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Chapter 12

Scientific considerations on a technique of ecological purification that made possible the cultivation of reef-building corals in Monaco

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ABSTRACT

For the last 50 years powerful mechanical filtration and water sterilization techniques have been driving the evolution of life support systems of most of the public seawater aquaria. Their recent progresses have made possible the keeping of fish as large as manta rays and whale sharks. However, by removing all of the plankton and failing to eliminate all of the wastes produced by the fish and the decomposition of organic detritus these techniques alter the composition of the water in the tank. Filtered and sterilized water does not meet the biological requirements of a number of plants and invertebrates that are much more demanding than fish. A few open-circuit aquaria built in pristine near-shore areas have overcome the above-mentioned inconveniences by pumping huge amounts of unpolluted water. But the number of places suitable to accommodate aquaria of this kind is and will remain very limited. Therefore, efforts have been made to adapt to public aquaria natural techniques for maintaining closed-circuit aquaria that hobbyists began to develop empirically in the middle of the fifties. The present paper analyses the processes that underpin the genesis and evolution of one of these techniques, which has been implemented in Monaco to cultivate reef-building corals. Cultivated corals were used to improve the exhibition in the aquarium of the Oceanographic Museum but also to carry out scientific experiments, which have resulted in major findings regarding the ecology and physiology of these organisms.

INTRODUCTION

Set up in 1956 by Dr. René Catala, the Nouméa's Aquarium was one of the first public aquariums in the world where reef-building coral were successfully cultivated and kept with a number of fishes among which one could see quite large specimens of Napoleon wrasses. It was an open-circuit aquarium situated close to the seashore. Water was pumped from a neighboring fringing reef and the life support system was reduced to a pumping station and a buffer pool that was feeding by gravity open-top tanks illuminated by natural light. In this regard the Nouméa's aquarium was a kind of extension of the New Caledonia lagoon. I met with Dr. Catala, in August 1973, during a stop over I had made on my way back home from Brisbane (Australia) where I had participated in the Second International Coral Reef Symposium. I took this opportunity to inform Dr. Catala that I had succeeded in keeping reef-building corals in closed circuit aquaria in the marine laboratory of Tulear (Madagascar). And he was surprised.

At that time all of the public aquarium curators that I knew were thinking that keeping live corals with fishes in closed circuit systems was looking like an inaccessible dream. However, I did not share this opinion because I had already obtained encouraging results. Yet, in the middle of the fifties, while I was living in Algeria, I had kept specimens of the zooxanthellate Mediterranean coral *Cladocora caespitosa* (Linnaeus, 1758) using by chance a simple water management method similar to that of Lee Chin Eng (Eng, 1961). Thirteen

years later, in Madagascar, the same technique enabled me to keep specimens of several species of hard and soft corals. These results convinced me that there was something to uncover and at the end of a 17 years-long trial and error process complemented by scientific experiments I perfected at the University of Nice (France) the method described in the paper I presented, in 1988, at the Second International Aquarium Congress (Jaubert, 1989). This method enabled me to set up a 40,000 L reef tank in the Monaco's oceanographic Museum and to resume research in this famous organization by establishing the European oceanographic center (affiliated to the Council of Europe) where the ecology and physiology of cultivated corals was investigated using the most advanced scientific techniques.

Research completed in this center resulted in major findings, particularly the mechanisms which underpin: (a) the uptake and transport of bicarbonate and calcium ions (Tambutté *et al.*, 1995 and 1996; Al Moghrabi *et al.*, 1996; Bénazet-Tambutté *et al.*, 1996; Goiran *et al.*, 1996); (b) the metabolic fractionation of stable isotopes of oxygen, carbon and nitrogen (Reynaud-Vaganay *et al.*, 1999 and 2001; Muscatine *et al.*, 2005); (c) the synthesis of the skeletal organic matrix (Allemand *et al.*, 1998); and (d) the uptake and excretion of organic carbon (Al Moghrabi *et al.*, 1993; Ferrier-Pagès *et al.*, 1998a and 1998b). Experiments carried out using reef mesocosms enabled us to show that elevated temperature and carbon dioxide partial pressure (pCO_2) have a synergetic action, which inhibits calcification (Leclercq *et al.*, 2002; Reynaud *et al.,* 2003). This result may explain why reefs, which are exposed to warming temperatures and surface water acidification associated to rising concentrations of atmospheric CO_2, degrade worldwide in remote areas where they are not directly impacted by human activities.

MILESTONES ON THE WAY OF A DISCOVERY

Old stories and souvenirs

I became an aquarium hobbyist in June 1948. I was seven years old and my anniversary gift was a freshwater aquarium. At that time, the most popular way to set up aquariums was extremely simple (Boucher, 1946). My 50 liters tank (Figure 1a) was made of glasses sealed to zinc coated steel angles using a putty containing minium (red lead oxide). The soil was composed of one layer of humus covered with one layer of quartz sand. With a dense population of plants the tank was a kind of naturally balanced micro pound. Water stirred by a flow of bubbles was not filtered but remained crystal clear.

The same year I had the opportunity to see the first film of Jacques Cousteau (18 m below

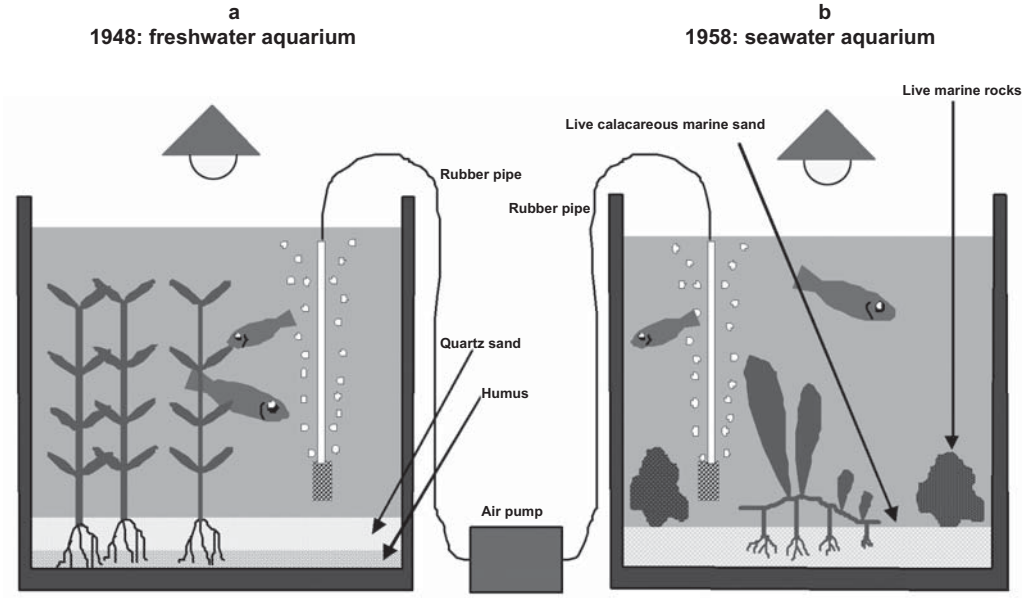


Figure 1: Freshwater and seawater aquarium, using the same basic natural principles, respectively set up in 1948 and 1958. The seawater aquarium set up in 1958 is similar to that of Lee Shin Eng.

the surface). Imitating Cousteau I started to snorkel in the Mediterranean Sea and admire the aquatic life, which was more attractive than that of my freshwater aquarium. I was fascinated and several years later I decided to try to set up a seawater aquarium by transposing the above-described freshwater aquarium technique. For this purpose I used live sand, live rocks and *Caulerpa prolifera* (Forsskål) Lamouroux 1809 (Figure 1b).

My first experiments quickly resulted in disasters. Their primary cause, the toxic compounds released by the corrosion of zinc, remained enigmatic during several years but became understandable when I used successfully, by chance, a small all-glass tank recovered from a dismantled lead battery. In this small tank I could easily keep small fishes, shrimps and anemones. Situated in front of a window the tank was receiving enough natural light to meet the requirements of the alga *Caulerpa prolifera* and of small colonies of the zooxanthellate coral *Cladocora caespitosa*.

At that time I did not know that my technique was similar to that of Lee Shin Eng who published in the Tropical Fish Hobbyist magazine an article entitled "Nature's system of keeping marine fishes" (Eng, 1961), which I uncovered two decades later.

Two years later, during summer vacations in France, I met by chance with René Coutant. This pioneering seawater aquarist (who founded in 1970 the Aquarium de La Rochelle, France) was manufacturing seawater tanks in a quite small shop situated close to the bank of river Charente in the city of Saintes. In the Coutant's tanks water was filtered trough glass wool, calcareous sand and activated carbon. When I said that I did not use any filter Coutant gave me a specimen of the little booklet he had published (Coutant, 1958). In this booklet I could read (page 2) "*Filtration, an important topic that will cause much ink to flow, is not indispensable in a well balanced tank*".
Thus, in the middle of the fifties at least 3 aquarists, living in different countries, Lee Chin Eng in Indonesia, René Coutant in France and Jean Jaubert in Algeria, had empirically discovered that a closed-circuit seawater aquarium could function very well without a filter and perhaps even better than with a filter. None of them knew why. They used simple techniques likely because polymers as well as sophisticated seawater-compatible equipment did not exist at that time and they were living close to the sea where they could collect live-rocks and live-sand. At that time I ignored and also Eng and Coutant ignored I presume, that, in the middle of the Victorian Era (nineteenth century), a number of amateurs were using almost the same technique to keep marine fish and invertebrates in glass jars where algae (especially *Ulva* sp.) illuminated by sunlight were the main source of oxygen. This technique is described in two handbooks (Gosse, 1855; Taylor 1910). According to (Verwey, 1930), quoted by Delbeek and Sprung (2005), the curators of an open-circuit aquarium built in 1928 in Indonesia were using live-rocks and full sunlight to keep corals and anemones and even breed clownfish.

My coral keeping experiments in France and Monaco

In 1973, Jacques Cousteau was preparing an exhibition dealing with reef-building corals in the Monaco's Museum. To make this exhibition more attractive, Cousteau who knew that I was keeping live coral in Nice asked me to set up a live coral tank in his Museum. At that time almost no coral was available on the market. Therefore I went to Eilat (Israel) with one of my colleagues (Dr. Marc Lafaurie) to collect colonies of various species. Thanks to the logistic support of the Weisman Institute and of the Heinz Steinitz Marine Biological Laboratory we succeeded in bringing back about fifty colonies belonging to fifteen different species of corals. Lafaurie and I had designed the 1,500 L closed-circuit tank inside which these corals were kept in Monaco. Height VHO daylight fluorescent tubes fixed inside a cover ventilated by electric fans provided enough light to meet the photosynthesis requirements of the corals. A prototype of chiller built in the Museum's workshop and a thermo regulated heater were used to maintain the temperature between 23 and 26 °C. But we made the error to equip this tank with an under-gravel filter operated with a powerful airlift. During one year, with almost no fish in the tank, the corals remained in good condition. But 6 month after we had added fish filamentous algae started to grow. The same phenomenon occurred in the tanks I had set up at the University of Nice. At the beginning this algal growth was discrete. I didn't pay enough attention to this sign of eutrophication it and wrote a too optimistic article regarding the performances of the system (Jaubert, 1976).

When the growth of nitrophilous algae became uncontrollable I understood that nitrate was causing the trouble. But I did not know how to get rid of it. If I had known the book of Peter Wilkens (Wilkens, 1973) I would most likely have used the technology promoted by the group that gave birth to the Berlin method and set up a powerful protein skimmer to reduce nitrate formation by eliminating dissolved organic matter before bacterial degradation. But I did not read the book and took another path.

Although I was keeping reef-building corals in the laboratory I could not use them to investigate their physiology because they were neither healthy enough nor enough numerous to allow valuable scientific experiments. In this regard I decided to carry out experiments on the seafloor. For this purpose I designed and built respirometers (Jaubert, 1977) and other underwater devices. During experiments carried out in Aqaba (Jordan) in 1979 I uncovered that reef sediments were sinks for nitrate (unpublished results). From the literature (Koike and Hattori, 1978; Sorensen, 1978) I understood that heterotrophic denitrifying bacteria living in hypoxic and anoxic layers of organic-rich marine sediments were responsible for this phenomenon. By "respiring" nitrate to oxidize organic compounds these bacteria were converting nitrate into molecular nitrogen. Immediately I thought that by creating a hypoxic sediment layer in my aquariums I could make use of this process to eliminate nitrate.

A simple way to create a hypoxic layer in my aquarium was to turn off the under-gravel filters. I disconnected the airlift of the under-gravel filter of one of the aquariums inside which I was keeping corals. But since this airlift was also used to stir and aerate the aquarium water I kept it and connected it to a strainer fixed above the sand (Figure 2). As PVC T connections were fixed to its upper extremity this airlift was also doing the work of a basic protein skimmer (Figure 2). This light skimming may have played a significant role. By discarding brownish foam it may have contributed to remove colored organic matter from the water of this aquarium (as well from that of other tanks I equipped later with this simple system), which never became yellowish although I never used activated carbon to filter it.

Eight months later the "miracle" occurred. The corals that were surviving in this aquarium started to grow. Measurements made with a spectrophotometer confirmed that nitrate had virtually disappeared from a concentration of 70 $mg.L^{-1}$ of NO_3^- (representing a theoretical rate of removal of 0.2 mg a day). To repeat this experiment I decided to dismantle a 2,000 liters tank and restock it from scratch with live sand, live rocks, corals and fish that I had collected in the Red Sea. The first results were excellent (Jaubert, 1981) but the tank

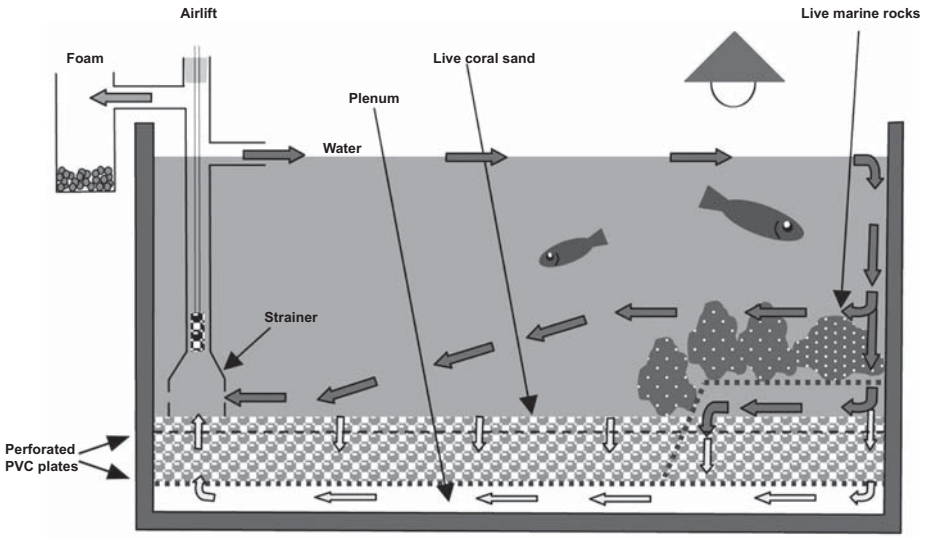


Figure 2: Airlift for water stirring and skimming in the Jaubert System (Jaubert, 1989). Arrows illustrate schematically water movements. Grey arrows simulate the circulation of the oxygen-rich water in the aquarium. White arrows simulate the circulation of pore-water throughout the sediment and that of the hypoxic water in the plenum.

needed more than 2 years of maturation to fully equilibrate.

THE JAUBERT'S SYSTEM

During 8 years I improved the system. I did this in a more or less empirical way because my academic responsibilities of research team leader, my *in situ* experiments in the Red Sea as well as the Pacific Ocean (French Polynesia) and my lectures at the University of Nice were consuming most of my time. In fact, my aquarium activities never fell within the scope of my "official" research activities and thus remained marginal. However, in 1988, I took the time needed to describe the system in Monaco at the Second International Aquarium Congress (Jaubert, 1989). At that time my aquarium was looking like a small Red Sea coral patch and in the opening address of the Congress, Jacques Cousteau acknowledged this achievement and its prospects (Cousteau, 1989).

Soft and hard corals have been thriving in this closed-circuit tank for 15 years and would be likely still thriving if I had not dismantled it in 1994 when I had to move full time to Monaco where I had founded the European oceanographic center.

Sand bed, plenum, stirring and pore-water circulation

The thick layer of sand and the plenum, which Lee Shin Eng did not use, characterizes the Jaubert's system (Figure 2 & 3). The plenum is the void space situated under the plastic screen that supports the coarse coral sand on the aquarium floor. The plenum contains a layer of hypoxic water. Its role is not substantiated by scientific data. I could never find enough time and money to carry out the experiments needed to compare the denitrification yield of aquariums functioning with and without plenums. Indeed, to comply with the statistical constraints of scientific experiments, I should have set up a minimum of 3 aquariums with a plenum and 3 aquariums without a plenum. Nevertheless I presume that the plenum facilitates pore-water circulation

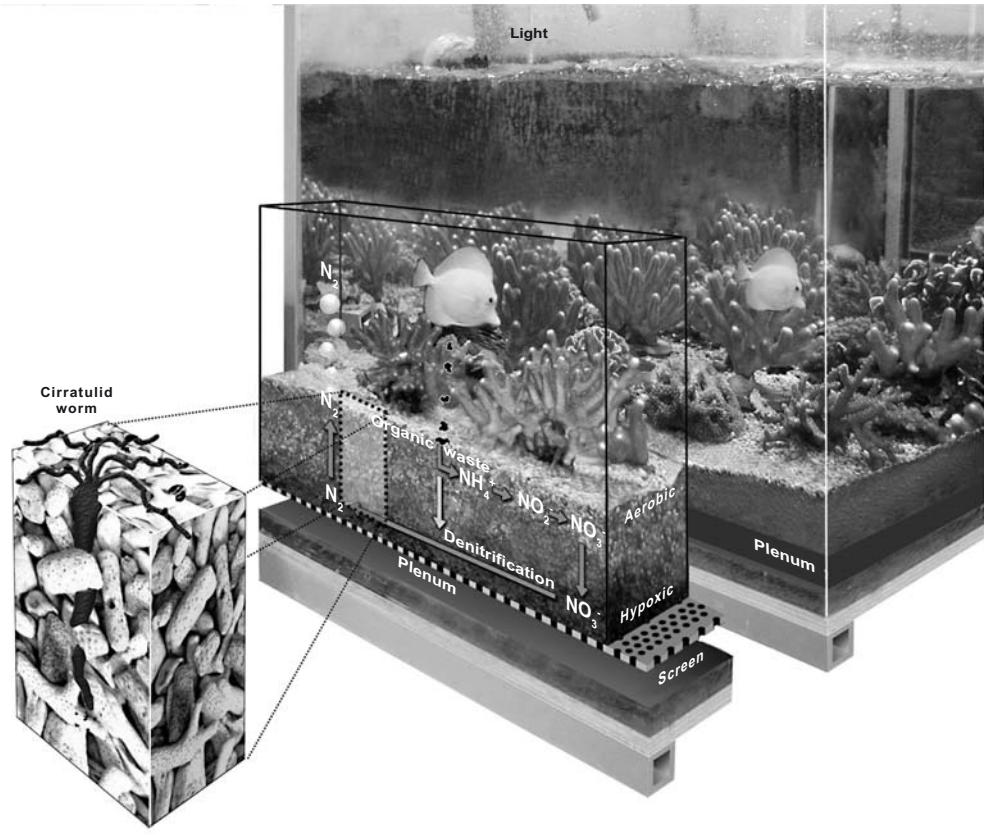


Figure 3: Basic functioning principles of the Jaubert NNR System.

through the sediment in aquaria within which stirring is moderate (Figure 2) and that pore-water circulation plays a key role. Indeed, by increasing fluid exchanges between the sediment and the overlying water several fold relative to simple molecular diffusion (Precht and Huettel, 2003):
(1) it brings to the deeper layers of the sediment some oxygen, which prevents them from becoming completely anoxic and by way of consequence toxic pockets of hydrogen sulfide from forming;
(2) it brings more nitrate to the bacteria and more calcium to the aquarium water than a simple process of diffusion.

During years I have underestimated both the intensity as well as the role of the stirring-induced pore-water circulation and the adjective "confined" I used to qualify the plenum water (Jaubert, 1989) was inappropriate. To keep the intensity of pore-water circulation within limits compatible with the maintenance of hypoxic conditions in the plenum (average concentration of oxygen: 0.5 $mg.l^{-1}$) stirring in the aquarium should be moderate. Indeed a strong stirring increases turbulences and pressure oscillations at the sediment-water interface and cause more seawater to infiltrate the sediment. Increased pore-water circulation brings too much oxygen to the deeper layers of the sediment breaking or stopping the work of the denitrifying bacteria.

Toonen and Wee (2005a) have completed an experimental comparison of sediment-based biological filtration designs for re-circulating aquarium systems and concluded that there was no detectable advantage to the inclusion of a plenum beneath sediments. The quality of this excellent work is not questionable. However, their experimental set up was too different from the system dealt with in the present paper to allow a relevant comparison. Firstly their tanks were significantly smaller. Secondly their "live" animal experiment was conducted under unspecified indoor ambient light condition, which I presume was significantly lower than that of current reef tanks. Thirdly their tanks might have not been sufficiently biologically mature. The sediment meiofauna may require more than 12 months to multiply and form a diversified population, which equilibrates according to the amount of organic matter on which they feed. This matter is composed of the debris that falls at the surface of the sediment and of the minute organic particles that are captured and incorporated into the sediment by pore-water circulation. In this regard generalizing the claim suggesting that the role of sediment in-fauna in nutrient processing is minimal (Toonen and Wee, 2005a) seems premature. On the other hand, inadequate stirring-induced pore-water circulation as well as limiting concentrations of organic carbon in the sediment of deep sand bed aquaria with or without a plenum may explain the relatively high final concentration of nitrate and low concentrations of dissolved calcium carbonate found by these authors. From the photos of the experimental set up published in the Advanced Aquarist's Online Magazine (Toonen and Wee, 2005b) one can infer that the current generated by the submersible pumps visible on these photos and the resulting pore-water circulation were too intense to maintain adequate hypoxic conditions in coarse sand-beds with or without a plenum. Pore-water circulation carries minute particles captured at the aquarium water sediment interface (Huettel *et al.*, 1996). This process generates some mechanical filtration, which may explain why water remains crystal clear in aquaria equipped with a deep coarse coral sand bed in spite of the absence of any classical mechanical filter.

In coastal areas, strong pressure oscillations generated by waves and currents control pore-water circulation and oxygen dynamics in permeable sediments. Precht and Huettel (2003) and Precht *et al.* (2004) have shown that coastal sediments form a gigantic filter, which recycles nutrients in permeable reef sediments (Wild *et al.*, 2004) and more generally purifies coastal waters. Extremely complex catalytic processes and metabolic pathways mediate the biogeochemical processes responsible for this purification. These processes and their ecological role are still poorly known because during many years scientists have been investigating fine muddy sediments with no or negligible pore-water circulation rather than permeable sediments. In this regard, elaborating on this topic in the present paper would be speculative and premature.

Mineralization of organic matter

During the day, zooxanthellae and macro-algae produce enough oxygen to create conditions of super-saturation in the water column and the contiguous layer of sand. Consequently, organic and inorganic nitrogen are converted

into nitrates and the aquarium remains virtually free of ammonium and nitrite. In a similar way particulate (POP) and dissolved organic phosphorus (DOP) are mineralized into dissolved inorganic phosphorus (DIP). DIP is an essential chemical for life. In many marine environments it is a bio-limiting macronutrient because its availability is related to the insolubility of phosphate salts (Pratt, 2006). However, in aquaria opposite conditions tend to prevail. DOP and DIP are highly undesirable substances, which promote blooms of cyanobacteria and algae. Since my aquarium never experienced such blooms during decades I presume that the basic skimmer discarded part of DOP before mineralization and that DIP was fixed in the sediments and/or in the plenum by biogeochemical processes. Anschutz *et al.* (2007) have found that a portion of the phosphate and ammonium released during mineralization does not escape the carbonated sediment of a coastal lagoon (Thau, near the city of Marseille, France), since the concentration gradient was close to zero between 1 and 25 cm depth below the sediment water interface. But since highly complex processes of adsorption and desorption controlled by magnesium, iron and other minerals drive the adsorption and desorption of DIC (Millero *et al.*, 2001) the above-mentioned presumption is a working hypothesis rather than an explanation. On the other hand, one has to consider that recent findings reviewed and put into an "*Earth-system perspective*" by Gruber and Galloway (2008) are being resulting in important revisions of the scientific concepts that underpin the current understanding of the global nitrogen cycle and of the way it interacts with the other major biogeochemical cycles, particularly that of carbon and phosphorus.

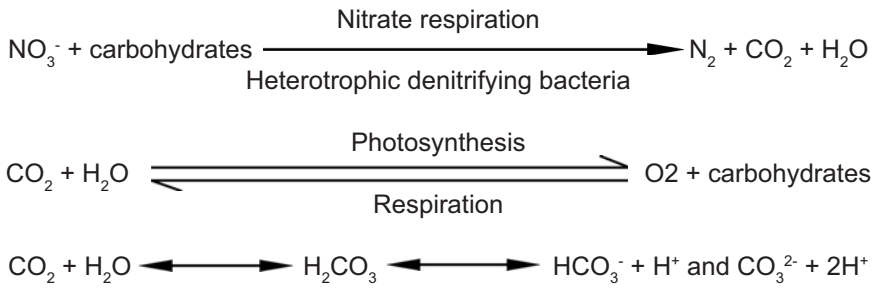
One of the most surprising discovery concerns new Crenarchaeota microorganisms bearing the ammonia monooxygenase encoding genes (*amoA*). Their abundance in shallow (300 m) marine waters and sediments is 1–2 orders of magnitude higher than those of the "classical" β- and γ-Proteobacteria, which are commonly thought to mediate nitrification in marine environments (Wuchter *et al.*, 2006). The exact role of these nitrifying Crenarchaeota is still unknown but may be important. Other recent findings reviewed by Op den Camp *et al.* (2006) and Kuenen (2008) have shown that more than 50 % of the molecular nitrogen lost by the Ocean is produced by the anaerobic oxidation of ammonium (anammox), a process mediated by autotrophic planktomycete-like micro-organisms, which were unknown 10 years ago. Annamox prokaryotes have been found in almost all of the terrestrial and marine environments but not yet in reef sediments. Hence, in the light of the above-mentioned considerations it is unlikely that they do not exist in a naturally purified reef tanks where they may participate with their heterotrophic counterparts in the reduction of the reactive forms of inorganic nitrogen.

Heterotrophic nitrate reduction, respiration and calcium dissolution

In addition to pore-water circulation, which carries minute particles originating from the aquarium water (Huettel *et al.*, 1996), the sediment fauna and meiofauna plays a key role in the nitrate reduction process by transferring to the hypoxic layers of the sediment organic matter on which denitrifying bacteria feed. This transfer results both from bio-turbation and the release of fecal pellets, which contain undigested organic compounds. By moving continuously sand grains, organisms responsible for bio-turbation transport and discard into the plenum microscopic mineral and stable organic particles, which otherwise would clog the sediment after a while. The most efficient animals are small cirratulid worms (Figure 3, first row) that collect and swallow, using their sweeping tentacles, all kinds of edible detritus that fall at the surface of the sand.

By respiring nitrate and oxidizing organic compounds the heterotrophic denitrifying bacteria release carbon dioxide into the interstitial water between the aragonite grains of the sand. This carbon dioxide hydrates and forms carbonic acid (H_2CO_3), which dissolves aragonite and releases calcium ions. Other, perhaps more important sources of carbon dioxide in the interstitial water are the respiration of the dense meiofauna that populates the sediment and that of the aerobic bacteria, which degrade their feces. In this regard, the oxygenated and hypoxic sand layers function like calcium reactors.
Carbon dioxide (CO_2) hydration forms carbonic acid (H_2CO_3). Carbonic acid dissociation forms bicarbonate ions (HCO_3^-) as well as carbonate ions (CO_3^{2-}) and releases protons (H^+). A thorough description of these processes and

The basic equations, which illustrate the above-mentioned chemical processes, are:



of the associated pH variations can be found in Andersen (2002).

Total alkalinity and pH show important diurnal variations. In the small volume of a reef aquarium these variations are higher than in nature (Jaubert *et al.*, 1995). They reflect those of the partial pressure of CO_2 (pCO_2) and O_2 (pO_2), which are correlated and depend on the metabolic activity of bacteria, algae, corals, fish, etc. At night calcium carbonate dissolution is higher than calcification. Total alkalinity, pH and pO_2 decrease while pCO_2 increases. Opposite conditions prevail during the day. In a balanced aquarium, dissolution releases enough calcium ions to replace those taken up by corals and calcareous algae. Empirically, I succeeded in establishing a robust and lasting balance in the 2,000 liters aquarium I had set up in my office at the University of Nice (Jaubert, 1989) by covering less than one third of the surface of its floor with live rocks supported by perforated shelves (Figure 2). The thickness of the sediment bed, made of coarse coral sand (2-4 mm) was 8-10 cm. A perforated PVC plate (hole diameter 1 cm) buried under 2-3 cm of sand prevented fishes from connecting the aquarium water with that of the plenum by digging holes in the aquarium floor. This aquarium has been functioning perfectly well for 15 years without any addition of calcium.

However, after a decade the calcium concentration of the aquarium water started to decrease. This depletion was likely due to the fact that dissolution was no longer able to compensate the calcium taken up by calcifying organisms, which had significantly increased their biomass, particularly branching *Acropora* (unpublished result). Fortunately, corals were able to adapt to this decreasing calcium concentration by decreasing their rate of calcification. Since the growth rate (branch elongation) of branching *Acropora* colonies was not significantly affected by the decrease in their rate of calcification, I uncovered this phenomenon by chance when I broke by mistake a branch of *Acropora hemprichii* (Ehrenberg, 1834) and noticed that the skeleton of this branch was abnormally porous. At that time the calcium concentration of the aquarium water was 350 $mg.l^{-1}$. This observation agrees with the experiment of Gattuso *et al.*, (2000) who showed that artificial seawater with a low calcium concentration lowered calcification rate of *Stylophora pistillata*. It suggests that the adaptation capacity of many reef corals to low calcium concentrations is higher than usually accepted. This assumption is supported by the experimental results of Fine and Tchernov (2007) who showed that *Oculina patagonica* (Angelis, 1908) and *Madracis pharensis* (Heller, 1868) were able to survive and recover from complete decalcification.

Oxygen balance, sediment fauna and fish biomass

Under conditions of reduced aeration gas exchanges at the atmosphere water-interface of a reef aquarium have a negligible effect on the oxygen concentration of the water, which depends essentially on the photosynthetic production and the heterotrophic consumption (Leclercq *et al.*, 1999). In this regard, the fact that in the above-described aquarium the minimum pO_2 (recorded at the end of the night) was stable and close to saturation indicated that this aquarium was balanced with respect to oxygen over a cycle of 24 hours. In other words, photosynthesis (essentially that of photo-calcifying organisms) was producing enough oxygen to meet the biological and chemical demands of the system. Decomposers (aerobic bacteria and sediment meiofauna) are major oxygen consumers. Since these organisms feed mostly on fish faeces and unconsumed particles of food their biomass reaches equilibrium slowly according to that of fish.

Maturation time

The development of the sediment meiofauna, which is a key component of the system, requires 12 months. Furthermore, in most of my aquaria a period of two years maturation was necessary to enable the system to reach robust and lasting balance. Then these aquaria were virtually maintenance free. Modifications of the permeability of the coral sand due to the slow accumulation of minute refractory particles between the sediment grains may also play a role in this long maturation process.

Applications to a public aquarium

In 1989, Professor François Doumenge who had become Director of the Monaco's Museum after the retirement of Jacques Cousteau asked me to design and set up a 40,000 L reef tank for its public aquarium. At that time it was impossible to find enough coral and live stones to stock such a large tank. Therefore I had to collect them in the sea. I completed this work in Djibouti with only 2 technicians of the Monaco's Museum (Gilles Pérez and Philippe Maurel) thanks to the strong logistic support of the local authorities. I also designed and set up a 12,000 L storage pool inside which corals were recovering during one week before being transferred to the reef tank. At the rate of one shipment a week, 2 months were needed to gather enough material to build and populate a nice reef. After the inauguration of the reef tank the storage pool became the first piece of a farm where corals were asexually propagated.

During 6 months this tank functioned in closed circuit. Then subsequently to an accidental influx of fresh water due to the mistake of the technician who was responsible for maintaining the salinity within the range 38-40 ‰ it was decided to operate it in a semi-closed mode with a rate of water renewal of 5 % a day.

Today, 18 years later, this reef tank is still a semi-closed tank full of coral and fish. Colonies of *Echinopora fruticulosa* (Ehrenberg, 1834), *Montipora danae* (Milne Edwards and Haime, 1851) and *Stylophora pistillata* (Esper, 1797) as well as several species belonging to the genus *Acropora* have tremendously increased in size and given birth to a several generations of cloned colonies that populate many other tanks.

Applications to scientific research

In 1990 I was commissioned by H.S.H. Prince Rainier to found the European oceanographic center. This center, which was part of a Council of Europe network, was dedicated to the physiology and ecology of reef-building corals. Most of the experiments were carried out using cultivated corals and reef mesocosms.

At the community level (reef) experiments were carried out using reef mesocosms. One of them was the above mentioned reef tank I had set up in the Monaco's Museum. Jaubert *et al.* (1995) showed that the global metabolic features of this captive reef kept in a semi-closed circuit tank were similar to those of the most productive natural reefs. This finding, which has been recently confirmed in a closed circuit Jaubert system (Ikeda *et al.*, 2004; Table 1), enabled researchers of the European center

Table 1. Global metabolic features of a captive reef kept in a closed circuit tank compared to those of natural reefs (from Ikeda et al.*, 2004). This closed circuit tank is a typical Jaubert NNR System set up under license by the organization Monaco Kenkyusho (Himonya 3-16-21 – Meguro-ku – Tokyo 152-0003) in the National Museum of Emerging Science and Innovation, Tokyo.*

	Net photosynthesis ($mmolC.m^{-3}.d^{-1}$)	Net calcification ($mmolC.m^{-3}.d^{-1}$)	Ratio of photosynthesis to calcification
The Monaco Aquarium	74.3 ± 7	56.6 ± 10.4	1.3
Reef Flat of Palau Barrier Reef (from Ikeda 1997)	83.5	65	1.3
Moat of Shirao, Ishigaki Island, Japan (from Hata 2003)	48.5-65.5	35-60.5	1.1-1.4

to use mesocosms of this type as models to investigate the consequences of increasing content of carbon dioxide in the atmosphere and the subsequent acidification of surface water on the metabolism of reefs (Leclercq *et al.*, 1999; 2000 and 2002; Reynaud *et al.*, 2003).

CONCLUSION

The Jaubert's system and systems derived from it have significantly contributed to the development of reef tanks in Monaco (Oceanographic Museum) and Taiwan (National Museum of Marine Biology and Aquarium). It has also contributed to make possible laboratory experiments that have resulted in striking improvements of the knowledge of the biology and ecology of reef-building corals. However, several aspects of the functioning of this system are still poorly known. In particular the pattern and intensity of pore-water circulation as well as the flux of chemicals and energy at the sediment- water interface are unknown and should be quantified. Recent findings concerning the filtration process driven by pore-water circulation in coastal sediments and new microbial processes involved in the nitrogen cycle have shed some light on the biogeochemical processes that may take place in the aquarium floor. A significant amount of work and time as well as fairly sophisticated equipment are needed to investigate these biogeochemical processes and to elucidate the exact role of the plenum. So far my scientific carrier never provided me with the opportunity to develop research on naturally balanced aquaria. Hence, developing this type of research was my major objective, in July 2004, when I became Director of the Monaco's oceanographic Museum. Unfortunately, at that time, the Museum was in a critical financial as well as social situation and I had to dedicate all my time and efforts to reorganize it and increase its incomes. At the end of 2006 the annual number of visitors had gone above **the** symbolic threshold of 600,000. Hence, I started to make plans to renovate the exhibitions and resume research, a major mission given to the Museum by its famous founder H.S.H. Prince Albert I. Sadly the project was rejected by the Board of the Oceanographic Institute, which owns the Monaco's Museum. When I realized that I had underestimated the bureaucratic weight of this French Foundation and that several years of an exhausting fight would likely be necessary to reach my objectives I decided to give up and resigned. Now and I will try to dedicate a significant part of my time and efforts to carry out scientific experiments aimed to improve methods for setting up naturally balanced and more self-sustainable aquaria. Popularizing this type of aquaria and explaining how they function might contribute to enhance public awareness on marine conservation as well as other key ecological issues and to change behaviors.

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