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Simulated climate change scenarios impact the reproduction and early life stages of a soft coral

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ABSTRACT

Coral reefs are threatened worldwide by global climate change, manifested in anthropogenic ocean warming and acidification. Despite the importance of coral sexual reproduction for the continuity of coral reefs, our understanding of the extent of the impact of climate change on coral sexual reproduction, particularly on coral reproductive phenology and early life stages, is limited. Here, we experimentally examined the effects of predicted end-of-the-century seawater conditions on the sexual reproduction and photosynthetic capacity of a Red-Sea zooxanthellate octocoral, *Rhytisma fulvum.* Sexually mature colonies were exposed to ambient temperature and pH conditions and to Representative Concentration Pathway (RCP) conditions (4.5 and 8.5), five weeks prior to their expected surface-brooding event. The reproductive phenology of the colonies under the simulated seawater conditions was compared to that on the natural reef. In addition, subsequent planulae development and their metamorphosis into primary polyps under the same RCP conditions as their parent colonies were monitored in a running seawater system. The results reveal that both RCP conditions led to a change in the timing of onset of the surface-brooding event and its synchronicity. In contrast, the surface-brooding event under ambient conditions co-occurred with that of the *in-situ* reef colonies and maintained its synchrony. Similarly, planula survival and polyp metamorphosis rate were significantly reduced under both RCP conditions compared to propagules reared under ambient conditions. In addition, the photosynthetic capacity of the parent colonies under both RCPs showed a reduction relative to that under the ambient conditions in the experiment, suggesting a reduction in carbon fixation during the late stages of gametogenesis. While our findings indicate that octocoral reproductive phenology is affected by environmental changes, further work is required in order to elucidate the long-term implications for the *R. fulvum* population in the northern Red Sea.

1. Introduction

Marine ecosystems are being challenged by increasing sea surface temperatures (SST) and reduced seawater pH, resulting from increased anthropogenic CO_2 in the atmosphere. The carbonate system is undergoing change too, with a 0.1 unit decrease in seawater pH having been reported between the pre-industrial period and the end of the 20th century (Hoegh-Guldberg et al., 2007; Doney et al., 2009). Ocean acidification (OA) (Doney et al., 2009) is predicted to continue, with an additional decrease of 0.2–0.4 pH units by the end of the 21st century (IPCC, 2013). SST is predicted to rise by a further 1–4 °C by the end of the 21st century (IPCC, 2013), increasing the frequency of extreme marine heatwaves (Smale et al., 2019). These global stressors are rapidly transforming the coral-reef ecosystems worldwide from scleractinian coral dominated habitats to octocoral dominated ones (Norström et al., 2009; Tsounis and Edmunds 2017; Sánchez et al., 2019). However, our knowledge regarding the impact of climate change on octocorals is both scarce and currently limited to specific species and locations (e.g. Kipson et al., 2012 (*Paramuricea clavata,* Northwestern Mediterranean); Gabay et al., 2014 (*Ovabunda macrospiculata*, Gulf of Aqaba); Ramsby and Goulet 2019 (*Briareum asbestinum*, Mexico, Caribbeans); Lasker et al., 2020 (multiple octocoral species, US Virgin Islands, Caribbeans). Understanding the combined effects of the predicted end-of-the-century SST and pH conditions on a greater number of octocoral species, including their early life stages, is therefore of importance for predicting – and mediating – the fate of coral reefs.

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While climate change, reflected in ocean warming (OW) and OA processes, is already severely affecting tropical reefs (Hughes 1994; Hughes et al., 2018), studies on their combined impact (OWA) on coral early life stages are scarce (Cumbo et al., 2013; Przeslawski et al., 2015). Timing is a key factor for successful sexual reproduction among marine invertebrates, including corals (e.g. Richmond 1997; Keith et al., 2016). It has been suggested that the seasonal increase in SST is the major environmental cue governing the timing of reproduction events in corals (Keith et al., 2016). Similarly, little is known on the effects of OWA on coral reproductive phenology, including the timing of onset of spawning events and their synchronicity (Babcock et al., 1986; Guest et al., 2008). The synchronicity of onset of spawning events within a given coral population commonly coincides with suitable environmental conditions, maximizing the reproductive yield (number of planulae-larvae) by enhancing fertilization rate and the survival of the sexual progeny (Babcock et al., 1986; Guest et al., 2008). It has been hypothesized that coral reproductive synchrony may face a disruption due to changes caused by OW, particularly among species for which seawater temperature acts as an external cue for their reproduction (Baird et al., 2009). Surface-brooding synchronicity is entrained by environmental cues (Benayahu and Loya 1983; Liberman et al., 2018), with the planulae exposed to seawater during embryogenesis (Benayahu and Loya 1983, Lasker et al., 2020). However, there is little information to date regarding how this might be impacted by OWA conditions. Such understanding is necessary in order to improve coral-reef management and conservation in an era of global climate change (Anthony et al., 2015; Hughes et al., 2017).

Studies on the effects of OW alone on the early life stages of stony corals have demonstrated adverse impacts, including reduced fertilization success(*Acropora millepora*: Negri et al., (2007), reduced planula survivorship (*Favia fragum* and *A. palmata:* Randall and Szmant 2009a; 2009b), and accelerated settlement followed by increased post-settlement mortality (A. solitaryensis: Nozawa and Harrison 2007). In addition, OW has been shown to cause embryonic abnormalities in both the stony coral *A. millepora* (Negri et al., 2007) and the octocoral *Paramuricea clavata* (Kipson et al., 2012). Similarly, OA seems to pose a severe threat to coral early life stages, including reduced fertilization success (*A. palmata*: Albright et al., 2010), reduced subsequent planula metamorphosis rate, reduced polyp growth rate, and a reduction in algal acquisition (*A. digitifera* and *A. tenuis*: Suwa et al., 2010). Interestingly, the combined effects of OW and OA on the sexual reproduction and early life stages of various marine invertebrates do not necessarily act synergistically (see also Supplementary Table S1). For example, no consistent effect of OWA conditions was noted on the fertilization, embryonic development, and survivorship of planulae and primary polyps of *A. millepora* and *A. tenuis*, whereas OW by itself accelerated their embryonic development (Chua et al., 2013). A significant decrease in the growth rate of primary polyps of *Porites panamensis* occurred under OWA conditions, but the settlement and survival rates of its planulae were not affected (Anlauf et al., 2011). No study has as yet examined the combined effect of OWA conditions on the early life stages of reef-dwelling octocorals or on their photo-physiological responses.

In the current study we hypothesized that the predicted end-of-the-century OWA conditions (RCPs 4.5 and 8.5, IPCC, 2013) may affect the reproductive phenology and photo-physiology of the octocoral *Rhytisma fulvum* in the Gulf of Aqaba (northern Red Sea). This species is a dioecious surface-brooder reproducing annually in June–July (Benayahu and Loya 1983), and it hosts *Cladocopium* photosymbionts across its entire depth of occurrence in the Gulf of Aqaba (Pupier et al., 2019). Shortly before the embryos appear on the colony surface, the spawned sperm drift to the female colonies and the mature oocytes are fertilized internally and then spawned. The embryos develop on the surface of the female colonies, entangled in the coral mucus, until planula maturation on day 6 following their release (Benayahu and Loya 1983). Interestingly, the timing of onset (the specific day) and synchronicity of a surface-brooding event (the simultaneous release of the fertilized eggs from the polyps at a given depth) may greatly resemble a spawning event. However, similar to internal-brooding corals, the dispersing propagules are competent planula larvae that are released from the parental colony into the water. Consequently, it has been suggested that this peculiar mode of reproduction represents an evolutionary link between broadcast-spawning and internal-brooding (Kahng et al., 2011). In an earlier study, we demonstrated that a thermal threshold acts as an external cue for the surface-brooding events in *R. fulvum,* which also leads to a delayed event in the upper mesophotic reefs (30–45 m) compared to in the shallower waters (Liberman et al., 2018).

Specifically, the current study tested the effects of predicted end-of-the-century OWA conditions, including the timing of surface-brooding onset and its synchronicity, on planula survival and metamorphosis of the primary polyps. In addition, the photosynthetic capacity of the parent colonies was examined by measuring their chlorophyll fluorescence. Our findings shed light on the possible effects of OWA conditions on a suite of life-history traits of this octocoral.

2. Material and methods

2.1. Experimental design

To examine the effect of predicted end-of-the-century OWA conditions on the synchronicity of sexual reproduction and on the photosynthesis of *R. fulvum,* combined seawater temperature and pH conditions were manipulated in a triplicate 40 L aquaria set-up (Table 1). Two treatments and a reference (ambient seawater conditions) were established in the Red Sea Simulator (RSS), at the Interuniversity Institute (IUI) for Marine Sciences in Eilat, Israel (northern Gulf of Aqaba, Red Sea) (Bellworthy and Fine 2018). Seawater temperature and pH conditions were selected according to the predicted end-of-the-century RCP conditions presented by the Intergovernmental Panel on Climate Change (IPCC, 2013). The treatments comprised present-day ambient seawater temperature and pH (24.7 °C, pH∼8.14); RCP 4.5 (seawater + 1 °C, pH − 0.2); and RCP 8.5 (seawater + 3 °C, pH − 0.4, Table 1). Temperature and pH were monitored in the RSS aquaria by an automated robot that continuously adjusted the system settings to reach the selected values (Bellworthy and Fine 2018). A total of 25 colonies of *R. fulvum* were sampled (6 × 2–6 × 4 cm) several weeks prior to the expected surface-brooding event (Liberman et al., 2018, Day 1, May 30, 2017) on the reef adjacent to the IUI and on another reef 2 km to the north (10–12 m, 29°32′ N 34°55' E). The colonies were individually labeled and a 1 × 1 cm fragment from each was preserved in 70% ethanol for sex determination. Sexual maturity was confirmed under a dissecting microscope according to the presence of male or female gonads in the polyp cavities, following a previously published method (Liberman et al., 2018). Fertile colonies (n = 21) were immediately transferred to nine RSS aquaria, each with at least one female and one male colony per aquarium, and were acclimated for five days under ambient seawater conditions. The remaining four colonies were returned to the IUI reef (10 m) and served as a reference for the translocation procedure. In the treatment RSS aquaria, seawater temperature was increased by 1 °C and pH was decreased by 0.1 units daily, until the above-desired values per treatment were attained. Colony viability was monitored daily for five consecutive weeks (i.e., colony growth vs. shrinkage and polyp expansion vs. retraction), and also in comparison to the translocated colonies on the natural reef.

2.2. Reproductive phenology

The parent colonies in the treatment RSS aquaria were examined twice a day in order to detect the onset of surface brooding in each colony and monitor the development of the planulae, in comparison to conspecific colonies at the IUI reef. Following their release to the colonies' surface, 50–100 embryos were carefully removed from each

Table 1

Rhytisma fulvum: Mean (±SE) of measured and calculated abiotic parameters under ambient, RCP 4.5 and RCP 8.5 conditions in Red Sea simulator aquaria. Experiment conducted in 2017. n indicates the number of measurements for each variable in each treatment.

	Ambient			RCP 4.5			RCP 8.5		
Treatment/Variable	June	July	August	June	July	August	June	July	August
Temperature	24.84 ± 0.01	26.53 ± 0.01	26.78 ± 0.01	26.19 ± 0.007	27.46 ± 0.009	27.08 ± 0.01	27.76 ± 0.003	29.1 ± 0.007	29 ± 0.005
N	2982	4447	4464	16013	44834	24315	45793	27727	31517
pH	8.13 ± 0.001	8.12 ± 0.001	8.12 ± 0.002	7.89 ± 0.0008	7.88 ± 0.001	7.91 ± 0.001	7.63 ± 0.001	7.69 ± 0.001	7.72 ± 0.003
N	853	1351	922	1585	2396	1799	1141	2279	2019

colony and introduced into duplicate 50 ml transparent PVC tubes (25–50 embryos per tube), sealed with a 200 μm mesh to allow continuous water flow. The tubes were placed in the RSS aquaria under the respective conditions of their parent colonies (i.e., ambient seawater conditions, RCP 4.5, and RCP 8.5). Algae and debris were removed daily from the tubes and nets in order to maintain transparency and consistent water flow. In order to compare the effect of OWA conditions on the reef-derived embryos with that on the RSS embryos, they were similarly collected upon release and introduced into tubes as described above, and divided into six experimental RSS aquaria (2 tubes per treatment). The embryos/planulae were individually counted on day 3 and day 7 following release for both the RSS and reef embryos. Subsequently, eight days after release of the embryos, terracotta tiles (2 × 2 cm), pre-conditioned under ambient RSS seawater conditions, were introduced into the tubes. Primary polyp development was examined using a stereoscopic microscope (Leica 165 F C), on day 18 and day 28 after release of the embryos to the colonies' surface. Metamorphosis was classified into categories following Freire et al. (2019): early metamorphosed - settled, underdeveloped, usually bell-shaped polyp; advanced metamorphosed - polyp with eight short tentacles, with no or few evident symbiont algal cells; fully metamorphosed - developed polyp with symbiont-bearing pinullate tentacles.

2.3. Photosynthetic capacity

To determine whether productivity (photosynthesis) under OWA condition has an impact on reproductive output (through energetic pathways), the photosynthetic capacities of the parent *R. fulvum* colonies were determined on day 48 following introduction of the colonies into the RSS aquaria (July 2017), using an imaging pulse amplitude modulated fluorometer (I-PAM, Walz GmbH, Effeltrich, Germany). Four-six maximal absorbance areas were selected as AOI (areas of interest) and averaged in order to measure the fluorescence of each of the parameters described below. Rapid light curves (RLCs) were generated to infer the sensitivity of photosystem II (PSII) to changing PAR. Each colony was dark-acclimated for a period of 15 min prior to measurements, and the colonies were then illuminated with 0–701 μmol photons m^{-2} s^{-1} (PAR) in 15 steps of 20 s, and a saturation pulse at the end of each interval. Distinctive parameters of RLC were calculated using the Imaging PAM software (ImagingWin), including the effective photosynthetic efficiency (Y_{II}) and non-photochemical quenching values (NPQ). Maximum photosynthetic yield (F_v/F_m) was calculated as $F_v/F_m = (F_m\text{-}F_o/F_m)$, where F_o and F_m are the minimum and maximum fluorescence after dark acclimation, respectively. Relative electron transport rate (rETR) was calculated as (Y_{II})*PAR (Ralph et al., 2005). The maximum electron transport rate (rETR) was obtained from the rETR curve as the maximum yield of each treatment. The light efficiency factor (α) of each treatment was calculated from the initial slope of the rETR curve. The minimum saturating irradiance (E_k) of each treatment was calculated by dividing the rETRmax with the α (Ralph et al., 2005).

2.4. Statistics

Raw temperature and pH data measurements were averaged per treatment and per month, as presented in Table 1. All statistical analyses were performed using the software R (R Core Computing Team, 2017). We used a mixed effects model approach to determine whether the posited end-of-the-century seawater conditions had influenced planula survival or the photosynthetic capacity of the parental colonies. The mixed-effects approach allowed us to account for random variation associated with the aquarium of each parent colony or settlement tube. Specifically, differences in planula survival rate between embryos derived from the RSS or the reef colonies and reared under the different treatment conditions were first analyzed and visualized using Kaplan-Meier (KM) log-rank survival analysis using 'survival' R package (Therneau and Lumley, 2015). A random-effects cox proportional hazard model was then performed using the 'coxme' package in R (Therneau 2015). Here, we treated the different seawater conditions and the source of the embryos (RSS-derived vs. reef-derived) as a fixed effect and the aquaria as a random effect. Additionally, we examined the development of *R. fulvum* primary polyps that had been reared and settled under end-of-the-century conditions. Differences in polyp metamorphosis stages score were evaluated with respect to treatment conditions at two time points, using a cumulative link model as implemented in the 'ordinal' package (Christensen, 2012), with a probit link function. A Wald test was also run, in order to evaluate factor significance for polyp development.

Linear mixed effect (LME) models fitted by restricted maximum likelihood were applied to compare the differences in the calculated RLC parameters (R package 'lmer'). Fv/Fm, α, rETRmax, E_k, and NPQmax for the colonies under the three different seawater conditions. Similar to the survival analysis, treatment was defined as a fixed effect and the running seawater aquarium that contained the polyps as a random effect. Dependent variables were tested for normal distribution using Shapiro-Wilk test and for homoscedasticity using Levene's Test.

3. Results

3.1. Reproductive phenology

The timing of onset of surface-brooding in colonies of *R. fulvum* maintained under ambient seawater conditions was analogous to the timing of the event on the IUI reef (July 5, 2017, Fig. 1). Similarly, the onset of surface-brooding in the two female colonies that were translocated in the reef remained in synchrony with that of the reef colonies. In contrast, an earlier and asynchronous surface-brooding occurred in the female colonies maintained under both of the predicted end-of the-century seawater conditions, RCP 4.5 and 8.5. Those colonies exhibited an earlier onset of surface-brooding, which occurred between 6 and 25 days prior to that of the colonies maintained under ambient conditions or that on the IUI reef. Specifically, under RCP 4.5 seawater conditions, three out of five colonies synchronously released their embryos on June 18–19, and the two other surface-brooding events occurred asynchronously: one colony on June 11; and the other colony repeatedly on June 15 and 27. Under RCP 8.5 seawater conditions, two out of the three colonies released their embryos asynchronously, on June 12 and 19, whereas no surface-brooding was observed in the third colony (Fig. 1). Embryos were observed to develop normally into planulae on the surface of the female colonies under the ambient seawater conditions, resulting in a synchronized development of elongate planulae on day 6

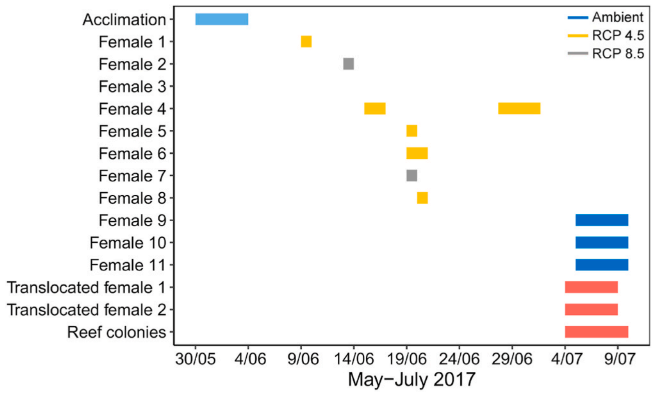

Fig. 1. *Rhytisma fulvum*: Timing of onset and duration of surface-brooding for colonies in the Red Sea simulator aquaria under ambient (blue), RCP 4.5 (yellow), and RCP 8.5 (gray) conditions during May-July 2017. Each rectangle represents a surface-brooding event of a single female colony. Red rectangle represents timing of surface-brooding event in transplanted colonies and naturally-growing colonies in the shallow reef. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

following their release. Concomitantly, the mucus entangling the brooded embryos degraded and the mature planulae detached into the aquaria water. However, atypically, under both RCP 4.5 and 8.5, 1-3-day-old surface-brooded embryos were observed to detach from their parental colonies and drift towards the water surface(see Fig. 2).

A significantly higher survival rate of planulae was recorded for the embryos collected from the IUI reef in all RSS treatments, compared to those released by the colonies maintained in the RSS (+36%, Coxme, $Z_1 = -7.21$, $p < 0.0001$, Fig. 3). End-of-the-century conditions thus had a significant effect on the survival rate of planulae that were collected and reared in the RSS (KM log-rank test: $p < 0.0001$), with a decrease of 21% and 27% under RCP 4.5 and RCP 8.5, respectively, compared to the planulae maintained under the ambient conditions. In contrast, seawater treatment conditions had no significant effect on the survival rate of planulae that were obtained from the reef colonies and reared in the RSS under ambient conditions or under RCP 4.5 and 8.5 (KM log-rank test: $p = 0.78$, Fig. 3).

The development rate of *R. fulvum* primary polyps was significantly influenced by RSS seawater treatment conditions ($P_{LRT} = 0.009$; Fig. 4), with both RCP 4.5 and RCP 8.5, leading to a lower number of fully developed primary polyps on both day 18 and day 28 post embryo release, in comparison to those in the ambient treatment. While time itself had no significant effect ($P_{LRT} = 0.37$) on the developmental stage of the primary polyps, the interaction between treatment conditions and time had a significant effect ($P_{LRT} = 0.002$, Fig. 4). It can therefore be concluded that the posited end-of-the-century seawater conditions had an ongoing effect on the development of the primary polyps over time.

3.2. *Photosynthetic capacity*

Five photosynthetic capacity estimators were compared at the end of the experiment (day 49) between *R. fulvum* colonies maintained under the different seawater conditions (\Table 2). The maximum relative electron transport rate was significantly reduced under end-of-the-century seawater conditions (Table 2, LME, $F_{2,\ 7} = 5.26$, $p < 0.04$), with a decrease of 11.5% and 17.9% for colonies under RCP 4.5 and RCP 8.5, respectively, in comparison to those under the ambient conditions (Fig. 5A). There were no significant differences between the values of Alpha, Fv/Fm, eK, and NPQmax in the colonies under the different RCPs, in comparison to those under the ambient conditions ().

4. Discussion

The current study has examined for the first time the effects of two potential end-of-the-century seawater conditions on the reproductive phenology and photosynthetic capacity of the octocoral *R. fulvum,* in comparison to present-day ambient conditions. Our results indicate that the combined elevated temperature and reduced pH could profoundly

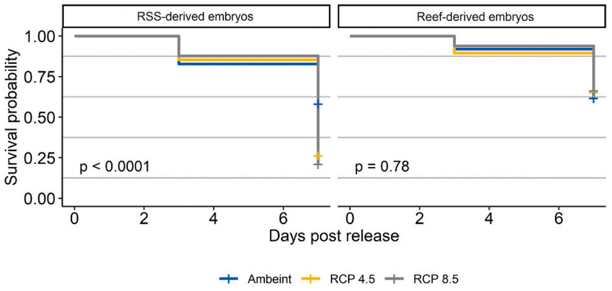

Fig. 3. *Rhytisma fulvum*: Kaplan-Meier log-rank survival probability of planulae under RSS seawater conditions. Left: embryos collected from RSS colonies; right: embryos collected from reef colonies. Presented p. values are of survival probability under treatment and seawater conditions. Cross marks represent survival probability of each treatment on day 7 after onset of surface-brooding event.

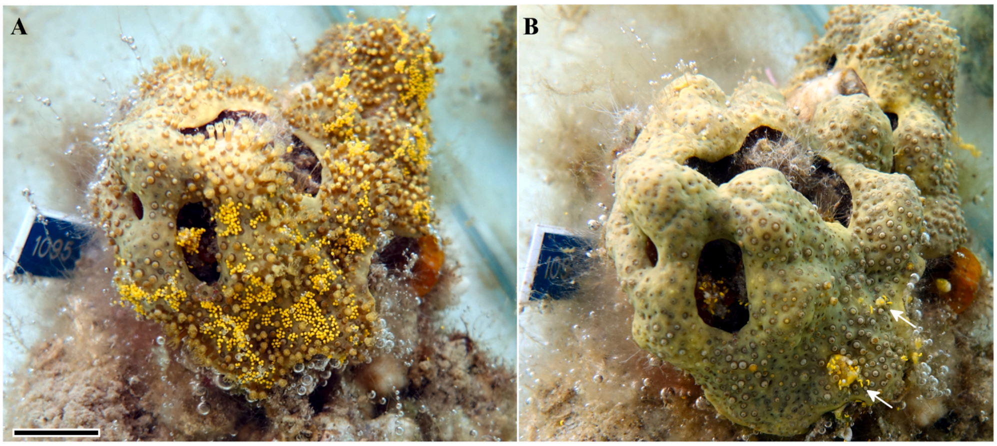

Fig. 2. *Rhytisma fulvum*: Surface-brooding under RCP 4.5: (A) Onset of surface-brooding featuring lemon-yellow embryos, (B) Sparse elongated planula-larvae entangled in mucus (arrows) on day 4 after onset of surface-brooding event; arrows point to arrows point to planulae entangled in mucus on the colony surface. Scale bar = 1 cm at A applies also to B. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

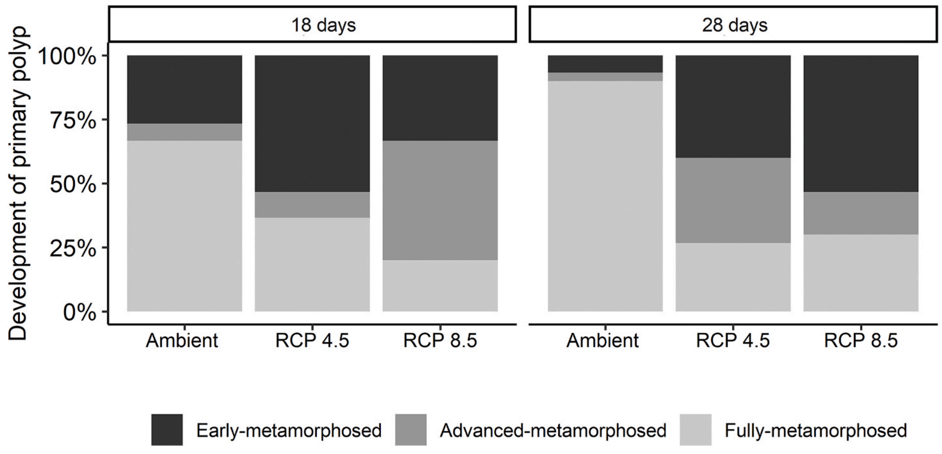

Fig. 4. *Rhytisma fulvum*: Metamorphic stages of primary polyps. Left: 18 days after onset of surface-brooding event; right: 28 days after onset of surface-brooding event. X axis represents the maintenance seawater conditions of primary polyps.

Table 2

Rhytisma fulvum: Curve fitting from RLC data of colonies under ambient and RCP treatments. Values are mean ± SE for seawater conditions ambient and RCP 8.5 (n = 3 for each) and for RCP 4.5 (n = 4). Asterisk indicates significant differences between treatments (LME, df = 2, $p < 0.05$).

Variable/ Treatment	Ambient	RCP 4.5	RCP 8.5	F Value	p-value
Fv/Fm	0.47 ± 0.03	0.46 ± 0.09	0.38 ± 0.15	0.63	0.57
rETRmax*	40.58 ± 1.22	29.02 ± 4.56	22.62 ± 3.44	5.26	0.04
A	0.23 ± 0.008	0.18 ± 0.02	0.17 ± 0.02	1.61	0.26
E_k	174.9 ± 10.55	162.56 ± 15.12	133.9 ± 3.4	2.69	0.13
NPQmax	0.32 ± 0.06	0.46 ± 0.09	0.48 ± 0.06	1.21	0.35

alter the timing of surface-brooding onset. In addition, we demonstrate that surface-brooding synchronicity might break down under the predicted future oceanic conditions (Fig. 1). The rates of change in both temperature rise and pH decrease were faster than in any climate change scenario posited to date, and do not account for any potential acclimatization processes by the affected corals that may occur over a longer time period. However, these rates simulate temporal deviations from long-term averages of seawater conditions, such as in the case of extreme marine heatwaves under low pH seawater conditions (Klein et al., 2017).

4.1. Reproductive phenology

Considerable changes in reproductive phenology as a result of global warming have now become evident in various organisms and ecosystems (Visser et al., 2010). Increasing evidence is presented from long-term studies of plants, birds, and insects, with many species documented as displaying changes in the timing of their breeding periods (Forrest and Miller-Rushing 2010). However, less is known about such shifts in marine ecosystems, and particularly in coral reefs (Baird et al., 2009; Shlesinger and Loya 2019; Shaffer et al., 2020). In addition, only a few studies have dealt with the challenging task of examining coral reproductive phenology under simulated seawater conditions. Nonetheless, it has been suggested that the increase in SST is likely to have major implications for coral reproductive phenology (Baird et al., 2009). Consequently, investigating the phenology of the surface-brooder *R. fulvum* offers an advantage, in that its phenological responses to seawater conditions, i.e. the release of its embryos and their development into planulae-larvae several days later, can be non-intrusively monitored. The results of the current study indicate that under a combination of higher temperature and lower pH (RCP 4.5 and 8.5), the timing of onset of surface-brooding will take place up to three weeks earlier relative to that in the present day. Other studies have suggested that increased temperature affects the reproduction of certain stony corals. For example, the gametogenic cycle and timing of spawning of the stony coral *Echiniopora lamellosa* in Taiwan were accelerated by elevated seawater temperature (Fan and Dai 1999), which also led to earlier planula release by *Pocilopora damicornis* there

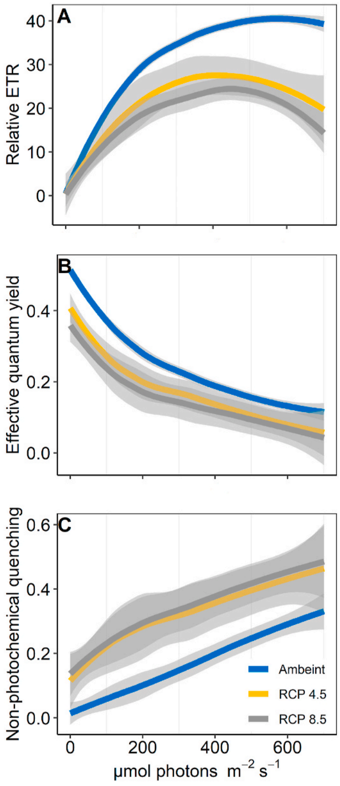

Fig. 5. *Rhytisma fulvum*: Chlorophyll fluorescence obtained by imaging-PAM for colonies under RSS seawater conditions: (A) relative electron transport rate (rETR) (B) effective PS II quantum yield (Y_{II}) (C) non-photochemical quenching (NPQ). Each line represents colonies under three treatment conditions (ambient: n = 3, RCP 4.5 and 8.5: n = 4 for each).

(Crowder et al., 2014). Furthermore, *Acropora digitifera* in Okinawa (Japan) spawned one day earlier in response to elevated temperature, suggesting that certain hormones are involved in accelerating coral bioregulatory processes (Paxton et al., 2016). However, the long-term implications of accelerated gametogenesis on coral populations are still unknown. Nonetheless, for *R. fulvum* colonies an earlier reproductive event during the usual reproductive season might suggest a longer window for consecutive surface-brooding events.

The synchronicity of gonad maturation and breeding events in both stony and soft corals is entrained to environmental cues such as photoperiod and seawater temperature (e.g. Harrison 2011; Kahng et al., 2011; Keith et al., 2016). Lunar periodicity and solar irradiance exhibit a predictable seasonal cyclicity (Kaniewska et al., 2015; Raible et al., 2017). The increasing SST and extreme thermal anomalies due to OW may have considerable implications for coral reproduction phenology, especially in species for which environmental cues synchronize their reproduction (Lawrence and Soame 2004; Baird et al., 2009). In the current study, whereas surface-brooding synchrony was partially disrupted under RCP 4.5 and 8.5, surface-brooding in all colonies maintained under ambient present-day conditions, as well as in the translocated reef colonies, remained synchronous with that of the reef population. A high level of surface-brooding synchrony within a *R. fulvum* population, at similar depths, has recently been suggested to be cued by an accelerated increase in seawater temperature (Liberman et al., 2018). It is therefore suggested that differences in the rate of increase in seawater temperature induced the asynchronous release of the embryos under the OWA conditions of the present study. A breakdown in reproductive synchrony was recently shown to have occurred in several stony-coral species in the Gulf of Eilat (Shlesinger and Loya 2019). These dramatic changes were also reported to have reduced the recruitment rate of the affected species on the reef. It has been hypothesized that OA may have led too to the reported disruption in reproductive synchrony of stony corals (Olischläger and Wild, 2020). However, unlike other marine taxa, there is limited evidence for such disruption of coral reproductive synchrony; and, therefore, the effect of OWA on coral reproduction is still unresolved (Guest et al., 2008). The findings from the current study nonetheless indicate that surface-brooding synchronicity will be affected by future changes in climate conditions; although whether this response will negatively affect these corals' reproductive success remains to be determined.

The findings from the current study reveal that all the embryos collected from parent colonies experiencing end-of-the-century conditions displayed a similar development rate by day 3 (blastula stage; Benayahu and Loya 1983), but a lower planula survival (i.e. the number of embryos that developed into planula by day 7), in comparison to embryos that were collected from reef colonies experiencing ambient conditions. It thus seems that fertilization rates within the RSS aquaria were not affected by the OWA conditions, as can be inferred from the high survival rate of embryos on day 3, in all treatments (Fig. 3). Consequently, we speculate that the lower number of RSS-derived embryos that successfully developed into planulae under OWA conditions was due to impairment that occurred during the final stages of gametogenesis (May–June). Elevated temperature has been shown to influence soft-coral gonad maturation. For example, the oocyte size in *Lobophytum compactum,* together with its lipid and protein concentrations, were significantly reduced under elevated temperature (Michalek-Wagner and Willis 2001a, 2001b). Although not significant, a reduction in planula survival was also recorded here under the ambient conditions in comparison to those collected from the natural reef. It is suggested that this might be a result of the environmental conditions in the RSS during the final stages of gametogenesis, such as light irradiance, which marginally differed from that in the natural reef. Additionally, our results indicate that the electron transport rate of parental colonies was reduced under both RCP conditions, in comparison to the colonies under the ambient conditions (Table 2), which may have caused a deficit in the energy available to the colonies and potentially limited their ability to invest in their progeny. However, the relationship between the photochemical damage of cnidarian species and the performance of their offspring is complex and more research is needed on this topic.

Post-settlement development of *R. fulvum* primary polyps was also impacted by end-of-the-century OWA conditions. The current results show that significantly higher numbers of fully developed primary polyps were recorded under the ambient conditions in comparison to those under both RCPs, at both of the studied time points. This could be attributed to the underdeveloped embryos and/or planulae as already discussed above. Similarly, it could have been caused by a slower developmental rate under the OWA conditions. However, the latter explanation is less likely since a faster post-settlement development under elevated seawater temperature has been recorded in several species, such as in the gorgonian *Paramuricea clavata* (Kipson et al., 2012) and in a number of stony coral species (Randall and Szmant 2009a, 2009b; Chua et al., 2013). Nonetheless, to the best of our knowledge the current results are the first to indicate the interactive effect of elevated seawater temperature and reduced pH on post-settlement development in an octocoral.

The current study has shown that under RCP 4.5 and 8.5 seawater conditions, most of the developing embryos detached from the colonies and drifted towards the water surface prior to maturation. Although detached embryos may continue to develop in suspension, as has been shown in this study, they are unlikely to escape predation in the natural environment. Such early detachment indicates a failure to complete normal development while being surface-brooded (Liberman et al., 2018). It is suggested that the predicted end-of-the-century OWA conditions applied here affected the mucus itself, resulting in detachment of the embryos and reducing planula yield. In support of this suggestion, elevated seawater temperatures were reported to significantly reduce the thickness of the surface mucus layer of *Diploria* spp. In the Caribbean (Pratte and Richardson 2014), and to cause a shift in the sugar content of the mucus in thermally-stressed *Acropora muricata* in Taiwan (Lee et al., 2016). Additionally, reduced seawater pH resulted in a shift in the bacterial community of the mucus in the Red-Sea stony coral *Acropora eurystoma* (Meron et al., 2011), which may alter the chemical features of the mucus. We further suggest that for species reproducing via surface-brooding, any impairment of the mucus may severely affect population recruitment.

The findings of the current study raise major concerns regarding the possible impact of future OWA conditions on the reproduction of the surface-brooder *R. fulvum*, and their implications for its recruitment. However, it should be noted that in internal-brooding species (Kahng et al., 2011), the developing planulae might undergo an advantageous preconditioning over time to changing seawater conditions (e.g. Marshall 2008; Cole et al., 2016; Bellworthy et al., 2019). In addition, internal-brooding species often reproduce asynchronously (Harrison 2011), with changes to external cues such as seawater temperature consequently being less likely to affect them. Further studies are needed in order to explore how end-of-the-century seawater conditions might affect coral reproduction; and to determine whether certain reproduction modes will be more advantageous than others.

CRediT authorship contribution statement

Ronen Liberman: Methodology, Data curation, Formal analysis, Investigation, Resources, Validation, Writing - original draft, Visualization. **Maoz Fine:** Methodology, Writing - review & editing, Project administration, Funding acquisition. **Yehuda Benayahu:** Funding acquisition, Writing - review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.105215.

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Ethics

Collection of animals complied with official permits issued by the Israel National Parks and Nature Authority.

Data availability

All data and r scripts used in the preparation of this manuscript are available at: https://github.com/ronenliberman/CC-disrupt-octocoral-reproductive-synchrony.

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