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Acquisition of obligate mutualist symbionts during the larval stage is not beneficial for a coral host

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Abstract

Theory suggests that the direct transmission of beneficial endosymbionts (mutualists) from parents to offspring (vertical transmission) in animal hosts is advantageous and evolutionarily stable, yet many host species instead acquire their symbionts from the environment (horizontal acquisition). An outstanding question in marine biology is why some scleractinian corals do not provision their eggs and larvae with the endosymbiotic dinoflagellates that are necessary for a juvenile's ultimate survival. We tested whether the acquisition of photosynthetic endosymbionts (family Symbiodiniaceae) during the planktonic larval stage was advantageous, as is widely assumed, in the ecologically important and threatened Caribbean reef-building coral *Orbicella faveolata*. Following larval acquisition, similar changes occurred in host energetic lipid use and gene expression regardless of whether their symbionts were photosynthesizing, suggesting the symbionts did not provide the energetic benefit characteristic of the mutualism in adults. Larvae that acquired photosymbionts isolated from conspecific adults on their natal reef exhibited a reduction in swimming, which may interfere with their ability to find suitable settlement substrate, and also a decrease in survival. Larvae exposed to two cultured algal species did not exhibit differences in survival, but decreased their swimming activity in response to one species. We conclude that acquiring photosymbionts during the larval stage confers no advantages and can in fact be disadvantageous to this coral host. The timing of symbiont acquisition appears to be a critical component of a host's life history strategy and overall reproductive fitness, and this timing itself appears to be under selective pressure.

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KEYWORDS
cnidarians, coral reefs, larval ecology, life history evolution, Symbiodiniaceae

1 | INTRODUCTION

Mutualisms between metazoan hosts and autotrophic symbionts, such as those between corals (Cnidaria:Hexacorallia:Scleractinia) and dinoflagellate algae of the family Symbiodiniaceae (Dinophyceae:Suessiales), allow both to survive in nutrient-poor environments such as tropical oceans (LaJeunesse et al., 2018; Muscatine & Porter, 1977). When hosts depend on symbiont-derived energy for survival, theory predicts that passing symbionts directly to offspring via vertical transmission (VT) is evolutionarily favourable (Bull, Molineux, & Rice, 1991; Doebeli & Knowlton, 1998; Ewald, 1987; Wilkinson & Sherratt, 2001). In VT coral species, photosymbiont cells are capable of producing energy for their host during its larval stage, potentially subsidizing the parental endowment of energetic lipids and providing larvae with additional time to search for a suitable settlement location (Alamaru, Yam, Shemesh, & Loya, 2009; Gaither & Rowan, 2010; Harii, Nadaoka, Yamamoto, & Iwao, 2007; Harii, Yamamoto, & Hoegh-Guldberg, 2010; Richmond, 1987). Despite the potential advantages provided by photosymbionts during the larval stage, fewer than 30% of coral species pass these symbiotic dinoflagellates vertically to their offspring (Baird, Guest, & Willis, 2009). Instead, the majority of coral species acquire their photosymbionts from the surrounding environment in a process known as horizontal acquisition (HA).

The prevalence of HA among coral species suggests that hosts might not widely benefit from acquiring photosymbionts at the time of release. Symbiodiniaceae species are most beneficial to their coral host within a set range of environmental conditions (e.g., Rowan & Knowlton, 1995; LaJeunesse, 2001; Baker, 2003), and HA is strongly associated with coral species capable of long-distance dispersal (Baird, Guest, et al., 2009). These observations led to the hypothesis that HA is advantageous by allowing dispersing larvae to acquire photosymbionts species that are optimal for the environmental conditions where they settle, rather than those most advantageous where they are produced (Baird, Guest, et al., 2009; LaJeunesse et al., 2004; Schwarz, Krupp, & Weis, 1999). Lacking photosymbionts can also protect larvae from deleterious environmental conditions during dispersal, such as high light and temperature, which can cause symbiont-induced stress to the larval host (Chamberland, Latijnhouwers, Huisman, Hartmann, & Vermeij, 2017; Hartmann, Baird, Knowlton, & Huang, 2017; Hartmann, Marhaver, Chamberland, Sandin, & Vermeij, 2013; Nesa, Baird, Harii, Yakovleva, & Hidaka, 2012; Yakovleva et al., 2009). These disadvantages suggest that delaying photosymbiont acquisition provides benefits to larvae of HA coral species, perhaps explaining the incongruence between theory and natural history. While the consequences of larval acquisition in HA species have been examined (e.g., Yakovleva et al., 2009, Nesa et al., 2012, Suzuki et al., 2013), we lack a complete assessment of the physiological, behavioural and ecological costs and benefits of HA (Mies, Sumida, Rädecker, & Voolstra, 2017).

Whether delayed photosymbiont acquisition is under positive selection remains an open question. Larvae of many coral species develop a mouth, making them capable of taking up photosymbionts in the family Symbiodiniaceae while in the plankton (Adams, Cumbo, & Takabayashi, 2009; Schwarz et al., 1999; Voolstra, Schwarz, et al., 2009). However, despite the ability to acquire photosymbionts, multiple factors may delay acquisition until after larvae move from the water column to the benthos. Free-living Symbiodiniaceae cells are an order of magnitude more abundant in sediments than in the water column (Littman, Oppen, & Willis, 2008), acquisition of these photosymbionts by coral larvae is greater in the presence of sediments than without (Adams et al., 2009; Cumbo, Baird, & Oppen, 2013; Nitschke, Davy, Cribb, & Ward, 2015), and the algal cells can act as a settlement cue on benthic surfaces (Vermeij, Frade, & Bak, 2013). While these and other phenomena may delay photosymbiont acquisition until after larvae contact the benthos, it is not known whether delaying acquisition until then is advantageous.

Taken together, previous work predicts two opposed consequences of larval photosymbiont acquisition in HA species. If Symbiodiniaceae provide energy to larvae, this may benefit the host by enhancing survival or prolonging larval longevity. In contrast, if negative consequences arise for larvae, even in environmental conditions favouring the mutualism (e.g., no light or temperature stress), this would suggest selection for delayed photosymbiont acquisition. The primary goal of this study was to determine which prediction best describes the larva–symbiont relationship in an HA coral species. Studies of multiple coral species, including that studied here, find mild changes in larval gene expression following photosymbiont acquisition (e.g., apoptosis and oxidative stress) that may be associated with suppression of the host's immune response (Mohamed et al., 2016; Schnitzler & Weis, 2010; Schwarz et al., 2008; Voolstra, Schwarz, et al., 2009). In contrast to larvae, when settled and metamorphosed corals acquire photosymbionts, central metabolism and symbiont handling processes are primarily upregulated (*Orbicella faveolata*, Reyes-Bermudez et al., 2009; *Acropora* sp. Yuyama, Harii, & Hidaka, 2012), suggesting that algal infection leads to different molecular responses across life stages. By simultaneously assessing larval host behaviour, energy use and gene expression, our experiments helped resolve the ecological consequences of photosymbiont acquisition and photosynthesis in coral larvae, shedding new light on the ramifications of HA in corals and generating testable hypotheses for why selection may favour HA in this foundational yet severely threatened taxon.

2 | MATERIALS AND METHODS

Three sets of experiments were carried out to determine whether acquiring algal photosymbionts during the planktonic larval stage was beneficial to the scleractinian coral *Orbicella faveolata* (Ellis

& Solander, 1786; formerly *Montastraea faveolata*; Budd, Fukami, Smith, & Knowlton, 2012). *O. faveolata*, a broadcast spawning species, can acquire algal symbionts within the family Symbiodiniaceae, including *Symbiodinium microadriaticum* and *Breviolum minutum* (formerly Symbiodinium type B1, LaJeunesse et al., 2018) via HA (see Supporting Information Figure S1 for an overview of the experiments).

2.1 | Experiment 1: The effect of photosymbiont acquisition (infection) on larval gene expression and energetic lipid use

Gamete bundles containing symbiont-free eggs and sperm were collected from seven colonies of *O. faveolata* during the annual mass spawning event on the leeward coast of Curaçao (12°6′33″ N, 68°57′15″ W) in September 2011. After fertilization, planula larvae were reared in the laboratory as described in Vermeij, Fogarty, and Miller (2006). On day 7 after spawning (d7), approximately 1,500 actively swimming larvae were placed into each of 20 clear polystyrene plastic clamshell bins (12.7 × 12.7 cm, Dart Container Corp., Mason, MI, USA) filled with 300 ml of 0.45 µm filtered seawater (hereafter referred to as FSW; Millipore HA filter; EMD Millipore, Billerica, MA, USA). These bins provided five replicates per treatment for a 2 × 2 factorial experiment: photosymbiont-infected larvae (Symb) versus photosymbiont-free larvae (NoSymb) and larvae exposed to a 12:12-hr light-dark cycle (Light) versus larvae exposed to constant darkness (Dark). This experiment was designed to reveal the effects of Symbiodiniaceae infection and photosynthetic activity on two types of response variables: host gene expression and host depletion of energetic lipids. Concurrently, carbon fixation was measured to confirm that light-exposed Symbiodiniaceae were indeed photosynthesizing.

Once larvae were distributed into the twenty bins, photosymbiont cells were added immediately afterwards. To achieve successful infection by larvae in the Symb treatments, we used a culture of *B. minutum* (previously *Symbiodinium minutum*, LaJeunesse et al., 2018; cp-type B184, ITS2 type B1, Mf1.05b.01.SCI.01 cultured by M.A. Coffroth), which is closely related to the wild species found in *O. faveolata* (Voolstra, Schwarz, et al., 2009). The infection protocol is detailed in the Supporting Information.

Following photosymbiont infection, larvae from each bin were rinsed on d17 with FSW through a 50-µm nylon mesh filter to remove any *B. minutum* cells that remained in the water column, and then, larvae were transferred to new bins with fresh FSW. Each of the ten bins per infection treatment was then randomly assigned to either the Light or Dark treatment group to begin the gene expression experiment (N = 5 bins/treatment). On the same day, two small batches of larvae were removed from each bin, one for measuring energetic lipid content and one for measuring symbiont photosynthetic activity. For the lipid analysis, 50 larvae were placed on a precombusted glass fibre filter (N = 20; GF/F; Whatman; Maidstone, Kent, UK) and frozen at −20°C (lipid time point t0). To quantify carbon fixation, 55 larvae were placed in a Petri dish that contained 30 ml of FSW with a final concentration of 2.3 mmol/L $NaH^{13}CO_3$ (N = 20; 99% ^{13}C, BD Biosciences, Billerica, MA). Sodium bicarbonate comprised almost entirely of the stable isotope of carbon, C-13, provided a traceable source of dissolved inorganic carbon for photosynthesis by *B. minutum*. The labelled carbon was later measured in the combined larval and *B. minutum* tissue to determine the extent of carbon fixation that had occurred.

All bins and Petri dishes were placed underneath two fluorescent aquarium lights (36”, Aqueon, Franklin, WI, USA) which were secured above the bins to produce irradiance levels equivalent to those experienced by the study species in situ (50–60 µmol m^{-2} s^{-1}, measured with a flat sensor; WALZ Mess und Regeltechnik, Germany; Vermeij & Bak, 2002). The locations of the 20 bins and 20 Petri dishes were randomly assigned underneath the light source. Bins and Petri dishes in the dark treatment were covered using aluminium foil, and the experiment was conducted for 48 hr.

At the conclusion of the 48-hr experiment, 50 larvae were sampled from each bin for lipid quantification (lipid time point t48) to compare with samples from the beginning of the experiment. For the gene expression analyses, the remaining larvae in each bin (~1,350) were consolidated on a 50-µm nylon mesh filter and transferred using a rubber scraper to a 2 ml cryovial (Simport Scientific, Beloeil, QC, Canada) containing RNAlater (Qiagen Sciences, Germantown, MD, USA). These samples were kept at 4°C for 18 hr and then placed at −20°C for less than 1 week. Samples were then stored at −80°C until they were analysed. Larvae in the 20 Petri dishes that were exposed to ^{13}C-enriched sodium bicarbonate were placed on a combusted GF/F and frozen at −20°C until analysis.

2.2 | Application of microarray technology to measure gene expression

For construction of cDNA probes, total RNA was extracted from ~1,350 frozen (−80°C) larvae per experimental replicate (N = 5) using QIAzol Lysis Reagent and an RNeasy Kit (Qiagen Sciences). Microarray slides were prepared following the methods of Closek et al. (2014); this process and the method for scanning the slides are described in detail in the Supporting Information. Genes were annotated using tBLASTx and BLASTx (E-value threshold = $1e^{-5}$) against the Swiss-Prot, UniProt and GenBank nonredundant DNA and protein databases (see the Supporting Information for more details about the reference design).

2.3 | Global gene expression profiling in response to symbiont infection and light

For each microarray, the data matrix was filtered to only the genes that successfully hybridized (i.e., produced data) in at least three replicates for each treatment. Missing values were imputed, and all values were normalized to a mean of zero and the same unit variance in each gene. Relative expression across all genes was compared among treatments using the machine-learning algorithm Random Forests (Brieman, 2001) with the *rfPermute* package in R (Archer, 2016). The importance of each

gene's expression for distinguishing between treatments was ranked based on the Random Forests mean decrease accuracy score, reflecting the decrease in the model's predictive power when that gene was removed (see the Supporting Information for more details).

2.4 | Change in lipid content in response to symbiont infection and light

Total lipids were extracted from larvae using the Bligh and Dyer (1959) method. Lipid classes were quantified via thin layer chromatography with flame ionization detection on an Iatroscan TLC-FID MK-5 (Iatron Laboratories, Inc., Japan) following the methods of Hartmann, Marhaver, and Vermeij (2018; see the Supporting Information for more details). A two-factor ANOVA was used to determine differences in lipid depletion among treatments based on the change in lipid content over the 48-hr experiment for each lipid class, with photosymbiont status and light exposure as the independent variables. If the change in lipid content was not normally distributed, the ANOVA was performed on log-transformed data (e.g., Δ wax esters (WE) = log WE t_{48} − log WE t_0). In addition, a paired *t* test was used to determine whether lipid content changed over the 48-hr experiment.

2.5 | Degree of symbiont infection and stable isotope analysis to estimate photosynthetic activity

To obtain quantitative measurements of photosymbiont infection, the total number of algal cells per larva was measured from larvae exposed to $NaH^{13}CO_3$-enriched seawater by imaging 10 larvae per filter (20% of the total larvae in the replicate) with an epifluorescence microscope (Leitz Orthoplan, GmbH, Hanau, Germany). The number of algal cells per larva was counted using ImageJ (NIH), and the mean number of cells per larva was calculated for each treatment (N = 10 larvae/treatment). After imaging, samples from Petri dishes containing larvae exposed to $NaH^{13}CO_3$ were acidified for 6 hr via fumigation in a desiccator containing a small open beaker of concentrated HCl (12 M) to remove residual $NaH^{13}CO_3$ and any other residual inorganic carbon. Filters were dried at 70°C and immediately placed in precombusted aluminium weigh boats for total C and $\delta^{13}C$ analysis at the UC Davis Stable Isotope Facility (see the Supporting Information for details).

2.6 | Experiment 2: Behavioural response of larvae to infection with local photosymbionts

Survival, swimming activity and settlement was quantified in larvae infected with photosymbiont communities isolated from conspecific adults on the natal reef (henceforth referred to as local photosymbionts) and in Light and Dark conditions. Survival was used to quantify lethal stress. Rates of swimming and settlement were used to estimate sublethal stress. Swimming is one of the most energetically costly behaviours for a larva, and the process of metamorphosis is similarly taxing. In *O. faveolata* in particular, cessation of swimming without prompt settlement can be a sign of energy exhaustion and low competency to metamorphose (Raimondi & Morse, 2000; K.L. Marhaver & M.J.A. Vermeij, pers. obs.). Larvae of this species also exhibit this behaviour in response to pathogen exposure (K.L. Marhaver, pers. obs.). While larvae of other coral species are more often observed in a motionless state due to the absence of water motion or settlement cues, *O. faveolata* tend to not be still for long periods until just prior to attachment and metamorphosis. Therefore, the number of individuals swimming in each dish was used here as a metric of typical behaviour of planktonic larvae across treatments.

In larval cultures of *O. faveolata*, settlement can be encouraged by providing positive settlement cues such as crustose coralline algae (CCA; Morse & Morse, 1991, Morse, Morse, Raimondi, & Hooker, 1994); however, settlement does occur in significant numbers in this species even in the absence of these cues (e.g., Marhaver, Vermeij, Rohwer, & Sandin, 2013). In this experiment, CCA was not added to the culture containers for two reasons. First, we wanted to maintain larvae in relatively clean conditions that were representative of the planktonic environment, during which photosymbionts might provide an important added energy source to larvae. Second, photosymbiont cells are found free-living on the benthos and could be transferred into the experimental replicates during the addition of CCA chips.

To determine the behavioural response of larvae to photosymbiont infection during the larval period, gametes of *O. faveolata* were collected at the same site as those in Experiment 1 (12°6′33″ N, 68°57′15″ W) 2 years later and they were reared as described above. As in Experiment 1, larvae were exposed to photosymbionts for 10 days beginning 7 days after spawning (d7). The local symbionts used for infection were comprised of the full Symbiodiniaceae community (containing one or more species) isolated from five conspecific adult corals (*O. faveolata*) collected from the same reef tract as in Experiment 1. Symbiodiniaceae were isolated from adult coral tissue, and larvae were exposed to these cells on the same day (d7; see the Supporting Information for details of the isolation protocol). Photosymbiont infection was monitored as described for Experiment 1.

On d17, all larvae were rinsed in FSW and allocated to the same four experimental treatments as in Experiment 1: a 2 × 2 factorial experiment of photosymbiont-infected larvae (Symb) vs. photosymbiont-free larvae (NoSymb) crossed with larvae exposed to a 12:12-hr light–dark cycle (Light) vs. larvae exposed to constant darkness (Dark). Each of the four treatments was replicated in five Petri dishes, each containing 40 actively swimming larvae in 40 ml of FSW. All Petri dishes were placed under the same light fixture used in Experiment 1; again, light intensity at the surface of the experimental replicates was measured between 50 and 60 μmol m^{-2} s^{-1}. Dark treatment Petri dishes were covered with aluminium foil to ensure complete darkness.

The number of larvae alive, swimming and settled was counted daily on d19–d25. The total number of surviving individuals was defined as the total number of larvae and settlers visible in the dish at a given time. Dead larvae appear as pale, fraying clusters of tissue or they are no longer visible (Marhaver et al., 2013). Dead larvae were

removed with a plastic pipet. The number of larvae swimming was determined based on the number of larvae moving (as opposed to sitting or floating motionlessly). The number of settlers was determined as the number of individuals that had attached to the Petri dish, formed a mouth and begun calcifying, that is, the number of individuals that had begun or completed metamorphosis.

Data were analysed in two ways. First, a per-day maximum-likelihood approach was used to assess survival, swimming and settlement behaviour (Hartmann et al., 2013). A binomial error distribution was used to estimate the most likely probability of survival, swimming and settlement at each time point for each treatment. A suite of models describing unique groupings of treatments was used to identify treatment groups with significantly different means for each factor (see Hartmann et al., 2013 for a detailed description of this method). Survival was calculated as the proportion of larvae or settlers alive each day relative to the starting number of larvae, swimming was defined as the proportion of larvae swimming relative to the number of larvae and settlers alive on that respective day, and settlement was defined as the proportion of individuals that had settled and begun metamorphosis relative to the total number of larvae and settlers alive on that respective day. The best-fit probabilities for a given set of treatment groupings were estimated using maximum likelihood, and models were compared with likelihood ratio tests or Akaike information criterion depending on nestedness.

Second, because behaviour was recorded daily, the differences in swimming behaviour and settlement could be compared as rates among treatments. To do this, the proportion of larvae either swimming or newly settled at each time point relative to the pool of larvae that could have performed that behaviour was calculated based on the previous time step (e.g., settled larvae at time t_1 cannot settle again nor swim at t_2). The proportional data were arcsine square root-transformed, and a repeated measures two-factor ANOVA was performed to compare means of larvae performing each behaviour by symbiont status and light treatment. This analysis was performed using seven time points (d19–25) as the repeated measures response variable.

2.7 | Experiment 3: Behavioural response to infection by two different Symbiodiniaceae species

Larvae of *O. faveolata* (from the same cohort used in Experiment 1) were exposed to two Symbiodiniaceae species: *S. microadriaticum* (cp-type A194, ITS2 type A1, *Cass*Kb8; cultured by M.A. Coffroth) and *B. minutum* (as was used in Experiment 1). Both photosymbiont species are known to be taken up by *O. faveolata* larvae, but *S. microadriaticum* elicits a stress response in larvae following uptake, while *B. minutum* does not (based on gene expression profiling; Voolstra, Schwarz, et al., 2009). Reference cultures were maintained in f/2 medium at a salinity of 38 PSU and 26°C under a 14:10-hr light:dark cycle (70–90 µmol m^{-2} s^{-1}, from 40 W fluorescent lights) and were characterized following the protocols of Santos, Gutierrez-Rodriguez, and Coffroth (2003). Symbiodiniaceae cultures were added to larvae on d6. Larvae were allocated to five replicate containers per treatment, each containing 40 larvae in 40 ml FSW, and replicates were assigned to one of three treatment groups: (a) photosymbiont-free larvae (control, NoSymb), (b) larvae exposed to *B. minutum* and (c) larvae exposed to *S. microadriaticum*. Larvae were exposed to daily cycles of 12:12-hr light:dark with the same fluorescent aquarium lights and irradiance levels used in Experiments 1 and 2. The number of individuals alive, swimming and settled was counted on d10, d16, d17 and d33. Larvae were continuously exposed to algal cells for the duration of the experiment, though infection was not visually confirmed. Data on survival, settlement and swimming rate were analysed separately by day using the approach from Hartmann et al. (2013) and the maximum-likelihood approach described above. There were no statistical differences between data collected on d16 and d17; therefore, only data from d16 are presented here.

3 | RESULTS

3.1 | Experiment 1: Photosymbiont infection and photosynthesis

After 10 days of exposure to photosymbionts of the family Symbiodiniaceae, larvae in the Symb/Light treatment contained 81.2 ± 14.2 algal cells/individual (N = 10; mean ± SE) and larvae in the Symb/Dark treatment contained 100.2 ± 49.0 cells/individual. Algal infection was not significantly different for larvae in the Symb treatments, whether exposed to Light or Dark treatments (t_6 = −0.372; p = 0.72). Mean $\delta^{13}C$ enrichment differed among the Symb/Light, Symb/Dark and NoSymb/Dark treatments ($F_{2,8}$ = 15.48; p = 0.002; NoSymb/Light were not analysed; see Materials and Methods). $\delta^{13}C$ enrichment was more than 100-fold greater in *Symb*/Light than in Symb/Dark (p = 0.003) and NoSymb/Dark (p = 0.005) demonstrating that the symbiont cells were photosynthesizing in the light treatment. Furthermore, enrichment was similar between individuals in the Symb/Dark and NoSymb/Dark treatments (p = 0.97), demonstrating that dark conditions effectively suspended photosynthesis in the photosymbiont-infected larvae that were not exposed to light. Consistently, there was no detectable difference in larval carbon enrichment due to symbiont photosynthesis, though the presence of the photosymbiont increased larval C content. Symb/Light larvae contained 0.62 ± 0.08 µg C per larva (mean ± SD) while Symb/Dark larvae contained 0.68 ± 0.04 µg C per larva (p = 0.23) and Symb larvae contained 0.65 ± 0.07 µg C per larva while NoSymb larvae contained 0.49 ± 0.03 µg C per larva (p = 0.002).

3.2 | Differential expression in response to photosymbiont infection and light environment

The presence or absence of photosymbionts in larvae caused a far greater change in overall host gene expression patterns than did light environment, as evidenced by distinct separation in gene expression profiles between Symb and NoSymb larvae, and poor discrimination between Light and Dark samples (Figure 1a, Supporting

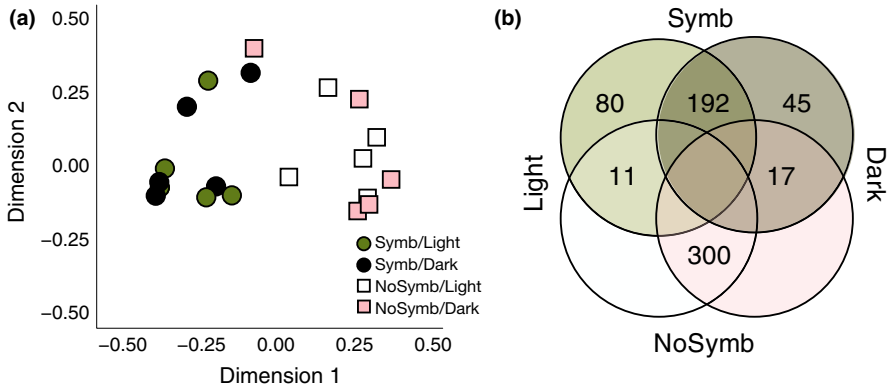

FIGURE 1 Comparisons of *Orbicella faveolata* larval gene expression patterns. The four treatment groups included in the comparison were as follows: larvae with Symbiodiniaceae photosymbionts in 12:12-hr light:dark (Symb/Light), larvae with photosymbionts in constant darkness (Symb/Dark), larvae with no photosymbionts in 12:12-hr light:dark (NoSymb/Light) and larvae with no photosymbionts in constant darkness (NoSymb/Dark). (a) The similarity of gene expression profiles among all samples from the four treatments, depicted based on the first and second dimensions of the Random Forests proximity projection. (b) A Venn diagram of the number of differentially expressed genes by treatment. Values represent the number of upregulated genes in the respective treatment [Colour figure can be viewed at wileyonlinelibrary.com]

Information Table S1). In larvae with photosymbionts, 192 genes were upregulated (62 annotated) and 300 were downregulated (125 annotated) relative to larvae without photosymbionts (Figure 1b). In larvae exposed to Light, 11 genes were upregulated (five annotated) and 17 genes were downregulated (four annotated) relative to larvae in Dark (Figure 1b).

To isolate the effect of symbiont photosynthesis, gene expression in Symb/Light was compared to Symb/Dark. In larvae with photosynthesizing symbionts (Symb/Light), 80 genes were upregulated (36 annotated) and 45 genes were downregulated (12 annotated) relative to photosymbiont-infected larvae in which the algal cells were not photosynthesizing. Thus, the largest number of differentially expressed genes was associated with photosymbiont infection and there was less of a response due to light environment or photosynthetically active photosymbionts. To determine whether photosymbiont infection was beneficial, neutral or harmful in larvae, we focused our analysis of gene expression on genes associated with three processes: cellular turnover (e.g., apoptosis), metamorphosis and central metabolism.

3.3 | Differential gene expression in response to photosymbiont infection

Photosymbiont infection resulted in gene expression changes consistent with the initiation of apoptosis and oxidative stress pathways, both of which may be activated during an immune response to an infecting agent, the preparation of larvae for metamorphosis and as a response to stress (Supporting Information Table S1). Six genes associated with oxidative stress were upregulated in photosymbiont-infected larvae: three genes for glutathione transferase activity (two genes associated with Glutathione S-transferase 5 and one associated with Glutathione transferase omega-1, Supporting Information Table S1) and three genes associated with the removal of oxygen radicals (peroxidasin-like protein; peroxiredoxin-6; and thioredoxin reductase 1, cytoplasmic). Furthermore, three genes associated with ubiquitin conjugation were upregulated (ubiquitin carboxyl-terminal hydrolase 5; ubiquitin conjugation factor E4 B; and ubiquitin carboxyl-terminal hydrolase 32) and two genes were downregulated (ubiquitin-conjugating enzyme E2 A and probable ubiquitin carboxyl-terminal hydrolase FAF-X).

Photosymbiont infection also led to the upregulation of genes promoting and opposing apoptosis, suggesting more active regulation of apoptosis pathways. The Bcl genes, which promote (Bax) or halt apoptosis (Bcl-2 and Bcl-X) via mediation of caspase pathways, were tightly regulated in photosymbiont-infected larvae but not in photosymbiont-free larvae, suggesting that apoptotic processes were active in infected larvae. Across all 10 Symb replicates, there was a positive correlation between the expression of Bax and the expression of a Bax inhibitor (transmembrane Bax inhibitor motif-containing protein 4; $R^2 = 0.76$, $p < 0.001$) and thus a positive correlation between the expression of genes with opposing protein functions. Additionally, the expression of Bax correlated negatively with expression of two anti-apoptotic proteins, Bcl-2 ($R^2 = 0.42$, $p = 0.04$) and Bcl-X proteins ($R^2 = 0.49$, $p = 0.01$). In no instance were similar correlations found among the NoSymb replicates. Lipopolysaccharide-induced tumour necrosis factor-alpha factor homolog was also upregulated in infected larvae; this is a known apoptosis-inducing factor in corals (Quistad et al., 2014). These results suggest that (a) apoptosis was promoted upon photosymbiont infection via the expression of the pro-apoptotic Bax protein and the downregulation of genes for related anti-apoptotic proteins and (b) cells were responding to and attempting to halt Bax-induced apoptosis by upregulating a Bax inhibitor and thus attempting to stave off cell death.

In contrast to its effect on genes related to cell cycling, photosymbiont infection had little effect on the expression of the majority

of core metabolism genes, although ATP synthase subunit beta, which catalyses the production of ATP, and glyoxylate reductase, which can enhance photosynthetic capability in plants, were both upregulated. Aldose reductase, which performs the first step in glucose oxidation, was downregulated in photosymbiont-infected larvae relative to photosymbiont-free larvae. This suggests that photosymbiont-carrying larvae may be using simpler carbon molecules than glucose such as glyoxylate for energy. Downregulation was also seen in 2-acylglycerol O-acyltransferase 2, which is involved in triacylglycerol (TAG) biosynthesis.

3.4 | Differential gene expression in response to symbiont photosynthesis

To examine larval gene expression in response to actively photosynthesizing algal cells, infected larvae were compared between the Symb/Light and Symb/Dark treatments. Compared to photosymbiont-infected larvae in the dark, larvae with photosynthesizing symbionts upregulated genes associated with light stress (Supporting Information Table S1b). Most notably, two genes associated with phospholipase A2 (phospholipase A2 AP-PLA2-I and phospholipase A2) were upregulated, as was calmodulin. These proteins work in concert to initiate the production of arachidonic acid, a precursor to a suite of inflammatory lipids (e.g., platelet-activating factor; PAF) that are found across many taxa and were recently found to occur in corals (PAF and Lyso-PAF; Galtier d'Auriac et al., 2018; Quinn et al., 2016). In addition, phosphatidylethanolamine-binding protein 4, which inhibits tumour necrosis factor-alpha-induced apoptosis, was upregulated in Symb/Light relative to Symb/Dark treatments. Thus, apoptotic processes that have been associated with stress in corals are upregulated when Symbiodiniaceae are photosynthesizing (rather than just present), though this effect could also be a response to the presence of light rather than the activity of the photosymbiont.

3.5 | Differential gene expression in response to light environment

While larvae exhibited a greater response to photosymbiont infection, 28 genes were differentially expressed in response to the light environment (between Light and Dark treatments; Figure 1b, Supporting Information Table S1c). Differential expression included genes associated with ATP synthesis. Specifically, compared to Symb/Dark larvae, Symb/Light larvae exhibited upregulation of two sequences associated with ATP synthase-coupling factor 6 mitochondrial, ATP synthase subunit delta mitochondrial and ATP synthase subunit O (mitochondrial). These expression patterns offer evidence that larvae in Light treatments were using more available energy than those in Dark treatments.

3.6 | Energetic lipids declined in photosymbiont-free larvae

Coral larvae are comprised of more than 50% lipid by weight; this lipid reserve is primarily comprised of WE and TAG, which provide energy and positive buoyancy, and phospholipids (PL), which provide structure (Arai et al., 1993). The abundances of these three lipid classes were quantified to determine the extent to which larvae exhausted parentally provisioned energetic lipids just after symbiont acquisition, as symbiont-derived energy could subsidize the exhaustion of these lipids. Compared to levels at the start of the experiment, TAG content was significantly lower in NoSymb/Dark larvae at the end of the 48-hr experiment ($t_4 = -3.64$; $p = 0.02$), whereas there were no significant changes through time in the other three treatments (NoSymb/Light $p = 0.62$, Symb/Light $p = 0.25$, Symb/Dark $p = 0.50$; Figure 2a). Across the same time period, WE content declined in the NoSymb/Light ($t_4 = -4.31$; $p = 0.01$; Figure 2b) and NoSymb/Dark treatments ($t_4 = -4.04$; $p = 0.02$), but did not change in either Symb/Light ($t_4 = -1.34$; $p = 0.25$) or Symb/Dark treatments ($t_4 = -0.97$; $p = 0.39$). Taken together, these results show that these lipid classes (which are used as energy reserves) declined in symbiont-free, but not in symbiont-infected, larvae during the 48-hr experiment regardless of whether the algal cells were photosynthesizing.

When the magnitude of change in lipid content among treatment groups was compared, photosymbiont-free larvae had lower TAG content regardless of light environment ($F_1 = 6.7$; $p = 0.02$). In contrast, there was no difference in the amount of change in WE when compared among the four treatments. There was also no difference in the amount of change in PL among treatments (Figure 2c), nor was there a change in PL through time in any treatment.

3.7 | Experiment 2: Larval behaviour in response to photosymbiont infection and light environment

After 10 days of exposure, larvae infected with local photosymbionts contained 172.9 ± 36.2 algal cells per individual ($N = 10$; mean ±SE). After one week (d26), survival was highest for larvae in NoSymb/Light (92% surviving; Figure 3), lower in NoSymb/Dark (85%) and lowest in Symb/Light and Symb/Dark larvae (76%; $p < 0.05$). Overall, the survival of photosymbiont-infected larvae (Symb/Light and Symb/Dark) was lower than the survival of photosymbiont-free larvae (NoSymb/Light and NoSymb/Dark; three-parameter model, $p < 0.05$ relative to the next-best model). In contrast, Light and Dark larvae exhibited no difference in survival ($p > 0.05$, two-parameter model), indicating that infection reduced larval survival regardless of light regime. Across the duration of the experiment, a greater percentage of photosymbiont-free larvae were actively swimming (50%) relative to photosymbiont-infected larvae (39%; Figure 3b; $F_{1,16} = 6.38$; $p < 0.05$). On d26, settlement of larvae in the NoSymb/Light treatment was significantly lower than in the other three treatments (10% vs. 19%, 19% and 20% settled in NoSymb/Dark, Symb/Dark and Symb/Light, respectively; Figure 3c; two-parameter model).

3.8 | Experiment 3: Larval behaviour in response to infection of B. minutum and S. microadriaticum.

Larvae infected with *B. minutum*, larvae infected with *S. microadriaticum* and photosymbiont-free larvae had similar decreases in survival

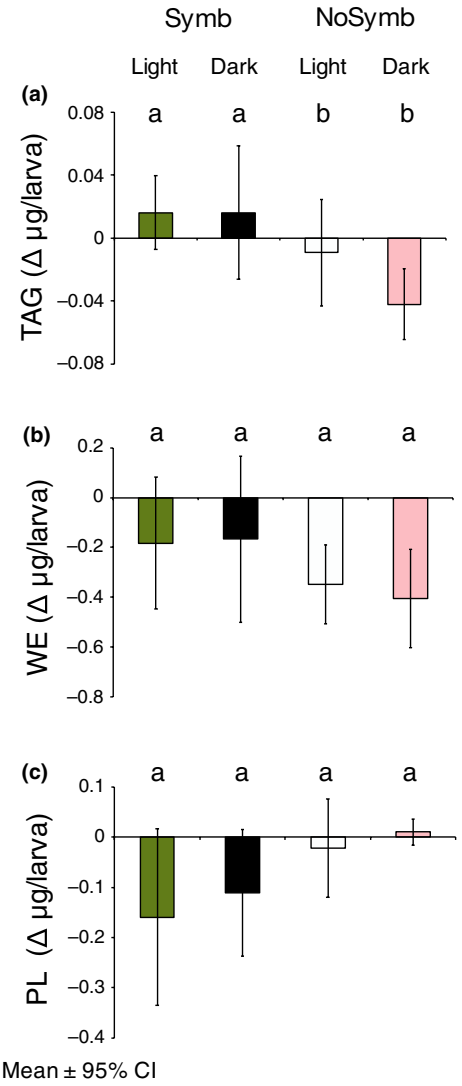

FIGURE 2 Changes in *Orbicella faveolata* larval lipid content over a 48-hr period among the four treatments depicted in Figure 1. Lipid content is reported as µg lipid/larva and shown for the energetic lipids (a) triacylglycerol (TAG) and (b) wax esters (WE), as well as (c) the structural phospholipids (PL). Values represent means with 95% confidence intervals. A two-factor ANOVA was used to assess significant differences in the change in lipid content between treatment groups. The results of this test were reported using lower-case letters: Treatment groups with the same letter were statistically similar, and treatment groups with different letters were statistically distinct ($\alpha = 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

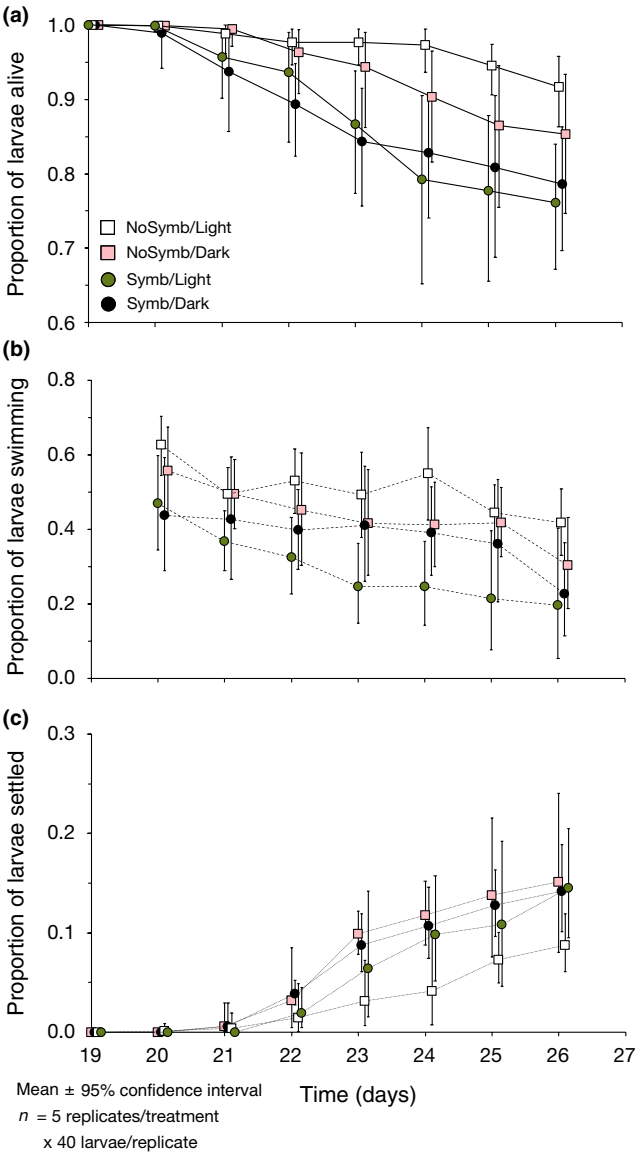

FIGURE 3 Survival, swimming activity and settlement of *Orbicella faveolata* larvae. Larvae were either infected with Symbiodiniaceae photosymbionts isolated from local conspecifics (light green and dark green circles) or not exposed to photosymbionts (white and pink squares). Reported are the proportion of *O. faveolata* larvae (a) alive, (b) swimming and (c) settled (c) while exposed to a 12:12-hr light:dark cycle (Light) or constant darkness (Dark). Data were collected on seven consecutive days, 19–26 days after spawning ($N = 5$ replicates/treatment combination). Arcsine square root-transformed means and 95% confidence intervals are shown in order to constrain the proportions between 0 and 1 [Colour figure can be viewed at wileyonlinelibrary.com]

through time ($p > 0.05$; Figure 4). Therefore, unlike in Experiment 2, neither the presence or absence of symbionts nor the species of Symbiodiniaceae had an effect on survival of larvae in this experiment.

Differences in swimming activity among treatments were more evident than differences in survival. On d10, the proportion of larvae swimming differed among the three treatments (three-parameter model; $p < 0.05$; Figure 4): 82% of larvae infected with *S. microadriaticum* were swimming, 59% of larvae infected with *B. minutum* were swimming, and 41% of NoSymb larvae were swimming. On d16, swimming activity was also different among treatments ($p < 0.05$), but the relative order changed, as larvae infected with *B. minutum* were the most active (74%), NoSymb larvae were intermediate (58%), and larvae infected with *S. microadriaticum* were the least active swimmers (20%). Similarly, on d33, larvae infected

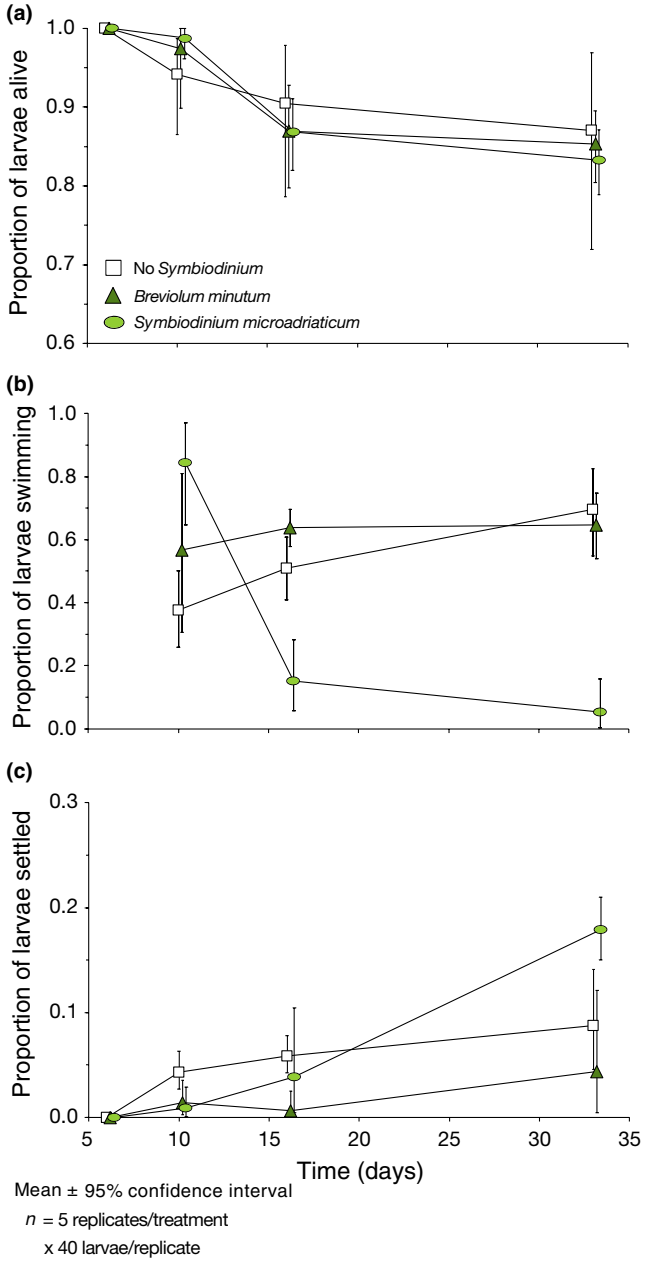

FIGURE 4 Survival, swimming activity and settlement of *Orbicella faveolata* larvae exposed to one of two Symbiodiniaceae species or to no Symbiodiniaceae (white squares). The two Symbiodiniaceae species were cultured strains of *Breviolum minutum* (dark green triangles) and *Symbiodinium microadriaticum* (light green ovals). Reported are the proportion of *O. faveolata* larvae (a) alive, (b) swimming and (c) settled on days 10, 16 and 33 after spawning, relative to the initial starting population (N = 5 replicates/treatment group). The larval cohort used here was the same one used in Experiment 1 (Figures 1 & 2). Arcsine square root-transformed means and 95% confidence intervals are shown in order to constrain the proportions between 0 and 1 [Colour figure can be viewed at wileyonlinelibrary.com]

with *S. microadriaticum* had lower swimming activity (10%) than larvae in the other two treatments (at least 78%; two-parameter model; $p < 0.0001$). In sum, as the experiment progressed, larvae infected with *S. microadriaticum* either settled or stopped moving, while photosymbiont-free larvae and larvae infected with the competent photosymbiont *B. minutum* continued to swim.

Some small but significant differences in the number of settled individuals were observed among treatments, with the greatest difference on d33 when settlement of *S. microadriaticum*-infected larvae was higher (18%) than in the other two treatments (4% and 9% for *B. minutum* and NoSymb, respectively; two-parameter model; $p < 0.01$).

4 | DISCUSSION

The mutualism between adult corals and their Symbiodiniaceae photosymbionts is characterized by algal carbon fixation and translocation to the host, providing the coral its primary source of energy. During the larval stage, corals instead depend primarily on parentally provisioned "energetic" lipids (WE and TAG; Arai et al., 1993, Harii et al., 2007). In some species, parents pass photosymbionts directly to their eggs and larvae (VT), which can provide an additional energy source that benefits the larval host (Alamaru et al., 2009; Gaither & Rowan, 2010; Harii et al., 2007, 2010; Richmond, 1987). In a coral species that does not pass photosymbionts to its offspring (HA), we found that following acquisition, symbiont photosynthesis did not offset host use of parentally provisioned lipids, nor did it lead to substantial changes in the expression of metabolic genes. These results suggest that larvae did not gain energy from symbiont photosynthesis as in the mutualism in adults and larvae of some VT species. After acquiring photosymbionts isolated from local adults or a species cultured in the laboratory, larvae exhibit behaviours that may reduce the likelihood they successfully recruit to the benthos. In response to another laboratory-cultured species, however, larval behaviours were similar to that of larvae without symbionts. Based on these findings, we conclude that symbiont acquisition during the larval stage has either negative or neutral consequences for larvae, suggesting that the timing of symbiont acquisition is a critical component of the host's life history.

4.1 | Symbiont photosynthesis does not offset the drawdown of parentally provisioned lipids

Energetic lipid use was similar in photosymbiont-infected larvae exposed to a light:dark cycle and those exposed to complete darkness, in which photosynthesis by was prevented. Total carbon content also did not differ between photosymbiont-infected larvae in Light and Dark. Therefore, while photosymbiont-infected larvae consumed less energetic lipid than photosymbiont-free conspecifics, this could not be the result of an energetic subsidy gained in the form of fixed carbon from their photosymbionts. Instead, differences in swimming behaviour between photosymbiont-infected and photosymbiont-free larvae may have contributed to differences in lipid use.

The energy required for active swimming in marine invertebrate larvae constitutes a substantial portion of their energy budget

(Bennett & Marshall, 2005; Wendt, 2000) and can come directly from lipid catabolism (Harii et al., 2007; Lucas, Walker, Holland, & Crisp, 1979). Larvae infected with photosymbionts isolated from local adults and a laboratory culture of *Symbiodinium microadriaticum* were less active swimmers than their symbiont-free counterparts, and these larvae may have consumed less energetic lipids as a result. Yet, larvae infected with a laboratory culture of another Symbiodiniaceae species, *B. minutum*, swam as much as photosymbiont-free larvae, suggesting that reduced swimming does not fully explain the lack of lipid use in infected larvae. Furthermore, gene expression patterns did not reflect differential metabolism of lipids following infection. Thus, while our data suggest larvae do not gain an energetic subsidy from acquiring Symbiodiniaceae, why photosymbiont-bearing larvae used less lipid than photosymbiont-free larvae would be an intriguing question for further study.

4.2 | Larvae infected with photosymbionts showed reductions or no change in swimming activity

The ability to swim allows *O. faveolata* larvae to search for and select settlement locations, rather than float passively until they encounter settlement cues (Marhaver et al., 2013; Vermeij et al., 2006, 2013). Thus, when individuals of this species stop swimming without a prompt transition to attachment and metamorphosis, it not only reduces the likelihood that they will encounter settlement cues but often signals an exhaustion of energy available to continue the search. In this species, motionlessness for long periods of time is rare, even in water lacking settlement cues, unless individuals are under stress. Motionless, un-metamorphosed individuals are often observed in larval cultures when larvae have failed to settle after extended periods of time or when larvae are exposed to pathogenic bacterial isolates (KLM & MJAV, personal observations).

Settlement cues were withheld in our experiments to mimic conditions encountered in the water column and simulate continued swimming (Grasso et al., 2011; Morse & Morse, 1991; Morse et al., 1994; Siboni et al., 2012; Tebben et al., 2011). Following the acquisition of photosymbionts isolated from local adults, the number of larvae that were swimming decreased by 23% (Experiment 2). In other words, they became motionless without promptly attaching and completing metamorphosis, indicating an interruption in the typical ontogenetic progression of this species. In response to exposure to two laboratory-cultured Symbiodiniaceae species, larvae were as likely to be swimming as their photosymbiont-free counterparts or reduced their swimming activity, as in Experiment 2. If acquiring photosymbionts was beneficial for larvae, we expected that more larvae would swim and perhaps would extend the larval period. The latter could occur if gaining an internal energy source from their photosymbionts allowed individuals additional time to search for suitable settlement substrate, as has been shown for VT coral species (Chamberland et al., 2017; Richmond, 1987). Instead, the opposite was observed in response to local symbionts and a laboratory-cultured species, potentially reducing rather than increasing the likelihood that larvae settle in an advantageous location.

Despite the lack of cues, settlement and metamorphosis were observed in our behavioural experiments. In Experiment 2, photosymbiont-free larvae exposed to light had lower settlement than photosymbiont-bearing larvae (both Light and Dark) and photosymbiont-free larvae kept in constant darkness (Dark). In Experiment 3, larvae infected with *S. microadriaticum* had higher settlement than photosymbiont-free larvae and larvae infected with *B. minutum* (Experiment 3). Thus, we found no evidence that photosymbiont acquisition led to delayed settlement. Instead, larvae that acquired photosymbionts were more or equally likely to settle as photosymbiont-free larvae.

4.3 | Photosymbiont acquisition led to the upregulation of apoptotic and oxidative stress pathways

Apoptosis and oxidative stress were upregulated in response to photosymbiont acquisition in the planktonic coral larvae studied here. Oxidative stress is commonly observed in coral larvae as a response to environmental stress. For example, high light and temperature induce oxidative stress in nonsymbiotic coral larvae (Aranda et al., 2011; Meyer, Aglyamova, & Matz, 2011; Polato et al., 2010; Voolstra, Schnetzer, et al., 2009), and the presence of photosymbionts exacerbates this response and leads to higher mortality (Yakovleva et al., 2009). Oxidative stress does not singularly indicate environmental stress, though, as it appears to play a role in the photosymbiont acquisition process and metamorphosis (Mohamed et al., 2016; Reyes-Bermudez et al., 2009). Consistent with the latter work, oxidative stress was upregulated here in response to photosymbiont acquisition regardless of the light environment (i.e., larvae in the Symb/Light treatment could have experienced oxidative stress but did not appear to). Interestingly, infected larvae in the light treatment upregulated phospholipase A2, which was recently found to initiate apoptotic cascades in response to light stress in corals (d'Auriac et al., 2018). Therefore, oxidative stress may be characteristic of the infection process and metamorphosis, while other apoptotic processes are activated in response to light stress in photosymbiont-bearing larvae.

Upregulation of apoptosis pathways in larval corals has previously been associated with suppressed immune responses to photosymbiont infection (Dunn & Weis, 2009; Mohamed et al., 2016; Voolstra, Schwarz, et al., 2009), preparing to metamorphose (Grasso et al., 2008, Grasso et al., 2011; Reyes-Bermudez et al., 2009) and responding to environmental stress (Olsen, Ritson-Williams, Ochrietor, Paul, & Ross, 2013; Polato et al., 2010; Voolstra, Schnetzer, et al., 2009). In contrast to the larval response to acquisition, newly metamorphosed polyps do not show upregulation of apoptotic pathways after they take up Symbiodiniaceae (Yuyama et al., 2012) suggesting it may be a uniquely larval response to infection. Given that apoptosis can arise due to multiple processes characteristic of the larval stage, the additional physiological and behavioural metrics studied here can aid in understanding the ramifications of elevated apoptosis in larvae. Previous work shows that photosymbiont acquisition at the time of settlement increases postsettlement survival (Suzuki et

al., 2013). In contrast, we found that acquisition by planktonic larvae decreases survival when they acquire photosymbionts isolated from local adults and has no effect on survival when they acquire two laboratory-cultured photosymbiont species, providing further evidence that the response to photosymbiont acquisition differs across life stages and that, depending on the source of symbionts, acquisition during the larval stage can be harmful.

Pro- and anti-apoptosis-associated genes were activated simultaneously and indicate heightened regulation of apoptosis following infection. The primary apoptotic pathways upregulated in infected larvae include the Bcl-2 gene family and ubiquitin proteases. The activation of pro- and anti-apoptotic Bcl genes suggests competitive pathways of cell death or survival, processes previously associated with photosymbiont acquisition (Mohamed et al., 2016) and metamorphosis (Grasso et al., 2011) in larval corals. Ubiquitin proteases mark and denature damaged proteins and have been associated with cellular, tissue and even whole organism death in other marine invertebrates (reviewed in Mykles, 1998). While seemingly harmful, the ubiquitin–protease pathway can have both pro- and anti-apoptotic roles, depending on the proteins it targets (Jesenberger & Jentsch, 2002). Cell death can be avoided by the ubiquitination of caspases (which carry out apoptosis), as well as mediators of caspases associated with the Bcl family of proteins (Jesenberger & Jentsch, 2002). Thus, upregulation of the ubiquitin–protease pathway during stress may signal increased apoptosis or, conversely, may stave it off.

While apoptosis is critical to basic physiological processes such as immune responses and metamorphosis, rampant and uncontrolled apoptosis is a feature of symbiotic failure in corals (i.e., bleaching) that can lead to death of the host (Ainsworth et al., 2011; Kvitt, Rosenfeld, Zandbank, & Tchernov, 2011; Tchernov et al., 2011). Significant decreases in survival of photosymbiont-infected larvae in Experiment 2 may have arisen due to the inability of some individuals to regulate apoptotic processes after photosymbiont acquisition. Furthermore, larvae bearing photosymbionts exhibited expression patterns consistent with light stress in the Light treatment (e.g., upregulation of phospholipase A2), perhaps explaining why the greatest discrepancy in survival was between photosymbiont-bearing and photosymbiont-free larvae exposed to light (Figure 3a). Acquisition-induced decreases in survival may highlight a selective mechanism that delays photosymbiont acquisition until the end of the larval period, after which photosymbionts enhance survival (i.e., after settlement and metamorphosis; Suzuki et al., 2013).

4.4 | Photosymbiont acquisition and species: The role of timing and partner

Larvae of HA corals and other cnidarians initially acquire multiple species of Symbiodiniaceae from the surrounding environment (Coffroth, Santos, & Goulet, 2001; Colley & Trench, 1983; Poland & Coffroth, 2017). The incorporation of the photosymbiont into host cells and continued maintenance of the association involves both the symbiont (Hill & Hill, 2012; Sachs, Essenberg, & Turcotte, 2011; Sachs & Wilcox, 2006) and the host (Baird, Bhagooli, Ralph, & Takahashi, 2009; Barott, Venn, Perez, Tambutté, & Tresguerres, 2015; Paxton, Davy, & Weis, 2013), whereby mixed symbiont communities are winnowed to one or a few dominant Symbiodiniaceae species, either through host selection mechanisms or competition among Symbiodiniaceae species (Bay et al., 2011; Coffroth et al., 2001; Dunn & Weis, 2009; Poland & Coffroth, 2017; Rodriguez-Lanetty, Wood-Charlson, Hollingsworth, Krupp, & Weis, 2006). The symbiont winnowing process can take much longer than the initial acquisition period (Mies et al., 2017; Poland & Coffroth, 2017). For example, metamorphosed recruits of a soft coral continue altering their symbiont communities over the course of multiple years, eventually harbouring communities similar to those found in their parents (Poland & Coffroth, 2017). Thus, while mechanisms to reduce noncompatible symbiont species (e.g., caspases; Dunn & Weis, 2009) may be initiated within the first days of life in a coral larva, the process by which compatible symbiont species are established continues beyond the larval period, through host selection or intersymbiont competition, and as host physiology and environmental conditions change through time.

In the first weeks of life, we found that the behaviour of *O. faveolata* larvae depended on the Symbiodiniaceae species to which they were exposed. When infected with a species not known to induce stress when taken up by *O. faveolata* larvae (*B. minutum*; Voolstra, Schwarz, et al., 2009), larvae showed behavioural patterns similar to symbiont-free conspecifics, that is, most individuals continued to swim. Yet, when exposed to a Symbiodiniaceae species known to induce stress (*S. microadriaticum*), the majority of individuals stopped moving as the experiment progressed. Unexpectedly, larvae showed decreased swimming activity and decreased survival following infection with the Symbiodiniaceae expected to be most compatible with them—those isolated from conspecific adults on their natal reef. The two laboratory-cultured species have not been found in adult *O. faveolata* in situ and presumably were not present in the Symbiodiniaceae we isolated from local adults. Evidence that Symbiodiniaceae from local adults were more harmful to larvae than the presumably less-compatible cultured species supports the theory that possessing symbionts from the natal reef is less favourable than sampling novel photosymbiont species from the environment. If future studies confirmed this observation and identified a mechanism that causes local photosymbionts to be particularly deleterious to progeny, it would add further evidence of positive selection for HA.

Our results suggest that taking up poorly compatible Symbiodiniaceae species during the planktonic larval stage can interrupt behaviours that are critical for transitioning into a metamorphosed settler, even despite the activity of winnowing mechanisms meant to remove or reduce the abundance of poor symbiotic partners. While settled polyps are able to slowly foster the most beneficial symbiont communities (Poland & Coffroth, 2017), a swimming larva may face consequences much greater than physiological stress alone, as acquisition may compromise the processes of settlement and metamorphosis. Thus, host selectivity of symbionts may only be beneficial during certain life stages despite being a common feature of HA across marine invertebrates (e.g., squid, Nishiguchi, Ruby, &

McFall-Ngai, 1998, Nyholm & McFall-Ngai, 2004; clams, Belda-Baillie et al., 1999; gastropods, García Ramos & Banaszak, 2014).

4.5 | Broader implications

Our results demonstrate that even in nonstressful environments, possessing symbionts during the planktonic larval stage is not always advantageous in an HA coral species. These results add to existing evidence that individuals can incur a cost when they acquire photosymbionts during the planktonic larval stage (harsh environments, Yakovleva et al., 2009; Nesa et al., 2012; Hartmann et al., 2013; Chamberland et al., 2017; possessing photosymbiont species inappropriate for the settlement location, Schwarz et al., 1999; LaJeunesse et al., 2004; Baird, Guest, et al., 2009). On the other side of metamorphosis, delaying photosymbiont acquisition well past the settlement stage can reduce survival relative to individuals that acquire Symbiodiniaceae at the time of settlement and metamorphosis (Suzuki et al., 2013). Thus, corals with HA strategies for photosymbiont acquisition are expected to be under selection to acquire symbionts neither too early or too late during ontogeny, but rather near to the location and time of settlement and metamorphosis, that is, the right type (for the place) at the right time. This provides a testable theory in which to examine the ecological ramifications of HA. Such a test could be carried out by infecting individuals at each early life stage—embryo, swimming larva, crawling larva, settler, recruit, juvenile— while measuring survival, behaviour, energy use and gene expression at each stage.

The physiological, immune and energetic costs associated with photosymbiont acquisition in coral larvae may very well occur in other marine invertebrate species with horizontal symbiont acquisition and a planktonic larval stage. The incidence of HA in marine, aquatic and subterranean environments and its near absence in aboveground terrestrial systems may be due in part to the fact that symbionts are more easily able to survive in the former environments where they can avoid desiccation (Kikuchi, Hosokawa, & Fukatsu, 2007). Together, the lack of overwhelming benefits and the multiple costs of symbiont acquisition during the pelagic stage suggest that the advantages adults gain from Symbiodiniaceae are not also conferred to larvae, perhaps explaining why HA is surprisingly common in corals.

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DATA ACCESSIBILITY

The raw data used to generate all figures are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.js42579.

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