NOTE

Differential regeneration of artificial lesions among sympatric morphs of the Caribbean corals *Porites astreoides* and *Stephanocoenia michelinii*

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ABSTRACT: Regeneration of artificial lesions was studied as an ecophysiological character in 2 morphs of *Porites astreoides* and *Stephanocoenia michelinii* to contribute to a better understanding of their presently unclear taxonomic status. As a reference, regeneration was also studied in 3 species/morphs of *Madracis*. *P. astreoides* consists of a green and a brown morph, while *S. michelinii* consists of an encrusting morph with widely spaced corallites and a massive morph with compacted polyps. Regeneration was significantly faster in the green *P. astreoides* and in the encrusting *S. michelinii*. No significant differences were found among the *Madracis* species/morphs. The energy required for faster regeneration in the 2 morphs was apparently not generated as a result of elevated densities of zooxanthellae or chlorophyll *a*. The green *P. astreoides* which occurs at more shallow depths, and perhaps also the encrusting *S. michelinii*, may be better adapted to the increased disturbances at shallow depths by means of, among other things, faster regeneration of lesions.

KEY WORDS: Regeneration · Lesions · Corals · Differentiation · *Porites astreoides* · *Stephanocoenia michelinii* · *Madracis*

The taxonomic status of scleractinian corals has mostly been based on skeletal features such as growth form and microstructural characteristics (e.g. Wells 1956). Most corals, however, display a high degree of morphological variation. This variation may occur in corallites within a colony, within different parts of a single colony, between colonies within the same biotope, between environmental zones, or within regions (Veron 1995).

Lang (1984) therefore suggested also studying non-skeletal traits, such as sexual and asexual reproduc-tion, external and internal morphology, behavior, ecology (e.g. non-scleractinian associates, distribution), and physiology (environmental tolerance limits), to distinguish closely related species when skeletal features fail to reveal differences. A multi-character approach is even more favored, because when a consistent pattern is found in several characters, debate over the adequacy of a particular characteristic as a taxonomic tool becomes less important (Weil & Knowlton 1994). This approach is now being used more often than before, and is frequently based on a combination of genetic and ecological or physiological characteristics (e.g. Ayre et al. 1991, Gattuso et al. 1991, Weil 1992, Potts et al. 1993, Van Veghel 1994, Weil & Knowlton 1994, Takabayashi & Hoegh-Guldberg 1995).

The 2 Caribbean corals *Porites astreoides* (Lamarck) and *Stephanocoenia michelinii* (Milne-Edwards and Haime) both possess at least 2 morphs which can be visually distinguished *in situ*. *P. astreoides* consists of a green (i.e. yellow-green to dark green; in Curaçao this morph is yellow-green) and a brown morph (i.e. light to dark brown) (Gleason 1993). *S. michelinii* consists of an encrusting morph with widely spaced corallites, and a massive morph with compacted polyps (see pictures in Humann 1994, p. 110–111). The encrusting morph is light-brown in colour and most of the pigment is restricted to the corallites, giving the coenosarc a pale-white appearance. The massive morph forms dome-shaped colonies and is green-brown in colour.

Some studies have found genetic, morphological or physiological differences between the *Porites astreoides* morphs (Gleason 1993, Potts et al. 1993), while others found no differences (Weil 1992). Very little

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attention has been paid to the morphs of *Stephanocoenia michelinii*. Scatterday (1974) mentioned a modification of growth in this and other coral species with depth or decreased light intensities, resulting in colonies with a lower corallite density and an increase in surface area of the coenosteum. Apparently, the taxonomic status of the *P. astreoides* and *S. michelinii* morphs remains unclear.

In this study we used the regeneration of artificial lesions as an ecophysiological character (see also Van Veghel & Bak 1994) to contribute to a better understanding of the presently unclear taxonomic status of the *Porites astreoides* and *Stephanocoenia michelinii* morphs.

Materials and methods. Colonies of *Porites astreoides* and *Stephanocoenia michelinii* morphs were selected haphazardly at Carmabi buoy 1 on the southwestern coast of Curaçao, Netherlands Antilles, at a depth of 5 to 7 m (light extinction coefficient $k = -0.117$ m^{-1}; Bosscher & Meesters 1992). Artificial lesions were inflicted on 18 green *P. astreoides*, 15 brown *P. astreoides*, 7 massive *S. michelinii*, and 9 encrusting *S. michelinii* colonies. On top of each colony a small artificial lesion was drilled using a hand-held drill equipped with a 2 mm steel bit. This resulted in cylindrical lesions with a diameter and depth of approximately 2 mm.

The initial lesion perimeter, determined with macrophotography, and the initial lesion depth, measured with a Vernier caliper, were used to calculate the total initial lesion area. Initial lesion sizes (mean ± SD) for the 4 morphs were: 15.1 ± 0.7 mm^2 for the green *Porites astreoides*, 13.7 ± 1.0 mm^2 for the brown *P. astreoides*, 16.1 ± 0.7 mm^2 for the massive *Stephanocoenia michelinii* and 15.1 ± 0.9 mm^2 for the encrusting *S. michelinii*. Tissue recovery following lesion infliction was determined over a time period of 9 d in *P. astreoides* and 21 d in *S. michelinii*, during December 1996. At each measuring interval (2 to 3 d) the progress of tissue recovery along the vertical lesion wall and lesion bottom was measured. The regeneration of the lesions is quantified as the mean percent of the total lesion area (i.e. t_0) recovered versus time.

For each lesion, the regression slope was calculated for the curve of lesion size versus time. Although this curve was exponential when the mean lesion size was depicted versus time (see Fig. 1), the curve was linear for individual lesions (mean r^2 per species > 0.83). The slopes were calculated using least squares linear regression and were compared between species with a 1-way ANOVA or Mann-Whitney U-test (Sokal & Rohlf 1995). All analyses were performed with the program SYSTAT (SYSTAT 1992).

To interpret differences in regeneration among the *Porites astreoides* and *Stephanocoenia michelinii*
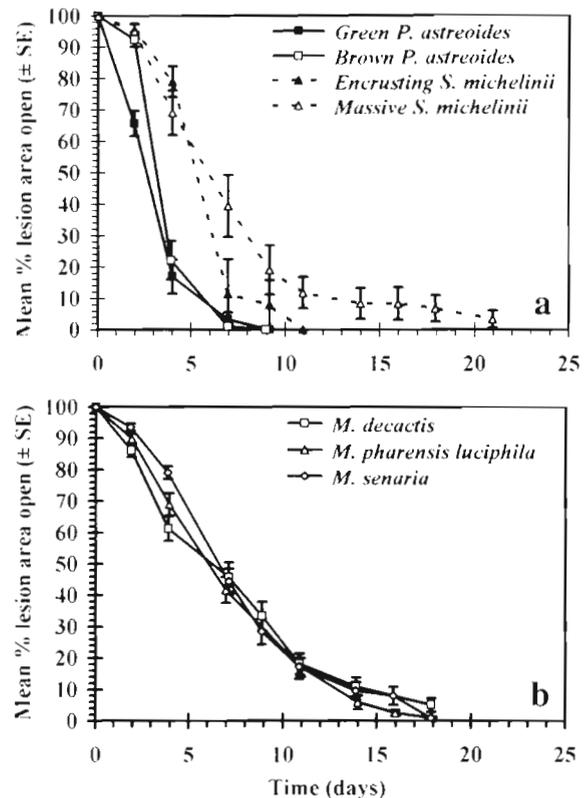

Fig. 1. (a) Regeneration of artificial lesions as a function of time for 2 morphs of *Porites astreoides* and *Stephanocoenia michelinii*. (b) Regeneration of artificial lesions as a function of time for 3 species/morphs of *Madracis*

morphs, the experiment was repeated in 3 species/morphs of *Madracis*, which served as a reference for differences between species/morphs within 1 genus. This genus also has species/morphs with relatively small polyps, and colonies occur in the same depth range as *P. astreoides* and *S. michelinii*. During October–November 1996, artificial lesions were inflicted on 27 *M. decactis* (Lyman), 29 *M. pharensis luciphila* (Heller) and 31 *M. senaria* (Wells) colonies (see key to species of *Madracis* in Wells 1973a, b).

At the end of the regeneration experiment a small tissue sample (ca 0.5×1.0 cm) near the lesion was collected from each colony. Zooxanthellae and chlorophyll *a* densities were determined as in Szmant & Gassman (1990).

Results. Differences in regeneration between the 2 morphs of *Porites astreoides* were observed during the first 4 d following lesion infliction (Fig. 1a). The mean regression slope of the green *P. astreoides* was significantly higher (p = 0.005, Mann-Whitney U-test) compared to that of the brown morph (Fig. 2). Nonetheless, all lesions had closed after 9 d in both morphs (Fig. 1a).

In an earlier experiment, during February–March 1993, the regeneration of larger lesions (diameter =

1 cm) was studied in *Porites astreoides* at a depth of 5 m at the Seaquarium reef (I. Nagelkerken, E. H. Meesters & R. P. M. Bak unpubl.). During this experiment the colour of the morphs was noted but no distinction was made during data analysis. Later recalculation of the data, however, also showed a significantly steeper mean regression slope (p = 0.049, Mann-Whitney *U*-test) for the green morph [slope = -0.066 ± 0.010 (SE), n = 19] than for the brown morph (slope = -0.037 ± 0.014, n = 6).

Differences in lesion closure between the 2 morphs of *Stephanocoenia michelinii* were observed after the first 4 d following lesion infliction (Fig. 1a). The mean regression slope for the encrusting *S. michelinii* was significantly steeper ($F_{1,14}$ = 9.576, p = 0.008, 1-way ANOVA) than for the massive morph (Fig. 2). The difference in regeneration between the 2 morphs was also expressed as a difference between the number of lesions closed at the end of the experiment. All lesions in the encrusting morph were closed after 11 d, while lesion closure in the massive morph took more than 21 d (Fig. 1a).

In contrast to the *Porites astreoides* and *Stephanocoenia michelinii* morphs, the regeneration of lesions in the 3 *Madracis* species/morphs was similar (Fig. 1b). No significant difference (p > 0.942, 1-way ANOVA and Tukey HSD test) was found between the mean regression slopes of *M. decactis*, *M. pharensis luciphila* and *M. senaria* (Fig. 2). The variation in the percentage of lesions closed among the 3 species/morphs was small, and most lesions were closed after 18 d.

Neither the zooxanthellae nor chlorophyll *a* densities varied significantly among the morphs of *Porites astreoides* and *Stephanocoenia michelinii* (Table 1).

Discussion. This study shows that the regeneration of artificial lesions differed significantly among the

Table 1. Mean zooxanthellae and chlorophyll *a* densities of *Porites astreoides* and *Stephanocoenia michelinii* morphs

	Zooxanthellae (cm^{-2})	Chl *a* ($\mu g\ cm^{-2}$)
Green *P. astreoides*	$3.1 \pm 0.2 \times 10^6$	7.7 ± 1.2
Brown *P. astreoides*	$3.1 \pm 0.3 \times 10^6$	7.5 ± 0.9
p-value (1-way ANOVA)	0.962	0.896
Encrusting *S. michelinii*	$4\ 0 \pm 0.4 \times 10^6$	7.9 ± 0.9
Massive *S. michelinii*	$3.3 \pm 0.3 \times 10^6$	5.1 ± 1.4
p-value (1-way ANOVA)	0.225	0.093

Porites astreoides and *Stephanocoenia michelinii* morphs. There is no conclusive data yet on the taxonomic status of the colour morphs of *P. astreoides*. Weil (1992) did not find any significant genetic or morphological variation between the 2 morphs. Likewise, Potts et al. (1993) did not find any differences in morphometric data. Other studies, however, did find differences between the 2 morphs. Fusion only occurs in colonies of similar colour (in Gleason 1993), while Potts et al. (1993) found a difference in allele frequencies between the 2 colour morphs. Furthermore, Gleason (1993) found a difference in both their vertical distribution and resistance to ultraviolet radiation. Although no morphological differences have yet been found and the genetic variation is unclear, the present study shows an ecological difference among the *P. astreoides* and *S. michelinii* morphs.

Scatterday (1974) reported a modification of growth in *Stephanocoenia michelinii* and other coral species with water depth. He observed that colonies living under reduced light conditions had larger corallites which were spaced wider apart. The existence of the 2 *S. michelinii* morphs cannot be attributed to this phenomenon, however. The corallites of the encrusting morph showed a similar modification in corallite spacing, but we observed this morph to be more abundant in shallow water. It is surprising that so little attention has been paid to these 2 morphs. The present study shows a differentiation in regeneration between the 2 morphs and care should therefore be taken when interpreting data from studies which have not separated these 2 morphs.

It is known that at the species level corals commonly show differences in regeneration (Bak & Steward-Van Es 1980, Meesters et al. 1992, Meesters & Bak 1993, Bak & Meesters 1997) and it is striking that there are differences between the *Porites astreoides* and *Stephanocoenia michelinii* morphs and not between the species/morphs of *Madracis*. This result is even more surprising as our experimental lesions were relatively small, and small lesions are less likely to show differentiation in regeneration rate than large ones
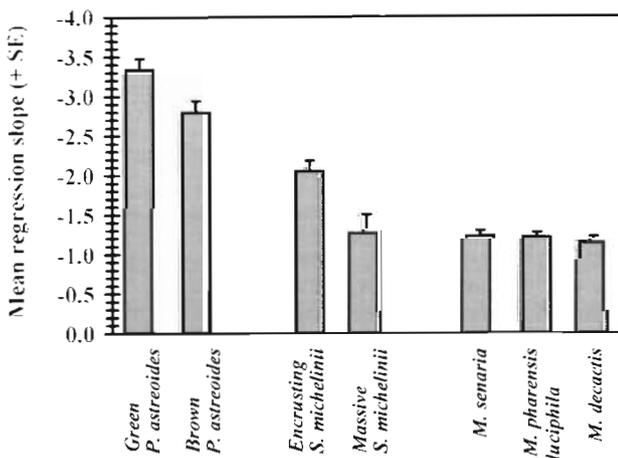

Fig. 2. Regression slopes of lesion recovery (lesion size vs time) for the different morphs of *Porites astreoides* and *Stephanocoenia michelinii* and species/morphs of *Madracis*

(Meesters et al. 1997). Large lesion regeneration may still show differences within *Madracis*, but our results with small lesions emphasize the significant difference between the *P. astreoides* and *S. michelinii* morphs. This relatively large difference in an important eco-physiological character highlights a distance between morphs of *P. astreoides* and *S. michelinii*.

Van Veghel & Bak (1994) studied regeneration in 3 morphs of the *Montastrea annularis* complex in an attempt to determine their taxonomic status and found that one of the morphs (bumpy or *Montastrea franksi*; Weil & Knowlton 1994) regenerated faster than the other. Weil & Knowlton (1994) regard the morphs as 3 species, based on a multi-character analysis. This underscores the use of regeneration in the evaluation of taxonomic distinction.

The differences in regeneration among the *Porites astreoides* and *Stephanocoenia michelinii* morphs cannot be explained by a difference in their zooxanthellae and chlorophyll *a* densities. Energy budgets of corals are often limited, and energy is partitioned over important processes such as respiration, growth, reproduction and regeneration (Bak 1983, Edmunds & Spencer Davies 1986, Spencer Davies 1991). Regeneration of tissue requires, apart from nutrients acquired through heterotrophic feeding, a considerable amount of energy which must be supplied by the polyps surrounding and in the vicinity of the lesion (Meesters et al. 1994, 1997, Oren et al. 1997). The energy available to polyps often largely originates from the symbiotic algae in the host tissue (Muscatine et al. 1984). Faster regeneration in the green *P. astreoides* and in the encrusting *S. michelinii* requires more energy, but this is apparently not generated as a result of elevated densities of symbiotic cells or chlorophyll *a* pigments. Likewise, Takabayashi & Hoegh-Guldberg (1995) found different growth rates in 2 colour morphs of *Pocillopora damicornis*, but no difference in their zooxanthellae densities.

The extra energy required for faster regeneration could be generated to the detriment of other physiological processes (e.g. Bak 1983, Meesters et al. 1994, Van Veghel & Bak 1994). In this study the effects on other processes were not investigated. Gleason (1993) found significantly higher concentrations of mycosporine-like amino acids (MAAs) in the green *Porites astreoides* compared to the brown morph. He showed, however, that the higher metabolic cost of production of specific MAAs was not to the detriment of growth and protein content in the green morph.

Aside from a potential difference among morphs in the allocation of energy to physiological processes, 3 other possibilities may explain a difference in energy supply. They are: different species of zooxanthellae (Rowan et al. 1997) which have a different rate of photosynthesis or translocation of photosynthetic energy to the coral; a difference in the rate and type of zooplankton captured (Wellington 1982, Sebens et al. 1996); or a difference in the storage or translocation of lipids (Stimson 1987, Harland et al. 1993). The possibility exists that such physiological traits which are strongly linked to the regenerative abilities are not fully independent among the morphs, but are linked to the specific colony colour and morphology of the morphs. For example, in *Porites astreoides* morphs, the difference in the animal pigmentation may lead to different light levels in the zooxanthellae, while in *Stephanocoenia michelinii* morphs the different colony morphology may lead to a difference in particle capture. Thus, even though the morphs occur in the same environment, differences in morphology and colour may lead to a different physiological response between the morphs, resulting in the observed differentiation in regeneration.

The morphs which show the fastest regeneration (i.e. the green *Porites astreoides* and the encrusting *Stephanocoenia michelinii*) also occur at shallower depths (Gleason 1993, authors' pers. obs.). Considering this and the higher concentration of MAAs in the green morph (Gleason 1993), it is possible that the green *P. astreoides*, and perhaps also the encrusting *S. michelinii*, are better adapted to the environmental conditions in shallow water. In shallow water, increased stresses and disturbances often exists as a result of, for example, elevated UV radiation levels and increased sedimentation rates. Thus, the shallower-occurring green *P. astreoides* is able to protect itself against the increased UV radiation through a higher production of MAAs (Gleason 1993), while a higher regeneration rate (this study) may be a strategy for coping with the increase in the number of naturally occurring lesions in shallow water.

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