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PERSPECTIVE

Len Muscatine (1932–2007) and his contributions to the understanding of algal-invertebrate endosymbiosis

O. Hoegh-Guldberg · G. Muller-Parker · C. B. Cook · R. D. Gates · E. Gladfelter · R. K. Trench · V. M. Weis

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Abstract The late Leonard (Len) Muscatine (1932–2007) played a key role in the development of the understanding of algal-invertebrate symbioses. For over 40 years (1958–2005), Professor Muscatine was an inspirational mentor and leader in this field, guiding both the ideas and lives of generations of scientists, many of whom are still active in this research area. His scientific contributions were instrumental in crafting the understanding of a fundamentally important part of our world; that of endosymbiosis, where two or more independent organisms live together in a cellular harmony that belies a complex set of molecular and evolutionary interactions. Muscatine's research career was defined by investigations aimed at unraveling these interactions, particularly the specificity, metabolism, regulation, and disintegration of algal-invertebrate symbiosis. His gentle interrogation of his students and colleagues as to "What is the question?" led more than often to the focused research that yielded the insightful answers that still resonate today as the most current in the field.

Keywords Endosymbiosis · Leonard Muscatine · Coral · *Hydra* · *Symbiodinium* · *Chlorella*

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O. Hoegh-Guldberg (✉)
Centre for Marine Studies, University of Queensland,
St. Lucia 4072, Queensland, Australia
e-mail: oveh@uq.edu.au

G. Muller-Parker
Shannon Point Marine Center and Biology Department,
Western Washington University,
Bellingham, WA 98225-9160, USA

C. B. Cook
Harbor Branch Oceanographic Institution, Inc.,
Fort Pierce, Florida 34946, USA

R. D. Gates
Hawaii Institute of Marine Biology,
University of Hawaii, Kaneohe, HI 96744, USA

E. Gladfelter
Marine Policy Center, Woods Hole Oceanographic Institution,
Woods Hole, MA 02543, USA

R. K. Trench
Department of Ecology, Evolution and Marine Biology,
University of California at Santa Barbara,
Santa Barbara, CA 93106, USA

V. M. Weis
Department of Zoology, Oregon State University,
Corvallis, OR 97331, USA

Introduction

Muscatine began his academic career as an English major and discovered biology in his senior year. After obtaining his BA (Lafayette College, PA), he moved to the University of California at Berkeley where he completed an MA (1956) and a PhD (1961) under the supervision of the invertebrate zoologist Cadet Hand. Even from those early stages, Muscatine began to make his mark on our understanding of algal-invertebrate symbiosis. Hand (1956) and Odum and Odum (1956), for example, were engaged in a vigorous debate about the trophic status of corals and their symbiotic algae, with both sides eventually conceding that clear experimental evidence was needed to resolve whether energy and nutrients were transferred between symbiotic partners. In a letter to Gisèle Muller-Parker (November 13, 2002) together with the original reprint of his 1956 paper, Cadet Hand recalled his suggestion to use radioisotopes to investigate the role of algae in corals, writing, "That

suggestion is what started Muscatine on his doctoral research and look where it took him!" From these beginnings, Len Muscatine developed a life-long passion for the symbiosis between algae and invertebrates.

Muscatine's career was exemplified by scientific excellence and creativity. His approach to science combined keen observational skills, clever experiments aimed at deciphering underlying mechanisms or processes, and a superior ability to define the core question within an area of inquiry. Muscatine placed an emphasis on functional aspects of algal-invertebrate symbioses from very early in his career. This strategy was central to his success in generating a lasting legacy of ideas and discoveries.

The present Perspective traces the origin and development of the cornerstone research undertaken by Len Muscatine. Muscatine's contributions have shaped our understanding of the cell biology, carbon metabolism, specificity and regulatory phenomena underpinning the complex, yet fundamentally important, endosymbioses between single-celled algae (particularly dinoflagellates in the genus *Symbiodinium*) and invertebrate animals.

Carbon budgets, CZAR and the nutritional biology of algal-invertebrate symbiosis

The first direct experimental evidence of a nutritional role for symbiotic algae in animal hosts came from Muscatine's doctoral work at the University of California, Berkeley. Using relatively novel radioautographic ^{14}C labeling techniques, Muscatine demonstrated that photosynthetically generated organic carbon was transferred from *Symbiodinium* to the tissues of the anemone host *Anthopleura elegantissima* (Muscatine and Hand 1958). In this seminal paper, he concluded, "the nutrition of anemones with symbiotic algae, and probably reef-building corals, is at least in part derived from excesses of the photosynthetic activities of the symbiotic algae." This statement heralded a new set of approaches to old problems, driving many of Muscatine's efforts and contributions (as well as those of his students and colleagues) over the next four decades in their attempt to understand algal-animal symbioses.

Muscatine held two post-doctoral appointments, one with Howard Lenhoff at the University of Miami and the other with Andy Benson at Scripps Institution of Oceanography. This work concentrated on green *Hydra*, a model organism for algal symbiosis that is amenable to the approaches that Muscatine, as well as his students and colleagues, would later apply to reef-building corals. In Miami, Muscatine demonstrated that the symbionts (*Chlorella*) provided *Hydra* with organic carbon fixed during photosynthesis (Muscatine and Lenhoff 1963) that enhanced both growth and survival when food was limiting (Muscatine and Lenhoff 1965a, b). This important finding has been substantiated in other algal-animal symbioses: although algal symbionts provide nutritional supplement, animal hosts do not rely exclusively on their algae for growth. In work with Benson at Scripps, Muscatine concentrated on the nature of the translocated carbon compounds, using paper radiochromatography to identify these compounds. This work led to the recognition that *Chlorella*, freshly isolated from *Hydra*, released a significant proportion of photosynthetically fixed carbon as maltose (Muscatine 1965), while those from *Paramecium* and freshwater sponges released glucose (Muscatine et al. 1967). The finding that specific, unique photosynthetic products were translocated from symbiotic algae would prove to be a general phenomenon of algal symbiosis.

Muscatine began to work with corals and other tropical dinoflagellate symbioses at the University of California, Los Angeles (UCLA) where he took a faculty position in 1964 and remained for the rest of his career. Using experimental techniques and methods adapted from his *Hydra* work, he showed that glycerol was the major product released by symbiotic dinoflagellates from reef-building corals and the giant clam *Tridacna*, and discovered that the release of these products was enhanced by host tissue homogenate (Muscatine 1967). He suggested that excretion of glycerol and other photosynthetic products by *Symbiodinium* to host tissue was the mechanism by which *Symbiodinium* and corals contribute to the productivity of coral reefs. Bob Trench, one of Muscatine's first graduate students, revealed the variety of products that could be found among various "strains" of *Symbiodinium*. The papers from Bob Trench's thesis set the stage for the exploration of the large diversity that exists within the genus *Symbiodinium* and which is currently a major research focus (Trench 1971a, b, c; LaJeunesse 2005; Stat et al. 2006).

Muscatine subsequently showed that about 40% of the total fixed ^{14}C was translocated by *Symbiodinium* and incorporated by coral tissues (in this case field and in situ studies with *Pocillopora damicornis*), with very little accumulating in the skeleton (Muscatine and Cernichiari 1969). In a now classic review, Smith et al. (1969) demonstrated that metabolite transport from symbionts to hosts was a general phenomenon in a wide range of mutualistic and parasitic associations. While the nature of host tissue "factors" in regulating this metabolite transport has not been fully elucidated (Muscatine et al. 1972; Gates et al. 1995, 1999; Cook and Davy 2001), it is clear that the co-evolution of host and symbiont has led to tight metabolic interactions that involve the transfer of metabolites between partners. In collaborations with Ruth Gates, Ove Hoegh-Guldberg, Karl Biel and others, Muscatine found that a suite of amino acids naturally occurring in the hosts' tissues elicited the same quantitative and qualitative response by

the algae as the original host homogenate (Gates et al. 1995, 1999; Biel et al. 2007). Whether these mixtures of amino acids actually constitute the original host "factor" still requires resolution.

The demonstration of translocation between symbionts and invertebrate hosts prompted Muscatine to turn to a critical ecological question: "To what extent does translocation satisfy coral animal tissue carbon requirements for daily maintenance respiration?" (Muscatine and Porter 1977). A major conceptual advance in this area came with the development of equations that estimated "CZAR" (Contribution of Zooxanthellae to Animal Respiration) and total carbon budgets (Muscatine et al. 1981, 1983). These calculations included estimates of host daytime respiration, symbiont respiration and carbon-specific growth rates of the algae. Underwater respirometers designed by Larry McCloskey and Jim Porter enabled calculations of daily integrated oxygen flux. Using this approach and others, Muscatine and his colleagues, including Zvy Dubinsky and Paul Falkowski, were able to produce carbon budgets for corals on the reef, including estimates of in situ translocation. As part of a series of papers in the Proceedings of the Royal Society, they demonstrated that symbiotic algae supplied over 100% of the host's daily carbon needs under optimal light conditions. They also demonstrated through these carbon budgets that *Symbiodinium* must release at least 95% of the photosynthetic carbon that it fixes to the coral host (for the budgets to remain balanced). These studies also concluded that corals in shallow, well lit habitats were likely to be photoautotrophic (with possible abundant or "junk food" carbon; Falkowski et al. 1984), while those in deep water and shaded habitats were necessarily obligate heterotrophs (McCloskey and Muscatine 1984; Muscatine et al. 1984; Porter et al. 1984) and hence more dependent on particle feeding. Interestingly, the observation of shallow water corals exuding copious quantities of organic carbon as mucus emphasized the discovery that Benson and Muscatine (1974) had made a decade before that mucus was important in the flow of energy from corals to fish in reef ecosystems.

Muscatine also led studies on the nitrogen metabolism in corals and subsequently related these to his work on carbon flux. This focus on nitrogen is of considerable interest given the conventional view that the high productivity of coral reefs was anomalous relative to the relatively oligotrophic waters that bathed them. His work on the translocation of photosynthate revealed that amino acids were among the photosynthetic products released by *Symbiodinium* (Muscatine 1967), leading to a potential mechanism which could underpin nitrogen recycling as a way of circumventing the low nutrient concentrations of sub-tropical and tropical waters (Lewis and Smith 1971). These ideas also suggested that an additional advantage of symbiosis was the recycling of nitrogen through the flow of ammonium ions in one direction (to *Symbiodinium*) and amino acids in the other (to the host), thus conserving nitrogen in the otherwise nutrient depleted tropical oceans (Muscatine and Porter 1977). Muscatine and colleagues demonstrated that corals take up and assimilate dissolved inorganic nitrogen (Muscatine and D'Elia 1978; Muscatine et al. 1979; Muscatine and Marian 1982; Wilkerson and Muscatine 1984). He was also among the first to use $\delta^{15}N$ techniques to assess nitrogen sources for corals (Muscatine and Kaplan 1994), and with Falkowski and Dubinsky, he developed a model for how carbon and nitrogen could regulate the relative biomasses of host and symbiont within coral symbioses (Falkowski et al. 1993). Central to that model was the finding that growth of *Symbiodinium* is potentially nitrogen limited and that host control of algal growth allows more photosynthate to be released to the host and/or the environment.

Coral calcification

One of the most impressive aspects of the symbiosis between scleractinian corals and *Symbiodinium* is the ability to make massive calcium carbonate skeletons and hence build the enormous reef frameworks that are characteristic of the world's coral reefs. Muscatine was drawn, as were others, to the intellectual challenges presented by unraveling how the symbiosis of corals and their algae contributed to calcification. Muscatine was consequently influenced by Thomas F. Goreau (1925–1970) whose pioneering work measured calcium deposition in the field as well as light-enhanced calcification by symbiotic corals (Goreau 1959; Goreau and Goreau 1959). Although much of Muscatine's subsequent research focused on the trophodynamics of reef corals (as described elsewhere in this Perspective) he was intrigued by the question as to the role that *Symbiodinium* might play in enhancing the biomineralization process in corals (Muscatine 1971).

Muscatine and Elsa Cernichiari found that illuminated corals acquire 2–3 times more ^{14}C in both skeletal carbonate and matrix fractions than corals incubated in the dark (Muscatine and Cernichiari 1969). They stated that "The detection of ^{14}C in components of the coral matrix may be a consequence of the relatively rapid growth rate of hermatypic corals." Muscatine and his students also went on to investigate the biochemical nature of the skeletal organic material (Young 1969; Young et al. 1971) and the morphology and physiology of calcification (Vandermeulen 1972; Vandermeulen et al. 1972) in *Pocillopora damicornis*. This work was the first to employ DCMU to test the effect of inhibiting the photosynthetic activity of intact algal-invertebrate symbioses. This led to the important discovery that light-enhanced calcification in corals depends on the

photosynthesis of *Symbiodinium* and hence was not due to other effects of light on the coral host.

Muscatine and one of his early post doctoral fellows, Vicki Pearse, investigated the role of *Symbiodinium* in stimulating light-enhanced calcification of the Caribbean coral *Acropora cervicornis*. This coral (as with most *Acropora* species) presented a paradox in that the highest calcification rates were found in areas of the colony (i.e., branch tips) which were largely devoid of symbiotic dinoflagellates. Using radioisotopes, Pearse and Muscatine (1971) discovered that the light enhancement of calcification within these tips resulted from photosynthetic products that were translocated from *Symbiodinium* populations located proximal to the branch tip. Vandermeulen and Muscatine (1974) tested the hypothesis that *Symbiodinium* enhances calcification in the light by augmenting matrix formation. They incubated *P. damicornis* in the dark with various organic products produced by *Symbiodinium* that might be precursors to matrix synthesis. These compounds, however, had no effect on the calcification rate. Prophetically, they concluded that "the next decade of research in this area will profit from more clearly defined conceptual models of coral calcification." These models for how calcification occurs in corals remain far from complete to this day; perhaps an indication of the complexity and diversity of calcification mechanisms apparent among reef-building corals and other marine calcifiers.

The next decade in Muscatine's laboratory saw two more graduate students focusing their energy on producing detailed morphological studies of skeletogenesis in *P. damicornis* (Johnston 1978, 1980) and *A. cervicornis* (Gladfelter 1983a). Ian Johnston used both transmission and scanning electron microscopy to define the relationship between coral tissue and the skeleton in *P. damicornis* and suggested a mechanism by which the production of the organic matrix mediated biomineralization (Johnston 1980). Betsy Gladfelter performed similar studies with *A. cervicornis* but was unable to detect a role for the organic matrix in driving calcification. Her study, however, described a diel cycle of crystal deposition (Gladfelter 1982, 1983b) as well as the circulation of fluids within the gastrovascular system of *A. cervicornis* (Gladfelter 1983c) which ultimately explained how *Acropora* tips gained the energy needed for fast growth while not having significant populations of *Symbiodinium* (Pearse and Muscatine 1971).

Muscatine returned to addressing questions surrounding coral calcification later in his career. Together with Denis Allemand and colleagues at the Musée Océanographique de Monaco in the late 1990s, Muscatine explored the architecture associated with calcification in corals. They clearly defined the morphology of the desmosomes which are the specialized epithelial cells responsible for anchoring the animal to the skeleton (Muscatine et al. 1997). The resulting paper was selected as "Best Paper" by the editorial board of this journal ("Coral Reefs"). Following this paper, Muscatine contributed two additional papers on coral calcification. In one, he showed with Australian colleagues that the skeletal matrix of corals could act as a store of information about the isotopic source of nitrogen in the environment (Hoegh-Guldberg et al. 2004). In a second study, he measured the stable isotope ratio (^{15}N) within the organic matrix of extant and fossil corals to investigate the origin of photosymbiosis in reef-building corals. Muscatine et al. (2005) found distinct signals within the organic matrix which allowed one to distinguish symbiotic from non-symbiotic corals, which permitted him to conclude that "symbiotic algae augment coral calcification by contributing to the synthesis of organic matrix and they may have done so as early as the Triassic."

Specificity and regulation of symbiont populations

Len Muscatine's fascination with how mutualistic symbioses achieve long-term stability can be traced to papers published around 1970. These interests led to dissection of the events that occur as symbiotic algae enter and establish populations within animal host cells. Muscatine saw a number of interesting questions within a process which somehow managed to circumvent the normal self–non-self recognition systems that normally prevent the invasion of animal cells by pathogens and other foreign entities. To become symbiotic, algae must enter the host cell and avoid digestion, integrate metabolically with the host, reproduce, and subsequently be transferred from one host to the next. Muscatine broke the problem down into a series of components and focused on experiments designed to demonstrate the nature of events in each case. Much of this work used the *Hydra-Chlorella* model and later led to studies pursued largely by his students and their students for symbioses involving *Symbiodinium* (e.g., Colley and Trench 1983, 1985; Weis et al. 2001; Rodriguez-Lanetty et al. 2006b). To a large extent, Muscatine's insights remain important in this area, especially to efforts aimed at understanding the flexibility of endosymbioses and their ability to (or not to) switch their partners following rapid changes in the environment (e.g., Baker 2002 versus Hoegh-Guldberg et al. 2002).

Muscatine's early work established that *Hydra* is selective in the uptake of symbiotic algae, with host cells recognizing only a subset of particles from the local environment (Pardy and Muscatine 1973). Intriguingly, symbiotic algae are able to evoke specific phagocytic processes from *Hydra* digestive cells (Muscatine 1974). One of the hallmarks of Muscatine's approach in this area was to combine experimental studies with structural analyses using tools

such as electron microscopy. This approach (cf. Muscatine et al. 1975a, b) resolved cellular events associated with the incorporation of the "correct" (homologous) cells into symbiotic hosts. With his students Paul McNeil and Tom Hohman, Muscatine also explored the surface properties of algae that triggered phagocytosis by the host cell. Their work using latex spheres revealed that phagocytosis by the host cells was enhanced by polyanions and highly inhibited by polycations. In addition, they manipulated polyanions and polycations on a variety of particles including freshly isolated symbionts, free-living *Chlorella* and heat killed symbionts and showed that ions affected the degree to which phagocytosis by digestive cells occurred (McNeil et al. 1981). These studies make up much of our current understanding of the first steps in the incorporation of algal symbionts into endosymbiotic associations.

In pondering the early steps of symbiosis, Muscatine also realized that there were post-phagocytic steps involved in host-symbiont recognition, such as the avoidance of digestion once a particle had been incorporated into a phagosome within the host cell. Hohman et al. (1982) found that phagosomes that contained live homologous symbionts persisted because lysosomes containing digestive enzymes failed to fuse with them. Interestingly, polycation polypeptides could override this avoidance and lead to fusion and destruction of phagosomes containing symbionts. These steps in establishing the partnership between host and symbiont were complemented by other events such as the movement of symbiotic algae preferentially to the basal portion of the digestive cells of *Hydra* (Pardy and Muscatine 1973; Muscatine et al. 1975a). These studies illustrated that a series of events, rather than simply the entry of the "correct" symbiont into a host cell, were necessary to establish populations of algae as endosymbionts.

Muscatine's thoughts about the incorporation of symbionts into host cells led him to consider a second issue related to temporal stability of mutualistic endosymbioses: the regulation of symbiont numbers within each host cell. Within the *Hydra* symbiosis, there were clearly several forces at play. Work that Muscatine undertook with student Nick Neckelmann (Muscatine and Neckelmann 1981) found that artificial increase of symbiont numbers in the host cell (by injecting algae into the host coelenteron) was followed by a reduction in symbiont numbers to control levels. This suggested that host cell and symbiont division cycles somehow interacted to regulate algal populations. Paul McAuley, working in Muscatine's laboratory, showed that non-dividing host cells with a full complement of *Chlorella* inhibited symbiont mitosis, and that this inhibition did not occur in growing host cells. Using flow cytometry to measure the DNA content of cells, McAuley and Muscatine (1986) showed that symbiotic algae are prevented from progressing all the way through mitosis in starved hosts. Similar observations were made for symbioses involving *Symbiodinium* (Smith and Muscatine 1986, 1999), which complemented work defining cell specific growth in *Symbiodinium* (Wilkerson et al. 1983, 1988; Muscatine et al. 1986). In the latter case, cell division rates were extremely low in symbiosis as compared with cultured or free-living S*ymbiodinium* (Muscatine 1989). These observations prompted Muscatine to conclude that premitotic controls (e.g., mitogenic factors, nutrients) might be more important in regulating algal numbers, especially given that evidence for post-mitotic control via digestion or expulsion of symbionts was scant (e.g., Hoegh-Guldberg et al. 1987).

Muscatine's investigations into the regulatory biology of cnidarian dinoflagellate symbiosis paralleled and reflected his interests in the nutrient and metabolic integration of these associations. For example, experiments with both *Hydra* and reef-building corals revealed that it was possible to adjust the population density of symbiotic cells by adding nutrients such as ammonium (Blank and Muscatine 1987; Hoegh-Guldberg and Smith 1989a; Muscatine et al. 1989), suggesting that the availability of nutrients played an important role in regulating the population density of symbionts in invertebrate host cells. In total, Muscatine's research in this area unveiled sophisticated and highly complex cellular processes involved in recognition, regulatory and integrative biology, reflecting high levels of specificity in endosymbioses between cnidarians and dinoflagellates.

Stability of symbioses in a time of global change

In the early 1980s, just as Muscatine was beginning to understand the factors that lead to the long-term stability of symbioses, disturbing reports of the destabilization of algal-invertebrate symbioses through widespread coral bleaching started to appear in the scientific literature. Working with Peter Glynn and Esther Peters following the 1983 coral bleaching event in the Caribbean, Muscatine examined the tissue architecture of bleached corals and collaborated on a series of manipulative experiments to evaluate whether infectious disease was involved in bleaching events (Glynn et al. 1985). Based on the inability to induce bleaching in a series of iso-, allo- and xenografts between healthy and bleached colonies, the team concluded that an infectious disease was not involved, and that bleaching was likely to be due to changes in the metabolism of corals driven by prolonged exposure to elevated temperatures associated with the El Nino. These ideas have been largely borne out by later studies (Brown 1997; Hoegh-Guldberg 1999).

Back in his laboratory at UCLA, Muscatine's students and postdoctoral fellows focused on the cellular mechanisms

responsible for the loss of symbionts when symbiotic anthozoans bleach (Steen and Muscatine 1987; Hoegh-Guldberg and Smith 1989b; Muscatine et al. 1991; Gates et al. 1992; Sawyer and Muscatine 2001). Studies undertaken by Hoegh-Guldberg and Smith (1989b) provided the experimental evidence that elevated temperature was the primary factor causing coral bleaching. Steen and Muscatine (1987) and Muscatine et al. (1991) exploited the experimental opportunities made possible by the discovery that a rapid bleaching response in the anemone *Aiptasia pulchella* was also induced by transient exposure to low temperatures. Interestingly, this discovery was made by accident while working with whole sea anemones in NMR spectroscopy. This anecdote highlights additional facets of Muscatine's scientific approach that contributed to his success; first, he read broadly outside the field and was always looking for newly emerging technologies and approaches to better address his scientific interests; and second, he was very open minded and paid attention to the unexpected, allowing observations and data to drive the direction of his science rather than the need to see his particular hypotheses proven right.

Using the rapid cold shock protocol, Muscatine and his students Grant Steen and Judy Doino demonstrated that symbionts were actively lost by exocytosis (Steen and Muscatine 1987) and that bleaching was directly related to the duration and magnitude of low temperature depression (Muscatine et al. 1991). Work with Ruth Gates and Garen Baghdasarian built on these findings by using more dynamic imaging techniques which helped to define the cellular products released by anthozoans in response to high and low temperatures. The rationale behind this work was that the nature of the products released should provide important insights into the cellular mechanisms leading to the dissociation of host and symbiont. In developing this argument, the research group introduced apoptosis, (or programmed cell death), necrosis (cell death), pinching off and host cell detachment, in addition to exocytosis as potential mechanisms that could account for symbiont loss (Gates et al. 1992). The results unexpectedly unveiled an important role for host cell detachment in the loss of symbionts from anemones and corals during bleaching. Research in this area by other groups has since demonstrated that, depending on the coral species and nature of the stressor, all of the mechanisms introduced by Gates et al. (1992) can mechanistically drive the loss of symbionts from corals (Brown et al. 1995; Huang et al. 1998; Dunn et al. 2002; Ainsworth et al. 2007).

Following up on the mechanisms underlying host cell detachment, Muscatine and his final student Sara Sawyer tested the hypothesis that dysfunction in host cell adhesion reflected thermotropic change in membrane fluidity and disturbances to calcium regulation and calcium dependent cell adhesion. After extensive work using electron paramagnetic resonance measurements of plasma membrane fluidity, coupled with dynamic imaging of intracellular calcium pools and pharmacological manipulations of intracellular calcium concentrations, the hypothesis was eventually rejected. True to form, Muscatine and Sawyer pursued an outlier in their data: treatment with caffeine that mimicked the effects of temperature by inducing host cell detachment. After confirming that the caffeine treatment had no effect on cellular calcium regulation, they used two-dimensional gel electrophoresis of ^{32}P labeled proteins to demonstrate that both temperature disturbances and caffeine altered protein phosphorylation in anemones, and that this cellular shift resulted in the detachment of host cells (Sawyer and Muscatine 2001).

The breadth and versatility of Muscatine's research program are highlighted in one additional piece of work on coral stress responses conducted during Muscatine's last years at UCLA. He and his student Calvin Nii demonstrated that the site of production of superoxide ions in temperature-stressed *Aiptasia* was in the host and not in the endosymbiont (Nii and Muscatine 1997), a finding corroborated by Levy et al. (2006) and others. These types of discoveries broadened our understanding of an important area of the biology of algal-invertebrate symbioses: the production and regulation of superoxide.

Cell biology as a critical tool for understanding algal-invertebrate endosymbiosis

Many of the questions that Muscatine and his colleagues posed 20–30 years ago are now being addressed with fast-moving molecular and genomic technologies such as genome sequencing, array studies and Expression Sequence Tag (EST) projects (deBoer et al. 2007; Kuo et al. 2004; Rodriguez-Lanetty et al. 2006a; Schwarz et al. 2006; Leggat et al. 2007). In concert with these rapid technological developments, there has been a resurgence of interest in the cellular biology of specificity and recognition after a lull of almost 30 years since Muscatine first posed questions in this areas. The application of these modern technologies to these fundamental questions is now revealing further intriguing insights into the biology of the two endosymbiotic partners. Genomic studies, for example, have revealed that cnidarians and higher metazoans (Kortschak et al. 2003; Kusserow et al. 2005) and likewise, dinoflagellates and apicomplexans, share an astonishing homology in their cellular pathways and mechanisms that are typical of other host/microbe symbioses (Leggat et al. 2007). Cellular studies of host innate immunity and the mechanisms used by symbionts to invade host cells, both during the onset of healthy symbiosis and its stress-induced breakdown (e.g., bleaching), are just

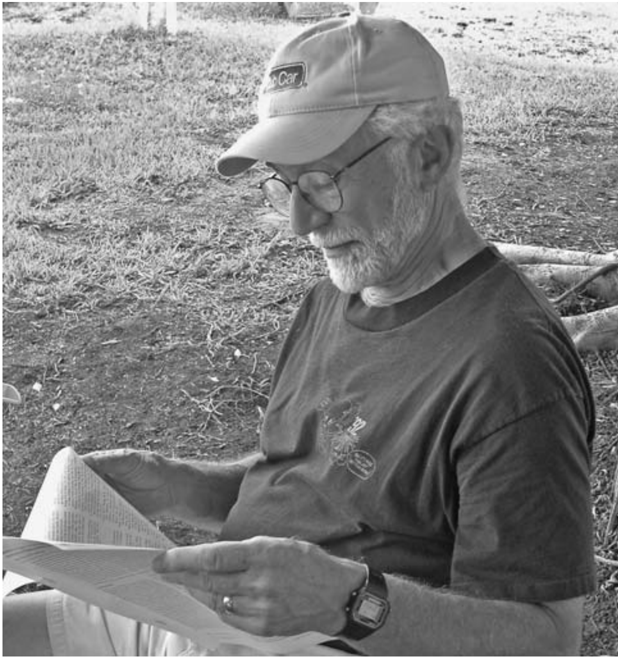

Fig. 1 Professor Leonard Muscatine enjoying a quiet moment during the Coral Reef Targeted Research workshop (http://www.gefcoral.org) in Puerto Morelos, Mexico (September 2002)

beginning, but appear set to unravel further exciting aspects of the biology of endosymbiosis (Perez and Weis 2006; Dunn et al. 2007).

In reviewing the lifetime work of Professor Leonard Muscatine (Fig. 1), the fundamental importance of understanding the cellular events involved in initiating and maintaining symbiotic associations becomes clear. The insights obtained from this level of inquiry remain crucial to any attempt to comprehend the complex biology of corals and the reefs they build. In addition, defining the cellular processes and mechanisms is central to any attempt to understand the stability of symbiosis, and hence the destabilization of coral reefs under global change. Researchers active in this area today continue to build on the strong discoveries and insights provided by Len Muscatine. In doing so, this research community has begun to unlock the secrets behind the highly successful but environmentally sensitive phenomenon of algal-animal symbiosis.

In addition to his scientific legacy, Len Muscatine also contributed extensively to the professional development of his students and colleagues. This was always done through a strong sense of humor and the use of wry reflections on life that often had import to a particular occasion or issue. He nurtured generations of students and colleagues by sharpening their critical thinking skills and their ability to articulate tractable questions. He taught his students to be critical of what they read, of how they and others did research, and also to be aware of how people conduct themselves in the context of their careers. Muscatine insisted that each student spend at least one summer in the field with him; these field experiences were usually accompanied by and enhanced greatly by his loving partner Draselle and their family of four children. His strong commitment to family, and his inclusion of students in family and social gatherings with colleagues, provided a model for his students as they became independent investigators.

Finally, while it is clear that the future will bring new and exciting discoveries in the area of algal-invertebrate endosymbiosis, we will miss the sharp mind, scientific excellence and warm personality of Len Muscatine, who will be remembered as one of our field's scientific champions. His legacy stands firm, as a foundation for the many future generations of scientists who will strive to understand one of the more fascinating areas of biology, that of the endosymbiosis between algae and invertebrates.

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