

**UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ**

**Colegio de Ciencias Biológicas y Ambientales**

**Thermal tolerance assessment of corals from Isabela Island,  
Galápagos: comparison of photosynthesis and respiration rates  
between a nursery and its donor colonies**

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**Biología**

Trabajo de fin de carrera presentado como requisito  
para la obtención del título de  
**BIÓLOGA**

Quito, 22 de mayo de 2023

# **UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ**

**Colegio de Ciencias Biológicas y Ambientales**

## **HOJA DE CALIFICACIÓN DE TRABAJO DE FIN DE CARRERA**

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Galápagos: comparison of photosynthesis and respiration rates  
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Quito, 22 de mayo de 2023

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## **AGRADECIMIENTOS**

I would like to thank Margarita Brandt and John Bruno, my thesis director and co-director, for their funding, guidance, and support throughout the development of this project. Nicolás Dávalos (founder of "Reef Revival), Favio Rivera, Katelyn Gould, and Hayley Capone for their assistance during the field and labwork. Esteban Agudo-Adriani and Abel Valdivia for their help with statistical analyses. The Galapagos Science Center for providing research facilities, and the Galapagos National Park for granting the research permit PC-27-22. My family, friends, and teachers for their love, encouragement, and support.

Thank you all for your contributions and belief in this endeavor.

## RESUMEN

En un mundo que enfrenta un calentamiento acelerado de los océanos debido al cambio climático, comprender la tolerancia térmica de los corales es crucial para predecir su supervivencia a largo plazo y el mantenimiento de funciones ecológicas clave. En este estudio, utilizamos un enfoque de Curvas de Rendimiento Termales (TPCs por sus siglas en inglés) para comparar las tolerancias térmicas de fragmentos de coral *Pocillopora* del vivero "Reef Revival" y sus colonias donantes, con el objetivo de entender qué tan bien se desempeñarán los fragmentos del vivero al ser trasplantados al fondo marino. Utilizamos un sistema de respirometría cerrado, donde sometimos a los corales a ocho temperaturas (20, 24, 26, 28, 29, 31, 33 y 35 °C) y medimos sus tasas de fotosíntesis y respiración. Al examinar el rendimiento de los fragmentos del vivero, las medianas de las temperaturas óptimas (Topts) para fotosíntesis y respiración no mostraron diferencias significativas y ambas superaron los 32°C, lo que es más alto que las temperaturas máximas que el vivero enfrentó en la estación cálida del 2022 (aproximadamente 31°C). Además, observamos un rango más amplio de las Topts para la fotosíntesis que para la respiración, lo que implica una gran variación en la susceptibilidad al blanqueamiento. Por otro lado, no pudimos hacer esta misma comparación para las colonias donantes, ya que fue imposible estimar los parámetros de las TPCs para estos fragmentos; sin embargo, en general, sus TPCs se mostraron muy parecidas a las del vivero. Específicamente, las tasas de fotosíntesis y respiración alcanzaron los mismos niveles en los dos tipos de fragmentos, y ambas TPCs mostraron tasas decrecientes de fotosíntesis a altas temperaturas. En conjunto, estos resultados brindan información importante que se puede usar en las iniciativas actuales de restauración de corales en Isabela ya que sugieren que los corales *Pocillopora* del vivero tendrán buenas tasas de supervivencia en futuros eventos de El Niño.

**Palabras clave:** tolerancia térmica, vivero, corales, colonias donantes, fotosíntesis, respiración

## ABSTRACT

In a world facing accelerated ocean warming due to climate change, understanding the thermal tolerance of corals is crucial for predicting their long-term survival and the maintenance of key ecological functions. In this study, we used a Thermal Performance Curves (TPCs) approach to compare the thermal tolerances of *Pocillopora* coral fragments from the "Reef Revival" nursery and their donor colonies, with the aim of understanding how well the nursery fragments will perform when transplanted to the seabed. We used a closed respirometry system, where we subjected the corals to eight temperatures (20, 24, 26, 28, 29, 31, 33 and 35 °C) and measured their rates of photosynthesis and respiration. When examining the performance of the nursery fragments, the median Thermal Optima (Topts) for photosynthesis and respiration did not show significant differences and both exceeded 32°C, which is higher than the maximum temperatures the nursery experienced during the warm season of 2022 (approximately 31°C). Furthermore, we observed a wider range of Topts for photosynthesis than for respiration, implying a large variation in susceptibility to bleaching. On the other hand, we were unable to make the same comparison for the donor colony fragments as it was impossible to estimate their TPC parameters; however, overall, the TPCs for these fragments showed remarkable similarity to those of the nursery. Specifically, the rates of photosynthesis and respiration reached the same levels in both types of fragments, and both TPCs exhibited decreasing rates of photosynthesis at high temperatures. Taken together, these results provide valuable information that can be used in ongoing coral restoration initiatives on Isabela, as they suggest that *Pocillopora* corals from the nursery will have good survival rates in future El Niño events.

**Key words:** thermal tolerance, coral nursery, donor colonies, photosynthesis, respiration.

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## INTRODUCTION

Research into the thermal tolerance of corals has gained widespread attention due to the devastating impact climate change has on these crucial habitat-building organisms (Douglas, 2003; Goodwin, 2011). Indeed, this is the primary reason why global coral cover has declined by 50% since the 1950s (Eddy et al., 2021). Over the last few decades, researchers have evaluated the effects of heat stress on more than 100 coral species worldwide (McLachlan et al., 2020). Among this extensive research, one of the most relevant topics is how temperature affects different physiological processes such as photosynthesis and respiration. Photosynthesis is directly linked to coral bleaching, and corals cannot endure a bleached state for extended periods of time. Therefore, understanding how these processes are impacted by temperature is essential for predicting the long-term survival of corals and the maintenance of key ecosystem functions on a larger scale, such as carbon storage (Oakley & Davy, 2018; Silbiger et al., 2019).

Since photosynthesis and respiration are regulated by distinct enzymatic systems, they are likely to respond differently to changes in temperature (Silbiger et al., 2019). Moreover, coral photosynthesis relies exclusively on zooxanthellae, while respiration is dependent on the entire holobiont. In theory, respiration should be less temperature-sensitive than photosynthesis as most corals can survive, at least temporarily, post-bleaching. Although many studies support this hypothesis (Silbiger et al., 2019; Tchernov et al., 2011), there is also heaps of evidence to the contrary, indicating that coral physiology is highly species-specific and depends on other factors, like fragment size, pH and food supply (Bahr et al., 2018; Edmunds, 2008; Edmunds & Burgess, 2016).

Corals, like any ectotherm, are able to adapt or acclimatize to different thermal regimes (Seebacher et al., 2015). This can occur through various mechanisms, either by genetics or physiology (i.e. phenotypic plasticity) of both the host coral and associated zooxanthellae (Chevin et al., 2010). Through these mechanisms, corals reduce the negative impacts that thermal stress has on their growth rates, reproduction, and other components of organismal performance, improving their overall fitness (Kern et al., 2015; Putnam et al., 2017). According to evolutionary thermal biology, corals from warmer environments should perform better during periods of elevated sea temperature than those from colder ones (Angilletta et al., 2006).

This phenomenon has already been documented across a wide range of species and ecosystems; for example, there is evidence that *Orbicella franksi* corals from Panama, a relatively warm location, have higher thermal tolerances than their conspecifics from cooler Bermuda (Silbiger et al., 2019). However, some researchers suggest that adaptation and/or acclimatization do not always occur and could depend on temporal and spatial scales. A recent study in Bermuda found that, contrary to expectations, there were no significant differences in thermal performance between upper-mesophotic and shallow reefs for four coral species (Gould et al., 2021).

The thermal sensitivity of ectotherms like corals can be quantified using the Thermal Performance Curves (TPCs) approach, which describes the relationship between environmental temperature and biological performance rates, such as respiration or photosynthesis. TPCs have a unimodal shape and are characterized by several parameters, including the thermal optimum, activation energy, and deactivation energy. Among these parameters, the thermal optimum ( $T_{opt}$ ) –temperature at which performance is maximized– is

mostly used for comparisons. A  $T_{opt}$  shifted to the right is indicative of greater tolerance to high temperatures (Angilletta et al., 2006; Huey & Kingsolver, 1989).

The 1982-1983 El Niño event had a devastating impact on the coral population in Galápagos, leading to a mortality rate of 95 to 99% (Glynn, 1994). However, subsequent El Niño events did not result in mortality rates as high as anticipated. Furthermore, recent years have shown a recovery of Galápagos coral communities (Glynn et al., 2009), suggesting that the remaining corals may have adapted or acclimatized to high temperatures (Glynn et al., 2018). Against this backdrop of renewed hope, a few coral restoration projects have emerged in the Galápagos, including "Reef Revival". This initiative is one of the only two coral nurseries in the Archipelago; it was created in 2021, and it is located on Isabela Island, in the bay of Puerto Villamil. Our project thus aimed to assess the thermal tolerance of coral fragments from "Reef Revival" and is of particular significance since thermal tolerance studies suffer from an uneven distribution (McLachlan et al., 2020) and, to date, none have been published for the Galápagos Islands, despite their extreme temperature regime. This archipelago is shaped by several marine currents, like the Cromwell and Panama currents, and heavily impacted by ENSO events, making it an ideal location for heat-stress studies (Glynn et al., 2016).

Our primary goal was to assess the thermal tolerance of *Pocillopora* spp. corals from the "Reef Revival" nursery on Isabela Island, Galápagos. To this end, we formulated two research questions: 1) Is photosynthesis more temperature-sensitive than respiration? and 2) Are nursery corals more thermally tolerant than corals from donor colonies? We posed the second question because the nursery is shallower and, therefore, exposed to a warmer thermal regime than the donor colonies. Thus, we wanted to understand whether nursery corals had undergone any acclimatization process as a result. With this study we hope to better predict the survival rates of these corals and ultimately contribute to the restoration of degraded reefs

through their use (Ishida-Castañeda et al., 2020; Morikawa & Palumbi, 2019; Suggett et al., 2019).

## METHODS

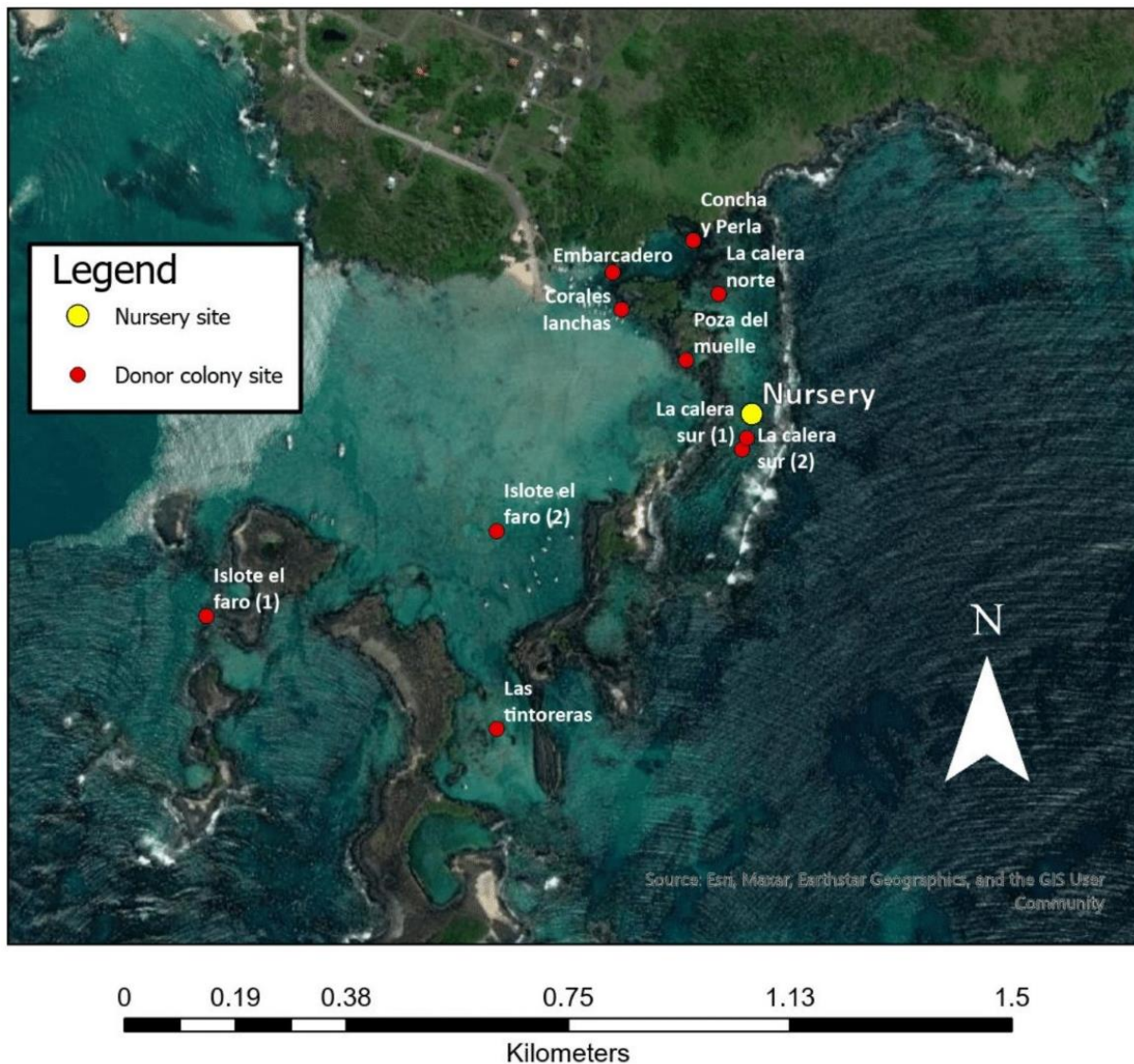
### Study area

Isabela is the largest island of the Galápagos archipelago and therefore temperature varies greatly from one place to another (Glynn et al., 2016). Because of its shallow depths, our study area does not receive direct influence from the cold Cromwell current but still has a relatively cold thermal regime in comparison to other places in the Galápagos (Glynn et al., 2016). Our study was carried out in the southeastern portion of the Island, in the bay of Puerto Villamil.

The nursery (Reef Revival) was located at coordinates S 0. 964769° W 90. 955805° at a depth of 1.5 m below sea level at low tide. The donor colonies, on the other hand, were scattered near the coastline in ten different places (Figure 1) at an average depth of 2.5 m below sea level at low tide. Therefore, it was assumed their thermal regime would overall be colder than that of the nursery since the latter was very shallow and exposed to a great deal of sunlight. Loggers from the nursery site indicate that in 2022 it had an average temperature of 25.67°C, a maximum of 31.17°C and a minimum of 20.47°C in the warm season and an average of 22.81°C, a maximum of 30.96°C and a minimum of 18.24°C in the cold one. Unfortunately, we were not able to place loggers in all the donor colonies, but we used the temperature data from “Concha y Perla” as a reference. In 2021, this site had an average of 25.54°C, a maximum of 29.94°C and a minimum of 21.29°C in the warm season and an average of 22.61°C, a maximum of 25.99°C and a minimum of 18.51°C in the cold one. From these data, it can be noted that maximum temperatures were higher in the nursery than in this donor colony.

Reef Revival was established in December 2021 and is actively engaged in coral conservation efforts. Currently, the project harbors four coral species, with 240 coral fragments

from the branching genus *Pocillopora* spp., and 10 fragments encompassing three distinct species of massive corals: *Pavona clavus*, *Pavona gigantea*, and *Porites lobata*.



**Figure 1.** Map showing the location of the study areas on Isabela Island, Galápagos. The nursery is represented by a yellow dot and the donor colonies by red dots. The spatial scale is shown at the bottom of the Figure.

### Study species

*Pocillopora* is a genus of branching corals that exhibits a wide distribution across tropical and subtropical marine environments. In fact, it is one of the four most abundant reef-building coral

genera in the Eastern Tropical Pacific (ETP), making it a good predictor of how reefs in this biogeographic region will perform in the face of climate change (Glynn et al., 2016). *Pocillopora* corals showcase remarkable plasticity in their coloration and structure, which can vary significantly depending on the environmental conditions they inhabit. Consequently, differentiating between species without molecular analysis poses considerable challenges. To date, this genus encompasses around 20 recognized species, though its taxonomy remains a subject of ongoing research and debate (Gélin et al., 2017).



**Figure 2.** *Pocillopora* spp. coral fragments in the nursery “Reef Revival”, photographer: Nicolás Dávalos.

### **Sample collection**

All the experiments were performed at the start of July of 2023. We collected 11 fragments from the nursery on July 2<sup>nd</sup> and 3<sup>rd</sup> and nine fragments from the donor colonies on July 8<sup>th</sup>. We wanted each coral fragment to represent a different genotype and intended to use the same



genotypes for both the nursery and donor colonies. However, two fragments (genotypes) could not be found from the donor colonies, but for the other nine we had matching fragments from the donor colonies and the nursery. Coral colonies were considered different genotypes if they were more than 100 m apart from each other or if they looked morphologically distinct (Baums, 2008); however we do recognize that genetic analyses are needed to confirm this.

Nursery fragments were small (2.5 x 2 x 1.5 cm in average) and easy to handle, so they were collected through snorkeling by bare hand. However, fragments from the donor colonies were collected (also through snorkeling) with a hammer and chisel to avoid causing damage to the colonies; the size of these fragments was 4 x 2.5 x 2 cm in average. The transportation period was very short (less than 1 hr) and during this time corals were placed in buckets with water from the collection sites. Once in the laboratory, fragments were kept in buckets with an aerator at ambient temperature (around 20 °C). The time lapse between the collection and the start of the experiments was never greater than 2 hrs. Before starting the experiments, it was necessary to reduce the size of some donor colony fragments so that they had similar sizes to those of the nursery. This was done using a hammer and a chisel. We also removed the algae, crabs and other small organisms that inhabited the corals with a toothbrush, being careful not to damage the coral tissue.

### **Thermal response measurements**

All experiments were run the same day the corals were collected. We used a closed system of ten 620-cm<sup>3</sup> acrylic chambers with magnetic stir bars to measure the thermal sensitivity of the coral fragments. Stir bars guaranteed continuous water circulation in each chamber and prevented oxygen super-saturation. This system was placed inside a cooler containing unfiltered sea water gathered each morning before the experiments. Our respirometry setup consisted of nine chambers with coral fragments randomly assigned to them and one randomly

selected “blank” chamber with only seawater as control (Figure 3). Blank readings were later used to account for the oxygen consumption and production of bacteria and other microorganisms present in the seawater. We took salinity and Photosynthetically Active Radiation (PAR) measurements before each trial, to a) ensure that the water showed the same salinity as the collection site (37ppt) and b) that all chambers received similar amounts of light.



**Figure 3.** Respirometry setup: acrylic chambers are shown containing the coral fragments and spinners, connected to the Presens probes, and placed inside the cooler.

Oxygen consumption / production and temperature inside each individual chamber were monitored using a fiber-optic oxygen probe (Presens) and a temperature probe (Pt1000), respectively. For each experiment, we used eight different temperature trials: 20°C, 24°C,

26°C, 28°C, 29°C, 31°C, 33°C and 35°C. Initially, we also used 38°C for nursery respiration; however, due to the observed bleaching and fragment disintegration this temperature was not employed in subsequent experiments. This range of temperatures was chosen because previous studies indicate the  $T_{opt}$  of tropical corals to be close to 30°C (Huffmyer et al., 2021). Temperatures below 20°C were not used to avoid cold coral bleaching at the beginning of the trials (Gould et al., 2021; Silbiger et al., 2019), and we decreased the range between temperatures around the  $T_{opt}$  (30°C) to improve curve fitting. Temperature was controlled using a thermostat system, bucket heaters, and a chiller.

For each temperature we performed two trials sequentially: one with light (photosynthesis) and one in the dark (respiration). During respiration trials a black tarp covered the chambers from any outside light sources. Conversely, a full spectrum aquarium light was suspended above the chambers during photosynthesis trials. Each photosynthesis / respiration trial lasted approximately 10 min (we spent 20 min at each temperature) and the oxygen concentration in the chambers was measured every 1 s during this time. The time spent ramping between one temperature and the next never surpassed 30 min, so that the corals would not a) acclimatize to a given temperature or b) suffer too severe stress / damage in the most extreme temperatures.

After finishing all the measurements, coral fragments were wrapped in aluminum foil along with a genotype ID and frozen at -17°C. When all the experiments were finished, we brought the fragments to the Marine Ecology Laboratory of the Galapagos Science Center (GSC) on San Cristóbal Island. There, the corals were first dried in an oven for 24 hrs at 60 °C and then burned in a muffle furnace for 4 hrs at 500 °C. This allowed us to calculate their ash free dry weight (AFDW) and normalize all the photosynthesis / respiration rates.

## Statistical data analysis

All the data analysis is based on the methodology established by (Silbiger et al., 2019) and uses an adapted version of their R code. First, we extracted metabolic rates at each temperature ( $\mu\text{mol} / \text{hr}$ ) from the raw changes in oxygen concentration recorded by the Presens software. Then, we normalized raw metabolic rates to individual coral AFDW and chamber volume ( $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ), and the rate from the blank was subtracted from all the other rates. After that, we modeled TPCs with a log-transformed Sharpe-Schoolfield modified equation (Padfield et al., 2017; Schoolfield et al., 1981; Silbiger et al., 2019):

$$\log (\text{rate}) = b (T_c) + E \left( \frac{1}{T_c} - \frac{1}{k * T_i} \right) - \log \left( 1 + e^{Eh \left( \frac{1}{K * Th} - \frac{1}{K * T_i} \right)} \right)$$

where: E= activation energy, Eh= deactivation energy, Tc= reference temperature (temperature at which inactivation is not experienced), b(Tc)= log rate at a constant temperature, K= Boltzmann constant ( $8.62 \times 10^{-5} \text{ eV K}^{-1}$ ), T<sub>i</sub>= temperature in Kelvin (K), Th= temperature in Kelvin (K) where the rate is half of the maximal rate.

Additionally, to find the thermal optima (Topt) of each TPC curve we utilized the following equation:

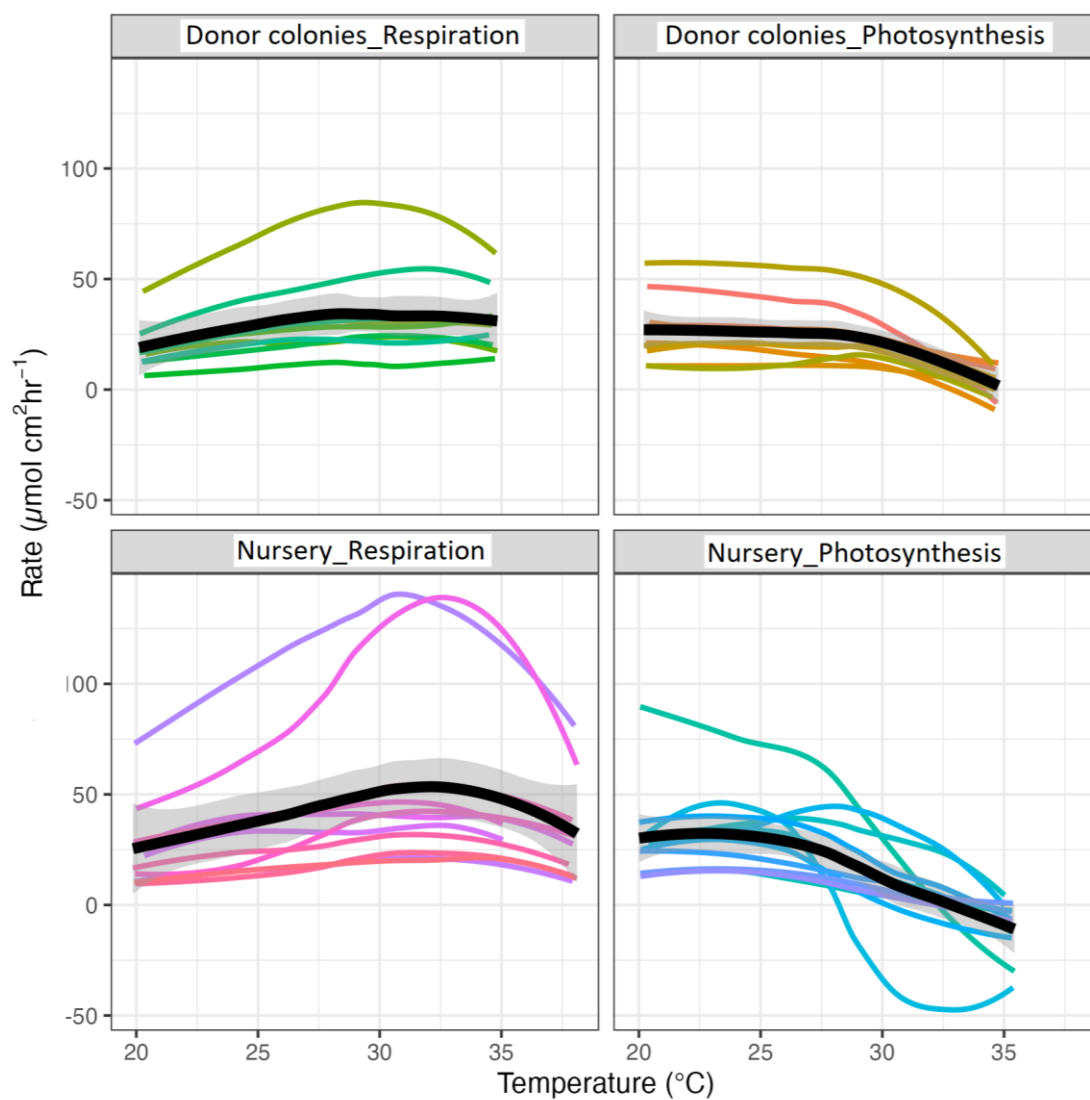
$$T_{opt} = \left( \frac{Eh * Th}{Eh + \left( K * Th_k * \log \left( \frac{Eh}{E} - 1 \right) \right)} \right)$$

Finally, to see if there were significant differences in thermal optima (Topt) among treatments, we ran a Bayesian analysis using the Hamiltonian Monte Carlo algorithm (MCMC method) in Stan, a probabilistic programming language. The output of this analysis were the median values and 95% credible intervals for the Topts. All the data and code used can be found here:

<https://www.dropbox.com/sh/6d5t4ivtwwc1ko7/AAAb0ikXY4UN69BSIW-Ow67Ka?dl=0>

## RESULTS

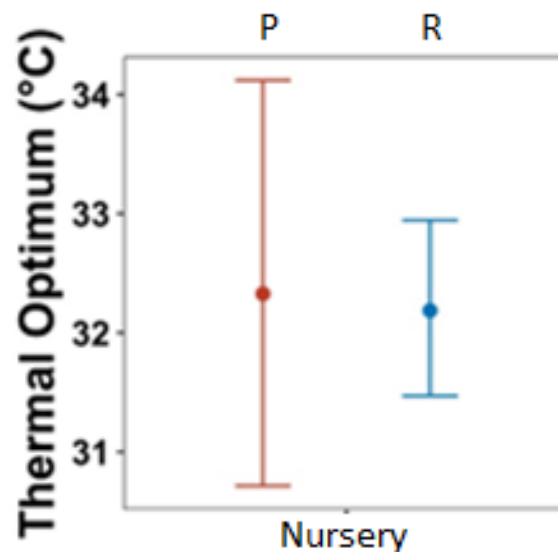
In general, corals from both donor colonies and the nursery displayed relatively similar respiration and photosynthesis rates (DCR = 20 – 35  $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ; NR = 25 – 50  $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ; DCP = 0 – 25  $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ; NP = -10 – 30  $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ). However, photosynthesis rates for both types of fragments (donor colonies and nursery) seem to have captured only the end of their TPCs, as they show declining rates at higher temperatures (Figure 4).



**Figure 4.** TPCs of respiration / photosynthesis rates ( $\mu\text{mol} / \text{cm}^2 * \text{hr}$ ) for *Pocillopora* spp. corals from Isabela Island, Galápagos. Individual colored lines are the estimated TPCs for each coral genotype

(nursery:  $n=11$ , donor colonies:  $n=9$ ,  $n$  total = 20). Thick black lines are the medians for each TPC  $\pm$  95% bootstrap confidence intervals (grey shaded bands) drawn from the posterior distribution.

On the other hand, the TPCs of both respiration and photosynthesis rates look similar between fragments from the donor colonies to those from the nursery (i.e., respiration rates from both look more unimodal than photosynthesis rates). Having said that, we were unable to estimate the  $T_{opt}$ s of both rates for the fragments from the donor colonies, as these TPCs were flatter than those for the nursery fragments (Figure 4). Of the latter, there were no significant differences between  $T_{opt}$ s of photosynthesis and respiration rates, with both median values being higher than  $32^{\circ}\text{C}$  (NP =  $32.33^{\circ}\text{C}$ ; NR =  $32.19^{\circ}\text{C}$ ). However, variability in the  $T_{opt}$  was considerably greater for photosynthesis, as shown by its broader 95% confidence interval (NP =  $30.71\text{--}34.12^{\circ}\text{C}$  [95% BCI]; NR =  $31.47\text{--}32.95^{\circ}\text{C}$  [95% BCI]) (Figure 5).



**Figure 5.** Comparison between photosynthesis and respiration thermal optima ( $T_{opt}$ ) of nursery corals. P = photosynthesis (in red); R = respiration (in blue). Dots represent the median of each parameter, and the ranges represent the 95% Credible Interval from the Bayesian analysis (BCI). If the ranges of two treatments fully overlap it means that there is no significant difference between them.

## DISCUSSION

Based on our findings, we successfully addressed our main research question: photosynthesis does not exhibit greater temperature sensitivity than respiration in coral fragments sourced from the "Reef Revival" nursery. However, our second research question (Do nursery corals demonstrate greater thermal tolerance compared to corals from donor colonies?) remains unanswered. The significance and implications of these results are discussed below.

Numerous studies have examined the thermal tolerance of the *Pocillopora* spp. complex, with results suggesting the genus thrives in warm waters ranging from 23°C to 30°C, with its  $T_{opt}$  being around the latter value. Nonetheless, some species can tolerate temperatures far outside of this range; for example, *Pocillopora damicornis* survives in temperatures as low as 14°C and as high as 33°C. Together, these outcomes suggest the thermal tolerance of this genus varies greatly depending on several factors, including species, genotype, type of zooxanthellae involved, and local temperature regime (Huffmyer et al., 2021).

Given this background, our study found the median  $T_{opt}$  for both photosynthesis and respiration of nursery corals was greater than 32°C, which is between 0.5 and 1°C higher than values reported for *Pocillopora* spp. corals in other locations like China, Hawaii and the Red Sea (Banc-Prandi et al., 2022; Clausen & Roth, 1975; Huffmyer et al., 2021; Jiang et al., 2021). This suggests corals from "Reef Revival" are likely to have good survival rates both in the wild and within the nursery. In fact, this was demonstrated during a recent period of elevated sea surface temperatures (average: 28-29°C); all corals from the nursery survived. The high thermal resistance these corals show may be due in part to selection pressures from previous El Niño events that favored colonies with a genetic predisposition to warmer temperatures (Glynn et al., 2018).

On the other hand, we did not find significant differences in  $T_{opt}$  between photosynthesis and respiration rates for the nursery corals (Figure 5). If the photosynthesis  $T_{opt}$  was significantly lower than that of respiration, corals could initially survive at high temperatures but would bleach quickly and have higher chances of dying afterwards (Douglas, 2003; Silbiger et al., 2019). In fact, *Pocillopora* corals rely on photosynthesis to fulfill 75% to 95% of their nutritional requirements (Huang et al., 2020). Consequently, their survival rates following bleaching events tend to be relatively low. Therefore, the fact these two processes have so similar sensitivities further indicates nursery corals will have good survival rates during El Niño events.

As highlighted in the introduction, the prevailing theory suggests that photosynthesis should exhibit greater thermal sensitivity compared to respiration. This expectation arises because corals can typically sustain respiration for short periods even when they are already bleached and therefore not actively photosynthesizing. Previous studies conducted in Panama and Bermuda with the TPC approach have lent support to this notion (Gould et al., 2021; Silbiger et al., 2019). However, the literature also reveals significant variability in the thermal tolerance of different physiological processes across study sites and species (Bahr et al., 2018; Edmunds, 2008; Edmunds & Burgess, 2016). Therefore, it is plausible that the zooxanthellae strains associated with the corals used in our study, after several generations, have undergone adaptation to high temperatures, potentially influenced by previous El Niño events (Glynn et al., 2018). This adaptation process may have led to the selection of coral-symbiont combinations with similar thermal tolerance capabilities.

Another interesting finding was that photosynthesis had a greater range of variation in its  $T_{opt}$  than respiration, suggesting nursery corals have considerable differences in their bleaching responses to elevated temperatures. This variability could be attributed to



physiological differences both in the host corals and in the zooxanthellae associated with them (Baird et al., 2009; Douglas, 2003; Oakley & Davy, 2018), thus, more research in this direction is required. In any case, a high phenotypic (and associated genetic) diversity is linked to better population responses to adverse or unexpected circumstances, such as El Niño or La Niña events (Hume et al., 2016; Van Oppen et al., 2011).

We were unable to compare the thermal tolerance of the nursery corals with that of the donor colonies: our donor colonies TPCs either captured the first half (photosynthesis) or second half (respiration) of the correspondent TPCs, and hence the TPCs' parameters could not be estimated (Figure 4). Although the respiration TPC for nursery fragments was also somewhat flat, some individual corals showed greater variability and hence the parameters were estimable. In any case, having used temperatures higher than 35°C (for photosynthesis) or lower than 20°C might have improved the actual TPCs of these corals. This should be considered in future studies of this coral nursery, as the response of corals to low temperatures is an indicator of cold bleaching, which has already occurred in several locations in Galápagos (such as Devil's Crown pocilloporid reef) during past La Niña events (Glynn et al., 2018).

In this experiment, we did not use temperatures below 20°C because there was a possibility that coral fragments would bleach at the beginning of the experiments and affect our findings. However, we recommend the use of other methods such as acute stress assays or zooxanthellae counts to assess coral response to colder temperatures. Additionally, more respirometry experiments could be performed with lower starting temperatures to obtain complete TPC curves for future comparisons. A previous study conducted in 2019 with the coral *Orbicella franksi* also failed to reach the lower end of the TPC due to a lack of cold temperatures, as their experimental starting points were 24°C and 26°C. In contrast, a 2021 study conducted in Bermuda, which started at 17°C, successfully captured complete TPC curves. These findings indicate that a minimum temperature of 16°C or 17°C should be

considered when conducting TPC studies to fully understand the thermal performance of corals.

Our experiment had some limitations worth mentioning. For instance, the sample size was relatively small, consisting of only nine fragments from the donor colonies and 11 from the nursery. As a result, our findings may not accurately represent the *Pocillopora* coral communities present in Isabela Island. Additionally, all the fragments we used were small, measuring less than 2.5 x 2 x 1.5 cm in average. This is important because the impact of size in the physiology of complex modular animals like corals remains poorly understood; thus, when extrapolated to predict the physiological response of large coral colonies, area-normalized measurements obtained using small fragments may not provide precise estimations (Edmunds & Burgess, 2016). In fact, previous studies on a nursery with *Porites lobata* and *P. compressa* showed a significant positive correlation between fragment size and growth / survival rate, and higher urchin damage for fragments smaller than 3 cm (Forsman et al., 2006). Hence, it would be worthwhile to explore the effects of different fragment sizes in coral thermal tolerance with future respirometry experiments. Doing so could aid in re-evaluating the optimal size of fragments to be kept in the nursery (Ishida-Castañeda et al., 2020).

The period between fragment collection and the start of the experiments was relatively short, so the corals may have been stressed by the handling and cleaning procedures performed prior to testing. Therefore, the thermal performances we obtained could be inferior to the actual ones (Silbiger et al., 2019). Despite this, our findings showed that the nursery corals had high thermal tolerances, and it can be assumed that all the fragments were subjected to similar levels of stress, thereby minimizing the impact of this limitation on our comparisons. Furthermore, it is important to mention that we measured cumulative thermal stress by sequentially subjecting each coral fragment to eight different temperatures. This should be taken into account when comparing our results with those of acute thermal stress studies, as coral may react differently

to sudden temperature changes as opposed to gradual ones (McLachlan et al., 2020). Having said that, coral bleaching events typically occur due to sustained high ocean surface temperatures over an extended period of time (Douglas, 2003). As a result, it would be valuable to explore the responses of coral to both acute and prolonged thermal stressors in future experiments.

Finally, it would be interesting to extend these studies to other common coral species found in the Eastern Tropical Pacific (ETP) region, such as *Pavona clavus* or *Porites lobata* (Glynn et al., 2016). This could aid in predicting how the composition of coral communities in the Galápagos will shift with climate change and, consequently, help “Reef Revival” prioritize restoration with coral species that will be mostly affected by. Previous studies show that coral morphology plays a crucial role in bleaching resistance and long-term survival, with species with massive growth (like *Pavona* or *Porites*) generally being more tolerant than branching corals like *Pocillopora* (Liang et al., 2017; Loya et al., 2001; Schlöder & D’Croz, 2004; van Woesik et al., 2012). In fact, the coral community in the Galápagos was once dominated by branching genera like *Pocillopora*, but this has shifted in favor of massive species like *P. clavus* or *P. lobata* (Glynn et al., 2018).

## CONCLUSION

Our study showed that *Pocillopora* spp. corals from the nursery “Reef Revival” have higher thermal tolerances than the literature suggests and a wide variability in their bleaching responses to high temperatures; indicating that they will probably perform well in the face of future El Niño events. Our findings showcase the importance of doing thermal tolerance assessments in understudied places such as the Galápagos Islands. In the future, the insights we gained from this study could help us perform similar research in other parts of the archipelago or in mainland Ecuador, where coral communities have been largely overlooked. Similarly, our findings will help increase the database of respirometry knowledge we have for corals, allowing comparisons with the results obtained in other parts of the world.

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**ANEXO A: COORDINATES OF THE DONOR COLONIES USED FOR FIGURE 1**

<b>Location</b>	<b>Latitude</b>	<b>Longitude</b>
Islote el faro (1)	S 0. 967840°	W 90. 964089°
Islote el faro (2)	S 0. 966549°	W 90. 963896°
Las tintoreras	S 0. 969554°	W 90. 959680°
La calera norte	S 0. 962945°	W 90. 956309°
Concha y Perla	S 0. 962131°	W 90. 956696°
Embarcadero	S 0. 962616°	W 90. 957919°
La calera sur (1)	S 0. 965308°	W 90. 955955°
La calera sur (2)	S 0. 965133°	W 90. 955882°
Poza del muelle	S 0. 963950°	W 90. 956803°
Corales lanchas	S 0. 963180°	W 90. 957786°

**ANEXO B: THERMAL OPTIMA (TOPT) VALUES USED FOR FIGURE 5**

<b>Treatment</b>	<b>Variable Name</b>	<b>Point</b>	<b>Value</b>	<b>Lower Range</b>	<b>Upper Range</b>
Nursery Photosynthesis	Topt	Median	32.33	30.71	34.12
Nursery Respiration	Topt	Median	32.19	31.47	32.95