Received Date : 11-Mar-2016
Revised Date : 23-Aug-2016
Accepted Date : 23-Aug-2016
Article type : Regular Article

The effects of *Symbiodinium* (Pyrrhophyta) identity on growth, survivorship, and thermal tolerance of newly settled coral recruits[1]

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Editorial Responsibility: S. Lin (Associate Editor)

Abstract

For many coral species, the obligate association with phylogenetically diverse algal endosymbiont species is dynamic in time and space. Here, we used controlled laboratory inoculations of newly settled, aposymbiotic corals (*Orbicella faveolata*) with two cultured species of algal symbiont (*Symbiodinium microadriaticum* and *S. minutum*) to examine the role of symbiont identity on growth, survivorship, and thermal tolerance of the coral holobiont. We evaluated these data in the context of *Symbiodinium* photophysiology for nine months post-settlement and also during a 5-day period of elevated temperatures. Our data show that recruits that were inoculated with *S. minutum* grew significantly slower than those inoculated with *S. microadriaticum* (occasionally co-occurring with *S. minutum*), but that there was no difference in survivorship of *O. faveolata* polyps infected with *Symbiodinium*. However, photophysiological metrics, such as effective quantum yield (ΔFv/F'm, the efficiency with which available light is used to drive photosynthesis and α, the maximum light utilization coefficient) were higher in those slower-growing recruits containing *S. minutum*. These findings suggest that light use (i.e., photophysiology) and carbon acquisition by the coral host (i.e., host growth) are decoupled, but did not distinguish the source of this difference. Neither *Symbiodinium* treatment demonstrated a significant negative effect of a five-day exposure to temperatures as high as 32° C under low light conditions similar to those measured at settlement habitats.

Keywords: symbiosis, stress, recruits, *Orbicella faveolata,* photophysiology

PAM - Pulse Amplitude Modulated

RLC - Rapid Light Curves

rETR - relative Electron Transport Rate

ΔFv/F'm - effective photochemical efficiency

α - maximum light utilization coefficient

Fv/Fm- maximum photochemical efficiency

ITS – internal transcribed spacer

cp –chloroplast

KML – Keys Marine Laboratory

FSW – filtered seawater

EtOH – Ethanol

CTAB - Cetyl trimethyl ammonium bromide

cp23S - chloroplast large subunit

PSII – photosystem II

rmANOVA – repeated measures analysis of variance

SA – surface area

Introduction

The symbiosis between scleractinian corals and their algal endosymbionts is a two-way exchange of resources that has allowed coral reefs to dominate in high-light, oligotrophic tropical oceans. With photosynthetically-derived energy, algal symbionts of the genus *Symbiodinium* provide their host corals with both essential and energy-rich metabolites that drive growth, calcification and survival of reef building corals. The light-rich host tissue environment promotes photosynthesis in *Symbiodinium*, which also receive recycled inorganic nutrients from their host (reviewed in Sheppard et al. 2009).

The genetically diverse genus *Symbiodinium* comprises at least nine deeply divergent "clades" (Pochon et al. 2014) within and among which there is a high level of species, physiological, and ecological diversity (reviewed in Baker 2003), including taxa that are host generalists and specialists, as well as free living strains (Pochon et al. 2010, Thornhill et al. 2014). Much research has focused on how this diversity relates to holobiont (coral and associated symbionts) fitness in the field (e.g., Glynn et al. 2001, Little et al. 2004, Baker et al. 2004, Warner et al. 2006, Abrego et al. 2008, Putnam et al. 2012, Kemp et al. 2014, Edmunds et al. 2014), particularly thermal tolerance. Horizontal transmission of symbionts, in which aposymbiotic larvae or newly settled polyps take up a diverse set of *Symbiodinium* strains from the environment de novo each generation, is common in scleractinian corals (Little et al. 2004, Coffroth et al. 2006, Baird et al. 2009, Cumbo et al. 2013, Poland et al. 2013). However, only a small portion of the available research has focused on coral juveniles in the first year(s) post settlement, the time period

during which the symbiosis is first established for horizontally transmitting corals and can be highly flexible (Coffroth et al 2001, Little et al. 2004, Abrego et al. 2009, Poland et al. 2013). While both host-mediated (Voolstra et al. 2009, Dunn and Weis 2009, Poland 2010) and environmentally-mediated (Abrego et al. 2012) changes in the dominant symbiont type occur as corals develop into adults, it is common that representatives from 2-4 *Symbiodinium* clades remain present within adult scleractinian host tissues at various levels of abundance (Finney et al. 2010, Silverstein et al. 2012), though usually with a particular symbiont phylotype (Rowan et al. 1997, Glynn et al. 2001, Goulet 2006, Kemp et al. 2008) or even isoclonal algal strain (Goulet and Coffroth 2003, Baums et al. 2014) dominating within the host or within a section of host tissue. In adult colonies, changes in the dominant symbiont type can enhance stress tolerance for the coral host (Buddemeier and Fautin 1993, Cunning et al. 2015, Silverstein et al. 2015).

Interestingly, changes in symbiont community structure unrelated to environmental conditions occur during coral ontogeny, suggesting essential, yet understudied, differences in the symbiosis of juveniles versus adult coral-*Symbiodinium* interactions that could include metabolic differences and/or immature endosymbiont recognition capacity. Juveniles of the octocoral *Briareum asbestinum* are dominated by one clade B *Symbiodinium* phylotype during the first 12-18 months but then transition to a different clade B *Symbiodinium* phylotype (Poland 2010) that is the dominant symbiont found in almost all adult *Briareum* sampled from several parts of the Caribbean and Bermuda (Poland 2010). In the Pacific coral *Acropora tenuis,* symbiont populations also switch from a dominant juvenile symbiont type to the adult homologous association after a period of 3.5 years (Abrego et al. 2009).

Although much research has focused on the ecological significance of symbiont identity in adult colonies within established reefs (reviewed by Baker 2003, van Oppen et al. 2009), information on how *Symbiodinium* strains affect the ecology of newly settled and juvenile corals is limited. Indo-Pacific *Acropora* have been the focus of the majority of these studies, and that research has revealed that variation among juvenile host-symbiont pairings leads to important differences in polyp budding rates (Little et al. 2004), host carbon acquisition (Cantin et al. 2009), skeletal deposition (Jones and Berkelmans 2010, Yuyama and Higuchi 2014), energetics (Jones and Berkelmans 2011),

and thermal stress responses (Abrego et al. 2008, Howells et al. 2012, Yuyama and Higuchi 2014). Importantly, associations of the same *Symbiodinium* species can have differing effects on juvenile vs. adult hosts. For example, adult *Acropora millepora* hosting *Symbiodinium* D1a are less sensitive to thermally induced bleaching compared to those hosting C2 (Berkelmans and Van Oppen 2006) while the opposite is true for juveniles of the closely related *Acropora tenuis* hosting *Symbiodinium* D1 or C2 (Abrego et al. 2008). In the Caribbean, the coral juvenile life stage has been shown to be an important factor in structuring reef communities (Miller et al. 2000) with growth within the first one to two years post-settlement having a critical influence on survival (Rylaarsdam 1983, Edmunds and Gates 2004). This emphasizes the importance of understanding the ecology of newly settled and juvenile corals especially for dominant Caribbean reef builders including *Orbicella* spp.

To better understand the mechanisms underpinning physiological differences among coral individuals hosting different *Symbiodinium* species, analyses of photophysiology have become common for both cultured and *in-hospite Symbiodinium*. Most common is the use of Pulse Amplitude Modulated (PAM) fluorometry to measure chlorophyll fluorescence and infer pathways of light-energy flow through the photosynthetic machinery. Various components of photophysiology, particularly the rate and efficiency with which light energy is used in photochemistry, can be assessed through PAM fluorometry, but, due to the complexities of photosynthesis (i.e., the conversion of that light energy into fixed carbon), studies that employ PAM fluorometry exclusively are limited in their ability to predict the quality of a symbiont with regard to the algae's contribution of carbon to their host. Examining emergent properties of particular host-symbiont associations (e.g., photophysiology, growth, survivorship, and thermal tolerance) in tandem with individual symbiont performance parameters is necessary to evaluate the quality and potential stability of different coral-algal associations (Cantin et al. 2009).

The star coral, *Orbicella faveolata* (previously *Montastraea*: Budd et al. 2012), a major constituent of Caribbean reefs, forms symbioses in the field and laboratory with an exceptionally diverse suite of *Symbiodinium* taxa, including representatives from at least four different *Symbiodinium* clades (Rowan and Knowlton 1995, Toller et al. 2001,

Kemp et al. 2008, Finney et al. 2010, Kemp et al. 2015). Yet little is known about how this diversity affects newly settled recruits and juveniles. For this experiment we used laboratory inoculations to focus on the specific influence of two different *Symbiodinium* species (*S. microadriaticum* and *S. minutum*) on host growth and survivorship during the first nine months following settlement. To understand the contribution of *Symbiodinium* photophysiology to the host parameters measured, we employed PAM fluorometry, using Rapid Light Curves (RLCs) to quantify effective photochemical efficiency (ΔFv/F'm), relative Electron Transport Rate through PSII (rETR), and the maximum light utilization coefficient (α). Furthermore, we compared the thermal bleaching response of coral hosts harboring different *Symbiodinium* and evaluated the maximum photochemical efficiency (Fv/Fm) of *Symbiodinium*, a measure particularly sensitive to thermally induced loss of photosynthetic function (reviewed in Warner et al. 2010).

Materials and Methods

Symbiodinium *Source*

Most of the *Symbiodinium* naturally found in adult *O. faveolata* colonies, including the symbionts which commonly dominate, are exceedingly difficult to culture (LaJeunesse 2001, Santos et al. 2001, Krueger and Gates 2012). As the dominant *in hospite Symbiodinium* species are currently not in culture, we used two well-studied, easily-cultured species of *Symbiodinium* that are known to form viable symbioses with juvenile *O. faveolata* (Coffroth unpublished): 1) *S. microadriaticum,* Freudenthal, a clade A species (Culture ID: CassKB8; cp-23S rDNA type A194; ITS type A1; Genbank: AY035405.1), which was originally isolated from *Cassiopea* sp. but has not been reported in *O. faveolata* adults, and 2) *S. minutum*, a clade B species (Culture ID: Mf1.05b; cp-23S rDNA type B184; ITS type B1; Genbank: JX213588.1), which was isolated from tissues of *O. faveolata*. *S. minutum* readily establishes a symbiosis with *O. faveolata* recruits (Voolstra et al. 2009,) although it has never been found as a dominant *in-hospite* species within *O. faveolata* adults (LaJeunesse et al. 2012). All *Symbiodinium* cultures used in this study originated from highly concentrated, isoclonal reference cultures maintained at the University at Buffalo – SUNY (BURR Culture Collection: http://www.nsm.buffalo.edu/Bio /burr/). The cultures used in the experiments were

maintained at the Keys Marine Lab (Florida) in f/2 medium (Guillard 1975), at ~27° C, under a 14:10 h light:dark regime.

Collection of Gametes, Rearing of Coral Larvae, and Experimental Set-up

Gametes of *O. faveolata* were collected during the mass spawning event of 19-20 August 2011 from multiple colonies at two reef locations in the Middle and Lower Keys, Alligator Reef (24° 48.771' N, 80° 40.167' W) and Looe Key Reef (24° 32.693' N, 81° 24.562' W), respectively. All eggs and sperm from a single site were mixed immediately after collection for fertilization following protocols of Miller and Szmant (2006), and transferred to Keys Marine Laboratory (KML), Long Key, FL. At KML, gametes and later embryos from all sites were mixed and maintained in filtered seawater (FSW, 1.0 μm) from the time of collection and thereafter in order to prevent exposure to exogenous sources of *Symbiodinium* spp. The resulting aposymbiotic larvae were placed into a series of six plastic tubs with 18-20 L filtered seawater (1.0 μm) at a density of approximately 3,000 larvae · L^{-1}. Thirty ceramic tiles (5 X 5 cm) that had been preconditioned for 10 d in filtered seawater were provided as settlement substrata.

For larval rearing, closed circulation tanks were maintained in a shaded outdoor laboratory with full seawater changes 1-3 times · d^{-1}. Beginning five days after collection and fertilization, when larvae began to settle and metamorphose, the frequency of water changes decrease to every 2-3 d, with each water change followed by inoculation with either *S. microadriaticum* or *S. minutum* cultures at 500 cells · mL^{-1}. A control tank containing randomly selected tiles with recruits were not inoculated and thus those recruits remained aposymbiotic.

Experimental Set-up

When visible infection (i.e., golden coloration within polyp tissues) was confirmed with 10X light microscopy (~2 weeks after settlement and 10 d following the start of inoculations) the tiles were transferred to the University of Miami Experimental Hatchery (Virginia Key, Miami, FL) where they were maintained for the following nine months without further exposure to cultured *Symbiodinium*. For each *Symbiodinium* treatment, 10-20 tiles were randomly distributed among four replicate tanks (40-80 tiles · $treatment^{-1}$) with flow-through, 0.2 μm filtered, UV-treated seawater from nearby Bear Cut (Virginia Key Beach Park, FL). Seawater filtration prevented exposure to environmental

sources of *Symbiodinium,* and allowed growth and survivorship to be monitored for specific symbiont-host pairings. Regular maintenance included weekly feeding with Zeigler ™ (Gardners, PA, USA) larval diet (<100 microns in size), regular tank cleaning, and random rotation of treatments among tanks that ranged in light exposure between 5-20 μmol photons · m^{-2} · s^{-1}. Light levels were similar to the low light levels measured in colony crevices and under ledges in the field (14-43 μmol photons · m^{-2} · s^{-1}; see Table S1 in the Supporting Information) the preferred settlement substrate of *O. faveolata* recruits (Szmant and Miller 2006, Miller 2014), and recruits remained visibly infected throughout the experiment. Twenty recruits per month from each tank were sampled to determine *Symbiodinium* identity in each inoculation treatment. The 8-month sampling timepoint was omitted to ensure there were sufficient recruits for the subsequent thermal tolerance experiment, which was conducted on 9-month old recruits.

Symbiont Genotyping

Samples were preserved in 95% ethanol (EtOH) for subsequent DNA extraction following a 2X Cetyl trimethyl ammonium bromide (CTAB) protocol for *Symbiodinium* DNA isolation (Coffroth et al. 1992). This protocol was slightly modified by the addition of a "bead-beating" step in which a 50-100 uL volume of glass beads (size: 425-600 um; Sigma) per tube were added and shaken on high on a Vortex Genie (Scientific Instruments) on the highest setting for 5 min to rupture the symbiont cell wall (Goulet and Coffroth 1997, Yuan et al. 2014). Symbiont species were distinguished via length heteroplasmy in domain V of chloroplast large subunit (cp23S) ribosomal DNA (Santos et al. 2003). The cp-23S gene was amplified with polymerase chain reaction (PCR) and the PCR product was then run on a 6.5% Long Ranger acrylamide gel using LI-COR's Long ReadIR 4200 DNA Sequencer along with size standards for fragment size analysis. PCR for each sample was repeated at least twice for scoring. In rare cases replicates differed in their banding patterns and were repeated a third time. Very faint bands were excluded if they occurred only once in three replicates, otherwise all bands from all replicates were included in the analysis.

Growth and Survivorship

Growth and survivorship of symbiotic *O. faveolata* recruits were recorded with monthly, high-resolution photographs of one designated tile from each tank (n=4 per

treatment) over the course of the nine-month experiment. Each of the selected tiles included 75-130 recruits at the start of the experiment. Recruits were considered as individual settlers beginning as single polyps with limited budding occurring throughout the duration of the experiment. For growth analysis, 20 individual recruits were initially randomly selected from the photographed tiles and the surface area of each recruit 'footprint' was measured by carefully tracing the base of the polyp using Adobe Photoshop™ and then quantifying the area with ImageJ (http://imagej.net/). Bimonthly measures of these same recruits were averaged for each tank replicate (n=4). Assumptions of normality were tested with a Shapiro-Wilk normality test, and the effects of time and symbiont treatment (and their interaction) on the log-transformed growth data were tested with a 2-way repeated measures ANOVA.

Survivorship was calculated as the proportion of recruits remaining at the end of the nine-month growth experiment relative to the number of recruits at the start. These proportional data were arcsine transformed and survivorship among symbiont treatments was compared using a one-way ANOVA. All statistical analyses were run in R (R Core Development Team 2012).

Symbiont Photophysiology

Measures of photophysiology for recruits harboring either *S. microadriaticum* or *S. minutum* were determined using an imaging-PAM (WALZ Mess- und Regeltechnik, Germany). All fluorescence parameters are described and abbreviated according to Cosgrove and Borowitzka (2010) and Enríquez and Borowitzka (2010). For the first six months following settlement (age in months 1-6) analyses were run monthly on one designated tile per tank (n=4 tiles). We measured the effective quantum yield (ΔFv/Fm', the efficiency of photochemistry in the light-adapted state) for six randomly selected recruits from each tile repeated monthly for six months. Data were analyzed using a 2-way ANOVA, with symbiont treatment and time as factors and tile nested within treatment. Additionally, Rapid Light Curve (RLC) analysis was performed on two month old recruits. RLC analysis measured ΔFv/Fm' as a function of increasing irradiance, in this case across eight steps of increasing PAR (0, 11, 21, 36, 56, 81, 111, and 146 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}), each of which was followed by a saturating pulse. RLC analysis examines the way in which algae use light to drive photosynthesis, by converting

ΔFv/Fm' into a measure of the rate of electron transport through the photosynthetic chain (rETR). Using SigmaPlot (Systat Software, San Jose, CA, USA), we fitted a hyperbolic tangent function (Platt et al. 1980) to the PAR vs. rETR data for each of 48 recruits (2 *Symbiodinium* treatments X 4 replicate tanks X 6 polyps), and calculated the maximum light utilization coefficient (α, the slope of the linear phase at subsaturating light). We used a 2-tailed Student's *t*-test to test for a significant effect of *Symbiodinium* treatment on α.

Thermal Tolerance

Tiles with nine-month old juvenile corals were transferred to KML to assess the photosynthetic response of *Symbiodinium* exposed to elevated temperatures *in hospite*. Tiles were selected from each of the symbiont treatments and randomly assigned to one of three temperature treatments: 27° C (ambient, non-stressful), 29° C, and 32° C. The highest temperature treatment of 32° C represents acute thermal stress that is expected to elicit bleaching within days of exposure, while 29° C is expected to elicit bleaching only when experienced over the long term (weeks to months; Manzello et al. 2007). Temperature treatments were carried out in aquaria with 10 L of filtered seawater, a submersible heater, a submersible pump for circulation, and a HOBO™ datalogger (Onset Computer Corporation, Cape Cod, Massachusetts, USA) that collected temperature data every 30 min. Juvenile corals were given 10 days to acclimate to laboratory conditions where light levels were ~20 μmol · photons · m^{-2} · s^{-1}, which was similar to the light levels that those polyps experienced at the UMH hatchery for the preceding 9 months. Temperatures were then raised by 1° C · d^{-1} until experimental temperatures were reached (after 5 d). Once temperatures had been established, pre-dawn (dark-acclimated) maximum quantum yield of PSII (Fv/Fm) was measured daily for 5 d to assess the thermal stress response within each *Symbiodinium* treatment. A diving PAM (WALZ Mess- und Regeltechnik, Germany) was used to collect data for 4-6 recruits of each *Symbiodinium* treatment selected haphazardly within each of three replicate aquaria (n=3). Since we were interested in the photophysiological response of each *Symbiodinium* treatment individually to the temperature treatments, we used two separate 2-way rmANOVAs to compare Fv/Fm readings for each tank averaged across

five days at experimental temperature, with temperature and time as factors in the analysis.

Results

Symbiodinium *Genotyping*

Although *S. microadriaticum* and *S. minutum* have not been detected in *O. faveolata*, the new recruits rapidly acquired these symbionts and maintained the symbiosis for the duration of the study (Fig. 1). The uninoculated controls had rare occurrences of each inoculum (2 with *S. microadriaticum* and 3 with *S. minutum* out of 123 polyps sampled over 7 months; Fig. 1A). Polyps inoculated with *S. microadriaticum* often had *S. minutum* in addition to the inoculum species (Fig. 1B). This occurred more frequently in some months than others. In contrast, polyps that were inoculated with *S. minutum* were found to harbor *S. minutum* almost exclusively throughout the experiment (Fig. 1C). Though our method of chloroplast genotyping does not resolve *Symbiodinium* species present at very low levels, the occurrence of only the *Symbiodinium* cp23S types in contaminated samples from inoculated and control treatments suggests that the source of contamination was likely our laboratory inocula, rather than environmental sources. Unfortunately, the small size of recruits meant that entire individuals were sampled for genotyping. Thus, we could not incorporate genotype information through time to specific physiological metrics of either the coral or symbiont, neither could we consider the mixed state separately. Rather we assume that all recruits within a treatment had a similar frequency of symbiont types as recorded from 20 randomly sampled recruits. The assumptions and limitations of this approach are considered within the Discussion.

Survivorship

The mean proportional survivorship of symbiotic *O. faveolata* recruits over nine months was relatively high (0.93 +.029/-.037 for *S. microadriaticum*; 0.91 +0.027/-0.032 for *S. minutum*; 0.79 +0.112/-0.141 for controls; average +/- 95% Confidence Interval) and there was a significant effect of inoculation treatment on survivorship (ANOVA ($F_{(2,9)}$=4.42, p=0.047). A Tukeys post hoc test indicated that recruits with *S. microadriaticum* had significantly higher survivorship than the controls (p=0.046).

Pairwise comparisons of *S. microadriaticum* vs. *S. minutum* and *S. minutum* vs. controls were not significantly different (p= 0.81 and p=0.12, respectively).

Growth

The small size and clear tissues of aposymbiotic recruits prohibited accurate area measures within the control treatment photographs thus growth was only compared between the two infected treatments. Recruit surface area measurements showed that *O. faveolata* polyp growth over the nine-month experiment was best fit by an exponential growth model (R^2= 0.998 and 0.948 for *S. microadriaticum* and *S. minutum,* respectively; Fig. 2), though this model may not be appropriate for long term growth studies. Consequently, growth data were transformed to the $\log(SA_T/SA_{T-1})$ for each time point (T) relative to the previous time point (T-1). The Shapiro-Wilk test showed no violations of normality for the transformed distribution ($p>0.05$) or within the residuals ($p>0.05$). There was a significant effect of *Symbiodinium* inoculation treatment on the transformed growth rate (2-way rmANOVA; Table 1), with no effect of time (Table 1), and no interaction (Table 1).

Symbiont Photophysiology

Symbiodinium acclimated to low light (20 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) differed significantly in their quantum yield (ΔFv/Fm'). *Symbiodinium* of the *S. minutum* treatment directed a higher proportion of available light to drive photochemistry compared to *Symbiodinium* of the *S. microadriaticum* treatment (ANOVA, Table 2; Fig. 3). This pattern was consistent as time progressed, i.e., there was no effect of time (ANOVA, Table 2; Fig. 3), even when *S. minutum* were occasionally detected in *S. microadriaticum* inoculation treatment polyps. There were no data for polyps at one month old. A separate *t*-test on data calculated from two month old polyp RLCs revealed that α values were significantly higher (p=0.024) in the *S. minutum* treatment (α =0.506 ± 0.02, mean ± SD) relative to the *S. microadriaticum* treatment (α =0.444 ± 0.04, mean ± SD), indicating that *S. minutum* is more efficient at higher irradiance than *Symbiodinium* of the *S. microadriaticum* treatment.

Thermal Tolerance

Temperature data collected every 30 min with HOBO™ dataloggers (Onset Computer Corporation, Cape Cod, Massachusetts) showed that the low and high

temperature treatments were maintained at their target values (29°C treatment: 28.4°C ± 0.62, 32°C treatment: 32.1°C ± 0.60; mean ± SD). However, as a consequence of experimental limitations, ambient temperatures were slightly lower than the target temperature (27°C treatment: 25.4°C ± 1.5; mean ± SD) likely due to laboratory conditions. Changes in Fv/Fm of *Symbiodinium* were relatively small for both symbiont treatments across all temperature treatments over the five days of this experiment. The data conformed to assumptions of homogeneity of variance in both *Symbiodinium* data sets (Bartlett test, K-squared=16.29, 15.57 (*S. microadriaticum* and *S. minutum*, respectively), df=14, p>0.05) and we confirmed graphically that the distribution of residuals did not violate normality for either data set. Separate ANOVAs showed that time was a significant factor in the analyses of both *Symbiodinium* treatments, with a slight decrease in Fv/Fm over time at all temperatures (Table 3; Fig. 4). There was no effect of temperature, nor any interaction with time, on Fv/Fm for either *Symbiodinium* treatment *S. microadriaticum* or *Symbiodinium* treatment *S. minutum* (Table 3). Despite an effect of time, the magnitude of the change in Fv/Fm over the course of the experiment (a maximum change of ~0.05 for both *Symbiodinium* treatments) suggests photoacclimation (Robison and Warner 2006), rather than photodamage (significant impairment of photosystem II accompanied by decreases in Fv/Fm > 0.1; Robison and Warner 2006, Warner et al. 2006).

Discussion

Most research on the effects of different algal symbionts on Caribbean scleractinian corals has examined the differences in fitness of adult corals with naturally-established symbionts (e.g., Rowan 2004, LaJeunesse and Thornhill 2011, Kemp et al 2014). This study is the first to focus on juvenile coral recruits from Caribbean taxa undertaken in a controlled laboratory environment in which the physiological contribution of symbionts can be assessed in isolation. To determine whether differences in symbiont composition might help corals compensate for changing environments, a condition of the Adaptive Bleaching Hypothesis (Buddemeier and Fautin 1993, Baker et al. 2004), the potentially different physiological responses of each developmental stage of the symbiosis to the thermal environment may be important. Our data show that the *Symbiodinium* composition of *O. faveolata* recruits may influence its recruitment success, and that

critical holobiont characteristics (i.e., polyp growth) were not well predicted by *Symbiodinium* photophysiology for the *Symbiodinium* species studied. Although some cross-contamination between *Symbiodinium* treatments was observed, the majority of polyps within the first four months sampled (polyps up to 5 months old) were found with only the intended inoculum (combined data from months 2-5 excluding polyps in which no symbionts were detected: 55 of 65 and 75 of 76 for *S. microadriaticum* and *S. minutum*, respectively). The frequency with which the non-inoculum *Symbiodinium* species was detected in the *S. microadriaticum* inoculated polyps increased over time. However, neither growth patterns nor photophysiological data collected throughout the experiment were significantly affected by time. Mixed symbiont communities of *O. faveolata* adults with *Symbiodinium* B1 and D1a had photophysiological measures intermediate to single strain in-hospite communities (Cunning et al. 2015). Assuming that symbiont frequencies found in recruits sampled for genotyping are reflective of symbiont communities within recruits monitored for growth and physiology over time, the stability of the mean and variance of photophysiology measured through time (Fig. 3) suggests that *S. microadriaticum* continued to dominate in abundance (although our methods cannot confirm this directly). Thus, although we discuss the data in terms of differences between *Symbiodinium* inoculum species, it is not possible here to determine the potential influence of a mixed infection state (two *Symbiodinium* species present) as we cannot assign symbiont genotyping data to specific physiology metrics. Had we been able to genotype the symbionts of those recruits used for physiology at the end of the experiment we would still lack the temporal resolution of determining when those polyps may have entered a mixed symbiont state.

High juvenile coral growth rates are an essential life history trait because juveniles need to quickly move to larger size classes to escape the risks of overgrowth and predation resulting in size-dependent mortality (Rylaarsdam 1983, Edmunds and Gates 2004). Settlement tiles placed in the field showed that *O. faveolata* recruits preferentially settled on the undersides of tiles as well as in cracks and crevices of the reef (Szmant and Miller 2006, Miller 2014). Our experiment found that, under low light conditions similar to those measured in preferred settlement habitats in our field site, polyp growth was significantly influenced by the particular host-*Symbiodinium* pairing, with polyps from

the *S. microadriaticum* treatment growing more quickly than those from the *S. minutum* treatment. There was no difference in survivorship between the two *Symbiodinium* treatments, which both remained well above survivorship rates reported from the field (~90% in the present study vs. ~20% in the field at two-months post settlement; Szmant and Miller 2006, Miller 2014). Such high survivorship is likely due to the controlled laboratory setting, eliminating the risks of predation and overgrowth.

Photophysiology also differed between the two *Symbiodinium* treatments, particularly the efficiency with which available light is used to drive photosynthesis, as indicated by the significant difference in ΔFv/F'm and α between the two *Symbiodinium* treatments. While these parameters do not measure photosynthetic efficiency in terms of production, they have been used as a measure of adaptation to low or high light (Ralph and Gademann 2005). In this study, the higher ΔFv/F'm and α of the *Symbiodinium* of the *S. minutum* treatment indicates that this symbiont species is able to use more of the available light to drive photosynthesis relative to the *S. microadriaticum* treatment (considering the contamination in parts). This suggests that *S. minutum* may be better adapted to low light relative to *S. microadriaticum*. While clade A *Symbiodinium* is rare in *O. faveolata* in Florida (Kemp et al. 2015), in adult colonies of *O. faveolata* in the lower Caribbean, *Symbiodinium* of clades A (ITS-type A3) and B (ITS-type B17) have similar distributions (Rowan et al. 1997, Kemp et al. 2014), often co-occurring within the tops and upper parts of the colonies. However, within the most shaded areas of the colony and at deeper depths, both Clade A and B are displaced by *Symbiodinium* of Clade C (ITS-type C7). In those colonies, measures of photophysiology under non-stress conditions were similar across the colony despite differences in light availability and *Symbiodinium* distributions (Kemp et al. 2014). While light use efficiency may have been maximized across the variations in light microenvironment across a colony surface via changes in *Symbiodinium* communities, in our study an increased efficiency in light use by *S. minutum* relative to *S. microadriaticum* did not translate to increased growth of the coral polyp. Thus in our laboratory setting with low light and regular feeding, it may be that polyps containing mainly *S. microadriaticum* symbionts are able to utilize additional sources of carbon metabolically for growth though how symbiont photophysiology and coral growth are linked in the field remains understudied.

Mixed results were found when the relationship between photophysiology and energy acquisition by the host was assessed in juvenile *Acropora* species. Juvenile *A. tenuis* containing *Symbiodinium* C1 have been found to grow significantly faster than those with *Symbiodinium* D (Little et al. 2004), despite the fact that there is no difference in gross photosynthesis (as measured by O_2 microprofiles) between these same host-symbiont pairings at their control (26ºC) and intermediate (29° C) temperatures (Abrego et al. 2008). However, *A. millepora* containing *Symbiodinium* C1 exhibit higher $rETR_{max}$ and increased carbon assimilation to the host in comparison to conspecifics containing D (Cantin et al. 2009).

The many steps between photophysiology and carbon production (i.e., photosynthesis) are complex, including both assimilatory and non-assimilatory electron flow (Jones et al. 1998). As a result, the efficiency with which light energy is used to assimilate and then translocate carbon is not necessarily equal among different *Symbiodinium*, which may decouple the link between light use (i.e., photophysiology) and carbon fixation and eventual carbon acquisition by the coral host (i.e., host growth). Therefore, although light use efficiency may be greater in one species than another as measured by α, the efficiency with which that energy is used to fix and/or translocate carbon may be lower, leading to differences in host growth (all other factors being equal). Thus, although *Symbiodinium microadriaticum* uses a lower proportion of the available light to drive photosynthesis, it may be able to use that energy to generate relatively more carbon-based sugars via more efficient pathways and/or by harboring higher densities of symbionts (Hoogenboom et al. 2010). Unfortunately, the low absolute number of cells in a single polyp inhibited our ability to accurately quantify symbiont abundance with standard hemocytometer based protocols (e.g., Edmunds et al. 2001, Goulet and Coffroth 2003, Coffroth et al. 2010). However, sugar compositions were shown to differ significantly between *S. microadriaticum* and *S. minutum* and also varied with changes in light levels and temperature, with significant changes in inositol (Klueter et al. 2015). A comparison of the genomes of *Acropora digitifera* and *S. kawagutii* suggests that the proportion of the photosynthetically derived resources translocated to the host depends on the match between what the host can import and what the symbiont can export (Lin et al. 2015). Thus, the more efficient light use of *S. minutum* may in fact result in a higher

amount of carbon per cell but a lower proportion of that carbon may be available to the host.

From our results, individual *Symbiodinium* types or a combination clearly has an effect on both photosynthesis and host growth, consistent with other studies (e.g., Little et al. 2004, Abrego et al. 2009) and further supporting the importance of biochemical complementation between the coral host and symbiotic dinoflagellate as critical for the success of the symbiotic relationship. Further studies that can relate photophysiology, photochemistry, and biochemistry, such as measures of respirometry, carbon fixation and/or metabolomics are needed to understand how photophysiology relates to host resource acquisition and thus holobiont fitness.

Short-term exposure (5 d) to elevated temperatures of 29° C and 32° C did not elicit a visible bleaching response in hosts from either symbiont treatment, nor did it instigate a biologically significant change in Fv/Fm (dark-adapted maximum quantum yield), a commonly used metric of thermal stress (see Warner et al. 2010). One factor that might have contributed to these results is the low light level under which these experiments were conducted. The synergistic role of temperature and light in the disruption of PSII (Jones et al. 1998) suggests that increased temperatures may be less inhibitory for coral polyps growing in low light environments, such as in cracks and crevices within the reef. This is consistent with findings of Robison et al. (2006) who found no damage to PSII in cultured isolates at low light levels (but higher than those of our experiment: 100 vs. ~20 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}). However, at higher light levels (600 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}), damage to PSII machinery (corresponding to decreases in Fv/Fm) was significant in *S. minutum* after five days of heat stress. In our study, temperature alone may have caused some impairment of the PSII apparatus, but if so, it does not seem to have exceeded the repair or re-synthesis capacity, such that it did not elicit a significant biological effect on overall photosynthetic function. Prolonged exposure to thermal stress may also increase the likelihood of seeing a stress response in these corals, as has been seen in other studies (e.g., Abrego et al. 2008). Data collected at NOAA's Long Key Buoy (http://www.ndbc.noaa.gov/station_history.php?station=lonf1) near our study site indicate the sea surface temperatures in 2011 reached over 32° C (with daily fluctuations)

for a period of 10 days approximately one month following spawning. Thus, it is possible that corals subjected to field conditions would be more negatively affected.

Here we have demonstrated the importance of *Symbiodinium* identity on the growth of *O. faveolata* polyps at a critical life history stage. Although derived from cultures, both of the *Symbiodinium* species used in this study are found in Caribbean cnidarian symbioses (*S. microadriaticum*: *Cassiopea xamachana*, Mellas et al. 2014; *S. minutum*: *Aiptasia* sp., LaJeunesse et al. 2012) and readily form a symbiosis with *O. faveolata* (as opposed to some species which are unable to associate with this host; Voolstra et al. 2009). Natural (field) growth rate data for *O. faveolata* acquiring environmental symbionts are lacking, but research on coral recruits suggests that polyps <3 mm in diameter after the first 2-3 months of growth have a low (<20%) chance of survival when predators are present (Rylaarsdam 1983). Thus, while our thermal tolerance experiments indicate these experimental coral-*Symbiodinium* pairings may survive at higher temperatures, both pairings exhibited growth rates in the laboratory, where light was limited, that fall short of those that promote survivorship in the field (*S. microadriaticum*: 0.94 ± 0.33 mm, *S. minutum* 0.66 ± 0.19 mm, mean diameter after nine months ± SD).

Although much attention has been paid to shifts in *Symbiodinium* communities in colonies exposed to various stressors, there remain several aspects of the symbiosis that are poorly understood, particularly the early stages of the symbiosis. The importance of post-settlement bottlenecks in structuring reef communities (Miller et al. 2000) and the implications of partner flexibility at this stage for adaptation to climate change emphasize the need to better understand the patterns and processes that shape coral-algal partnerships in the early stages of their life history.

Acknowledgments

This work was funded by NSF OCE-09-26822 (MAC). We would like to thank peer reviewers whose feedback strengthened the manuscript; FKNMS for permission to work in the Florida Keys (FKNMS-2009-081- A3); the Keys Marine Laboratory staff, N. Leigh, E. Keister, R. Mellas, and all of the volunteers who made the collection and rearing of coral larvae not only possible but enjoyable.

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Table 1. Analysis of Variance (2-way) investigating the effects of *Symbiodinium* treatment and time on surface area changes in footprints of *Orbicella faveolata* polyps.

Source of Variation	df	Mean Square	F-ratio	P-value
Symbiont	1	0.05111	10.81	0.012 *
Time	3	0.00127	0.507	0.682
Symbiont X Time	3	0.00344	1.373	0.283

*Significant at the p<0.05 level

Table 2. Analysis of Variance (2-way) investigating the effects of *Symbiodinium* treatment and time on ΔFv/F'm.

Source of Variation	df	Mean Square	F-ratio	P-value
Symbiont	1	0.037	16.79	0.006 *
Month	4	0.002	1.043	0.406
Symbiont X Month	4	0.001	0.669	0.620

*Significant at the p<0.05 level

Table 3. Analysis of Variance (2-way) investigating the effects of temperature (27, 29, or 32º C) and time (5 days) on Fv/Fm (n=3) for *Orbicella faveolata* polyps inoculated with *Symbiodinium microadriaticum* or with *S. minutum*.

S. microadriaticum

Source of Variation	df	Mean Square	F-ratio	P-value
Temperature	2	0.003	0.576	0.59
Time	4	0.001	5.964	<0.005*
Temperature X Time	8	0.0004	2.087	0.08

S. minutum

Source of Variation	df	Mean Square	F-ratio	P-value
Temperature	2	0.008	5.112	0.051
Time	4	0.002	9.177	<0.005*
Temperature X Time	8	0.0002	1.046	0.37

*Significant at the p<0.05 level

Figure Legends

Figure 1. *Symbiodinium* identity in *Orbicella faveolata* polyps inoculated with **A** no symbionts, **B** *Symbiodinium microadriaticum*, or **C** *S. minutum* cultures.

Figure 2. Growth of *Orbicella faveolata* polyps as measured by surface area of a polyp's footprint determined through images collected over nine months (n=4). Grey and black markers show data for polyps initially inoculated with *Symbiodinium microadriaticum* or *S. minutum,* respectively, with error bars indicating 95% CI. Lines represent exponential growth functions fit to data (R^2= 0.998 and 0.948 for *S. microadriaticum* or *S. minutum,* respectively).

Figure 3. Effective quantum yield (ΔFv/Fm') of *Orbicella faveolata* polyps measured over nine months (n=4). Grey and black markers show data for polyps initially inoculated with *Symbiodinium microadriaticum* or *S. minutum* respectively, with error bars indicating 95% CI.

Figure 4. Maximum quantum yield (Fv/Fm) of *Orbicella faveolata* polyps exposed to three different temperatures (27, 29, or 32º C) prior to and over a period of five days at temperature (n=3). **A** measurements for polyps initially inoculated with *Symbiodinium microadriaticum* and **B** measurements for polyps initially inoculated with *S. minutum*. Error bars indicate 95% CI.

Figure 1

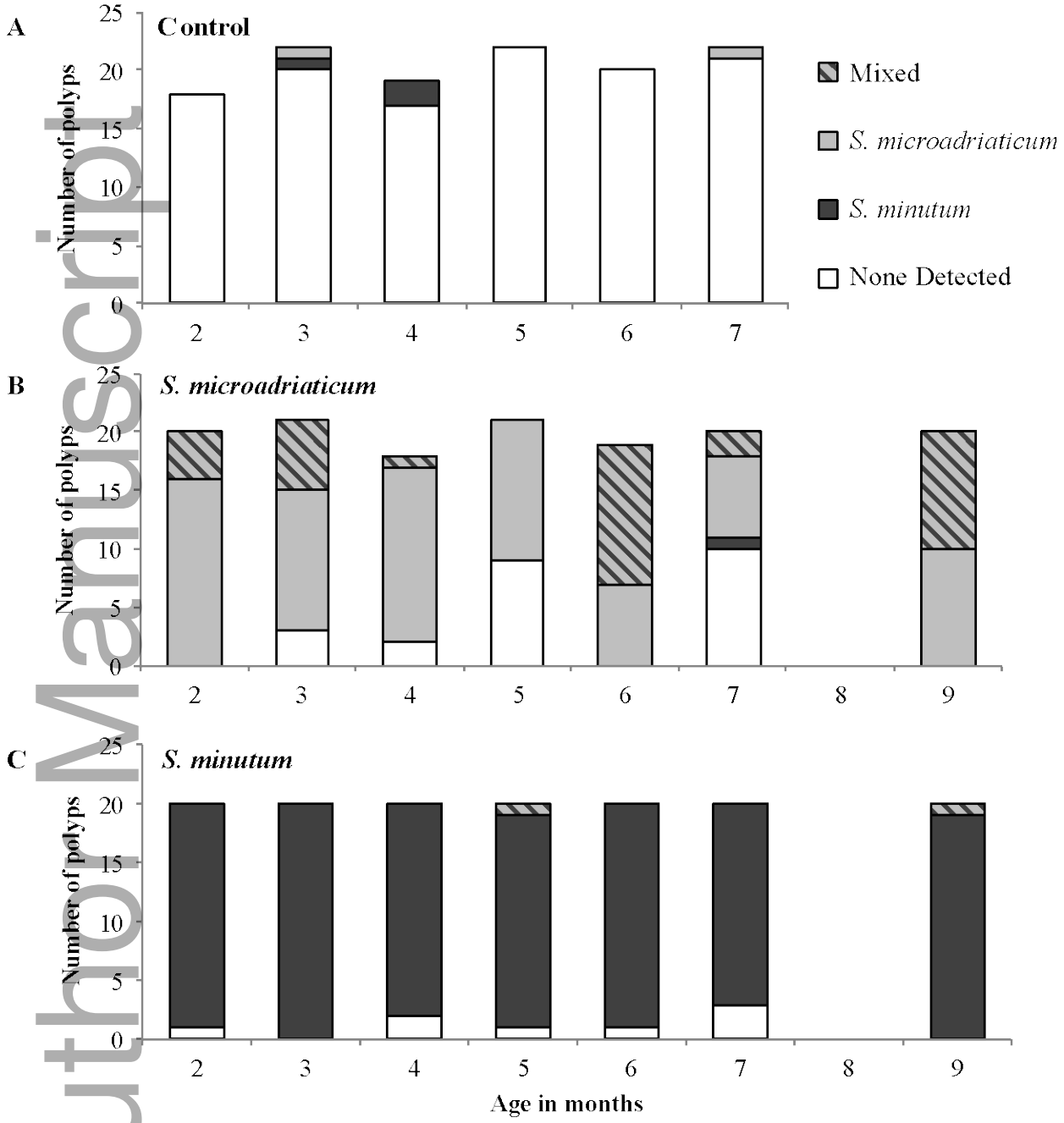


Figure 2

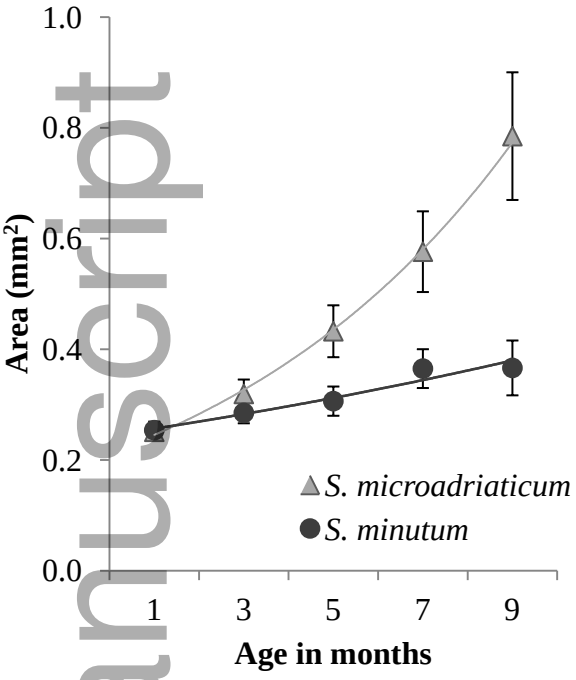


Figure 3

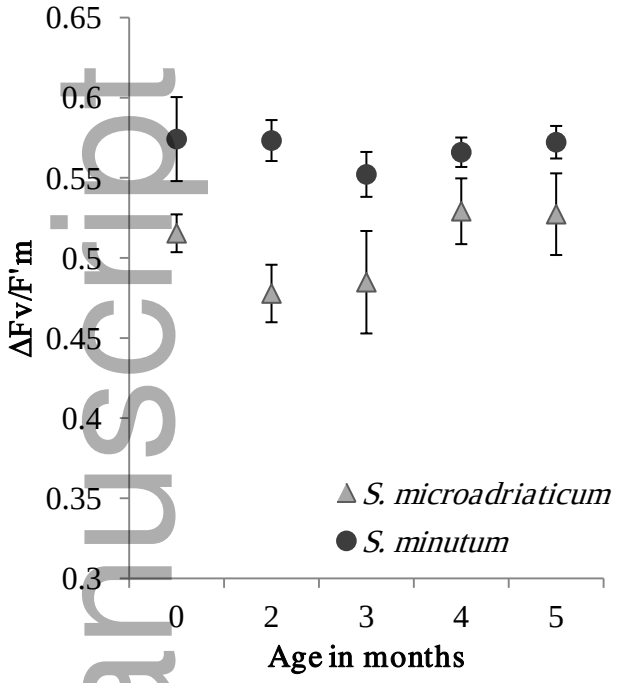
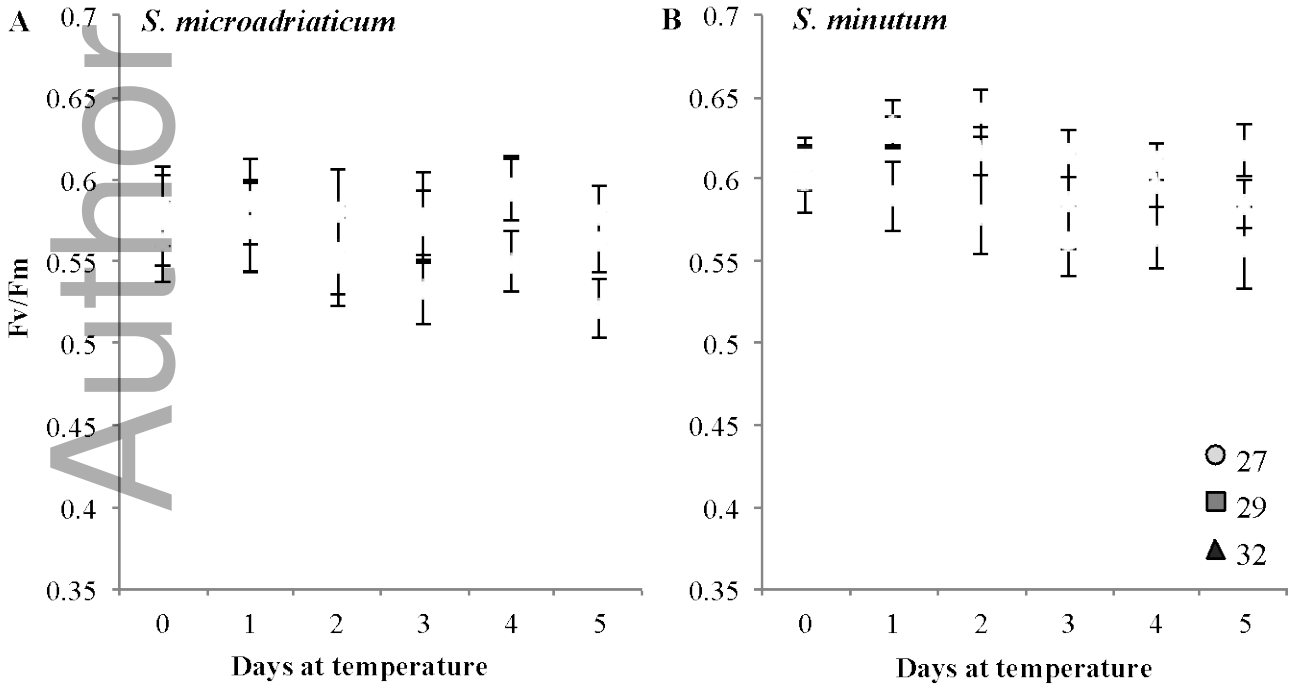
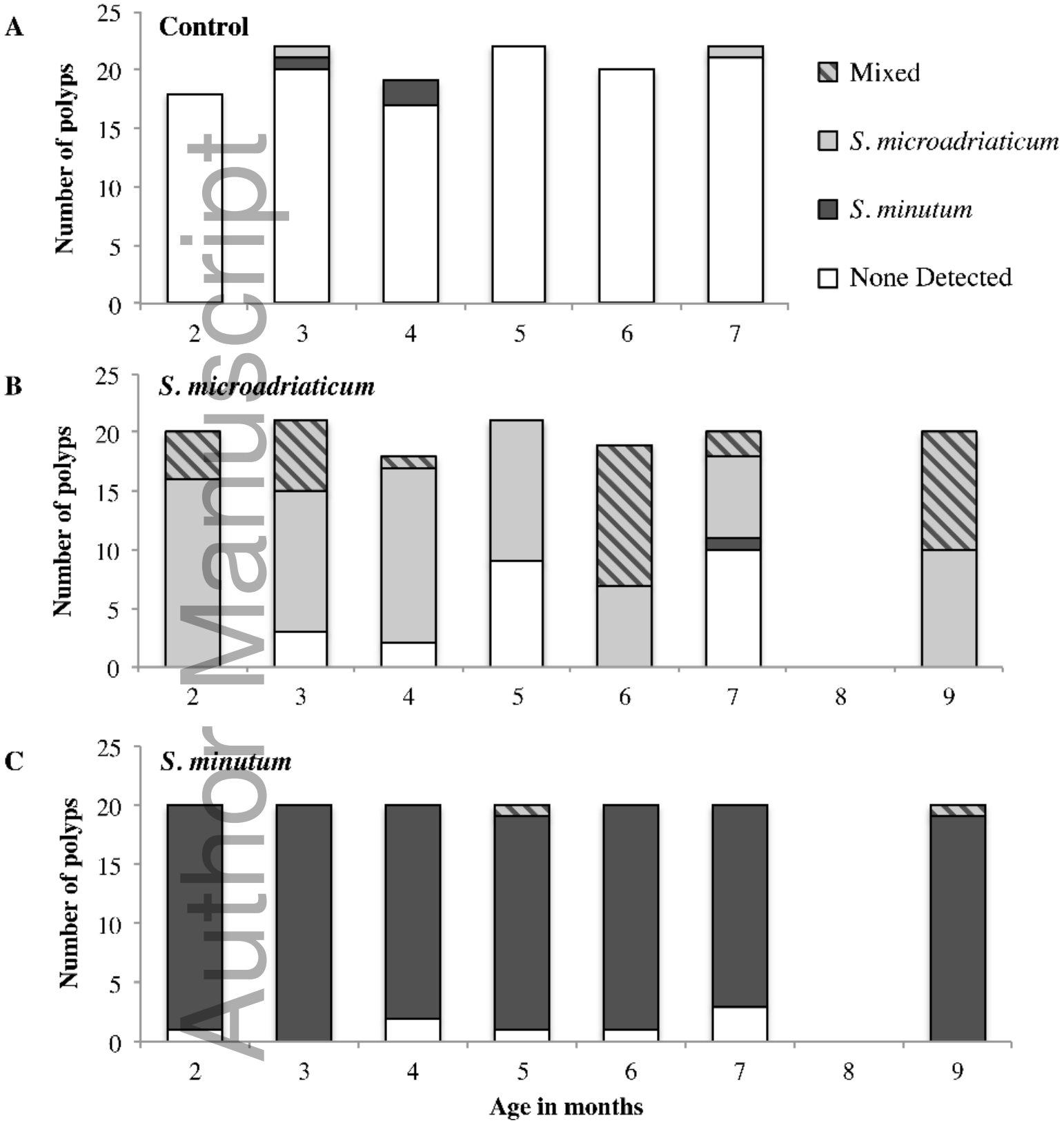


Figure 4





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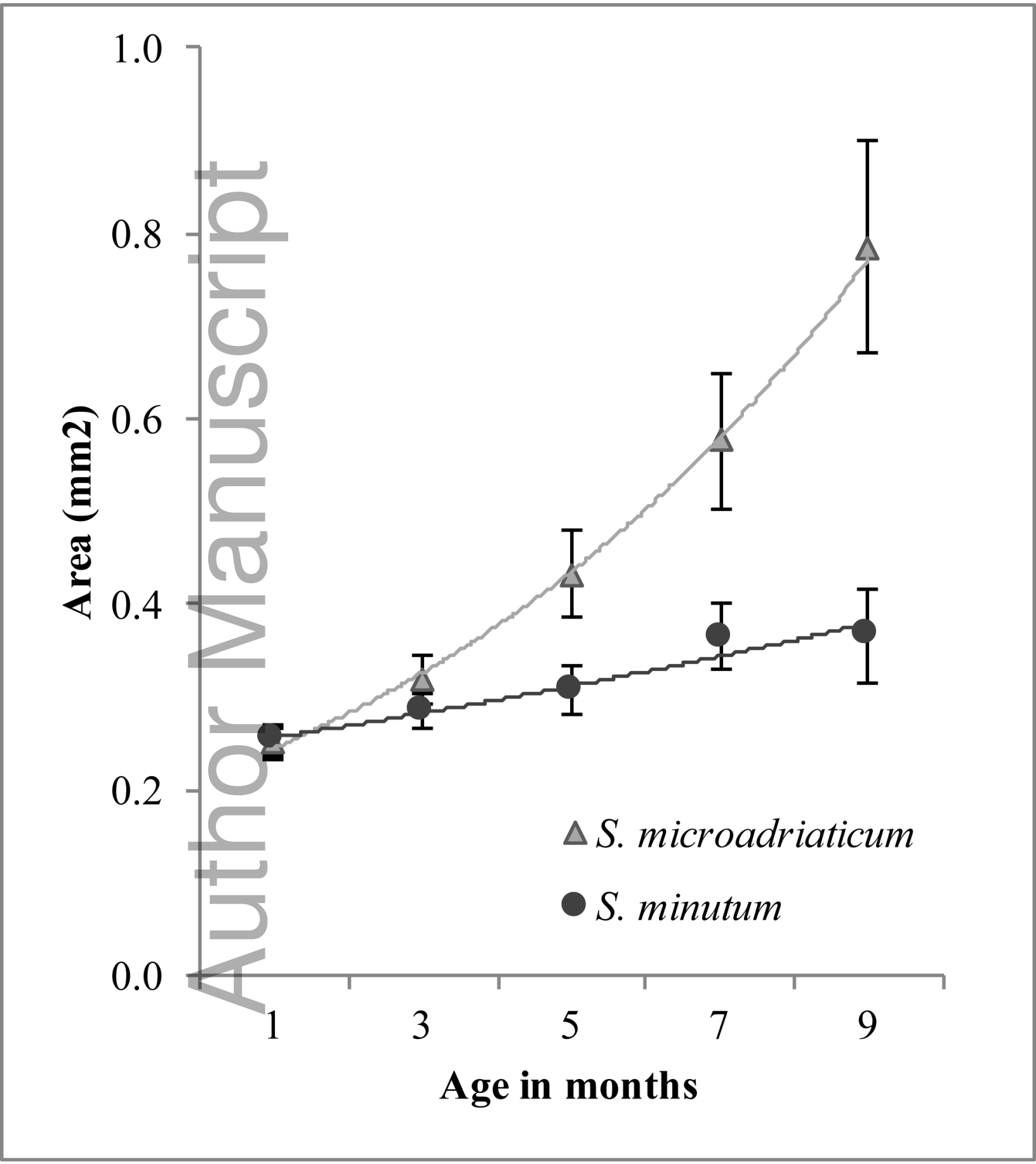

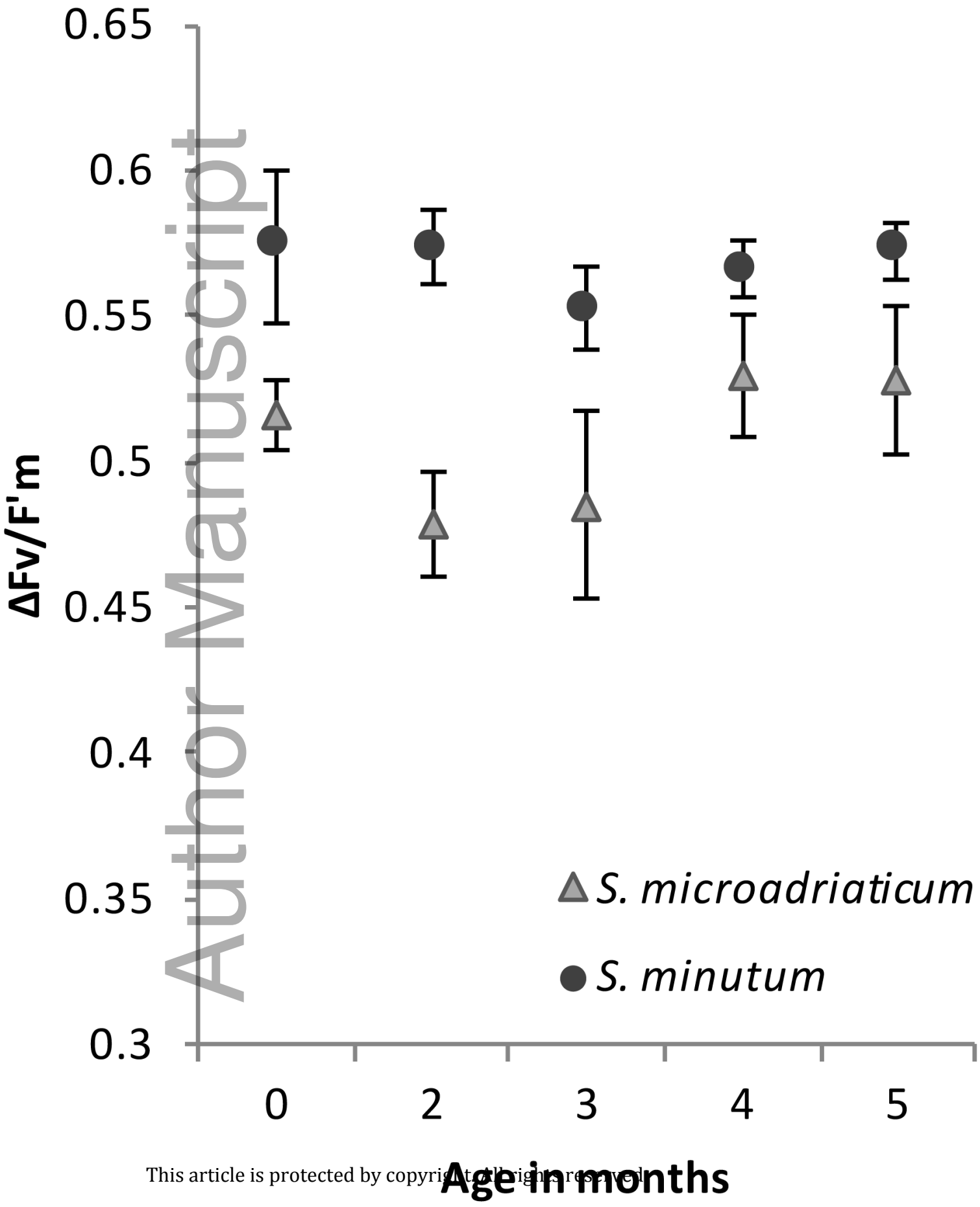

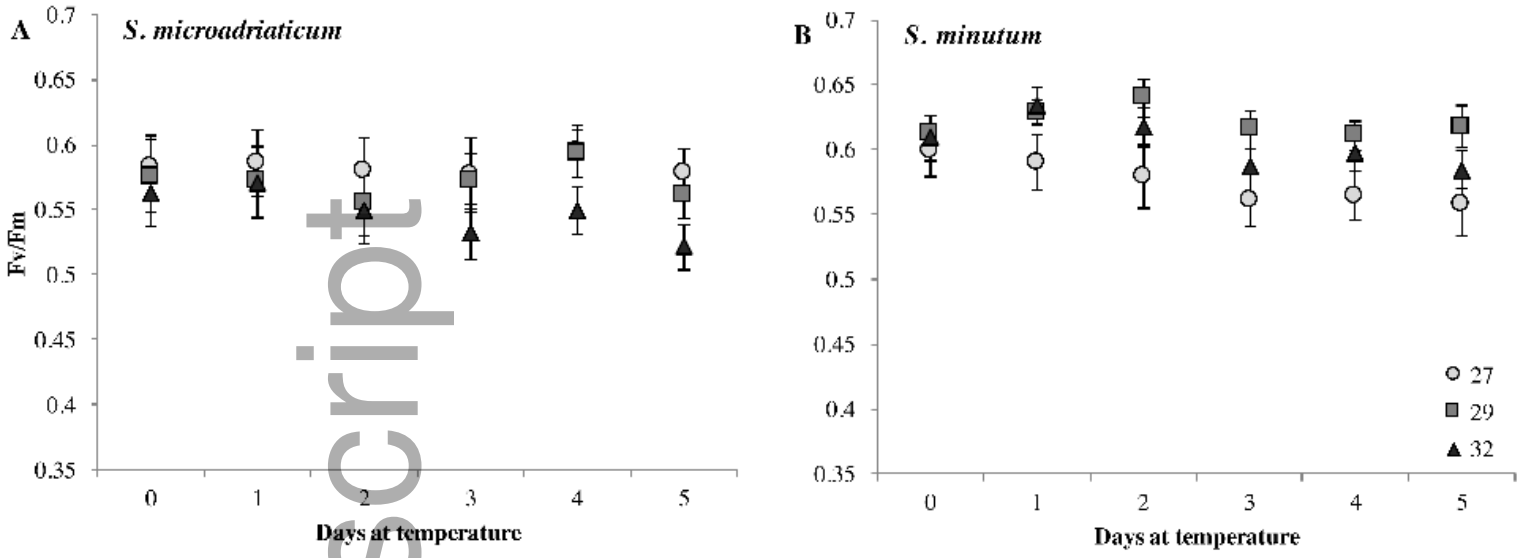

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