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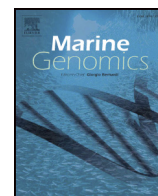SEE PROFILE

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1 Review

Circadian clocks in symbiotic corals: The duet between *Symbiodinium* algae and their coral host

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ABSTRACT

To date, the association and synchronization between two organismal circadian clocks ticking in parallel as part of a meta-organism (termed a symbiotic association), have rarely been investigated. Reef-building corals exhibit complex rhythmic responses to diurnal, lunar, and annual changes. Understanding circadian, circatidal, and annual regulation in reef-building corals is complicated by the presence of photosynthetic endosymbionts, which have a profound physiochemical influence on the intracellular environment. How corals tune their animal-based clock machinery to respond to external cues while simultaneously responding to internal physiological changes imposed by the symbiont, is not clear. There is insufficient molecular or physiological evidence of the existence of a circadian pacemaker that controls the metabolism, photosynthesis, synchronized mass spawning, and calcification processes in symbiotic corals. In this review, we present current knowledge regarding the animal pacemaker and the symbiotic-algal pacemaker. We examine the evidence from behavioral, physiological, molecular, and evolutionary perspectives. We explain why symbiotic corals are an interesting model with which to study the complexities and evolution of the metazoan circadian clock. We also provide evidence of why the chronobiology of corals is fundamental and extremely important for explaining the biology, physiology, and metabolism of coral reefs. A deeper understanding of these complex issues can help explain coral mass spawning, one of the earth's greatest and most mysterious behavioral phenomena.

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1. Introduction

Circadian clocks are endogenous systems that allow a wide variety of organisms to maintain their activity in conjunction with external environmental conditions, typically on a daily cycle and might be linked to and seasonal periodicity (Pittendrigh, 1993; Imaizumi et al., 2007; Harmer et al., 2001). Circadian rhythms are generally characterized as free-running with a periodicity of ~24 h under constant stimulus or in the absence of external cues [e.g., at constant light (LL), constant darkness (DD), or constant temperature]. Endogenous clock systems are believed to include three major mechanisms: 1) the input pathway that perceives environmental entraining, such as light and temperature; 2) the central oscillator, based on transcriptional/translational feedback loops comprised of positive and negative elements (Roenneberg and Merrow, 2005). The positive elements activate the expression of the clock genes, and the negative elements inhibit the expression of the positive elements (Bell-Pedersen et al., 2005); and 3) the output pathway, which governs the expression of genes participating in a variety of circadian-controlled processes and that are collectively known as clock-controlled genes (CCG) (Mittag, 1996; Somers, 1999; Johnson and Golden, 1999; Bell-Pedersen et al., 2005; Quecini et al., 2007) (see Fig. 1). The structure of the feedback loops was found to be a common mechanism in all investigated eukaryotic organisms (Dunlap et al., 2004); however, the core molecular components recruited to form the clock are less known and vary among organisms (McClung, 2006; Edgar et al., 2012). Therefore, there is apparently no common core set of clock components across phylogenetic kingdoms, suggesting that daily timekeeping evolved independently within different lineages. Orchestration of the endogenous clock with the surroundings provides many advantages and enables organisms to anticipate and prepare their metabolic, behavioral, and physiological states for future changes, such as light/dark (LD) cycling, changes in the light spectra or ambient temperature, and alterations in nutrient availability. Many advantages of an environmentally synchronized endogenous circadian clock have been demonstrated in organisms, ranging from prokaryotes, such as Synechococcus (Ouyang et al., 1998), to mammals (Panda et al., 2002). During the past two decades, extensive research has greatly improved our understanding of the transcription/translation feedback-loop mechanism that controls the endogenous circadian clock and has identified the molecular components of several model organisms, such as cyanobacteria (Ishiura et al., 1998; Ditty et al., 2003), Neurospora (Lee et al., 2000), Drosophila (Glossop et al., 1999), Arabidopsis (Alabadi et al., 2001; Schaffer et al., 2001), and mammals (reviewed in Reppert and Weaver, 2002).
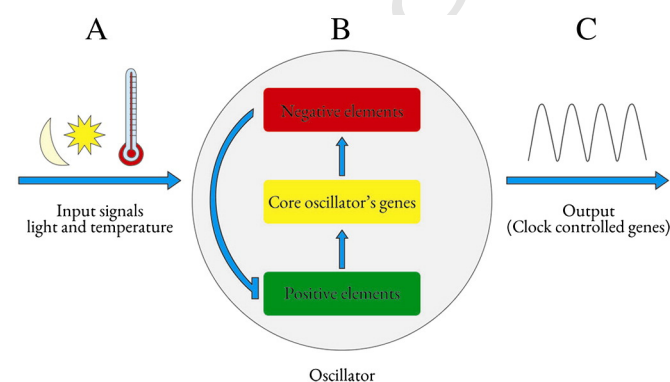

Fig. 1. The circadian clock system. The circadian clock includes three components: (A) input pathways, regulating the oscillator response to various external stimuli, such as light and temperature; (B) an oscillator, which generates and sustains rhythm, the oscillator − the molecular core architecture composed positive and negative elements, which form the feedback loop and maintain a 24 h cycle. The oscillator is responsible for governing the expression of genes participating in a variety of circadian-controlled processes and known as clock-controlled genes (CCGs). (C) Output pathways, which convey rhythmic information from the oscillator to other physiological systems.

2. Symbiotic corals as known model organisms with which to study circadian-clock evolution

Corals belong to the class Anthozoa within the phylum Cnidaria and include 6100 species. Scleractinian corals constitute the most abundant order of polypoidal (polyp-forming) marine invertebrates, which mainly populate shallow water in tropical and subtropical reefs. Scleractinian corals provide the framework for the entire coral-reef ecosystem and create one of the most massive biogenic structures in the world, generating shelter and food for a great number of fish and invertebrate species (McAllister, 1991; Reaka-Kudla, 1997, 2001). The continued success of scleractinian corals is due to the presence of symbiotic dinoflagellate algae, primarily Symbiodinium spp., in their tissues. Symbiodinium are located in vacuoles (symbiosomes) within the endodermal cells of the coral that line the gastrovascular cavity (Stat et al., 2006). The mechanisms that enable the anthozoan host to recognize Symbiodinium are not well understood, but likely include the recognition of specific host–symbiont combinations by certain glycoprotein patterns on the surface of the algal cell, with colonization and tolerance of the symbiont's presence by the subsequent downregulation of the host's immune system (Weis, 2008). Symbiodinium provide their coral hosts with energy. Both the host and its symbionts benefit from this interaction: the coral receives photosynthetic carbon products from the algae and, in turn, provides the symbionts with essential nutrients, such as nitrogen and phosphorus compounds (Yellowlees et al., 2008).

To date, molecular research in chronobiology has focused almost exclusively on models of terrestrial organisms. However, we still do not know the molecular components of the circadian clock of most eukaryotic marine organisms. The significance of the marine milieu to chronobiology research is tremendous, as the marine environment is home to 14% of all known species. The marine environment also represents the most ancient ecosystem, and organisms there have adapted closely to its rhythms over millions of years (Tessmar-Raible et al., 2011). Another unique characteristic of the marine environment is that it is governed by many cycles influenced by the position of the sun and the moon. In contrast, most terrestrial model organisms display circadian and seasonal rhythms that are controlled almost exclusively by the sun (Tessmar-Raible et al., 2011). The marine environment is governed by diverse rhythms, such as the day/night cycle, the tides, the lunar/semi-lunar cycles, and the seasonal cycle. The influence of the moon on marine-organism physiology and behavior affects a wide range of phenomena, including the influence of lunar cycles on gonad maturation and spawning synchronization. In addition, tidal cycles affect the motility and behavior of many invertebrates living in the tidal zone. As residents of the marine environment, corals are known to be influenced by several periodic cycles that are determined by the sun and moon. Additional rhythms in corals are linked to factors such as salinity, current, and temperature. The endosymbiotic Symbiodinium in scleractinian corals are exposed to the same environmental oscillations as their host, allowing the light/dark cycle to dominate the metabolic rhythm of the algae.

Theories addressing the origin of the circadian clock suggest corals as a relevant system to study (Reitzel et al., 2010). Firstly, scleractinian corals belong to an early branching metazoan lineage, thus, their study can offer insights not only into the origins of the circadian molecular machinery that regulates temporal patterns in cnidarians, but also into how circadian rhythms operated in the ancestral eumetazoan lineage. Secondly, reef-building corals are also fascinating because of the tightly coupled symbiosis with their endosymbiotic algae. These algal symbionts can cause major diurnal changes to their cellular environment based on the influence of oxygen tension, pH, and other variables. While we can examine circadian clocks in each organism independently, taking on the challenge of inspecting coral and Symbiodinium clocks simultaneously promises to shed light on new areas of chronobiology. Symbiosis between corals and algae requires metabolic synchronization and coordination as well as "time sharing" between parallel processes in the host and the symbiont (Fig. 2). The optimization of this interaction
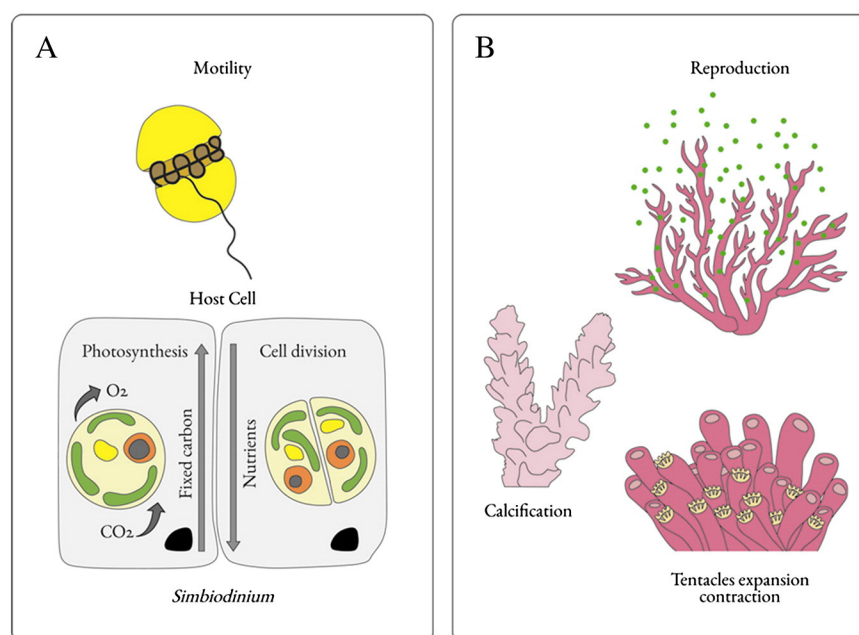
Fig. 2. Known rhythmic outputs that were observed among corals and their symbiotic algae. A — Diel rhythms were observed for *Symbiodinium* photosynthesis and cell division in culture and in symbiotic form, while cell motility possesses a diel rhythm in culture. B — Some coral species possess diel phenotypic rhythm of tentacle expansion/contraction, diel and annual rhythm for coral calcification, and circalunar and circannual behavior of coral reproduction. The interaction between the two clocks (of the host and of the symbionts) is not clear yet.

may have driven the evolution of an internal pacemaker (Reitzel et al., 2013). To date, the unique association/synchronization relationship between two circadian clocks in one single meta-organism (Bosch and McFall-Ngai, 2011) (a symbiotic association) ticking in parallel has hardly been investigated, with the exception of squid–vibrio symbiosis (Wier et al., 2010; Rader et al., 2012). There is not yet sufficient molecular and physiological evidence to conclusively demonstrate the existence of a dual circadian pacemaker that controls organism metabolism, algal photosynthesis, and the coral calcification process in a synchronized fashion. This review aims to present the current knowledge regarding the physiological, behavioral, and molecular aspects of coral rhythmicity in terms of both the host and its algal symbionts. We discuss whether the rhythms depend on exogenous cues or endogenous mechanisms.

It has become increasingly apparent that symbiosis is a ubiquitous trait of multicellular taxa, which rely on microbial symbionts to meet certain physiological requirements that could not be met otherwise (Bosch and McFall-Ngai, 2011). A relevant question is, therefore, how symbiotic organisms measure time and synchronize themselves to their environmental conditions. Of particular relevance is how two pacemakers, the host and the symbiont, function together in one organism. It is also important to understand how the interactions between these two clocks mutually increase organismal survivorship. Our goal herein is to address these important questions in symbiotic systems using the scleractinian–*Symbiodinium* symbiosis as a target model.

3. What is known and not known about coral rhythms

Corals are known to possess several rhythmic phenomena, but it is not yet clear whether most are directly cued by environmental signals (such as light/dark cycles) or by endogenous circadian clocks. These phenomena include calcification, tentacle expansion–contraction, and reproduction.

3.1. Coral calcification

Calcification is a critical process in the growth and stabilization of the coral-reef structure. The calcification process of corals is assumed to be rhythmic because the coral skeleton contains high-density bands and low-density bands. These skeletal bands are correlated with an annual periodicity (Neville, 1967). In addition to the annual records kept in coral skeletons, finer bands, which seem to correlate with a daily periodicity, can also be observed (Risk and Pearce, 1992; Gill et al., 2006). Fossil corals (not scleractinians) from 350 to 400 million years ago show between 365 and 410 daily growth lines per annual ring, and these can be used as geochronometers (Wells, 1963). Among these fossil corals are tabulates and rugosans, while there is some evidence of corals from the Triassic period, known as modern corals or scleractinian corals, which have been extensively documented since the mid-Ordovician period. There is significant empirical information available on the growth rates of recent forms of these organisms but very little on the link between the endogenous clock that control their growth (Risk and Pearce, 1992). Astronomers generally agree that while the period of the earth's revolution around the sun has been constant, its period of rotation on its polar axis (currently 24 h) has not been constant throughout the earth's history. Indeed, a deceleration of approximately 2 s per 100,000 years is estimated to occur and can be attributed to the dissipation of rotational energy by surface and internal tidal forces. It, thus, appears that day length has been increasing throughout geological time and the number of days in the year has been decreasing. At the beginning of the Cambrian period, day length would have been 21 h (Wells, 1963). Similarly, the monthly cycle during the Devonian period has been estimated to be 30.6 days based on fossil coral lines (Scrutton, 1965). The calcification process is energy-consuming, and at least some of the energy is used at the expense of symbiont photosynthesis (Pearse and Muscatine, 1971; Barnes and Chalker, 1990; Furla et al., 2000). Calcification is also light-dependent (i.e., modulated by the corals photosynthetic symbionts), and it is known that symbiotic corals calcify much faster in light than in darkness. Corals that have lost their symbiotic algae calcify at slower rates (Kawaguti and Sakumoto, 1948; Goreau, 1959; Goreau and Goreau, 1959). These experimental observations led to the knowledge that light enhances calcification. Al-Horani et al. (2003) showed that light triggers calcium uptake; although the photosynthetic process supplies energy, it is not the driving force of calcification. Chalker (1977) showed that calcification in the coral *Acropora cervicornis* occurs rhythmically under a light/dark cycle and has a diel pattern of 24 h under constant darkness. However, calcification can proceed in the dark for a short period of time due to the internal

metabolic sources of the coral. Changes in calcification rates of *Galaxea fascicularis* during the day have been observed under constant laboratory conditions, with a constant spectral composition and constant light intensity (Al-Horani et al., 2007). These observations indicate that calcification is likely to be controlled by the endogenous circadian clock, although more solid evidence is needed. In contrast, another study found no changes in the calcification rate of *Stylophora pistillata* coral under laboratory conditions, and the rate of calcification was instead observed to be constant (Moya et al., 2006). Therefore, the study by Moya et al. (2006) indicates that calcification appears to depend only on light and is not regulated by the endogenous clock. Levy et al. (2011) showed that at the transcriptome level, the α-type carbonic anhydrase cluster potentially underlies the phenomenon of light-enhanced coral calcification. Putative scleractinian biomineralization genes, known as SCRiPs, have also shown differential gene expression in the corals *Orbicella faveolata* and *Acropora millepora* that seem to correlate with calcification processes in different developmental and physiological states on a daily cycle associated with the onset and breakdown of symbiosis (Sunagawa et al, 2009; Reyes-Bermudez et al., 2009). The question of whether calcification is light-dependent/gated or controlled by an endogenous clock and whether it relies on the circadian rhythm of photosynthesis, remains open. More information at the molecular level will assist in elucidating the mechanisms underlying calcification rhythms.
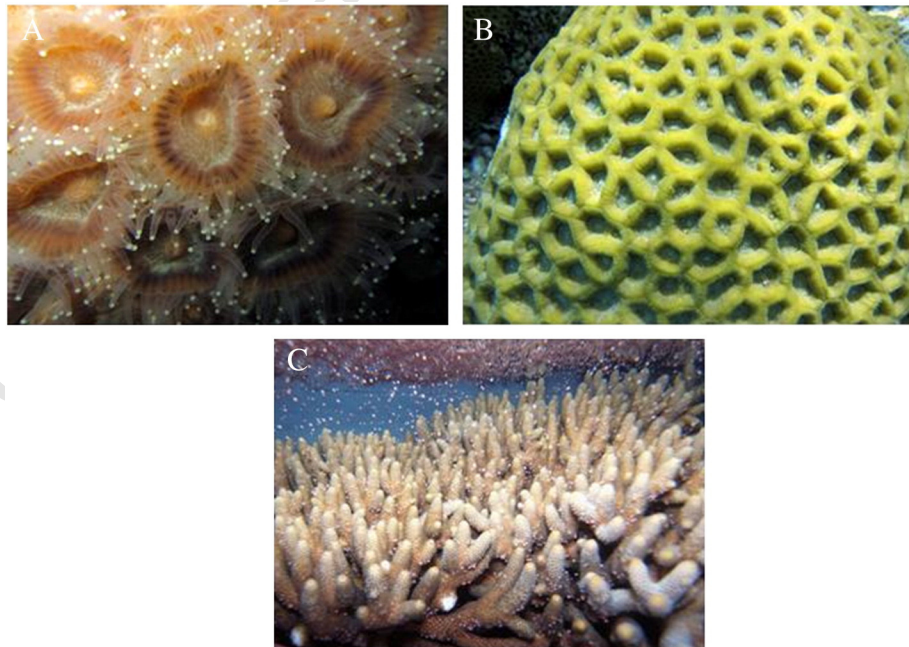
3.2. Tentacle expansion and contraction

Corals expand their tentacles to capture prey. This behavior occurs mostly during the night, although in some corals, the tentacles expand during the day or at all times when they are not spawning (Abe, 1939; Wells, 1966; Porter, 1974; Sebens and Deriemer, 1977; Lasker, 1979). Differences in prey availability, photosynthetic activity, and the concentration of symbionts within the tentacle are responsible for the fact that some species of coral exhibit diurnal rather than nocturnal activity (Vareschi and Fricke, 1986; Levy et al., 2001, 2003). In some of the investigated species of *Fungia*, tentacle behavior followed a diel rhythm both under ambient light and in continuous darkness (Sweeney, 1976). Hoadley et al. (2011) observed a rhythmic pattern, with tentacle contraction during the day and expansion during the night in the coral *Favia fragum*, but they observed a diminished rhythmicity of the tentacles under constant light. These phenomena were investigated in other Cnidaria and were found to be caused directly by light (Taddei-Ferretti and Musio, 2000; Hendricks et al., 2012). The expansion–contraction behavior of tentacles (Fig. 3A, B) is still poorly understood and, based on inconsistencies in the research, is likely to be species-dependent. This phenotypic behavior can be induced by different factors in different coral species. Factors including the availability of prey, the presence of monochromatic light, and the availability of currents (Levy et al., 2001), can mask circadian behavior. One recent paper demonstrated that net photosynthesis in the soft coral *Hallaxa fuscescens* is higher during the coral's pulsation phase than during its rest phase (Kremien et al., 2013), which may imply that the symbionts control tentacle rhythms in symbiotic corals.

3.3. Coral reproduction: it's all about the timing

Extensive research in the last several decades has greatly increased our data on scleractinian and other coral reproductive processes from a broader geographic range. One of the most important features of coral reproduction is its timing. Timing is essential for both reproductive strategies in corals, broadcast spawning, and brooding, not only for successful fertilization but also for the larvae to find a suitable place to settle and undergo metamorphosis. The timing for the release of gametes and larvae often varies geographically within a given coral species. This variation is likely due to the responses of different populations to varying environmental conditions (Oliver et al., 1988; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Babcock et al., 1994; Richmond, 1997; Loya, 2004). Mass-spawning events occur when many coral species spawn simultaneously. Those events are especially well known for two coral reefs, the Great Barrier Reef (GBR) and Western Australia reefs (Houtman Abrolhos Islands and Ningaloo). The biggest mass-spawning event occurs in the GBR, when almost 130 coral species spawned together in remarkable coordination (Harrison et al., 1984; Willis et al., 1985; Babcock et al., 1986) (Fig. 3C). A smaller-scale mass-spawning event occurs in Western Australia (Simpson, 1991). Other mass-spawning events occur at smaller scales (medium-scale events) and involve the combined spawning of many different species. The Gulf of

Fig. 3. A, B — Coral phenotypic rhythmic behavior showing tentacle expansion/contraction in the symbiotic coral *Favia favus*, Gulf of Aqaba, Red Sea. Tentacles usually expand after dusk and contract after sunrise (pictures by O. Levy). C — Colonies of the coral *Acropora millepora* synchronously release bundles of eggs and sperm during mass-spawning events during nighttime five days after November full moon in the Great Barrier Reef (GBR), Australia (picture by O. Levy).

Mexico and the Caribbean region are well known for their multi-species spawning events (Gittings et al., 1992; Hagman et al., 1998; Beaver et al., 2004; Bastidas et al., 2005; Harrison and Booth, 2007). Medium-scale synchronization was also observed in Japan, Taiwan, the Philippines, Singapore, Indonesia, Papua New Guinea, Solomon Islands, French Polynesia, the Egyptian Red Sea, and several other sites (review in Harrison, 2011). In contrast to these events, other reefs show little, if any, reproduction synchronization among species. Reefs without synchronization between different species include those in the north Red Sea, the Central Pacific, Hawaii, and Okinawa. Synchronized spawning within species has the advantages of increasing the chances of fertilization, cross-fertilization, and genetic mixing between colonies. The advantage of mass-spawning events is the reduction of the levels of overall predation, while the disadvantages of synchronized mass spawning include the possible formation of non-viable, inter-species hybrids (Willis et al., 1985; Oliver et al., 1988).

How can synchronization differences among different species and within the same species at different sites be explained? Reproduction in corals is influenced mainly by three types of time cycles. The first, the annual/seasonal rhythm, is mainly related to seasonal variations in sea temperature. Changes in sea temperature are most likely involved in triggering egg and sperm maturation in scleractinian corals (Yonge, 1940; Kojis and Quinn, 1981; Harrison et al., 1984; Stoddart and Black, 1985; Willis et al., 1985; Babcock et al., 1986). The second cycle is a monthly lunar rhythm, which is involved in coordinating the timing of mass-spawning events. Tidal cycles are important, and it is common for corals to spawn during low-amplitude neap tides (Oliver et al., 1988; Simpson, 1991; Babcock, 1995; Mendes and Woodley, 2002); tidal cycling is also correlated with cues from the lunar phase and the amount of visible moonlight (Jokiel, 1985). The third cycle involved in the timing of the gamete release is the diel light cycle, which acts as a zeitgeber ("time giver") and induces spawning to occur after a precise period of darkness (Harrison and Wallace, 1990).

The mechanism of synchronized mass spawning is still considered enigmatic, and many other factors have been suggested as possible influences on the timing of coral reproduction. The proposed factors include sea temperature (Glynn et al., 1991; Hayashibara et al., 1993), tidal changes (Wyers et al., 1991), darkness levels (Harriott, 1983; Babcock et al., 1986; Hunter, 1989), a near-zero solar insolation derivative (van Woesik et al., 2006), wind-pattern changes (van Woesik, 2009), food availability (Fadlallah, 1984), rainfall levels (Mendes and Woodley, 2002), moonlight availability (Guest et al., 2002), twilight chromaticity (Sweeney et al., 2011), and salinity (Jokiel, 1985). However, the question of how and whether endogenous circadian mechanisms are involved in coral reproduction synchronization remains open. Controlled laboratory experiments may provide an answer. Many researchers have demonstrated the influence of light on spawning rhythm. Jokiel et al. (1985) kept *Pocillopora damicornis* corals under constant full-moon light conditions and constant new-moon light conditions, and found that the synchronization of their monthly larval production was disrupted. Some published evidence (Babcock, 1984, 1988; Hunter, 1989) indicates that coral spawning times may be shifted by an early artificial sunset cue given a few days prior to spawning. Recently, Brady et al. (2009) showed that in the coral *Montastraea franksi*, shorter, controlled light cycles on the day of spawning without previous adaptation caused the coral to spawn earlier. These results support the theory that spawning is not controlled by a circadian endogenous mechanism but is instead directly controlled by local solar light cycles, at least for the precise tuning to the day and hour. Boch et al. (2011) analyzed the contributions of separate components of light dynamics (spectral changes of moonlight during twilight, decreasing twilight-intensity rate, and lunar photoperiod changes), since the effects of twilight and lunar skylight on coral spawning synchrony have been controversial. The results with *Acropora humilis* coral under controlled conditions show that lunar photoperiod cues are most likely the major driver of synchronized spawning on a given night, whereas far-reaching differences in the spectral dynamics have secondary effects on spawning. Whether synchronized spawning is environmentally gated or controlled by endogenous mechanisms, remains unknown, and will be discussed below. Understanding how corals perceive and integrate information about environmental factors in order to regulate their reproductive cycles will require further behavioral and molecular research.

3.4. Molecular knowledge regarding the circadian clock in corals

Despite extensive research in the past few years, there are many gaps in our knowledge regarding the perception of external signals, the molecular components (genes and proteins), and the mechanism of circadian-clock feedback loops in corals. Gorbunov and Falkowski (2002) suggested that detection of the blue region of the spectrum of moonlight might cue the specific night of spawning in corals because several species were found to be sensitive to this spectrum. Molecular research on the coral *A. millepora* revealed a family of cryptochrome genes with high similarity to their vertebrate counterparts. Coral *cry1* belongs to the mammalian-type (m-type) CRY group. Both *cry1* and *cry2* are only distantly related to the *Drosophila*-type CRYs. While *cry2* more closely resembles the *Danio* photoreceptor candidate *cry4* type and was found to exhibit rhythmic transcript expression only under ambient LD cycles but not under DD conditions (Levy et al., 2007), it may induce an intrinsic clock on a monthly basis since its expression increases on full-moon nights compared to new-moon nights (Levy et al., 2007). These findings suggest that the cryptochromes may act as photoreceptor genes and can mediate environmental signals, such as moonlight, in synchronizing the central pacemaker (Levy et al., 2007). In contrast, Hoadley et al. (2011) did not find a correlation between the elevated expression of the cryptochrome family members and the precise day of spawning in the brooder coral *F. fragum*. Bioinformatic analysis has shown that the orthologs of many core circadian genes in mammals and *Drosophila* are present in the basal metazoan phylum Cnidaria, specifically in the coral *A. millepora* and in the sea anemone *Nematostella vectensis* (Reitzel et al., 2010; Vize, 2009). After the recent sequencing of the *Acropora digitifera* genome (Shinzato et al., 2011), a genome-wide survey identified many photoreceptors and circadian genes based on sequence homology to the mammalian and *Drosophila* orthologs (Shoguchi et al., 2013). The *clock* and *cycle* genes are the most conserved core circadian genes found in bilaterian animals and serve as positive elements in a feedback loop. These genes were identified in cnidarians (Reitzel et al., 2010; Brady et al., 2011; Hoadley et al., 2011). Although present in some bilaterian animals, cnidarians lack the *timeless* and *period* genes in their feedback loop (Reitzel et al., 2010; Brady et al., 2011; Hoadley et al., 2011; Shoguchi et al., 2013). Quantitative PCR of core circadian genes in the coral *F. fragum* revealed a diel rhythm in the mRNA that was abundant during the LD cycle of *cry1*, *cry2*, and *clock* genes but absent in the rhythm for *cycle*. The rhythm was absent for all of the genes under DD conditions (Hoadley et al., 2011). Brady et al. (2011) showed that in larvae of the coral *A. millepora*, *clock*, *cry2*, and *cycle* demonstrated diel patterns under 24 h of both LD and DD conditions, whereas *cry1* lost its rhythmicity under DD conditions. Recent work by Levy et al. (2011) revealed complexities in the diurnal changes of gene expression in the coral *A. millepora* using cDNA microarrays. Coral colonies under both LD and DD conditions yielded rhythmic transcripts. Clustering analysis of the LD and DD data identified genes possessing similar cellular pathways with the same temporal expression patterns. Among these rhythmic expression groups were genes that were rigorously light-coupled, including the coral cryptochromes, which were expressed under conditions of maximum illumination (typically at midday). Another cluster of genes, also expressed at midday, was identified as antioxidant genes associated with the presence of extreme oxygen tension in the coral tissue due to the high rate of photosynthesis of the endosymbiotic algae. Levy et al. (2011) also showed that in corals, there is a nighttime preference for

DNA replication and that it is rhythmic and light-dependent, occurring only under LD cycles. The differences in the expression of core circadian clock genes among different coral species will require much more research.

4. *Symbiodinium* rhythms

Symbiodinium, the symbiotic dinoflagellates that live within the coral tissues, are considered to be responsible for the flourishing of the reef system. Free-living dinoflagellates have been well studied for their diel/circadian rhythms of photosynthesis, motility, cell division, UV sensitivity, and bio-illumination (Hastings, 2007). Corals control a few aspects of the life cycles of their symbiotic algae, such as limiting growth rate by preventing translocation from the host of essential nutrients for algal cell cytokinesis (Smith and Muscatine, 1999) or maintaining symbionts in a non-motile state (Stat et al., 2006). Diversion of photosynthates from *Symbiodinium* towards the host is executed through putative 'host factors' (chemical agents) that are found in host homogenates (Gates et al., 1995, 1999). These factors, identified as a group of free amino acids, induce the release of fixed carbon by freshly isolated symbionts from several cnidarian hosts (Gates et al., 1995, 1999; Cook and Davy, 2001). Host factors include taurine (a non-protein-free amino acid) (Wang and Douglas, 1997) or other chemical compounds (Withers et al., 1998). There is still substantial uncertainly regarding the nature and the composition among species. It is of interest to understand the influence of the rhythmic behavior of *Symbiodinium* within coral tissues compared to free-living *Symbiodinium* under culture conditions.

4.1. Symbiodinium cell division and motility

Symbiodinium have been tested for the circadian control of cell division (Fitt and Trench, 1983), and for circadian rhythms in motility (Fitt et al., 1981; Yacobovitch et al., 2004). Cultured *Symbiodinium* under normal LD-cycle conditions undergo karyokinesis, cytokinesis, and motility according to a diurnal pattern. Under LL or DD conditions, the patterns of cytokinesis and motility are circadian and maintain the rhythm in the absence of external signals. These patterns are evident for up to seven days in constant dim light but tend to dampen out with time; under conditions of darkness, the rhythm tapered out after approximately the same time (Fitt and Trench, 1983). The fact that non-motile cells are seen in stationary cultures (dense culture, net growth = zero) of free-living *Symbiodinium* may imply that *Symbiodinium* do not develop flagella in high concentrations in the same way as in the coccoid non-motile stage, which predominates in the host tissues. Alternatively, it may be that flagella are synthesized during cell division in the host but are soon lost (Trench, 1979, 1980; Schoenberg and Trench, 1980). Although some research has reported the presence of motile *Symbiodinium* in freshly extruded pellets (Ricard and Salvat, 1977), the influence of the host on motility is not yet clear.

### 4.2. Symbiodinium photosynthesis

Many photosynthetic organisms demonstrate a diel photosynthetic rhythm, which is also maintained under constant light conditions. In some organisms, such as the dinoflagellate *Lingulodinium*, the diel rhythm can be maintained under constant light for periods of weeks (Hastings et al., 1961). The continuation of a diel rhythm under conditions of constant artificial light, combined with the rhythmic behavior under LL conditions, supports the hypothesis that an endogenous circadian clock controls photosynthesis. Many photosynthetic regulatory processes have been investigated in *Symbiodinium*, including oxygen evolution, PSII quantum yield (Sorek and Levy, 2012a,b; Sorek et al., 2013), carbon assimilation (Chalker, 1977), and pigment content (Sorek et al., 2013). Oxygen evolution under LL conditions followed diel oscillation, with almost the same rhythm per cycle of LD; this

cycling lasted for more than four days both in the culture and inside the coral tissues (e.g. diel rhythm of 48 h under LD and LL see Fig. 4). The PSII yield was also observed in both models and found to possess a diel rhythm under LL conditions. At the molecular level, photosynthetic circadian rhythms were revealed in the gene OEE1 (oxygen evolving enhancer 1), suggesting a diel rhythm under LD and LL conditions (Sorek et al., 2013). Although photosynthesis is rhythmic both in cultured symbionts and within the coral host, it is still unclear how the circadian mechanisms in coral are affected by the symbionts and whether/how the ability of the algae to maintain circadian rhythms under constant light affects coral molecular regulation and behavior.

4.3. Molecular aspects of the circadian clock in Symbiodinium

Unlike the well-studied circadian clock of *Arabidopsis* and cyanobacteria, little is known about the mechanism in other photosynthetic lineages. Given that photosynthesis in eukaryotes has evolved through multiple secondary endosymbiotic events, the attempt to elucidate the circadian clock mechanisms in multiple, independently evolved alga lineages can fill a void in our knowledge. In turn, such information would help us identify whether there is a core circadian clock as well as core clock-controlled genes among photosynthetic eukaryotes. Two major models, *Chlamydomonas* (green algae) and *Lingulodinium* (dinoflagellate), have revealed that circadian clocks control various phenomena, but the mechanisms and genetic components remain unknown. The putative genes of the circadian clock in the green unicellular algae *Ostreococcus* (green algae) were recently characterized by Corellou et al. (2009). Photosynthetic algae reveal details of the evolution of circadian clocks in plants, and two master components of the plant circadian clock, *toc1* (timing of Cab expression 1) and *cca1* (circadian clock associated 1), were found to be part of the main feedback loop in *Ostreococcus*. By evaluating the global transcripts of unicellular algae and dinoflagellates, many groups of clock-controlled genes were identified as having circadian rhythms in terms of their photosynthesis, metabolism, cell cycle, DNA, protein, and lipid biosynthesis (Okamoto and Hastings, 2003; Van Dolah et al., 2007; Monnier et al., 2010). Using cultured *Symbiodinium microadriaticum*, clade A1 (KB8), microarray containing 853 features, we successfully showed that 30.1% of the genes possess diel oscillation patterns during the LD period, when the peak is two-fold more than in the other time points.

These genes are made up of three groups with different expression patterns that were identified using SOM (self-organization map)
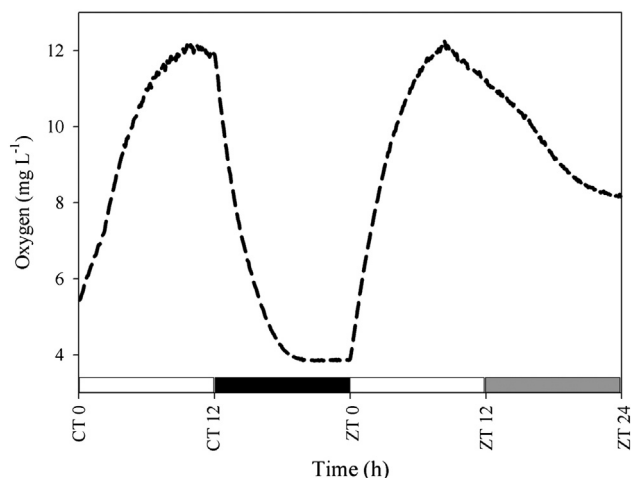
Fig. 4. Oxygen evolution during the LD cycle and LL cycle in *Stylophora pistillata* coral under 100 μmol quanta m^{-2} s^{-1} at 22 °C. The oxygen concentration was measured with a four-channel oxygen meter (OXY4) equipped with four optical oxygen sensors (OPTODs; PreSens, Germany) connected to four dipping probes (DP-PSts). White bars indicate the light period, black bars indicate the dark period, and gray bars indicate subjective night under constant light (for each time point, N = 4).
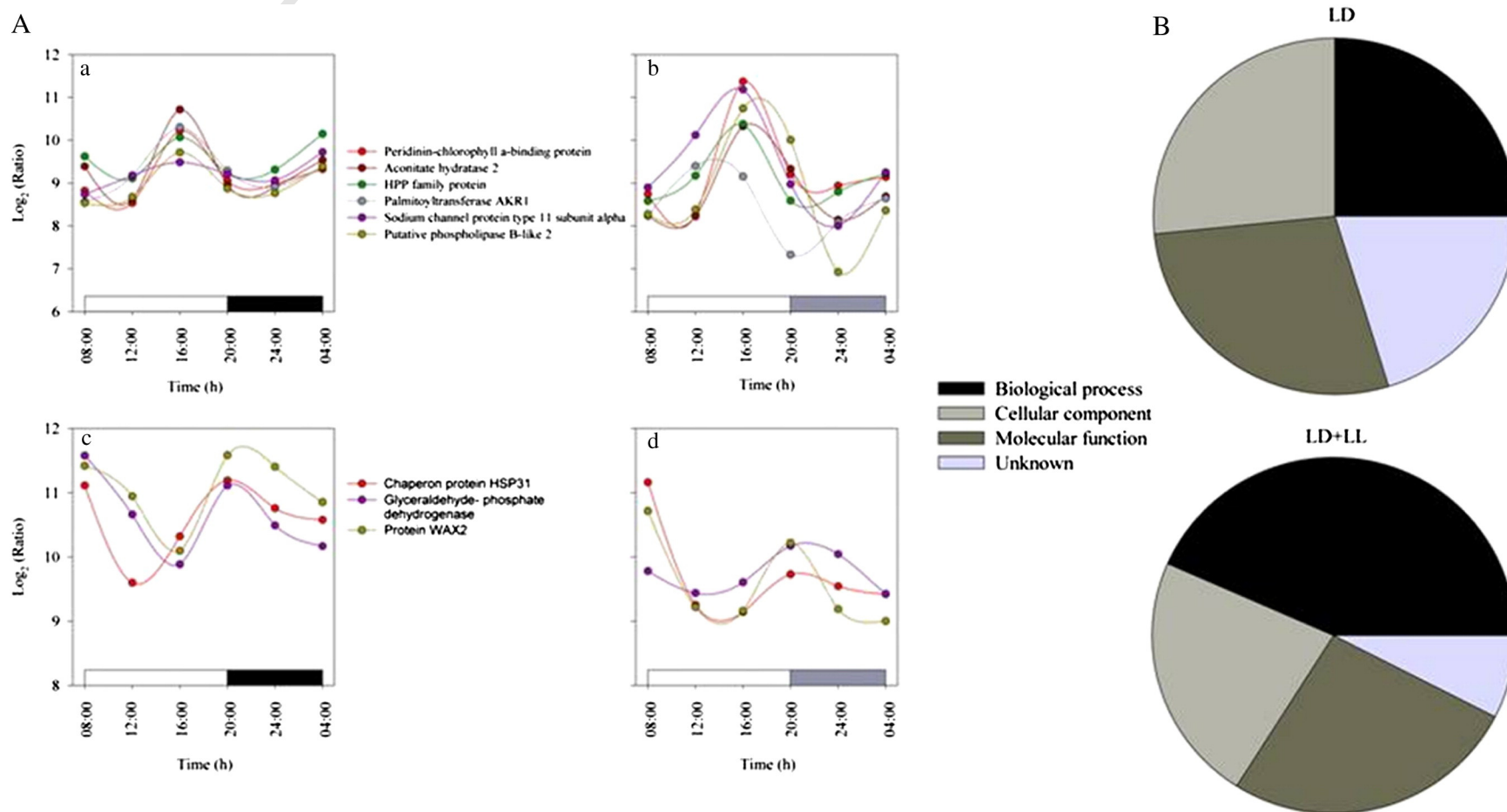
Fig. 5. A — Representative genes identified by microarray analysis with circadian oscillation that peaked at 16:00 (8 h of light exposure) in both LD and LL. White bars indicate the light period, black bars indicate the dark period, and gray bars indicate subjective darkness. B — Gene ontology divided the genes possessing diel rhythms into four categories: molecular function, biological process, cellular structure, and unknown function. Upper panel — genes with a diel rhythm under LD. Lower panel — genes with a diel rhythm under both LD and LL.

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clustering. Of the identified genes, 7.1% were found to have daily patterns under both LD and LL conditions (Fig. 5, Table 1). These genes are most likely under circadian control. The small number of annotated genes from our list of differentially expressed genes does not allow us to reach any conclusions regarding the diel control of biochemical pathways in *Symbiodinium*. Microarray studies on *Arabidopsis* (Schaffer et al., 2001; Harmer et al., 2000) have revealed that 11% of genes have daily oscillation patterns under LD conditions. However, circadian rhythms under both LD and LL conditions were observed for only 2% of the genes. Similar numbers were found for *Chlamydomonas* (Kucho et al., 2005). For other dinoflagellates (Van Dolah et al., 2007), approximately 9.8% of the genes were found to oscillate under LD conditions, and the circadian rhythm (LD + LL)-controlled genes accounted for approximately 3–3.3% of the total genes (Van Dolah et al., 2007; Okamoto and Hastings, 2003). Our research revealed that over a third of the genes in *Symbiodinium* (30.1%) have diel oscillations during the LD period from the microarray containing 853 features. This number is high when comparing to what was found in other plants and algae but, at the moment, it is still preliminary estimation due to a low number of expressed sequenced tags available on the array. Although these preliminary results showing high levels of transcriptional processing found in the *Symbiodinium* they are consistent with the results of other studies on dinoflagellate circadian rhythms (Van Dolah et al., 2007). Although we can conclude that there are genes under circadian control in different photosynthetic eukaryotes, due to the paucity of data and the deep phylogenetic divergences of the organisms for which we have data at hand, we cannot determine whether these circadian-regulated genes and gene networks are homologous across the eukaryotic tree.

5. How do the host and symbiont clocks operate in concert?

Extensive work on the circadian system of corals and their symbiotic algae contributes to our understanding of how these systems use internal pacemakers to associate environmental cues with an array of distinct behavioral, physiological, morphological, and molecular responses. Much less is known about the possible interplay of the two endogenous clocks ticking in one organism. The few published studies dealing with the rhythm of symbiosis have mostly focused on human gastrointestinal microbes (Barnett et al., 2004; Guerfali et al., 2008),

Table 1
List of genes with diel rhythm during LD and LL.

Peak	GO IDs	New annotation	Uniseq	Clone
08:00	–	–	CAXB1808.b2_c_s	CAXB1808
08:00	–	–	CAXB1178.b2_c_s	CAXB1178
08:00	?	?	?	CAXB1689
08:00	GO:0005783; GO:0016021; GO:0005886; GO:0005774; GO:0005506; GO:0016491; GO:0006723; GO:0055114; GO:0048235; GO:0010025	s:Q8H1Z0; Protein WAX2	Contig403	CAXB462
08:00	?	?	?	CAXB736
08:00	GO:0020015; GO:0004365; GO:0051287; GO:0006096; GO:0055114	s:P22513; Glyceraldehyde-3-phosphate dehydrogenase, glycosomal	CAXB1281.b2_c_s	CAXB1281
08:00	–	t:Q5ENK4; Putative uncharacterized protein (Fragment)	CAXB2415.b2_c_s	CAXB2415
08:00	?	?	?	CAXB1179
08:00	–	–	CAXB1392.b2_c_s	CAXB1392
08:00	–	–	CAXB1774.b2_c_s	CAXB1774
08:00	GO:0005625; GO:0008233; GO:0005515; GO:0006950	s:Q04432; Probable chaperone protein HSP31	CAXB1786.b2_c_s	CAXB1786
08:00	?	?	?	CAXB2501
08:00	?	?	?	CAXB446
08:00	GO:0055085	t:B5Y4E0; Predicted protein	CAXB587.b1_c_s	CAXB587
08:00	GO:0016021	s:Q0VCH8; Transmembrane protein 65	CAXB418.b1_c_s	CAXB418
16:00				CAXB631
16:00	–	–	CAXB801.b1_c_s	CAXB801
16:00	GO:0009507; GO:0030076; GO:0016168; GO:0018298	s:P51874; Peridinin-chlorophyll a-binding protein, chloroplastic	Contig104	CAXB1100
16:00	?	?	?	CAXB1417
16:00	–	–	CAXB1423.b2_c_s	CAXB1423
16:00	GO:0003676; GO:0000166	t:C5LD99; Putative uncharacterized protein	CAXB454.b1_c_s	CAXB454
16:00	?	?	?	CAXB2477
16:00	?	?	?	CAXB864
16:00	–	–	Contig472	CAXB2324
16:00	–	–	Contig387	CAXB1331
16:00	?	?	?	CAXB866
16:00	GO:0047456; GO:0051539; GO:0003994; GO:0003994; GO:0046872; GO:0003730; GO:0005515; GO:0019629; GO:0006417; GO:0006099	s:P36683; Aconitate hydratase 2	Contig137	CAXB490
16:00	GO:0016758; GO:0016999	s:O05496; Uncharacterized UDP-glucosyltransferase ydhE	CAXB1329.b2_c_s	CAXB1329
16:00	–	t:D2L8J9; HPP family protein	CAXB1170.b2_c_s	CAXB1170
16:00	GO:0043202; GO:0016787; GO:0016042	s:Q2KIY5; Putative phospholipase B-like 2	Contig381	CAXB1453
16:00	?	?	?	CAXB930
16:00	–	t:A8I1Z6; Putative uncharacterized protein	CAXB984.b1_c_s	CAXB984
16:00	GO:0004013; GO:0005488; GO:0006730	s:P68173; Adenosylhomocysteinase	CAXB2197.b2_c_s	CAXB2197
16:00	GO:0031901; GO:0000139; GO:0016021; GO:0008415; GO:0008270	s:Q4P6L3; Palmitoyltransferase AKR1	CAXB573.b1_c_s	CAXB573
16:00	GO:0001518; GO:0005248	s:O88457; Sodium channel protein type 11 subunit alpha	Contig485	CAXB2348
16:00	–	–	CAXB1290.b2_c_s	CAXB1290
16:00	–	t:C1FGJ3; Predicted protein	Contig402	CAXB461
16:00	?	?	?	CAXB758

and on squid and the luminous bacteria that colonize their complex light-emitting organs (Wier et al., 2010; Rader et al., 2012). These symbioses are extracellular and therefore do not involve the same cellular control of symbiont proliferation as in the obligate intracellular symbiosis of corals and unicellular algae. In intracellular symbiosis, special mechanisms for nutrient transfer between symbiont and host have been developed. The host cell strictly regulates population size (cell division), metabolism, growth rate, and the non-motile stage of the symbionts using chemical signaling (Koike et al., 2004; Stat et al., 2006). Our experiments with oxygen evolution in *Symbiodinium* demonstrated that the algae maintain their rhythm even in symbiotic form. Under conditions of constant light, this rhythmic ability is not negatively affected by host control. However, cultured free-living algae maintain the rhythm for a longer period than those living inside coral tissue. In contrast, other known, in-culture circadian rhythm regulators, such as cell division and motility, fall under the control of the coral once *Symbiodinium* are *in hospite*.

The coral is also influenced by its symbionts in many ways. Dramatic diurnal physiological changes occur independently of the clock as a consequence of their obligate association with photosynthetic endosymbionts. This association enables the high rates of calcification, which are characteristic of reef-building corals, to occur. The presence of the symbionts, however, is a double-edged sword. *Symbiodinium* photosynthesis keeps the coral tissue significantly above oxygen saturation by day but allows it to be nearly hypoxic during the night, when respiration dominates (Kuhl et al., 1995). In their recent work, Levy et al. (2011) discovered a cluster of genes that are expressed in midday. They were identified as antioxidant genes and are associated with the presence of extreme oxygen tension in the coral tissue due to the high photosynthesis rate of the endosymbiotic algae. As has been recently suggested, such findings can provide a link between the cellular redox state and the ancestral traits of the circadian clock (Rosbash, 2009; Edgar et al., 2012). It has also been shown that corals have a nighttime preference for DNA replication that is rhythmic and light dependent, occurring only under LD cycles. It has been suggested that DNA replication is light-dependent/gated, meaning it occurs only under light–dark cycles, while the replication process is scheduled for nighttime in order to reduce the dangerous effects of UV radiation that could impede daytime replication (Levy et al., 2011). Furthermore, rhythmic glycolytic enzymes are most likely regulated by the expression of the hypoxia-inducible factor (HIF) in a clock-independent manner, as indicated by symbiont-imposed hypoxia after sunset. Given the evolutionary success of the reef-building coral symbiosis and its persistence over geological time, such subtle molecular adaptations are perhaps to be expected.

However, it is not yet known whether the host and the symbiont sense the environment differently, with different mechanisms, cycles, and periods, or whether they sense and respond in the same manner with their internal endogenous clocks. Of note, corals possess photoreceptors, including cryptochromes (Levy et al., 2007) and opsins (Mason et al., 2012; Shoguchi et al., 2013) however, some light signals might be perceived by the symbionts and indirectly influences the host. It is possible that the coral controls the algal endogenous clock in symbiosis (or vice versa), such that there is one main "master clock" and a second "slave clock". This area of study has been only recently initiated, and there is much to be learned about how two clocks can work in parallel in a single meta-organism.

6. Future directions

The solar-based circadian clock mechanism, the circannual period and the genes that regulate it, remain poorly understood. Even less is known about the lunar clock, which is governed by the moon and is responsible for circatidal and monthly lunar rhythms. Tides have a significant impact on the organisms occupying tidal zones. Large changes in humidity, salinity, temperature, oxygen levels, water current, sun irradiation, food availability, hydrostatic pressure, and predator exposure,

occur twice a day with the tides. In many marine organisms living in tidal zones, the circatidal clock primarily affects their locomotor activity (reviewed in Palmer, 2000; Naylor, 2010; Tessmar-Raible et al., 2011). However, circatidal rhythms have been minimally investigated in corals. Unfortunately, few coral behaviors have a clear diel pattern, and much remains to be learned about their circadian rhythm behaviors. It is not known whether the coral has one clock, such as the circadian clock, or that multiple clock-like circannual and/or circatidal systems that might work together to sense the environment and respond to its signals. The lunar cycle is connected to coral reproduction. For instance, the coral *A. millepora* harbors at least three members of the cryptochrome genes family, *cry-dash*, *cry1*, and *cry2* (Levy et al., 2007). The *cry2* RNA and specific antibody of CRY2 protein are affected by exposure to lunar light; therefore, *cry2* might mediate responses to lunar light. However, additional research will be required to determine whether *Acropora cry2* can act as a light receptor that mediates lunar light stimulus.

Finally, an understanding of coral chronobiology is highly important for developing an understanding of the evolution of circadian clocks. Because cnidarians are closely related to bilaterian animals, a comparison of cnidarian and bilaterian clocks will provide insight into the origin and nature of the ancestral animal clock. The same can be argued for *Symbiodinium* chronobiology, since photosynthesis is clearly linked to diurnal light oscillations. Photosynthetic eukaryotes have a complex evolutionary history, and comparative work can only shed light into whether circadian regulation is a conserved mechanism across these lineages. Information on both circadian clocks will impact our understanding of the ecology, regulation, and preservation of coral reefs.

References

Abe, N., 1939. On the expansion and contraction of the polyp of a reef coral *Caulastrea furcata Dana*. Palao Trop. Biol. Stn. Stud. 1, 651–670.

Alabadi, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P., Kay, S.A., 2001. Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. Science 293, 880–883.

Al-Horani, F.A., Al-Moghrabi, S.L., de Beer, D., 2003. The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. Mar. Biol. 142, 419–426.

Al-Horani, F.A., Tambutté, É., Allemand, D., 2007. Dark calcification and the daily rhythm of calcification in the scleractinian coral, *Galaxea fascicularis*. Coral Reefs 26, 531–538.

Babcock, R.C., 1984. Reproduction and distribution of two species of *Goniastrea* (Scleractinia) from the Great Barrier Reef Province. Coral Reefs 2, 187–195.

Babcock, R.C., 1988. Age-structure, survivorship and fecundity in populations of massive corals. Proc 6th int coral Reef Symp, 2, pp. 625–633.

Babcock, R.C., 1995. Synchronous multispecific spawning on coral reefs: potential for hybridization and roles of gamete recognition. Reprod. Fertil. Dev. 7, 943–950.

Babcock, R.C., Bull, G.D., Harrison, P.L., Heyward, A.J., Oliver, J.K., Wallace, C.C., Willis, B.L., 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar. Biol. 90, 379–396.

Babcock, R.C., Wills, B.L., Simpson, C.J., 1994. Mass spawning of corals on a high latitude coral reef. Coral Reefs 13, 161–169.

Barnes, D.J., Chalker, B.E., 1990. Calcification and photosynthesis in reef building corals and algae. In: Dubinsky, Z. (Ed.), Ecosystems of the World. Coral Reefs, vol. 25. Elsevier, pp. 109–131.

Barnett, M.J., Toman, C.J., Fisher, R.F., Long, S.R., 2004. A dual-genome Symbiosis Chip for coordinate study of signal exchange and development in a prokaryote–host interaction. Proc. Natl. Acad. Sci. U. S. A. 101, 16636–16641.

Bastidas, C., Croquer, A., Zubillaga, A.L., Ramos, R., Kortnik, V., Weinberger, C., Marquez, L.M., 2005. Coral mass- and split-spawning at a coastal and an offshore Venezuelan reef, southern Caribbean. Hydrobiologia 541, 101–106.

Beaver, C.R., Earle, S.A., Tunnell Jr., J.W., Evans, E.F., de la Cerda, A.V., 2004. Mass spawning of reef corals within the Veracruz Reef System, Veracruz, Mexico. Coral Reefs 23, 324.

Bell-Pedersen, D., Cassone, V.M., Earnest, D.J., Golden, S.S., Hardin, P.E., Thomas, T.L., Zoran, M.J., 2005. Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat. Rev. Genet. 6, 544–556.

Boch, C.A.B., Ananthasubramaniam, A.M., Sweeney, F.J., Doyle, I.I.I., Morse, D.E., 2011. Effects of light dynamics on coral spawning synchrony. Biol. Bull. 220, 161–173.

Bosch, T.C., McFall-Ngai, M.J., 2011. Metaorganisms as the new frontier. Zoology 114, 185–190.

Brady, A.K., Hilton, J.D., Vize, P.D., 2009. Coral spawn timing is a direct response to solar light cycles and is not an entrained circadian response. Coral Reefs 28, 677–680.

Brady, A.K., Snyder, K.A., Vize, P.D., 2011. Circadian cycles of gene expression in the coral, Acropora millepora. PLoS One 6, e25072.

Chalker, B.E., 1977. Daily variation in the calcification capacity of Acropora cervicornis. In: Taylor, D.L. (Ed.), Proceedings: Third International Coral Reef Symposium. Rosenstiel School of Marine and Atmospheric Science, Miami, pp. 417–423.

Cook, C.B., Davy, S.K., 2001. Are free amino acids responsible for the host factor effects on symbiotic zooxanthellae in extracts of host tissue? Hydrobiologia 461, 71–78.

Corellou, F., et al., 2009. Clocks in the green lineage: comparative functional analysis of the circadian architecture of the picoeukaryote Ostreococcus. Plant Cell 21, 3436–3449.

Ditty, J.L., Williams, S.B., Golden, S.S., 2003. A cyanobacterial circadian timing mechanism. Annu. Rev. Genet. 37, 513–543.

Dunlap, J.C., Loros, J.J., DeCoursey, P., 2004. Chronobiology: Biological Timekeeping. Sinauer Associates, Sunderland, MA 406.

Edgar, R.S., et al., 2012. Peroxiredoxins are conserved markers of circadian rhythms. Nature 485, 459–464.

Fadlallah, Y.H., 1984. Sexual reproduction, development and larval biology in scleractinian corals. Rev. Coral Reefs 2, 129.

Fitt, W.K., Trench, R.K., 1983. The relation of diel patterns of cell division to diel patterns of motility in the symbiotic dinoflagellate Symbiodinium microadriaticum Freudenthal in culture. New Phytol. 94, 421–432.

Fitt, W.K., Change, S.S., Trench, R.K., 1981. Motility patterns of different strains of the symbiotic dinoflagellate Symbiodinium (=Gymnodinium) microadriaticum (Freudenthal) in culture. Bull. Mar. Sci. Gulf Caribb. 31, 436–443.

Furla, P., Galgani, I., Durand, I., Allemand, D., 2000. Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. J. Exp. Biol. 203, 3445–3457.

Gates, R.D., Hoegh-Guldberg, O., McFall-Ngai, M.J., Bil, K.Y., Muscatine, L., 1995. Free amino acids exhibit anthozoan "host factor" activity: they induce the release of photosynthate from symbiotic dinoflagellates in vitro. Proc. Natl. Acad. Sci. 92 (16), 7430–7434.

Gates, R.D., Bil, K.Y., Muscatine, L., 1999. The influence of an anthozoan "host factor" on the physiology of a symbiotic dinoflagellate. J. Exp. Mar. Biol. Ecol. 232, 241–259.

Gill, I.P., Dickson, J.A.D., Hubbard, D.K., 2006. Daily banding in corals: implications for paleoclimatic reconstruction and skeletonization. J. Sediment. Res. 76, 683.

Gittings, S.R., Boland, G.S., Deslarzes, K.J.P., Combs, C.L., Holland, B.S., Bright, T.J., 1992. Mass spawning and reproductive viability of reef corals at the east Flower Garden Bank, Northwest Gulf of Mexico. Bull. Mar. Sci. 51, 420–428.

Glossop, N.R., Lyons, L.C., Hardin, P.E., 1999. Interlocked feedback loops within the Drosophila circadian oscillator. Science 286, 766–768.

Glynn, P.W., Gassman, N.J., Eakin, C.M., Cortes, J., Smith, D.B., Guzman, H.M., 1991. Reef coral reproduction in the eastern Pacific: Costa Rica, Panama, and Galapagos Islands (Ecuador). I. Pocilloporidae. Mar. Biol. 109, 355–368.

Gorbunov, M.Y., Falkowski, P.G., 2002. Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight. Limnol. Oceanogr. 47, 309–315.

Goreau, T.F., 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol. Bull. 116, 59–75.

Goreau, T.F., Goreau, N.I., 1959. The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. Biol. Bull. 117, 239–250.

Guerfali, F.Z., et al., 2008. Simultaneous gene expression profiling in human macrophages infected with Leishmania major parasites using SAGE. BMC Genomics 9, 238.

Guest, J.R., Chou, L.M., Baird, A.H., Goh, B.P.L., 2002. Multispecific, synchronous coral spawning in Singapore. Coral Reefs 21, 422–423.

Hagman, D.K., Gittings, S.R., Deslarzes, K.J.P., 1998. Timing, species participation and environmental factors influencing annual mass spawning at the Flower Garden Banks (Northwest Gulf of Mexico). Gulf Mex. Sci. 16, 170–179.

Harmer, S.L., Hogenesch, L.B., Straume, M., Chang, H.S., Han, B., Zhu, T., Wang, X., Kreps, J.A., Kay, S.A., 2000. Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science 290, 2110–2113.

Harmer, S.L., Panda, S., Kay, S.A., 2001. Molecular bases of circadian rhythms. Annu. Rev. Cell Dev. Biol. 17, 215–253.

Harriott, V.J., 1983. Reproductive seasonality, settlement, and post-settlement mortality of Pocillopora damicornis (Linnaeus), at Lizard Island, Great Barrier Reef. Coral Reefs 2, 151–157.

Harrison, P.L., 2011. Sexual reproduction of scleractinian corals. In: Dubinsky, Z., Stambler, N. (Eds.), Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht, pp. 59–85.

Harrison, P.L., Booth, D.J., 2007. Coral reefs: naturally dynamic and increasingly disturbed ecosystems. In: Connell, S.D., Gillanders, B.M. (Eds.), Marine Ecology. Oxford University Press, Melbourne, pp. 316–377.

Harrison, P.L., Wallace, C.C., 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky, Z. (Ed.), Ecosystems of the World. Coral Reefs, vol. 25. Elsevier, Amsterdam, pp. 133–207.

Harrison, P.L., Babcock, R.C., Bull, G.D., Oliver, J.K., Wallace, C.C., Willis, B.L., 1984. Mass spawning in tropical reef corals. Science 223, 1186–1189.

Hastings, J.W., 2007. The Gonyaulax clock at 50: translational control of circadian expression. Cold Spring Harb. Symp. Quant. Biol. 72, 141–144.

Hastings, J.W., Astrachan, L., Sweeney, B.M., 1961. Persistent daily rhythm in photosynthesis. J. Gen. Physiol. 45, 69–76.

Hayashibara, T., Shimoike, K., Kimura, T., Hosaka, S., Heyward, A.J., Harrison, P.L., Kudo, K., Omori, M., 1993. Patterns of coral spawning at Akajima Island, Okinawa, Japan. Mar. Ecol. Prog. Ser. 101, 253–262.

Hendricks, W.D., Byrum, C.A., Meyer-Bernstein, E.L., 2012. Characterization of circadian behavior in the starlet sea anemone, Nematostella vectensis. PLoS One 7, e46843.

Hoadley, K.D., Szmant, A.M., Pyott, S.J., 2011. Circadian clock gene expression in the coral Favia fragum over diel and lunar reproductive cycles. PLoS One 6, e19755.

Hunter, C.L., 1989. Environmental cues controlling spawning in two Hawaiian corals. In: Verrucosa, Montipora, Dilatata, M. (Eds.), Proc 6th Int Coral Reef Symp. , 2, pp. 727–732.

Imaizumi, T., Kay, S.A., Schroeder, J.I., 2007. Circadian rhythms — daily watch on metabolism. Science 318, 1730–1731.

Ishiura, M., Kutsuna, S., Aoki, S., Iwasaki, H., Andersson, C.R., Tanabe, A., Golden, S.S., Johnson, C.H., Kondo, T., 1998. Expression of a gene cluster kaiABC as a circadian feedback process in Cyanobacteria. Science 281, 1519–1523.

Johnson, C.H., Golden, S.S., 1999. Circadian programs in cyanobacteria: adaptiveness and mechanism. Annu. Rev. Microbiol. 53, 389–409.

Jokiel, P.L., 1985. Lunar periodicity of planula release in the reef coral Pocillopora damicornis in relation to various environmental factors. Proc 5th Int Coral Reef Symp, 4, pp. 307–312 (Tahiti).

Jokiel, P.L., Ito, R.Y., Liu, P.M., 1985. Night irradiance and synchronization of lunar release of planula larvae in the reef coral Pocillopora damicornis. Mar. Biol. 88, 167–174.

Kawaguti, S., Sakumoto, D., 1948. The effect of light on time calcium deposition of corals. Bull. Oceanogr. Inst. Taiwan 4, 65–70.

Koike, K., Jimbo, M., Sakai, R., Kaeriyama, M., Muramoto, K., Ogata, T., Kamiya, H., 2004. Octocoral chemical signaling selects and controls dinoflagellate symbionts. Biol. Bull. 207, 80–86.

Kojis, B.L., Quinn, N.J., 1981. Aspects of sexual reproduction and larval development in the shallow water hermatyphic coral Goniastrea australensis (Edwards and Haime 1857). Bull. Mar. Sci. 31, 558–573.

Kremien, M., Shavit, U., Mass, T., Genin, A., 2013. Benefit of pulsation in soft corals. Proc. Natl. Acad. Sci. U. S. A. (doi:10.1073). **Q5**

Kucho, K., Okamoto, K., Tabata, S., Fukuzawa, H., Ishiura, M., 2005. Identification of novel clock controlled genes by cDNA macroarray analysis in Chlamydomonas reinhardtii. Plant Mol. Biol. 57, 889–906.

Kuhl, M., et al., 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH, and light. Mar. Ecol. Prog. Ser. 117, 159–172.

Lasker, H.R., 1979. Light dependent activity patterns among reef corals: Montastrea cavernosa. Biol. Bull. 156, 196–211.

Lee, K., Loros, J.J., Dunlap, J.C., 2000. Interconnected feedback loops in the Neurospora circadian system. Science 289, 107–110.

Levy, O., Mizrahi, L., Chadwick-Furman, N.E., Achituv, Y., 2001. Factors controlling the expansion behavior of Favia favus (Cnidaria:Scleractinia): effects of light, flow, and planktonic prey. Biol. Bull. 200, 118–126.

Levy, O., Dubinsky, Z., Achituv, Y., 2003. Photobehavior of stony corals: responses to light spectra and intensity. J. Exp. Biol. 206, 4041–4049.

Levy, O., Appelbaum, L., Leggat, W., Gothlif, Y., Hayward, D.C., Miller, D.J., Hoegh-Guldberg, O., 2007. Light-responsive cryptochromes from a simple multicellular animal, the coral Acropora millepora. Science 318, 467–470.

Levy, O., et al., 2011. Complex diel cycles of gene expression in coral–algal symbiosis. Science 331, 175-175. **Q7**

Loya, Y., 2004. The coral reefs of Eilat—past, present and future: three decades of coral community structure studies. In: Rosenberg, E., Loya, Y. (Eds.), Coral Health and Disease. Springer-Verlag, Berlin, pp. 1–29.

Mason, B., Schmale, M., Gibbs, P., Miller, M.W., Wang, Q., Levay, K., hestopalov, V., Slepak, V.Z., 2012. Evidence for multiple phototransduction pathways in a reef-building coral. PLoS ONE 7, e50371.

McAllister, D.E., 1991. What is the status of the world's coral reef fishes? Sea Wind 5, 14–18.

McClung, C.R., 2006. Plant circadian rhythms. Plant Cell 18, 792–803.

Mendes, J.M., Woodley, J.D., 2002. Timing of reproduction in Montastraea annularis: relationship to environmental variables. Mar. Ecol. Prog. Ser. 227, 241–251.

Mittag, M., 1996. Conserved circadian elements in phylogenetically diverse algae. Proc. Natl. Acad. Sci. U. S. A. 93, 14401–14404.

Monnier, A., et al., 2010. Orchestrated transcription of biological processes in the marine picoeukaryote Ostreococcus exposed to light/dark cycles. BMC Genomics 11, 192.

Moya, A., Tambutté, S., Tambutté, E., Zoccola, D., Caminiti, N., Allemand, D., 2006. Study of calcification during a daily cycle of the coral Stylophora pistillata: implications for 'light-enhanced calcification'. J. Exp. Biol. 209, 3413–3419.

Naylor, E., 2010. Chronobiology of Marine Organisms. Cambridge University Press, Cambridge, UK.

Neville, A.C., 1967. Daily growth layers in animals and plants. Biol. Rev. Camb. Philos. Soc. 42, 421–441.

Okamoto, O.K., Hastings, J.W., 2003. Genome-wide analysis of redox-regulated genes. Gene 321, 73–81.

Oliver, J.K., Babcock, R.C., Harrison, P.L., Willis, B.L., 1988. Geographic extent of mass coral spawning: clues to ultimate causal factors. Proceedings of 6th International Coral Reef Symposium. , vol. 2, pp. 803–810 (Townsville).

Ouyang, Y., Andersson, C.R., Kondo, T., Golden, S.S., Johnson, C.H., 1998. Resonating circadian clocks enhance fitness in cyanobacteria. Proc. Natl. Acad. Sci. U. S. A. 95, 8660–8664.

Palmer, J.D., 2000. The clocks controlling the tide-associated rhythms of intertidal animals. Bioessays 22, 32–37.

Panda, S., Hogenesch, J.B., Kay, S.A., 2002. Circadian rhythms from flies to human. Nature 417, 329–335.

Pearse, V.B., Muscatine, L., 1971. Role of symbiotic algae (zooxanthellae) in coral calcification. Biol. Bull. 141, 350–363.

Pittendrigh, C.S., 1993. Temporal organization — reflections of a Darwinian clock-watcher. Annu. Rev. Physiol. 55, 16–54.

Porter, J., 1974. Zooplankton feeding by the Caribbean reef-building coral *Montastrea cavernosa*. Proc. 2nd Int. Coral Reef Symp, 1, pp. 111–125.

Quecini, V., Zucchi, M.I., Baldin, J., Vello, N.A., 2007. Identification of soybean genes involved in circadian clock mechanism and photoperiodic control of flowering time by in silico analyses. J. Integr. Plant Biol. 49, 1640–1653.

Rader, B.A., Kremer, N., Apicella, M.A., Goldman, W.E., McFall-Ngai, M.J., 2012. Modulation of symbiont lipid A signaling by host alkaline phosphatases in the squid–vibrio symbiosis. MBio 3, e00093–e.

Reaka-Kudla, M.L., 1997. Global biodiversity of coral reefs: a comparison with rainforests. In: Reaka-Kudla, M.L., Wilson, D.E. (Eds.), Biodiversity II: Understanding and Protecting Our Biological Resources. Joseph Henry Press.

Reaka-Kudla, M.L., 2001. Known and unknown biodiversity, risk of extinction and conservation strategy in the sea. Waters Peril 19–33.

Reitzel, A.M., Behrendt, L., Tarrant, A.M., 2010. Light entrained rhythmic gene expression in the sea anemone *Nematostella vectensis*: the evolution of the animal circadian clock. PLoS One 5, e12805.

Reitzel, A.M., Tarrant, A.M., Levy, O., 2013. Circadian clocks in the cnidaria: environmental entrainment, molecular regulation, and organismal Outputs. Integr. Comp. Biol. 1–13. http://dx.doi.org/10.1093/icb/ict024.

Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418, 935–941.

Reyes-Bermudez, A., DeSalvo, M.K., Voolstra, C.R., Sunagawa, S., Szmant, A.M., Iglesias-Prieto, R., Medina, M., 2009. Gene expression microarray analysis encompassing metamorphosis and the onset of calcification in the scleractinian coral *Montastraea faveolata*. Mar. Genomics 2, 149–159.

Ricard, M., Salvat, B., 1977. Faeces of *Tridacna maxima* (Mollusca–Bivalvia), composition and coral reef importance. Proceedings of the Third Coral Reef Symposium, pp. 495–501.

Richmond, R.H., 1997. Reproduction and recruitment in corals: critical links in the persistence of reefs. In: Birkeland, C. (Ed.), Life and Death of Coral Reefs. Chapman & Hall, New York, pp. 175–197.

Richmond, R.H., Hunter, C.L., 1990. Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar. Ecol. Prog. Ser. 60, 185–203.

Risk, M.J., Pearce, T.H., 1992. Interference imaging of daily growth bands in massive corals. Nature 358, 572–573.

Roenneberg, T., Merrow, M., 2005. Circadian clocks — the fall and rise of physiology. Nat. Rev. Mol. Cell Biol. 6, 965–971.

Rosbash, M., 2009. The implication of multiple circadian clock origins. PLoS Biol. 7, e10000062.

Schaffer, R., Landgraf, J., Accerbi, M., Simon, V., Larson, M., Wisman, E., 2001. Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. Plant Cell 13, 113–123.

Schoenberg, D.A., Trench, R.K., 1980. Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. Proc. R. Soc. Lond. Ser. B 207, 405–427.

Scrutton, C.T., 1965. Periodicity in Devonian coral growth. Paleontology 7, 552–558.

Sebens, K.P., Deriemer, K., 1977. Diel cycles of expansion and contraction in coral reef anthozoans. Mar. Biol. 43, 247–256.

Shinzato, C., Chuya, E., et al., 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. Nature 476 (7360), 320–323.

Shoguchi, E., Tanaka, M., Shinzato, C., Kawashima, T., Satoh, N., 2013. A genome-wide survey of photoreceptor and circadian genes in the coral, *Acropora digitifera*. Gene 515, 426–431.

Simpson, C.J., 1991. Mass spawning of corals on Western Australian and comparisons with the Great Barrier Reef. J. R. Soc. West. Aust. 74, 85–91.

Smith, G.J., Muscatine, L., 1999. Cell cycle of symbiotic dinoflagellates: variation in G1 phase-duration with anemone nutritional status and macronutrient supply in the *Aptasia pulchella–Symbiodinium pulchrorum* symbiosis. Mar. Biol. 135, 405–418.

Somers, D.E., 1999. The physiology and molecular bases of the plant circadian clock. Plant Physiol. 121, 9–19.

Sorek, M., Levy, O., 2012a. Influence of the quantity and quality of light on photosynthetic periodicity in coral endosymbiotic algae. PLoS ONE 7, e43264.

Sorek, M., Levy, O., 2012b. The effect of temperature compensation on the circadian rhythmicity of photosynthesis in *Symbiodinium*, coral-symbiotic alga. Sci. Rep. 2. http://dx.doi.org/10.1038/srep00536.

Sorek, M., Yacobi, Y.Z., Roopin, M., Berman-Frank, I., Levy, O., 2013. Photosynthetic circadian rhythmicity patterns of *Symbiodinium*, the coral endosymbiotic algae. Proc. R. Soc. B 20122942. http://dx.doi.org/10.1098/rspb.2012.2942.

Stat, M., Carter, D., Hoegh-Guldberg, O., 2006. The evolutionary history of *Symbiodinium* and scleractinian hosts — symbiosis, diversity, and the effect of climate change. Perspect. Plant Ecol. Evol. Syst. 8, 23–43.

Stoddart, J.A., Black, R., 1985. Cycles of gametogenesis and planulation in the coral *Pocillopora damicornis* from south Western Australia. Mar. Ecol. Prog. 23, 153–164.

Sunagawa, S., DeSalvo, M.K., Voolstra, C.R., Reyes-Bermudez, A., Medina, M., 2009. Identification and gene expression analysis of a taxonomically restricted cysteine-rich protein family in reef-building corals. PLoS One 4 (3), e4865.

Sweeney, B.M., 1976. Circadian rhythms in corals, particularly Fungiidae. Biol. Bull. 151, 236–246.

Sweeney, A.M., Boch, C.A., Johnsen, S., Morse, D.E., 2011. Twilight spectral dynamics and the coral reef invertebrate spawning response. J. Exp. Biol. 214, 770–777.

Taddei-Ferretti, C., Musio, C., 2000. Photobehaviour of Hydra (Cnidaria, Hydrozoa) and correlated mechanisms: a case of extraocular photosensitivity. J. Photochem. Photobiol. B Biol. 55, 88–101.

Tessmar-Raible, K., Raible, F., Arboleda, E., 2011. Another place, another timer: marine species and the rhythms of life. Bioessays 33, 165–172.

Trench, R.K., 1979. Cell biology of plant animal symbiosis. Annu. Rev. Plant Physiol. 30, 485–531.

Trench, R.K., 1980. Integrative mechanisms in mutualistic symbioses. In: Cook, C.B., Pappas, P.W., Rudolf, E.D. (Eds.), Cellular Interactions in Symbiosis and Parasitism. Ohio State University Press, Columbus, pp. 275–297.

Van Dolah, F.M., Lidie, K.B., Morey, J.S., Brunelle, S.A., Ryan, J.C., Monroe, E.A., Haynes, B.L., 2007. Microarray analysis of diurnal and circadian regulated genes in the Florida red-tide dinoflagellate *Karenia brevis* (Dinophyceae). J. Phycol. 43, 741–752.

van Woesik, R., 2009. Calm before the spawn: global coral spawning patterns are explained by regional wind fields. Proc. R. Soc. B 277, 715–722.

van Woesik, R., Lacharmoise, F., Koksal, S., 2006. Annual cycles of solar insolation predict spawning times of Caribbean corals. Ecol. Lett. 9, 390–398.

Vareschi, E., Fricke, H., 1986. Light responses of a scleractinian coral (*Plerogyra sinuosa*). Mar. Biol. 90, 395–402.

Vize, P.D., 2009. Transcriptome analysis of the circadian regulatory network in the coral *Acropora millepora*. Biol. Bull. 216, 131–137.

Wang, J.T., Douglas, A.E., 1997. Nutrients, signals and photosynthate release by symbiotic algae: the impact of taurine on the dinoflagellate alga *Symbiodinium* from the sea anemone *Aiptasia pulchella*. Plant Physiol. 114, 631–636.

Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. J. Exp. Biol. 211, 3059–3066.

Wells, J.W., 1963. Coral growth and geochronometry. Nature 197, 948–950.

Wells, J.W., 1966. Evolutionary development in the scleractinian family Fungiidae. In: Rees, W.J. (Ed.), The Cnidaria and their evolution. Symposia of the Zoological Society of London, 16. Academic Press, London, pp. 223–246.

Wier, A.M., et al., 2010. Transcriptional patterns in both host and bacterium underlie a daily rhythm of anatomical and metabolic change in a beneficial symbiosis. Proc. Natl. Acad. Sci. U. S. A. 107, 2259–2264.

Willis, B.L., Babcock, R.C., Harrison, P.L., Oliver, T.K., 1985. Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. Proc. 5th Int. Coral Reef Cong., Tahiti, 4, pp. 343–348.

Withers, K.J.T., Grant, A.J., Hinde, R., 1998. Effects of amino acids on the isolated symbiotic algae of the coral *Plesiastrea versipora* (Lamarck): absence of a host release factor response. Comp. Biochem. Physiol. A 120, 599–607.

Wyers, S.C., Barnes, H.S., Smith, S.R., 1991. Spawning of hermatypic corals in Bermuda; a pilot study. Hydrobiologia 216, 109–116.

Yacobovitch, T., Benayahu, Y., Weis, V.M., 2004. Motility of zooxanthellae isolated from the Red Sea soft coral *Heteroxenia fuscescens* (Cnidaria). J. Exp. Mar. Biol. Ecol. 298, 35–48.

Yellowlees, D., Alwyn, T., Rees, V., Leggat, W., 2008. Metabolic interactions between algal symbionts and invertebrate hosts. Plant Cell Environ. 31, 679–694.

Yonge, C.M., 1940. The biology of reef building corals. Sci. Rep. Great Barrier Reef Expedition 1, 353–391.