Endobiotic microalgae in molluscan life

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Abstract

Endobiotic microalgae inhabit various groups of organisms, including bivalves. In this group, the association between the giant molluscs *Tridacna* and *Symbiodinium* is one of the most recognizable. This consortium allows hydrobionts to survive in oligotrophic waters by regulating their metabolism. The available research has provided an understanding of the interaction and adaptation of these symbionts, but the problem of the beginning of the formation of these relationships remains unresolved. In the case of Tridacninae, symbiosis is essential for the survival of bivalves, in contrast to representatives of the Mytilidae and the *Coccomyxa* found in them. A few works devoted mainly to the morphological aspects of invasion have shown that endobiont causes inflammation and pathology. Having data to clarify the exact "diagnosis" of the interaction of these organisms is not enough. It is possible that the relationship between bivalves and *Coccomyxa* is in the early stages of being established, which may lead to mutualism or parasitism in the future. We assume that the analysis of works on the symbiosis of *Symbiodinium* and bivalves will facilitate the course of research for the less studied *Coccomyxa* and their hosts. By postulating the *Coccomyxa* represent a unique evolutionary model for the formation of a symbiotic system, it is possible to use this system to study the interaction of organisms during their initial contact. The identified signalling pathways and mechanisms that allow the photobionts to evade host immunity can be useful for constructing new forms of symbiosystems.

Keywords: Bivalve molluscs, Endobiotic microalgae, *Coccomyxa*, *Symbiodinium*, Symbiosis

Introduction

Biotic interactions between invertebrate and algae are widespread in freshwater and marine communities and can vary in terms of mutualism, commensalism, and parasitism, involving a large variety of partner species (Davy et al., 2012; Gontier, 2015; Hinde, 1987; Mies et al., 2017a; Yellowlees et al., 2008). However, a microalgal intruder found in the tissues of bivalve molluscs is not an ordinary finding. This phenomenon became known after Goetsch & Scheuring (1926) described the microalgae *Chlorella* from the freshwater bivalves *Anodonta cygnea* and *Unio pictorum*. Kawaguti (1941) then reported on photosymbiotic algae found in *Corculum cardissa* (Cardiidae), and in Wiborg first described flagellated unicellular green algae found in the siphon and mantle of *Modiolus modiolus*. In 1967, Rowell also reported small (4.6 μm) oval algae with microflagella infesting the gills and mantle of *M. modiolus* (Naidu & South, 1970). Naidu & South, from the green colouring of the adduc-

tor muscles, gonads and mantle of *Placopecten magellanicus* and based on the morphology of phytobionts, concluded that these bivalves become infested with algae different from that which infests *Modiolus*; these microalgae are described as endozoic algae (living inside the animal), and are classified as symbiotic zoochlorellae (Naidu & South, 1970). In further studies of algae-infested scallops, Naidu (1971) reported the parasitic status of the involved algae but failed to define their systematic status. Later, Stevenson & Robin South (1974) described a new member of the Coccomyxaceae family called *Coccomyxa parasitica*; in 1978, Boraso-De-Zaixso & Zaixso recorded green infection in *Mytilus edulis*, suggesting that it was also caused by *C. parasitica* (Boraso-De-Zaixso & Zaixso, 1978). The biotic status of *Coccomyxa* invaders has been discussed for a long time and its parasitic effects on the organism have recently been described in detail (Sokolnikova et al., 2016).

However, the evidence given above has received less attention than other algae and mollusc associations such as those found in the family Cardiidae (Trench et al., 1981; Vermeij, 2013). Several species of Cardiidae have symbiotic dinoflagellates of the genus *Symbiodinium* (occasionally called as zooxanthellae) (Kawaguti, 1983). These include all species of the subfamily Tridacninae, including the well-known genera of the giant molluscs *Tridacna* and *Hippopus*, and the lesser known genera of smaller molluscs of the subfamily Fraginae, including *Fragum*, *Corculum*, *Lunulicardia* (Kawaguti, 1950; Kawaguti, 1983; Vermeij, 2013) and *Fluviolanatus* of the Trapeziidae family (Morton, 1982). For many years, photosymbionts in tridacnines were assumed to be located in haemal spaces, where they are culled by amoebocytes and digested in the digestive gland; undigested components had to reside in the kidneys (Goreau et al., 1973; Reid et al., 1984; Yonge, 1981). However, in 1946, Mansour described a system of tubules arising from a diverticular duct of the stomach of *Tridacna*, ramifying through most clams and containing symbionts. Yonge (1953) opposed Mansour's view, and most authors largely supported Yonge (Fitt & Trench, 1981; Morton, 1978) until Norton et al. (Norton & Jones, 1992; Norton et al., 1992) re-evaluated Mansour's results and confirmed that epithelial tubes with zooxanthellae are directly connected to the stomach and that blind-ended tertiary tubes do not connect with the haemocoel; therefore, algae are not found in the haemolymph compartment (Yonge, 1981). The same zooxanthellal tubes have also been found in *Corculum* (Farmer et al., 2001; Kawaguti, 1950) and *Fragnum* (Kawaguti, 1983; Morton, 2000; Ohno et al., 1995). Oocytes and embryos

of Cardiidae do not possess microalgae. Molluscs acquire them at the veliger or juvenile stage (Hirose et al., 2006; Jameson, 1976; Neo & Todd, 2009).

Currently, at least more 30 species of bivalves maintain symbiosis with microalgae. *Symbiodium* are dinoflagellates that inhabit animals and are colloquially called zooxanthellae. Whereas zooxanthellate are animals that form symbiosis with these microalgae (these terms are currently controversial). Zooxanthellae are highly specialized algae that mostly live as endosymbionts within a wide marine invertebrate phylogeny (Vermeij, 2013). Numerous strains of *Symbiodium* inhabit tridacnids, making each algae-host species combination unique and displaying its own particular level of ecological adaptation to light, metabolites, and other conditions (Ambariyanto & Hoegh-Guldberg, 1997; Belda et al., 1993; Belda-Baillie et al., 1998; Jantzen et al., 2008). As a result of the fact that dinoflagellates are able to donate some of the products of photosynthesis to molluscs, their role is especially great in meeting the energy needs of the host. While molluscs provide zooxanthellae with inorganic nutrients and protect them (Ambariyanto, 2002; Holt et al., 2014).

In bivalves, the mechanisms underlying their invasion by microalgae *Coccomyxa* (Chlorophyta) remain unknown. Also, the causes of algae settlement (physiological conditions, environmental conditions, or the activity of invaders) are not clear. Rodríguez et al. (2008) via genetic analysis showed that algae first isolated by Stevenson & Robin South (1974) and stored in the Culture Collection of Algae and Protozoa (CCAP) strain 216/18 was not *Coccomyxa*, but rather that all algae isolated from mussels collected from different areas belong to the *Coccomyxa* clade. Most studies have suggested that *Coccomyxa* are facultative parasites that spend some stages of their lifecycle within hosts (Crespo et al., 2009; Gray et al., 1999; Mortensen et al., 2005; Naidu & South, 1970; Sokolnikova et al., 2016; Stevenson, 1972; Stevenson & Robin South, 1974; Vaschenko et al., 2013; Vázquez et al., 2010). According to studies, algal invasion causes pathologies, including abnormal histomorphology and dysfunction of the affected organs, showing an expressed inflammatory and tissue degeneration. These pathologies lead to further developmental delay, organ dystrophy and deterioration of filtering ability (Gray et al., 1999; Mortensen et al., 2005; Sokolnikova et al., 2016).

Although not confirmed, according to early reports, clam *Clinocardium nutalli* belonging to Cardiidae has symbionts morphologically similar to those of Chlorella (Cooke, 1975;

Hartman & Pratt, 1976; Jones & Jacobs, 1992). At present, the algal infection of bivalves *C. nutalli* (Cooke, 1975), *Panopea abbreviate* (Vázquez et al., 2015) and *Fluviolanatus subtorta* (Morton, 1982) remains largely unexplored, and information on symbiosis in *Lunulicardia retusa* (Kirkendale, 2009; Schneider & Carter, 2001) and *Ensis macha* (Vázquez et al., 2010) bivalves is lacking.

The varying publication rates of research studies addressing microalgal symbiotic organisms do not always appear to reflect their significance. Here, we analysed peer-reviewed literature published from the initial description of endobiotic algae to identify potential milestone discoveries or achievements that may have driven the intensity of research conducted in subsequent years. Although several papers have provided excellent overviews of interactions between some microalgae and invertebrates, to our knowledge, there has been no comprehensive review of all associations of endobiotic microalgae and marine bivalve molluscs.

We focus on green microalgae *Coccomyxa* currently ascribed parasitic or pathogenic status (Sokolnikova et al., 2016; Zuykov et al., 2018a), likely due to the intermediate evolutionary stage in the development of relationships between these organisms. However, available data for establishing an exact "diagnosis" of the interactions of these organisms are not sufficient. We performed an analysis of satisfactory works to form a consortium of studies on *Symbiodinium* and on representatives of Cardiidae to facilitate the course of research on less studied green algae *Coccomyxa* and their hosts.

Factors contributing to the colonization of bivalve molluscs by microalgae

Although symbiosis between bivalves and photobionts have repeatedly arisen, their history, mechanisms and prevalence remain poorly understood (Hinde, 1987; Vermeij, 2013). Since the first description of a microalgae in *C. cardissa* (Kawaguti, 1941; Kawaguti, 1950), photosymbiosis has been identified in more 30 living species. At least most of these cases (approximately 27) are concentrated within a single family, Cardiidae (Vermeij, 2013), and 5 host species belong to Mytilidae (Zuykov et al., 2018b), indicating strong patterns of phylogenetic clumping. All tested representatives of three fragine genera (*Lunulicardia*, *Fragum* and *Corculum*) (Kirkendale et al., 2021), *Tridacna* and *Hippopus* are entirely photosymbiotic while sampled members of the other seven fragine genera and subgenera (*Trigoniocardia*,

Americardia, *Apiocardia*, *Ctenocardia*, *Papillicardium*, *Parvicardium* and *Microfragum*) are not identified as photosymbiont hosts (Vermeij, 2013).

The Tridacnidae family includes two genera (*Hippopus* and *Tridacna*), but at present there are disputes regarding the phylogenetic status of *Tridacna* (Cai et al., 2019; Lizano & Santos, 2014; Penny & Willan, 2014). These animals serve as effective filter feeders and are at the same time autotrophic due to their symbiosis with zooxanthellae, which affords them a nutritional and growth advantage over typical heterotrophic molluscs and may have enabled this molluscs to reach the largest size of any bivalves (Hernawan, 2008; Trench et al., 1981). Distribution ranges vary among giant clam species (Munro, 1993; Vermeij, 2013). These are epifaunal molluscs which inhabit isolated waters of the Indo-Pacific oligotrophic coral reefs (Neo & Todd, 2009). Due to their phototrophic features, giant clams usually found in shallower habitats (1–20 m) except for *Tridacna tevoroa*, which inhabits deep waters (Klumpp & Lucas, 1994). *Tridacna maxima* covers the widest area, which runs from the Red Sea to Polynesia and East Africa (Hernawan, 2012; Lucas, 1994; Othman et al., 2010). *T. maxima* is mostly found in shallow waters (reef flats and edges) while *Tridacna squamosa* can be found on lower fore-reef slopes (Jantzen et al., 2008). *Tridacna crocea* (boring giant clam) is the smallest species with shell length of 15 cm. It has been proven that the distribution of molluscs in depth depends on their nutrition (Dolorosa et al., 2014). For example, data suggest that *T. squamosa* is a mixotroph whose photoautotrophic range is extended by heterotrophy whereas *T. maxima* is a strictly functional photoautotroph limited by light.

In the giant molluscs Tridacnidae, an enlarged and fleshy mantle surrounds the siphons. The outer shell ribs of the *T. squamosa*, *T. maxima* and *T. crocea* periodically form concave or flattened shields (extensions) that support the edges of the mantle (Norton & Jones, 1992; Norton et al., 1992; Trench et al., 1981). The colourful pigmentation of varied patterns on the mantle are attributable to the iridophores (Ghoshal et al., 2016; Griffiths et al., 1992; Kawaguti, 1966) that distribute photosynthetically productive wavelengths by the lateral- and forward-scattering of light into the tissue and reflecting non-productive wavelengths (Ghoshal et al., 2016; Holt et al., 2014). Under the iridocytes lying on the surface of the mantle tissues, photosymbiotic microalgae are located in high density vertical columns, which leads to a uniform redistribution of incoming light along the sides of the columns and, thus, makes photosynthesis possible deep inside the tissue (Ghoshal et al., 2016;

Holt et al., 2014; Kawaguti, 1966). The visceral organs are little modified, and only the anus moves from the hind surface of the adductor to the upper surface, maintaining its relationship with the anteriorly displaced exhalant aperture (Norton & Jones, 1992; Munro, 1993).

Hippopus (*Hippopus hippopus* and *Hippopus porcellanus*) has colloquial names, including strawberry clam and horse's hoof clam. The way of life of *Hippopus* differs significantly from *Tridacna*, despite the fact that its morphology has significant similarities with the morphology of other members of this family. While these bivalves can be mistaken for *Tridacna derasa* due to their similar shell, the mantles of *Hippopus* lack hyaline organs and are coloured brown, grey or green (however, we observe the same immediate reaction to shadows), and the incurrent siphon includes prominent guard tentacles (Lucas, 1994; Munro, 1993). Mantle does not project beyond the margins of their shells. Their siphons are also hypertrophied, diverge widely, exposing an area of transparent olive-green tissue patterned with fine parallel wavy lines. Zooxanthellae are less common than tridacnae, and their middle and outer folds are even smaller and especially the first ones, also pigmented (Hernawan, 2012; Lucas, 1994; Norton & Jones, 1992). The final habitats of *H. hippopus* are located on the sand of the lee of the reefs. Probably, the initial attachment of molluscs occurs on the seaward side, and then there is an early freedom and subsequent rolling on the reef surface. *H. hippopus* is similar to *Tridacna* in that it also loses its ability to attach to a substrate with age (Munro, 1993). *H. porcellanus* inhabits shallow reefs along the edges of lagoons and intertidal zones (Dolorosa et al., 2014; Hernawan, 2012).

Bivalve *C. cardissa* has a peculiar type of shell and a more transparent upper valve. They are heavily pressed antero-posteriorly, forming a distinct heart shape, which is the source of its common name. Moreover, unlike other representatives, it includes several zooxanthellae within its gill filaments, labial palps, mantles and digestive gland. The peculiar shape and habits of this mollusc can be attributed to its association with *Symbiodinium* (Farmer et al., 2001; Kawaguti, 1950; Vermeij, 2013). Molluscs are distributed in the tropical Indo-Pacific, excluding the Red Sea and shallow waters off the coast of East Africa (ter Poorten, 2009). Molluscs have short siphons and therefore live close to the sedimentary water interface. The normal positioning of internal organs is somewhat displaced in accordance with the deformation of shells (Farmer et al., 2001). The upper side of the mantle is pale, as it contains few microalgal cells. The lower side, however, is dark brown in colour due to large accumula-

tions of zooxanthellae. The mantle is extremely delicate and thin except at its edge. Such a combination can facilitate effective light penetration into the gill filaments, where zooxanthellae accumulate in great abundance. On the lower side, however, there is no need for light to penetrate through the mantle, as there are no more zooxanthellae beneath the mantle (Kawaguti, 1950). Numerous pigment granules are found on the outer surface of the mantle. They are red, reddish purple, or blue in colour with considerable fluorescence. They are slightly larger than those of zooxanthellae. Whether these pigment granules filter excessively strong light remains unknown (Li et al., 2018). Like giant clams, this bivalve possesses a zooxanthellal tubular system (Farmer et al., 2001; Kawaguti, 1950).

All species of *Fragnum* are medium to small sized (10–45 mm) with the exception of the relatively large *Fragnum unedo* (reaching up to 65 mm in length). The mantle edge of the posterior and siphonal area extends broadly, especially near the ventral margins. Marginal part of the mantles is fringed with a line or with lines of thin processes and are ornamented with a fairly dense distribution of whitish glittering iridophores close to the surface (Kawaguti, 1983; Morton, 2000; Ohno et al., 1995). The siphonal area, gill lamellae and mantle are brown in colour due to heavy loads of zooxanthellae, but microalgae exhibit different patterns of distribution in species of *Fragnum* (these species also differ is size and mass). These features suggest that even in closely allied species, modifications of shell textures are correlated with differentiation in patterns of symbiosis with zooxanthellae. *Fragnum erugatum* has a tubular zooxanthellae system, but unlike giant clams, it connects the kidneys to the digestive diverticula of the stomach (Berry & Playford, 1997; Morton, 2000). Ingested microalgae cells from the gut, along with senescent zooxanthellae from the haemocoel, were found to be carried through this tube to the renal tubules for lysosomal hydrolysis and further elimination from the organism. This recently discovered fact forces us to reconsider the functioning of the zooxanthellae tubule system.

The heart cockle, *Clinocardium nuttalli*, is widely distributed in littoral settings from San Diego, California; north to the Nunivak, Pribiloff, and Commander islands; and south from the eastern Pacific to Japan (Hartman & Pratt, 1976; Jones & Jacobs, 1992). The heart cockle with green-mantle due to the presence of microalgae are regularly observed on the Pacific coast of North America (probably, *Chlorella*) (Cooke, 1975). Infected individuals usually inhabit the muddy bottom of rather turbid water bodies. An infaunal specimen of *C. nuttalli* found

in the upper reaches of the intertidal zone does not contain endobiotic algae. These specimens are on average smaller than their semi-infaunal or epifaunal counterparts. Semi-infaunal clams live at or below low mean water levels and all contain symbionts. They are noticeably larger than infaunal clams and include more annual bands. Epifaunal clams, all containing photosymbiotic algae, are larger and older (Jones & Jacobs, 1992). Semi-infaunal molluscs contain symbionts mainly in the tissues of the mantle and siphon adjacent to the shell. However, in some individuals, accumulations of microalgae are observed on the surface of the foot facing the siphon part. The effect of photosymbionts on the rate of shell growth or the isotopic composition of oxygen and carbon in the shell carbonate was not found in these molluscs (Hartman & Pratt, 1976; Jones & Jacobs, 1992). Perhaps the *C. nuttalli*, like other members of the family Cardiidae, still contains microalgae *Symbiodinium*.

F. subtorta (Trapeziidae) is found in the coastal lagoons of northern, western, southeastern and eastern New South Wales, Australia and estuaries across the continent (Morton, 1982). The shell is thin, the mantle is expanded with an enormous haemocoel with large algal cells demonstrating all of the characteristics of "zooxanthellae" (e.g., *Symbiodinium microadriaticum*) (Morton, 1982; Vermeij, 2013). Like that of *Corculum*, the thin shell of *Fluviolanatus* reflects a morphological compromise between two opposing demands: first, the protection of the animal's body and second, light penetration for photosynthesis by algae. Studies of this bivalve conducted by Morton in 1982 were not continued.

Southern geoduck *Panopea abbreviata* lives buried deep in sand and mud sediments to 70 cm, leaving only the tips of its long pair of fused siphons visible from the low intertidal zone to at least 75 m (Van der Molen et al., 2007). In a survey of the health status of bivalves in the Northern Patagonian gulfs, Argentina (Vázquez et al., 2010), some geoducks possess green siphons tips resembling infections of algae *Coccomyxa* (Chlorococcales: Coccomyxaceae) described by Rodríguez et al. (2008) for *M. edulis*. The alga has only been found in haemocytes that infiltrate connective tissues of the geoduck siphons. An examination of histological sections of the digestive gland, gills, gonads, mantle and kidney failed to detect algae. *P. abbreviata* extends its siphons roughly 20 cm above the sediment-water interface, supplying light exposure levels adequate for algal survival (Vázquez et al., 2010). Infected geoducks had lower condition index values than non-infected ones (regardless of the age of the molluscs, the season and their location) (Vázquez et al.,

2015).

A single case of the infection of *C. parasitica* in razor clam *E. macha* has been registered but has not been described in any way (algae was found in only one of 480 specimens examined) (Vázquez et al., 2010).

The deep-sea scallop, *P. magellanicus*, is found from the northern shores of the Gulf of St. Lawrence to Cape Hatteras, North Carolina. The bivalves is usually found at depths of less than 18 m in the northern portion of its range to more than 55 m off the coast of North Carolina. Like all scallops, *P. magellanicus* has photoreceptive eyes along the edges of its pinkish mantle (Naidu, 1971). Naidu & South in 1970 described green algae infesting shallow-water scallops of *P. magellanicus* inhabiting areas further north than its normal range. The main areas of infection were the mantle folds, the base of the adductor muscle, and the distal end of the gonad. Later (Stevenson & Robin South, 1974), additional sites of algal colonies were found in the adductor muscle (posterior and anterior), anus, gill filaments and amoebocytes in smears of stomach and haemolyth. The colour of the infected areas of the body depended on the size and shape of the colonies: small irregular colonies appeared greenish yellow while larger compact colonies were deep green. The shells of infested molluscs take an anomaly form. A direct dependency has been found between impaired shell growth resulting in the stunting of one or both scallor valves and the presence of algae: sometimes the shell edges are laminated into distinct layers with two or more lips or laminae; sometimes, however, all three layers of the shell are recessed, giving rise to an indentation in its outline (Stevenson, 1972). According to the latest genetic data (Rodríguez et al., 2008), algae isolated from a scallop, stored in a CCAP, and predetermined exclusively by morphological recognition is a sister sequence to *Nannochloris*, differing considerably from the *Coccomyxa* clade.

Representatives of the genus *Mytilus* (*Mytilus galloprovincialis*, *Mytilus trossulus*, *M. edulis*, *Mytilus edulis chilensis* and the hybrid *M. edulis-M. trossulus*) usually have a smooth shell and are found everywhere. The shell is triangular in shape, with rounded edges and thin concentric growth lines without divergent ribs. Whereas the shell of *C. parasitica*-infected mussels is often highly eroded and the growth lines on the posterior margin are irregular (Zuykov et al., 2014; Zuykov et al., 2018b). A large collection of pearls is occasionally found in the mantle tissues. Pearls are inorganic and organic particles (e.g., dead parasites) coated with nacre (Rodríguez et al., 2008). The scars of the posterior adductor muscle on the inner surface of the shell in about half of all molluscs

are partially or even completely faceted with extra calcium carbonate material (Zuykov et al., 2014). *Modiolus kurilensis* ("horse mussel") is a bivalve mollusc of the Mytilidae family. The molluscs includes brown periostracum grown into long, soft bristles close to the margins of the shell, especially in small individuals (Lutaenko & Noseworthy, 2012). *Coccomyxa*-infested *Modiolus* have deformed, round, heart-shaped shells with blunt, jagged edges and wide gaps between valves. The shells of some specimens have strongly pronounced concentric lines on both the inside and outside of the valves, reflecting discontinuous growth (Sokolnikova et al., 2016). *Modiolus* are found in western and northwestern areas of the Sea of Japan and along the coast of Sakhalin. *Mytilus* is usually found in intertidal and subtidal beds of rocky shores (Lutaenko & Noseworthy, 2012). *M. kurilensis* is found in bays with silty and sandy-silty sediments with scattered boulders and large pebbles (Sokolnikova et al., 2016). *Coccomyxa*-infested Mytilidae are typically found in the middle or lower intertidal zone at the depths of 0.3–0.7 m (Gray et al., 1999; Mortensen et al., 2005; Rodríguez et al., 2008), of 2–5 m in eutrophication water (Sokolnikova et al., 2016; Syasina et al., 2012) and of 15 m in benthic ecosystems with sandy or silty bottoms (Vázquez et al., 2010). Some authors (Gray et al., 1999; Naidu & South, 1970; Vázquez et al., 2010) note that the degree of infection of different species of molluscs decreases with increasing water depth, since algae require access to light for photosynthesis. Also, some studies (Crespo et al., 2009; Mortensen et al., 2005; Rodríguez et al., 2008; Stevenson & Robin South, 1974; Zuykov et al., 2014) report on the infection of molluscs older than two years. However, Sokolnikova et al. (2016) observed infection in horse mussels only older than 6 years. Some authors (Mortensen et al., 2005; Zuykov et al., 2014) have also suggested that the degree and frequency of algal infestation increase with age, but to a certain extent because very old individuals have not been found. This likely reflects the early mortality of infested molluscs.

The only factor that influences the appearance of algae in the tissues of molluscs mentioned in the literature is light. Other limiting conditions have not been investigated. No morphological features found in Mytilidae organs for placing algae in their tissues, distinguishing them from "healthy" bivalves (with the exception of a poorly closing shell), have been observed.

Two groups of bivalves, *Clinocardium* (Cooke, 1975; Jones & Jacobs, 1992) and freshwater *Unio* and *Anodonta* (Goetsch & Scheuring, 1926), also fail to show obvious signs of specialization.

Thus, symbiosis is characterized by one or more features: (1) overgrown tissues bearing algae (especially in the back of the mantle); (2) a wide gap between shell valves, which is usually thick and heavily weighted; (3) the light-conducting microstructure of the shell is organized by long prisms located above the tissues with algae on the upper dorsal or posterior side of the valves, which is sometimes accompanied by lateral expansion and dorsoventral flattening of the shell.

Environmental changes can cause the loss of the symbionts (Belda-Baillie et al., 1998; Buck et al., 2002; Fitt & Rees, 1995; Junchompoo et al., 2013; Leggat et al., 2003), which provokes the whitening of the clam's mantle tissue. Thus, three environmental factors limit the relationship between these organisms. The first factor is light intensity and elevated temperature (Buck et al., 2002). It is believed that a global increase in seawater temperature to 28℃–34℃ leads to the destruction of the zooxanthellae photosynthetic apparatus or through dark reactions of photosynthesis and, ultimately, to the disappearance of algae from the host organism through a phenomenon called bleaching (Buck et al., 2002; Hernawan, 2008; Junchompoo et al., 2013; Leggat et al., 2003). Gomez & Mingoa-Licuanan (1998) have reported on the high mortality of giant clams during unusual ocean current "El Nino". Within one year, seven species (*Tridacna gigas*, *T. derasa*, *T. squamosa*, *T. maxima*, *T. crocea*, *H. hippopus*, and *H. porcellanus*) were observed to be bleached and died in unusually high-water temperatures. The bleaching has led to a decrease in the zooxanthellae population by almost 30 times (Leggat et al., 2003). Buck et al. (2002) have also shown that light intensification and temperature rises of 4℃–6℃ are the main causes of mollusc bleaching. Dubousquet et al. (2016) found that although the mantles of giant molluscs remained coloured throughout the heat stress experiment, *Symbiodinium* residing in these tissues show morphological changes: algal cells gradually show signs of damage and increase in size with increasing temperature. Control cells had a diameter ≤ 13 µm. At a temperature of 31℃, the diameter reached 16 µm, and at 32℃–17.5 µm. In addition, aggregates were found in the cytosol of some cells (< 1%) at 31℃ (the frequency of these aggregates increased at 32℃). Cell rupture and cell debris were observed after 5 days at 32℃. Even after lowering the temperature to 26℃ (within 12 days), this phenomenon was still observed, and new *Symbiodinium* cells showed a new phenotype with large dark granulations.

The second limiting factor concerns the availability of phosphate and ammonium to photobionts (Belda & Yellowlees, 1995; Belda et al., 1993), which increases in eutrophic water. Ambariyanto & Hoegh-Guldberg (1997) observed that nitrogen

addition significantly changes the ultrastructure of zooxanthellae inhabiting clams. Molluscs exposure to elevated ammonium increases microalgae density, reduces algal cell size, down-regulates N uptake by zooxanthellae newly isolated from their hosts, and reduces glutamate levels in the haemolymph with increased pools of certain free amino acids (methionine and tyrosine) found in the zooxanthellae. These data confirm that the photobionts in molluscs are N-limited *in situ* and have free access to inorganic ammonium from seawater. Similar data are available for phosphorus. The P-N ratio of the host reflects the content of nitrogen and phosphorus in the habitat, while this is not observed in zooxanthellae (Belda-Baillie et al., 1998). Four major aspects of bleaching were in turn identified: (1) the loss of photosymbionts, (2) the decline of chl a, c1 in remaining symbiotic algae, (3) the retention of small zooxanthellae in tissue, and (4) the release of ammonium (NH_4^+) into the water column alongside the blockage of ammonium nutrient uptake.

In addition to elevated temperatures, light intensity, ammonium and phosphate levels, factors such as marine pollution, reduced salinity levels, the presence of parasitic, bacterial or viral infections, etc. may also influence. Further studies are needed to understand limiting factors and the stress tolerance of giant clam to restock wild populations of these incredible molluscs and to provide useful information for conservation management.

Characteristics of endobiotic microalgae

Dinoflagellates have a wide range of adaptive capabilities, which are associated with a high level of diversity of morphological and genetic forms, and allow them to inhabit both marine and freshwater reservoirs. Because *Symbiodinium* are active swimmers and capable of vertical migration, they can thrive in conditions unsuitable for many species of phytoplankton (Hackett et al., 2004; Kooistra et al., 2007). Most photosynthetics have chlorophylls a and c2, the carotenoid beta-carotene, and a group of xanthophylls, which are unique to dinoflagellates: peridinin, dinoxanthin, and diadinoxanthin. However, some dinoflagellates have acquired other pigments through endosymbiosis, such as through fucoxanthin (Hackett et al., 2004; Jeffrey & Haxo, 1968). Algal cells have a single nucleus, a cup-shaped or single band chloroplast (chloroplast thylakoids usually arrange in distinct stacks), one to four mitochondria, and several ribosomes but few endoplasmic reticulum. Pyrenoids are limited, and only a few starch grains can be observed. Two different cell types can be distinguished on the basis of the cell-wall covering or theca

(Lee et al., 2015; Posten & Walter, 2012). Darkly stained reticulate bodies, likely the polyphosphate bodies are found in most cells (most conspicuously in apparently older, degenerating cells). "Naked" cells are fragile and have an outer plasmalemma that surrounds a layer of flattened vesicles. "Armored" cells have cellulose or other polysaccharides within each vesicle, giving the cells a more inflexible and rigid wall. These cellulose plates are arranged in patterns called "tabulation" and used as taxonomic "fingerprints" (Hackett et al., 2004; Posten & Walter, 2012). Outside, the cell wall is usually surrounded by a thick amorphous layer. It is likely that this layer is of algal origin, since some authors indicate that it often occurs between groups of algal cells that are still surrounded by a cell wall, or as a kind of matrix in which single algal cells and free cell walls are found. The surface of *Symbiodinium* cells is varied: some cells are smooth and spherical, while others have elaborate structures or outgrowths (Hackett et al., 2004; Kooistra et al., 2007).

The core of dinoflagellates is also unique: the chromosomes are clearly visible at all stages of growth because they do not go through the packaging, as is common with other phytoplankton, but instead remain permanently condensed; the nucleosomes associated with their DNA are few or none; the pattern of mitosis is exceptional (Hackett et al., 2004; Lee et al. 2015).

Another distinguishing feature of dinoflagellates is that motile cells include two unequal flagella: one is ribbon-shaped, flattened, lies in a transverse groove, surrounds the cell, ensuring its rotation and movement; the other flagellum acts as a rudder and is directed backward along the longitudinal groove (Carty, 2003; Posten & Walter, 2012).

At the moment, traditional biological methods have not provided useful information for the identification and classification of *Symbiodinium* (though zooxanthellae have traditionally been difficult to classify). Until recently (Pochon & Pawlowski, 2006), *S.* (= *Gymnodinium*) *microadriaticum* was considered the only pandemic species of symbiotic dinoflagellates. However, the use of complex analysis tools (genetic, biochemical, physiological, morphological and behavioral) has led to a reassessment of the taxonomy of these zooxanthellae, revealing a wide diversity within the genus *Symbiodinium*. Based on numerous studies (Carlos et al., 2002; Coffroth & Santos, 2005; Fitt & Rees, 1995; Lajeunesse, 2001; Lee et al., 2015; Pochon et al., 2014), a new classification scheme for the genus has been developed, which divides it into several large groups called clades (A–H). Clades A, B, C and D are symbionts of molluscs (Pochon et al., 2014).

Previously, it was assumed that one host species contains a taxonomically homogeneous population of symbionts (one host-one symbiont). However, recent molecular studies have shown that some giant clams can form symbiosis with heterogeneous *Symbiodinium*, which includes at least 4 taxa of zooxanthellae (Carlos et al., 2000). For example, phylogenetic analysis of rRNA small subunits has shown (Carlos et al., 2002) that the *Symbiodinium* A and C clades can be isolated from six species of giant bivalves. Baillie et al. (2008) found the same result for allozyme and random amplified polymorphic DNA patterns. Regarding the latter, *Symbiodinium* clade D has been isolated from *T. crocea* (Ishikura et al., 2004). However, the exact determination of species of genus *Symbiodinium* has still not yet been achieved, as it is not clear which molecule(s) differentiate(s) the species in this genus.

Zooxanthellae present different lifecycles in symbiosis and culture conditions (Hinde, 1987; Yellowlees et al., 2008; Zahl & McLaughlin, 1957). Studies show that a number of morphological and physiological changes occur shortly after isolation of zooxanthellae from the host. In animals, algal cells are found in all growth and division stages, including cells that appear as aplanospores with young cells encased within a single cell wall. Inside the host, microalgae are in a vegetative state, which is characterized by the absence of flagella or a distinct band of cells (non-motile or coccoid form). Outside the host, cells are usually motile (flagellated) (Zahl & McLaughlin, 1957) and phototaxis (Domotor & D'Elia, 1986; Taylor, 1969). In culture, there is an alternation of mobile and non-motile forms (Domotor & D'Elia, 1986; Muscatine, 1980).

All strains of *Symbiodinium* are engulfed by molluscs during feeding, but only a particular strain will eventually dominate a particular host. The "selection" of a strain by a mollusc depends entirely on how that strain can survive, compete, and grow with other strains within the host (Ambariyanto, 2002; Fitt & Trench, 1981).

Difficulties are also experienced in research on green microalgae *Coccomyxa*. In 1971, Naidu reported on the parasitic status of algae-infesting *Pecten* but failed to define their taxonomy. Later, Stevenson & Robin South (1974) described a new member of the Coccomyxaceae family named *C. parasitica*; in 1978, Boraso-De-Zaixso & Zaixso found green invasion in *M. edulis* and suggested that it was also caused by *C. parasitica*. Bala (1995), Gray et al. (1999), Mortensen et al. (2005), and Rodríguez et al. (2008) described *Coccomyxa* in *M. edulis* from different water areas of the Atlantic Ocean. Rodríguez et al.

(2008) showed that all microalgae isolated from mussels collected in different areas (except strain CCAP 216/18) belong to the *Coccomyxa* clade. Whereas the algae isolated by Stevenson and Robin South and stored in the Collection of Algal and Protozoal Cultures (CCAP 216/18) are not actually *C. parasitica*. In 2009, Crespo et al. subsequently observed *C. parasitica* in *M. galloprovincialis*; later, Vazquez et al. (2010) discovered *C. parasitica* in *P. abbreviate* and *E. macha*. A phylogenetic analysis of algae from *Modiolus* performed by Sokolnikova et al. (2016) clearly suggests that *M. kurilensis* from the Sea of Japan is also infested by *C. parasitica*, similar to *C. parasitica* from Flensburg fjord 2 (EU127471) and Flensburg fjord 1 (EU127470) (Rodríguez et al., 2008). Then (Zuykov et al., 2014; Zuykov et al., 2018a), these microalgae were found in *M. edulis*, *M. edulis-M. trossulus* hybrid and *M. trossulus* in the St. Lawrence Estuary, Canada.

C. parasitica is a eukaryotic round or oval unicellular microalgae of approximately 2–10 μm without pyrenoids and flagella, and with one or two parietal cup-like chloroplasts (Crespo et al., 2009; Gray et al., 1999; Rodríguez et al., 2008; Sokolnikova et al., 2016; Syasina et al., 2012; Vázquez et al., 2010). Pigments of *C. parasitica* include chlorophyll a and b and the carotenoids: neoxanthin, violaxanthin, lutein, zeaxanthin, and b,b-carotene (Crespo et al., 2009; Rodríguez et al., 2008). In addition, Crespo et al. found alloxanthin (a typical cryptophytes) and diadinoxanthin and diatoxanthin (typical of chrysophytes) (Crespo et al., 2009).

Coccomyxa reproduces through di- and tetra-autospores 2–8 μm in size (Crespo et al., 2009; Gray et al., 1999; Rodríguez et al., 2008; Sokolnikova et al., 2016; Syasina et al., 2012). The culture obtained by Stevenson & Robin South (1974) had different shape of cells (ranging from round to drop-shaped with various outgrowths) and 8-, 16-cell sporangia proving the difference between the microalgae infesting the tissues of scallops and *C. parasitica*.

Hartman & Pratt (1976), Naidu & South (1970), Rodríguez et al. (2008), Stevenson & Robin South (1974) attempted to isolate microalgae from mollusc tissues in various experiments. Despite the variety and complexity of methods for isolating *Coccomyxa*, the resulting cultures were contaminated with bacteria and died. Belzile & Gosselin (2015), Sokolnikova et al. (2016) obtained a pure (likely axenic) culture of *Coccomyxa* from the tissues of molluscs and analysed its lifecycle via laboratory cultivation. They established that a culture of microalgae develops at a temperature of 15 ℃–18 ℃ or 20 ℃–22 ℃, respectively. A medium with an f/2 composition was an optimal medium for a

culture of *C. parasitica* from *Modiolus* (Sokolnikova et al., 2016), free-living *Coccomyxa* sp. CPCC 508 (Verma et al., 2009) and *Coccomyxa* sp. from *M. edulis* (Belzile & Gosselin, 2015). Naidu & South (1970), Stevenson & Robin South (1974), Hartman & Pratt (1976) also provided an list of suitable media for cultivating endobiotic algae that are similar in composition to f/2. The 18/6 h or 16/8 h photoperiods included an optimal sequence of light and dark phases for growing *Coccomyxa* in culture (without exposure to direct light) (Belzile & Gosselin, 2015; Sokolnikova et al., 2016). Young cells were small (3.7 ± 1.1 μm) with a single cup-shaped chloroplast, while old cells were much larger (8.6 ± 1.4 μm) with a single diffused chloroplast.

In 1995, Bala studying the spread of *Coccomyxa* in Mytilidae species *Brachidontes rodriguezi*, *Aulacomya atraatra*, *Perumytilus purpuratus* and *Mytilus edulis platensis* from the San Jose Gulf found these algae only in mussels. Vazquez et al. (2010) observed *Coccomyxa* only in *Panopea* among all collected molluscs (*Ostrea puelchana*, *P. abbreviata*, *Pododesmus rudis* and *Aequipecten tehuelchus*).

The data described above, and the fact that Sokolnikova et al. (2016) also failed to detect invasion of other mollusc species in mixed drusen, except for the case of *M. kurilensis*, suggest that the microalgae *C. parasitica* exhibits specificity for Mytilidae.

Description of microalgae infested *Clinocardium* is very limited (Cooke, 1975; Hartman & Pratt, 1976; Jones & Jacobs, 1992). It is known that microalgal cells had many ribosomes, a single cup-shaped or band chloroplast, few starch grains, and a small endoplasmic reticulum, but did not have pyrenoids and flagella. The cell wall of these algal cells is usually thicker than that of *Chlorella* and is surrounded by a wide amorphous layer (when algae in molluscs [Cooke, 1975] and culture [Hartman & Pratt, 1976]). Microalgae reproduce by simple division and the formation of autospores (4, 8 or 16 daughter cells) (Hartman & Pratt, 1976).

Microalgae (approximately 1–11 μm in size) described as a parasite of the marine scallop, *P. magellanicus* (Naidu & South, 1970) have highly variable cell morphology (from elliptical to spherical, sickle-shaped or oblong), characterized by the possession of a distinct hyaline tip which is reduced in culture. There are no pyrenoids in the cells, but there are 1–3 parietal cup-shaped chloroplasts. Pigment profile consisted of chlorophyll a and b, b,b-carotene and neoxanthin. Reproduction is carried out by 2, 4 or 8 autospores in the host, and in culture by 16 more autospores (with a predominance of 2- and 8-cell forms). As the culture ages, algal cells become more spherical, vacuolated, and decrease in size, the cell wall thickens, large refractile cell inclusions develop, chloroplasts decrease in size, oil-like storage products are formed, and the rate of autospore formation decreases. Vacuolization and the formation of cytoplasmic inclusions were observed mainly in old liquid cultures, and spherical cells in agar cultures (Stevenson, 1972; Stevenson & Robin South, 1974).

Relationship bivalves and microalgae

Establishment of associations

Dinoflagellate symbionts by most animal hosts is often achieved through the reinfection of offspring with each successive generation (Hirose et al., 2006). Ingested zooxanthellae by larval tridacnids are present in the stomach for more than a week, and after metamorphosis, the cells move into channels within the developing mantle a duct, which marks the start of the zooxanthellal tubular system (Fitt & Trench, 1981; Fitt et al., 1986; Hernawan, 2008; Mies et al., 2017a; Neo & Todd, 2009). Microalgae are found in the hypertrophied tissues of the mantle, and also in the stomach, digestive gland, intestine, heart (Goreau et al., 1973; Fitt & Trench, 1981; Fitt et al., 1986; Mansour, 1946; Morton, 1978) and faeces of clams (Fitt et al., 1986; Maruyama & Heslinga, 1997). Since they are viable morphologically intact and photosynthetically functional, Trench et al. (1981) suggested that microalgae cannot be digested by molluscs as previously thought (Fankboner, 1971). Dinoflagellates are capable of transferring some of their products of photosynthesis to the host (Ambariyanto, 2002; Ambariyanto & Hoegh-Guldberg, 1997; Fitt & Rees, 1995; Goreau et al., 1973; Hernawan, 2008; Leggat et al., 2000). The number of zooxanthellae and their contribution to the host metabolism primarily depend on the light intensity and the size of the mollusc (Ambariyanto, 2002).

It has been found that there are two mechanisms by which zooxanthellae appear in the next generation of their symbiotic host. Zooxanthellae can be acquired directly from parents through eggs or from the environment (Gontier, 2015). Endobionts were not found in trochophores (Fitt & Trench, 1981), which confirms that microalgae are not transmitted to larvae (Jameson, 1976; Fitt et al., 1984; Fitt et al., 1986; Hernawan, 2008). However, some authors have shown that larvae acquire zooxanthellae *de novo* from the environment shortly after metamorphosis (Ambariyanto, 2002; Fitt et al., 1984; Hernawan, 2008; Jameson, 1976; Trench et al., 1981). Despite the fact that all strains of *Symbiodinium* sp. are eaten by molluscs, only a par-

ticular strain will eventually dominate a particular host (Ambariyanto, 2002; Fitt & Trench, 1981). The selection of a strain depends on how it can survive, grow and compete with other strains in the host (Carlos et al., 2000; Fitt & Rees, 1995; Fitt et al., 1986; Mies et al., 2017a), implying that each generation must acquire complementary symbionts from the environment through a process called horizontal ("open" system) transmission (Coffroth & Santos, 2005).

Despite numerous studies, there is still no information about the relationship between dinoflagellates and host larvae in the process of their development. This is especially true for the selection of a "suitable" strain of zooxanthellae. It is likely that bivalves select strains of *Symbiodinium* through the interaction of lectins and glycans, as evidenced by similar studies of the interaction of *Symbiodinium* with gastropods and corals (Mies et al., 2017b).

Mansour's observation (1946) of a tubular system originating from the stomach of molluscs was perceived by Young (1953) and Morton (1978) as an impossible phenomenon for molluscs. Later, Norton & Jones (1992) and Norton et al. (1992) confirmed Mansour's discovery using histological methods. In their studies, they showed that *Symbiodinium* is mainly found in the blindly closed terminals of the intestinal diverticulum located in the mantle. This system of tubules, first in the form of one primary tube, departs from the diverticular duct of the stomach, and then passes to the dorsal side of the digestive organ. Further, the tube system, passing over the digestive organs, diverges into left and right tubes. Each tube pierces the kidney, enters the root of the middle ctenidial support ligament, then goes around the adductor muscle and flows into the base of the siphon mantle. After that, the tubes, branching, form a secondary system of tubules. Further, these tubes, reaching the upper border of the inner fold of the siphon membrane, form multiple branches of tertiary zooxanthelic tubules. These tubes, making a convolution, are already blindly ending. In addition to the siphonal mantle, secondary tubules also form branches in the pericardium, heart, connective tissue of the arterial bulb, ctenidium, and lateral mantle (Norton et al., 1992; Hernawan, 2008). The tertiary tubes contain the large numbers of algal cells. When bleached giant clams, the mantle lacks algae, and the tertiary zooxanthellal tube atrophies (Farmer et al., 2001; Hernawan, 2008; Norton et al., 1992). Anatomical and histological data show no interaction between the zooxanthellae tubule system with the haemolymphatic system, which is the reason for the absence of algae in the haemal sinuses (Norton et al., 1992). The mor-

phology observed is similar to that of the Cardiidae, *C. cardissa*, which has a zooxanthellal system observed in tridacnid clams. *Symbiodinium* in the lumen of the tubules are often observed pressed against the membrane of the cells that form the wall of the tubules (which is why some authors assumed that algae are intracellular) (e.g., Kawaguti, 1966). The algae are separated from the haemolymphatic system by tissue of one cell layer thick (Morton, 2000; Farmer et al., 2001). Like giant clams, *F. erugatum* possesses a zooxanthellal tubular system but links digestive diverticulitis from the stomach with the kidneys. The cells of the tubules are contain algae in different stages of degeneration, intact or vacuolated cells, and lumina contain their disintegration products (Morton, 2000). The functioning of the recently identified zooxanthellal tubular system in the Tridacnidae is likely in need of more detailed re-examination.

Symbiodinium is located in thin membranous tubules underneath the iridocytes, which are organized into vertical columns roughly parallel to the direction of the incident photons (Ghoshal et al., 2016; Holt et al., 2014; Kawaguti, 1966). In the mantle epithelium of the molluscs *T. crocea*, *T. maxima* and *T. derasa*, phytobionts are densely packed into vertical quasi-ordered microcolumns, the long axis of which is parallel to the downward light, as described above. The columns have a diameter of 50–100 mm and are located at a distance of approximately 200 mm from each other. Holt et al. (2014) reconstructed the three-dimensional arrangement of microalgae cells: it turned out that the surface area of the columns is about 10 times larger than the area of the surface of the mantle tissue containing them. Since iridocytes are somewhat reflective, some authors suggest that they may play a photoprotective role (Ghoshal et al., 2016). However, the data obtained by Holt et al. (2014) show that that back-scattering from iridocytes the backscatter from iridocytes is most intense in the green to yellow portion of the visible spectrum, in which the absorption of *Symbiodinium* is minimal, since they more efficiently use the red and blue visible light for photosynthesis. For example, even yellow and green wavelengths are scattered back by iridocytes about 20% of all incident light. Thus, due to backscattering of light, any photoprotection by the iridocytes of the algae is minimal. The arrangement of columns of microalgae in the mantle geometrically affects the angle and wavelength of light, and allows it to scatter evenly, reaching deep tissue layers (Holt et al., 2014).

To date, mechanisms that adjust algal population size remain unclear. Initially, Fankboner (1971) proposed that microalgal cells are selectively culled by amoebocytes within the

mantle margin and then digested by lysosomes in the circulatory system and in the interdiverticular spaces of the digestive gland. This process was defined as "the low systematic removal and utilization of degenerate zooxanthellae" (Hernawan, 2008). Some researchers have observed the excretion of dark brown faeces with viable microalgae (Fitt et al., 1986; Lucas, 1994; Maruyama & Heslinga, 1997; Trench et al., 1981). Lucas (1994) described that microalgae move through the zooxanthellae system into the digestive tract and are excreted from there undigested. Maruyama & Heslinga (1997) estimated the number of faecal dinoflagellates at up to 1.46% of the population in the mantle. Approximately 64% to 89% of newly formed microalgae from the population were missing and remained undefined (further researches must identify this missing population).

With regard to *Coccomyxa*, reliable literature results concerning the mechanisms through which algae enter the bodies of molluscs are absent. Initially, in 1970, Naidu & South proposed that algae penetrated bivalves through damaged shells and outer epithelial layers. Although experimental infection, in which algal cells were injected into the marginal mantle fold, were the most successful, Stevenson (1972), after long-term observations of naturally infected individuals at an early age, found that not every infested individual had damage to the body. After that, this group of scientists inclined to the assumption that algae penetrate the body of molluscs in the process of feeding through the digestive system. This assumption was confirmed by the fact that Stevenson & Robin South (1974) observed hemocytes with phagocytosed, undigested algae in the stomachs of molluscs. The authors found that the cell wall of microalgae is resistant even to 75% H_2SO_4. Therefore, they decided that hemocytes, not having sufficiently effective mechanisms for the destruction of microalgae, by engulfing them, only contribute to the spread of parasites throughout the body (Stevenson & Robin South, 1974). The histological analysis performed by Sokolnikova et al. (2016) revealed the ubiquitous distribution of algae in the body of molluscs (except for gills and adductor muscles). The most intense hemocyte infiltration was observed in the mantle, gonads, kidneys, digestive gland, and gills. Encapsulation of microalgae cells with large foci of fibrosis and necrosis was also often observed in these organs. Such an extensive inflammatory reaction, according to the authors, indicates the pathogenic effect of *Coccomyxa* on bivalve molluscs (Crespo et al., 2009; Gray et al., 1999; Mortensen et al., 2005; Rodríguez et al., 2008; Vázquez et al., 2010) that are commonly observed during parasitic invasion (Villalba et al.,

1997; Zuykov et al., 2014). Pathological alterations in the mantle of *M. kurilensis* (Sokolnikova et al., 2016) were similar to those in the siphon tissues of *P. abbreviate* (Vázquez et al., 2010), and were expressed in a change in the orientation of muscle and connective fibers, the appearance of cellular debris, and infiltration with haemocytes with engulfed algae. According to the researchs, *Coccomyxa* invasion leads to general tissue deformation and haemocyte infiltration, and adductor muscle weakening and atrophy (Gray et al., 1999), increased mucus in the mantle (Sokolnikova et al., 2016), shell erosion (Mortensen et al., 2005; Sokolnikova et al., 2016; Zuykov et al., 2014; Zuykov et al., 2018b), increase in concretions number of kidney (Sokolnikova et al., 2016), reproductive cycle disorder, due to underdevelopment of the gonads and replacement of their connective tissue (Gray et al., 1999; Vaschenko et al., 2013). These disturbances then lead to developmental delays, organ dystrophy, and impaired filtration. A recent paper (Zuykov et al., 2018b) also shows that the degree of shell deformations increases with an increasing concentration of microalgae in the haemolymph/extrapallial fluids. *Coccomyxa* affects calcification in infested bivalves *M. edulis*. Authors propose that as the concentration of algal cells in biological fluids reaches high levels, bivalves lose metabolic CO_2 and alter acid-base regulation at the calcification site, where an initial portion of extra shell material is deposited onto the shell-growing edge. Thus, molluscs' intense defensive reactions against algal infestation and pathogenic effects indicate that the relationship between green algae and molluscs is characterized by parasitism (Gray et al., 1999; Rodríguez et al., 2008; Sokolnikova et al., 2016; Stevenson & Robin South, 1974; Vázquez et al., 2010; Zuykov et al., 2018b). Further, no ultrastructural data on the tissue dislocation of algae yet clarify histological rearrangement of molluscs in the presence of algae.

Unlike tridacnines, *Coccomyxa* of all stages from "aplanospores" to older cells is observed in the mollusc haemocytes (Sokolnikova et al., 2016; Syasina et al., 2012). However, the mechanism for recognizing microalgae from haemocytes and the cause of their non-digestion remain unclear. Algal cells are also often found in *Clinocardium* haemocytes (Hartman & Pratt, 1976), which resemble acidophilic granular haemocytes of an oyster *Crassostrea*. Thus, at present, the morphological appearance of bivalves and the cellular conditions of *Coccomyxa* differ markedly from those of known mutualistic algae found in other hosts. Second, the resulting effects on the host and on its responses are much more typical of those of a parasitic combination.

Physiological aspects of interactions between algae and bivalves

The importance of symbiosis in the life of molluscs can be inferred from the patterns of their growth and survival. The first studies concerning the role of zooxanthellae in the life of molluscs were made by Fitt & Trench (1981). According to many researchers (Ambariyanto, 2002; Ambariyanto & Hoegh-Guldberg, 1997; Belda-Baillie et al., 1998; Buck et al., 2002; Dubousquet et al., 2016; Goreau et al., 1973; Grice & Bell, 1999; Jantzen et al., 2008), larvae survive grow larger and longer with *Symbiodinium* was than those without it. Fitt et al. (1986) have shown that juveniles that have algal symbionts can survive for more than 10 months using only light as their sole source of energy.

The number of zooxanthellae has been considered one of the most basic factors contributing to bivalve survival (Griffiths & Klumpp, 1996). Molluscs lacking a large number of microalgae are known to exhibit significantly inhibited fitness, resulting reduced growth, weight, size, fecundity and survival (Ambariyanto & Hoegh-Guldberg, 1997; Buck et al., 2002; Grice & Bell, 1999; Griffiths & Klumpp, 1996; Jantzen et al., 2008; Leggat et al., 2003). For example, Griffiths & Klumpp (1996) described logarithmic increases in bivalves length for *H. hippopus*, *T. crocea*, *T. derasa*, *T. gigas* and *T. squamosa* with increasing total algae numbers. For small body sizes (2 cm length), microalgae numbers were found to be much lower in *T. squamosa* and *T. gigas* than in *T. crocea*, *T. derasa* and *H. hippopus*. However, once body sizes reached 30 cm, *T. squamosa* and *T. gigas* had the largest *Symbiodinium* populations as reflected by their intensive relative photosynthesis. Maruyama & Heslinga (1997) investigated the daily population dynamics of microalgae in the mantle and established that mitotic index increased after sunset and reached a maximum value after 3 to 5 hours. The growth of the mantle and shell of molluscs has been found to be directly correlated with zooxanthellae populations.

Researches using radioactively labelled dinoflagellates from *Tridacna* sp. showed that glucose sugars, glycerol and amino acids are among the compounds translocated from algae to the host (Goreau et al., 1973; Ip et al., 2017; Ishikura et al., 1999; Muscatine, 1980; Yellowlees et al., 2008). Carbon budgets have revealed that 90%–95% of the carbon fixed daily by zooxanthellae is translocated to the host (Ambariyanto, 2002; Belda & Yellowlees, 1995; Belda et al., 1993; Mies et al., 2017a). This carbon is enough to meet the daily energy and growth needs of the mollusc, except for recently metamorphosed individuals, which have more needs but fewer microalgae (Fitt et al., 1986; Ip et al., 2017; Mies et al., 2017a). However, molluscs are able to absorb dissolved nutrients from seawater in addition to those obtained from their symbionts (Jantzen et al., 2008). Intact bivalves deplete seawater of phosphorous (Belda & Yellowlees, 1995; Belda et al., 1993; Belda-Baillie et al., 1998) and inorganic nitrogen (Wilkerson & Trench, 1986). Additions of nitrate and ammonia lead to the intensification of the processes of division of zooxanthellae, pigmentation and growth of clams (Belda et al., 1993; Fitt & Rees, 1995; Hastie et al., 1992). Additions of phosphate with dissolved inorganic nitrogen also increase growth rates (Fitt & Rees, 1995; Grice & Bell, 1999; Wilkerson & Trench, 1986). Additions of phosphorous and inorganic nitrogen lead to decreased calcification and the altered crystal formation of calcium carbonate. Phosphate, in particular, is known to inhibit calcium carbonate crystal growth (Ambariyanto & Hoegh-Guldberg, 1997). It has been assumed that photosymbionts are the components responsible for the uptake and assimilation of inorganic nutrients (Muscatine, 1980). However, data of high concentrations of enzymes in host tissue involved in assimilating nitrogen in corals and clams has led to alternate interpretations of such results (Belda-Baillie et al., 1998). It probable that host tissues are at least partially involved in the uptake of these dissolved nutrients. Apparently, the absorption of dissolved carbon compounds from the environment can supplement the main carbon obtained from microalgae (Goreau et al., 1973). Algae, storing lipids, can be a source of additional energy, and can also be used to composition organic skeletal matrices, mucus and new tissues (Ambariyanto, 2002; Ambariyanto & Hoegh-Guldberg, 1997; Belda-Baillie et al., 1998).

Trench et al. (1981) found that in *T. maxima*, algae provide most of the molluscs' need for respiratory carbon, which ranges from 62% to 84% on cloudy and sunny days, respectively (assuming 40% translocation). The genetic varieties of dinoflagellates found in the family Cardiidae are partly determined by the growth and species characteristics of the host. Ikeda et al. (2017) revealed that zooxanthellae zooxanthellae in clams use CO_2 as their main source of Ci (whereas coral symbionts use bicarbonate).

For *Coccomyxa*, the intense defensive reactions of molluscs against algal infestation, major pathology like alterations histomorphological patterns and infested organ dysfunction accompanied with tissue degeneration allow us to conclude that *C. parasitica*, which invades bivalves and which is capable of reproduction regardless of the host involved is a facultative

parasite. However, the data of histological studies serve as the only argument in favour of the parasitic status of algae. At the moment, no biochemical research has been conducted on the relationship between *Coccomyxa* and molluscs with the exception of studies on the intense calcification of infested bivalve shells (Zuykov et al., 2014; Zuykov et al, 2018b).

Conclusions and avenues for future research

Associations between two distinct species reflect ecologically and evolutionary influential phenomena facilitating the survival of both partners in diverse habitats. All extant symbiotic associations are the result of a series of events bringing two originally genetically independent organisms together to produce a more or less integrated unit.

For example, such cooperation, combined with efficient nutrient recycling, reflects adaptation to life in oligotrophic environmental conditions. In members of the subfamily Tridacninae, symbiosis with dinoflagellates leads to increased shell growth, increased lifespan, and gigantism. In *F. erugatum*, this alliance contributes the rapid growth and size of the bivalves population (but short lifespan), allowing them to dominate their unique oligotrophic and hypersaline environment. The formation of symbiotic systems is a complex multifaceted process that includes several stages: absorption of microalgae in the stomach cavity, phagocytosis by host cells, integration into host functions, long-term persistence and proliferation. Each step, alone or in conjunction with others, plays a role in selecting the suitable mollusc/microalgae combinations. At the same time, specific molecular mechanisms and physical factors can influence each stage. The mechanisms of selectivity underlying the expressed specificity of microalgal-invertebrate symbioses remain unknown, but evidence supporting the presence of molecular signalling between hosts and endobionts is accumulating.

The fact that different species of bivalves undergo varying degrees of morphological modification to accommodate the *Symbiodinium* suggests that host-symbiont interactions are not the same in all bivalve species.

There is much less information about the origin and biology of such associations between *Coccomyxa* and bivalves. In molluscs known to be associated with *Symbiodinium*, special morphophysiological features of shell structure/adaptation, internal organs (mostly the mantle), digestive and haemal systems maintain microalgae and effective cohabitation for survival in oligotrophic waters. Of all of the foregoing components, bivalves

inhabited by *Coccomyxa* are characterized by pronounced anomalies within the shell (its density, structure, and shape), allowing light to penetrate to illuminate internal organs (mostly the mantle) containing microalgae. The internal reorganization of molluscs with *Coccomyxa* are catastrophic and lead to organ dysfunction, but in spite of this it also allows hydrobionts to survive in oligotrophic water. From an evolutionary perspective, *Coccomyxa* may infect bivalves in an intermediate stage on an evolutionary path that may lead to mutualism or to parasitism in the future. At present, there are no data on portal(s) through which this parasite enters its host or on physiological and biochemical adaptations that result in consortium. The larvae of these molluscs have also not been tested for the presence of microalgae. There are also no data on the factors that maintain a stable relationship leading to benefits for the host and interventionist, including physiological interactions that underlie energy and nutrient transfer. There have been no detailed studies on how invertebrates defend against algae. Studies on the immunity of the host and on it is role in the establishment, regulation and breakdown of symbiosis will be key to understanding host-endobiont recognition and specificity. From a purely speculative viewpoint, oxygen produced by algae in periods of active photosynthesis may be used by molluscs. Carbon dioxide produced by a mollusc may in turn be taken up by alga during carbon fixation. However, at this stage of evolution, inflammatory reactions and extensive histopathological disorders of the bivalve's tissues to infecting parasites, with their overall adverse effects observed when algae are present at large concentrations; serve as sufficient cause for assigning *Coccomyxa* a parasite. In light of the fact that there are more and more reports of algae infecting new species of commercial molluscs, in the long run there is some threat of the subsequent spread of this viable parasite, which is especially dangerous for aquaculture farms. Further studies must focus on the relationship between *Coccomyxa* populations and various physiological features of molluscs such as their growth and nutrition performance, filter feeding, respiration patterns, and photosynthetic responses.

Investigation of the physiological aspects of associations should allow for further insight into the inception, maintenance, evolution and control of algal-invertebrate symbioses. Data show that symbiosis between marine molluscs and algae enhances metabolic capabilities and thus opportunities to occupy ecological niches of both symbiotic eukaryotes and host.

Achieving an understanding of the fundamental principles of this phenomenon will be difficult due to its complexity: it is

likely to be influenced by bivalve taxa and their developmental stages, differences in bivalve and symbionts microhabitat conditions, and the availability of partners. Understanding these trends should help researchers prioritize their research efforts on these algae together with their development as model systems for further research on the formation of symbiotic associations.

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