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► **To cite this version:**

Marc Besson, William E Feeney, Camille Gache, Duncan A O'brien, Cécile Berthe, et al.. Anemone bleaching impacts the larval recruitment success of an anemone-associated fish. *Coral Reefs*, 2022, <10.1007/s00338-022-02323-x>. <hal-03872411>

HAL Id: hal-03872411

<https://hal.science/hal-03872411v1>

Submitted on 25 Nov 2022

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Anemone bleaching impacts the larval recruitment success of an anemone-associated fish

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Received: 7 September 2021 / Accepted: 5 November 2022
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Abstract In marine environments, mutualisms such as those between corals or sea anemones and their algal symbionts (Symbiodiniaceae) play a key role for supporting surrounding biodiversity. However, as the breakdown of the mutualism between corals and/or anemones and Symbiodiniaceae (i.e. bleaching) become increasingly frequent and severe, the risk of losing the additional species that rely on them may also increase. While the effects of anemone bleaching on the biology and ecology of anemone-associated fishes have been the subject of recent research, relatively little is known about the impacts that anemone bleaching might have on the recruitment of larval fish. Here, we report

that climate change-induced anemone bleaching impairs a secondary mutualism between anemones and an anemone-associated fish species, the threespot dascyllus (*Dascyllus trimaculatus*). Field-based monitoring over a 1-year period showed anemones that bleached experienced decreased recruitment of larval *D. trimaculatus* compared to those that did not bleach, with abundances of newly settled *D. trimaculatus* three times lower in bleached versus unbleached anemones. A visual choice experiment showed that this pattern is associated with fish being less attracted to bleached anemones, and a predation experiment demonstrated that fish associated with bleached anemones experienced higher mortality compared to those associated with unbleached anemones. These results suggest that the decreased recruitment of *D. trimaculatus* observed in bleached anemones may be driven by hampered pre-settlement (habitat selection) and post-settlement (survival to predation) processes for larval *D. trimaculatus* in bleached hosts. This study

Marc Besson and William E. Feeney have contributed equally to this work.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00338-022-02323-x>.

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highlights the risk of cascading mutualism breakdowns in coral reefs as conditions deteriorate and stresses the importance of protecting these mutualisms for the maintenance of coral reef biodiversity.

Keywords Mutualism breakdown · Climate change · Coral reef · Symbiosis · Predator–prey interactions

Introduction

The world's ecosystems are changing at an unprecedented rate (Pugnaire et al. 2019), with human activities and climate change impacting species and their interactions (Baker et al. 2018; Guiden et al. 2019). Consequently, the breakdown of ecologically important relationships, such as mutualisms (i.e. cooperative interactions between species), has become more common in recent years (De Fouw et al. 2016; Hector et al. 2022). These breakdowns can result in large-scale biodiversity losses, threatening ecosystem structure and function (Stuart-Smith et al. 2018; Vidal et al. 2021), and highlight the need to further examine how these cooperative relationships fare in the face of change.

Across the world's coral reefs, biodiversity is supported by the mutualisms that exist between organisms such as corals or sea anemones, and their algal endosymbionts Symbiodiniaceae (Goulet and Goulet 2021). In addition to supporting diverse species assemblages through the creation of habitat and resources (Brooker et al. 2019; Chomicki et al. 2019), these relationships also support secondary mutualisms, such as those between fishes and anemones (Fautin 1991). Anemone-associated fishes provide increased aeration and nutrition to anemones (Szczebak et al. 2013; Lim et al. 2016), which in turn provide protection to fishes from predators (Mariscal

1970; Fautin 1991; Feeney et al. 2019). Similar to the relationships between corals or anemones and Symbiodiniaceae, the symbioses between anemone-associated fishes and anemones support surrounding biodiversity, for example by facilitating the coexistence of multiple fish and crustacean species around the anemone (Fautin 1991; Schmitt and Holbrook 2003; Brooker et al. 2019). These compounding positive effects highlight how mutualism breakdown might affect the relationships and ecological processes that rely on them.

The past decade has seen increasingly recurrent and widespread mutualism breakdowns between corals or anemones and Symbiodiniaceae (i.e. bleaching) across the world's coral reefs (Hughes et al. 2018). Such bleaching events cause declines in the abundance and diversity of fishes associated with bleached hosts (Pratchett et al. 2011, 2018). In anemones, bleaching events have detrimental impacts on the abundance, behaviour, fecundity, growth rate, hormonal pathways, and metabolism of anemone-associated fishes (Saenz-Agudelo et al. 2011; Beldade et al. 2017; Norin et al. 2018; Cortese et al. 2021; Hoepner and Fobert 2022). However, relatively little is known about the impact that anemone bleaching has on the recruitment of larval anemonefishes (Saenz-Agudelo et al. 2011) and other damselfish species that live in obligate association with anemones during their settlement and juvenile stages (e.g. threespot dascyllus, *Dascyllus trimaculatus*; Schmitt and Holbrook 1996, 2000, 2003; Feeney et al. 2019). Given the importance of larval recruitment for the resilience of fish populations (Jones et al. 2009), and the importance of fish recruitment for the resilience to bleaching of their coral and anemone hosts (Chase et al. 2018; Pryor et al. 2020), it is key to better understand whether and how larval recruitment processes of anemone-associated fishes can be impacted by anemone bleaching.

In this study, to examine whether temperature-induced anemone bleaching was associated with reduced rates of fish recruitment, we first performed a 1-year in situ survey to monitor the abundance of newly settled and juvenile *D. trimaculatus* in unbleached and bleached magnificent sea anemone (*Heteractis magnifica*). We then conducted two laboratory experiments to determine whether pre-settlement processes (e.g., habitat selection and fish preferences for unbleached vs. bleached anemones) and/or post-settlement processes (e.g., predation and differential survival in unbleached vs. bleached anemones) drive *D. trimaculatus* recruitment patterns. In the first experiment, we performed a visual choice experiment using newly settled *D. trimaculatus* to determine their visual preference for unbleached versus bleached *H. magnifica*. In the second experiment, we conducted a predation test to investigate whether the protective benefits typically conferred to newly settled *D. trimaculatus* through their association with *H. magnifica* were reduced in bleached versus unbleached anemones.

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Materials and methods

Study species

The threespot dascyllus (*Dascyllus trimaculatus*) is a common coral reef fish in the Indo-West Pacific that is an obligate associate of the magnificent anemone (*Heteractis magnifica*) in French Polynesia during its settlement and juvenile stages (Bernardi et al. 2001; Feeney et al. 2019). *Dascyllus trimaculatus* breed year-round; however, settlement principally occurs twice a month around quarter moon phases, with pulses lasting between 3 and 5 days and peaking in June, July and August at Moorea, French Polynesia (our study location) (Schmitt and Holbrook 1999). Newly settled individuals are morphologically distinct from juveniles: their tail remains transparent for seven days following settlement (Schmitt and Holbrook 1996). Therefore, there is a 5-day period, spanning from 2 days before to 3 days after the new moon and full moon phases, during which it is possible to distinguish newly settled fish from juveniles that settled in a previous settlement pulse.

Study site and anemone monitoring

Research was conducted at Moorea (17° 29' S, 149° 49' W), French Polynesia, between August 2015 and August 2016. Field monitoring and sampling were conducted around the island, and aquaria experiments were conducted at CRIOBE research station. Periodic monitoring of 17 discrete anemone patches was conducted in an 800 m² area (depth: 1–3 m) at the north-east of the island (17° 29.854' S–149° 45.247' W to 17° 29.864' S–149° 45.253' W). Each anemone patch consisted of one to five individual anemones in contact with one another (minimum distance between two patches: 2 m). Given the narrowness of the lagoon at this location and the consequent strong water current, all anemone patches experienced similar temperatures (<http://observatoire.criobe.pf/CRIOBEData/displaySondeLive.jsp>). Patches were monitored at every full and new moon during the study period ($n = 25$ monitoring events). During each monitoring event at each anemone patch, newly settled and juvenile individuals were counted by observers on snorkel. Each patch was classified as bleached if at least one individual anemone was bleached (determined using a colour score; Siebeck et al. 2006), or unbleached if all anemones within a patch were unbleached.

Sampling and husbandry of anemones and fishes

Anemones (~ 20 cm diameter, orange colour morph), either fully unbleached or naturally fully bleached, but otherwise similar (tentacles extended, solid attachment to

substrate, closed mouth), were collected from shallow reef flats (depth: 1–5 m) on the north and southeast coasts of the island. Following collection, anemones were held in two 125 L (50 × 50 × 50 cm) aquaria (one for unbleached and one for bleached anemones) with ambient water flow for 48 h before being used in experiments. Each anemone was fed with a 1 cm² piece of mashed prawn every 48 h when not being used in an experiment. More than 200 newly settled fish individuals were collected from the same southeast location as the anemones (i.e. from a mix of unbleached and bleached anemone patches located very nearby from one another), using hand nets. This sample size (see visual choice and predation experiments below) could only be achieved at this location, which unfortunately did not allow us to determine the previous settlement choice of these individuals (the vicinity of all unbleached and bleached anemones patches in this location permitted the newly settled *D. trimaculatus* to move freely from one patch to another while we sampled them). After sampling, newly settled individuals were held together in a 125 L aquarium with ambient water flow for 48 h before being tested in either the visual choice or predation experiments. Newly settled individuals were not fed during this acclimation period. Blacktail snappers (*Lutjanus fulvus*, a common predator of settlement-stage reef fishes; Besson et al. 2020) of ~ 15 cm standard length were collected at night using hand nets on shallow reef flats (depth: 1–10 m) on the north coast of the island. Snappers were kept in a 125 L aquarium where they were fed daily with prawns to satiation, but were not fed for 48 h prior to the predation experiments. All holding tanks had a constant supply of fresh seawater and aeration, and shelters for fish. Different fish and anemones were used for each trial within each experiment. Following experimentation, all organisms were released back to their collection location.

Visual choice experiment

We conducted a choice experiment to assess whether newly settled individuals could distinguish between visual cues from bleached versus unbleached anemones. During each trial ($n = 10$ trials), 10 newly settled fish were placed into a 500 L visual choice aquarium (250 × 80 × 25 cm) where they acclimated for 5 min in a 40 × 40 cm area, enclosed with fine mesh, on one side of the aquarium (Supplementary Fig. 1). After the acclimation period, the mesh was removed, enabling the fish to navigate the full aquarium. At the opposite side of the aquarium, two transparent 18 L aquaria (30 × 20 × 30 cm), separated by 40 cm, presented two distinct visual stimuli: an unbleached anemone and a bleached anemone (Supplementary Fig. 1). Anemones were presented in separate aquaria to ensure

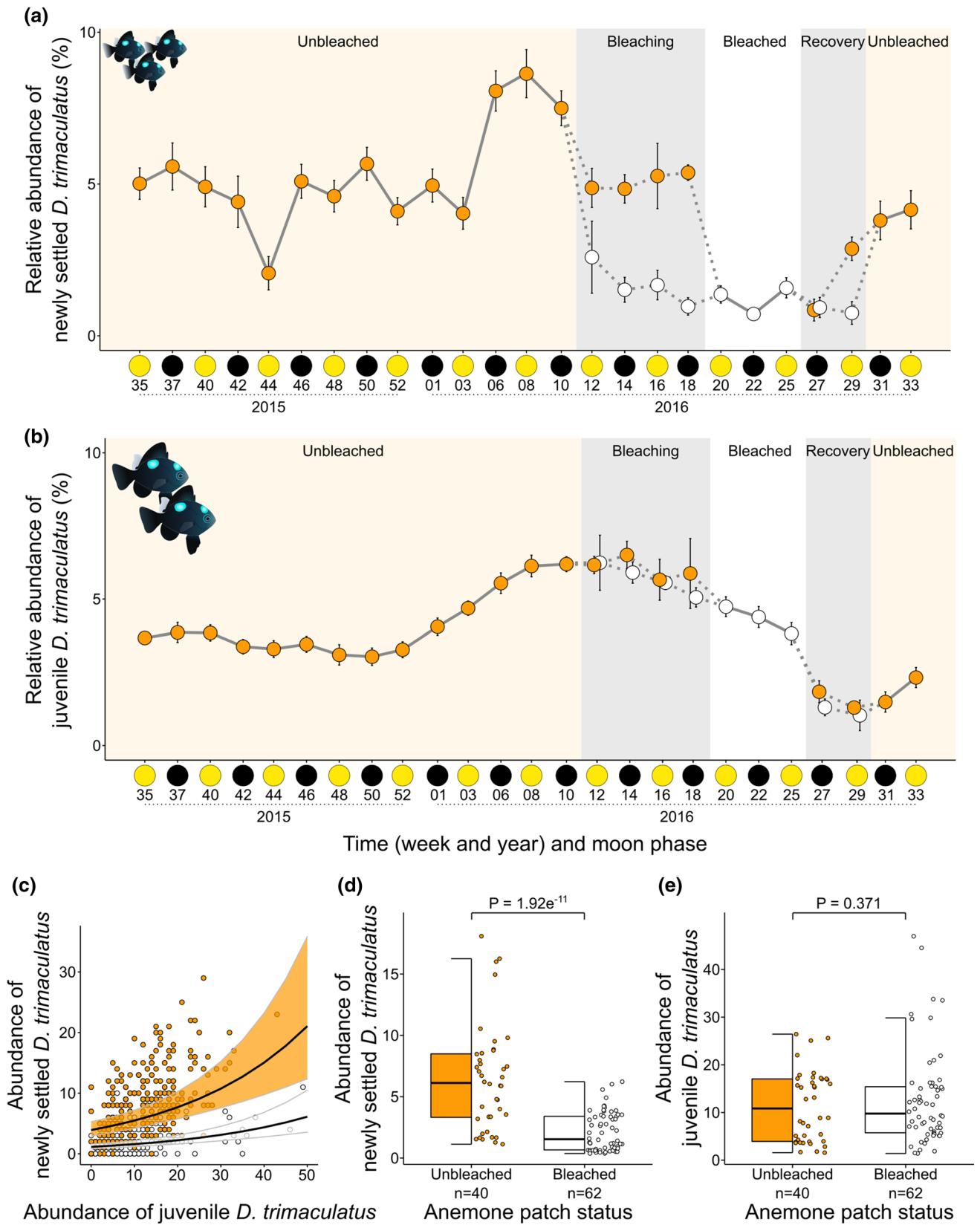


Fig. 1 Relative abundance of newly settled (a) and juvenile (b) *Dasycyllus trimaculatus* in unbleached and bleached anemones over a 1-year period at Moorea, French Polynesia. Orange and white circles indicate mean (\pm se) for unbleached and bleached anemone patches, respectively. Respective orange, grey and white backgrounds indicate periods when all anemone patches were unbleached (orange), some anemone patches were unbleached and others were bleached (i.e. bleaching and recovery periods) (grey), or all anemone patches were bleached (white). On the x-axis, yellow and black circles, and their associated number, indicate full and new moon, and their associated week, respectively. **c** Fitted values of newly settled *D. trimaculatus* abundance according to juvenile abundance and anemone patch status (negative binomial generalized linear mixed-effects model \pm 95% confidence intervals). **d** and **e** Abundance of newly settled and juvenile (respectively) *D. trimaculatus* in unbleached versus bleached anemone patches during the “bleaching and recovery periods” (i.e. weeks 12–18, and 27–29, when both types of anemones were simultaneously present). Boxplots indicate the minimum ($Q1 - 1.5 \times$ Interquartile Range (IQR)), first quartile ($Q1$), median, third quartile ($Q3$), maximum ($Q3 + 1.5 \times$ IQR), and circles indicate each fitted value. P values correspond to Tukey post hoc test outputs

that only visual cues were available to the fish. Visual preference was assessed by counting the number of fish located within 10 cm of the edge of the aquaria where the visual stimuli were presented, at 1 min and 5 min after release. These aquaria were switched between each trial to account for any potential side bias.

Predation experiment

We conducted a predation experiment to test whether the protection benefits conferred through anemone associations changed in bleached versus unbleached anemones. Prior to each trial ($n = 10$ trials per treatment), a bleached or unbleached anemone was placed in a 125 L aquarium in an open seawater circuit. The anemone was then left for 30 min to acclimate, after which 10 newly settled fish were introduced. After allowing the fish to habituate to the anemone (30 min), two snappers were introduced into the aquaria for 24 h. Each tank had a shelter (concrete block) for the snappers to hide. Following the 24 h experimental period, we counted the number of fish remaining in the aquaria.

Statistical analyses

All statistical analyses were conducted using R version 3.5.3 (R Core Team 2020). We used negative binomial generalised mixed-effects models using the *glmmTMB* package (Brooks et al. 2017) to assess if anemone patch status (unbleached/bleached), moon phase (new moon/full moon), the abundance of juveniles and the interactions between the abundance of juveniles and the categorical factors influenced the abundance of newly settled fish in anemone patches. Anemone patch identity was included as a random factor

to account for potential inter-patch differences (e.g. number of anemones in the patch, patch surface area). Similarly, a first-order auto-regression covariance structure (AR1) was included to control for residual serial dependence between temporally observed counts (Zuur et al. 2009; Mitchell et al. 2020). The resulting negative binomial model formulation was therefore given as follows:

$$y_i \sim \text{NB}(y_i | \mu_i, \theta)$$

$$\log(\mu_i) = X_i\beta + Z_i\mathbf{b}$$

where X is the $i \times p$ matrix of the p predictor variables/fixed effects, β is the $p \times 1$ vector of predictor regression coefficients, Z is an $i \times q$ design matrix for the random effect variables, and \mathbf{b} is the final $q \times 1$ vector of random effects. In this model, however, \mathbf{b} is composed of two parts, the random effect of patch identity ($b_{0,i,q}$ where q is the patch index and i is the count index) and the autoregressive covariance matrix between counts (ϵ_{ij}), where ρ is the estimated correlation coefficient. This means that as time between counts increases, the count variability become less similar, whereas temporally adjacent counts are more strongly correlated.

$$b_{iq} = b_{0,iq} + \epsilon_i$$

$$\epsilon_i = \begin{bmatrix} 1 & \rho & \rho^2 & \dots & \rho^{n-1} \\ \rho & 1 & \rho & \dots & \rho^{n-2} \\ \rho^2 & \rho & 1 & \dots & \rho^{n-3} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho^{n-1} & \rho^{n-2} & \rho^{n-3} & \dots & 1 \end{bmatrix}$$

A top-down approach was then used to arrive at the best descriptive model (Supplementary Tables 1–2), using Likelihood Ratio Tests and the Akaike Information Criterion (AIC) to assess the loss of explanatory power following the removal of an interaction or single-term predictor (Akaike 1973). Following this, Tukey’s post hoc tests were carried out using the *glht* function in the *multcomp* package (Hothorn et al. 2008). Using the same procedure, we evaluated if the anemone patch status and the moon phase influenced the abundance of juveniles in anemone patches. Once these models were established using the data from the entire monitoring period, the same models were fitted on a subset of the data from weeks 12 to 18 and 27 to 29, representing a shorter period of time when both unbleached and bleached anemone patches were present. This was used to control for any potential effect of season conflating the effect of anemone bleaching across the entire 1-year monitoring period.

The number of newly settled fish that chose the visual stimulus of the unbleached anemone versus the bleached anemone at 1 min and at 5 min, and the number of survivors

in bleached versus unbleached anemones were compared using one sided, paired and unpaired, respectively, Wilcoxon rank-sum tests.

Ethics statement

This study was carried out in accordance with the French Polynesia *Code de l'Environnement* for animal ethics and scientific research (<https://www.service-public.fr/diren/partager/code/>).

Results

Effect of anemone bleaching on fish recruitment

No anemones were bleached when monitoring started (August 2015) and until week 10 (February 2016) (“unbleached” period, Fig. 1a–b). Anemone bleaching was first detected in week 12 (March 2016) and the number of anemone patches being bleached persistently increased until week 18 (April 2016) (“bleaching” period, Fig. 1a–b). All anemone patches were bleached from week 20 (May 2016) to week 25 (June 2016) (“bleached” period, Fig. 1a–b). Recovery began at week 27 (July 2016) with the number of anemone patches being bleached constantly decreasing until week 29 (July 2016) (“recovery” period, Fig. 1a–b). No anemone exhibited evidence of bleaching by week 31 (August 2016) and until the end of the survey: second unbleached period (Fig. 1a–b).

The number of newly settled and juvenile *D. trimaculatus* varied across the study period (Fig. 1a–b), with the number of newly settled fish being positively influenced by the number of juveniles present on an anemone patch ($\chi^2_1 = 31.441$, $P = 2.056e^{-08}$, Fig. 1c; Supplementary Table 3). Anemone patch status also had a significant effect on the abundance of newly settled fish, which was three times lower per patch

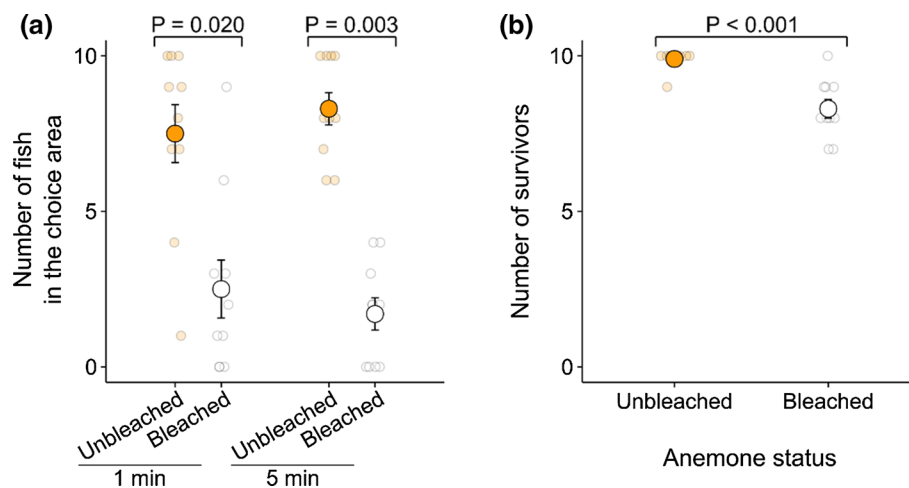
in bleached versus unbleached anemone patches, when considering either the full monitoring period ($\chi^2_1 = 158.317$, $P < 2.2e^{-16}$; Supplementary Fig. 2; Supplementary Table 3) or the “bleaching” and “recovery” periods (during which both bleached and unbleached anemones were observed, i.e. weeks 12–18 and 27–29; $\chi^2_1 = 45.051$, $P = 1.920e^{-11}$ Fig. 1d; Supplementary Table 4). However, we found no interaction between juvenile abundance and anemone patch status on new settler abundance (likelihood ratio test, $\chi^2_{-1} = 0.150$, $P = 0.699$), which is consistent with the absence of effect of anemone patch status on the abundance of juveniles, whether we consider the full period ($\chi^2_1 = 3.639$, $P = 0.056$; Supplementary Fig. 3; Supplementary Table 5) or the bleaching/recovery period ($\chi^2_1 = 0.801$, $P = 0.371$; Fig. 1e; Supplementary Table 6). We also observed no effect of full moon and new moon on the abundance of either newly settled or juvenile fish (Supplementary Tables 1–2).

Visual preferences and survival

For the visual preference experiment, in each trial, all newly settled fish immediately moved to the side of the aquarium near the choice areas and no individuals remained near the acclimation area (Supplementary Fig. 1). After both 1 min and 5 min, all fish were in a choice area, and significantly more individuals selected the visual stimuli of the unbleached anemone over the bleached anemone ($V = 48$, $P = 0.020$, and $V = 55$, $P = 0.003$, respectively; Fig. 2a).

For the predation experiment, after 24 h of exposure to two predators (blacktail snappers), *D. trimaculatus* that were associated with an unbleached anemone exhibited significantly higher survival ($99 \pm 1\%$) than those that were in a bleached anemone ($83 \pm 3\%$) ($W = 93$, $P < 0.001$, Fig. 2b).

Fig. 2 Number of fish associated with bleached and unbleached anemones following 1 min and 5 min visual choice experiment (a), and number of fish remaining on unbleached or bleached anemone following 24 h predation experiment (b). In both a and b, orange and white circles indicate mean \pm se, and transparent circles indicate each data point



Discussion

Our long-term anemone monitoring indicates that temperature-induced anemone bleaching significantly reduces the recruitment of larval *D. trimaculatus*. Our laboratory experiments suggest that this recruitment pattern may be driven by hampered pre- and post-settlement processes in bleached anemones. Indeed, our visual choice experiment showed that newly settled fish have decreased visual preferences for bleached anemones; however, it is possible that the initial settlement choice made by these fish (before we sampled them) influenced their second (laboratory) choice. Nevertheless, our predation experiment indicates that fish associated with a bleached anemone experienced significantly higher predation compared to those associated with an unbleached anemone. Taken together, these results suggest that bleached anemones are less attractive and confer less protection to newly settled *D. trimaculatus* than unbleached anemones.

While recent research suggests that anemone bleaching does not impact nematocyst toxicity (Hoepner et al. 2019), past research demonstrated that bleached anemones can decrease in body size, potentially reducing their ability to provide shelter and increasing the likelihood of predation for associated fish (Saenz-Agudelo et al. 2011). The shelter characteristics of bleached anemones are further hampered by the white colour of the bleached host, which likely make newly settled and juvenile *D. trimaculatus* individuals (mostly black) more conspicuous, and thus more prone to predation (Coker et al. 2009). In the anemonefish *Amphiprion chrysopterus*, diminished growth and metabolic rates were observed in juvenile individuals associated with bleached *H. magnifica* anemones (Norin et al. 2018; Cortese et al. 2021). Moreover, *A. chrysopterus* juveniles were shown to spend more time out of their *H. magnifica* anemone when bleached (Cortese et al. 2021). While we did not measure these variables, these results in another anemone-associated fish species are consistent with the higher predation risk and decreased recruitment success observed in bleached anemones in our study. Additionally, increased stress hormone levels have been observed in *A. chrysopterus* associated with bleached *H. magnifica* (Beldade et al. 2017). The alteration of such endocrine pathways is known to impair larval fish sensory organ maturation and sensory abilities during recruitment (Besson et al. 2020), which may also help explain the increased vulnerability to predation of newly settled *D. trimaculatus* in bleached anemones. Furthermore, the visual preference of new settlers for unbleached anemones compared to bleached anemones suggests that the decreased recruitment of *D. trimaculatus* in bleached anemones result from a combination of impaired pre-settlement (habitat selection) and post-settlement (survival in the selected habitat) processes. Additionally, the increased temperatures that can cause anemones to bleach can also impair anemonefish

physiology (Pankhurst and Munday 2011), regardless of if they settled in a bleached or unbleached anemone, which could explain the decreasing abundance of juvenile *D. trimaculatus* observed in our study over the bleaching and bleached periods.

Given that the key benefits conferred through the fish-anemone relationship, to both the anemones and fish, are diminished by bleaching, our results suggest that the initial breakdown of the mutualism between anemones and Symbiodiniaceae impairs the secondary mutualism between anemones and fish. It is well-established that the breakdown of mutualisms that support biodiversity can lead to decreased availability of ecologically important resources and negatively impact the biodiversity and ecological processes that relies on those resources (Hughes et al. 2018). Our results show that the presence of juveniles increases recruitment rates of new settlers, that anemone bleaching reduces the likelihood of habitat selection, and that fish associated with a bleached anemone do not receive a key benefit typically conferred through this partnership (i.e. refuge from predation; Feeney et al. 2019). Likewise, as the benefits conferred to anemones (e.g., increased aeration and nutrition, Szczebak et al. 2013; Lim et al. 2016; and promoted growth, Holbrook and Schmitt 2005) rely on the presence of anemonefishes, a decrease in the recruitment of larval anemonefishes, and potentially other anemone-associated fishes such as *D. trimaculatus*, could decrease these benefits. In corals, the presence of symbiotic fishes, through the service they provide, positively influenced the host colonies susceptibility to bleaching, and recovery from bleaching (Chase et al. 2018). Given that the same positive effects have been demonstrated for anemonefishes on the resilience of anemones to bleaching (Pryor et al. 2020), our results suggest that a reduced recruitment of larval fishes into bleached anemones may trigger a negative feedback loop. Indeed, a reduced recruitment of fish into bleached anemones could decrease their resilience and recovery from bleaching, further decreasing their ability to recruit new fish. Therefore, in addition to supporting a broad literature that shows biodiversity costs associated with mutualism breakdown, our results are consistent with the hypothesis that mutualism breakdowns and impairments may compound coral reef degradation through the progressive reduction in, or removal of, the resources that each mutualism creates.

In conclusion, our findings show that the breakdown of one mutualism (i.e. anemone bleaching) impair ecological processes (i.e. pre- and post-settlement processes and recruitment of a larval fish) that could ultimately lead to the breakdown of a secondary mutualism (i.e. between anemones and anemone-associated fishes). Our results therefore support the idea that one mutualism breakdown may precede cascading mutualism breakdowns through the progressive loss of ecologically important resources that are produced

as a by-product of these mutualistic relationships (Kiers et al. 2010). Considering that virtually all organisms rely on mutualisms for their survival (Bronstein 2015), this study highlights the importance of protecting Symbiodiniaceae-anemone and fish-anemone mutualisms, as this may confer disproportionate benefits for maintaining coral reef biodiversity and ecosystem functions.

Acknowledgements We thank J-O Irisson and D Cortese for their help inputting the statistic models. We thank I Moniz, L François, A Savura, L Latry, P Schep, D Van Oosten, H Dore, T Lorin and M Calvez for their help in the field.

Author contributions MB, WEF, and DL conceived the study, with important contributions from CG, RMB, ZLC, and VL; field data and experiments were conducted by MB, CG, and CB; statistical analyses were conducted by MB and DAO; MB and WEF wrote the manuscript with input from all authors.

Funding MB was supported by Fondation Bettencourt-Schueller; WEF, ZLC and RMB were supported by IRCP-OPT-ECO Research Grants, and WEF by the Alexander von Humboldt Foundation; DL and VL were supported by Fondation de France (2019-08602), ANR-19-CE34-0006-Manini, and ANR-19-CE14-0010-SENSO.

Data availability Data and code supporting the results are available at <https://doi.org/10.5281/zenodo.6657184>.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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