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RESEARCH ARTICLE

Mutualistic damselfish induce higher photosynthetic rates in their host coral

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ABSTRACT

Coral reefs are amongst the most diverse ecosystems on Earth where complex inter-specific interactions are ubiquitous. An example of such interactions is the mutualistic relationship between damselfishes and branching corals in the Northern Red Sea, where the fish use corals as shelter and provide them with nutrients, enhance the flow between their branches, and protect them from predators. By enhancing the flow between the coral branches, the fish ventilate the coral's inner zone, mitigating hypoxic conditions that otherwise develop within that zone during the night. Here, we tested, for the first time, the effects of the damselfish *Dascyllus marginatus* on photosynthesis and respiration in its host coral *Stylophora pistillata*. Laboratory experiments using an intermittent-flow respirometer showed that the presence of fish between the coral branches under light conditions augmented the coral's photosynthetic rate. No effect on the coral's respiration was found under dark conditions. When a fish was allowed to enter the inner zone of a dead coral skeleton, its respiration was higher than when it was in a live coral. Field observations indicated that damselfish were present between coral branches 18–34% of the time during daylight hours and at all times during the night. Considering the changes induced by the fish together with the proportion of time they were found between coral branches in the field, the effect of the fish amounted to an augmentation of 3–6% of the coral's daily photosynthesis. Our findings reveal a previously unknown positive contribution of coral-dwelling fish to their host's photosynthesis.

KEY WORDS: *Dascyllus*, Mutualism, Physiology, Red Sea, Respiration, *Stylophora*

INTRODUCTION

Coral reefs are highly complex ecosystems with numerous inter-specific mutualistic interactions (e.g. Castro, 1976; Patton, 1976; Holbrook et al., 2008). A ubiquitous example is the mutualistic relationship between damselfishes and branching corals. In this interaction, the coral is used by the fish as a shelter from predators during the day and night, and as a place for social interactions and egg laying (Sale, 1971a,b; Fishelson et al., 1974; Coates, 1980; Sweatman, 1985; Liberman et al., 1995). The fish in return remove sediment and other objects from the coral surface (Stewart et al., 2006), protect it from predators such as butterflyfish or crown-of-thorns starfish (Weber and Woodhead, 1970; Pratchett, 2001; Chase et al., 2014), excrete nutrients rich in nitrogen and phosphorus that the coral takes up, and effectively ventilate the colony during the night (e.g. Meyer and Schultz, 1985a,b; Liberman et al., 1995; Holbrook and Schmitt, 2002; Goldshmid et al., 2004; Pinnegar and Polunin, 2006; Holbrook et al., 2008). Consequently, coral colonies that are inhabited by mutualistic damselfish grow faster, have significantly more tissue biomass and zooxanthellae, and produce more eggs than conspecific colonies from which the fish were experimentally removed (Meyer and Schultz, 1985b; Liberman et al., 1995). The effect of damselfish on the growth rate of their host is a function of the fish biomass (Holbrook et al., 2008). The effect on coral growth rate increases at greater depths and under lower illumination, but diminishes under conditions of high nutrient supply or strong flow (Chase et al., 2014). Damselfish species for which the mutualistic relationship with live corals is obligatory spend the night between the branches of their host coral and vigorously ventilate it with rapid fin strokes while maintaining a nearly stationary position (Goldshmid et al., 2004). This ventilation enhances the flow between the coral branches while they are respiring and greatly mitigates the development of severe hypoxic conditions inside the colony during the night (Shashar et al., 1993; Goldshmid et al., 2004). During the day, strong water flow augments photosynthesis by removing excess oxygen from the coral tissue (Mass et al., 2010; Kremien et al., 2013; Wild and Naumann, 2013), although this effect has not yet been demonstrated for fish fanning.

The rhythmic behavior of the mutualistic damselfish is governed by ambient light. At dawn, the fish emerge from their shelters between the coral branches and feed on drifting zooplankton until dusk (Rickel and Genin, 2005). During the day, the fish retreat to the inner coral shelter when threatened, where they start exhibiting fin strokes immediately after entering the space between the coral branches. However, the effects of ventilating the coral during the day, when photosynthesis-generated oxygen is plentiful, are unknown. Here, we experimentally examined the effect of mutualistic damselfish on the respiration and photosynthesis of their host coral under dark and light conditions. *In situ* observations were used to assess the relevance of our laboratory findings for natural conditions. We expected the damselfish to enhance coral photosynthesis by aeration of their host when located between its branches during the day. Similarly, if low oxygen in the inner space between the coral branches limits coral respiration, we expected that the ventilation of that space by the fish (Goldshmid et al., 2004) would enhance the coral respiration rate. Such enhancement, if it occurs, could explain the higher growth rates of fish-inhabited colonies (Meyer and Schultz, 1985b; Liberman et al., 1995; Goldshmid et al., 2004; Holbrook et al., 2008).

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MATERIALS AND METHODS

The study was conducted between October 2013 and March 2014 at the Interuniversity Institute for Marine Science (IUI) in Eilat (29°30′N, 34°54′E), Northern Red Sea, Israel. The experiments with fish and corals and the *in situ* observations were carried out under a permit from the Israel Nature & Park Authorities (permit no. 2013/40071), strictly adhering to animal treatment regulations.

Adult damselfish *Dascyllus marginatus* (Rüppell 1829) and small colonies of the branching coral *Stylophora pistillata* Esper 1797 were collected at 5–8 m depth in the reef off IUI. Prior to capture, the fish were partially anesthetized using clove oil and immediately caught and transferred to a plastic bag using a small hand net, assuring gentle handling throughout. Once out of the sea, one pair consisting of a single colony (length 10–12 cm, width 9–10 cm) and a single fish was placed per 12 liter tank in an open running seawater system. The captured fish were acclimatized for at least 1 week prior to experiments, maintaining the same light cycles in the laboratory as in their natural environment. Acclimation was inferred when the fish appeared relaxed, readily fed on brine shrimp nauplii, and appeared to swim normally around the coral, finding shelter between its branches when threatened and during the night.

Coral and fish experiments

An intermittent-flow metabolic chamber, 2.76 liters in volume, was used to determine respiration and photosynthesis as described by Zimmermann and Kunzmann (2001) (Fig. 1). Prior to measurements, the metabolic chamber and the pipes were thoroughly cleaned with sodium hypochlorite solution and 70% ethanol, and then rinsed with distilled water to effectively remove possible contamination by bacterial growth. After cleaning, the entire system was filled with seawater filtered using a 0.8/0.2 µm filter (AcroPak™ 1500 with Supor membrane; Pall Corporation, New York, USA), and a blank trial before and after the experiment was run to ensure zero change in oxygen concentration in the animal-free chamber. Occasional observations on small suspended particles placed in the chamber to visualize water flow (between runs) indicated a flow of ∼2–3 cm s^{-1} around the corals driven by the chamber's pump, a value similar to the conditions at the Eilat reef. The gradual oxygen change in the resulting time series indicated no variability of oxygen in the system within a run.

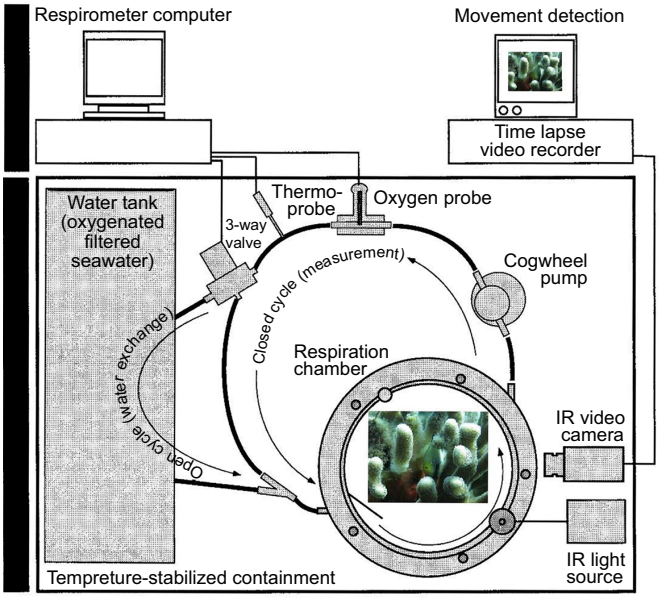

Fig. 1. Experimental setup of the intermittent system. Adapted from Zimmermann and Kunzmann (2001), which was adapted from Zimmermann and Hubold (1998).

Measurements were replicated with haphazardly determined pairs (n=4 light experiments; n=5 dark experiments) maintaining the original pairs during the experiments. Five different treatments were used with each pair: (i) fish alone, (ii) coral alone, (iii) fish+live coral, (iv) fish+dead coral and (v) fish/live coral. Treatments iii and iv were carried out with the fish permitted access 'inside' the colony, i.e. to the space between the coral branches, whereas the fish/live coral treatment was carried out with the animals physically separated using a plastic net (1 cm mesh size). The combination of these five treatments allowed us to test whether the presence of one animal affected the rate of respiration or, for the coral, photosynthesis of the other animal. The fish/live coral treatment was carried out only in light conditions, and the fish+dead coral treatment was tested only in darkness. The treatments fish alone, coral alone, fish+live coral and fish/live coral were replicated three times under light conditions. In darkness, each pair was measured once for each treatment. Dried, bare skeletons of *S. pistillata* of similar size and shape were used in the treatment fish+dead coral. Coral skeletons were dried outdoors for at least 1 month before the laboratory experiments started. Prior to each run, they were again exposed to sunlight for at least 12 h and thoroughly brushed to remove fouling organisms that may have settled on them during preceding trials in order to prevent the presence of a biofilm that could potentially influence respiration. Once the dead coral was placed in the chamber, it was vigorously shaken in order to remove any air bubbles that could have been trapped in the skeleton. All colonies, including the dead skeleton, were sufficiently large for the fish to readily enter the space between the branches. For illumination, a photosynthesis-fit metal-halide lamp (230 V, 150 W; Dragon Source Ltd, NingBo, China) was positioned ∼50 cm above the chamber, resulting in light intensity of 183 µmol quanta m^{-2} s^{-1}, as in Kremien et al. (2013). The order of the treatments within each pair was randomly determined. Visual monitoring of the behavior of the fish during the trials indicated that in all treatments the fish constantly moved their fins, as they do in the reef.

Routine metabolic rate (RMR) was used to assess respiration of both fish and coral. RMR measures the metabolism of an organism during normal spontaneous activity including locomotion and digestion (Brett and Groves, 1979). RMR (mg O_2 h^{-1}) was calculated from the rate of decline in dissolved oxygen and the volume of the respirometer (Steffensen, 1989; Dowd et al., 2006) as $RMR=(\Delta O_2 \text{ per interval} \times V_r)/\Delta t$, where ΔO_2 is the change in oxygen concentration (mg l^{-1}), V_r is the respirometer volume of the system (l) and Δt is the change in duration of the change in oxygen concentration in the respirometer water (h). Oxygen concentration and temperature were concurrently recorded using an oxygen dipping probe (DP-PSt3; PreSens, Regensburg, Germany) and a temperature sensor (PT 1000) at a sampling rate of 10 s. Water temperature was taken into account for each analysis of the oxygen changes, in both the respiration and photosynthesis experiments, being 22–25°C for the entire period. The temperature range for the duration of the experiments (approximately 3 months each) was 2°C for the respiration experiment and 1°C for the photosynthesis experiment. When respiration by the fish was normalized to the animals' tissue biomass in order to compare our values with those of other authors, the standardization was calculated based on measurements of the RMR and divided by fish wet mass. For normalization of the oxygen changes in the coral, the total length of two different fragments from two different colonies was oven-dried

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at 60°C for 48 h, weighed, and burned at 450°C for 6 h to remove metabolically active tissue, then re-weighed (Schoepf et al., 2013).

Once a blank trial, lasting 30 min, showed no 'contamination' (i.e. change in oxygen when no animal was present in the system), the experimental trials began, with enough time for the acclimatization of the animal, typically lasting 1.5–2 h each. Throughout, temperature was kept constant and oxygen concentration was maintained at around 80–90% saturation by intermittent replacement of the water in the chamber using an automatic 3-way valve, effectively preventing hypoxia during dark trials. For a given pair, all four treatments in which photosynthesis was measured were conducted in a single day, while treatments of respiration measurements were carried out on different days. Changes in oxygen were derived from the slope of oxygen curves over intervals of 10 min. The last 7–10 intervals were used for each pair per measurement to calculate mean rates per pair. A post-blank run of 30 min was carried out at the end of the day, to account for bacterial respiration, after transferring the coral and fish back to the holding tank. Upon the completion of all trials with a pair, both animals were weighed and the fish length measured, and the animals were released at their site of origin in the reef.

In situ observations

The length of time *D. marginatus* spent inside *S. pistillata* during the daytime was measured for four coral colonies at 5–6 m depth in the coral reef off the IUI. An underwater video camera was positioned on a tripod ∼1 m away from the target coral. To allow fish habituation to the camera and tripod, the system was deployed at least 1 day prior to data collection. The camera was connected to a computer in the laboratory using a 100 m-long power and data cable. Data were recorded at a rate of 1 frame every 20 s using HandyAvi software (Anderson's AZcendant software, http://www.azcendant.com/), thereby producing time-lapse series lasting the entire daytime hours. The images of the time-lapse series were inspected individually and the fish outside and inside the coral were counted for posterior percentage calculations. A total of four *S. pistillata* colonies, hosting 2–5 *D. marginatus* each, were recorded for one full day each. The records were processed to obtain the percentage of time at least one fish from the group was found inside the coral, i.e. when the fish could completely enter the colony and was swimming among the branches of the coral colony. For posterior analysis, irradiation data were taken from the meteorological data set provided by The Israel National Monitoring Program at the Gulf of Eilat (NMP, 2015) in order to see whether light levels influenced the hiding behavior of fish and, as a consequence, changes in the coral's photosynthesis. Furthermore, a positive effect of fish ventilation on photosynthesis is only likely to occur when the photosynthesis rate is limited due to build-up of oxygen in the coral tissues, i.e. at times of high irradiation.

Data analysis

Rates of respiration (RMR) and photosynthesis were calculated as the second quartile of the whole data set from each of the pairs independently for the measured treatment, reported in mg O_2 h^{-1} (Davoodi and Claireaux, 2007; Chabot and Claireaux, 2008; Dupont-Prinet et al., 2010; Lefevre et al., 2011).

Statistical analyses were performed using *R* (version 3.1.1). Linear models were used to assess oxygen fluxes under different treatment groups accounting for repeated measures and nestedness. Therefore, a nested general linear mixed-effect model was fitted by restricted maximum likelihood (REML) with multiple random-effect terms. Pair, replicate and interval were included as nested random factors and treatment was included as a fixed factor [treatment+(1|pair/replicate/interval)]. Repeatability analysis using the standard error of measurement was conducted in this experiment, and model assumptions were tested using diagnosis plots of the model for normality, and homoscedasticity of the data and Cook's distance for leverage. No data transformation was required for the REML model as model assumptions were met. As data from the dark experiment showed a non-normal distribution, a generalized linear mixed model fitted by maximum likelihood (Laplace approximation) was applied, in which treatment was considered a fixed factor, and pair and interval were included as random factors (Table 1). Because of the non-normal and continuous distribution of the data, a transformation with the Gamma family and a log-link function was applied in the model. Type II Wald chi-square tests were used to test for significance of treatment effects. Tukey contrasts, which account for multiple comparisons, were used to assess significant differences between treatments. Both statistical models were run using the package lme4 (Bates et al., 2015), while the package multcomp (Hothorn et al., 2008) was used for the *post hoc* tests.

RESULTS

Net oxygen production in the fish+live coral treatment was on average 0.9 mg O_2 h^{-1} (22%) higher than in the fish/live coral treatment (Fig. 2, Table 2), indicating substantial enhancement of oxygen production when the fish was present between the branches of the coral during the day. This effect was highly significant ($P<0.001$), with the R^2 value for the model of 84.38 accounting for the treatment with different fish–coral pairs, the replicates within pairs, and the 10 intervals within each replicate, after verifying compliance with the model assumptions of normal distribution and homoscedasticity. A visual assessment of the Cook's distance showed only one point with relatively high leverage. Note that this

Table 1. Details of the type II Wald chi-square test for the linear mixed model assessing treatment effects on net oxygen production in light conditions and of the generalized linear mixed model assessing treatment effects on respiration measured in the dark

	Light		Dark		
Fixed effects	Mean±s.d.	*t*-value	Mean±s.d.	*t*-value	*P*-value
Fish alone	−1.180±0.146	−40.590	−0.963±0.066	−14.612	<0.001
Coral alone	4.750±0.635	7.490	−2.659±0.125	7.330	<0.001
Fish+dead coral	–	–	−1.148±0.067	−11.108	<0.001
Fish+live coral	3.963±0.129	−6.070	−3.539±0.064	4.949	<0.001
Fish/live coral	3.087±0.133	−12.510	–	–	–
Fish alone+coral alone	3.528±0.147	−8.340	−3.597±0.065	4.931	<0.001

Mean(±s.d.) values for net O_2 production are given in mg O_2 h^{-1}. The *P*-value of the dark experiment represents the significance of the parameters included in the model with respect to the *t*-value.

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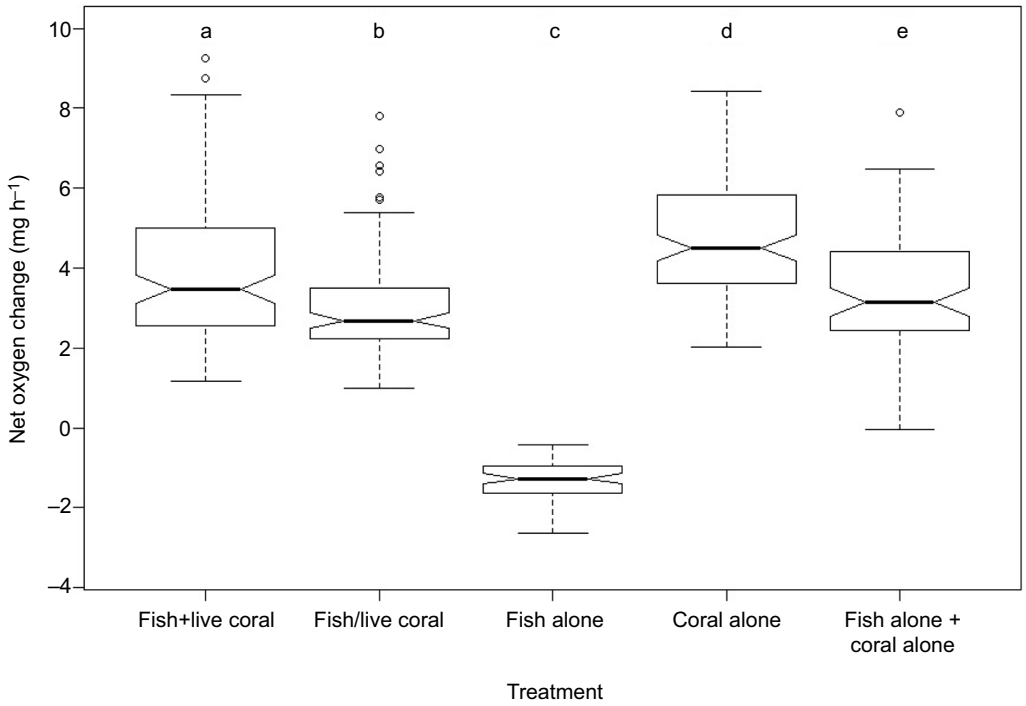

Fig. 2. Net oxygen production and consumption by the fish and coral (*n*=4) under light conditions when the fish and coral were alone, together, and in the same chamber but separated by a plastic mesh. In the box plots, the length of the box from top to bottom is the interquartile range (IQR), with the median represented by the bold line, and the maximum/minimum value of the third and first quartile minus 1.5 times the IQR indicated by the top/bottom whisker, respectively. Circles represent outliers. Significant differences (*post hoc* tests) are indicated by different letters above each box (Tukey contrast, $P<0.05$).

result is a conservative estimate because in one of the four pairs, the introduction of the fish between the coral branches did not affect the coral's net oxygen production; the extent of living tissue in this specific coral was relatively low and the fish in that pair was found inside the coral only part of the time (<0.5). Furthermore, the average net oxygen production in the treatment fish+live coral was 0.36 mg O_2 h^{-1} (9%) higher than the summed values of fish and coral measured alone (fish alone+coral alone). Surprisingly, the sum of fish alone and coral alone treatments under light conditions was 0.51 mg O_2 h^{-1} (14%) higher than the treatment fish/live coral, in which both were measured in the same chamber, but without contact between the two organisms (Fig. 2, Table 2). Taking into account the average biomass of the coral, the net oxygen production of the coral alone was 0.794±0.043 mg O_2 h^{-1} g^{-1} (mean±s.d., standardized by its wet mass).

In contrast, there were no significant differences between the respiration of fish and coral when measured together and individually (Table 2). The average RMR in the fish+live coral treatment was −3.54±0.10 mg O_2 h^{-1} compared with −3.60± 0.14 mg O_2 h^{-1} in the fish alone+coral alone treatment (means±s.d.; Fig. 3). Thus, there was no influence of the presence of fish on the respiration rate of the coral at night. However, the presence of the dead coral skeleton did significantly influence the respiration of the fish (Table 2). The RMR for the fish+dead coral treatment was 0.22 mg O_2 h^{-1} (19%) higher than the respiration for the fish alone treatment (Fig. 3), indicating a higher stress level in response to the dead coral. The model for this experiment displayed an R^2 value of 94.80, residuals were normally distributed, and while there was higher heteroscedasticity and leverage than in the previous experiment, it remained non-significant. When accounting for the biomass of the organisms (ranging from 2.9 to 5.7 g for the wet mass of the fish and 3.5 to 6.5 g for the dry mass of the coral), the RMR of (1) the fish alone was on average 0.288±0.027 mg O_2 h^{-1} g^{-1} (mean±s.d., standardized by its wet mass), and (2) the coral alone was on average 0.526± 0.012 mg O_2 h^{-1} g^{-1} (mean±s.d., standardized by its dry mass).

Table 2. Results of Tukey contrasts testing for relevant contrasts between treatments in the light and dark experiments

Comparison	Light $P(>\|z\|)$	Dark $P(>\|z\|)$
Fish+live coral versus fish/live coral	<0.001	–
Fish+live coral versus fish alone+coral alone	0.0236	1.000
Fish/live coral versus fish alone+coral alone	0.0246	–
Fish+dead coral versus fish alone	–	0.0084

In situ observations

At dawn, *D. marginatus* emerged from the coral and started to feed, retreating back into the coral before darkness (Fig. 4). During the crepuscular period (05:00–06:00 h, 16:30–17:30 h), *D. marginatus* spent on average more than half of its time (57%) inside the corals. When sun illumination was strong (08:00–15:00 h), the fish spent most of their time foraging in the waters surrounding their host colonies. Occasionally, the fish were observed retreating inside their corals, sometimes for protection and sometimes for no obvious reason. On average, the fish spent 19% of their time inside the corals during full daylight hours (08:00–15:00 h; Table 3). Furthermore, we found that the fish spent on average 7% more time inside the colony on clear days than on overcast ones (Fig. 4B,C versus A,D, respectively).

DISCUSSION

Coral and fish experiments

The influence of water flow on the photosynthetic process in diverse organisms has been systematically studied (e.g. Koehl and Alberte,

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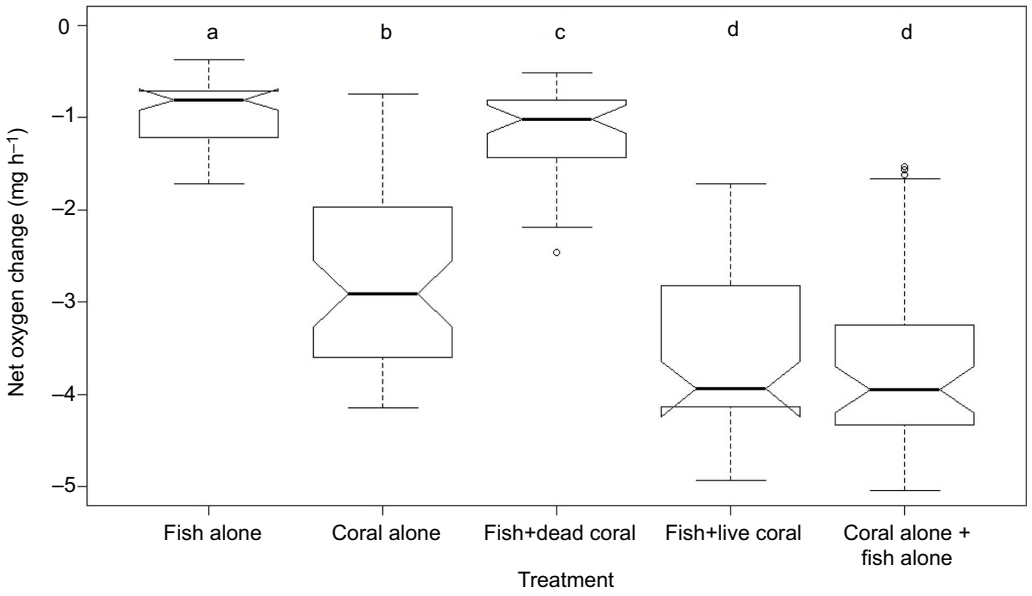

Fig. 3. Routine metabolic rate (RMR) of the fish and coral (*n*=5) measured in darkness when the fish and coral were alone or together in one chamber and when the fish was positioned in a dead coral skeleton. Box and whisker plots as for Fig. 2. Significant differences (*post hoc* tests) are indicated by different letters above each box (Tukey contrast, $P<0.05$).

1988; Patterson et al., 1991; Rickel and Genin, 2005; Enríquez and Rodríguez-Román, 2006; Mass et al., 2010; Kremien et al., 2013). Coral photosynthesis is strongly related to the thickness of the diffusive boundary layer (DBL) (Shashar et al., 1993; Kühl et al., 1995), which is reduced with higher water motion (Dennison and Barnes, 1988). In our study, when a fish was inside the coral colony, day or night, it was constantly moving its fins and continually ventilating and swimming between the coral's branches, thus reducing the DBL. Under these circumstances, we found that the coral photosynthesized almost one-quarter more compared with when fish and coral were physically separated by a plastic net. When the fish was not able to ventilate the colony (i.e. separated spatially), there was no augmentation of coral photosynthesis. As the sample size in this experiment was low, the likelihood of committing a type II error became more relevant regarding this lack of significance. However, the positive effect observed when coral and fish were measured together could also have been influenced by the production of carbon dioxide by the fish. This could have been taken up by the coral, thus increasing its photosynthesis. Our laboratory experiments, where fish ventilation resulted in an effect on coral photosynthesis, sought to simulate environments with low water currents (as in the Gulf of Eilat). In environments where water currents are stronger, the contribution of the fish could be reduced (Chase et al., 2014). Strong flow augments photosynthesis among benthic autotrophs, including corals, sea grass and algae (Mass et al., 2010). However, Shapiro et al. (2014) stated that when currents are weak, corals can actively enhance the mass transport near the DBL by up to 400% through strong vertical flows driven by motile epidermal cilia. We found that the coral displayed high rates of oxygen production when it was placed alone in the respirometer chamber. The net oxygen production of the combined treatment, calculated based on individual results of fish and coral, was significantly more positive than when the coral was physically separated from the fish. It is possible that when a fish detected a nearby coral in which it sought shelter, but could not enter, it became stressed and respired more, lowering the photosynthesis-driven net oxygen accumulation in the water. However, the fish seemed physically relaxed during the whole experiment: they swam normally as they would in the reef, the operculum was not opening faster than naturally, and they did not turn black as they do when stressed.

Under flow conditions, Mass et al. (2010) found that corals displayed lower respiration rates compared with those measured in the absence of flow. However, in response to the presence of fish, Goldshmid et al. (2004) expected to find higher rates of coral respiration amongst other factors, such as higher primary production (Patterson et al., 1991), calcification (Dennison and Barnes, 1988) or coral growth (Holbrook et al., 2008). Their expectation was based on the movement of the damselfish's fins, which produce a modulation in the hydrodynamic conditions surrounding the coral, reducing the thickness of the DBL and allowing the exchange of oxygen with the surrounding waters. Nevertheless, in this study we did not find significant differences in respiration when the fish and coral were in a chamber together, compared with the combined value for fish and coral calculated based on individual results. Future studies are needed to quantify the water flow inside the coral colonies using specialized equipment.

In this experiment, filtered seawater was used; thus, the coral did not have any food available for heterotrophy. Feeding in most animals is a metabolically demanding process (e.g. Szmant-Froelich and Pilson, 1984; Klumpp et al., 1992; Titlyanov et al., 2001; Borell et al., 2008; Naumann et al., 2011). Under non-feeding conditions, the oxygen concentration between the branches, even without fish, may not have limited the coral's low oxygen demand; therefore, enhancing the oxygen by fish ventilation did not have an effect. According to Borell et al. (2008), starving *S. pistillata* resulted in a significant decrease in respiration. Unfortunately, in this study, adding plankton to the water was impossible as it would have caused the measurements to be 'contaminated' by the plankton respiration. We always made sure that the filtered water in the tank would not affect respiration rates.

As a consequence of fish and coral respiration, the oxygen concentration inside a coral colony varies from supersaturation during the day to hypoxia at night (~10% of air saturation), while it remains constant in ambient water (Shashar et al., 1993; Kühl et al.,

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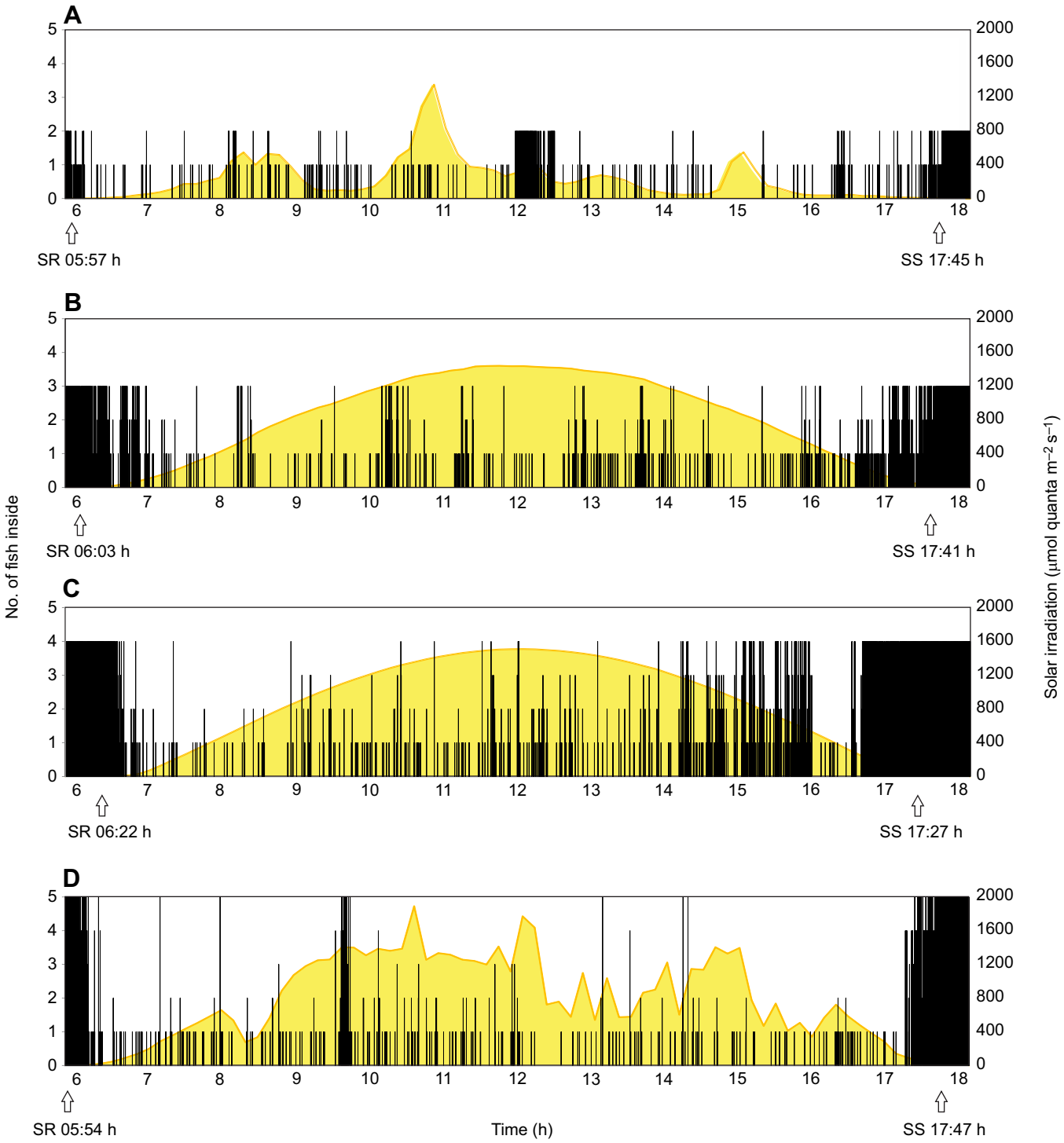

Fig. 4. Natural frame of the diurnal behavior of damselfish with reference to the solar radiation on a particular day. Times when fish were present inside the host colony are represented by black bars (left axis). Solar irradiation (yellow area, right axis) was measured from sunrise (SR) to sunset (SS). Plotted are representative randomly selected days. The measurements were carried out *in situ* with four different corals inhabited by 2 (A), 3 (B), 4 (C) and 5 (D) fish.

1995). It is exactly during this hypoxic period that damselfishes spend most of their time inside the coral colonies (Rickel and Genin, 2005; this study), and low oxygen levels during the night prompt higher ventilation by the damselfish (Berenshtein et al., 2014). The calculated respiration rates of *D. marginatus* (RMR=0.288± 0.027 mg O_2 h^{-1} g^{-1}) were comparable to those in Nilsson and Östlund-Nilsson (2004), who reported respiration rates of 0.306± 0.037 mg O_2 h^{-1} g^{-1} for *Dascyllus aruanus*, indicating that the fish were relaxed during the experiments. As damselfish are able to withstand very low oxygen saturation levels (~20%) in the water (Nilsson and Östlund-Nilsson, 2004), the resilience of the fish to the hypoxic conditions created in coral reefs during the night could be an adaptive trait of damselfish associated with their inhabitation of branching corals (Berenshtein et al., 2014).

As a coral-dwelling species, *D. marginatus* belongs to the fish group most vulnerable to coral degradation (Pratchett et al., 2008),

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Table 3. Results from *in situ* observations of coral colonies inhabited by damselfish, showing the percentage of time for which at least one fish was inside the coral

	Day			
% Time	A	B	C	D
Crepuscular period	40.4	76.6	55.2	55.9
Highest solar radiation	15.8	17.6	27.7	14.8
Whole experiment	18.7	30.4	34.3	23.4

A, 9 March 2014 (*n*=2); B, 4 March 2014 (*n*=3); C, 12 February 2014 (*n*=4); D, 12 March 2014 (*n*=5). See Fig. 4.

driven by the loss of habitat and the increment of stress due to the presence of potential predators, increase of intraspecific competition and changes in the environment (Lima and Dill, 1990; Feary et al., 2009; Coker et al., 2015a,b). Shelter and shoaling have tangible benefits for fishes that are detectable in reduced routine metabolism (Millidine et al., 2006; Nadler et al., 2016). We thus expected the presence of shelter provided by the coral colony to lead to reduced respiration by *D. marginatus*. Surprisingly, we found that a fish placed in the chamber with a dead coral skeleton respired significantly more than when alone. In choice experiments, juveniles of coral-associated damselfishes are able to discriminate between living and dead coral colonies (Feary et al., 2007). It is possible that the presence of a dead coral resulted in increased stress for the damselfish in our experiment, leading to increased respiration rates. Alternatively, residual microbial activity in the dead coral may have contributed to this observation, but the thorough treatment of the coral skeleton prior to the experiment renders this likelihood low.

One potential caveat of our experimental measurements is that water inside the chambers was flowing at a very low rate during measurements, and that under strong natural flow conditions, the effect of fish may be less than that observed here. Future studies should test the influence of different flow levels on damselfish effects by providing artificial flow inside the measurement chambers. However, in many reefs, including the one in Eilat, corals live in habitats where currents are weak (Goldshmid et al., 2004). Under such conditions, autotrophic organisms need to develop strategies to increase the water flow (Mass et al., 2010; Wild and Naumann, 2013; Kremien et al., 2013). The occupation by fish of a particular colony depends greatly on the inter-branch space of that specific coral, and the metabolic effects of fish–coral mutualism vary with local environmental conditions (Chase et al., 2014). Colonies in low-flow conditions, where increased ventilation by fish is advantageous, should feature inter-branch spaces most suitable to habitation by damselfish. It therefore seems reasonable to suggest that in the presence of fish, *S. pistillata* has developed an evolutionary strategy through which *D. marginatus* are 'invited' to inhabit the shelter between its branches and thereby benefit the coral by enhancing water motion inside the colony.

In situ recording of fish behavior

In this study, the effect of aeration by the fish was found to be directly related to the coral's tissue biomass. We found that, under aeration by fish, corals with larger tissue biomass showed higher photosynthetic rates. This result matches conditions in the natural environment, where healthy coral colonies generally have higher tissue biomass and host more than one damselfish. Under these conditions, aeration by the fish would be greater than that observed in laboratory experiments, where only one individual fish per colony was used. Our results showed that approximately 19–34% of the daytime, at least one fish was found between the coral branches. The amount of time at least one fish spends inside a coral colony might be influenced by the size of the colony and the fish group size, as in a colony with a larger group of fish the probability of finding an individual inside the coral would be higher. Our photosynthetic measurements indicated that ventilation by a single fish can increase photosynthesis by about 22%. When accounting for the amount of time that at least one fish was inside a colony under natural conditions, the overall augmentation of photosynthesis by fish ventilation during strong illumination hours would be small: 3–6% over the course of a full day. Further observations are needed to test the ecological relevance of this small increase in photosynthesis. However, our results probably are conservative estimates, as discussed above. At the population level, even small benefits in terms of shelter (for the fish) and metabolism (for the coral) are likely to confer evolutionary advantages. Considering both the *in situ* observations and the laboratory measurements, the presence of fish appears to be more beneficial to corals during the middle of the day, when flow, rather than light intensity, limits coral photosynthesis. Recent observations (A.G., unpublished data) indicated that fish tend to enter the coral and stop feeding on drifting zooplankton when currents are weak, reducing the risk of feeding out of the shelter. The absence of currents during the daytime implies conditions when water does not flow through the inner part of the coral, leading to accumulation of oxygen around the coral tissue; this is exactly when ventilation of the coral by the fish becomes more critical. The occurrence of the fish between the coral branches during the day, albeit intermittent, may become more important in stressed corals, such as colonies recovering from tissue injuries, actively competing neighbors and those stressed by water warming.

The main benefit for the fish of living in mutualism with a branching coral is the protection from predators (Holbrook et al., 2008). Our video records clearly showed that when a predator approached the coral, the fish rapidly sought refuge between the branches of their coral host. Further studies are needed to test whether there are links between the shelter-seeking behavior of coral-associated damselfishes and predator abundance, and to test possible effects on coral physiology.

The present study provides the first evidence of positive effects by an obligate coral-associated fish on coral photosynthesis. The results add to our growing understanding of fish–coral mutualism, and provide direction for future studies. These should test the theory that colonies in low-flow conditions, where increased ventilation by fish is advantageous, feature particularly favorable inter-branch spaces for optimal inhabitation by damselfish. Furthermore, the potential effect of predator removal on damselfish behavior, and subsequently on coral physiology, requires further study. The extent to which other coral-dwelling fishes, such as hawkfishes (Coker et al., 2015a,b), have similar effects on coral physiology should be assessed. Finally, given that water temperature influences both coral photosynthesis (e.g. Borell et al., 2008) and damselfish movement (Johansen and Jones, 2011; Beyan et al., 2015), the effects of rising water temperatures on the physiological aspects of fish–coral mutualisms are in need of further investigation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
N.G.-H., A.G., S.C.A.F. and A.K. designed research; N.G.-H. and A.G. performed research; N.G.-H. and S.C.A.F. analyzed data; and N.G.-H., A.G., S.C.A.F., A.K. wrote the paper.

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