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Senescence and dying signals in a reef building coral

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Summary. Decreases in two physiological processes (reproduction and growth) have been shown to precede the advent of colony death in the branching coral *Stylophora pistillata*. These diminutions were sometimes detectable even 6 months and more before any first visible sign of tissue mortality was observed.
Key words. Coral; dying signals; growth rate; reproduction; senescence; *Stylophora pistillata.*

There is little disagreement with the principle that an individual organism eventually accumulates physiological decrements that increase its likelihood of dying[2]. However, there appears to be some controversy regarding the presence of aging and dying processes in the phylum Coelenterata. For example, Strehler[3] indicates that among the coelenterates 'there are probably nonaging species, animals which may not undergo a regular senescence and animals which quite regularly and systematically die as individuals but their contents being returned to the colony'. He[3] summarizes that coelenterates of the class Anthozoa may fit into a category of organisms which fail to show aging as a whole because of a continual replacement regimen. Moreover, regarding reef building corals, it was long ago stated and has never been disproved that a coral 'is a living thing that knows no time of youthful vigour, no waxing to a period of adult life, no waning to senility – it knows no age – it practically knows no natural death'[4]. Therefore, when the death of a coral was recorded in field experiments, while other neighbor colonies still survived, it was suggested to be a result of competitive interactions and natural enemies[5], or was related to both physical and biological disturbances[6] rather than to internal aging and dying processes. In this report we describe decrements of two important physiological processes (reproduction and growth rate) in the branching coral *Stylophora pistillata,* preceding the advent of colony death. These diminutions could sometimes be detected even 6 months and more before any visible sign of damage or partial tissue mortality was observed. We conclude that such physiological decline may be the first harbinger of forthcoming natural mortality of the whole colony.

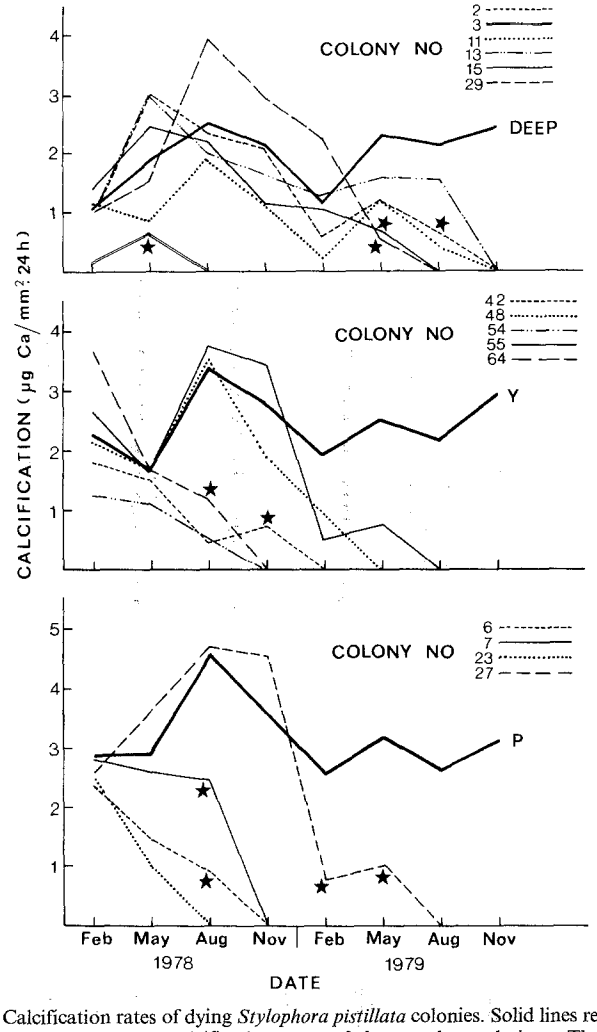
Materials and methods. Two sets of experiments were conducted on the reproduction and growth rate in the reef building coral *Stylophora pistillata.* The reproduction of *S. pistillata* was studied extensively in Eilat, Gulf of Eilat, Red Sea during 1974–1982[7–9]. During the course of this study we also followed the reproductive status of 20 mature colonies first sampled in Dec. 1976, and thereafter sampled 2–3-times each year during three reproductive seasons. These colonies were carefully chosen for their size (large colonies, more than 20 cm in diameter) and for their state of health (without dead branches or tissue damage). This study was ended 39 months later with the death of the last colony. Gonads were regularly examined every month using histological sections and planula-larvae were collected in situ with plankton nets or in the laboratory by putting mature colonies in aerated aquaria[7,8]. *S. pistillata* has a long period of reproduction, lasting approximately 8 months (from December to July). During the reproductive period synchronization in breeding exists in different branches within a single colony. However, no such synchronization has been found among colonies within the population. *S. pistillata* is a hermaphroditic species. Ovaries and testes are situated in the same polyp and attached to the septae by stalks. In the first year of reproduction the vast majority of the population contains male gonads only. With increase in size there is a tendency towards an increase in the percentage of hermaphroditic colonies[7,8].

Rates of growth of *S. pistillata* populations were studied during 1978–1981 in shallow (3–5 m) and deep water (27–30 m) colonies by using a radioisotopic tracer, ^{45}Ca[9,10]. In one set of experiments we traced the calcification rates of three different populations, sampled regularly every three months, during a period of 21 months, between Feb. 1978 and Nov. 1979: deep water colonies (n = 41), shallow purple (n = 30), and shallow yellow (n = 30). In deep water colonies it is impossible to distinguish between the color morphs, and these colonies exhibit a dark-brown color resulting from the zooxanthellae's pigments. All incubations were carried out in front of the Marine Biology laboratory at Eilat. Samples, each containing several branches, were carefully cut from colonies using pliers and incubated underwater with $^{45}CaCl_2$ (0.05 μ Ci/ml) for 24 h in closed transparent tanks. Tip branches were sampled and the surface area of each segment was calculated. The tissue was removed with a solution of 30% hydrogen peroxide[9,10].

Results and discussion. The modes of reproduction in 20 examined colonies are presented in the table. The results indicate a remarkable decrease in the rate of reproduction, which appears in many cases several months before the natural death of the colonies, and precedes any visible sign of partial death or damage. In colonies 6 and 14 (table) this dramatic decrease was recorded even 10–12 months before the first visible degeneration of tissue appeared. Of 6 colonies which were found dead following a sampling period of high reproductive rate (h+ or h++,

Reproductive state of 20 colonies of *S. pistillata* sampled during Dec. 1976–Feb. 1980. December and June months represent the beginning and the end of the reproductive season respectively. February and April represent the peak of the reproductive season[7–9]. Abbreviations: D, colony found dead; h, hermaphrodite, an average of less than 0.8 eggs per polyp; h^+, hermaphrodite, an average of 0.8–1.5 eggs per polyp; h^{++}, hermaphrodite, an average of more than 1.5 eggs per polyp; m, only male gonads present; S, destroyed by storm activity; –, sterile colony; asterisk, first sign of branches with tissue damage.

Coral no.	Sampled date Dec 76	Apr 77	Dec 77	Apr 78	Jun 78	Dec 78	Apr 79	Dec 79	Feb 80
1	–	h*	D						
2	h	h	D						
3	h	h^{++}	h^+	h^{++}	h	–	D		
4	h^{++}	h^+	h	m	D				
5	h	h	D						
6	h	h^{++}	m	–	–	–*	D		
7	h^{++}	h^+	h	D					
8	–	D							
9	h	h^+	h	h^{++}	S				
10	–	h	m	m	–	h^+	h	–*	D
11	–	D							
12	–	D							
13	–	h^{++}	h^{++}	h^{++}	–	h^{++}	h^+*	D	
14	–	h	–	h^{++}	–	–	–*	D	
15	–	h^{++}	h^+	S					
16	h^+	h^+	h^{++}	S					
17	–	D							
18	–	h*	D						
19	–	h^+	–	h^{++}	S				
20	h	h^+	m	h^{++}	–	h^+	D		



Calcification rates of dying *Stylophora pistillata* colonies. Solid lines represent the average calcification rates of the tested populations. Three populations were tested: deep water colonies, shallow yellows (Y) and shallow purples (P). Asterisk represents observed partial tissue damage to the colony.

table), in 4 of them (colonies 9, 15, 16, 19; table) the death was clearly related to storm activity, an external variable which destroyed the colonies. Although attention had been given at the time of the first sampling to choosing 'healthy' and unharmed mature colonies, none of the colonies which were found dead 4 months after the beginning of the study (Nos. 8, 11, 12, 17; table) contained gametes when first sampled. We assume that these colonies were already in the preadvent process of reduced reproduction which cannot be detected by the methods of field observation. In several cases (such as colonies 1, 2, 4, 5, 7; table) the colonies were, to a certain extent gametogenetically active (h, table). Possibly these colonies experienced a different cause of mortality.

As in the first experiment, during the second set of experiments many of the colonies died (fig.). In most of these colonies, a remarkable decrease in their calcification rates, compared to the average calcification rates of nearby conspecifics, was recorded 3–6 months before any visible tissue damage to the colony was reported (deep colonies Nos. 2, 13, 29; shallow colonies 6, 23, 42, 48, 54, 55; fig.). A partial death of branches was first recorded later, mostly about three months before the complete death of the colony was reported. This indicates that the partial damage to the colony was not the outcome of external variables (predation, competition or abiotic conditions) but was an internal process of failure which began at least 6–9 months before the death of the colony (expressed by a decrease in the calcification rate), continued by a progressive degeneration and ended in the death of the entire colony.

These two independent experiments (table, fig.) suggest that physiological processes can undergo gradual diminutions which are detectable several months before the colonies die. However, it has long been believed[11] and never disproved that a coral-colony cannot be exterminated by a natural death. For reef building corals this concept is supported by the well-documented phenomena of propagation through fragmentation[12–17] and the processes of partial mortality, fission and fusion[18].

Aging which occurs in animals who reach a fixed size after maturity is beyond any dispute[2]. However, this is not accepted for many coelenterates, for which it has been stated that there are animals which clearly never have an opportunity to age, because they divide and give rise to new growing organisms before age changes can become determinative, or they fail to show aging as

a whole because of a continual replacement regimen[3,4]. This assumption is also based on some known observations in which several sea anemones were maintained in aquaria for a very long time, and underwent no obvious change during 80–90 years of continuous observations[3]. In contrast, few indications point to the existence of aging and that individuals of coelenterates really die. In one case, a specimen of the sea anemone *Sagartia troglodytes* was kept in captivity for 66 years. This animal died naturally, after appearing to become weaker for several months[19].

In the scleractinian corals there is no direct evidence for the existence of old age, decay and natural death. Only in one case was it suggested that reproduction in corals could be followed by subsequent senescence and death[20]. Thus, it is generally believed that within a colony 'death and life are going on together by asexual reproduction and cease of the old polyps'[21]. The present study, therefore, provides evidence for the first time that the death of a coral colony could follow natural decremental processes and not only result from storm activities, sedimentation, changes in salinity or temperature, low tides, changes in light intensities, predation, competition[22] and disease[23]. This brings us face to face with one of the most difficult problems in physiology; the nature of the origin of death in corals, which leads to the changes recorded in the calcification and the reproductive processes. We conclude that as in higher multicellular animals, corals accumulate physiological failures which lead to a natural death of the whole colony (which is different form the partial mortality recorded in the past[18]). These failures could also be an indication of the senescence or aging processes of corals. The question of the universality of the aging process, and the question of whether all organisms age by essentially the same biological process, are critical in choosing an organism for research into aging in an attempt to obtain an understanding of human aging processes[24]. Although the present study characterizes processes occurring only at the level of the organism it could be of help in understanding aging phenomena. Further work is needed to elucidate the dying processes at other biological organization levels in corals such as in the tissue layer, and at the cellular, subcellular and molecular levels.

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A mitochondrial DNA polymorphism in honeybees (*Apis mellifera* L.)

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Summary. Mitochondrial DNA (mtDNA), isolated from worker honeybee larvae, was digested by each of seven 6-base restriction enzymes. Only one enzyme (*Bgl* II) showed a mtDNA difference between the three tested races (*Apis mellifera carcia, A.m. ligustica, A.m. caucasica*). Both *A.m. carnica* and *A.m. ligustica* showed the same pattern, differing from *A.m. caucasica*. The degree of fragment pattern similarity revealed that there is only a small level of mtDNA variation between the three races tested. This is in line with previous investigations of enzyme polymorphisms.
Key words. Mitochondrial DNA; genetic variation; *Apis mellifera* L.; male haploidy; cytoplasmic inheritance.

The analyses of polymorphisms in nuclear DNA, and in extranuclear DNA, is an important technique for describing variation in populations. In population genetic studies, genomic variation has usually been documented by isozyme studies whereas extranuclear DNA variability is mainly revealed by restriction enzyme analysis of mitochondrial DNA (mtDNA). Both methods are useful for discriminating between different populations and in tracing the evolution of natural diploid populations.

In most hymenopteran populations, which are male-haploid, there are problems because of limited isozyme polymorphisms. Theoretical approaches indicate that the potential for genomic variation in haplo-diploid populations should be reduced in comparison with diploid populations[1-3]. The findings for honeybees are consistent with this view, in that honeybee isozyme polymorphisms seem to be extremely rare[4-7], with very few polymorphic enzyme systems documented for *Apis mellifera* L.[8-14]. However the potential of mtDNA variation should not be reduced in male-haploid populations. Because mitochondria are thought to be inherited maternally[15], there should be no effect of haploid males, and the principles of mtDNA transmission