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In situ effects of simulated overfishing and eutrophication on settlement of benthic coral reef invertebrates in the Central Red Sea

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Abstract

In the Central Red Sea, relatively pristine coral reefs meet intense coastal development, but data on the effects of related stressors for reef functioning are lacking. This *in situ* study therefore investigated the independent and combined effects of simulated overfishing and eutrophication on settlement of reef associated invertebrates on light-exposed and -shaded tiles over 4 months. Findings revealed that at the end of the study period invertebrates had almost exclusively colonized shaded tiles, indicating that algae were superior settling competitors on light-exposed tiles. On the shaded tiles, simulated overfishing prevented settlement of hard corals, but significantly increased settlement of polychaetes, while simulated eutrophication only significantly decreased hard coral settlement relative to controls. The combined treatment significantly increased settlement of bryozoans and bivalves compared to controls and individual manipulations, but significantly decreased polychaetes compared to simulated overfishing. These results suggest settlement of polychaetes and hard corals as potential bioindicators for overfishing and eutrophication, respectively, and settlement of bivalves and bryozoans for a combination of both. Therefore, if investigated stressors are not controlled, phase shifts from dominance by hard corals to that by other invertebrates may occur at shaded reef locations in the Central Red Sea.

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Introduction:

Overfishing and eutrophication are among the most serious local stressors for coral reefs, worldwide and in the Red Sea (Burke et al. 2011). These threats can strongly affect invertebrate settlement. Settlement (i.e. the permanent attachment to the substrate) of sessile invertebrate larvae is an irreversible process and is thus of critical importance for reproduction, feeding, and survival of invertebrates (Harrison & Wallace 1990).

Invertebrate settlement can be influenced by numerous factors such as water flow (Mullineaux & Garland 1993), abundance and composition of microbial biofilms (Hadfield 2011; Sawall et al. 2012; Tran & Hadfield 2011), benthic macroalgae (Arnold et al. 2010; Harrington et al. 2004; O'Leary et al. 2012), conspecific adult invertebrates (Osman & Whitlatch 1995), predators and grazers (Connell & Anderson 1999; Glynn 1990; Lewis & Anderson 2012), or changing environmental conditions that provide competitive advantages to certain species (Hallock & Schlager 1986).

Eutrophication or the increase in nutrient availability influences biofilm diversity and composition (Kriwy & Uthicke 2011; Webster et al. 2004; Witt et al. 2012a; Witt et al. 2012b). Further, eutrophication and overfishing (of herbivores) can also increase growth of benthic macroalgae such as filamentous algae (Jessen et al. 2013a), thereby providing the faster growing algae with a competition advantage over invertebrates and allow them to take over suitable settlement substrates. In contrast, some slow growing algae such as crustose coralline algae (CCA) can trigger coral recruitment (Harrington et al. 2004; Heyward & Negri 1999), though they can be suppressed through reduced grazing (Jessen et al. 2013a). Additionally, the increase of certain filter feeders was linked to eutrophication and concomitant increase in organic matter in the water column that made them able to outcompete and prevent settlement of adjacent organisms (Chadwick & Morrow 2011; Hallock & Schlager 1986).

Further, overfishing can influence trophic interactions in two ways. Either, by reducing the number of herbivores and invertebrate predators and therefore freeing macroalgae and certain invertebrates of their top-down control (Birkeland 1977; Birrell et al. 2005; Diaz-Pulido et al. 2010; Osman & Whitlatch 1995; Vine 1974). Or, by the reduction of predators, that can result in the release of top-down control of invertebrate feeders such as sea urchins (Hay 1984; McClanahan & Shafir 1990). As a consequence, the amount of invertebrate settlement can be strongly reduced (Myers et al. 2007), sometimes even down to almost zero (Vine & Bailey-Brock 1984). Overfishing can even lead to increased bioerosion rates (Tribollet & Golubic 2011) that reduce suitable settlement habitat for new invertebrate settlement.

Although the top-down and bottom-up effects of overfishing and eutrophication have been intensively studied for benthic reef algal growth and development (e.g., Burkepile & Hay 2006, Smith et al. 2010, Jessen et al. 2013a), there are few studies available that investigated the individual or combined impact on tropical sessile invertebrate settlement in this context. Only (Tomascik 1991) and (Hunte & Wittenberg 1992) looked at coral settlement patterns along an eutrophication gradient, although it is not clear if the observed influence was due to altered larval supply. Additionally, our understanding of the ecology of coral reefs in the Red Sea is largely focused on studies conducted in the Gulf of Aqaba, but not in the remaining Red Sea (Berumen et al. 2013).

This study simulated overfishing and eutrophication over 4 months in an offshore reef in the Central Red Sea to answer the question which influence do the individual and combined effects of overfishing and eutrophication have on settlement of main sessile invertebrate groups.

Materials & Methods

Study site

The study was carried out over 16 weeks from June to September 2011 at the patch reef Al-Fahal that lies about 13 km off the Saudi Arabian coast in the Central Red Sea (N22.18.333, E38.57.768; see Jessen et al. 2012 for a map of the location). We selected this reef because of its relatively large distance from shore and presumably low impacts from potential fishing and land-derived nutrient import. The reef is characterized by high herbivore fish (22 g m^{-2}) and sea urchin biomass (38 g m^{-2}), low ambient concentrations of inorganic (DIN: 0.9 – 1.8 µmol L^{-1}; SRP: 0.06 – 0.10 µmol L^{-1}) and organic nutrients (DOC: 55 – 67 µmol L^{-1}), and relatively high live coral cover (49% hard and soft coral cover; for full results see Jessen et al. 2013a).

Experimental setup

Ten terracotta tiles each 10 x 10 cm (100 cm^2) were mounted on stainless steel screws at an angle of 45 degrees on each of 16 polyvinyl chloride (PVC) frames (50 x 75 cm) approximately 10 cm above the reef substrate at 5-6 m water depths and accessible to invertebrate herbivores (pers. obs.). Tiles were installed in 2 rows with a distance between 3 to 50 cm between. PVC frames were separated by 2-5 m. Prior to the start of the experiment, tiles were autoclaved to remove any interfering compounds that could have accumulated during tile production. Tiles were installed pairwise on top of each other with unglazed sides facing outside, resulting in an upper (light exposed) and lower (shaded) tile. We applied four different treatments to the frames (each with n = 4): (1) control (only the equipped frame), (2) fertilizer (see nutrient enrichment section), (3) cage (hemispherical zinc galvanized cages with a mesh size of 4 cm and a diameter of 100 cm), and (4) a combination of cage and fertilizer tubes.

The cages served to exclude larger predators and herbivores, but smaller fish (small damselfish, parrotfish, wrasses and surgeonfish) were able to access the insides of the cages

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Cage controls were not used, since studies showed that similar cages even with a lower mesh size did not affect water movement, light availability, and sedimentation rates (Burkepile & Hay 2007; Miller et al. 1999; Smith et al. 2001).

Eutrophication was simulated by deploying four fertilizer tubes around the frame, consisting of perforated PVC tubes filled with approximately 580 g Osmocote fertilizer (Scotts, 15 % total nitrogen (in form of nitrate & ammonium), 9 % phosphate (phosphoric pentoxide), and 12 % potassium oxide) embedded in 3 % agarose. Fertilizer was deployed once without replenishments, but regular monitoring of nutrient concentrations assured continuous release rates.

Treatments were not randomly assigned to the frames, instead the sequence control, fertilizer, cage, combination was repeated four times along the reef to control for potential biases such as microhabitats.

One pair of tiles (light-exposed and shaded) per frame was collected after 1, 2, 4, 8, and 16 wk(s) using SCUBA. Tiles were pre-scored and upon sampling divided in half (each 50 cm^2; an area which had been shown to be large enough from asymptotes of species-area curves by Hixon & Brostoff 1996) and then wrapped separately in ziplock bags. They were brought on board within 30 min where half of them were immediately flash frozen in liquid nitrogen for subsequent microbial analyses (results reported elsewhere), while the other half was handled as described below.

To test the success of fertilization, water samples (5 L) were taken directly before collecting tiles at each time point with large ziplock bags directly from above each frame (in total n=40 for nutrient enriched as well as non-enriched samples). From this stock 50 mL were filtered on pre-combusted Whatman-GF/F filters and used for inorganic nutrient measurements. The analyses of dissolved inorganic nitrogen (DIN = NH_4^++NO_3^-+NO_2^-) and soluble reactive phosphorous (SRP = PO_4^{3-}) were performed using continuous flow analyzer (FlowSys Alliance Instruments).

Invertebrate identification and enumeration

To remove attached sediment, precipitates, and mobile invertebrates, light-exposed and shaded tiles were rinsed with fresh water. Invertebrate classification was conducted with a dissection microscope (Zeiss Stemi 2000; 7.7-fold magnification). Briefly, all sessile invertebrates visible under the dissection microscope were identified with the help of (Vine 1986) and grouped to the following easily distinguishable categories: Scleractinia (Cnidaria), Bivalvia (Mollusca), Bryozoa, Polychaeta (Annelidae). We counted single animals (scleractinia, bivalvia, polychaetes like *Spirorbis* sp. or *Pomatoceros* sp.) or colonies (bryozoa, other polychaetes like *Filograna* sp.) on each tile to assess the number of individual settlement events. It could be argued that other factors than settlement such as competition, predation, and overgrowth affect the number of organisms in the course of the study. By considering only sessile and calcareous organisms and thoroughly searching the surface with a dissection microscope, we tried to minimize these potential biases as much as possible. Nevertheless, numbers can be slightly underestimated, since we cannot rule out that settlers arrived but did not persist.

Algal composition and algal biomass (only light-exposed tiles) was determined in the laboratory after invertebrate counting by taking pictures of submerged tiles and analyzing them using 100 randomly overlaid points in Coral Point Count with Excel extensions (CPCe) 4.1 (Kohler & Gill 2006). Primary algal groups were filamentous algae and non-coralline crusts on light-exposed tiles and crustose coralline algae (CCA) and non-coralline red crusts (such as *Peyssonnelia*) on shaded tiles. Foliose macroalgae such as *Padina*, *Lobophora*, or *Halimeda* were not found. See Jessen et al. (2013a) for full results of algal cover.

Data of 1 of 16 frames (No. 4, combined treatment) was removed from the dataset, as cage pictures and tile appearance indicated access of large predators and herbivores to this setup.

158 ***Statistical data analysis***

159 T-tests were used for analyzing inorganic nutrient concentrations at each sampling point. To
160 meet assumptions of normal distribution DIN-data were inverse square root (1/sqrt(x))
161 transformed. All invertebrate groups were tested for the individual and interactive effects of
162 cage, fertilizer, and time with a 3-factorial generalized linear model (GLM) in R (R
163 development core team 2012). As data showed over- and underdispersion, either quasi-GLM
164 models (hard corals, polychaetes) or negative binomial model (bivalvia, bryozoa) were used
165 for correction, depending which model fitted the data better based on pseudo-R^2 scores (Zuur
166 et al. 2009). For comparison of the different treatments, we used Tukey post hoc tests ('glht'
167 function) of the 'multcomp' package.

168

Results

The simulation of eutrophication constantly and significantly increased SRP concentrations compared to the controls (Fig. S1). DIN concentrations were also constantly increased, but did not always significantly differ from the controls (Fig. S1). Both, ambient and enriched treatments, experienced a peak in DIN concentrations after 4 weeks.

Over the sampling period of 16 weeks, 99.9 % of all observed sessile invertebrates settled on the shaded tiles. The exceptions were 1 hard coral recruit (control 2 wks), 5 polychaetes (1x control 2 wks; 3x fertilizer 4 wks; 1x combined 8 wks), and 2 bryozoan colonies (cage 16 wks).

Therefore, the following results stem exclusively from invertebrate observations of the shaded tiles (total 6,862 counts, and an average of 91 counts per shaded tile).

On a temporal scale, polychaetes occurred first after 1 week, bryozoans after 2 weeks, hard corals after 4 weeks, and bivalves after 8 weeks, however, there was no treatment-specific pattern when first settlement occurred (Fig. 1B, D, F, H). Other potential sessile invertebrate groups such as sponges, soft corals, crustaceans, and ascidians were not observed on the analyzed tiles, however the latter group appeared once on a spare tile after 16 weeks.

In the controls, all observed invertebrate groups were present at their lowest abundance compared to the other treatments, except hard coral settlement which was highest in this treatment. (Fig. 1A).

Simulated overfishing reduced hard coral numbers to zero relative to controls (Fig. 1A), significantly increased settlement of polychaetes (Fig. 1G), but did not show any significant effects on settlement of bryozoans and bivalves (Fig. 1C & E).

Under simulated eutrophication, hard coral settlement was significantly decreased by 11-fold relative to controls (Fig. 1A), while bryozoans, bivalves, and polychaetes were not significantly affected by this treatment (Fig. 1C-H).

The combination of manipulated eutrophication and overfishing significantly increased settlement of bryozoans and bivalves 7 and 11-fold relative to controls (Fig. 1C & E). Relative to simulated overfishing, the combined treatment significantly increased settlement of bryozoans 4-fold and that of bivalves 11-fold, but decreased settlement of polychaetes 2-fold, while settlement of hard corals was not affected. Relative to simulated eutrophication, the combined treatment significantly increased settlement of bryozoans 3-fold and bivalves 7-fold, while settlement of hard corals and polychaetes was not affected.

Except for bryozoans, all other groups showed significant interaction effects, i.e. their response to one manipulated factor depended on the level of the other factor (Fig. 1, Table S1).

Discussion

Sessile invertebrate groups responded heterogeneously to the simulation of overfishing and eutrophication with coral settlement declines in all treatments in comparison to controls. Interestingly, no additive effects were observed for the simultaneous treatment of both stressors compared to the single treatments.

Simulated overfishing increased settlement of polychaetes compared to controls. These observations are concordant with (Vine 1974), who observed increased spirorbid settlement in caged treatments. Interestingly, the positive effect of simulated overfishing on settlement of polychaetes was not visible in the combined treatment with increased nutrient availability and can therefore not be explained by predator exclosure. A possible explanation could be the presence of conspecific adult invertebrates that can suppress settlement in their vicinity (Osman & Whitlatch 1995). This hypothesis is supported by the fact that the different polychaete settlement responses between simulated overfishing and combined treatments were not visible before the occurrence of bryozoans and bivalves started after 8 weeks.

Simulated eutrophication alone only caused decrease of hard coral settlement, while all other invertebrates were neither positively nor negatively affected by this treatment. This finding is confirmed by the studies of (Tomascik 1991) and (Hunte & Wittenberg 1992), who also observed less hard coral settlement in eutrophic reefs and suggest that eutrophic conditions may alter the complex set of physical, chemical and/or biological signals that trigger settlement of coral larvae. However, it is not clear if such differences were caused by negative settlement behavior, post-settlement mortality, or reduced larval supply (i.e. reduced coral fecundity) as observed by (Loya et al. 2004) as a response to eutrophication. Large differences in functional algal cover between simulated eutrophication and control treatments did not exist (Jessen et al. 2013a). However, algae species were not identified on the species level, but potential differences on that level therefore may have occurred and influenced the settlement as shown for coralline algae by (Harrington et al. 2004). Furthermore, as shown for

231 coral fragments in a parallel experiment (Jessen et al. 2013b), increased nutrient
232 concentrations may have altered the microbial community structure of biofilms, thereby
233 changing chemical and structural cues that influence settlement.
234 The combination of manipulated overfishing and eutrophication resulted in the highest
235 settlement numbers of bivalves and bryozoans, that were both significantly increased
236 compared to manipulated overfishing and eutrophication treatments. However, algal cover, as
237 important settlement cue, did not substantially vary between combined and simulated
238 overfishing treatments (Jessen et al. 2013a), therefore we propose that indirect interaction
239 effects of predator/herbivore exclusion together with effects of microalgae that can benefit
240 from increased nutrients (Posey et al. 2002) and differences in bacterial and diatom biofilm
241 composition (Dahms et al. 2004; Yang et al. 2013) were causing the observed differences.
242 In this study, sessile invertebrates settled almost exclusively on shaded, compared to light-
243 exposed tiles. This light exposure-specific pattern has been confirmed for corals by studies
244 from other reefs (Birkeland 1977; Harrison & Wallace 1990; Sawall et al. 2013), and
245 contrasts the presence of algae biomass and abundance of filamentous algae that was highest
246 on light-exposed tiles during the present study (Jessen et al. 2013a). While these filamentous
247 algae can prevent invertebrate settlement (Arnold et al. 2010; Glasby & Connell 2001;
248 Virgilio et al. 2006), encrusting algae, i.e. CCAs, often facilitate and induce invertebrate
249 settlement (Arnold et al. 2010; Heyward & Negri 1999; Morse et al. 1996; Negri et al. 2001;
250 Whalan et al. 2012). Correspondingly, encrusting algae were not observed on the light-
251 exposed tiles, but were abundant on the shaded tiles, particularly in non-caged treatments
252 (Jessen et al. 2013a). Nevertheless, invertebrates were obviously present on light-exposed
253 substrate in natural reefs. It may be that adequate settlement substrates such as CCA exhibit
254 delayed growth on light-exposed underground (Smith et al. 2010) and thereby delaying sessile
255 invertebrate settlement. This suggests the need for studies over longer time spans to study
256 invertebrate settlement on light-exposed substrate. While other invertebrate groups that are
257 typically associated with coral reefs including sponges, soft corals, crustaceans, and ascidians

were absent in this experiment, they were found in other, though longer lasting, settling tiles experiments (e.g., Sawall et al. 2013). Their lack in this study may be either explained by the absence of reproduction events during the study period or delayed settlement on artificial substrate as suggested by the observation of ascidians on a spare tile after 16 weeks.

The absence of all hard coral settlement in the simulated overfishing treatments may be caused by the presence of more competitive invertebrates that prevented settlement or covered corals (Birkeland 1977; Sawall et al. 2013), filamentous algae (Arnold et al. 2010; Birrell et al. 2005; Kuffner et al. 2006), as well as the lower abundance of coralline algae (O'Leary et al. 2012), as these factors were significantly influenced by simulated overfishing on the same tiles (Jessen et al. 2013a).

In a recent review Cooper et al. (2009) summarized and evaluated potential bioindicators for coral reef health and water quality ranging from species presence and composition to physiological and isotopic parameters. Although their review included coral recruitment, other sessile invertebrates haven't been considered. At least for the study area, the findings of the present study suggest settlement of coral reef associated sessile invertebrates as specific bioindicator for overfishing and a combination of it with eutrophication. For overfishing, this may be an increase in polychaete settlement and a decrease for that of hard corals. For eutrophication the sole decrease of hard coral settlement, and for a combination of both stressors this may be an increase in bryozoan and bivalve settlement. Advantages of this approach would be the cost-effective and relative easy measurement together with low systematic knowledge that is needed to identify the taxonomic groups.

Although the reef appears to be in healthy condition, non-coral invertebrates such as polychaetes (under overfishing) or bivalves and bryozoans (combination with eutrophication) may rival hard coral dominance at shaded reef locations if simulated threats are not controlled in the study area, This can lead to potential alternative stable states as described for other invertebrates in (Norström et al. 2009).

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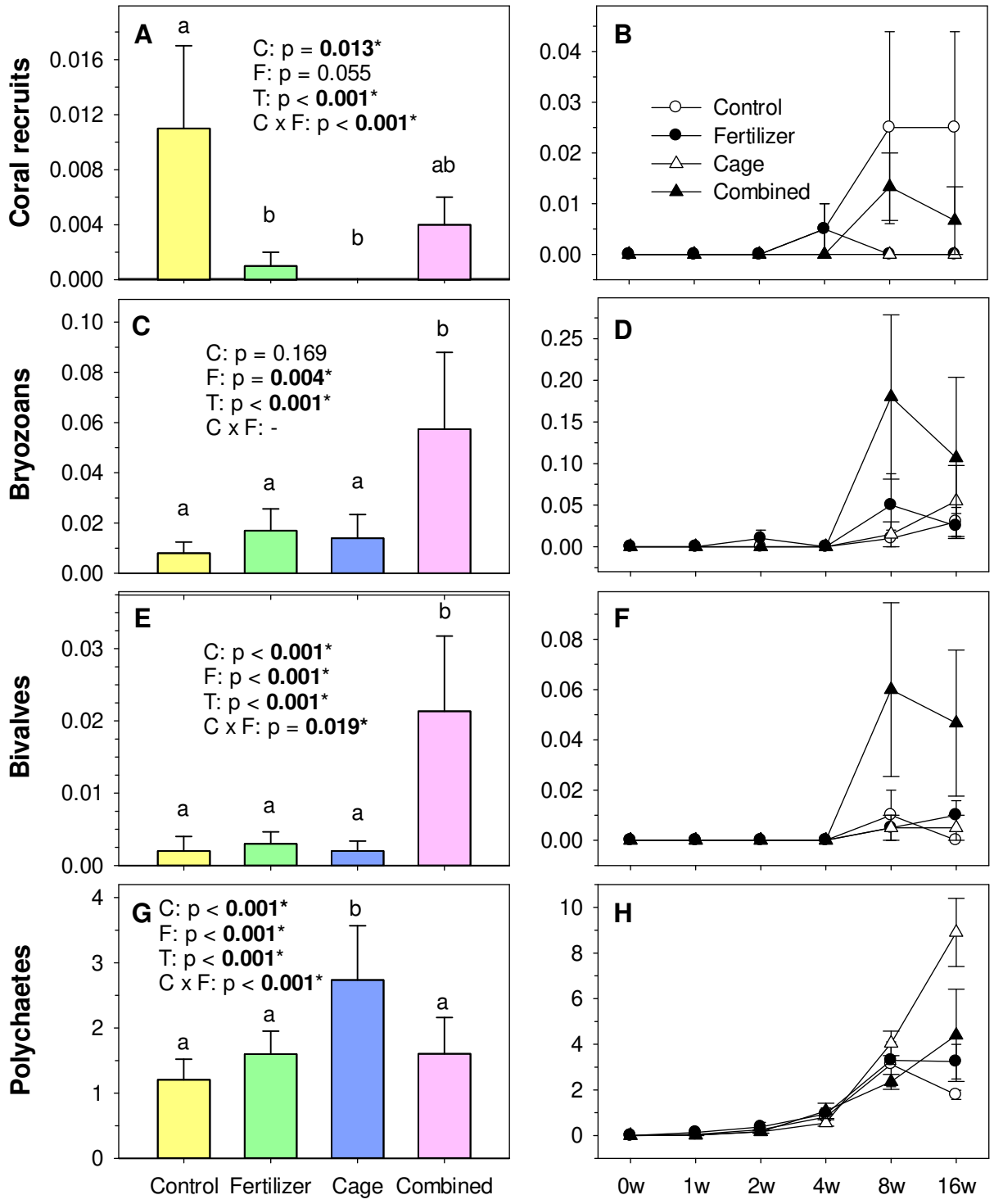

Fig. 1: Invertebrate settlement numbers (depicted per cm^{-2}; mean ± SE) on shaded tiles. Left column (A, C, E, G) shows settlement numbers per treatment averaged over all tiles and right column (B, D, F, H) shows temporal development of counted recruits of all 4 treatments. P-values were calculated from a 3-factorial GLM and originate from analysis across the whole study period (see Table S1 for full results). Dashes represent factors that have been excluded by the model reduction. Abbreviations: C=Cage, F=Fertilizer, T=Time. Treatments with same small letters are not significantly different (p>0.05) in post hoc pairwise comparisons.

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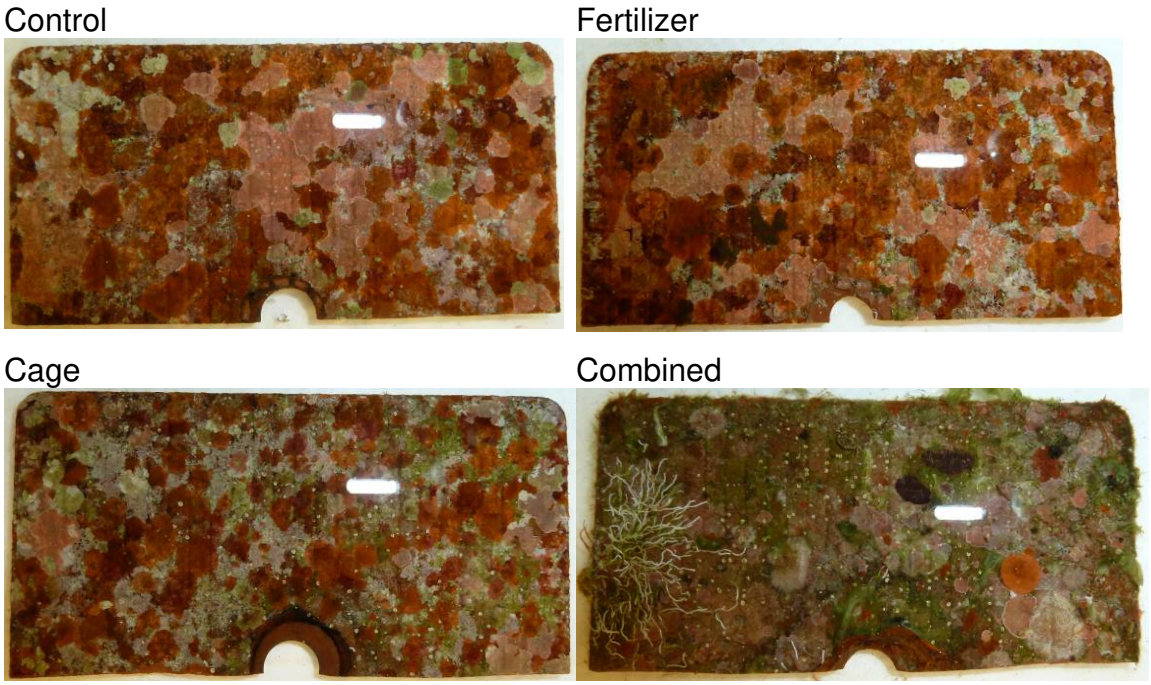

452 Fig. 2: Representative photographs of light-shaded tiles after 16 weeks of deployment in the reef. White
453 bars in the central upper right area of each picture are reflections caused by a camera flash. Hemi-circle
454 holes at the central lower edge were used for screws to attach tiles.
455

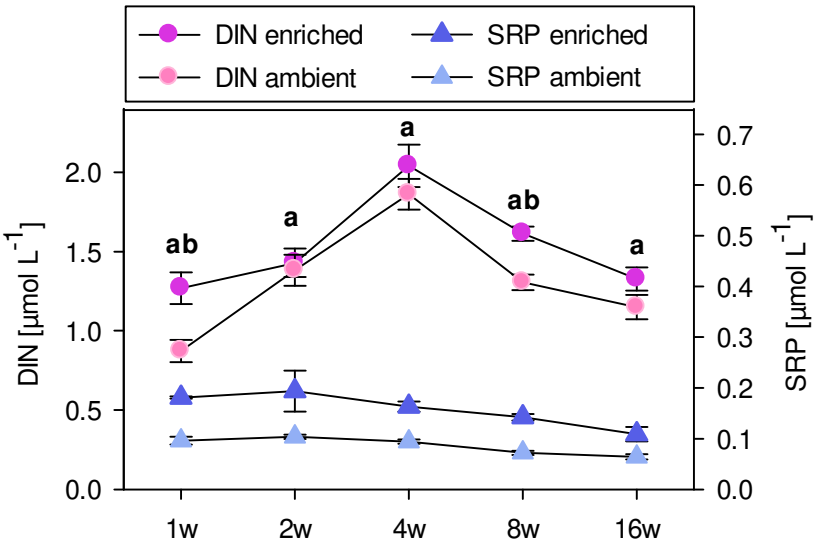

Fig. S1: Inorganic nutrient concentrations. Dissolved inorganic nitrogen (DIN) and soluble reactive phosphate (SRP) concentrations (µmol L^{-1}; means ± SE) in the nutrient enrichment treatments (fertilizer & combined) and the non-enriched treatments (control & cage). Small letters (a - SRP; b - DIN) indicate statistical significant differences of $p<0.05$ (t-test).

Table S1: Results of the 3-factorial GLM of invertebrate groups. Abbreviations: Cage (C), Fertilizer (F), and Time (T). Significant results are indicated in bold by asterisks. P-values of 0.000 represent values <0.001. Dashes represent factors that have been excluded by the model reduction.

		Scleractinia		Bryozoa		Bivalvia		Polychaetes	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
C	1	6.47	**0.013***	1.94	0.169	6.02	**0.000***	17.21	**0.000***
F	1	3.80	0.055	8.80	**0.004***	8.42	**0.000***	2.43	**0.000***
T	4	5.84	**0.000***	22.29	**0.000***	33.16	**0.000***	90.24	**0.000***
C x F	1	14.88	**0.000***	-	-	2.17	**0.019***	6.05	**0.000***