UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI BIOLOGIA

Corso di Laurea magistrale in Marine Biology

TESI DI LAUREA

Comparative assessment of different fragmentation methods on three ornamental soft corals of the order Malacalcyonacea (Octocorallia)

Relatore: Prof.ssa Daniela Bertotto
Dipartimento di Biomedicina Comparata e Alimentazione

Correlatore: Drssa Martina Bortoletti
Dipartimento di Biomedicina Comparata e Alimentazione

Laureando: Victorio Codotto

ANNO ACCADEMICO 2023/2024

INDEX

1. Abstract

Corals are often associated with words such as ecosystem engineer or reef-building corals, this is because these animals with their growth are able to shape the surrounding environment creating a complex habitat that allows life for thousands of species: from the simplest algae to top predators such as sharks. The main reef building corals are the so-called stony corals. Soft corals, although to a lower extent collaborate in this biodiversity and have often been shown to be more resilient than hard corals to the major hazards that can endanger a reef ecosystem. The most well-known hazards which are often directly linked to an anthropogenic component are ocean acidification, heat waves, and overfishing, which in turn can lead to increased stress that reduce the ability of corals to recover after a possible bleaching event (expulsion of zooxanthellae in autotrophic corals). Soft coral production is particularly important for the pharmaceutical sector, for the ornamental market, and for replenishment of damaged reefs. Regarding restocking campaigns due to higher genetic variability, sexual reproduction is often preferred, which, however, presents greater difficulties than asexual reproduction. Coral fragmentation, one of the asexual reproduction ways, is preferred in the ornamental market given the possibility of obtaining many clones of a single mother colony presenting good ornamental traits. Marine ornamental trade is based on the global movement of animals often wild caught mainly in the tropical Indo-Pacific. In recent years, several advances have been made in creating protocols for breeding fish and invertebrates for the ornamental market, unfortunately much of this know-how remains in the grey literature or is not easily shared by ornamental aquaculture facilities, thus not reaching the scientific literature. Following the example of Chaitanawisuti and Kritsanapuntu (2019), the aim of this thesis was to establish scientifically which is the best method to asexually reproduce three species of soft corals: *Litophyton* sp., *Lobophytum* sp. and *Klyxum* sp.. Two different methods were compared in a recirculating aquaculture system during a period of four weeks. The evaluation of the treatments was done by measuring the survival rate, adhesion time to the substrate, first healing time, state of well-being/stress through contraction or not of the animals. Survival analysis has been performed and a p-value <0.05 was considered significant. Statistically significant values indicated a lower survival of impaled *Litophyton* sp. compared with all other possible associations. Regarding healing time between the two different fragmentation methods, the tying method performed better than the impaling one. Again, a statistically significant difference was obtained for the time of adhesion to the ceramic support, the impaled *Litophyton* sp. proved to need longer time to adhere to the ceramic support. In conclusion, we suggest for future artificial propagation of these three species the tying method since it performed overall better than the impaling one.

2. Introduction

While a large set of popular definitions has been used to group corals, they are often discordant to taxonomical groups. Moreover, not all definitions currently used are universally approved. In summary, all corals belong to the class Anthozoa and most of them belong to the orders Scleractinia and Alcyonacea (recently rearranged as Subclass Octocorallia) (McFadden et al., 2022). Scleractinian corals (hard corals or stony corals) build a calcareous skeleton and are usually known as hermatypic or reef-building corals. Alcyonacean corals are usually known as soft corals and contain spiny skeletal sclerites rather than a hard skeleton. Other common definition concerns the presence of photosynthetic dinoflagellates within the genus *Symbiodinium* in the coral tissue (popularly known as zooxanthellae). These corals are known as symbiotic corals, while corals without zooxanthellae are named asymbiotic corals (Leal et al., 2017).

Coral reefs exist in tropical areas worldwide. In general, reefs are abundant in areas with shallow coastlines and clear, warm water where riverine discharge of sediments is low. Large coral reefs are rarely found in areas above 29° latitude where ocean temperatures fall below 18°C for extended periods as this slows coral growth and their capacity to build large reefs; however, zooxanthellate corals can be found in areas with water temperatures as low as 11°C. However, when the physical and ecological criteria are met, the results can be phenomenal. For example, the most biologically diverse reefs occur in the tropical Indo-Pacific in the areas around Indonesia and the Philippines and house over 550 species of coral and thousands of species of fish. The Great Barrier Reef off northeastern Australia is the largest reef in the world with more than 2800 individual reefs occupying over 1800 km of the Australian coastline (Burkepile and Hay, 2008).

Indonesia, with five other countries, Timor Leste, Malaysia (Sabah), The Philippines, Papua New Guinea, and the Solomon Islands, are known as the Coral Triangle area. The word Coral Triangle is used to name 16 ecoregions with the highest corals diversity in the world. In this region, there are at least 605 species of zooxanthellate which are 76% of the world's corals (Putra et al., 2022). Corals are simple, clonal invertebrates that serve as ecosystem engineers, building living structures (reefs) so large that they can be seen from space. These structures, which rival the greatest feats of human engineering, are powered through symbiosis with single-celled algae that are housed within the coral animal. This coral–algal cooperation facilitates a productive ecosystem that can grow in the nutrient-poor `desert' of isolated tropical seas. This cooperation is thought to be the most obvious positive interaction of reefs (Burkepile and Hay, 2008).

There are more than 100 genera of shallow-water soft coral from the Indo-Pacific that have been described. All these genera are distributed in marine environments and are present mainly in shallow tropical reefs and deep-sea habitats like seamounts (Putra et al., 2022).

Soft corals and sea fans are one of the major fauna components of coral reef ecosystems, long-time recognized to play important roles due to their quite big size (up to 2–2.5 m tall and across) and ability to form substratum for numerous organisms including bacteria, crustaceans (Dautova and Kiyashko, 2017), bryozoans, echinoderms, fish, molluscs, polychaetes, sponges and cnidarians (Goh et al., 1999). Soft corals Alcyonacea can occupy wide areas at the coral reef and produce skeletons suitable to input the calcite to the reef frame after death (Dautova and Kiyashko, 2017).

2.1. Coral biology

The mutualistic relationship between the cnidarian host and its endosymbiotic dinoflagellate algae of the family Symbiodiniaceae is vital for the persistence and continuity of coral reefs. Through inorganic carbon fixation, the photosynthetic symbionts provide their host with sugars and amino acids, a crucial asset in oligotrophic seas. In return, the host provides the resident algae with light-rich conditions, a sheltered environment, and a supply of inorganic nutrients. Consequently, the cnidarian host and its associated dinoflagellate algae are critical partners in the diverse assemblage of eukaryotic and prokaryotic taxa that comprise the coral holobiont. Endosymbiotic dinoflagellates of the family Symbiodiniaceae are abundant on coral reefs from very shallow water down to the upper mesophotic zone (30–70 m). Notably, it was found that the genetic lineages of the algal symbionts may modulate the phenotype of the holobiont in response to temperature rise or to poorly lit-environments, such as those affected by depth (Liberman et al., 2022). Zooxanthellae were initially assumed to represent one species; recent molecular evidence shows that there are at least seven distinct types or clades (referred to as clades A–G). Many corals house multiple clades of zooxanthellae, setting the stage for possible competition among symbionts and for symbiont selectivity by the host. Clades of zooxanthellae differ in their photosynthetic capacity and their tolerance of light, temperature, and other stressors, making them differentially useful to their hosts under changing environmental conditions (Burkepile and Hay, 2008). However, little is known regarding octocoral photosymbionts, and in particular regarding those found deeper than 30 m. Based on a study on 19 mesophotic octocoral species most of them hosted the genus *Cladocopium*. *Litophyton* spp. and *Klyxum utinomii* were exceptions, as they harboured *Symbiodinium* and *Durusdinium* photosymbionts, respectively. While the dominant algal lineage of each coral species did not vary across depth, the endosymbiont community structure significantly differed between host species, as well as between different depths for some host species (Liberman et al., 2022).

Corals are ecosystem engineers in that the growth of their calcium carbonate skeleton creates the biogenic structure on which the entire ecosystem depends. The calcification and growth of reef corals depends on a mutualism between corals and their intracellular photosynthetic dinoflagellates. Photosynthesis by zooxanthellae enhances calcification in corals and increases coral growth rates, ultimately leading to reef accretion and the massive reef framework found in many tropical seas (Burkepile and Hay, 2008). Compared to stony corals and gorgonians, soft corals possess a high proportion of cellular material and a low proportion of calcareous skeletal mass (generally in the form of free microscopic sclerites), as a result of which they appear soft. The calcifying cells and sclerites can be found throughout the bulk of the colony while the zooxanthellae, when present, are concentrated in the gastrodermis of their superficial polyps (i.e. cortical polyps) (Tentori et al., 2004). In soft coral *Lobophytum crassum* for example the protein MPL-2 has been found responsible for CaCO3 nucleation and crystal growth (Rahman and Oomori, 2008**)**. Thus, the physical structure of live and dead corals created by the coral–zooxanthellae mutualism provides heterogeneity and habitat complexity, facilitating the coexistence of diverse plant and animal assemblages. When corals are stressed by increasing light levels or temperatures, they often expel their zooxanthellae and become pale in colour (called coral bleaching). This process of bleaching may allow corals to take up new clades of zooxanthellae that are better adapted to the new environmental conditions. However, corals that fail to re-acquire zooxanthellae or acquire the wrong clades may ultimately die from the stress, suggesting that a failure of corals to acquire appropriate symbionts can be fatal under changing environmental conditions. Such alterations in the coral–zooxanthellae mutualism may allow corals greater flexibility in adapting to global climate change, which is a major threat to the health of coral reefs and the integrity of the coral–zooxanthellae mutualism (Burkepile and Hay, 2008). The results from Hartle-Mougiou et al. (2012) indicate that the specific host– symbiont association occurring in nature appears to persist over 2 years timescales in captivity, with no mixing of symbionts between hosts maintained in the same aquarium or apparent selection of stress-tolerant symbiont strains such as clade D.

Shallow water scleractinian corals and octocorals consume a wide range of prey items, from dissolved organic matter and bacteria to zooplankton and detrital particulate organic matter. Tropical scleractinian corals are mostly considered autotrophic as they rely mainly on photosynthesis-derived nutrients transferred from their zooxanthellae. A great amount of tropical Octocorallia (such as abundant genera *Sinularia*, *Sarcophyton* and some gorgonians) also belong to zooxanthellate corals, but many of them (such as gorgonians *Menella* and *Ellisella*) do not contain zooxanthellae in their tissue. Corals are also able to capture and ingest suspended particulate organic matter and small prey, so heterotrophy can be an important supplementary feeding mode to optimize coral diet (Dautova and Kiyashko, 2017). Soft coral feeding preferences are linked to their physiology, with polyp size and structure influencing their ability to capture various planktonic sources (Larkin et al., 2023). Coral heads that harbour fish schools receive nutrient supplements from fish excretion, grow up to 23% faster, and have more nitrogen and zooxanthellae per unit area than do corals without resident fishes (Burkepile and Hay, 2008).

Competition for limiting resources such as nutrients, space, light, or food is often a strong mechanism limiting the distribution and abundance of species in communities. On many coral reefs, the limiting resource for most benthic organisms is space or light, as most of the reef structure is often occupied. Consequently, corals have evolved a variety of competitive mechanisms including sweeper tentacles, digestive filaments, and rapid growth rates that allow them to fight neighbours for new space or protect the space they already occupy. Slow growing, massive corals often have the most potent direct competitive mechanisms (i.e., sweeper tentacles and digestive filaments that can sting and directly harm neighbouring corals) while branching corals such as many *Acropora* spp. rely on their high growth rates to overtop and shade competitors (Burkepile and Hay, 2008).

2.1.1. Polyps and stress

The respiration rate of octocorals is largely determined by the rate of diffusion of oxygen through ectoderm and endoderm and by polyp activity. Polyp activity affects respiration in two ways: polyp expansion maximizes the diffusion and consumption of oxygen in the tissue, whereas the contraction of polyps reduces feeding efficiency and respiration (Previati et al., 2010). Many reef-building corals exhibit daily cycles of tentacle expansion and contraction associated with prey capturing and nutrient acquisition. Corals with "nocturnal behaviour" expand their tentacles at night to capture prey to sustain their requirements for growth and reproduction by feeding on zooplankton, bacteria and suspended matter. During the day, tentacles are contracted which reduces the coral's metabolic expenditure. Corals exhibiting "diurnal behaviour", expand their tentacles mostly during the day. These corals often meet their energy requirements mainly through the translocation of C-rich photosynthates from their symbionts by maximising the sunlight exposure of their photosynthetic partners through the extension of their tentacles (Mardones et al., 2023). Polyp structures containing dense populations of zooxanthellae respond positively to light (expansion, positive orientation) and those with few or no zooxanthellae respond negatively (contraction, negative orientation) (Sebens and DeRiemer, 1977). Some other species pursue mixed nutritional strategies and tend to expand their polyps and tentacles continuously. The mechanisms regulating tentacle expansion and contraction remain unknown, but it is assumed that the endogenous circadian clock, and exogenous cues such as light, nutritional stimuli (prey size and density) and flow speed could be involved in the regulation (Mardones et al., 2023).

Colony stalks consist of a flexible hydroskeleton that expands and contracts periodically throughout the tidal cycle. Expansion occurs when water flow is moderate, probably indicating when colonies are feeding. Contraction occurs when water flow is either weak or extremely strong, probably owing to timing when planktonic food is minimally available, or when there is a high risk of being uprooted (Larkin et al., 2023).

Other causes related to polyp contraction have been reported: chronic brushing of corals by macroalgae in water currents (Brown et al., 1994), sediment overload (Vargas-Ángel et al., 2006), high concentrations of iron in seawater (Brown et al., 1994), the developing gonads occluded the gastric cavity for four months, during which time the polyps remained contracted and did not feed (Harrison and Wallace 1990), Previati et al. (2010) observed that polyps reduced their activity and the oxygen consumption above the optimal temperature for the corals species they studied, Zaragoza et al. (2014) in their publication related to bacterial infection of the sea anemone *Aiptasia pallida*, indicated darkening of the polyp tissue, retraction of the tentacles, and polyp mortality as the main observable signs of the infection, Larkin et al. (2023) saw a different polyp behaviour in corals fed with different diets.

Polyp retraction as a stress response has been extensively documented (Brown et al., 1994). For scleractinians, polyp contraction is one of the primary physiological responses when they are exposed to both abiotic and biotic stressors (e.g., desiccation and attack of predators) (Shikina et al., 2020). This behaviour has been used as a metric of health, wellbeing and willingness to feed (Larkin et al., 2023) (Vargas-Ángel et al., 2006) (Chaitanawisuti and Kritsanapuntu, 2019), even though many other macroscopic signs has been used as a sign of coral condition: polyp swelling, unusual appearance of oral disk (enlargement, contraction, or protrusion), changes in coloration (intensification, and/or bleaching), increased mucus production, active/inactive sediment removal, loss of natural texture lines (apparent smoothing-out of tissue), extrusion of mesenterial filaments, algal overgrowth, appearance of lesions and tissue necrosis and increased mucous production (Vargas-Ángel et al., 2006). Costa et al. (2021) studied stress in corals produced by common shipping practices in ornamental trade, they measured at arrival and after three months: oxidative stress (catalase – CAT, glutathione S-transferase - GST, and total glutathione - tGSH), oxidative damage (lipid peroxidation - LPO), energy reserves (lipids, proteins, carbohydrates) and electron transport system (ETS).

To minimize stress during nubbins maintenance, Larkin et al. (2023) recommend that they are provided with continuous water flow and aeration, with limited emersion, physical handling, or manipulation.

2.2. Threats to corals

In many regions of the world, coral reefs are mere remnants of what they were only a few decades ago. These changes to reefs are not adequately appreciated due to the problem of the `shifting baseline syndrome' – reefs that are deemed `normal' today are not what was `normal' only a few decades ago, much less a century or more ago (Burkepile and Hay, 2008).

Coral reefs are endangered around the world because of the compounding effects of multiple stressors such as overfishing, pollution, climate change, and change in coastal land use. Although biotic interactions (e.g., competition and herbivory) are emphasized as having important consequences for coral reef structure as well, abiotic disturbances such as hurricanes, temperature fluctuations, sedimentation stress, and sea-level change also produce long-lasting effects on reefs. Coral reefs are one of the hallmark ecosystems strongly influenced by disturbance as the frequency and intensity of hurricanes or disturbance events determines how many species of corals coexist on reefs. If disturbance is very frequent or very intense, then only species that can recolonize disturbed areas quickly or that can withstand intense disturbances will persist. If disturbance is infrequent and mild, then the most competitive species eliminate the less competitive species and come to dominate. However, if disturbance is of an intermediate frequency and intensity, then species with different life-history characteristics (i.e., good colonizers vs. good competitors) can coexist because the disturbance-intolerant species are not displaced frequently, and the poor competitors are not outcompeted. Reefs often recover from acute disturbances such as storms but infrequently recover from chronic disturbances. The coupling of acute natural disturbances with chronic anthropogenic disturbances often leads to precipitous declines in coral reef health driving coral reefs to alternate states such as seaweed-dominated reefs or sea urchin barrens (Burkepile and Hay, 2008). An example of anthropogenic disturbance can be found in the publication of Mardones et al. (2023) which work suggest that artificial light at night (ALAN) has the potential to affect nutrient acquisition mechanisms of symbiotic corals which may in turn result in exposed areas.

Although most early studies of competition on reefs focused on coral–coral competition, more recent studies have examined coral–seaweed competition because reefs are now more commonly overgrown by seaweeds that periodically seem to be killing corals. The conventional wisdom is that seaweeds are competitively superior and can overgrow and kill most corals. Although not all seaweeds are harmful to corals, most studies of coral–algal competition show that direct competition from seaweeds reduces the growth, survivorship, fecundity, and recruitment of many corals. Small, filamentous seaweeds, which are not as directly harmful to corals as are larger, foliose seaweeds, often trap sediments next to coral tissue, and this can smother and kill corals. In addition, seaweeds have disproportionately high negative effects on smaller coral colonies, particularly newly recruited corals, and large stands of seaweed can prevent juvenile corals from recruiting to reefs at all. Because seaweeds can overgrow and kill corals, herbivores are critical for coral reef function because they keep reefs free of seaweeds, thus facilitating the recruitment, growth, and resilience of corals. Fishes and urchins are typically the dominant herbivores on coral reefs. When in sufficient numbers, either fishes alone or sea urchins alone can remove greater than 90% of the daily primary production on reefs. When herbivores are removed by experimentation, overfishing, or disease, seaweeds replace corals, and the biogenic structure of the reef degrades. Experimental manipulations of herbivorous fish diversity demonstrate that species-richness is important for reef function because complementary feeding by different herbivorous fishes suppresses upright seaweeds, facilitates crustose corallines and turf algae, reduces coral mortality, and promotes coral growth. Hence not only are herbivores critical for coral reefs, but herbivore species-richness is also essential as a range of feeding strategies and physiologies allows efficient removal of seaweeds and promotes coral health (Burkepile and Hay, 2008).

On many Pacific coral reefs, outbreaks of the crown-of-thorns starfish, *Acanthaster planci*, cause loss of many square kilometers of coral reefs. These starfish are voracious coral predators that forage in large groups that can decimate large stands of coral, and their outbreaks have become more frequent since the 1960s when they were first documented. Research in the Fiji Islands has shown that outbreaks of *Acanthaster* are correlated to fishing pressure on reefs. High densities of *Acanthaster* decrease cover of reef-building corals and crustose coralline algae while increasing cover of filamentous algae. Thus, the removal of large predators is associated with explosions of *Acanthaster* populations that then have strong cascading effects on the organization of reef communities (Burkepile and Hay, 2008).

Zoantharians and octocorals are among the most important components of the sessile fauna of coral reefs. Most of them are much more opportunistic than scleractinians and are less vulnerable to natural and anthropogenic stress. They often replace stony corals on reefs and in reef zones after catastrophic events such as Acanthaster plagues, hurricanes, low tides, or pollution. The high ability of octocorals to compete with scleractinian corals in reef benthic communities could be based mostly on their chemical defence capacity and their high growth rate rather than on their trophic activity or their trophic opportunism in relation to sources of heterotrophic feeding (Sorokin, 1991). About this topic, Hoang et al. (2022) proved that in *Sarcophyton* soft corals, the effect of fish predation prevention was most pronounced in the combination of both chemical (crude coral extract) and physical (coral sclerites) defence factors, followed by the chemical and then the physical factor alone. Hence, both chemical and physical factors of defence against predation may contribute to the *Sarcophyton* abundance on reefs.

Pathogens can also be a threat for coral reefs, in 1983–84 an unknown pathogen swept through the Caribbean and killed approximately 99% of the then abundant sea urchin *Diadema antillarum*. In many areas of the Caribbean, *D. antillarum* had been the dominant herbivore keeping reefs free of most fleshy seaweeds and facilitating recruitment and growth by corals. Another outbreak that altered the structure of Caribbean reefs was the epidemic of white band disease among acroporid corals in the mid to late 1980s (Burkepile and Hay, 2008). Strong shifts in species composition in response to local and global threats have been reported for coral reefs worldwide. In the Caribbean, many reefs historically dominated by reef-building species such as *Acropora* spp. and *Montastraea* spp. are currently dominated by non-framework-building species with opportunistic and stress-resistant life-history strategies (Chamberland 2018). After a pathology survey on stony and soft corals Work et al. (2014) states that tissue loss was the most common gross lesion sampled followed by discoloration, growth anomalies, bleaching, and flatworm infestation. Moreover, they suggest the importance of including histopathology as an integral component of baseline coral disease surveys, because a given gross lesion might be associated with multiple potential causative agents.

Coral bleaching is probably the most known threat for corals. It occurs when corals degrade or expel their dinoflagellate symbionts in response to environmental stressors such as heatwaves, increased UV radiation and other environmental stressors. Although corals can reacquire symbionts and recover in weeks to months, recovered corals may grow slower and have reduced fecundity as compared to previously unbleached corals, giving bleaching-resistant corals an ecological advantage after bleaching events. Given that climate change models suggest an increase in sea surface temperatures of 1–3°C over the next 50–100 years, coral bleaching events may become an intense, annual stress on coral (Burkepile and Hay, 2008; Liberman et al., 2022). Despite the main reports are related to stony corals, there are some reports also in soft ones such as the one reported by Baran and Baria-Rodriguez (2021) in *Lobophytum*.

Trade in ornamental coral reef wildlife supports a multi-million-dollar industry but in some places threatens vulnerable coral reef species and ecosystems due to unsustainable practices and lack of effective regulation. To supply this trade, fishers sometimes deplete fish populations and rely on bad practices that harm coral reef organisms and habitats.

Many species are targeted to supply this trade, primarily based on their size and aesthetics. Examples include brightly-coloured juvenile or male fishes; stony corals with attractive skeletons or large, colourful polyps; and molluscs with colourful, ornate shells. The effects of the collection and trade in ornamental wildlife are less studied than other threats to coral reefs.

Collection has reduced certain populations, introduced invasive species, and in rare cases caused localized extirpations.

Invertebrates collected for trade also exhibited population declines; for instance, densities of symbiotic giant anemones in the Cebu region of the Philippines declined over 80%. Stony corals, the foundation of coral reef ecosystems, are also collected. Their collection can reduce coral cover and alter species compositions and population demographics.

Despite the potential impacts of collection, the stock status and sustainable harvest levels of most ornamental species remain largely unknown and unmonitored. Beyond the unevaluated status of targeted species and potential population declines, destructive practices, including cyanide fishing, are sometimes used to collect coral reef wildlife for trade. Cyanide is dispensed onto coral colonies to anesthetize and easily capture fish. Within minutes, these toxins kill an estimated 50% or more of exposed species, with additional deaths occurring hours to days later. Non-targeted species like corals, anemones, and other habitat forming species are also exposed and can be injured or killed. Cyanide blocks respiration in corals, causing coral bleaching and mortality. The current prevalence of cyanide fishing is unknown. Cyanide fishing occurs in at least 15 countries, including major exporters like Indonesia and the Philippines, though its use is apparently less prevalent today than 20 years ago. Other prevalent fishing methods also injure and kill both targeted and non-targeted species, including abrasive nets, spears, and crushing of corals to capture fish. The coral reef wildlife trade can also affect importing countries through the introduction of exotic and invasive species. The most prominent example is the introduction of lionfish (*Pterois volitans* and *P. miles*).

The coral reef wildlife trade exhibits both notable similarities and differences from other capture fisheries, including characteristics that can impede resource management. Organisms from nearly every trophic level are collected, making identification and monitoring of collected species exceptionally challenging. The life history, demographic, and population data required for traditional stock assessments are typically unavailable.

Many locations have few (if any) regulations addressing the coral reef wildlife trade. In countries where regulations exist, enforcement is hindered by inaccurate reporting of landings and illegal collection. A complex supply-chain creates challenges for managing the trade, especially in Southeast Asia. Similar to capture fisheries, many countries supply the trade and animals change hands many times between collection and export with no system to monitor this chain of custody. At each stage of the supply chain, animals from various sources are pooled, so separating sustainably harvested wildlife from their unsustainable counterparts is difficult. This supply chain complexity complicates reform in several ways. First, high supply-chain mortality can lead to market inefficiencies and drive overharvesting. Second, the complex supply chain presents challenges for reducing destructive collection practices, like cyanide fishing, since cyanide is increasingly difficult to detect with time. Third, import-documentation requirements are challenging when organisms change hands many times. All of these challenges would benefit from a shorter and more vertically integrated supply chain.

Methods such as gear restrictions; entry, size, and catch limits; fishing bans; spatial management; and size limits are commonly used, with mixed success, in this trade. Voluntary certification approaches, such as the Marine Aquarium Council, have also been attempted, but these programs were not viable. Conversely, stock assessments, scientifically-set total allowable catches (TACs), and rights-based fisheries management approaches (e.g., individual transferable quota systems and exclusive fishing zones) remain underutilized. The limited employment of these management techniques is largely due to data, management capacity, and resource limitations (e.g., finances or enforcement and monitoring personnel), as well as a lack of attention to ornamental fisheries.

TACs set limits on the overall collection of a species or group of species in a given year. Although TACs are not widely utilized, species-specific TACs are currently used in the coral fishery in Australia, whereas aggregate TACs (in the form of export quotas) are used in several island nations including the Maldives and Tonga.

Other potential strategies to reform the trade include aquaculture and industry reform. Aquaculture holds promise for alleviating collection pressure on wild populations. Aquaculture has already reduced the collection of wild fishes and invertebrates, including clownfish, seahorses, tridacnid clams, and several hard corals.

Despite the difficulties in managing the coral reef wildlife trade, some examples of successful management demonstrate that sustainable reform of the trade is possible. Some programs in exporting countries, such as Florida's moratorium on corals, Hawaii's fish replenishment areas, and the Maldives' and Tonga's no-take zones and tiered quota systems, represent steps towards reforming the trade. Similarly, legislation in importing countries, such as the E.U.'s Wildlife Trade Regulation and Australia's "whitelist," can also improve the trade's environmental sustainability by proactively restricting and monitoring imports when there are concerns about the conservation status, disease risk, or invasiveness of certain species. In contrast to the U.S.'s reactionary approach to managing the trade, the precautionary approach adopted by the E.U. and Australia allows importing countries more control and oversight. Examples of regulation and management in the E.U. and Australia highlight promising ways for importing countries to proactively steer the trade towards sustainability. Different combinations of these management and regulation strategies, in conjunction with emerging data-poor fisheries management approaches, aquaculture, and the other underutilized management tools identified here, offer considerable promise for the future (Dee et al., 2014).

One of the saving graces of coral reefs over the next few decades may be the creation and enforcement of marine reserves that protect reefs from overfishing. Overfishing is one of the most devastating threats to reefs, as fishers preferentially remove the large bodied fishes that are the strongest interactors in these ecosystems, resulting in fundamental changes to the food webs of reefs. The establishment of marine reserves limits or prevents the harvesting of fishes and invertebrates from areas of reef and theoretically allows populations of overharvested species to rebound, reestablishing viable populations of fishes and crucial ecosystem processes on reefs. Marine reserves can also restore trophic linkages that enhance the recovery of coral reefs such as the balance between herbivores and algae cover (Burkepile and Hay, 2008). Recently, much work on larval recruitment dynamics has been applied to the design and implementation of marine reserves aimed at preserving threatened populations of marine fishes and invertebrates (Chamberland 2018). Although the benefits of reserves to conservation and fisheries are promising, one of the main challenges to the success of marine reserves is the enforcement of no-harvesting policies once the reserve is established. However, if marine reserves can be implemented and enforced, they will be one of the best tools that conservation science currently has to protect, and hopefully resurrect, many coral reefs (Burkepile and Hay, 2008).

Many of the shallow reefs studied by Chamberland (2018) became dominated by brain coral species after the die-off of *Acropora palmata* populations. Several of these reefs were located in front of densely populated parts of the island, where they were exposed to frequent anthropogenic impacts including overfishing, coastal development, nutrient pollution from faulty sewage treatment facilities, and chemical pollutants from a nearby oil-refinery. Despite of aforementioned stressors, some of the reefs dominated by brain corals still had a high cover of corals (~30%) and support a large number and high diversity of other marine organisms. Such ecosystems have recently been described as "novel ecosystems". Per definition, a novel ecosystem is an ecosystem that "(…) has species compositions and relative abundances that have not occurred previously within a given biome. The key characteristics are (1) novelty: new species combinations, with the potential for changes in ecosystem functioning; and (2) human agency: ecosystems that are the result of deliberate or inadvertent human action, but do not depend on continued human intervention for their maintenance." (Hobbs et al., 2006). Chamberland (2018) proposed to apply this concept for reef restoration, whereby coral species targeted for restoration in degraded areas must be selected for their natural ability to recruit, grow and reproduce in impacted or degraded areas, rather than insisting on the return of once dominant species that may no longer be capable of thriving under the conditions typifying modern reefs.

Several studies have shown that certain octocorals appear more resilient than scleractinians, showing resistance to ocean acidification, rising temperatures, eutrophication, or extreme storms. Phase shifts to octocoral dominance may therefore lead to alternative communities with greater resilience to climate change (Nadir et al., 2023).

2.3. Corals and natural environment

The rich structural complexity provided by the coral's hard bodies gives shelter to many other species of plants and animals making coral reefs among the Earth's most biologically diverse ecosystems, harbouring hundreds of thousands to millions of species worldwide (Burkepile and Hay, 2008). Octocorals constitute a significant component of reef frameworks and provide structural complexity, habitats for associated organisms, and the transfer of energy between plankton and benthos (Nadir et al., 2023). In addition, reefs give us a glimpse of the spectacular record of Earth's history because the hard skeletons of corals fossilize to provide a long record of changes in coral distribution and abundance and also record chemical signals of past climatic events, like temperature and sea-level changes. Thus, reefs not only feed and protect humans and other species, but also provide a valuable window into our past, including how our present activities may be changing our environment, and possibly our future (Burkepile and Hay, 2008).

Large-scale research about soft coral distributions should be executed soon before extinction occurs. The coral reef as the habitat of soft coral is rapidly declining worldwide. Sadly, coral reefs are rapidly disappearing all over the world. This is primarily due to the mass extinction of many foundation species brought on by disease outbreaks, frequent intense bleaching events, increasing storm frequency and intensity, and ocean acidification caused by global climate change. These factors are all made worse by local/regional anthropogenic stressors like pollution, coastal development, and overfishing. Coral reef composition, structure, and function have changed as a result of significant losses in live coral cover, abundance, and diversity. Soft corals are also negatively affected by environmental degradation and diseases (Putra et al., 2022).

Coral propagation represents one of few economic opportunities for sustainable livelihood diversification in islander communities. Asexual coral propagation can cater to the poor and to all genders, enabling communities to actively participate in ornamental trade or reef restoration. In turn, these practices can restore tourism to proximal reefs, increase biodiversity and provide more local food sources. Indonesian regulations require ten per cent of corals cultured in Indonesia be used in reef restoration efforts. Refining government programmes to best direct these restoration efforts could further improve their effectiveness. It is critically important for a balance to be maintained between ex situ and in situ production in regions such as Indonesia, which exports the majority of aquacultured coral. If *ex situ* propagation becomes dominant in these areas, the economic incentive to maintain high coastal water quality (e.g. limit pollution) is likely to be reduced. Furthermore, the CITES Resolution Conference 16.6 suggests that this shift may favour ex situ over in situ propagation would also reduce revenue in the rural communities that depend on these natural resources (Barton et al., 2017).

2.4. Reproduction

Most corals spawn eggs and sperm for external fertilization and development; reproduction is usually seasonal, with breeding occurring during brief annual periods: many species participate in predictable mass spawning events. In addition to sexual reproduction, various asexual processes of reproduction can result in the formation of new colonies or solitary corals. These include fragmentation of established colonies, budding and transverse or longitudinal fission, single polyp bail-out, detachment of groups of polyps as drifting polyp balls, and asexually produced planulae. The diversity of reproductive methods employed by corals and other anthozoans is testament to the extraordinary plasticity of cnidarian tissue (Harrison and Wallace, 1990). Many corals reproduce both asexually through fragmentation and sexually by the production of gametes. Important reef-building corals such as acroporids are extremely successful at reproducing asexually and are dispersed when storms break apart parent colonies and spread the fragments to new portions of a reef where they can reattach and grow. Sexual reproduction in corals is also variable in that corals are typically either brooders or spawners. Brooders

release fertilized larvae into the water column while spawners release sperm and eggs into the water column, where they fertilize and disperse with the ocean currents (Burkepile and Hay, 2008). Concerning the corals used in this study, Baran and Baria-Rodriguez (2021), state that a species of the genus *Lobophytum* is a gonochoric broadcast spawner as the majority of octocoral species (trait that seems to be highly conserved), gametes matured and spawned in April, coinciding with increasing sea surface temperature.
The best predictor of differences in recruitment rates among reefs was the fecundity, not abundance, of adult corals and explained 72% of the variation in recruitment for acroporid corals. Recruitment rates decreased dramatically as the fecundity of adults decreased, but this decrease was not linear; a small decrease in the fecundity of adults resulted in a dramatic decrease in juvenile recruitment (Burkepile and Hay, 2008). Similar evidence was found on the octocoral *Pseudopterogorgia elisabethae* (Page and Lasker 2012). A key component to the replenishment of populations of coral reef organisms is the extent to which reefs are connected to other reefs (i.e., whether juveniles recruit to reefs from local or distant sources). Coral reefs, and marine ecosystems in general, differ from many terrestrial systems in that juvenile organisms have the potential to ride ocean currents and be dispersed over wide distances potentially connecting geographically distant populations, that's why they are thought to be demographically open (Burkepile and Hay, 2008; Chamberland 2018). Using currently available climate change models, in combination with relevant biogeographical and biological data, Wilson et al (2016) predicted that climate-driven oceanographic changes will either enhance or reduce species dispersal by strengthening, weakening, and altering the structure of oceanic dispersal pathways.

Corals produce non-feeding (i.e., lecitotrophic) larvae that disperse for hours to weeks in the plankton and must settle and metamorphose into their feeding benthic form before depleting their energetic resources. Settlement occurs when a larva moves from the water column and attaches to the benthos. Subsequent metamorphosis involves a developmental process during which a larva undergoes a series of morphological and physiological transformations from their larval form into a primary polyp or settler. Substrate selection for settlement by coral larvae depends on a myriad of factors such as light availability, substrate colouration and micro-topography, but is foremost driven by the presence of positive and negative chemical cues. Positive cues that trigger settlement are generally released by other organisms that indicate appropriate habitats for survival and growth. Crustose coralline algae and associated bacteria are for example known to induce settlement and metamorphosis in corals. Without such positive cues, larvae may fail to settle and metamorphose, and consequently, to successfully recruit (Chamberland 2018).

Restoration efforts were initiated using asexual propagation or "coral gardening" approaches whereby fragments are cultured from donor colonies in nurseries before they are outplanted on the reef. While asexual propagation has been successful, it requires that fragments are harvested from otherwise healthy colonies. It also limits the formation of new genotypes through genetic recombination, which may hamper the generation of genotypes better adapted to the altered environmental conditions on modern-day reefs. Using sexually- instead of asexually-produced offspring avoids these issues. Moreover, the use of eggs rather than fragments could yield a much larger number of individuals that can be reared for restoration efforts. Reared coral larvae are generally settled onto artificial substrates and kept in land-based or ocean nurseries for several months to years before they are outplanted. Land-based nurseries are generally assumed to offer stable and more protected environments for coral settlers relative to actual reef environments due to reduced fish predation, algal competition, and sedimentation. Because increased size corresponds to lower mortality in recently settled corals, extended grow-out periods are expected to increase the success of restoration efforts by allowing settlers to grow before they are outplanted on a reef. Alternatively, outplanting coral offspring soon after settlement might select for genotypes capable of acclimatizing to the conditions at the outplant site. Exposing recently settled corals to moderate stress conditions could also lead to increased tolerance to more severe stress conditions experienced later in life. Keeping settlers in nurseries for long periods of time or by outplanting them soon after settlement are consequently two different approaches that each have specific advantages for restoration purposes. Reduced nursery periods would also aid to making restoration efforts more economically viable. Large scale restoration efforts are currently extremely expensive due to the high costs associated with nursery maintenance and the outplanting of artificial substrates by hand. The costs to rear and outplant one artificial substrate containing at least one coral recruit currently range from $5.40 USD to $163 USD (Chamberland, 2018).

2.4.1. Aquaculture techniques

Soft corals, either for biotechnological research on marine natural products or in particular the dynamic appearance and colouration, have made them important additions in marine trade, particularly in reef tanks, which are gaining worldwide popularity. However, most of the soft corals used in the marine aquarium trade are collected from the wild, which in the long run will not be sustainable and will have negative impacts on the biodiversity and coral reef ecosystems. The increased demand for these organisms has led to their massive harvesting and has raised the need for efficient farming methodologies. The development of reliable and cost-effective methods for the propagation and culture of soft corals in hatcheries is the only solution for meeting the demands of hobbyists for aquarium trade as well as other markets and help in the restoration of degraded reefs that have already been adversely affected by natural and anthropogenic disturbances. In this context, coral aquaculture can be a potential solution for a continuous and sustainable supply of soft coral biomass. However, there is a lack of scientific studies on soft coral culture and very few species are artificially cultured (Chaitanawisuti and Kritsanapuntu, 2019; Rocha et al., 2013). The rearing of coral fragments in aquaria, prior to transplantation, generally mimics asexual reproductive processes. Corals can be broken up, or ‘fragmented’, in nature, primarily because of physical disturbances or stress (Larkin et al., 2023). Coral propagation by asexual reproduction is a relatively simple and inexpensive process, which has been commonly used for the production of new colonies, with a high survival rate of fragments and a reduced impact on mother colonies. Coral fragments can be produced either in situ or ex situ. *In situ* fragmentation and grow-out may benefit from natural environmental conditions and requires no adaption to artificial propagation systems. However, fragments are exposed to potential deleterious factors, such as sedimentation, pathogens, predators, competitors, and other natural hazards, which can reduce survival (Rocha et al., 2013). *In situ* coral aquaculture has been proved to be economically viable and a good way to support conservation of endangered natural coral reefs as well as local economy. The common coral farming techniques are adaptable to the coastal villages, using locally available materials for coral aquaculture (Todinanahary et al., 2017). In contrast, *ex situ* fragmentation has the advantage of maximizing survival and growth rates through the manipulation of culture conditions, such as light, flow and food availability (Rocha et al., 2013). Furthermore, it is possible to control the origin of the corals in *ex situ* aquaculture and thus avoid mass culturing single genotypes, which may ultimately lead to genetic pollution of natural populations.

Aquarium hobbyists and several companies that are farming corals have been improving coral propagation and culture techniques. Their expertise is sometimes disseminated, but most information is unsystematically distributed and often available in grey literature, such as aquarium magazines, web, forums, etc. (Leal et al., 2017).

Some of the first corals to be propagated were various members of the Alyconacea. Simply slicing a parent colony into parts using a razor blade or similar sharp cutting instrument can asexually propagate these corals. For example, branches can be snipped from *Sinularia* spp., or either vertical or horizontal sections cut from *Sarcophyton* spp. Cut areas heal rapidly, often within days. Propagated colonies can be made by slicing colonies in the process of longitudinal fission, having branches sliced off, or even by removing individual polyps. Another method used to separate daughter colonies of many soft corals is the use of rubber bands to exert continuous pressure across a parent colony to be ‘cut’. The coral heals as the pressure exerted by the rubber band slowly cuts through the parent colony. This decreases the likelihood of necrosis by invading microorganisms following direct cuts. It is, however, a much slower procedure. Encrusting species can also be ‘trained’ onto substrate placed adjacent to their spread, simply cutting the tissue once the colony has attached to the desired material. Flaccid or slimy soft corals, such as *Xenia* spp., are somewhat more difficult to manage because they lose much water when cut and cannot be glued with success (Borneman and Lowrie, 2001). Nadir et al. (2023) working on *Xenia umbellata* saw that excised single polyps successfully reattached to tissue-culture plates within 2-3 days and started budding within 10 days.

Amputation of the oral disc led to full regeneration within 7-10 days, with budding continuing throughout this period. Moreover, amputated tentacles developed into polyps within 21 days, demonstrating an unusual capacity for whole-body regeneration. Another publication about the same species tested four fragmentation methods and the stress related to each one, no evidence about a different stress level was found but they proved the "plug mesh method" as the most efficient (95 ± 5%) in terms of labour intensity and attachment rate to the ceramic stand (plug or coral plug) (Kim et al., 2022).

Because of their rapid rate of growth and healing capacity, a single Alcyonacean such as a *Sinularia* sp. can produce dozens or hundreds of cuttings a year with nearly 100% survival even by beginning aquarists. As with the Scleractinia, very small fragments of tissue can be grown without difficulty. Many of the asymbiotic octocorals are very difficult to maintain in captivity because of their high plankton requirements but given a plentiful source of food coupled with strong foam fractionation, similar success may be possible with asexual propagation methods. If tissue is blotted dry prior to placement on substrate, the cyanoacrylate adhesives work well for attachment. This procedure can be used for virtually any soft coral with substantial spicule support, including the Neptheids. However, calluses formed, heavy mucus, and the ability of many species to substantially expand their tissue volume renders glue bonds tenuous in some cases. Fishing line has been used to sew glue-resistant species onto substrate until attachment. Toothpicks can also be used to impale the tissue firmly against substrate material, or alternately, to affix the impaled toothpick to substrate with ties or rubber bands which would normally cut through the coral. Nubbins frequently become loose at the piercing location, and the use of wires (without any piercing) cannot secure them effectively as nubbins are amorphous and can change in shape and size in response to environmental conditions, feeding and stress. Some of these methods involve frequent handling to re-attach nubbins when they come loose, resulting in further stress.

Pea gravel can be used to fill a tray that is removed from strong water movement. The tissue attaches to the pea gravel, which can then be affixed to substrate using conventional adhesive techniques (Borneman and Lowrie, 2001; Larkin et al., 2023).

For soft corals, the physiological process of fragmentation usually entails the formation of root-like-processes (RLPs), which subsequently attach to the substrate**.** Occasional guidance of nubbins can be required to ensure their RLPs face downward, enabling the attachment process (Larkin et al., 2023). In *Litophyton arboretum* stems and polyps showed their lowest cell growth rate immediately after injury due to cutting (Days 1 to 3), and their highest cell growth rate during recovery time (Days 7 to 49). In all experiments, the cell growth rate of stems was significantly higher than that of polyps (Tentori et al., 2004).

A nursery phase is commonly used post-settlement or post-fragmentation to allow colonies to grow to a suitable size with reduced competition from fouling organisms

and protection from predation. Following the nursery phase, coral colonies can be transplanted onto denuded reefs or used to supply demand from hobbyists in the live coral trade (Barton et al., 2017).
The octocorals have proven to be extremely durable in captivity and very tolerant of propagation techniques. The success of asexual propagation methods could easily allow for a drastic reduction of wild collected corals, although there is still some resistance due to the total variety and value per size of collected vs captive-grown corals (Borneman and Lowrie, 2001).

Substrate selection should be tailored to the species being cultured and the suitability of the substrate for ornamental trade or reef restoration. Factors to be considered include the ability of substrate to be cleaned of fouling organisms, propensity to resist settlement and growth of fouling organisms, source materials available, space available for culture and whether or not the fragment will be transplanted onto a denuded reef. Consumer perception of coral substrate should be considered when propagating coral to supply the live ornamental trade. Although a large proportion of reef aquarium hobbyists may remove the coral from its substrate, aesthetics and dimensions of the substrates are likely to influence consumer perception. Aragocrete is a common fragment substrate generally well received by hobbyists. It is made by mixing equal parts of Portland cement and aragonite sand, which can then be moulded to provide a flat surface area for adhesion of the coral fragment. Crustose coralline algae readily proliferate on the surfaces of aragocrete, instead of unwanted filamentous algae forms, making it more aesthetically pleasing to ornamental enthusiasts. Basalt or coral gravel has been suggested as a substrate for soft coral which can be held in place with wooden toothpicks until successful attachment. Other viable options exist for soft coral species. Fusion of coral fragments to substrates plays an important role in their long-term survival. Some experiments have shown significant loss of fragments due to detachment from the substrate, with detachment rates surpassing direct fragment mortality rates in some cases (Barton et al., 2017).

The interaction between corals and fouling organisms, particularly filamentous algae, is generally detrimental for the health of adult corals and settlement of coral larvae. The addition of natural biocontrols in conjunction with manual removal of biofouling organisms reduces competition faced by coral recruits on their respective substrates. Antifouling paint can also reduce fouling coverage and cleaning procedures. Herbivorous sea snails, urchins and fish that consume macroalgae and anemones can improve the efficiency of ex situ nursery phase (Barton et al., 2017).

Chaitanawisuti and Kritsanapuntu (2019) tested different fragging methods on soft corals. They measured effects on healing time, self-attachment time, survival and development of the cuttings. Since zooxanthellate corals were used, feeding was not provided. In this study, the survival of a cutting was defined as the presence of the cutting completely attached to the substrata after the experiment had been started on each treatment and the loss of a cutting that was detached from the substrata or partly decaying was defined as dead. The healing time was recorded as the time needed to completely heal the cutting wounds. The wound was considered healed when it was completely closed and covered with pigmented tissue and the first polyp had grown inside the wounded area. The self-attachment time was determined as the time needed for the cuttings that were permanently fixed over the substrata and they did not fall down from the substrata when flipped upside down. The cuttings had already healed the wound area and started to attach by day 3-5, The complete attachment was achieved between day 9 and 12 in different methods. The best results for self-attachment were obtained using the “impaling method”. The highest average final survival of *Sarcophyton* sp*.,* *Cladiella* sp., and *Sinularia* sp. cuttings was obtained using the “impaling method”, “containing method 1” and “containing method 2” respectively. This study indicated suitable methods of attachment for producing cuttings available for use in targeted propagation farms for restoration purposes.

Other publications noted that cuttings can attach to substrate in 2 weeks and achieve a survival rate of 100% at the end of 80 days (Chaitanawisuti and Kritsanapuntu, 2019). Cuttings of *Lobophytum pauciflorum* of 6 cm length in a static seawater system can attach firmly to the substratum by approximately 2 weeks and new polyps were clearly visible on the cut portion by 25 days (Varghese et al., 2012).

The increasing interest in coral culture for reef restoration, biotechnological applications and to supply the marine aquarium trade has prompted researchers to optimize coral culture protocols, with emphasis on ex situ production. The fact that the soft coral cuttings successfully attached to the substratum and showed growth of numerous polyps within a short span of 2 months under hatchery conditions points to the possibility of transplanting the soft corals in the wild as a first step toward conservative soft coral farming. This may ultimately lead to appropriate strategies for conservation and the sustainable utilization of this resource (Chaitanawisuti and Kritsanapuntu, 2019).

2.4.2. Light

Light is a key factor for symbiotic corals due to their association with photosymbiotic unicellular dinoflagellates from genus *Symbiodinium*. The photosynthates produced by the zooxanthellae are transferred to the coral host and fulfil a significant part of its energetic requirements. Light variation is known to affect zooxanthellae density, photosynthetic pigment concentration and photosynthetic efficiency. Ultimately, changes in the density of zooxanthellae can affect coral physiology and its response to stress. As the fragmentation process per se induces stress to both coral mother colony and produced fragments, it is expected that light can play an important role on the post-fragmentation photophysiological processes and, therefore, on coral recovery.

An excessive increase in light levels is known to commonly damage the photosynthetic apparatus of zooxanthellae, while an increase in zooxanthellae density is usually recorded when corals are exposed to suboptimal light intensities. High and low light levels will ultimately lead to an adaptive response of the coral holobiont, either through the action of photoprotective mechanisms (such as the increase of photoprotective pigments) or adapting the photosynthetic apparatus to maximize light capture. Acclimation to low light involves the maximization of the light harvesting capacity through 1) the increase of photosynthetic pigment concentration in zooxanthellae; and 2) the multiplication of zooxanthellae (increased density). However, while changes in pigment concentrations present in zooxanthellae usually occur within 2–4 days, changes in the number of zooxanthellae only commonly occur within 40 days.

The effect of different light intensities in the physiology and photobiology of the soft coral *Sinularia flexibilis* following ex situ fragmentation have been studied trough the measurement of photosynthetic performance, zooxanthellae density, photosynthetic and accessory pigments concentration and coral fragments growth. No significant effects were recorded on coral growth between the different light treatments (50, 80 and 120 μmol quanta m−2 s−1). Therefore, the result suggest that the use of lower light levels can be a suitable option following fragmentation (Rocha et al., 2013). Other experiments in soft corals used 100 to 200 μmol quanta m−2 s−1 as photosynthetic active radiation (PAR) value (Chang et al., 2020; Rocha et al., 2015).

PAR refers to the spectral range (400 to 700 nanometres) of solar radiation that is used in photosynthesis. This is relevant for primary producers such as seagrass and phytoplankton as well as for most reef-building corals which contain photosyntheticalgae (zooxanthellae) (DES, 2018). The study from Rocha et al. (2013) showed that keeping *S. flexibilis* fragments under the same light conditions as their mother colonies seems to be photobiologically acceptable for a short-term husbandry (e.g. when producing a large number of small sized fragments for research studies), lower light intensities than those used for mother colonies may favour the photobiological performance of coral fragments intended to be stocked for longer periods and contribute to a reduction of production costs (e.g. when producing large sized colonies that can yield a larger biomass production for biotechnological applications and need to be stocked in captivity for several months).

2.4.3. Water flow

In nature, corals are adapted to their environment, and it is common to observe different species in different reef areas. Therefore, different coral species have different water flow preferences, and it is not possible to define a single ideal water flow regime to use in coral aquaculture systems. Nevertheless, the relation between corals and water flow has been thoroughly investigated, and the available information can help to predict how corals respond to water flow changes. The increase in flow speed will maximize the particle delivery rates to the coral surface. However, particle capture efficiency often decreases with increasing flow.

Therefore, particle capture rates are often highest at intermediate flow speeds, where particle delivery rate is high, but coral polyps are still capable of retaining food particles. There are also species-specific differences as branching and cylindrical coral species increase food capture with increasing flow. In contrast, flat coral species, such as the plate coral *Montipora* sp., show no effect of flow speed on particle capture. The effect of water flow on coral growth is reflected by the sum of the flow effects on particle capture, nutrient uptake, photosynthesis and respiration. Water flow enhances photosynthetic rates of in hospite *Symbiodinium* and increases respiration rates of coral tissue, as well as calcification rates, growth rate, uptake of dissolved nutrients and waste disposal from coral surfaces. Water flow interacts synergistically with light because it determines both the rate of supply of nutrients needed for photosynthesis and the efflux rate of oxygen and oxygen radicals that may inhibit photosynthesis (Leal et al., 2016).

2.5. Coral market

Coral reefs are amongst the most productive ecosystems on Earth. They provide habitat to 35% of all marine species and food, livelihoods and coastal protection to at least 500 million people. The latest estimates suggest that coral reefs on average provide $352,250 USD ha-1 annually through fisheries, coastal protection, tourism and as a source of new medicines, as many of the plants and animals that live on coral reefs produce chemicals that are useful as pharmaceuticals (Chamberland 2018; Burkepile and Hay, 2008) such as anti-cancer, anti-viral, anti-bacterial, anti-fungal drugs, antimalarial activity, anti-inflammatory activity (Ellithey and Ahmed, 2018; Dautova and Kiyashko, 2017; Lee and Su, 2011; Jahajeeah et at., 2023; Sheu et al., 2019), skin care products (Page and Lasker, 2012), algae growth inhibition (Coll et al., 1987). The work from Peng et al., (2018) indicated that the development of an efficient aquaculture protocol for soft corals led to the discovery of new secondary metabolites with unique structural features. Such protocols can lead to a sustainable supply of biologically active compounds in enough quantities for the pharmaceutical industry.

Additionally, these extremely productive and biodiverse ecosystems provide hundreds of species for the marine ornamental trade, which has been increasing over the last decades to a point that is becoming a threat for reef organisms. Corals are included in the list of principals exploited species for the marine ornamental trade, mostly because of their attractive colours and forms (Leal et al., 2017). Over one third of reef-building coral species (Scleractinia) are currently threatened by extinction (IUCN 2017) due to habitat destruction, overexploitation and anthropogenically-driven climate change (Chamberland 2018).

2.6. Description of the corals used in this experiment

The anthozoan clade Octocorallia comprises over 3500 species of soft corals, sea fans, and sea pens, including some of the ocean's most familiar and ecologically

important benthic fauna. Octocorals occur in all marine habitats worldwide. Their diversity is highest in the deep sea where they serve as important foundation species, generating structurally complex three-dimensional "forests" that support many other invertebrate and fish taxa.
They are also diverse and abundant on tropical coral reefs where they are often the dominant sessile space-occupiers, a trend that may be increasing on some reefs as scleractinian corals decline disproportionately in response to ongoing environmental change (McFadden et al., 2022).

Recently the old Alcyonacea order has been rearranged due to molecular phylogenetics which have revealed that almost all of the recognized families of octocorals are also non-monophyletic.
The traditional classification of octocorals into families and sub-orders or orders has been based largely on shared gross morphological characters such as the presence/ absence and composition of a skeletal axis and the overall growth form of the colony. Molecular phylogenetic analyses that have revealed the non-monophyly of these higher taxa also imply that these gross morphological characters are labile and subject to widespread homoplasy.
While Malacalcyonacea includes a majority of taxa previously considered to belong to the subordinal groups Holaxonia and Alcyoniina, it also includes many taxa formerly classified as Stolonifera and Scleraxonia. The vast majority of taxa in Malacalcyonacea have either a largely proteinaceous or no skeletal axis (McFadden et al., 2022).

2.6.1. *Lobophytum* sp.

Lobophytum sp., one of the animals used for this experiment is included in the family Sarcophytidae. The type species of this family is *Sarcophyton*, a well known soft coral. McFadden et al. (2022) describes this family as Octocorals without a skeletal axis, colonies lobate, plate-like or capitate with a conspicuous stalk, not highly branched. Polyps monomorphic or dimorphic, fully retractile into thick coenenchyma. The members of this family are among the most familiar, diverse and abundant species found in the shallow water of Indo-Pacific coral reefs. They have long been classified in Alcyoniidae along with temperate and cold-water species with which they share similar sclerites and massive, fleshy growth forms that are not highly branched. All species of Sarcophytidae are, however, zooxanthellate; their sclerites are never coloured; and the sclerites of the colony surface are almost always well-formed clubs while the colony interior has only tuberculate spindles that may be very large. Four of the five genera in this family (*Anastromvos, Lobophytum, Lohowia, Sarcophyton*) have dimorphic polyps.
The genus *Lobophytum* was originally described from Von Marenzeller (1886) as not mushroom-shaped, dimorphic. Autozooids and point-shaped siphonozooids only on the upper surface of the zoanthodema, which has grown into flaps, lobules or finger-shaped processes, which barely protrudes above the stem part. The surface

of the tough, resistant, polyp-bearing part is shagreen-like due to the mouths of the massive siphonozooids. The small club-shaped spicules of the bark layer not so pronounced, only abundant on the stem. The spicula of the cenenchyme of the polyp-bearing part of the zoanthodemes numerous spindles (double spindles) covered with many large belt-forming warts, usually over 0.05 mm.

2.6.2. *Litophyton* sp.

Litophyton sp., the second genus used for this experiment is the type genus of the family Nephtheidae. McFadden et al., (2022) describes this family as octocorals without a skeletal axis, colonies arborescent, often highly branched or umbellate and with a conspicuous stalk. Polyps arise from terminal branchlets, rarely on main branches or stalk polyps monomorphic, non-retractile, with weak or well-developed supporting bundle of sclerites. Sclerites of branch and stalk surface are spindles and irregular radiate-like forms, few sclerites in colony interior. Sclerites often brightly coloured. Zooxanthellate or azooxanthellate with a presence in shallow to moderately deep waters of tropical Indo-Pacific. In the original description by Forskål (1775) it is mainly described as yellow-veined coloured, with smooth alternate papillose, supported, ramified, oblong, fleshy branches and it stays erected in the water. As stated from Van Ofwegen (2016) the original descriptions of this species revealed hardly any characters, that's why here is reported her description: Nephtheids with bushy and arborescent colonies. Polyps clustered at the end of the terminal branches, forming catkins. Polyps non-retractile, without or with supporting bundle, sometimes completely unarmed. Sclerites of surface layer of branches, stem and stalk are spindles and unilateral spinose spindles, the colony stalk also contains capstans and derivatives of capstans. Interior of the stalk has sparsely tuberculated spindles.

2.6.3. *Klyxum* sp.

The last species used for this experiment is *Klyxum* sp. from Cladiellidae family, the type genus of this family is *Cladiella*, another well-known coral. McFadden et al. (2022) describes this family as octocorals without a skeletal axis, colonies lobate to digitate, lobes often subdivided but not highly branched. Polyps monomorphic, retractile or non-retractile but highly contractile, evenly distributed over lobe surfaces. Polyp sclerites minute discs, figure-eights or flattened rods. Sclerites of colony surface and coenenchyme double-heads, blunt spindles with conical protuberances, or minute figure-eights and granular rods. Some species may lack sclerites entirely. Zooxanthellate or (rarely) azooxanthellate. It can be found in the shallow waters of the tropical Indo-Pacific. The species used in this experiment has a more recent establishment compared to the previous ones; it comprehends several genera previously described by several authors. Alderslade (2000) describes these animals as lobate colonies, that, when expanded, generally have rather long lobes that subdivide. Colonies are usually quite small, but at least one species grows to about 50 cm in height, and in his experience, they are always soft and fleshy. Polyps

are clustered on the lobes, often very densely arranged, and are non-retractile. It is worth remarking that the polyps in species of *Klyxum* are capable of extreme contraction until they are more or less flush with the surface, but the author found no invagination in any of the specimens he examined. The sclerites in the coenenchyme are predominantly narrow or plump spindles, with relatively large, rounded, cone-shaped prominences. They are commonly shorter than 0.4 mm. The spindles are usually pointed, their abundance is variable, and in some species they only occur in the basal part of the colony stalk. There is no distinct layer of sclerites in the colony surface, but it is not unusual to find the sclerites that are near the surface to be slightly smaller, narrower, and smoother than those that lie deeper within. Polyps are usually dark brown, while the general colony colour is lighter, such as cream, or pinkish brown. In some species both polyps and colony are brown. Sclerites are always colourless. Abundant zooxanthellae. The genus has been found to occur in Madagascar, Maldives, Thailand, Indonesia, Great Barrier Reef, Papua New Guinea, Solomon Islands, New Caledonia, Fiji, Tonga, Palau, Philippines, and Japan. It can be found both in clear, coral reef waters, and turbid coastal regions.
All the corals used in this experiment were zooxanthellate.

3. Purpose of the thesis

The aim of this thesis is to establish scientifically which is the best method for creating cuttings through fragmentation of the three soft corals *Litophyton* sp., *Lobophytum* sp. and *Klyxum* sp., important for the ornamental and pharmaceutical markets and potentially for repopulation campaigns. The impaling method and the tying method are compared by measuring the survival rate, adhesion time to the substrate, first healing time, state of well-being/stress evaluated by means of contraction or not of the animals. The development of an aquaculture protocol is of great importance for further reduction of the fishing pressure on already endangered coral reefs.

4. Materials and methods

4.1. Experimental design

Two different coral propagation methods were applied to three species of soft corals. The parameters evaluated during a four-week period were: survival, adhesion time to the ceramic support, time necessary for the first wound healing, presence or absence of at least three extroflected polyps. Three replicates with five specimens for each genus, for a total of ninety corals in total were used for the test.

4.2. Facility context where the test took place

RecifAtHome vpc marin is the largest marine ornamental species shop in France, its main activities concern the sale of aquarium equipment, fish and marine invertebrates imported and cultured on site. Eight different recirculation aquaculture systems (RAS) are arranged within the company: one dedicated to macroalgae and growth of fish reproduced directly in the farm, one dedicated to larval breeding, one dedicated to fish and decapod broodstock, three dedicated to culturing hard corals and maintaining bivalves (mainly from the *Tridacna* genus), one dedicated to the quarantine of imported stony corals (mainly from Indonesia, the Philippines and Australia), one dedicated to soft corals, fish, decapods, molluscs and annelids.

4.3. Description of the system and its management method

The diversity of experimental systems employed to investigate ex situ coral production may be a bottleneck to the advance of the state of the art, as it impairs reliable comparisons between experiments, as well as the replication and optimization of culture protocols. One modular system which can be successfully used in medium to long-term experiments has been proposed. By employing standardized culture conditions, researchers from different institutes worldwide could be able to compare collected data in a more reliable way and advance the current state of the art on this research field (Rocha et al., 2015). Even if many similarities between the proposed system and the one used in this experiment exist, due to time and economic constraints was not possible to use exactly the same system.

The aquariums where the experiment was carried out are part of the RAS where soft corals and fish are present. The total volume of water including the aquariums and the filtering system is approximately 8000 litres. The following are connected in parallel to the system: a 400-litre aquarium, one 600-litre aquarium and 52 tanks measuring 40x150x30cm (filled with approximately 120 liters each); three of which were used for the three replicates of the experiment. Inside most of these tanks there are live rocks and coralline sand, these materials offer the ideal environment for bacteria and other important organisms to maintain excellent water quality. The filter system consists of: roller filter (Turtle system, The Frog Roll-Filter), sulfur reactor for nitrate reduction (Pacific Sun, SR-250) filled with sulfur (Carib Sea, LSM Live Sulfur Media) and aragonite, twelve 80W UV lamps (Aqua Medic), skimmer (Royal Exclusiv, Bubble King 500), a reactor for phosphate reduction (Pacific Sun, Algae Reactor AR PRO XL-size) filled with 5Kg of iron-based resin (D-D The Aquarium Solution, ROWA Phos). Inside the filtration system, a pump (Jecod, DCP-20000M) set at 110W is used to feed the reactors and the skimmer, a pump (Jebao, TSP-30000) set at 398W is used to feed the UV lamps, two pumps (Jebao, TSP-30000) set at 399W and positioned in parallel are used to supply water to all the aquariums. The system works so that the water passes completely through the roller filter and (unless there is a power failure) at least once through the UV lamps before returning to the aquariums. The water temperature is maintained at 26 ± 2°C via an air conditioning system that produces warm air in winter and cool air in summer.

The sea water used in this system is prepared by mixing water produced through a reverse osmosis system and specific aquarium salt (Red Sea) until obtaining a density of 1022 (approximately 33.2 ppt) measured with a floating densimeter (Aqua Medic) at a temperature of 25°C. At the end of each working day, the water level that has dropped due to evaporation, tank cleaning operations or the water accompanying sold animals, is re-established by adding directly into the filter system salt water or just freshwater coming from the reverse osmosis system as needed.

Every day in this system 25ml of an iodine supplement (Triton, Iodine), 30 sugar cubes (approximately 150g) are dosed as a carbon source to stimulate bacterial growth useful for the reduction of nitrates and phosphates, 24 liters (one liter each hour) of saturated calcium hydroxide solution (namely Kalkwasser) useful for integrating calcium and raising alkalinity and pH (which is reduced by the normal use of the sulfur reactor). Based on the tests carried out, a product to increase alkalinity (Seachem, Reef Builder), a calcium supplement (Seachem, Reef Advantage Calcium) and a magnesium supplement (Seachem, Reef Advantage Magnesium) are also used.

The aquariums used for the experiments are equipped with a flow pump (Tunze, Turbelle Nanostream 6045) rated 1500-4500l/h continuously switched on, an LED light (SuperFish, SF Slim LED 74cm) positioned 14cm from the water level which via a mechanical timer (Otio, T-10) produces a photoperiod equal to 11:13h light: dark.

During normal shop activities, various tests are carried out on all systems. In particular, as regards the system linked to the experiment, the alkalinity was measured five days a week using a portable photometer (Hanna Instruments, Checker HI755), the nitrate concentration twice a week using a portable photometer (Hanna Instruments, Checker HI782), the concentration of phosphates twice a week via portable photometer (Hanna Instruments, Checker HI774), the concentration of calcium twice a week via manual colorimetric test (Salifert, Calcium Ca Profi Test), the concentration of magnesium instead was measured only once during the experiment period via manual colorimetric test (Salifert, Magnesium Mg Profi Test). Furthermore, in the individual replicates the temperature was measured every day using an infrared thermometer (Sovarcate, HS960D) and dissolved oxygen using a digital oximeter (Milwaukee, MW605). Two measurements of photosynthetically active radiation (PAR) were carried out using a quantum-meter (Apogee Instruments, MQ-650), taking care to measure the two extreme values within the area intended for corals positioning.

4.4. Preliminary operations

4.4.1. Preparation of aquariums

The day before the beginning of the experiment, the tanks (which were used for normal shop activities) were emptied and cleaned, the coralline sand present was rinsed multiple times with water coming directly from the filtering system so as not to compromise its filtering capacity and then repositioned in the tanks. After filling, a stand made of black plastic egg crate raised about 5cm from the bottom of the tank was built and added, which is useful for inserting coral supports and allowing water to circulate underneath them too. It was then possible to set the water exchange at approximately 7.5l/min (90 exchanges per day) via a ball valve present on the inlet of each tank.

4.4.2. Preparation of ceramic supports for corals

Round coral plugs approximately 20mm in diameter, were used as support for the animals during the experiment. For those used to test impaling, prior preparation was necessary before the start of the test: briefly they were positioned on a egg crate, toothpicks were cut in half and glued using specific coral glue (Maxspect, Coral Glue) in the center of the support, after a few minutes when the glue was sufficiently hardened all the plugs (with and without the glued toothpick) were placed in water coming from the reverse osmosis system until they were used a few days later.

4.4.3. Cuttings preparation from mother colonies

The mother colonies used for this experiment were originally imported from Indonesia and kept for a long time in tanks belonging to the same system of the experiment and therefore acclimatized to chemical-physical conditions similar to those of destination as suggested from Rocha et al. (2013). The mother colonies of the species *Litophyton* sp. had a length of about 15-20cm, those of the species

Lobophytum sp. had a diameter of 6-8cm, that of the species *Klyxum* sp. was oval in shape, about 15x7cm. For each of the three species of coral used in this test, a tray was prepared filled with water from the filtering system and with a treatment at a concentration of 1ml/l against parasitic worms potentially present (DVH Aquatic, Coral Protec Coral Dip), the previously selected coral colonies were transferred to the aforementioned trays and kept for three minutes after which they were moved to similar trays without the treatment against parasites. A specific iodine-based disinfectant for corals (Seachem, Reef Dip) was added to the tray with the mother colonies according to the doses suggested by the manufacturer. One at a time the mother colonies were removed from the water and placed on the laboratory work surface to be cut with a scalpel into pieces of similar size while trying to respect the morphology of the colony and in greater numbers than necessary, finally they were placed in the tray of origin. As regards *Litophyton* sp., the length of the cuttings used for the test was approximately 30mm after cutting from the mother colony, while the *Lobophytum* sp. and *Klyxum* sp. cuttings had a square shape at the base and a size of approximately 16x16mm. From this point, we proceeded one genre at a time until transfer into the aquariums before proceeding with the next species. Half of the samples were intended for the method called " impaling " and half for the " tying " one, this two methods were similar to those described by Chaitanawisuti and Kritsanapuntu (2019) and the one mentioned by Larkin et al. (2023) respectively. One at a time, the corals intended for tying were extracted from the water and positioned on one of the plugs previously soaked in water coming from the reverse osmosis system, they were subsequently tied using common fishing line tight enough to keep the coral in position but not too much so that the thread penetrates inside the coral itself, knotted firmly, cut the excess thread and placed in the tray. In a similar way to the previous ones, the corals intended for impalement were extracted individually, pierced with a sterile needle of 2mm diameter (www.crazy-factory.com) commonly used to practice body piercing on people and subsequently inserted into the plugs with toothpicks previously glued (Figure 4). In order to help the adhesion of *Klyxum* sp. and *Lobophytum* sp., one side that presented one of the wounds just produced was specifically positioned in contact with the plug as greater growth was expected in these areas, similarly it was avoided to position the pigmented part that showed the presence of polyps in a poorly-lit way. As regards *Litophyton* sp., in the technique called impalement, the specimens were impaled axially, while in the tying method the plug was positioned radially respectfully the main axis of the coral. If some animals or plugs were damaged during these procedures, they were discarded and replaced with those previously prepared in excess. Once finished, the samples of the same genus were randomly selected and divided into various replicates and treatments, they were then precisely arranged on the egg crate present in the various tanks in order to obtain a balanced distribution and not disturb the nearby corals (Figure 5). We then proceeded to repeat the same process for the remaining two species. During the processes just described and the previous preparation of the plugs intended for impalement, the necessary time was also measured as a potentially useful data in case no differences were found in terms of survival between the two techniques applied.

It took an average of 0,8 minutes to prepare each coral plug with the toothpick glued on top before the beginning of the experiment. The mean time needed to impale one *Klyxum* sp. fragment was recorded in 1 minute, 1,6 minutes for *Lobophytum* sp. and 1,1 minutes for *Litophyton* sp..
The mean time needed to tie one *Klyxum* sp. fragment on a ceramic support was recorded in 3,7 minutes, 4 minutes for *Lobophytum* sp. and 3,5 minutes for *Litophyton* sp..

Figure 1: Particular of the slicing procedure applied on *Lobophytum sp.*. The mother colony (left) is cut in the middle of the stalk, thus dividing the rock used as base with the attached foot (middle specimen) by the upper part. The upper part is then split into several square-shaped nubbins (right) which has been used alternatively for the impaling or tying method.

Figure 2: Mother colony of *Litophyton sp.* and freshly cut coral fragments. To be noted the great difference in size between the nubbins and the mother colony's branches due to enormous fluid loss shown by this coral after an injury.

Figure 3*: Klyxum sp.* nubbins right after the slicing from the mother colony, coral plug and a 1-Euro coin (23mm diameter) as reference.

Figure 4: Left: a specimen of *Litophyton sp.* right after the assembling with the coral plug for the tying method.; Center: Particular of the impaling procedure: a needle is used to practice a piercing trough the coral nubbin, secondly, the toothpick-plug group is inserted into the needle and the coral is pushed into its final position (right picture).

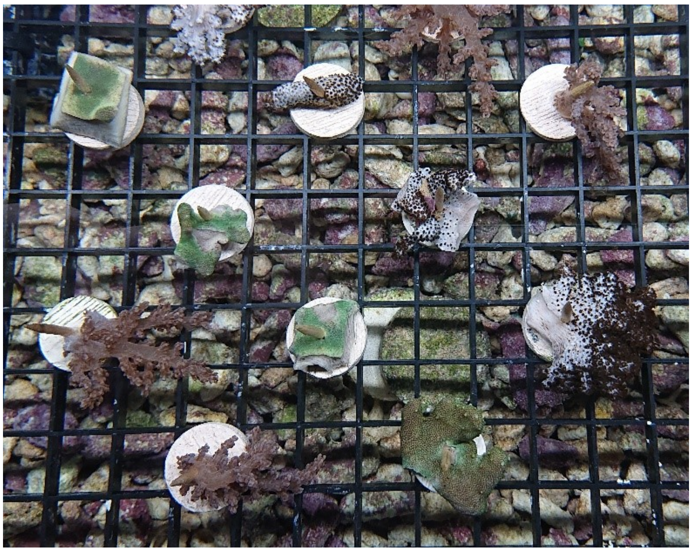

Figure 5*: Exaple of positioning of the impaled specimens at the beginning of the experiment.*

4.5. Measurement of parameters during the test

During a 28-day period several parameters were measured once a day between 2pm and 7pm in a similar manner to Chaitanawisuti and Kritsanapuntu (2019). The presence or absence of at least three everted polyps regarding *Klyxum* sp. (Figure 6) and *Lobophytum* sp. (Figure 7), or if the coral was "inflated" as regards *Litophyton* sp. (Figure 2 and 4). The healing of the wounds, caused during the cutting of the mother colonies to create the different samples was evaluated based on the possibility of observing the internal structure of the coral not yet completely healed or the complete coverage of the wound with new polyps (Figure 7). The number of days necessary for the first adhesion to the ceramic support was evaluated by manually trying to turn the coral from its position and taking note about the presence or absence of resistance. If the action of testing the adhesion had caused itself the detachment of an otherwise attached specimen,it would have been considered as adherent. Similarly, if any manipulation necessary for the measurement of parameters or for the production of pictures had modified the state of a coral (for example freeing it from the fishing line or toothpick) it would have been restored in the same way in which it was before the handling. Finally, the survival of the corals was evaluated, in particular not only death but also just detachment from the support was counted as death following Chaitanawisuti and Kritsanapuntu (2019). In temporal order from the first to the third replicate, survival was assessed first, then the presence of inflated polyps/animals and finally healing and adhesion to the support. On the sixth day from the start of the test, to keep under control the growth of filamentous algae that could have suffocated the small corals, three specimens of similar size of the gastropod *Dolabella auricularia* and *Strombus* sp. were added into each replicate. During the entire experiment no type of food was administered to the corals. On the last day of the experiment, after evaluating all the parameters, all the fishing lines used to tie the corals to the supports were delicately cut and removed, thus avoiding damaging the cuttings or having them incorporate during growth.

Figure 6: Already healed and adhered *Klyxum sp.* specimen. The same specimen with contracted

polyps (left) and not contracted (right). To be noted the fishing line completely embedded in the coral tissue due to growth of the baby-colony during the experiment.

Figure 7*:* Left: Tied Lobophytum sp. already adhered to the coral plug and completely healed with retracted polyps. To be noted the tense fishing line which is starting to be embedded in the baby-colony due to coral growth; Right: Lobophytum sp. with extended polyps.

4.6. Statistical analysis

Microsoft Excel and R Studio with the survival analysis has been used to analyse the gathered data and draw the plots. Survival analysis studies the time until an event occurs, in this case the death or release from the support, the healing time and the adhesion time. Cox proportional hazards regression was applied and a p value < 0.05 was considered statistically significative. A correlation analysis has been used to evaluate the relation between the number of contracted polyps and time, species or treatment (impaling or tying method).

5. Results

5.1. Water quality

Regarding the water quality of the recirculating aquaculture system and specifically in the three tanks used for the experiment, here are reported the chemical and physical parameters of the water, measured as previously described in the materials and methods section.

Parameter	Mean	SE	Parameter	Mean	SE
PAR r1	143	±18	Temp r1	27	±0.1
PAR r2	135	±15	Temp r2	27	±0.2
PAR r3	147.5	±2.5	Temp r3	26.7	±0.2
Alkalinity	153.3	±1.4	DO r1	5.96	±0.08
Phosphate	0.03	±0.01	DO r2	5.82	±0.11
Nitrate	4.1	±0.9	DO r3	5.82	±0.06
Calcium	441.3	±5.8	Magnesium	1290	

Table 1: Main water parameters measured during the experiment. SE: standard error; r1, r2, r3: replicate 1, replicate 2, replicate 3; PAR: Photosynthetic Active Radiation (µmol m-2 s-1); Alkalinity (mg/l); Phosphate (mg/l); Nitrate (mg/l); Calcium (mg/l); Magnesium (mg/l); Temp: Temperature (°C); DO: Dissolved Oxygen (mg/l).

5.2. Survival

At the end of the period of 28 days chosen as duration of the experiment, the final survival rate taking into account the three species and two aquaculture methods was 81±4%.

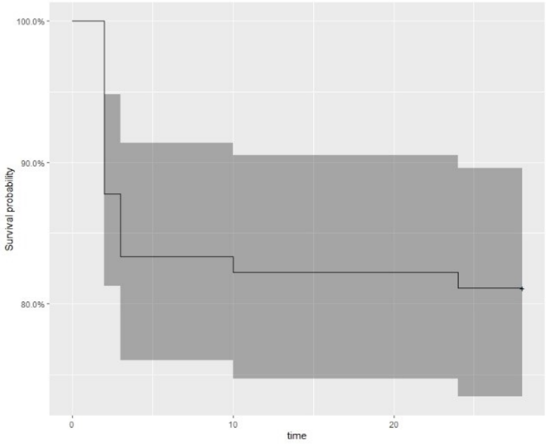

Figure 8: Survival probability for a coral fragment during the experimental period of 28 days (R survival analysis).

The survival rate for the different methods was measured: the tying method had the best result with 96±3% of the corals that reached the end of the trial. The impaling method on the contrary reached only 67±7% of survival rate.

Cox regression showed a statistically significant difference between the tying method and the impaling method.

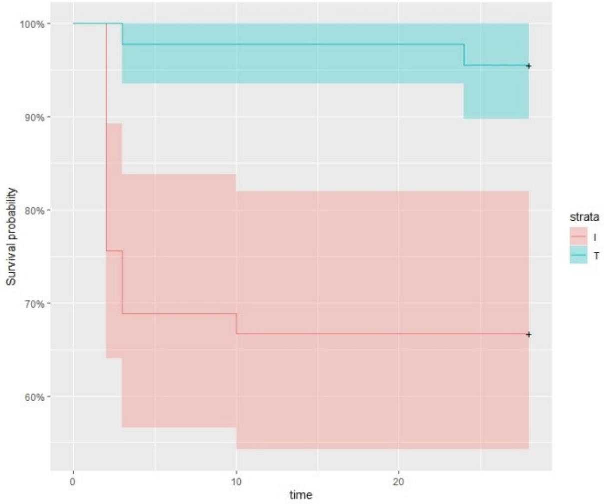

Figure 9: Survival probability for a coral fragment related to the impaling or tying method (R survival analysis)..

Considering both the aquaculture methods applied, *Lobophytum* sp. achieved a survival rate of 100%. Similarly, 90±5% of *Klyxum* sp. fragments survived. The lowest survival rate was obtained by *Litophyton* sp. which by the end of the experiment presented only 53±9% alive nubbins.

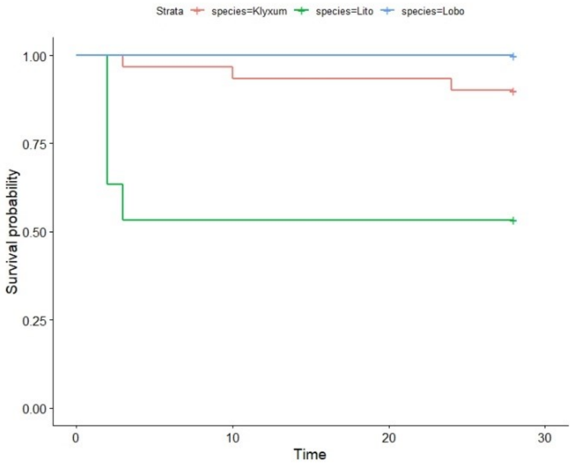

Figure 10: Survival probability for a coral fragment related to the species (R survival analysis).

More specifically, *Klyxum sp.* showed a survival rate of 93±6% for the impaling method and 87±9% for the tying method. *Lobophytum sp.* instead, achieved 100% of survival in both methods. *Litophyton sp.* in contrast obtained only 7±6% of survival with the impaling method and 100% with the tying method.
Cox regression showed a significant difference for the impaling method regarding *Litophyton sp.* which performed worse compared to the other species. We additionally found that the species is the main component regarding the survival probability in this experiment.

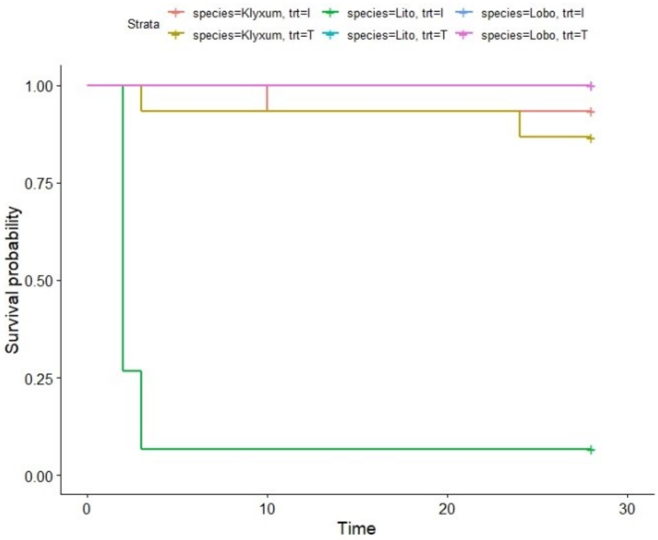

Figure 11: Survival probability produced by the different aquaculture methods applied to the three coral species used in this study (R Survival analysis).

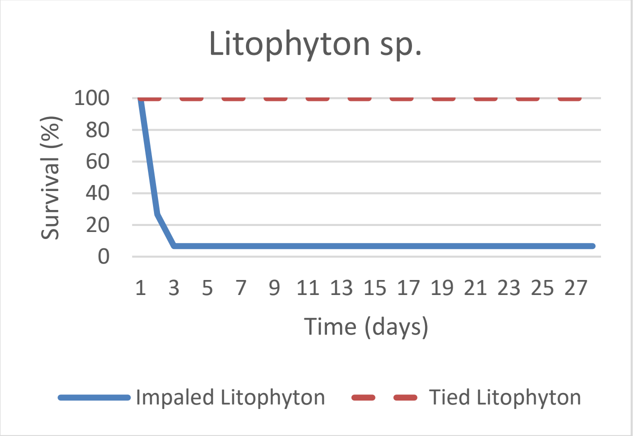

Figure 12: Survival trend during the experimental period of *Litophyton sp.* subjected to the tested aquaculture methods.

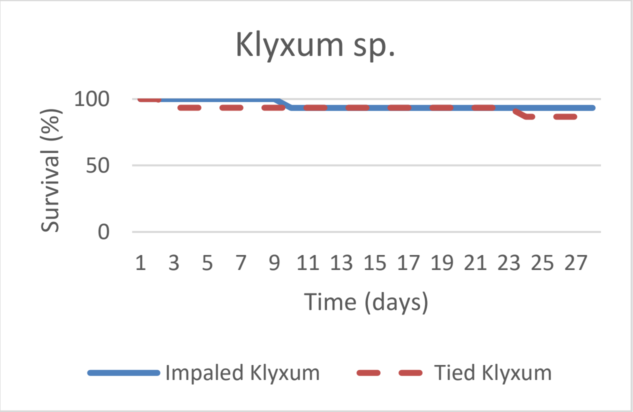

Figure 13: Survival trend during the experimental period of *Klyxum* sp. subjected to the tested aquaculture methods.

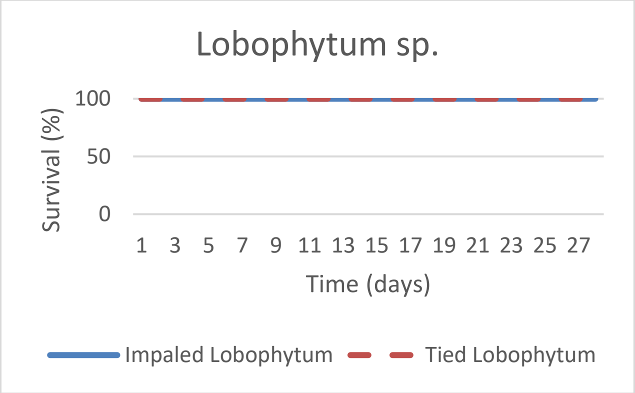

Figure 14: Survival trend during the experimental period of *Lobophytum sp.* subjected to the tested aquaculture methods.

5.3. Healing

At the beginning of the trial all the specimens were considered wounded because of the slicing of the mother colonies which was necessary for the production of the nubbins. The general healing rate during the total time length of the experiment was 78±4%.

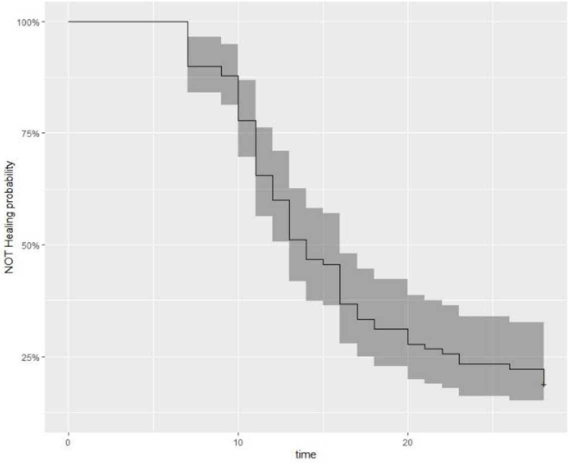

Figure 15: The survival analysis showed the NOT healing probability of the specimens. By the end of the period 22% of all the coral nubbins were not able to heal the wounded area.

The healing rate for the different methods was measured: the tying method showed the best result with 96±3% of the corals that healed by the end of the trial. The impaling method on the contrary reached 60±7% of healing rate.

Cox regression showed significant difference between the different methods. Moreover, the treatment was found as the main component affecting the healing time.

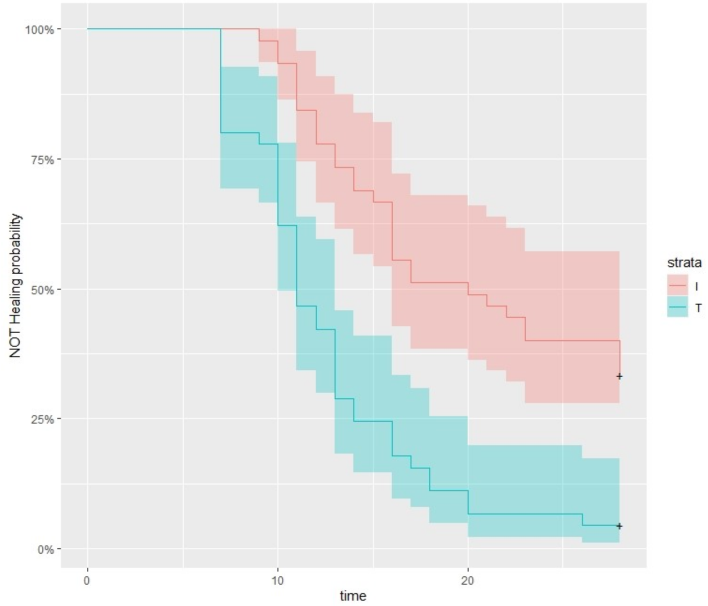

Figure 16: The survival analysis showed the NOT healing probability of the different treatments. By the end of the examined period 40% of all the impaled corals were not able to heal, in the tying method instead only 4% did not heal.

Considering both the aquaculture methods applied, 90±5% of all *Lobophytum* sp. were able to heal by the end of the experiment, the same result was achieved by *Klyxum* sp.. A different result was recorded for *Litophyton* sp. where only 53±9% healed the wounded area.

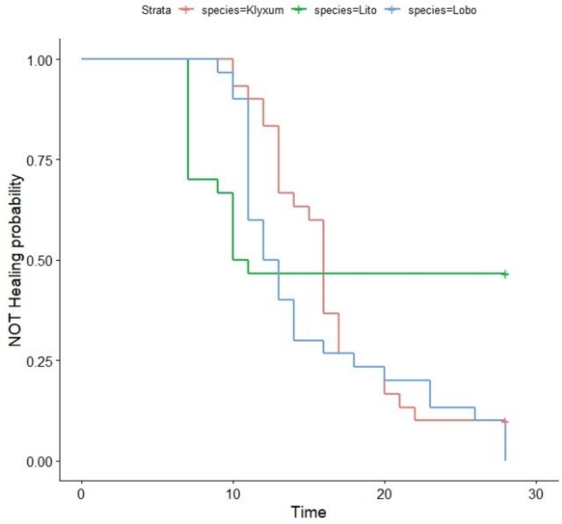

Figure 17: The survival analysis shows the NOT healing probability of the different species. By the end of the examined period 10% of all the *Klyxum* sp. , 10% of all the Lobophytum sp. and 47% of all the *Litophyton sp.* were not able to heal.

More in detail, 93±6% of all the impaled *Klyxum* sp. were able to heal by day 22. 87±9% of the tied *Klyxum* sp. completed the healing process by day 20.7±6% of the impaled *Litophyton* sp. completed the healing process by day 10. 100% of the tied *Litophyton* sp. completed the healing process by day 11. 80±10% of the impaled

Lobophytum sp. completed the healing process by day 23, the remaining 20% completed the healing between day 34 and 40 but for statistical purposes were assigned as they healed the last day of the experiment (censored). 100% of the tied *Lobophytum* sp. completed the healing process by day 26.
No statistical evidence was found to claim a difference among the different species.

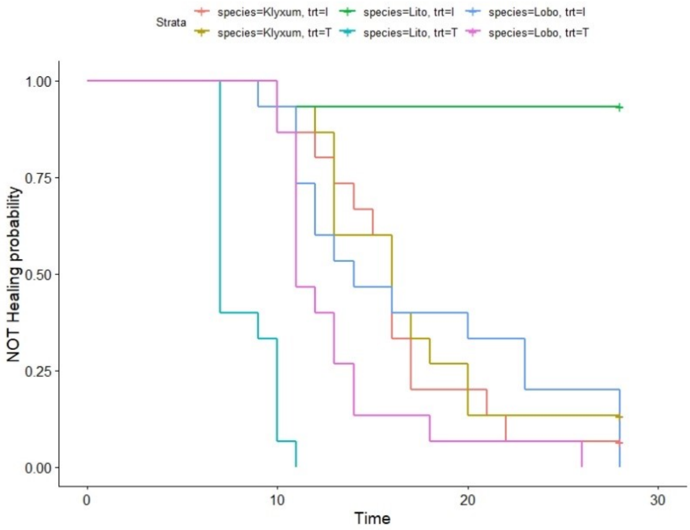

Figure 18: The survival analysis showed the NOT healing probability of the different species and treatments. By the end of the examined period the corals that were not able to heal were: 7% of the impaled *Klyxum* sp., 13% of the tied *Klyxum* sp. , 93% of all the impaled *Litophyton* sp. sp., 20% of all the impaled *Lobophytum* sp., while there is 0% probability that tied *Litophyton* sp. and *Lobophytum* sp. not heal during the 28 days period.

5.4. Adhesion

At the beginning of the experiment all the corals were considered not adhered to the ceramic support as explained in the materials and methods section. All the corals that were not considered as dead were able to adhere to the coral plug in the period taken into consideration, thus reaching an adhesion rate of 81±4%. For statistical purposes those considered as dead were assigned as adhered on day 28 (end of the test).

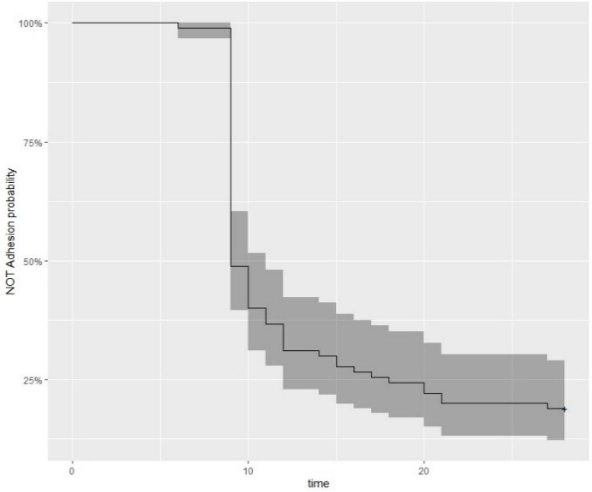

Figure 19: The survival analysis showed the NOT adhesion probability of the specimens. By the end of the period 19% of all the coral nubbins were not able to be permanently fixed on the coral plug.

The adhesion rate for the different methods was measured: the tying method had the best result with 96±3% of the corals that attached to the plug by the end of the trial. The impaling method on the contrary reached 67±7% of adhesion rate.
Cox regression showed a significant difference between the different methods regarding the time needed to the corals to adhere to the ceramic plug.

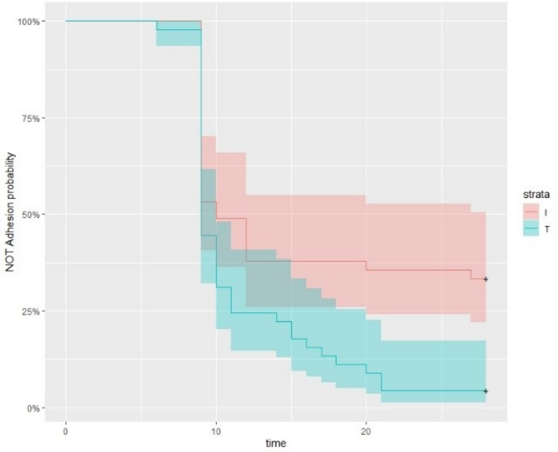

Figure 20: The survival analysis showed the NOT adhesion probability of the specimens. By the end of the period 33% of all the impaled and 4% of all the tied coral nubbins were not able to be permanently fixed on the coral plug.

Considering both the aquaculture methods applied, 100% of all *Lobophytum* sp. were able to adhere by the end of the experiment, 90±5 of *Klyxum* sp. fragments attached to the plug before day 28. A different result was recorded for *Litophyton* sp. where only 53±9% completed the adhesion.

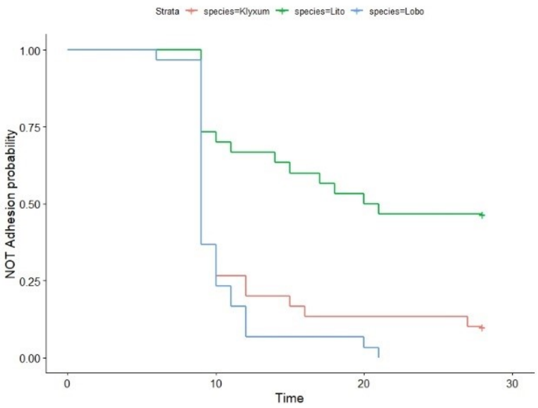

Figure 21: The survival analysis showed the NOT adhesion probability of the different species. By the end of the period 10% of all the *Klyxum* sp. , 47% of the Litophyton sp. and 0% of all the Lobophytum sp. were not able to be permanently fixed on the coral plug.

More in detail, 93±6% of all the impaled *Klyxum* sp. were able to adhere to the support by day 27. 87±9% of the tied *Klyxum* sp. completed the adhesion process by day 16. 7±6% of the impaled *Litophyton* sp. completed the adhesion process by day 9. 100% of the tied *Litophyton* sp. completed the attachment process by day 21. 100% of the impaled *Lobophytum* sp. adhered to the coral plug by day 20. 100% of the tied *Lobophytum* sp. were completely attached to the ceramic stand by day 21.

The species resulted as the main component regarding the adhesion probability in this experiment. Cox regression showed a significant difference among *Lobophytum* sp. and the other species.

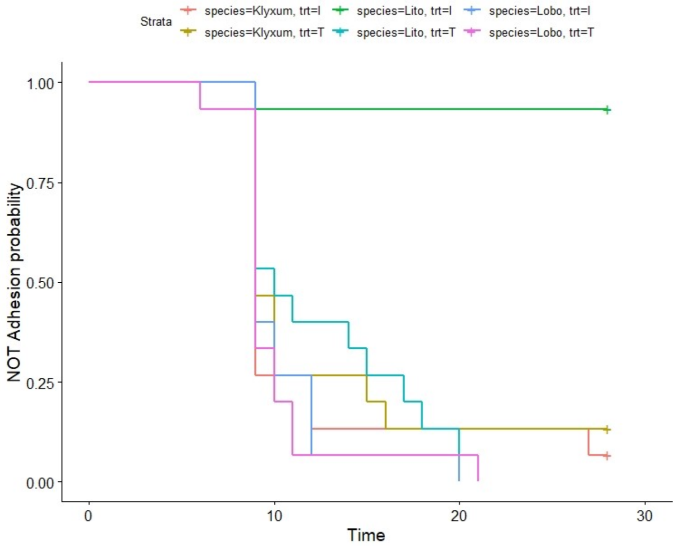

Figure 22*:* The survival analysis showed the NOT adhesion probability of the different species and treatments. By the end of the examined period the corals that were not able to be adhered to the coral plug were: 7% of the impaled *Klyxum* sp.*,* 13% of the tied *Klyxum* sp., 93% of all the impaled Litophyton sp., 0% of all the impaled or tied Lobophytum sp..

No statistically significant relation between the different measured variables was identified other than the obvious relation between time of death and time of healing or attachment.

The following table comprises the main data obtained during the period of 28 days in which two different aquaculture methods have been tested on three coral species important for the marine ornamental trade, pharmaceutical industry and conservation initiatives.

	Klyxum sp		*Litophyton* sp		*Lobophytum* sp	
	Tying	Impaling	Tying	Impaling	Tying	Impaling
Survival	86.7±8.8	93.3±6.4	100	6.7±6.4	100	100
Adhesion	10.2±0.7	10.7±1.3	12.6±1.2	9	10±0.8	10.5±0.7
Healing	15.2±0.9	15.4±0.9	8.2±0.4	10	13.1±1.1	17.3±1.8

Table 2: Rates of survival, adhesion and healing in the different species and methods. Survival: percentage of coral nubbins which survived for the whole experimental time (% ± standard error); Adhesion: time needed from a live coral to permanently attach to the coral plug (days mean ± standard error), Healing: time needed from a live coral to heal the wound produced at the beginning of the experiment (days mean ± standard error).

5.5. Polyp status

The condition of polyp contraction or the inflation of the whole baby-colony was recorded every day for all the specimens. The correlation between the state of the corals (everted polyps or inflated coral) and the species or treatment was evaluated and the main output are resumed in Table 3. Impaled *Litophyton* sp. and tied *Lobophytum* sp. have a negative trend while tied *Litophyton* sp. and impaled *Lobophytum* sp. have a positive one. *Klyxum* sp. instead has a too high p-value. This correlation can be graphically appreciated in Figure 23.

Sp+T	Corr.	p-value
Kl-I	0.183	>0.05
Kl-T	0.218	>0.05
Li-I	-4.04	<0.05
Li-T	0.604	<0.05
Lo-I	0.624	<0.05
Lo-T	-4.18	<0.05

Table 3: Correlation table.; Sp+T: species+ treatment; Kl= *Klyxum sp.*; Li= *Litophyton sp.*; Lo= *Lobophytum sp.*; I= impaling method; T= tying method; Corr.= correlation

An adjusted trend line (Figure 23) shows the mean percentage of corals with open polyps along the period taken into account for the different species and treatments. Both *Klyxum* sp. treatment after the first days with a lower value, start to rise and stay high up to the ent of the experiment. *Litophyton* sp. for the whole period doesn't change its status in both the treatments, even if a big difference is present between the two treatments. Impaled *Lobophytum* sp. has a growing trend, on the other hand the tied *Lobophytum* sp. has a decreasing pattern.

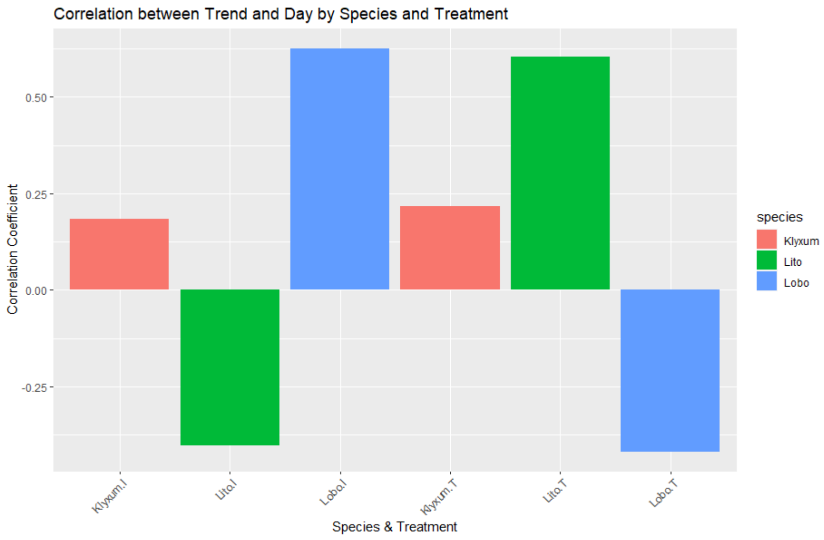

Figure 23: Correlation between number of specimens with expanded polyps and different species-treatment associations.

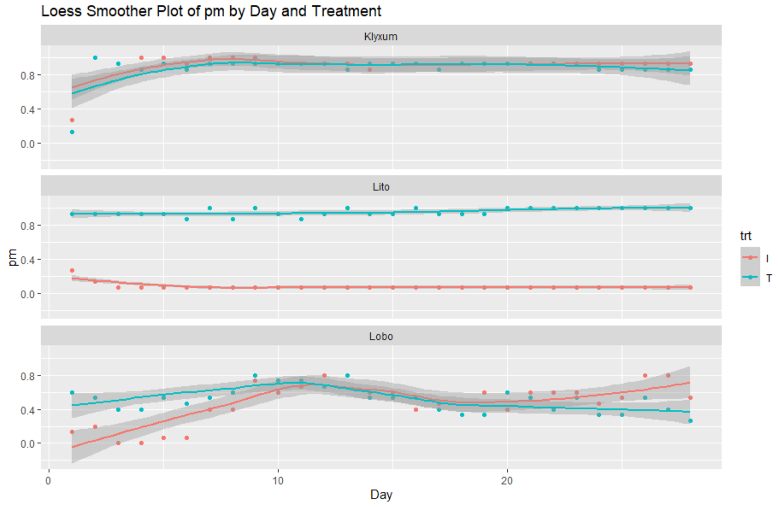

Figure 24: Loess smoother plot showing the trend of the number of polyps in the different species and treatment associations; pm=polyp mean; I=impaled; T=tied.

6. Discussion

This study tried to set the scientific bases for *ex-situ* aquaculture of the corals *Lobophytum* sp., *Litophyton* sp. and *Klyxum* sp. investigating which is the best aquaculture protocol between the impaling and the tying method.

The tying method produced a good survival rate among all the species. On the other hand the impaling method had a very low survival rate when associated with *Lithophyton* sp. and a good result with *Klyxum* sp. and *Lobophytum* sp..

The time needed to fix one coral on the plug was shorter in the impaling method even if it requires two different operations (gluing the toothpick and impaling the coral). However, if a square plug and a skilled operator are used the time needed for the tying method can easily be reduced as well.

No water quality issues were recorded, and the mean values obtained are in concert with the normal activity of this recirculating aquaculture system.

As suggested by Barton et al. (2017), the addition of grazers was effective in controlling filamentous algae and reducing coral-algae competition.

Contrary to what was reported by Larkin et al. (2023), during this experiment, although no piercings were performed in the tying method to sew the cuttings, a large number of corals characterized as dead due to displacement from the plug was not detected. In any case, this number could probably be further reduced if square-shaped plugs were used, which would allow lower initial loosing of the fishing line. It was noted in some subjects that during growth the fishing line when reached the maximum tension began to be encased by the coral but was pulled out at the end of the test without any apparent damage to the cutting. In other samples the fishing line became loose, but the coral nubbins had already completed the adhesion at the time, thus not being a problem.

Chaitanawisuti and Kritsanapuntu (2019) considered permanently fixed over the substrate the fragments that did not fall from the substrate when flipped upside down, in our case this was not possible as in the tying method the coral was firmly attached to the substrate. Whereas for impalement, more specifically for *Lobophytum* sp. and as early as day 1 the coral remained in place without slipping off the toothpick. This situation could be explained by the fact that the impalement was assisted by the use of a needle that produced a cleaner and more precise hole and, the following interference fit between the toothpick and the coral was sufficient to keep it in place but without being able to consider it attached to the base of the plug.

6.1. Survival

The survival rate was high in all the different associations other than the impaled *Litophyton* sp. which performed poorly. Our results are comparable to those obtained by Chaitanawisuti and Kritsanapuntu (2019) even if in different species and in smaller scale (this study).

Litophyton sp. and the other dead corals were considered dead following Chaitanawisuti and Kritsanapuntu (2019) however, in reality, many of them started to wander around the aquarium following the water flow, eventually attaching themselves to the coral sand present on the bottom without actually dying. As reported by Barton et al. (2017), the situation where a significant loss of fragments due to detachment from the substrate, with detachment rates surpassing direct fragment mortality rates is possible in some cases.

All the corals used in this test are soft corals and in fact in the description of the species in this thesis they are described as flashy, in particular Forskål (1775) speaking about *Litophyton* sp. states that these corals have fleshy branches and that they stay erected in water (indirectly suggesting their inability to do the same outside of it). In addition, during the preparation of the cuttings a huge loss of internal fluid was noted during cutting from the mother colony. As reported by Borneman and Lowrie (2001) this feature can be a problem also in another fragging method, also Larkin et al. (2023) reports that nubbins frequently become loose at the piercing location. Probably these characteristics made it unsuitable for impalement where this "inconsistency" did not allow it to remain in place. The other two species tested, on the other hand, showed little fluid loss during cuttings and much greater texture to the touch than *Litophyton* sp., Von Marenzeller (1886) actually describes the surface of the polyp-bearing part of *Lobophytum* sp. as tough and resistant.

6.2. Adhesion

We recorded a great adhesion rate on day 9 between all the species and the treatment considered other than for the impaled *Litophyton* sp., after this initial spike a slow conforming trend up to almost the end of the test was noted. We saw that the main part of the corals that survived were able to complete the adhesion by around day 20.

In *Klyxum* sp. the first adhered fragment was seen on day 9 and the last on day 27.

In *Lobophytum* sp. the first adhered fragment was seen on day 6 and the last on day 21.

In *Litophyton* sp. the first adhered fragment was seen on day 9 and the last on day 21.

We found a statistically significant difference between the two aquaculture methods applied regarding the adhesion time. Deeper statistical analysis showed that this difference was given by the difference between species, especially the impaled *Litophyton* sp. which dies in high number, failing in this way the adhesion to the ceramic stand. This is also confirmed by the main component analysis which indicates the species as more important than the treatment.

Nadir et al. (2023) working on single polyps of a different species of soft coral, reports an adhesion time of 2-3 days, while Chaitanawisuti and Kritsanapuntu (2019) reported the complete adhesion between day 9 and 12 which is similar to the two weeks reported by Varghese et al. (2012) in *Lobophytum* sp.. In our experiment, however, the time required for adhesion was longer, this finding could probably be explained by the difference between the species and the experimental system as stated by Rocha et al. (2015).

6.3. Healing

In *Klyxum* sp. the first healing was observed on day 10 in both treatments, and the last one on day 22. In *Lobophytum* sp. the first healing was observed on day 9. The last one regarding the tying method was observed on day 26. In *Litophyton* sp. the first healing was observed on day 7 and the last one on day 11.

Chaitanawisuti and Kritsanapuntu (2019) reported that in their experiment the healing time was from 3 to 10 days in different species in all the tested methods. Varghese et al. (2012) working with *Lobophytum* sp. saw new polyps on the cut portion by day 25. In our experiment, the same species behaved similarly but not all the samples were able to complete the healing process during the experimented time. Actually, the corals that didn't healed on time were in the impaling method which generally speaking performed worse than the tying method regarding the time needed to heal the wounded area. Indeed, we found a statistically significant difference between the two aquaculture methods applied. Moreover, the analysis of the main component affecting the healing time showed that the treatment is more important than the species.

The time needed by *Litophyton* sp. to heal in our experiment agrees with the findings of Tentori et al. (2004) since they state that after an injury due to cutting the highest cell growth rate in this species was recorded between days 7 and 49. Moreover, during the healing process it was possible to see the RLPs.

Probably the best result scored by the tying method is because it is less invasive than the impaling method where, in addition to the stress of being cut, the coral is also permanently impaled. For future studies is possible to test different impaling materials.

Since no valuable relation was identified between adhesion time, healing time and survival, they can be considered independent.

6.4. Polyps eversion

As mentioned in the introductory chapter, the contraction or the expansion of corals have been mentioned by several authors as a indicator of wellbeing or stress in these animals. Unfortunately, there are no scientific protocols to evaluate this behaviour and a lot of different factors can trigger the contraction in these animals, making it even more difficult to decipher.

Usually, a strong correlation is obtained when the correlation value is greater than ± 0,8. The values obtained showed only a weak correlation. In particular, this relation cannot be seen in *Klyxum* sp.. The significance obtained by *Litophyton* sp. is probably due to the very low survival rate in the impaling method. It is quite interesting the difference between the treatments on *Lobophytum* sp. since they have an opposite trend. If the behaviour of the polyps in this species is linked to its wellbeing, it is possible that the impaling method injured more the animal at the beginning of the experiment and thus during time it recovered. On the other hand, the tying method created less stress at the beginning but during the growth the fishing line started being tighter, up to the point of being encased into the coral tissue.

Probably as suggested by Larkin et al. (2023), the daily manipulation required to check the adhesion may have affected the amount of stress to which the corals were subjected. Similarly, the presence of the algae control grazers may have affected whether or not the polyps opened. In a possible production context, the last factor cannot be removed however the daily manipulation that was necessary for this study can, indicating a possible stress reduction in a productive context.

7. Conclusions

Coral reefs harbour great biodiversity and produce direct and indirect revenues for people leaving in the same area. Often these ecosystems are endangered by anthropogenic factors such as overfishing and climate change that can cause their collapse to a simpler environment with loss of biodiversity. Corals are an important source of income for tropical Indo-Pacific populations such as Indonesia and Philippines. These animals are mainly important for tourism, pharmaceutical, and ornamental sectors. Coral farming mirroring the food-producing species sector seems to be the best solution to cover market demands and possible restocking campaigns. Unfortunately, the know-how for ornamental aquaculture resides in grey literature and among specialized companies that hardly have the will to disseminate their culturing protocols. In fact, there have been just a small number of pioneering studies to figure out the best protocol for the reproduction of these animals. Unlike the purpose of restocking, for the ornamental and pharmaceutical markets asexual reproduction is often preferred as it is easier and capable of creating clones of the mother colony with the most important traits.

The purpose of this study was to determine which, among some of the most widely used soft coral fragmenting techniques was the best in terms of survival, healing time, and time to adhesion to the ceramic substrate. Data were also collected on the contraction status of polyps or the entire colony as a possible proxy for assessing animal welfare. Three different species and two aquaculture methods were tested during a four-week period.

Statistically significant values indicated a difference in survival of impaled *Litophyton* sp. compared with all other possible associations. Regarding healing time, a significant difference between the two different fragmentation methods has been achieved. About the adhesion time to the coral plug a statistically significant difference was obtained, the impaled *Litophyton* sp. proved to be different from the other species-treatment associations.

The overall performance of the impaled *Litophyton* sp. was worse than the other coral-treatment associations, its death compromised the opportunity to adhere and heal. Probably its soft texture and its high amount of water content did not allow it to achieve a good result with the impaling method. In the healing test some impaled specimens were not able to heal on time even if they survived, suggesting as confirmed by statistics the best result of the tying method.

We suggest for future propagation attempts of these three species the tying method since it performed overall better than the impaling one.

With this study we tried to transfer some of the know-how present in the coral farm where this experiment took place to the scientific literature. Ornamental aquaculture is a poorly exploited research field and harbours great opportunities for the researchers which are interested in this topic, especially considering the importance this research may have in the future in light of climate change and its consequences on coral reefs.

8. References

Alderslade P., (2000). Four new genera of soft corals (Coelenterata: Octocorallia), with notes on the classification of some established taxa. Edited by Van Ofwegen L. P., 237-249.

Baran C. C., Baria-Rodriguez M. V., (2021). Sexual reproduction in the soft coral *Lobophytum* schoedei in Bolinao-Anda Reef Complex, Pangasinan, northwestern Philippines. Invertebrate Biology, 140: 1-10.

Barton J. A., Willis B. L., Hutson K. S., (2017). Coral propagation: a review of techniques for ornamental trade and reef restoration. Reviews in Aquaculture, 9: 238-256.

Borneman E. H., Lowrie J., (2001). Advances in captive husbandry and propagation: an easily utilized reef replenishment means from the private sector?. Bulletin of Marine Science, 69(2): 897-913.

Brown B. E., Le Tissier M. D. A., Dunne R. P., (1994). Tissue retraction in the scleractinian coral *Coeloseris mayeri*, its effect upon coral pigmentation, and preliminary implications for heat balance. Marine Ecology Progress Series, 105: 209-218.

Burkepile D. E., Hay M. E., (2008). Coral Reefs. In Encyclopedia of Ecology (Second Edition), Edited by Fath B., Elsevier. Vol. 2: 426-438

Chaitanawisuti N., Kritsanapuntu S., (2019). Asexual propagation of the soft corals *Sinularia* sp., *Cladiella* sp. and *Sarcophyton* sp. (Octocorallia: Alcyonacea) using different methods of attachment in a recirculating seawater system. Aquaculture Research, 50: 2047-2053.

Chamberland V. F., (2018). Environmental Drivers of Recruitment Success in Caribbean Corals: Applications to Aid the Recovery of Threatened Coral Populations.

Chang TC., Mayfield A. B., Fan TY., (2020). Culture systems influence the physiological performance of the soft coral *Sarcophyton glaucum*. Scientific reports, 10: 20200.

Coll J. C., Price I. R., König G. M., Bowden B. F., (1987). Algal overgrowth of alcyonacean soft corals. Marine Biology, 96: 129-135.

Costa A. P. L., Silva D. A. M., Rodrigues A. C. M., Marques C. R., Soares A. M. V. M., Rocha R. J. M., (2021). Aquaculture Reports, 19: 10063.

Dautova T. N., Kiyashko S. I., (2017). Feeding mode diversity in Octocorallia can reflect their evolutionary determined taxa and morphology diversity. In

Life-Supporting Asia-Pacific Marine Ecosystems, Biodiversity and Their Functioning. Edited by Dautova T. N., Sun X., Sun S., Adrianov A. V., Science Press Beijing. 51-57

Dee L. E., Horii S. S., Thornhill D. J., (2014). Conservation and management of ornamental coral reef wildlife: Success, shortcomings, and future directions. Biological Conservation, 169: 225-237.

DES, 2018. Monitoring and Sampling Manual: Environmental Protection (Water) Policy. Brisbane: Department of Environment and Science Government. Queensland Government, 1-4.

Ellithey M. S., Ahmed H. H., (2018). Bioactive marine-derived compounds as potential anticancer candidates. Asian Journal of Pharmaceutical and Clinical Research, 11: 464-466.

Forskål P., (1755). Descriptiones Animalium, Avium, Amphibiorum, Piscium, Insectorum, Vermium; quae in Itinere Orientali Observavit Petrus Forskål. Post Mortem Auctoris editit Carsten Niebuhr. Adjuncta est materia Medica Kahirina. Mölleri, Hafniae, 19 + XXXIV + 164.

Goh N. K. C., Ng P. K. L., Chou L. M., (1999). Notes on the shallow water gorgonian-associated fauna on coral reefs in Singapore. Bulletin of Marine Science, 65(1): 259-282.

Harrison P. L., Wallace C. C., (1990). Reproduction, dispersal and recruitment of scleractinian corals Ecosystems of the world. Coral Reefs, 7: 133-196.

Hartle-Mougiou K., D'Angelo C., Smith E. G., Burt J., West P., Wiedenmann J., (2012). Diversity of zooxanthellae from corals and sea anemones after long-term aquarium culture. Journal of the Marine Biological Association of the United Kingdom, 92(4): 687-691.

Hoang X. B., Thai M. Q., Minh-Thu P., Pham X. K., Tung N. N., Dao H. V., (2022). Antipredator Defenses in Soft Corals of the Genus *Sarcophyton* (Octocorallia; Alcyoniidae) from Coastal Waters of Central Vietnam. Russian Journal of Marine Biology, 58 (2): 122-128.

Hobbs R. J., Arico S., Aronson J., Baron J. S., Bridgewater P., Cramer V. A., Epstein P. R., Ewel J. J., Klink C. A., Lugo A. E., Norton D., Ojima D., Richardson D. M., Sanderson E. W., Valladares F., Vilà M., Zamora R., Zobel M., (2006). Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography, 15: 1-7.

Jahaajeeah D., Ranghoo-Sanmukhiya M., Schäfer G., (2023). Metabolic Profiling, Antiviral Activity and the Microbiome of Some Mauritian Soft Corals. Marine Drugs, 21: 574.

Kim S., Wild C., Tilstra A., (2022). Effective asexual reproduction of a widespread soft coral: comparative assessment of four different fragmentation methods. PeerJ, 10: 1-15.

Larkin M. F., Davis T. R., Harasti D., Benkendorff K., Smith S. D. A., (2023). Substantial advancement in aquaria rearing methods to assist recovery of an Endangered soft coral. Aquatic Conservation, 33: 1-14.

Leal M. C., Ferrier-Pagès C., Petersen D., Osinga R., (2017). Corals. In Marine Ornamental Species Aquaculture. Edited by Calado R., Olivotto I., Planas Oliver M., Holt G. J., John Wiley & Sons Ltd. 406-429.

Leal M. C., Ferrier-Pagès C., Peterson D., Osinga R., (2016). Coral aquaculture: applying scientific knowledge to ex situ production. Reviews in Aquaculture, 8: 136-153.

Lee NL., Su JH., (2011). Tetrahydrofuran Cembranoids from the Cultured Soft Coral *Lobophytum crassum.* Marine Drugs, 9: 2526-2536.

Liberman R., Benayahu Y., Huchon D., (2022). Octocorals in the Gulf of Aqaba exhibit high photosymbiont fidelity. Frontiers in Microbiology, 13: 1-12.

Mardones M. L., Lambert K., Wiedenmann J., Davies T. W., Levy O., D'Angelo C., (2023). Artificial light at night (ALAN) disrupts behavioural patterns of reef corals. Marine Pollution Bulletin, 194: 1-8.

McFadden C. S., Van Ofwegen L. P., Quattrini A. M., (2022). Revisionary systematics of Octocorallia (Cnidaria: Anthozoa) guided by phylogenomics. Bulletin of the Society of Systematics Biologists, 1(3): 8735, 1-79.

Nadir E., Lotan T., Benayahu Y., (2023). *Xenia umbellata* (Octocorallia): A novel model organism for studying octocoral regeneration ability. Frontiers in Marine Science, 10: 2-9.

Page C. A., Lasker H. R., (2012). Effects of tissue loss, age and size on fecundity in the octocoral *Pseudopterogorgia elisabethae*. Journal of Experimental Marine Biology and Ecology, 434-435: 47-52.

Peng BR., Lu MC., El-Shazly M., Wu SL., Lai KH., Su JH., (2018). Aquaculture Soft Coral *Lobophytum crassum* as a Producer of Anti-Proliferative Cembranoids. Marine Drugs, 16: 1-12.

Previati M., Scinto A., Cerrano C., Osinga R., (2010). Oxygen consumption in Mediterranean octocorals under different temperatures. Journal Of Experimental Marine Biology and Ecology, 390: 39-48.

Putra A. W., Priawandiputra W., Litayy M., Atmowidi T., (2022). Comparison of shallow water soft coral (Octocorallia) diversity and distribution among three islands in Makassar Strait, Indonesia. Biodiversitas, 23(11): 5951-5961.

Rahman M. A., Oomori T., (2008). Identification and Function of New Proteins in Calcified endoskeleton: a New Insight in the Calcification Mechanism of Soft Corals. Oceans, 1-7.

Rocha R. J. M., Serôdio J., Leal M. C., Cartaxana P., Calado R., (2013). Effect of light intensity on post-fragmentation photobiological performance on the soft coral *Sinularia flexibilis*. Aquaculture, 338-391: 24-29.

Rocha R. J., Bontas B., Cartaxana P., Leal M. C., Ferreira J. M., Rosa R., Serôdio J., Calado R., (2015). Development of a Standardized Modular System for Experimental Coral Culture. Journal of the World Aquaculture Society, 46(3): 236-248.

Sebens K. P., DeRiemer K., (1977). Diel Cycles of Expansion and Contraction in Coral Rccf Anthozoans. Marine Biology, 43: 247-256.

Sheu JH., Peng BR., Fang LS., Hwang TL., Su JH., Wu YC., Sung PJ., (2019). Hydroperoxyditerpenoids from Octocorals. Israel Journal of Chemistry, 59: 403-413.

Shikina S., Chiu YL., Zhang Y., Yao YC., Liu TY., Tsai PH., Gaudin-Zatylny C., Chang CF., (2020). Involvement of GLWamide neuropeptides in polyp contraction of the adult stony coral *Euphyllia ancora*. Scientific reports, 10: 9427.

Sorokin Y. I., (1991) Biomass, Metabolic Rates and Feeding of Some Common Reef Zoantharians and Octocorals. Australian Journal of Marine and Freshwater Research, 42: 729-741.

Tentori E., Allemand D., Shepherd R., (2004). Cell growth and calcification result from uncoupled physiological processes in the soft coral *Litophyton arboreum*. Marine Ecology Progress Series, 276: 85-92.

Todinanahary G. G. B., Lavitra T., Andrifanilo H. H., Puccini N., Grosjean P., Eeckhaut I., (2017). Community-based coral aquaculture in Madagascar: A profitable economic system for a simple rearing technique? Aquaculture, 467: 225-234.

Van Ofwegen L. P., (2016). The genus *Litophyton* Forskål, 1775 (Octocorallia, Alcyonacea, Nephtheidae) in the Red Sea and the western Indian Ocean. ZooKeys, 567: 1-128.

Vargas-Àngel B., Riegl B., Gilliam D. S., Dodge R. E., (2006). An Experimental Histopathological Rating Scale of Sedimentation Stress in the Caribbean Coral *Montastraea cavernosa.* Nova Southeastern University, 49: 1168-1173.

Varghese M., Joshi K. K., Ignatius B., Kavungal V., (2012). Culture of the soft coral, *Lobophytum pauciflorum* (Family: Alcyoniidae) under captive conditions at Kochi, India. Marine Fisheries Information Service T&E Ser, 214: 11-12.

Von Marenzeller E., (1886). Ueber die *Sarcophytum* bennanten Alcyoniiden. *Zoologische Jahrbucher*. 1: 341-368, plate 9.

Wilson L. J., Fulton C. J., McC Hogg A., Joyce K. E., Radford B. T. M., Fraser C. I., (2016). Climate-driven changes to ocean circulation and their inferred impacts on marine dispersal patterns. Global Ecology and Biogeography, 25: 923-939.

Work T. M., Aeby G. S., Lasne G., Tribollet A., (2014). Gross and microscopic pathology of hard and soft corals in New Caledonia. Journal of Invertebrate Pathology, 120: 50-58.

Zaragoza W. J., Krediet C. J., Meyer J. L., Canas G., Ritchie K. B., Teplitsky M., (2014). Outcomes of Infections of Sea Anemone *Aiptasia pallida* with *Vibrio* spp. Pathogenic to Corals. Microbial Ecology, 68: 388-396.