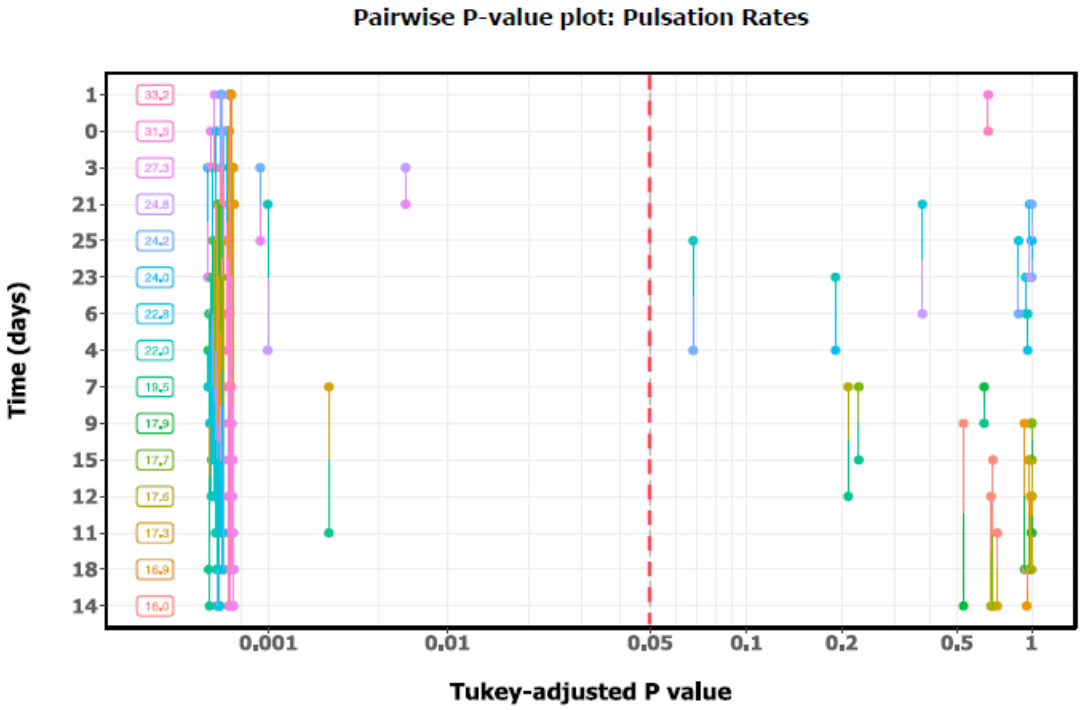

Figure S4.2 | Time factor pairwise comparisons for *X. umbellata* pulsation rates. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S5. Growth rates pairwise comparisons for the temperature factor. Tukey plots representing adjusted P-values of the calculated marginal means for coral growth rates (mm^2 day^{-1}) of *X. umbellata* corals under sustained warming.

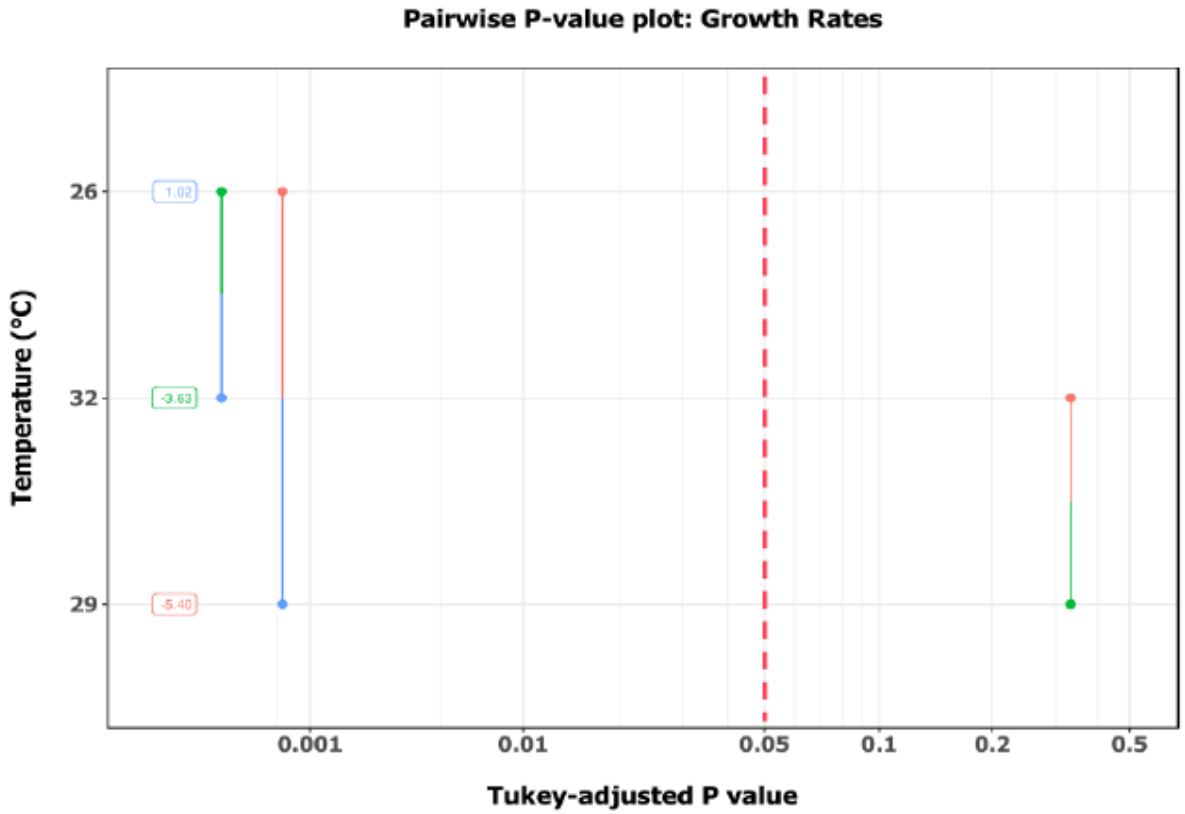

Figure S5.1 | Temperature factor pairwise comparisons for *X. umbellata* growth rates. The red segmented line indicates the P-value threshold for statistical significance ($\alpha = 0.05$).

Supplementary Information S6.

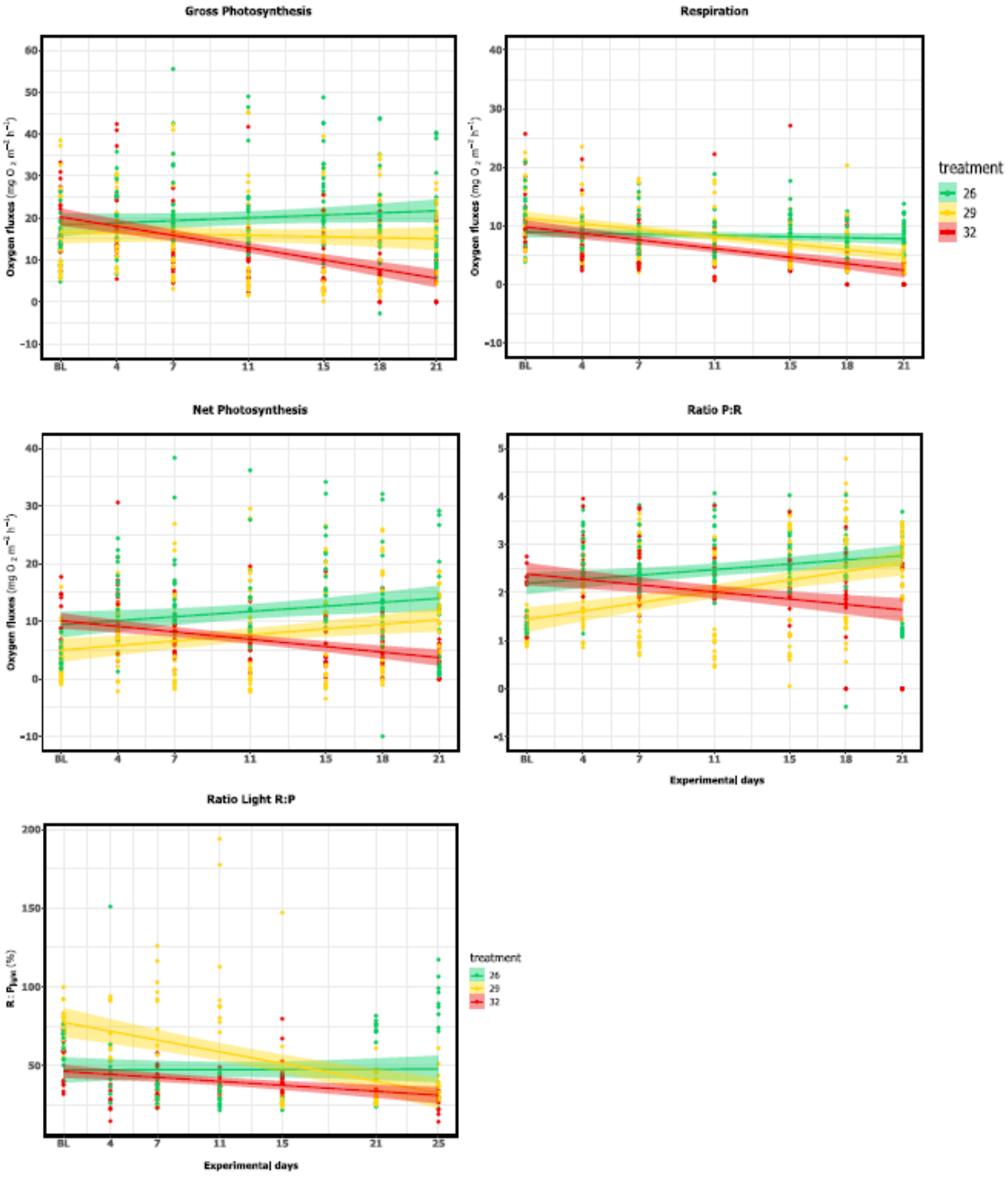

Figure S6.1 | *X. umbellata* gross photosynthesis, respiration, net photosynthesis rates, (mg O_2 m^{-2} h^{-1}), P:R and light R:P ratios under sustained warming. Linear fit portraying the parameters behavior as a function of temperature where 26°C represents the control condition (green), and 29°C and 32°C heat-stressed conditions (yellow and red respectively). Data depicts n = 8 replicates per treatment, with 3 corals measured over time per tank, and by each treatment.

Supplementary Information S7. Oxygen flux parameters pairwise comparisons for the temperature factor. Tukey plots representing adjusted P-values of the calculated marginal means for: gross and net photosynthesis, respiration rates and the P:R ratio of *X. umbellata* under sustained warming.

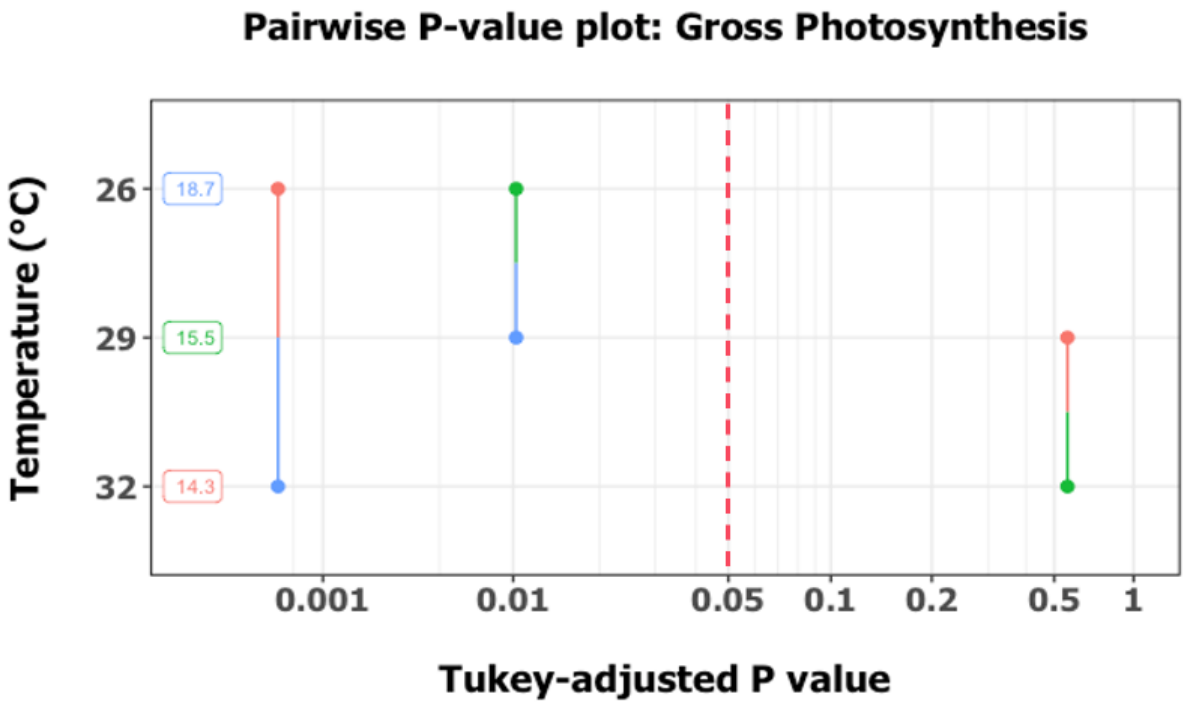

Figure S7.1 | Temperature pairwise comparison for *X. umbellata* gross photosynthesis. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

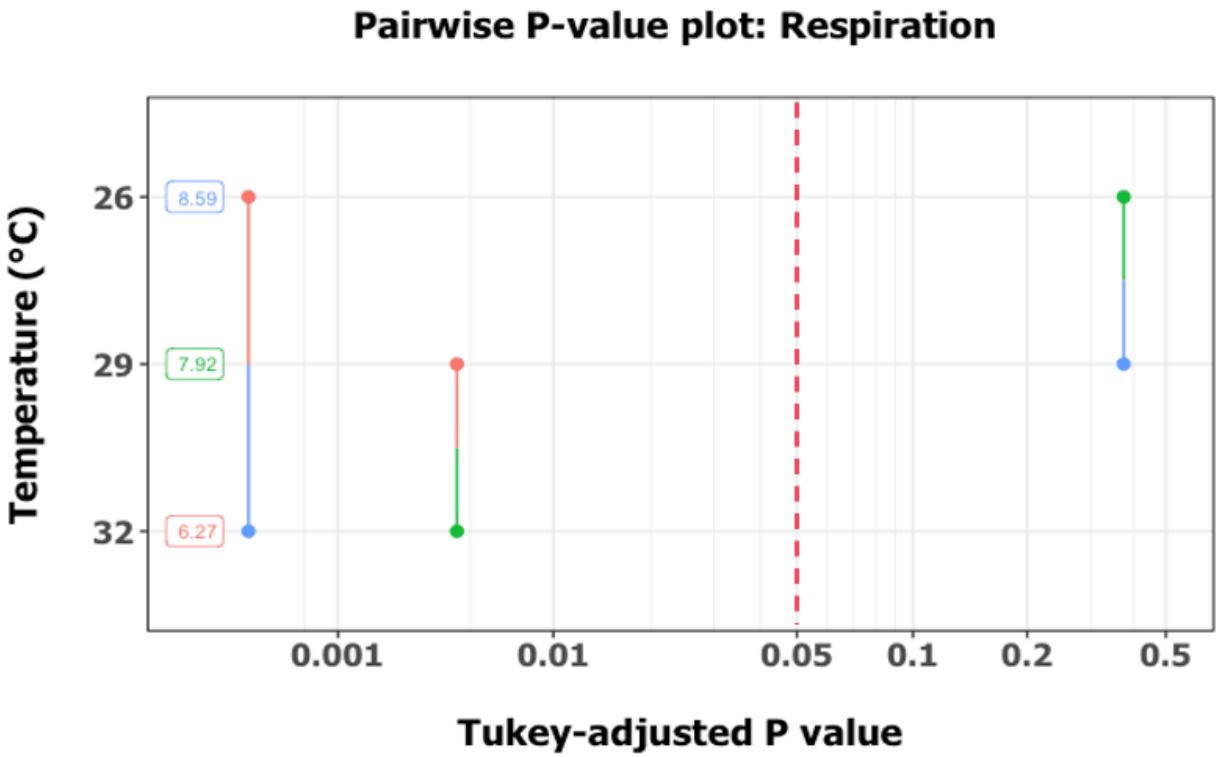

Figure S7.2 | Temperature pairwise comparison for *X. umbellata* respiration. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

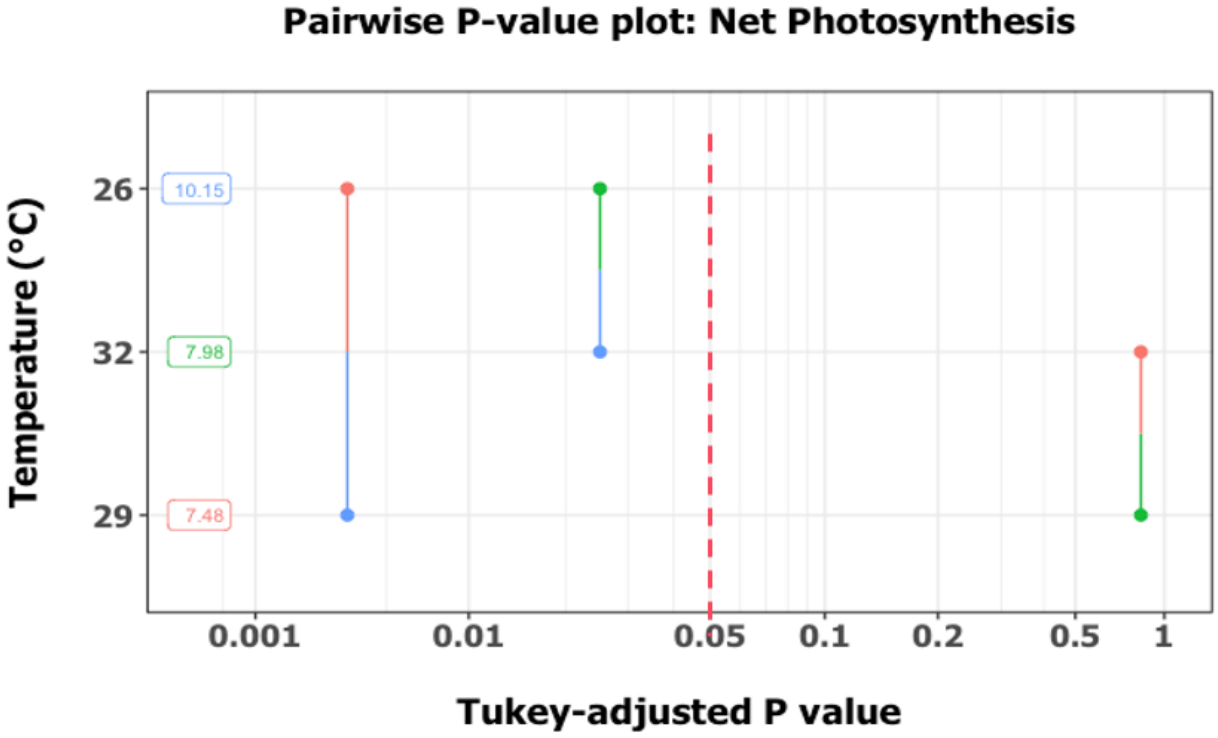

Figure S7.3 | Temperature pairwise comparison for *X. umbellata* net photosynthesis. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

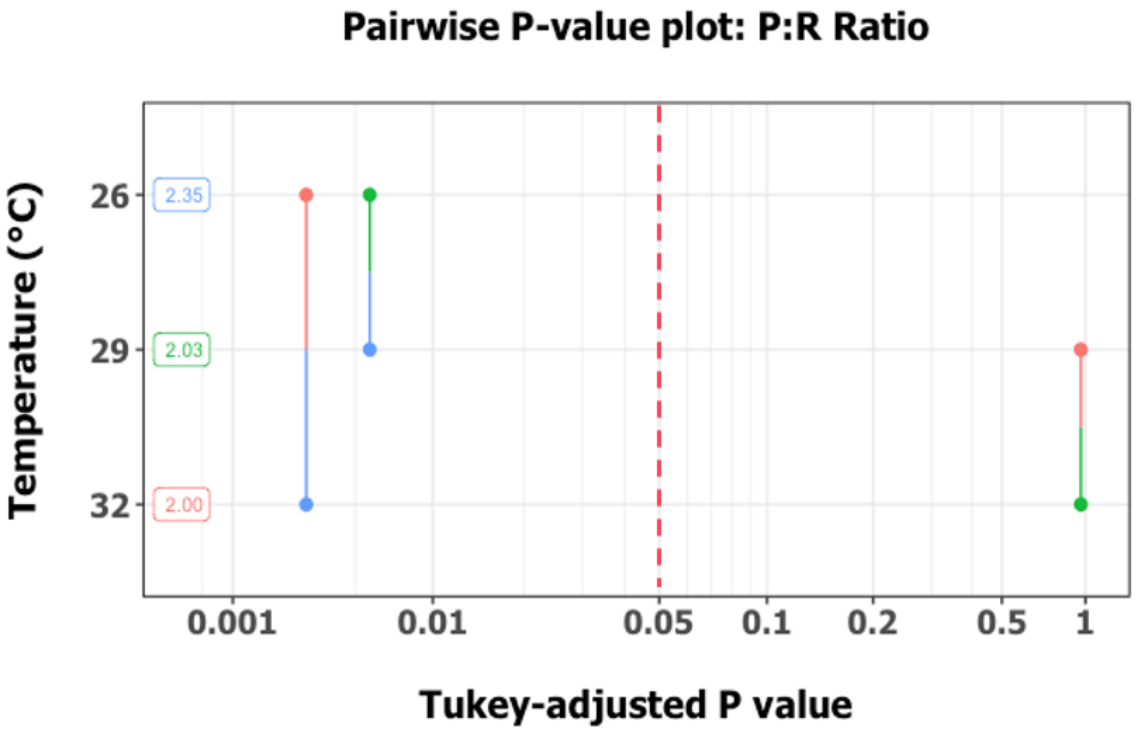

Figure S7.4 | Temperature pairwise comparison for *X. umbellata* P:R ratio. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

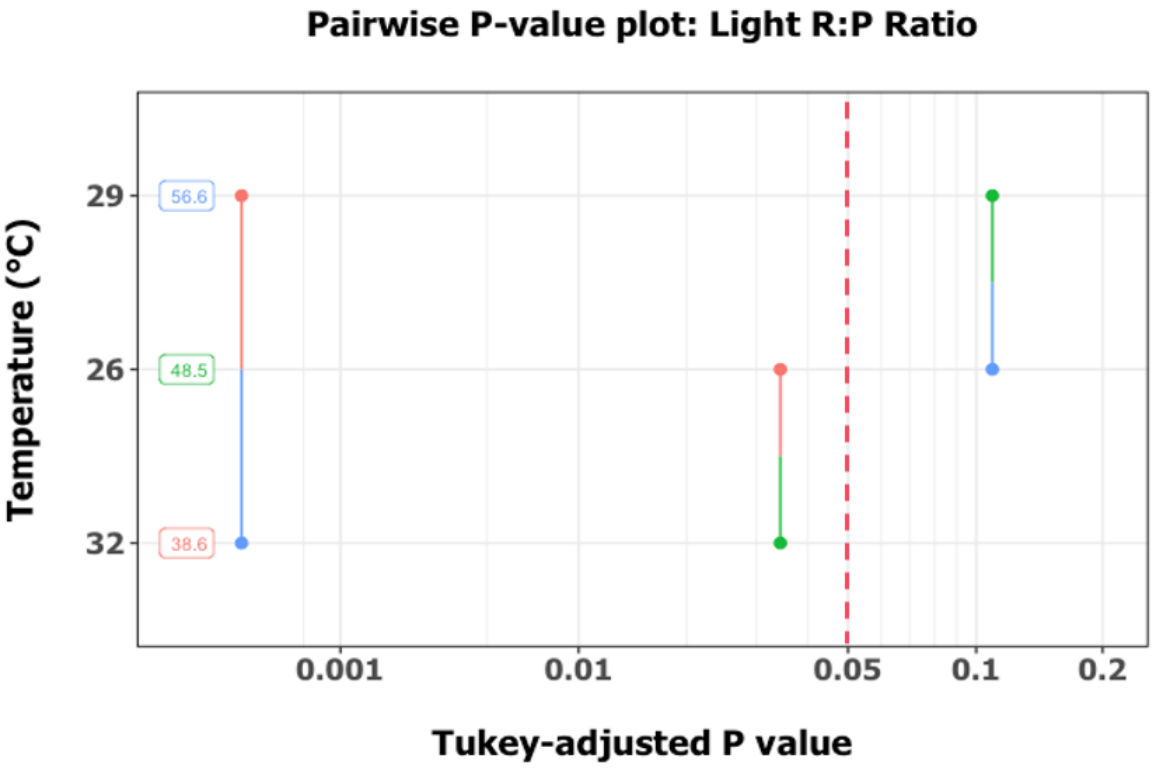

Figure S7.5 | Temperature pairwise comparison for *X. umbellata* light R:P ratio. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S8. Oxygen flux parameters pairwise comparisons for the time factor. Tukey plots representing adjusted P-values of the calculated marginal means for gross and net photosynthesis, respiration rates and the P:R ratio of *X. umbellata* corals under sustained warming.

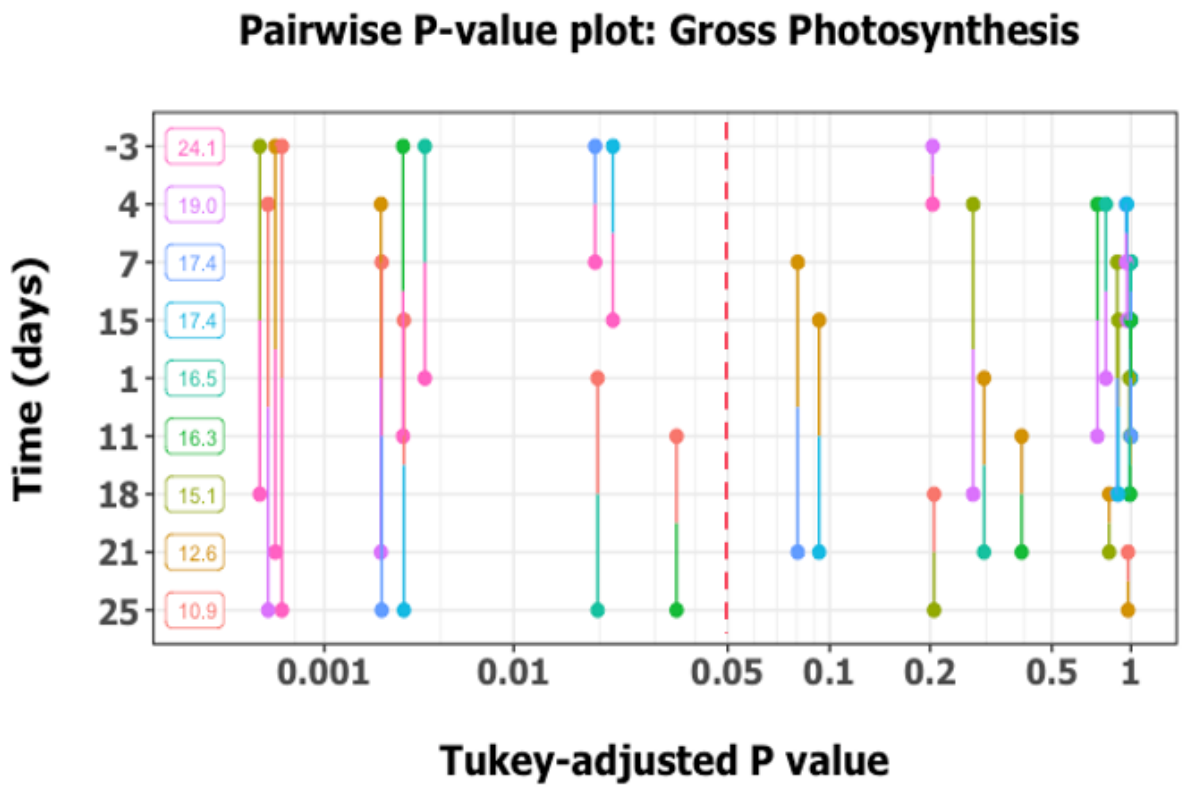

Figure S8.1 | Time factor pairwise comparisons for *X. umbellata* gross photosynthesis. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance ($\alpha = 0.05$).

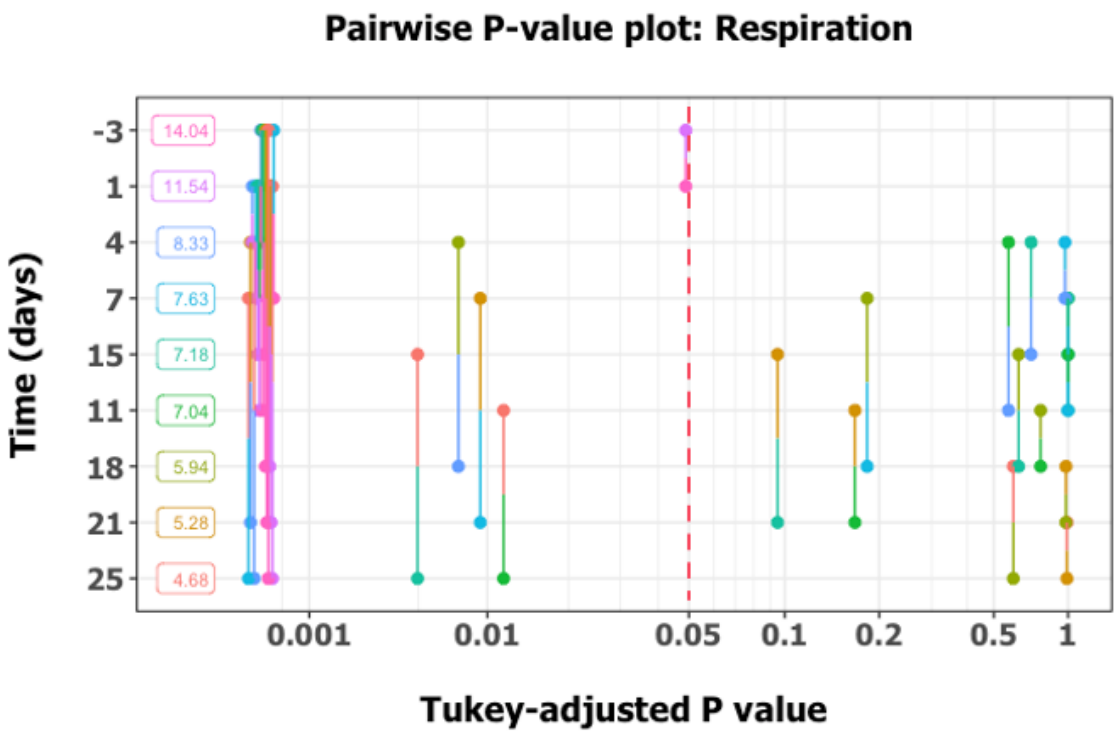

Figure S8.2 | Time factor pairwise comparisons for *X. umbellata* respiration. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance ($\alpha = 0.05$).

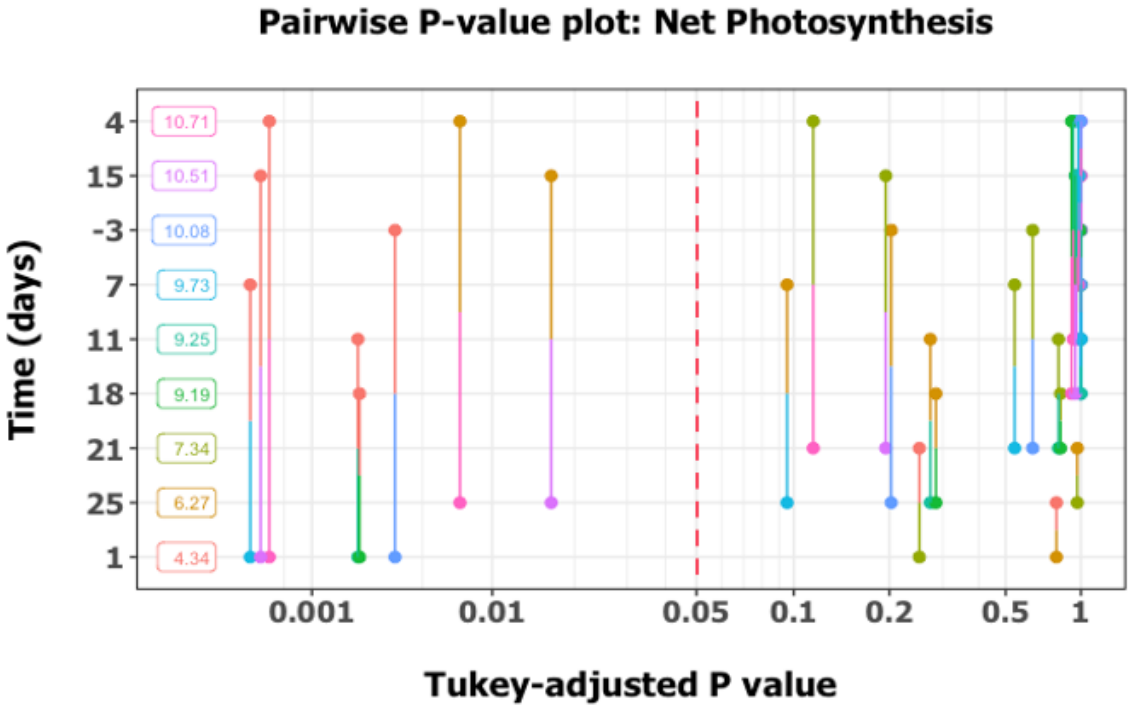

Figure S8.3 | Time factor pairwise comparisons for *X. umbellata* net photosynthesis. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance ($\alpha = 0.05$).

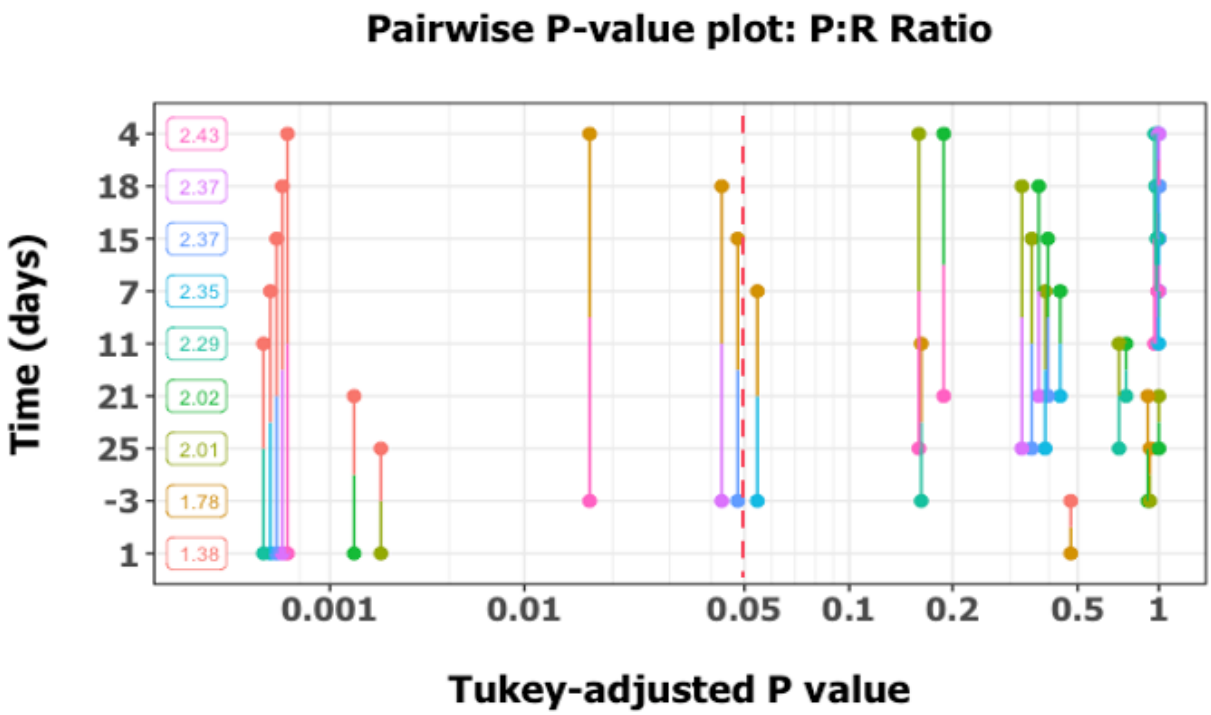

Figure S8.4 | Time factor pairwise comparisons for *X. umbellata* P:R ratio. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

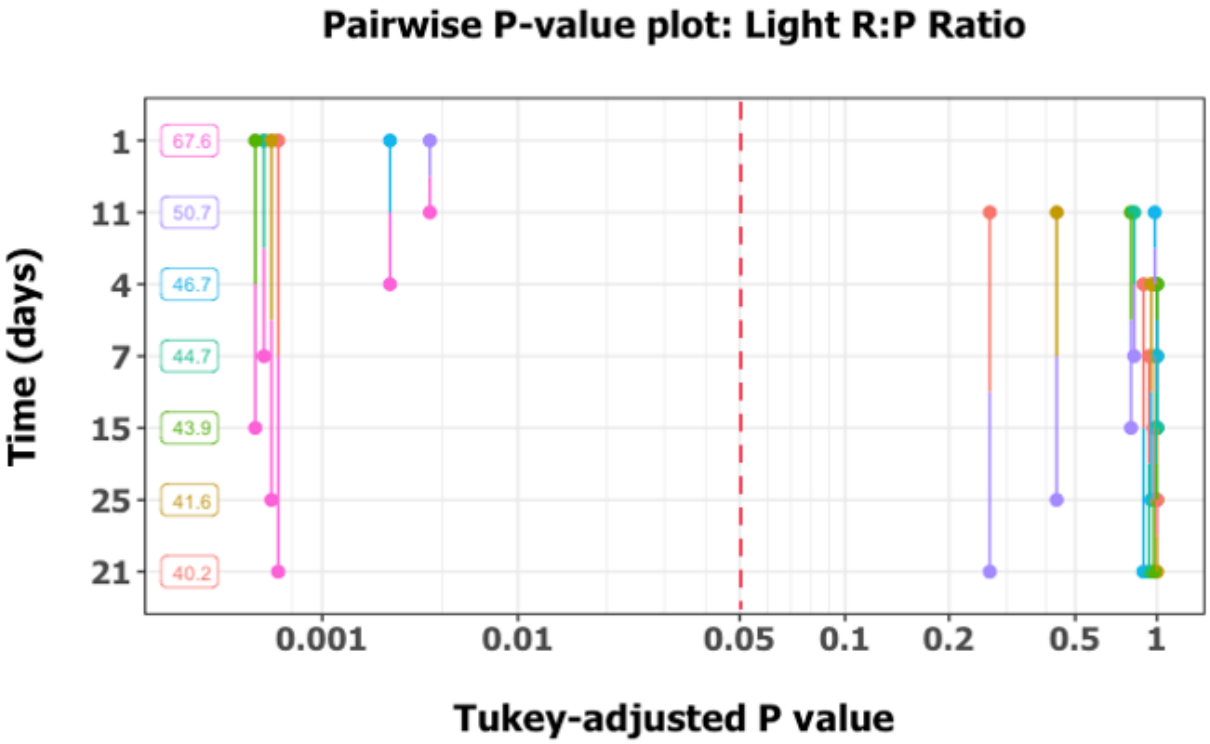

Figure S8.5 | Time factor pairwise comparisons for *X. umbellata* light R:P ratio. Tukey adjusted P-value plot. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S9.

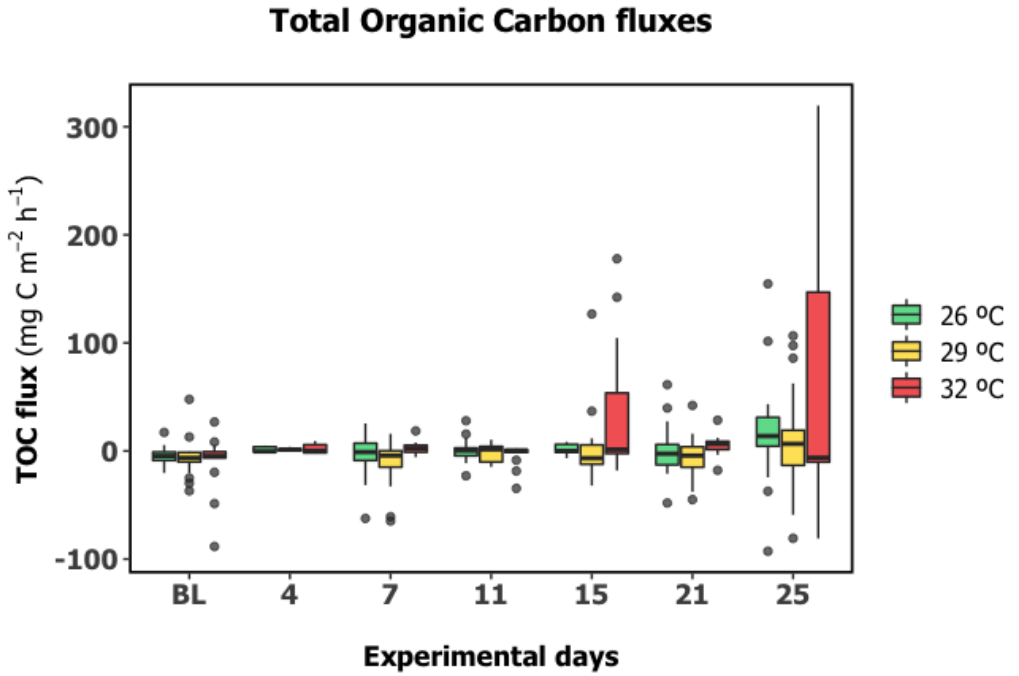

Figure S9.1 | Total Organic Carbon (TOC) fluxes over time. *X. umbellata* TOC fluxes calculated from dark incubations samples. The control condition for the temperature treatments is represented by 26°C (green), and heat-stressed conditions by 29°C (yellow) and 32°C (red). Each treatment included n = 6 replicates, with 3 corals measured per tank.

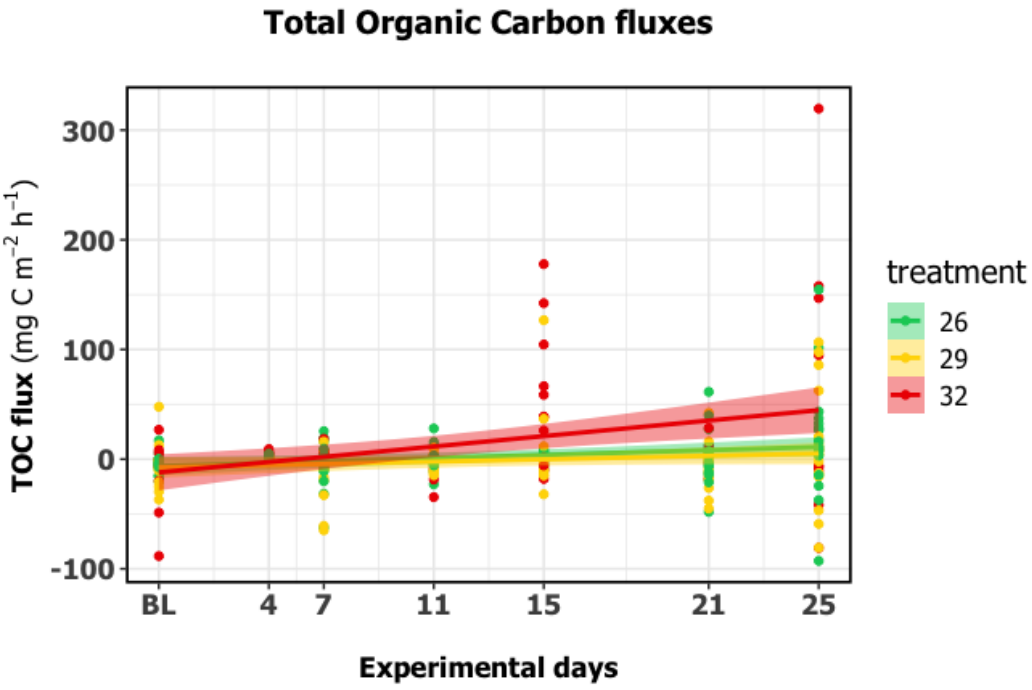

Figure S9.2 | Total Organic Carbon (TOC) fluxes over time. Linear fit depicting TOC behavior as a function of temperature with 26°C representing the control condition (green) and 29°C and 32°C representing heat-stressed conditions (yellow and red respectively). Each treatment included n = 6 replicates, with 3 corals measured per tank.

Supplementary Information S10. Temperature and time factor pairwise comparisons. Tukey plots representing adjusted P-values of the calculated marginal means for *X. umbellata* TOC fluxes under increased temperature.

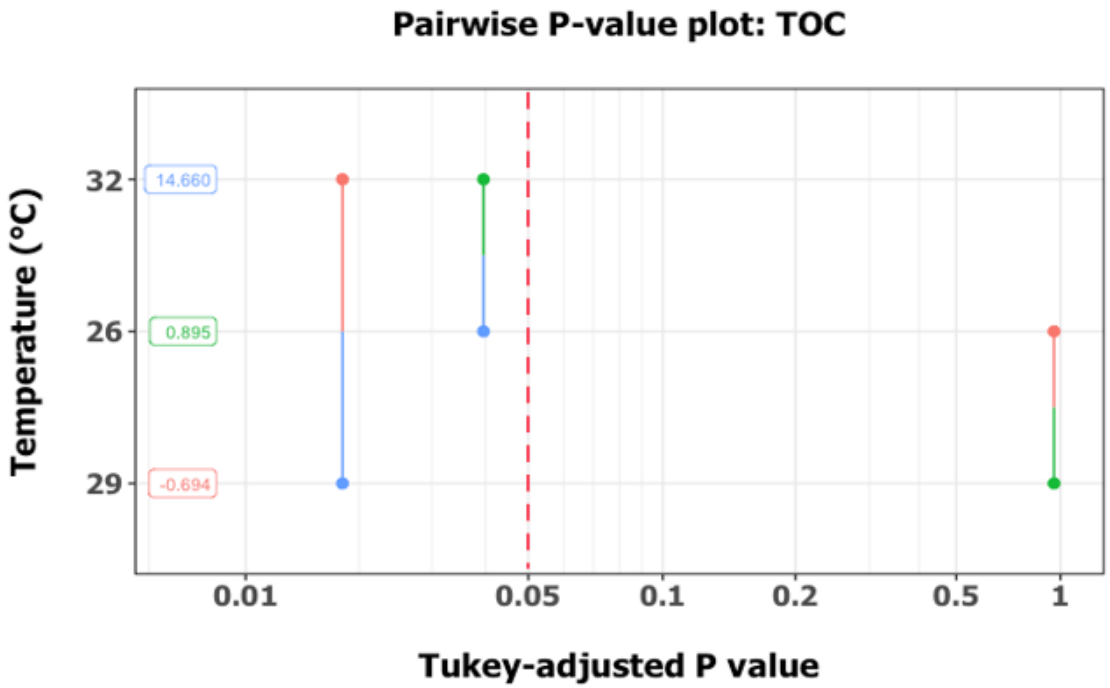

Figure S10.1 | Temperature factor pairwise comparison plot for *X. umbellata* TOC fluxes. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

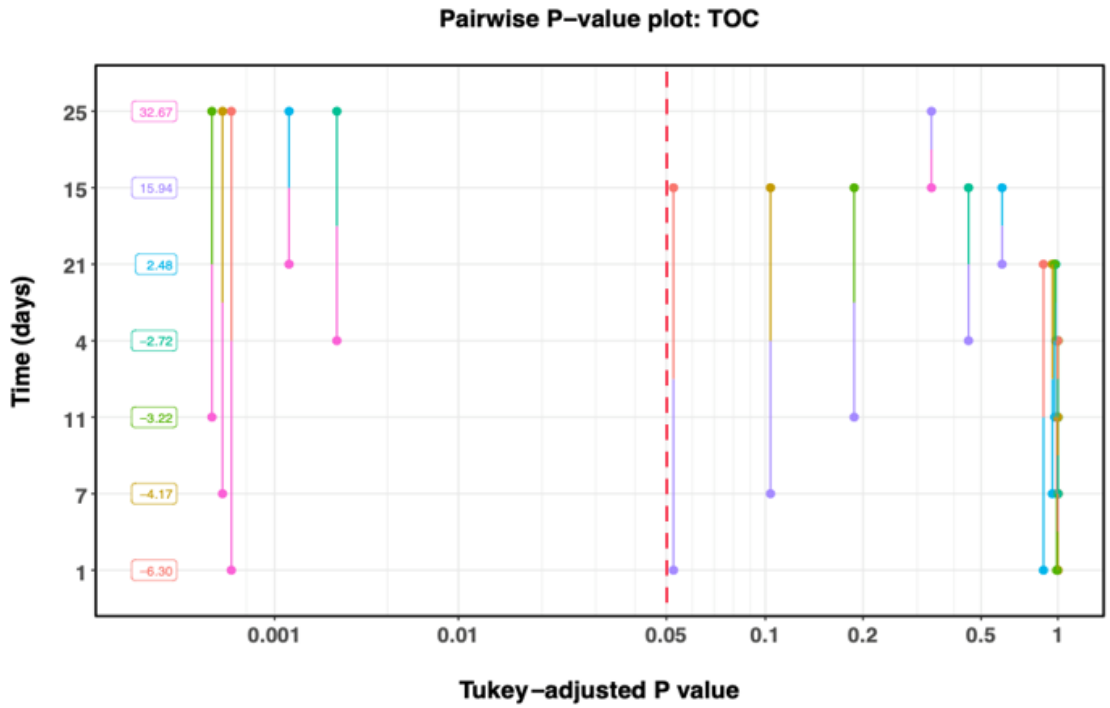

Figure S10.2 | Time factor pairwise comparison plot for *X. umbellata* TOC fluxes. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S11.

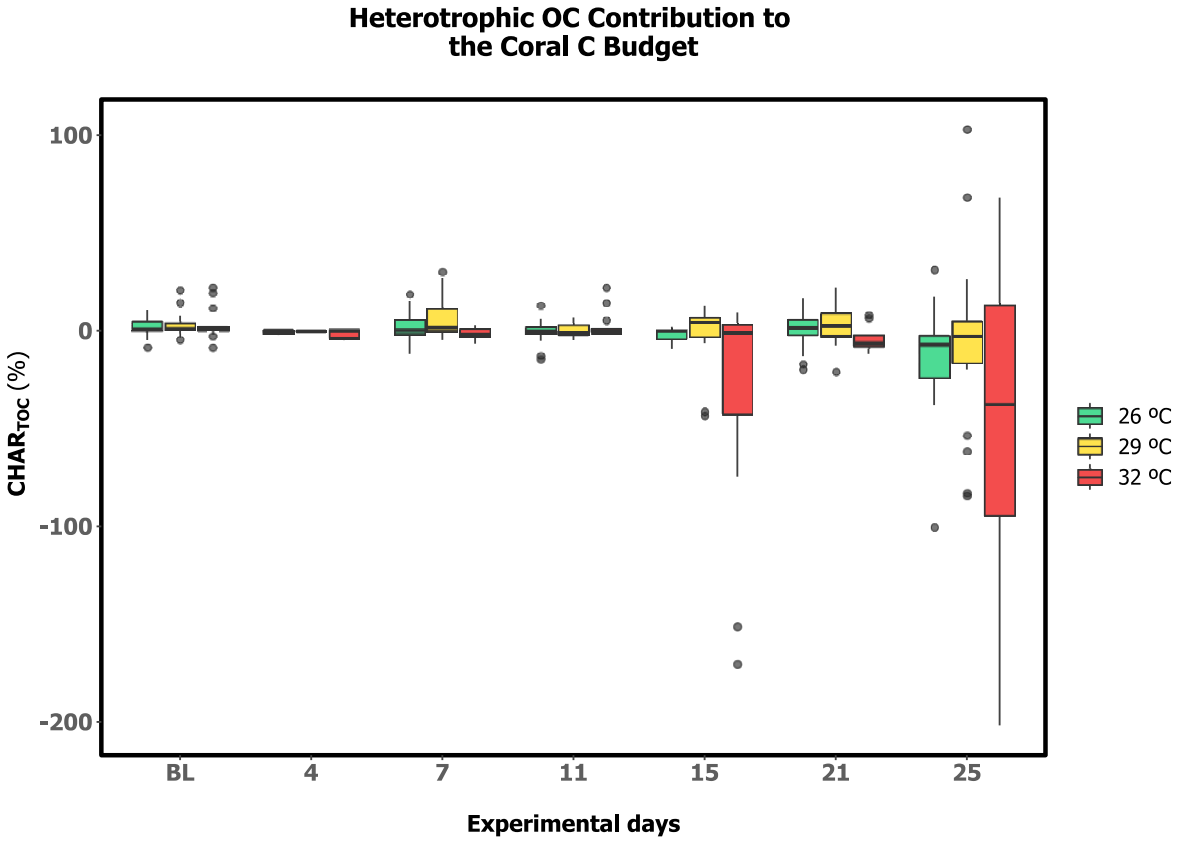

Figure S11.1 | Contribution of heterotrophically derived total organic carbon to animal respiration ($CHAR_{TOC}$) for *X. umbellata* corals in each treatment. Measurements correspond to daytime per cent contribution, where negative values indicate carbon losses. 26°C (green) represents the control condition for the temperature treatments, whereas 29°C (yellow) and 32°C (red) represent heat-stressed conditions. The data depicts n = 6 replicates per treatment with 3 corals measured per tank.

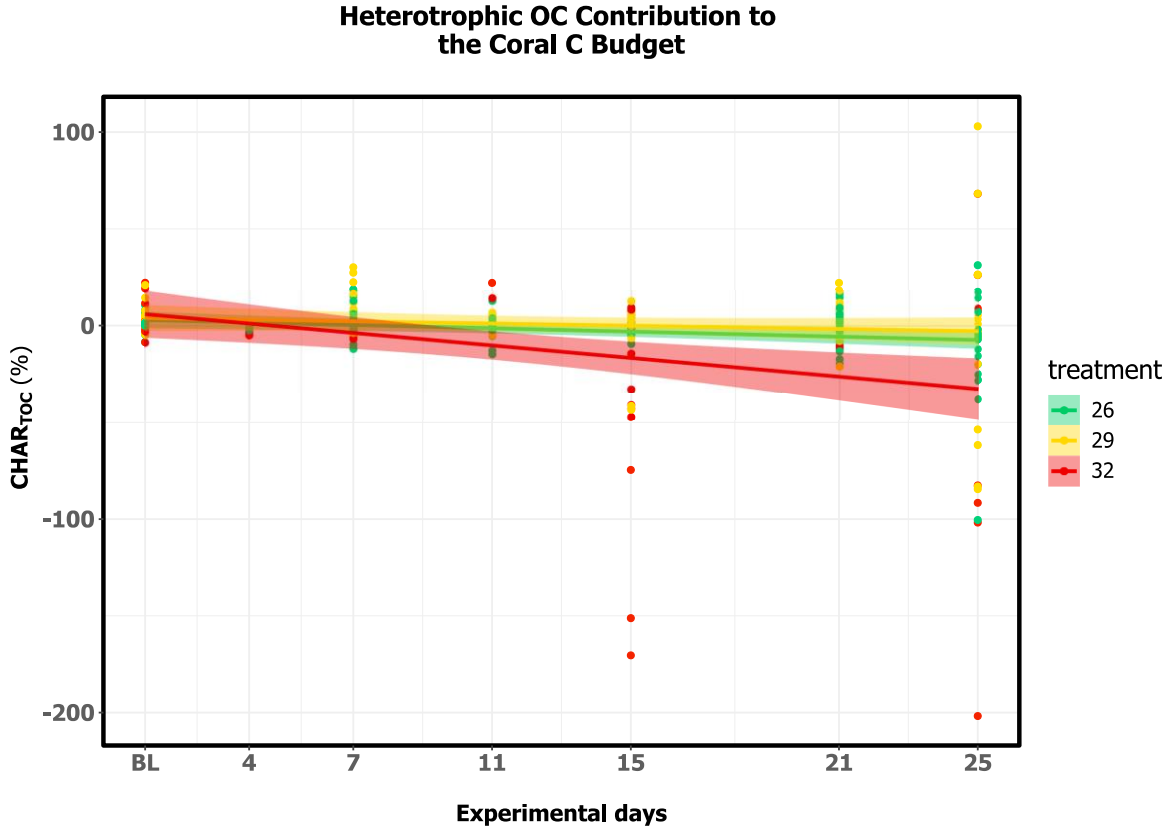

Figure S11.2 | Linear fit for heterotrophically derived total organic carbon ($CHAR_{TOC}$) over time. Detail on model fit for the percent contribution of $CHAR_{TOC}$ for *X. umbellata* corals in each treatment. Negative values indicate carbon losses whilst positive ones indicate carbon uptake. 26°C (green) represents the control condition for the temperature, whereas 29°C (yellow) and 32°C (red) indicate heat-stressed conditions (red). Each treatment had n = 6 replicates, with 3 corals measured per tank.

Supplementary Information S12. Temperature and time factor pairwise comparisons. Tukey plots representing adjusted P-values of the calculated marginal means for $CHAR_{TOC}$ contribution percentages to *X. umbellata* corals under increased temperature.

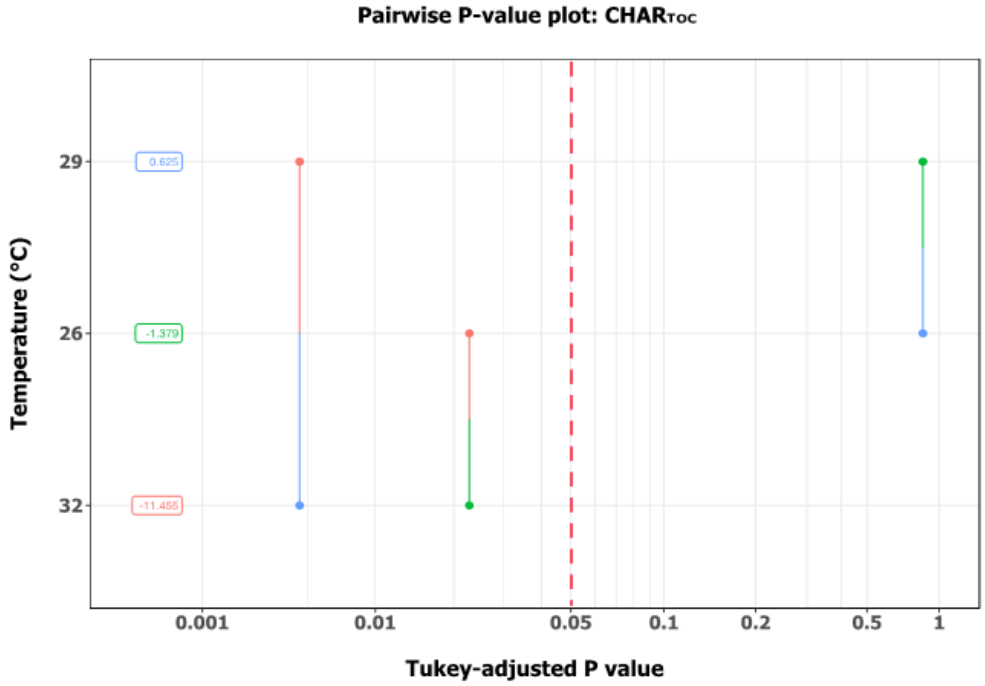

Figure S12.1 | Temperature factor pairwise comparison plot for $CHAR_{TOC}$ contribution percentages to *X. umbellata*. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

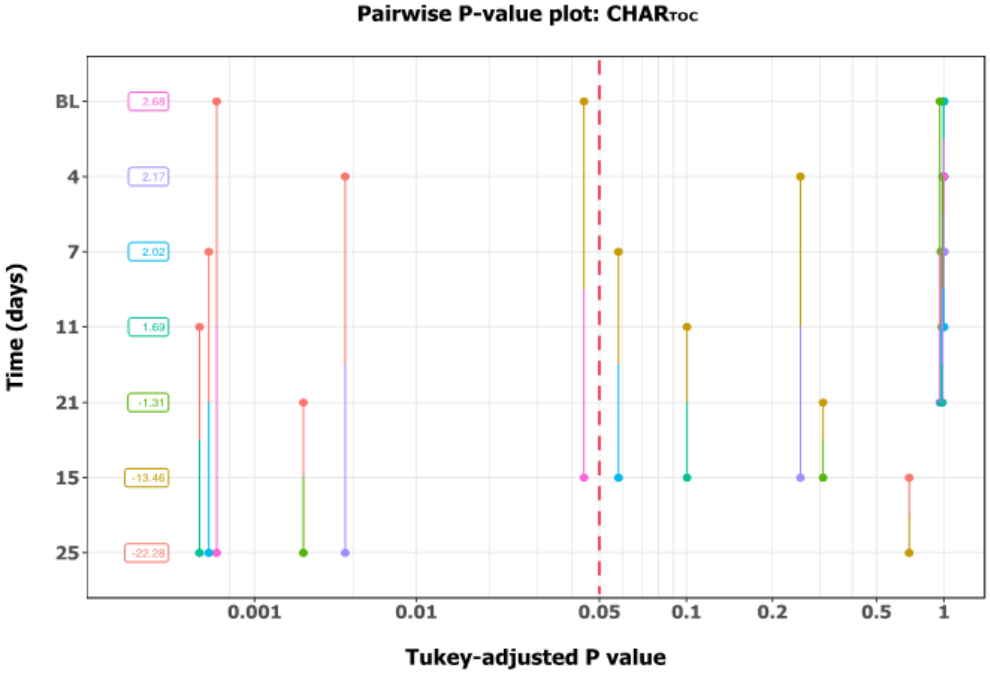

Figure S12.2 | Time factor pairwise comparison plot for $CHAR_{TOC}$ contribution percentages to *X. umbellata*. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S13.

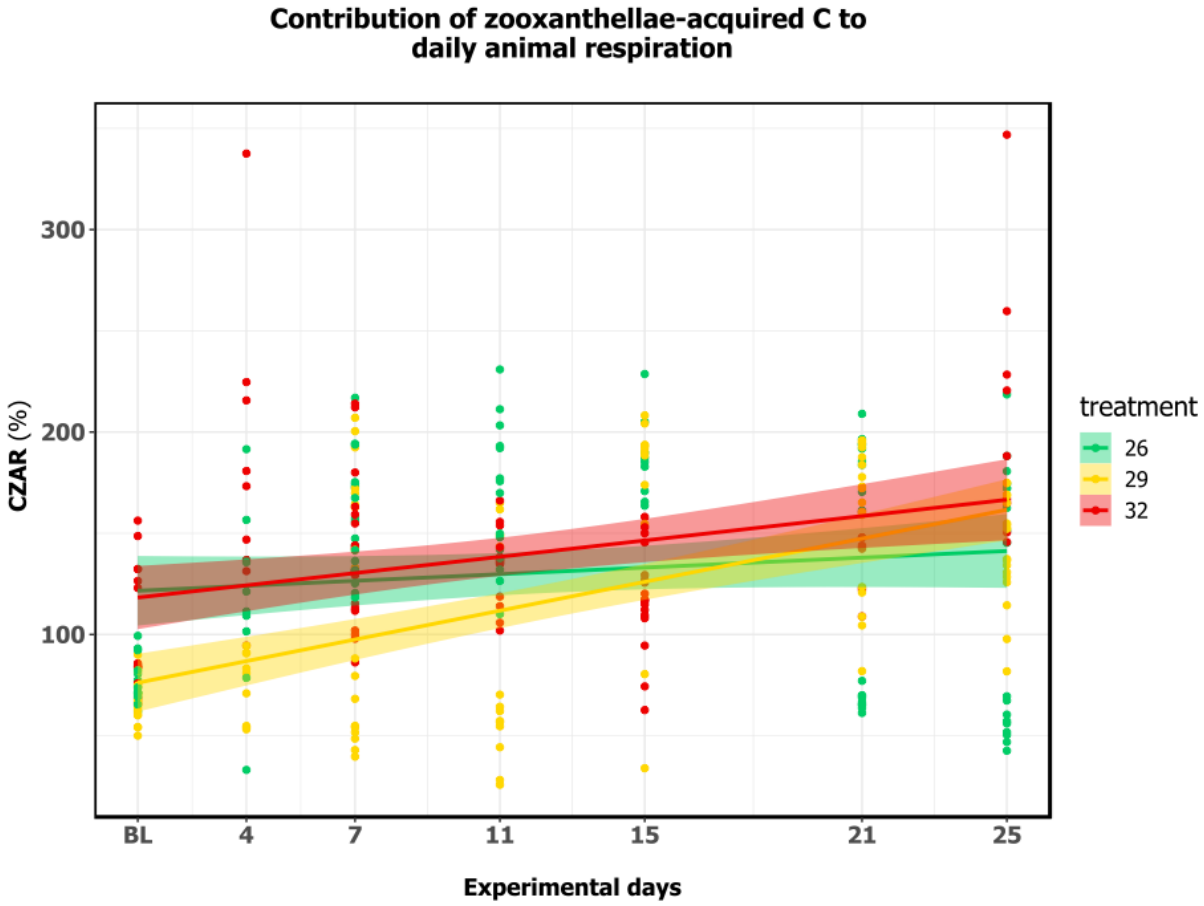

Figure S13.1 | Linear fit for the zooxanthellae-acquired carbon contribution percentage to daily animal respiration (CZAR) overtime. Detail on model fit for the percent contribution of CZAR for *X. umbellata* corals in each treatment indicating autotrophically acquired carbon available to the coral energy budget over time. 26°C (green) represents the control condition for the temperature, whereas 29°C (yellow) and 32°C (red) indicate heat-stressed conditions (red). Each treatment had n = 6 replicates, with 3 corals measured per tank.

Supplementary Information S14. Temperature and time factor pairwise comparisons. Tukey plots representing adjusted P-values of the calculated marginal means for *X. umbellata* CZAR under increased temperature.

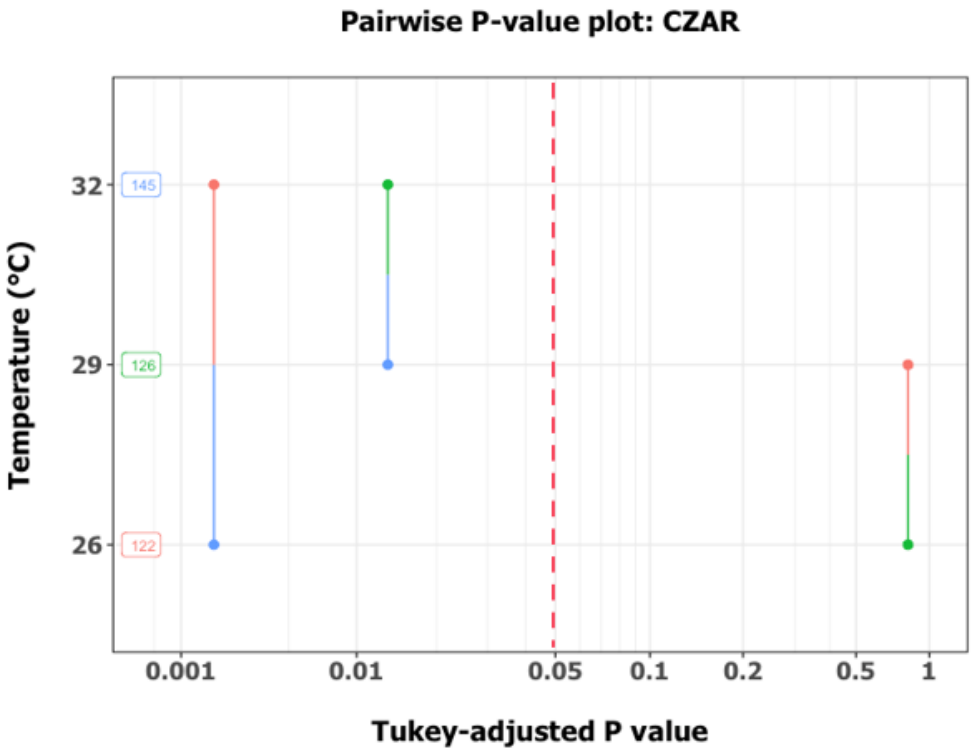

Figure S14.1 | Temperature factor pairwise comparison plot for *X. umbellata* CZAR. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

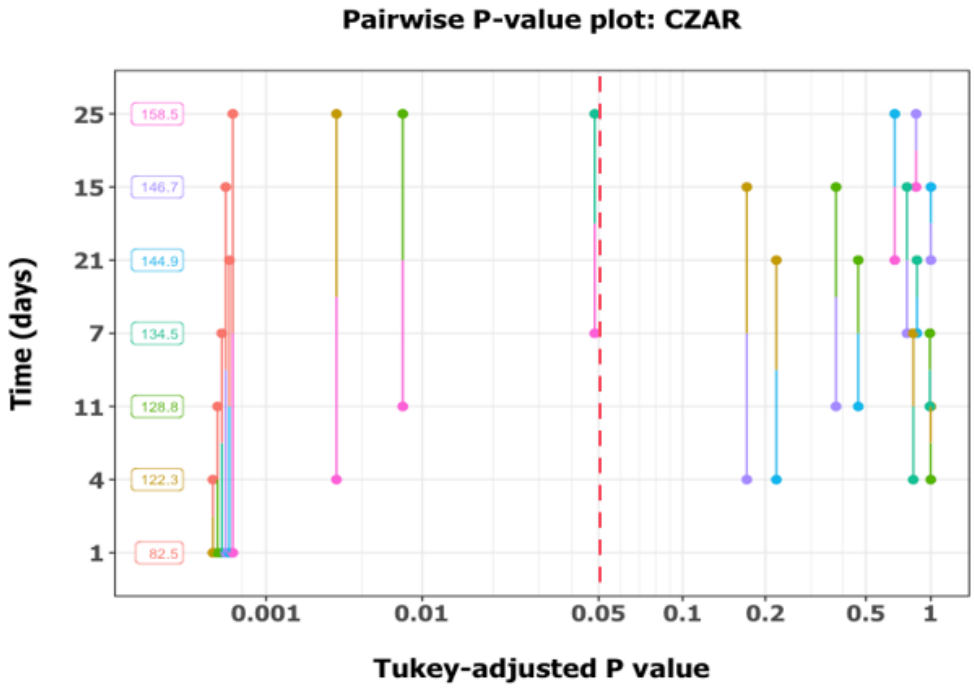

Figure S14.2 | Time factor pairwise comparison plot for *X. umbellata* CZAR. The red segmented line indicates the P-value threshold for statistical significance (α = 0.05).

Supplementary Information S15.

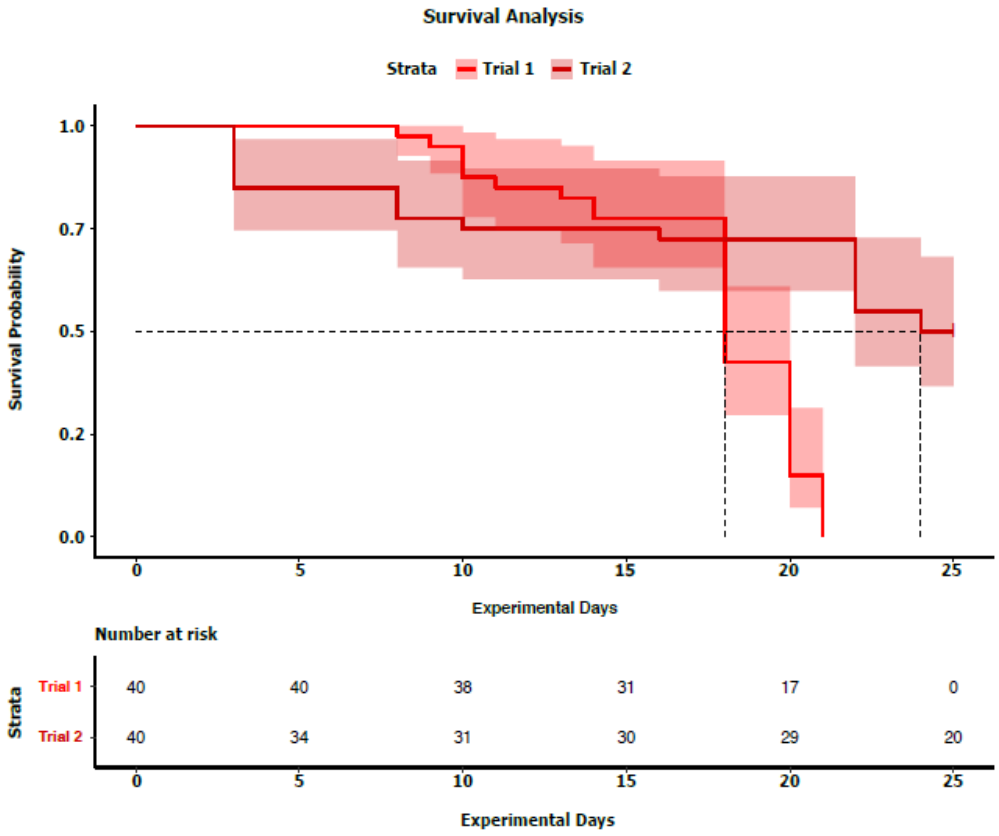

Figure S15.1 | Detail on survival probability functions of *X. umbellata* corals exposed to the 32°C temperature treatment over time. Lines depict the detailed survival probability functions for *X. umbellata* corals under the 32°C treatment for the two experimental trials conducted in the study (i.e., trials 1 and 2). Each trial included n = 40 individuals by the start of the experimental manipulation with 10 initial individuals per tank.

Supplementary Information S16.

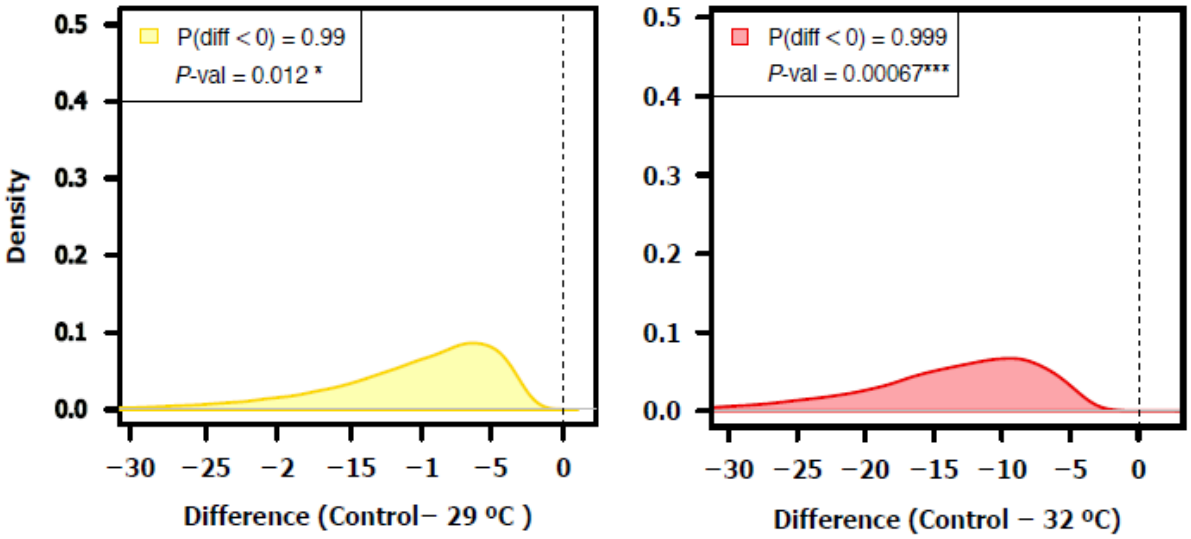

Figure S16.1 | Bayesian density plots for *X. umbellata* bleaching response difference between groups. The density plots show the difference between the controls and the heat-stressed temperature treatment groups at 29°C (yellow) and 32°C (red), respectively. The probability that both treatments have a negative effect on the coral colonies is above 90% in both cases and equals the proportion of the distribution below zero.

Supplementary Information S17. Summary tables: Tukey contrasts for gross and net photosynthesis, respiration rates and the P:R ratio of *X. umbellata* corals, under sustained warming temperatures over time.

Table S17.1 | Temperature pairwise mean comparisons for *X. umbellata* gross photosynthesis. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-3.238	1.089	-2.974	**0.00587 ****
32 - 26	-4.414	1.108	-3.985	**0.00020 *****
32 - 29	-1.176	1.119	-1.051	0.29313

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S17.2 | Temperature pairwise mean comparisons for *X. umbellata* respiration. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-0.6725	0.4914	-1.369	0.17112
32 - 26	-2.3161	0.4999	-4.633	**1.08e^{-05} *****
32 - 29	-1.6435	0.5046	-3.257	**0.00225 ****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S17.3 | Temperature pairwise mean comparisons for *X. umbellata* net photosynthesis. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-2.6746	0.8050	-3.323	**0.00268 ****
32 - 26	-2.1743	0.8189	-2.655	**0.01586 ***
32 - 29	0.5003	0.8274	0.605	0.54542

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S17.4 | Temperature pairwise mean comparisons for *X. umbellata* P:R ratio. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-0.3220	0.09988	-3.224	**0.00253 ****
32 - 26	-0.3508	0.10239	-3.426	**0.00184 ****
32 - 29	-0.0288	0.10257	-0.281	0.77872

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S17.5 | Temperature pairwise mean comparisons for *X. umbellata* light R:P percentages. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
26 - 29	8.035	3.944	2.037	0.1034
26 - 32	-9.933	3.940	-2.521	**0.0314 ***
29 - 32	-17.968	3.933	-4.569	**< .001 ****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Supplementary Information S18. Summary tables: Tukey contrasts for *X. umbellata* observed bleaching under sustained warming.

Table S18.1 | Temperature pairwise mean comparisons for *X. umbellata* observed bleaching response. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	0.6125	0.0949	6.455	**$2.16e^{-10}$ *****
32 - 26	0.9321	0.1293	7.208	**$1.71e^{-12}$ *****
32 - 29	0.3196	0.1293	2.471	**0.0135 ***

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Supplementary Information S19. Summary tables: Tukey contrasts for *X. umbellata* pulsation and growth rates under sustained warming.

Table S19.1 | Temperature pairwise mean comparisons for *X. umbellata* pulsation rates. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-4.954	1.179	-4.20	**$2.67e^{-05}$ *****
32 - 26	-18.988	1.220	-15.56	**$< 2e^{-16}$ *****
32 - 29	-14.034	1.218	-11.52	**$< 2e^{-16}$ *****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S19.2 | Temperature pairwise mean comparisons for *X. umbellata* growth rates. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	-6.420	1.184	-5.420	**$1.19e^{-07}$ *****
32 - 26	-4.645	0.526	-8.835	**$< 2e^{-16}$ *****
32 - 29	1.775	1.191	1.490	0.136

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Supplementary Information S20. Summary table: Tukey contrasts for *X. umbellata* TOC fluxes and, $CHAR_{TOC}$ and CZAR percentages and under sustained warming over time.

Table S20.1 | Temperature pairwise mean comparisons for *X. umbellata* TOC fluxes. Tukey contrast P-values.

Contrast	Estimate	Std. Error	t-ratio	p-value
26 - 29	1.59	5.49	-4.20	0.9549
26 - 32	-13.76	5.59	-15.56	**0.0395 ***
29 - 32	-15.35	5.57	-11.52	**0.0182 ***

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S20.2 | Temperature pairwise mean comparisons for *X. umbellata* $CHAR_{TOC}$ percentages. Tukey contrast P-values.

Contrast	Estimate	Std. Error	t-ratio	p-value
26 - 29	-2.0	3.64	-0.551	0.8463
26 - 32	10.1	3.77	2.672	**0.0227 ***
29 - 32	12.1	3.75	3.221	**0.0045 ****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S20.3 | Temperature pairwise mean comparisons for *X. umbellata* CZAR percentages. Tukey contrast P-values.

Contrast	Estimate	Std. Error	t-ratio	p-value
26 - 29	-4.07	6.53	-0.624	0.8069
26 - 32	-22.83	6.61	-3.454	**0.0018 ****
29 - 32	-18.76	6.60	-2.843	**0.0133 ***

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Supplementary Information S21. Summary table: Tukey contrasts for *X. umbellata* carbon (%C) and nitrogen (%N) contents after sustained warming exposure.

Table S21.1 | Temperature pairwise mean comparisons for *X. umbellata* carbon (%C) content. Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	28.644	3.393	8.443	**< 2e^{-16} *****
32 - 26	54.661	4.091	13.362	**< 2e^{-16} *****
32 - 29	26.017	2.287	11.376	**< 2e^{-16} *****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Table S21.2 | Temperature pairwise mean comparisons for *X. umbellata* nitrogen content (%N). Tukey contrast P-values.

Contrast	Estimate	Std. Error	z value	p-value
29 - 26	3.862	0.327	11.80	**< 2e^{-16} *****
32 - 26	7.727	0.395	19.57	**< 2e^{-16} *****
32 - 29	3.865	0.221	17.51	**< 2e^{-16} *****

Note: *P*-values defined as significant at a threshold of P ≤ 0.05 are highlighted in bold.

Acknowledgements

Acknowledgements

If you are reading this, it can only mean that we are standing at the very end of this project, so most certainly, the instructions worked out and I have succeeded in reaching the end of "the staircase", just as in Cortazar's "Instructions on how to climb a staircase". This means, that it is time for me to thank everyone that has accompanied me on this endeavor. Without all of you, this achievement would have not been possible and thus, I will be forever thankful for your support and companionship.

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Other publications during the candidature

Short periods of decreased water flow may modulate long-term ocean acidification in reef-building corals

Catarina P.P. Martins*[1], **Susana M. Simancas-Giraldo[2]**, Patrick Schubert[1], Marlene Wall[3], Christian Wild[2], Thomas Wilke[1], Maren Ziegler[1]

[1]Department of Animal Ecology & Systematics, Justus Liebig University Giessen, 35392 Giessen, Germany
[2]Marine Ecology Department, Faculty of Biology and Chemistry, University of Bremen, 28359 Bremen, Germany
[3]GEOMAR, Helmholtz Centre for Ocean Research, 24148 Kiel, Germany

Abstract

Ocean acidification (OA) poses a major threat to reef-building corals. Although water flow variability is common in coral reefs and modulates coral physiology, the interactive effects of flow and OA on corals remain poorly understood. Therefore, we performed a three-month OA experiment investigating the effect of changes in flow on coral physiology. We exposed the reef-building corals *Acropora cytherea*, *Pocillopora verrucosa*, and *Porites cylindrica* to control (pH 8.0) and OA (pH 7.8) conditions at moderate flow (6 cm s^{-1}) and monitored OA effects on growth. Throughout the experiment, we intermittently exposed all corals to low flow (2 cm s^{-1}) for 1.5 h and measured their respiration and photosynthesis under low and moderate flow. On average, corals under OA calcified 18 % less and grew 23 % less in surface area than those at ambient pH. We observed species-specific interactive effects of OA and flow on coral physiology. Photosynthesis:Respiration ratios decreased after 12 weeks of OA in *A. cytherea* (22 %) and *P. cylindrica* (28 %) under moderate flow, but under low flow they were unaffected by OA. In *P. verrucosa*, P:R ratios were stable. These results suggest that short periods of decreased water flow may modulate OA effects on some coral species, indicating flow variability as a factor to consider when assessing long-term climate change impacts.

Keywords
ocean acidification, reef-building corals, water flow, coral physiology.

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Universität Bremen

Universität Bremen | Fachbereich 02 | Postfach 33 04 40, 28334 Bremen

Universität Bremen
Fachbereich 2
Prüfungsamt Chemie
z. Hd. Frau Frauke Ernst
Leobener Straße

28359 Bremen
Deutschland

Prüfungsamt
Chemie

Frauke Ernst
Geschäftsstelle
Fachbereich 02
Leobener Str. / NW2
D-28359 Bremen

Verwaltungspavillon 06
Tel. 0421 218-62802
Fax 0421 218-9862802
frauke.ernst@uni-bremen.de
www.fb2.uni-bremen.de

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