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Greater Mitochondrial Energy Production Provides Resistance to Ocean Acidification in "Winning" Hermatypic Corals

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Coral communities around the world are projected to be negatively affected by ocean acidification. Not all coral species will respond in the same manner to rising CO_2 levels. Evidence from naturally acidified areas such as CO_2 seeps have shown that although a few species are resistant to elevated CO_2, most lack sufficient resistance resulting in their decline. This has led to the simple grouping of coral species into "winners" and "losers," but the physiological traits supporting this ecological assessment are yet to be fully understood. Here using CO_2 seeps, in two biogeographically distinct regions, we investigated whether physiological traits related to energy production [mitochondrial electron transport systems (ETSAs) activities] and biomass (protein contents) differed between winning and losing species in order to identify possible physiological traits of resistance to ocean acidification and whether they can be acquired during short-term transplantations. We show that winning species had a lower biomass (protein contents per coral surface area) resulting in a higher potential for energy production (biomass specific ETSA: ETSA per protein contents) compared to losing species. We hypothesize that winning species inherently allocate more energy toward inorganic growth (calcification) compared to somatic (tissue) growth. In contrast, we found that losing species that show a higher biomass under reference pCO_2 experienced a loss in biomass and variable response in area-specific ETSA that did not translate in an increase in biomass-specific ETSA following either short-term (4–5 months) or even life-long acclimation to elevated pCO_2 conditions. Our results suggest that resistance to ocean acidification in corals may not be acquired within a single generation or through the selection of physiologically resistant individuals. This reinforces current evidence suggesting that ocean acidification will reshape coral communities around the world, selecting species that have an inherent resistance to elevated pCO_2.

Keywords: ocean acidification, hermatypic corals, mitochondrial electron transport activity, biomass, resistance

INTRODUCTION

Ocean acidification and ocean warming are threatening the existence of coral reefs. The latest IPCC report warns that if meaningful reductions in CO_2 emissions does not happen soon, 99% of coral reefs could disappear by the end of the century (IPCC, 2018). The effects of ocean warming on hermatypic corals are readily observable with a dramatic increase in the frequency and severity of massive coral bleaching events in recent years (Hughes et al., 2018). Recent studies have shown that ocean acidification has already reduced the community calcification rates of reefs. For instance, Albright et al. (2016) chemically manipulated seawater carbonate chemistry of a section of a reef to attain pre-industrial carbonate concentration and aragonite saturation state levels, increasing reef net calcification by *ca.* 7%. Ultimately ocean acidification may cause some reefs to shift to net dissolution in the near future (Eyre et al., 2018). Hermatypic corals and other calcifying organisms are considered among the most vulnerable to ocean acidification, as the decrease in oceanic pH and carbonate ions concentration makes the precipitation of calcium carbonate (aragonite in the case of corals) more energetically costly (Cohen and Holcomb, 2009).

In hermatypic corals, the calcification process occurs in a semi-isolated space located between the calicoblastic tissue and the already formed skeleton. At this site of calcification, corals elevate the aragonite saturation state by expelling protons, pumping in calcium ions and maintaining a high concentration of carbonates. This tight regulation of the calcifying fluid chemistry allows corals to maintain high calcification rates and for some species to even maintain an elevated saturation state of the calcifying fluid under reduced seawater $\Omega_{aragonite}$ (Holcomb et al., 2014; McCulloch et al., 2017). However, this regulation comes at a cost as the active ion transport required to maintain the elevated pH (Holcomb et al., 2014) and/or calcium ions (DeCarlo et al., 2018) is achieved via the activity of Calcium-ATPase (Zoccola et al., 2004). In addition, bicarbonate ion concentration and the resulting aragonite saturation state are also tightly regulated through the activities of multiple enzymes and ion transporters (Bhattacharya et al., 2016). The energy required for the activity of those enzymes and transporters is produced by the coral host in the form of ATP through the mitochondrial electron transport system (ETSA) and oxidative phosphorylation of the respiration process (Chalker and Taylor, 1975; Galli and Solidoro, 2018).

The dependence of calcification on the capacity of energy (ATP activities) production is supported by the observation of a high number of mitochondria in the calicoblastic tissue (Tambutté et al., 2007), the suppression of light calcification by oxidative phosphorylation inhibitors (Chalker and Taylor, 1975), the expression of the enzymes and transporter involved in calcification which is limited to calicoblastic cells, and the correlation between the ETSA activities with calcification rates in both healthy and bleached corals (Agostini et al., 2013, 2016; Higuchi et al., 2018). Electron transport system activities can be measured by isolating fragmented mitochondrial membranes of the host tissue, and measuring the reduction of an artificial tetrazolium substrate (by the mitochondrial complex II) in the presence of a saturating concentration of the coenzyme NADH (Packard, 1971; Agostini et al., 2013). The transfer of electrons from complex II is deemed to be the slowest reaction in the chain and therefore would limit the following reaction including the production of ATP. Therefore, ETSA can be used to assess the coral potential for energy production (i.e., ATP).

Most studies on the effects of ocean acidification on coral calcification are conducted in laboratories under highly controlled environments and over relatively short timescales (Gattuso et al., 2015). Short experimental durations (days to weeks) do not allow corals to acquire complete acclimation to high pCO_2 conditions and are clearly insufficient for achieving adaptation (multi-generation evolution) or ecological adaptation (selection of resistant species and/or individuals) to these conditions (Riebesell et al., 2010). Natural analogues such as volcanic CO_2 seeps have increasingly been used to complement laboratory experiments (Hall-Spencer et al., 2008), allowing the physiological study of corals that have fully acclimatized to elevated pCO_2 throughout at least their entire post-settlement lives (Noonan et al., 2018). At such natural analogues for future conditions, ocean acidification has drastically decreased the coverage and diversity of corals (Fabricius et al., 2011; Inoue et al., 2013; Enochs et al., 2015; Agostini et al., 2018). Since corals found in the acidified areas of CO_2 seeps are ecologically adapted to the elevated pCO_2 conditions (i.e., capable of surviving), those species for which individuals are found in reasonable numbers have been designated as winning species, while those that are very rare or absent from the elevated pCO_2 areas are the losing species (*sensu* Fabricius et al., 2011).

The dichotomy of a species being either a "winner" or a "loser" may be inappropriate to encapsulate the range of effects of ocean acidification on hermatypic corals, but it has facilitated the distinction between traits that contribute toward the resistance of corals to ocean acidification. Here we present a study using two CO_2 seeps, one located in the coral reefs off the coast of Normanby Island in Papua New Guinea, and the other in the coral communities off the coast of Shikine Island in Japan. The aim of the study was to highlight general physiological traits that distinguished winning species that are resistant to ocean acidification (present in the elevated pCO_2 areas) from losing species that are sensitive to ocean acidification (almost or entirely absent in the elevated pCO_2 areas but common in the control sites at ambient pCO_2). The physiological traits studied were protein contents as a proxy for tissue biomass, and coral host mitochondrial electron transport system activities (ETSA) as a proxy for energy production, either normalized by coral tissue surface area or coral host protein contents. ETSA normalized by tissue surface area (surface area-specific ETSA) represents the potential energy production while ETSA normalized by protein contents of coral tissue (biomass-specific ETSA) represents the biomass specific potential energy production. Three hypotheses were tested (**Figure 1**).

(1) *Inherent physiological traits of coral species resistant to ocean acidification:* Are inherent physiological traits related to biomass and energy production linked with the resistance to ocean acidification? This hypothesis was

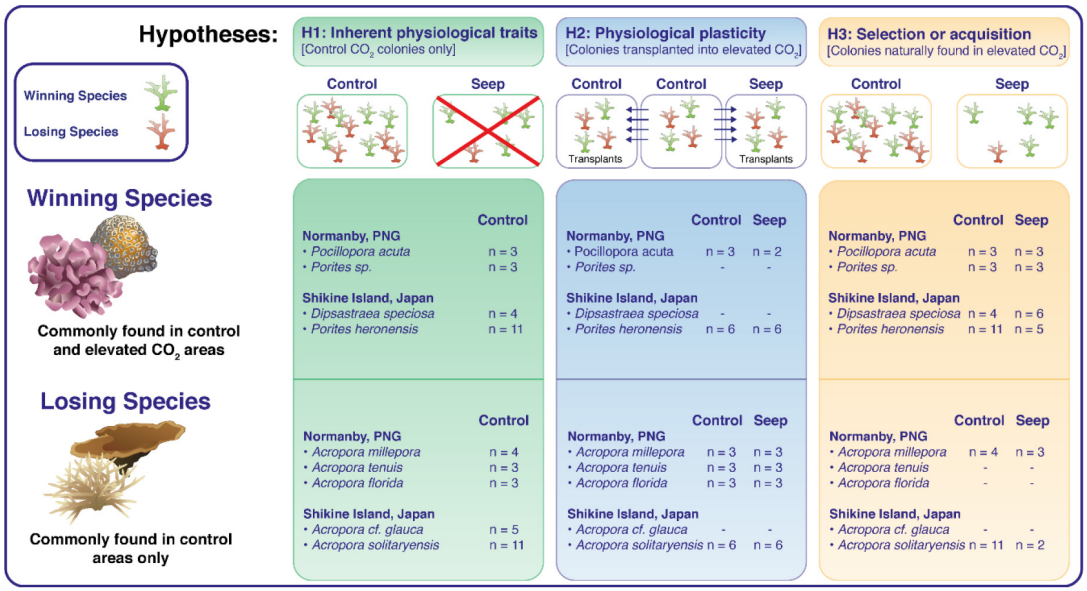

FIGURE 1 | Physiological traits (Protein per surface, surface area-specific ETSA and biomass-specific ETSA) of coral specimens belonging to winning species and losing species were used to test three hypotheses. (1) Inherent physiological traits of coral species resistant to ocean acidification: Physiological traits of corals in control areas; (2) Physiological plasticity as an acclimation response to ocean acidification: Physiological traits of corals transplanted from control areas into control and elevated pCO_2 areas (seep); (3) Selection or acquisition of resistance to ocean acidification: Physiological traits of corals found naturally in both elevated pCO_2 (seep) and control areas (*n* indicates the number of coral specimens used for each species and each CO_2 condition).

tested by measuring traits in individuals sampled in control areas only, thereby excluding potential stress responses and any acclimation/adaptation to elevated pCO_2 conditions.

(2) *Physiological plasticity as an acclimation response to ocean acidification:* Can resistance to ocean acidification be acquired over a short timescale (4–5 months) *via* physiological plasticity? This was tested by conducting transplantation experiments of winning and losing coral species from the control areas to the same control areas, and from the control areas to the elevated pCO_2 areas.

(3) *Selection or acquisition of resistance to ocean acidification:* Can the physiological traits required to tolerate ocean acidification be acquired either during the lifetime of individuals or through the selection of resistant individuals? This was tested by assessing the changes in protein contents and ETSA of winning and losing species that are naturally found in both the elevated pCO_2 areas and nearby (non-acidified) control areas.

MATERIALS AND METHODS

Sampling Sites

The study was conducted at two well-known CO_2 seeps. Previous studies at these two sites showed that the main driver of the difference in benthic communities between control and elevated pCO_2 sites was the increase in pCO_2. No difference in depth, total alkalinity, and temperature were found between sites (Uthicke and Fabricius, 2012; Fabricius et al., 2014; Agostini et al., 2018; Harvey et al., 2019; Witkowski et al., 2019; Cattano et al., 2020). At both seeps, the released gas is composed of more than 97% CO_2 with minimal concentrations in toxic gas such as hydrogen (Fabricius et al., 2011; Agostini et al., 2015). The first seep is located on the coast of Normanby Island in Papua New Guinea, and it has been extensively described in Fabricius et al. (2011) and following papers. The control area close to the CO_2 seep is a well-developed fringing reef showing a high diversity and coverage of corals. This control site experienced pH_T (mean ± SD) of 7.96 ± 0.03, corresponding to a pCO_2 of 468 ± 37 ppm and $\Omega_{aragonite}$ of 3.25 ± 0.17. In comparison, only few species of corals were found in the elevated pCO_2 area, with the coral community clearly dominated by massive *Porites* spp., although a few massive or sub-massive Favids, and a few branching corals of different species were present where space was available. The carbonate chemistry at this elevated pCO_2 area was: pH_T (mean ± SD) 7.66 ± 0.22, corresponding to a pCO_2 of 1,429 ± 1,027 ppm and $\Omega_{aragonite}$ of 1.83 ± 0.69. The second seep system used in this study is located off the shore of Shikine Island in Japan which was first described in Agostini et al. (2015, 2018) and following papers. Situated at a latitude of 34°N, this site is at the transition between sub-tropical and temperate zones. Due to the oceanographic context of the region, waters off Shikine Island show low CO_2 concentrations with pCO_2 often dropping down to 300 ppm. Large tabular Acroporids, sub-massive and encrusting corals (e.g., Favids and Porites) inhabit the control areas of these warm temperate reefs. In contrast, the area close to the seeps shows a very low abundance of corals and an ecosystem mostly dominated by turf algae (Harvey et al., 2019). There, the only corals recorded are a few small colonies of *Porites heronensis*, *Dipsastraea speciosa* and two small colonies of *Acropora solitaryensis*. This

area close to the seeps, hereafter "elevated pCO_2 area" has the following carbonate chemistry conditions: pH_T (mean ± SD) of 7.81 ± 0.09 corresponding to a pCO_2 of 769 ± 225 ppm and a $\Omega_{aragonite}$ of 1.76 ± 0.28. In contrast, the control areas located in an adjacent bay to the seeps are at ambient seawater carbonate chemistry: pH_T (mean ± SD) of 8.14 ± 0.06, pCO_2 of 309 ± 46 ppm and $\Omega_{aragonite}$ of 3.30 ± 0.35 (Harvey et al., 2018). Coral sampling and transplantation were conducted at both the Normanby and Shikine study sites, and in both the elevated pCO_2 and control areas.

Coral Samples

A total of 107 coral samples across 12 species were used, with 47 (across 7 species) sampled in Papua New Guinea under CITES permit 016132, and 60 (across 5 species) sampled in Shikine Island under permit Tokyo Prefecture 29-12. Fragments (5–10 cm^2 in size) of individual colonies were randomly sampled using chisel and hammer by a SCUBA diver. Care was taken to remove potentially attached algae or fauna. All colonies sampled were healthy and did not show signs of bleaching or tissue necrosis. Each coral species was categorized as a winning species if they were commonly found in the elevated CO_2 zones, or as a losing species if they were commonly found in the control zones but were absent or very rare from the elevated CO_2 zones. Previous literature available on the distribution of coral species for the two locations was also taken into consideration, namely Fabricius et al. (2011) and Agostini et al. (2018) for the Normanby Island and Shikine Island seeps, respectively. **Figure 1** summarizes all the species and coral specimens that were used.

(1) Inherent physiological traits of coral species resistant to ocean acidification: Physiological traits of corals in control areas

For the analyses designed to test hypothesis (1) Inherent physiological traits of coral species resistant to ocean acidification, fragments from colonies of the losing species *Acropora millepora*, *Acropora florida*, and *Acropora tenuis* for the Normanby site, and *Acropora cf glauca* and *Acropora solitaryensis* for the Shikine site, were sampled from the control areas. These were compared to traits measured on specimens from winning species sampled always at the control areas, *Pocillopora acuta* and massive *Porites* spp. for the Normanby site and *Dipsastrea speciosa* and *Porites heronensis* for the Shikine site.

(2) Physiological plasticity as an acclimation response to ocean acidification: Physiological Traits of corals transplanted from control areas into control and elevated pCO_2 areas

To test hypothesis 2, we transplanted corals from the control areas into the elevated CO_2 areas as well as into the same control site, the latter to test for transplantation effects. In Normanby, three losing species: *A. millepora* (control n = 4 and elevated pCO_2 n = 3), *A. tenuis* (control n = 3 and elevated pCO_2 n = 3), *A. florida* (control: n = 3 and elevated pCO_2 n = 3), and one winning species: *P. acuta* (control n = 3 and elevated pCO_2 n = 2), were transplanted from the 1st October 2016 to the 23rd January 2017. At the Shikine site, 12 individuals from the losing species *A. solitaryensis* and 12 individuals from the winning species *P. heronensis* were transplanted from the 22nd February 2018 to the 19th July 2018 into each site (n = 6 at each site for each species). Large fragments (>10 cm^2 in surface area) of healthy colonies of the various species were haphazardly sampled from the control areas using chisel and hammer, avoiding the base of the colonies and parts with clearly visible lesions. The fragments were glued to PVC tiles using epoxy glue and randomly attached to metallic grids (90 × 60 cm) that were fixed 10–20 cm above the seabed using metallic anchors at a depth of 3–6 m at the respective sites. In Normanby, the samples were collected and immediately transplanted. In Shikine, the transplantation field setup was done 1 month after sampling the coral fragments. During that month, the fragments were allowed to recover from the sampling in outdoor aquarium with running seawater at the Shimoda Marine Research Center, University of Tsukuba, Japan. At the end of the transplantation period, coral fragments were retrieved and measured on board the research vessels.

(3) Selection or acquisition of resistance to ocean acidification: Physiological traits of corals found naturally in both elevated pCO_2 and control areas

For the analyses designed to test hypothesis 3, we compared the physiological traits of winning and losing species where colonies could be found in both the control and elevated pCO_2 area. Although winning species could be found and collected easily in both areas (*P. acuta*, massive *Porites* spp. at the Normanby site, and *D. speciosa* and *P. heronensis* at the Shikine site), only the losing species *A. millepora* (Normanby site) and *A. solitaryensis* (Shikine site) could be collected in both areas. Physiological traits of the specimens found in control and elevated pCO_2 areas were compared. In addition, the traits of the specimens naturally found in the elevated pCO_2 area were compared to the traits of corals transplanted from the control areas to the elevated pCO_2 areas during the transplantation experiment.

ETSA and Protein Measurements Methods

The sampled fragments of colonies were immediately treated after collection, either aboard the M/B Chertan in Normanby, or at the Shikine Island Field Station (Shimoda Marine Research Center, University of Tsukuba) on Shikine Island. Coral tissue was removed using a custom air-gun connected to a source of compressed air in 10 ml of 0.2 μm filtered ice-cold seawater. Symbiodiniacea were pelleted by centrifugation (600 g, 15 min) and the coral host ETSA and protein contents were measured using the supernatant. All steps were conducted on ice to limit the degradation of the mitochondrial ETSAs. ETSA were measured under saturating concentrations of the NADH and NADPH coenzymes, in the presence of the artificial substrate INT ([4-iodophenyl]-3-[4-nitrophenyl]-5-phenyl-2H-tetrazolium chloride), following the protocols detailed in Agostini et al. (2013) with the following modifications. Filtered seawater instead of phosphate buffer saline was used for the blanks and for dissolving the reagents, and ETSA were extracted by placing

1 ml of the tissue slurry supernatant in 1.5 ml microtube in a sonic bath at 100 KHz for 10 min. For protein contents, 1 ml of the supernatant was stored at −20°C until measurement, which was carried out using the Bradford method (Bradford, 1976). Coral surface areas were determined by the wax method (Stimson and Kinzie, 1991).

Statistical Analysis

For all hypotheses the same physiological traits were used as response variables: protein contents (coral surface area normalized protein concentration), surface area-specific ETSA (coral surface area normalized ETSA), and biomass-specific ETSA (ETSA normalized by the host protein contents). For hypothesis 1, we tested for differences in those physiological traits between winning and losing species using a generalized linear mixed model (Gamma, link: identity) with resistance as a fixed effect (two levels: "Winning" and "Losing") and location (two levels: "Shikine," "Normanby") as a random effect in order to account for the associated variability. For hypothesis 2, we assessed the effect of a short-term exposure to elevated pCO_2 (4 months at the Normanby and 5 months at the Shikine seeps) compared to control conditions, for both winning and losing species. This used a generalized linear mixed model (Gamma, link: identity) with resistance (two levels: "Winning" and "Losing") and CO_2 condition (two levels: "Control," "elevated pCO_2") as fixed effects, and species as a random effect.

For hypothesis 3, we tested for the differences in physiological traits for coral specimens found naturally in the elevated pCO_2 areas compared to those found in the control areas, for both winning and losing species. This used a generalized linear mixed model (Gamma, link: identity) with resistance (two levels: "Winning" and "Losing") and CO_2 condition (two levels: "Control," "elevated pCO_2") as fixed effects, and species as a random effect. Finally, the response to elevated pCO_2 observed for coral specimens used in hypothesis 2 ("short exposure") and hypothesis 3 ("life-long exposure") were compared using a generalized linear mixed model (Gamma, link: identity) with resistance (two levels: "Winning" and "Losing") and exposure duration (two levels: "short exposure," "life-long exposure") as fixed effects and species as a random effect. All analysis and visualizations were performed with the R statistical programming languages (R Core Team, 2020) using the RStudio IDE[1] and the following packages: tidyverse (Wickham et al., 2019), ggplot2 (Wickham, 2016, p. 2), rstatix (Kassambara, 2020), patchwork (Pedersen, 2019), and lme4 (Bates et al., 2015). The code and raw data are available at https://gitlab.com/agoremix/oa-resistance.

RESULTS

(1) Inherent physiological traits of coral species resistant to ocean acidification: Physiological traits of corals in control areas

The amount of protein normalized per coral surface area was more than twofold higher in losing species compared to winning species (175 ± 20 and 73 ± 15 μg cm^{-2}, respectively; GLMM, resistance: t-value = 5.510, $p < 0.001$; **Figure 2A** and **Supplementary Tables 1, 2.1**). Mean surface area-specific ETSA was higher for losing species compared to winning species

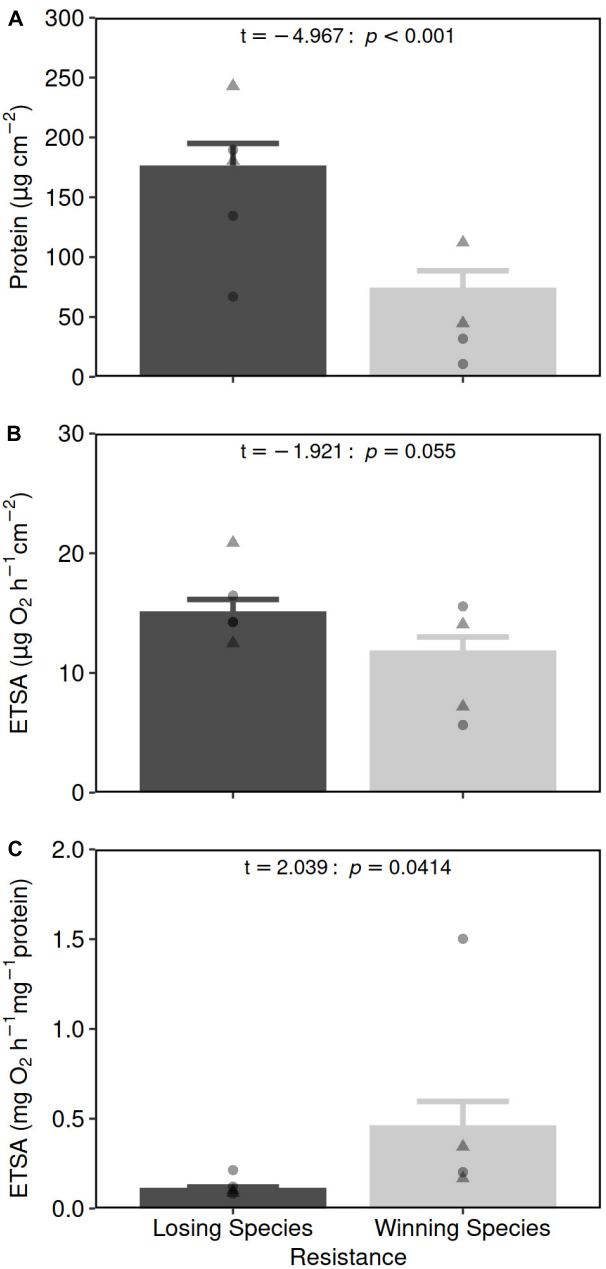

FIGURE 2 | Protein per surface **(A)**, ETSA per coral surface area **(B)** and ETSA per protein contents **(C)** (mean ± SE) in losing (black bars, sensitive to OA, n = 26) and winning coral species (**gray** bars, resistant to OA, n = 21), collected in the control areas. Individual points show the mean value per species with circles for species from the Normanby site and triangles for species from the Shikine site (see **Supplementary Table 1**). Two-way GLMM (Resistance with Location as a random factor) results are presented in the top-right of each panel (see **Supplementary Table 2** for more detailed statistics).

[1]https://rstudio.com/

(15.0 ± 1.1 and 11.8 ± 1.2 μg O_2 h^{-1} cm^{-2}, respectively, **Figure 2B** and **Supplementary Table 1**) but the difference was marginally non-significant (GLMM, resistance: t-value = −1.921, p = 0.055; **Supplementary Table 2.2**). This resulted in the biomass-specific ETSA being fourfold greater in the winning species compared to the losing species (0.46 ± 0.14 and 0.11 ± 0.01 mg O_2 h^{-1} mg^{-1} protein, respectively, GLMM, t-value = 2.039, p = 0.041; **Figure 2C** and **Supplementary Tables 1, 2.3**).

(2) Physiological plasticity as an acclimation response to ocean acidification: Physiological traits of corals transplanted from control areas into control and elevated pCO_2 areas

The four losing species tested (*Acropora millepora*, *Acropora tenuis*, *Acropora florida*, and *Acropora solitaryensis*) on average showed ∼40% decrease in their protein contents (**Figure 3A** and **Supplementary Table 3**) following a 4 or 5 months acclimation to elevated pCO_2 conditions (from 147 ± 17 μg cm^{-2} at the control sites to 87 ± 13 μg cm^{-2} at the elevated pCO_2 sites). The two winning species did not exhibit such a decrease, maintaining their protein contents at 87 ± 17 and 97 ± 15 μg cm^{-2} after transplantation to the control and elevated pCO_2 sites, respectively (GLMM: CO_2 condition x resistance, t-value = 2.064, p = 0.039; **Supplementary Table 4.1**).

The response of surface area-specific ETSA (**Figure 3B** and **Supplementary Table 3**) following a 4–5 months acclimation to elevated pCO_2 conditions showed a similar pattern to protein contents, with losing species showing a decrease in activity (from 13.3 ± 1.4 μg O_2 h^{-1} cm^{-2} at the control areas to 9.6 ± 1.5 μg O_2 h^{-1} cm^{-2} at the elevated pCO_2 areas), and winning species showing relatively little change (10.1 ± 1.5 μg O_2 h^{-1} cm^{-2} at the control sites to 9.2 ± 1.1 μg O_2 h^{-1} cm^{-2} at the elevated pCO_2 sites). The different responses exhibited by winning and losing species following transplantation into elevated pCO_2 conditions resulted in a significant interaction (GLMM: CO_2 condition x resistance, t-value = 811.0, $p < 0.001$; **Supplementary Table 4.2**).

For biomass-specific ETSA (**Figure 3C** and **Supplementary Table 3**), two patterns were observed with either a trend of increasing activity (*Acropora tenuis*, *Acropora florida*, and *Pocillopora acuta*) or remaining at similar levels of activity (*Acropora solitaryensis, Dipsastraea speciosa*, and *Porites heronensis*). However overall there was no significant response following transplantation into elevated pCO_2 conditions, regardless of the resistance of the species (GLMM: CO_2 condition, t-value = 1.747, p = 0.081; resistance, t-value = 0.393, p = 0.695; CO_2 condition × resistance, t-value = −0.573, p = 0.567; **Supplementary Table 4.3**).

(3) Selection or acquisition of resistance to ocean acidification: Physiological traits of corals found naturally in both elevated pCO_2 and control areas

For those coral specimens found naturally, only the losing species showed a difference in the amount of protein per surface area between control and elevated pCO_2 areas (**Figure 4A** and **Supplementary Table 5**). The mean amount of protein for those specimens collected at the elevated pCO_2 areas (86 ± 12 μg cm^{-2}) was half that of the specimens collected at the control areas (183 ± 26 μg cm^{-2}). This decrease was consistent for the two losing species tested: *A. millepora* and *A. solitaryensis*, at the Normanby and Shikine CO_2 seeps, respectively. The

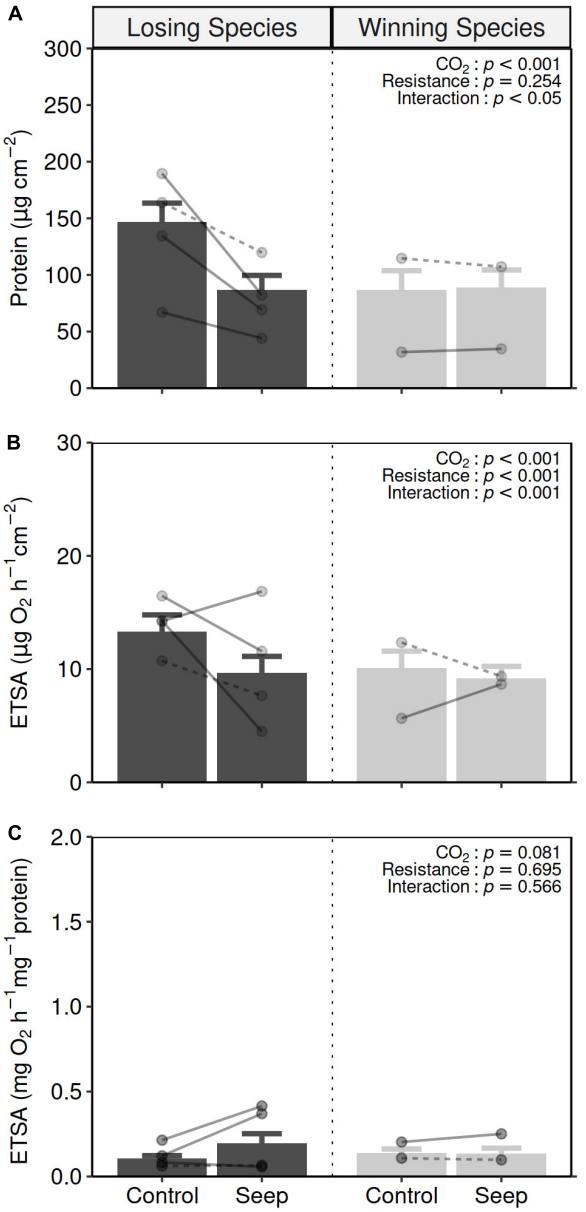

FIGURE 3 | Mean (±SE) protein per surface **(A)**, ETSA per coral surface area **(B)** and ETSA per protein contents **(C)** in losing species (black bars), and winning species (gray bars) specimens transplanted from the control areas to the control areas (control) and elevated pCO_2 areas (seep) for 4–5 months (losing species specimens: control n = 14, seep n = 5; winning species specimens: control n = 20, seep = 16). Points and lines show the mean and response per species with dashed and plain lines representing species from Shikine and Normanby CO_2 seeps, respectively (see **Supplementary Table 3**). Two-way GLMM: CO_2 condition x resistance with Species as a random factor) results are presented in the top-right of each panel (see **Supplementary Table 4** for more detailed statistics).

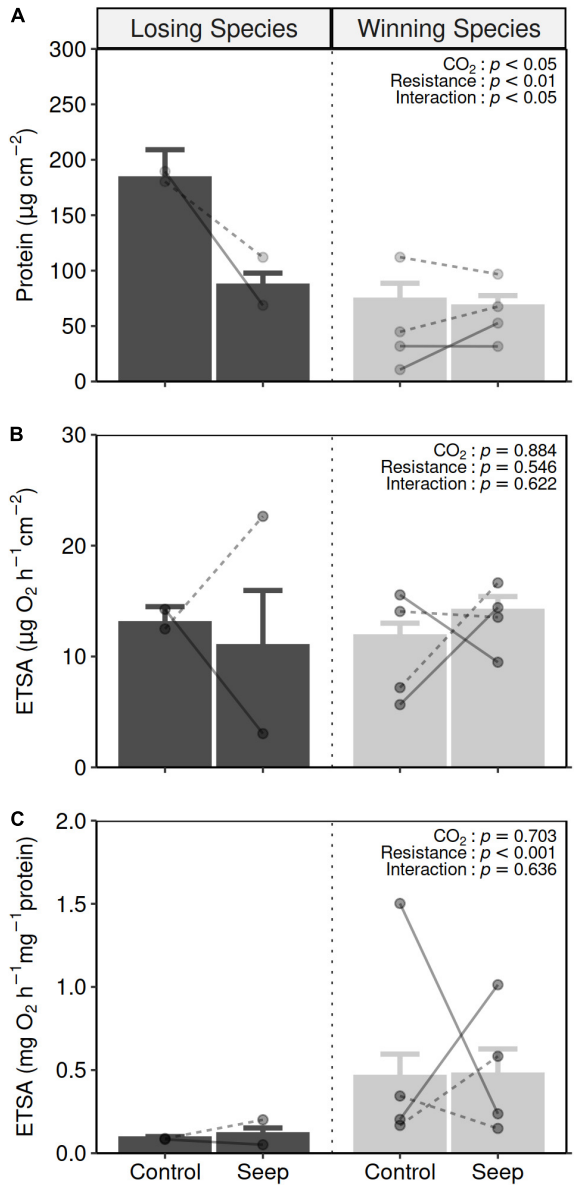

FIGURE 4 | Mean (±SE) protein per surface **(A)**, ETSA per coral surface area **(B)** and ETSA per protein contents **(C)** in losing species (black bars) and winning species (gray bars) species sampled at the control areas (control) and elevated pCO_2 areas (seep) (losing species specimens: control $n = 14$, seep $n = 5$; winning species specimens: control $n = 20$, seep $= 16$). Points and lines show the mean and response per species with dashed and plain lines representing species from Shikine and Normanby sites, respectively (see **Supplementary Table 5**). Two-way GLMM (CO_2 condition x resistance with Species as a random factor) results are presented in the top-right of each panel (see **Supplementary Table 6** for more detailed statistics).

mean amount of protein for winning species remained the same between specimens collected at the elevated pCO_2 and control areas (GLMM, CO_2 condition x resistance, t-value = 2.435, $p = 0.015$; **Supplementary Table 6.1**).

The response of ETSA in coral specimens exposed to life-long elevated pCO_2 conditions varied greatly among species. The amount of surface area-specific ETSA (**Figure 4B** and **Supplementary Table 5**) did not differ between specimens sampled in either control or elevated pCO_2 areas (GLMM, CO_2 condition: t-value = −0.146, $p = 0.884$) for both the winning nor losing coral species (GLMM, resistance: t-value = −0.603, $p = 0.546$) with no significant interaction (CO_2 condition x resistance: t-value = 0.493, $p = 0.622$; **Supplementary Table 6.2**). Biomass-specific ETSA (**Figure 4C** and **Supplementary Table 5**) were five times higher in the winning species (0.46 ± 0.14 mg O_2 h^{-1} mg^{-1} protein) compared to the losing species (0.093 ± 0.014 mg O_2 h^{-1} mg^{-1} protein) (GLMM, resistance, t-value = 3.519, $p = 0.0004$) but did not differ between CO_2 conditions for neither winning or losing species (GLMM, CO_2 condition: t-value = 0.381, $p = 0.703$; CO_2 condition x resistance: t-value = −0.473, $p = 0.636$; **Supplementary Table 6.1**).

The response to life-long exposure to elevated pCO_2 (hypothesis 3) was consistent with the response observed during the transplantation of specimens from control areas to elevated pCO_2 areas (hypothesis 2) (**Supplementary Tables 3, 5** and **Supplementary Figure 1**). No significant differences were observed in the response of coral specimens to the different periods of exposure to elevated pCO_2 for the mean amount of protein (GLMM, Exposure Condition, t-value = 0.685, $p = 0.4936$; **Supplementary Figure 1A** and **Supplementary Table 7.1**), surface area-specific ETSA (GLMM, Exposure Condition, t-value = −1.400, $p = 0.1615$; **Supplementary Figure 1A** and **Supplementary Table 7.2**), or biomass-specific ETSA (GLMM, Exposure Condition, t-value = −0.433, $p = 0.665$; **Supplementary Figure 1A** and **Supplementary Table 7.3**), in either losing or winning species (no significant interactions; GLMM, $p > 0.05$; **Supplementary Tables 5, 7**).

DISCUSSION

Climate change and ocean acidification will likely transform coral communities leaving only the most resistant species (Hughes et al., 2018) and individuals (Matsuda et al., 2020). Natural analogues such as CO_2 seeps can reveal ecological shifts in the composition of coral communities and highlight which physiological traits are associated with a higher resistance to ocean acidification. Here we found that resistant winning species had a lower biomass per coral surface area compared to losing species, while at similar levels of mitochondrial electron transport activities (ETSA). This resulted in a higher ETSA per biomass in the winning species. These traits were interpreted as the capacity of winning species to allocate more energy toward inorganic growth compared to losers which allocate more energy toward somatic growth. These traits are inherent to winning species and were not acquired following exposure to elevated pCO_2 since they were observed in specimens that were found in both the control and elevated pCO_2 areas. This capacity could generally not be acquired by the losing species, whether through physiological plasticity (following 4–5 months or lifelong acclimation to elevated pCO_2 conditions) or through the selection of specimens that show physiological traits associated with resistance to ocean acidification (a high biomass-specific energy production

potential) (see **Figure 5**). The responses following acclimation to elevated pCO_2 differed among species, with some winning species showing changes in their energy allocation depending on the pCO_2 levels they were maintained under. This demonstrates that winning species are still affected by ocean acidification, with their response either corresponding to some acclimation mechanisms that aims to increase their fitness under elevated pCO_2 or a negative impact of ocean acidification on their physiology. This highlights that the dichotomy of "winner" versus "loser" as previously proposed is too restrictive, and while informative, it does not accurately reflect the variety of responses and acclimation strategies of corals to ocean acidification.

A higher biomass or tissue thickness can provide more protection at the site of calcification (Jokiel, 2011; Rodolfo-Metalpa et al., 2011; Strahl et al., 2015; Kline et al., 2019) which allows for a better control of the aragonite saturation state and maintenance of inorganic growth under elevated pCO_2 conditions (Trotter et al., 2011; Holcomb et al., 2014). Individuals that can survive within the naturally acidified environment represent individuals whose physiological traits conferred them with sufficient resistance to ocean acidification as well as being ecologically capable of competing for primary space. In our study, the winning species exhibited a lower biomass in terms of their protein content normalized per coral tissue surface area. For example, despite their imperforated skeleton and low tissue biomass Pocilloporids are considered as resistant to ocean acidification (Comeau et al., 2014). Conversely, the higher protein contents found in losing species suggests that these species may require a larger amount of energy for somatic growth and the maintenance of this biomass (Kaniewska et al., 2015). This suggests that the amount of biomass covering the skeleton does not directly relate to a species' resistance to ocean acidification, instead the costly maintenance of biomass could reduce the ecological fitness of losing species under elevated pCO_2.

The mitochondrial ETSA, which represents the maximum potential rate of the respiratory electron transfer and therefore production of ATP, was similar or even slightly higher (albeit non-significantly) for the losing species compared to winning species when normalized by coral surface area. This finding reinforces the unexpected result that winning coral species do not achieve a greater resistance to ocean acidification through an increased biomass and/or availability of energy. Instead, the lower biomass and similar ETSA per coral surface area resulted in higher ETSA per biomass in the winning species. The resistance of winning coral species to ocean acidification could be linked with a different allocation strategy of energy between inorganic growth and somatic growth, with winning species allocating a greater amount of energy toward inorganic growth. As ocean acidification increases the energetic cost of calcification (Cohen and Holcomb, 2009), a higher energy production potential (ETSA) could allow for a stronger maintenance of the aragonitic saturation state at the site of calcification, enabling the maintenance of inorganic growth even under elevated pCO_2 (McCulloch et al., 2012). This increased energetic potential relative to biomass would additionally support other metabolic processes important for the resistance against ocean acidification, such as high fecundity (Albright, 2011), protein synthesis (Edmunds and Wall, 2014), or the maintenance of other anabolic pathways (Kaniewska et al., 2015).

In the present study, coral species that are sensitive to ocean acidification showed a decrease in their biomass regardless of whether they were acclimatized for 4–5 months or exposed for their entire lifetime to elevated pCO_2. Similar decreases in biomass have been observed across multiple studies (Edmunds and Wall, 2014; Strahl et al., 2015; Wall et al., 2017) with the response attributed to an impairment of protein anabolism due to elevated pCO_2. Two of the losing coral species (*A. florida* and *A. tenuis*) transplanted into the elevated pCO_2 conditions exhibited similar traits to the winning species, which themselves

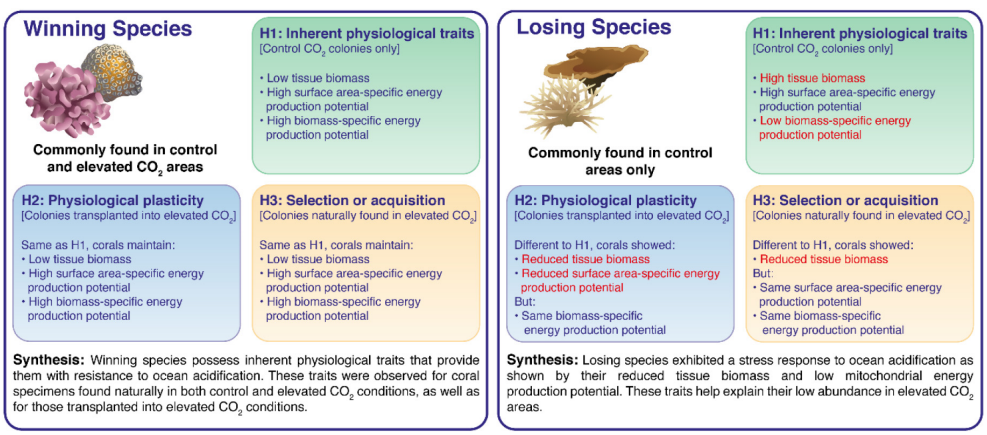

FIGURE 5 | Synthesis and interpretation of the main results for hypothesis (1) Inherent physiological traits of coral species resistant to ocean acidification: Physiological traits of corals in control areas, Hypothesis (2) Physiological plasticity as an acclimation response to ocean acidification: Physiological traits of corals transplanted from control areas into control and elevated pCO_2 areas, and Hypothesis (3) Selection or acquisition of resistance to ocean acidification: Physiological traits of corals found naturally in both elevated pCO_2 and control areas.

were unaffected by the change in pCO_2. This suggests that certain losing species are capable of demonstrating some physiological plasticity related to energy allocation. However this was not observed for the two other species (*A. solitaryensis* and *A. millepora*) tested and this plasticity did not translate into an overall increase in the biomass-specific energy production potential. Our observations support that resistance to ocean acidification cannot be acquired within a single generation (Comeau et al., 2019). Physiological traits of corals transplanted to elevated pCO_2 areas did not differ from those exhibited by corals that were found and sampled in the elevated pCO_2 areas: losing species exhibited a decrease in biomass which did not translate into an increase in biomass-specific energy production potential compared to winning species that were mostly unaffected by the elevated pCO_2. As specimens from losing species are rarely found in the elevated pCO_2 areas as well as often being of smaller size, this suggest that such short-term plasticity through the downregulation of metabolic processes may not be viable in the long term.

Although the different acclimation periods to elevated pCO_2 altered the specific responses of the species, the general patterns observed here were mostly consistent for corals tested in two radically different biogeographic regions, the coral reefs of Papua New Guinea and the marginal warm-temperate coral communities of Japan. The lower aragonite saturation state found in high latitudes suggests that the coral species there could have a higher tolerance to ocean acidification (Kleypas, 1999; Yara et al., 2012). However, the parameters tested in our study did not reveal a remarkable difference in terms of energy allocation between tropical and temperate coral species. This suggest that other traits, either physiological or ecological, may contribute toward the resistance to ocean acidification.

The importance of calcification and somatic growth in determining the resistance of scleractinian corals to ocean acidification remains poorly understood. Our findings suggest that species resistant to ocean acidification have a lower maintenance cost for biomass compared to more sensitive species. This could allow for more energy to be allocated toward inorganic growth providing them with an ecological advantage under high CO_2. The losing species did not exhibit these physiological traits and could not even acquire them through physiological plasticity or the selection of individuals. Instead, losing species exhibit a stress response under elevated pCO_2 whereby their biomass cannot be maintained. Taken together, unless adaptation rapidly occurs, losing species will lack the resistance required to survive future acidification, leaving only those species that possess an inherent resistance to ocean acidification. Such radical selection will likely result in a major loss of diversity and associated functioning in coral communities around the world.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://gitlab.com/agoremix/oa-resistance and Zenodo repository (https://doi.org/10.5281/zenodo.4415127).

AUTHOR CONTRIBUTIONS

SA, MM, FH, and RR-M contributed to conception and design of the study. SA, MM, FH, RR-M, and TB conducted the experiment and field survey in Papua New Guinea. SA, BH, RT, JH, and WY conducted the experiment and field survey in Japan. SA wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.600836/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.