Dispersal of *Symbiodinium* by the stoplight parrotfish *Sparisoma viride*

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Environmental reservoirs of zooxanthellae are essential for coral larvae settlement; understanding where they occur and how they are maintained is important for coral reef ecology. This study investigated the dispersal of *Symbiodinium* spp. by the stoplight parrotfish *Sparisoma viride*, which had high mean densities of viable and cultivable *Symbiodinium* (3207–8900 cells ml⁻¹) in faeces. Clades A, B and G were detected using amplified chloroplast ribosomal sequences (cp23S-HVR), and corresponded with diet preferences of fish and the environmental *Symbiodinium* diversity of the region. Cells are constantly dispersed in the water column and deposited in the substrate at a local level (86 ± 17.8 m²), demonstrating that parrotfishes are vectors for short-distance dispersal of zooxanthellae. Such dispersal could constitute a key role in the maintenance of environmental *Symbiodinium* reservoirs.

**Keywords:** endozoochorous; zooxanthellae; environmental *Symbiodinium*; *Sparisoma viride*; parrotfishes; cp23 gene

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1. INTRODUCTION

The majority of species of reef-building corals spawn aposymbiotic gametes. The non-symbiotic larvae depend on environmental zooxanthellae (*Symbiodinium* spp.) for their survival as they are dependent on photosynthetically derived nutrition [1]. The type of *Symbiodinium* they acquire influences the resilience of reef-building corals to environmental stresses, as each type differs in terms of its physiological ability to affect coral environmental tolerances [2].

There is great diversity among the types of free-living *Symbiodinium* [3], and the expulsion of viable symbionts from corals [4] and via the faeces of corallivorous fishes and other invertebrates [5] has been hypothesized to be the predominant source of environmental reservoirs of *Symbiodinium*. *Sparisoma viride*, one of the most abundant parrotfish in Caribbean reefs, is a large generalist herbivore [6] that carries viable *Symbiodinium* cells in its faeces [7]. Furthermore, dietary components of this species [8–10] host a great diversity of *Symbiodinium* [7,11,12]. Therefore, parrotfishes transporting and dispersing substantial levels of diverse zooxanthellae into the environment could contribute to maintaining free-living *Symbiodinium* reservoirs that are potentially available for non-symbiotic coral larvae. *Sparisoma viride* was evaluated as a vector for *Symbiodinium* dispersion by quantifying the grazed substrate, the dispersal area, and the genetic diversity and viability of *Symbiodinium*-cell densities extruded in faeces.

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2. MATERIAL AND METHODS

Three regions of the Caribbean Colombian coast were investigated (see the electronic supplementary material). During grazing experiments, adult *S. viride* were tracked until they deposited an intact pellet directly over sediments. The faeces were collected and pellets that could not be separated from the sediment were discarded. Faeces located over other substrates or in the water column were not collected to avoid the inclusion of external *Symbiodinium*. The presence of *Symbiodinium* was investigated in 68 samples that were sieved and examined using compound microscopy. Cell densities from 23 random samples were calculated using a haemocytometer. *Symbiodinium* cells were cultured in 10× liquid 1/2 media prepared with filtered sea water, and enriched with vitamins (thiamine 0.25 μg m⁻³) and antibiotics, penicillin-G (100 μg m⁻³), streptomycin (50 μg m⁻³) and kanamycin (50 μg m⁻³) to prevent contamination with bacteria. Using 50 ml flasks, 2 ml aliquots of each sample were cultured in 25 ml of 1/2 media and maintained in an incubator at 24°C under a 12L:12D cycle, and densities of each culture were estimated once a month.

Grazing and excreting patterns were evaluated by observing specific fish for 5 and 15 min time periods and recording the number of bites, substrate grazed and the number of faeces released, including the substrate type where they were deposited. Substrate cover of reef components was measured using photo-quadrants and bite frequencies were determined using Pearson correlations.

DNA was extracted from all fresh faeces and positive cultures collected during grazing experiments, following the CTAB protocol [13]. The hypervariable region of domain-V in the large subunit of the chloroplast ribosomal array (cp23S-HVR) was amplified using PCR [3]. Amplification products from cultures were sequenced directly, whereas those from fresh faeces were cloned (Promega pGEM-T Easy-Vector; see the electronic supplementary material). BLAST sequences with high match scores (greater than or equal to 90%) to *Symbiodinium* were assembled into alignments for reconstructing maximum-parsimony (MP) and maximum-likelihood (ML) trees.

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3. RESULTS

(a) Grazing patterns and dispersal of *Symbiodinium*

Substrate selection for grazing was determined for 146 adult individuals. They exhibited similar feeding preferences but the proportional use of each substrate type differed among regions (table 1, ANOVA: *F* = 5.25; *p* < 0.05, Levene’s test of variance homogeneity: *p* > 0.05). A positive correlation between substrate availability and grazing preference was identified at Cartagena and Santa Marta, but no significant relationship was evident at Isla Fuerte (Cartagena: *R* = 0.97, *p* < 0.01; Santa Marta: *R* = 0.95, *p* < 0.05 and Isla Fuerte: *R* = 0.19, *p* > 0.05). Fish released faeces over different substrates and the water column within the reef territory (table 2, no variation among regions, *F* = 0.00; *p* > 0.05, Levene’s test of variance homogeneity: *p* > 0.05), covering areas between 13.5 and 191.6 m² (see the electronic supplementary material).

(b) *Symbiodinium* cells in *Sparisoma viride* faeces

The presence of *Symbiodinium* cells was confirmed in 63 of 68 samples (93%), with highly variable densities
among individuals (from 73 to 38.3 × 10³ cells ml⁻¹). Mean densities were 3207 ± 1327 cells ml⁻¹ at Isla Fuerte, 5204 ± 2232 cells ml⁻¹ at Cartagena and 8900 ± 4568 cells ml⁻¹ at Santa Marta, but these differences were not significant (ANOVA: \( F = 0.51; p > 0.05 \), Levene’s test: \( p > 0.05 \); see the electronic supplementary material). There was a steady density increase (from 81.93 ± 11.87 cells ml⁻¹ to 119 ± 15.5 × 10³ cells ml⁻¹) of Symbiodinium in cultures (figure 1). Cells at different reproductive stages (coccoid, motile and mitotic) were identified, demonstrating that they were viable in terms of asexual reproduction.

Successful amplifications were obtained for uncultured (17) and cultured (five) samples. However, because of sequencing limitations, only 39 sequences were recovered (see the electronic supplementary material). Phylogenetic analyses grouped the strains identified in faeces with sequences of endosymbiotic strains from different Caribbean hosts and free-living strains. Clade A was the most prevalent type in uncultured and cultured samples, and included divergent
Figure 2. Phylogenetic tree (MP) of *Symbiodinium* cp23S-rDNA. Sequences in bold lettering correspond to direct sequenced cultures, fresh samples (uncultured3) and environmental samples (water sample SM). Clones are cloning products from positive amplification of fresh faeces. Published sequences from different hosts and environmental samples are in grey lettering with accession number from GenBank. Values above lines represent 1000 replicates bootstrap support (MP/ML). MP Phylogenetic trees were obtained (TBR) in PAUP. ML was obtained with the GTR + G model in RAxML, both analyses with gaps as missing.
sequences. Clade G, which is related to symbionts of clionaid sponges, was the second most prevalent clade in uncultured samples. Clade G was not, however, detected in cultures. Clade B was closely related to strain B184, normally present in gorgonians and some scleractinian corals (figure 2 and table 1).

4. DISCUSSION
This study presents compelling evidence for the role of parrotfishes as endozoochorous vectors for zooxanthellae in coral reefs. There are notable densities of viable and diverse zooxanthellae in parrotfish faeces that are dispersed in the reef, contributing to the maintenance of significant levels of diverse environmental Symbiodinium populations.

Sparisoma viride carries at least three clades, A, B and G, in its gut, and this corresponds to its diet preferences and the zooxanthella diversity present in the environment. Successful cultures suggested that Symbiodinium cells from faeces were alive and viable after passing through the digestive tract of the fishes. The regular presence of divergent strains of Symbiodinium clade A in cultures and fresh faeces explains why they are frequently detected in benthic environmental samples [16–18]. The presence of clade G in faeces was surprising, as it has only been identified as an endosymbiont of two clionaid sponges [11] and has not been detected in the environment. Clade G was not detected in cultures, probably because of technical difficulties relating to culturing various strains under the same conditions [19]. The limited detection of clade B, and the absence of clade C in all samples were unpredicted, as each is very common in this clade B, and the absence of clade C in all samples [16]. The limited detection of clade B, and the absence of clade C in all samples were unpredicted, but as such, is very common in this region. The potential for these zooxanthellae to establish symbiosis with asymbiotic coral larvae requires confirmation.

Mean densities (3207 ± 1327 to 8900 ± 4568 cells ml⁻¹) in parrotfish faeces correlated with those identified in sediments (1000–4000 cells ml⁻¹) [20], but greater concentrations (up to 38.3 × 10⁶ Symbiodinium cells ml⁻¹) were identified in individual faeces samples, suggesting that fishes carry significantly higher levels of Symbiodinium and that the continuous deposition of faeces on the substrate constitutes an important source of free-living cells [17].

Sparisoma viride carrying viable Symbiodinium move from the original site of ingestion. Therefore, faeces are constantly ejected into new sites, demonstrating viable cell transport at a local level. The maximum distance between faeces (35 m) correlates with the maximum movement distance of S. viride estimated in Barbados [21]. Therefore, the mean area of Symbiodinium dispersion is comparable with the territory size for this species. This supports the reef endemism of Symbiodinium populations [14,22] and suggests that the mechanism of dispersal by fishes contributes to the short-distance movement of Symbiodinium.

Spearfishing has resulted in reduced population sizes of Sparisoma viride at Isla Fuerte, where the lowest Symbiodinium counts were detected. Moreover, densities of environmental Symbiodinium vary among reef zones and are greatest at depths where parrotfishes are usually more abundant [6,10,20]. The findings of this study serve to demonstrate the ecological potential of parrotfish involvement in the dynamics of environmental reservoirs of Symbiodinium.

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Fitt, W. K. 2010 Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. *J. Biogeogr.* 37, 785–800. (doi:10.1111/j.1365-2699.2010.02273.x)


