UNIVERSITY OF PORTSMOUTH

SCHOOL OF BIOLOGICAL SCIENCE

PhD Marine Biology

Effects of macroalgae, with emphasis on *Sargassum* spp., on coral reef recruitment processes in Martinique (French West Indies).

By Marie Thabard

'The thesis is submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy of the University of Portsmouth.'

Submitted in 2012

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ABSTRACT

Many coral reef ecosystems have undergone profound ecological changes over the past decades leading sometimes to a shift from coral to macroalgal-dominated areas. In Martinique (Caribbean region), the proliferation of macroalgae is an important phenomenon. Coral reef resilience, involving reef building species recruitment, might be modified by macroalgal presence. This work aimed at understanding reef recruitment processes in areas dominated either by macroalgae, coral or intermediate, based on scuba diving observations, manipulative experiments and laboratory studies. Particular attention was given to the physical and chemical effects of *Sargassum* (one of the most represented species: 100-200 $g.m^{-2}$ (wet weight) in algal beds) on benthic invertebrates' larvae recruitment. Further experiments focused on the effects of surface molecules and of the waterborne cues produced by *Sargassum polyceratium* on the development of marine invertebrates' embryos.

This study demonstrated that juvenile coral diversity and density vary between the considered habitat types (i.e: dominated by algae, coral or intermediate with numerous sea urchins). It was low in *algal* areas (0.9-1.4 $recruit.m^{-2}$) as compared to *coral* ones (7-8 $recruit.m^{-2}$) and intermediate in urchin zones (2-3.2 $recruit.m^{-2}$). Moreover, species recruiting differed according to their reproductive mode. Brooders recruited more in coral areas, which suggested that they settled in the vicinity of their parent colonies. Settlement and recruitment experiments demonstrated the barrier effect of *Sargassum* species on settlement but no allelochemical impacts could be identified *in situ*. However, the laboratory based experiments demonstrated that *S. polyceratium* surface molecules were active against the early stages of development of *Arenicola brasiliensis* (annelid), *Codakia orbicularis* (bivalvia) and *Diadema antillarum* (sea urchin, a reef key stone species) (LC_{50} between 25 and 51 $\mu g.mL^{-1}$).

These results give insight into the coral recruitment capacities in several habitats, which is of major importance for reef managers.

CONTENT LIST

DECLARATION

"Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award."

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ABBREVIATION

AF: Antifouling.
APA: Alkaline phosphatase activity
C: Coral
CCA: Crustose coralline algae
CNP: Carbon nitrogen phosphorus
DEAL: Direction de l'aménagement et du logement
DIN: Dissolved inorganic nitrogen
DMSO: Dimethyl sulfoxide
DOP: Dissolved organic phosphorus
HCPC: hierarchical clustering on principal component
FWI: French West Indies
FSW: Filtered SeaWater
G: Gorgonian
GCRMN: Global coral reef monitoring network
GCMS: Gas chromatography-mass spectrometry
IFRECOR: Initiative française pour les récifs Coralliens
LIT: Line intercept transect
LPT: Line point transect
Ma: Macroalgae
MIC: minimum inhibitory concentration
mOD: Mili optical density
MNP: Marine Natural Products
MRT: multiple range test
N: Nitrogen
NMR: Nuclear magnetic resonance
OD: Optical density
P: Phosphorus
PCA: Principal component analysis
PCB: Polychlorobiphenyls
PERMANOVA: Permutation based non-parametric analysis of variance.
S: Sponge
SEM: Scanning electron microscopy
SPR: Substratum (sand, pavement rubble)
SRP: Soluble reactive phosphorus
SW: Seawater
TBT: tributylin
Tu: Turf
UV: Ultra violet
WFD: Water framework directive
Z: zooanthid

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DISSEMINATION

Publication

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M. Thabard, C. Hellio, V. Stiger, A. H. Engelen, L. Mattio, R. L. Fletcher, J.P. Maréchal. *Sargassum* proliferation success and its impact in tropical and subtropical regions: a review. Submitted journal of phycology.

Presentation of results at congress

M. Thabard, O. Gros, C. Hellio, J.P. Maréchal (2009) "Screening of biological activities of *Sargassum polyceratium* (Phaeophyceae, Fucaceae) extracts.". Marine Biotechnologies in the marine value chain. Concarneau (France).

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M. Thabard, C. Hellio, O Gros, L. Thompson, S. Debray, A. Dauchy, JP Marechal (2011) "Sargassum polyceratium chemical impact on coral reef invertebrate recruitment in Martinique (FWI): effects of surface molecules and conditioned waters." Trends in natural products research a PSE young scientists'meeting, Kolymvari (Crete).

Chapter I: General introduction: Coral reef health status, phase shift phenomenon and macroalgal reef colonisation.

I.1. Coral reef: an overview

I.1.1. Ecological description and world health status

Coral reefs support some of the most diverse and productive communities in the marine environment. In Martinique, 45 coral, 300 fish, 35 gorgonian, 370 mollusc and 70 sponge species have been reported on coral reefs and seagrass beds from 0 to 40 m depth (Legrand *et al.,* 2008). Coral reef economical value has been estimated annually at 245 M € in Martinique (Failler *et al.,* 2010). They provide important economic resources (food, tourism) to millions of people and their loss would be tragic for the economy of many countries (Cesar *et al.,* 2003). They are built by a variety of species that precipitate either calcium carbonate (such as coral and various calcareous algae including the coralline algae) or silicate (such as some marine sponges). Coral species are the main reef builders (with crustose coralline algae (CCA)) and live in symbiosis with microalgae called zooxanthellae (*Symbiodinium* spp.). The majority of coral reefs are found within a strip of 60˚ around the Equator (Figure 1). Their development is usually restricted to environments that fulfil several physical and chemical conditions, such as low nutrient concentrations, clear water, and narrow ranges of temperature. Slight modifications of environmental parameters (natural or anthropogenic) can induce major disruptions of this ecosystem and cause the loss of important families and key species thus influencing the reef function, resilience and stability (Bellwood and Hughes, 2001).

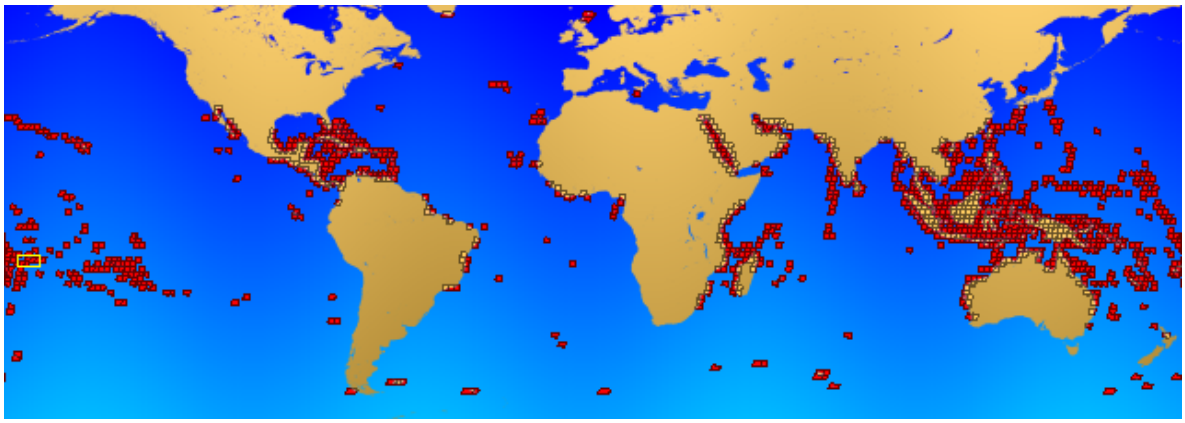


Figure 1: Distribution of coral reefs around the world (http://oceancolor.gsfc.nasa.gov/cgi/landsat.pl).

The development of macroalgae, as well as their proliferation is often limited on coral reefs exhibiting high grazing conditions (urchins and fishes) and low nutrient concentrations (Lapointe, 1997).

However, coral reefs have undergone severe changes over the past 30 years, which have sometimes lead to a "phase shift" phenomenon from coral to algal dominated reefs (Knowlton, 1992, McClanahan *et al.,* 1999). This coral loss was regional dependent (Figure 2). As an example, coral cover decreased from 52% to 3% while algal cover increased from 4 to 92% between 1977 and 1993 along the North coast of Jamaica (Hughes, 1994). Wilkinson (2008) estimates that 35% of coral reefs may be lost in the next 40 years and Caribbean reefs (Figure 2) are among the most degraded in the world (McManus and Polsenberg, 2004).

Figure 2: Threatened reefs in the Caribbean (Reefs at Risk revisited in the Atlantic/Caribbean; World Resources Institute, 2011).

I.1.2. Causes responsible for coral reef degradation

Both acute natural disturbances and chronic anthropogenic factors were demonstrated to play a role in reef degradation (Aronson *et al.,* 2003, Hughes *et al.,* 2003, McManus and Polsenberg, 2004) (Figure 3). Over the last decades, the development of tourism, the rise in coastal populations and human activities, have lead to an increase in the anthropogenic impacts on reefs and the decline of this ecosystem.

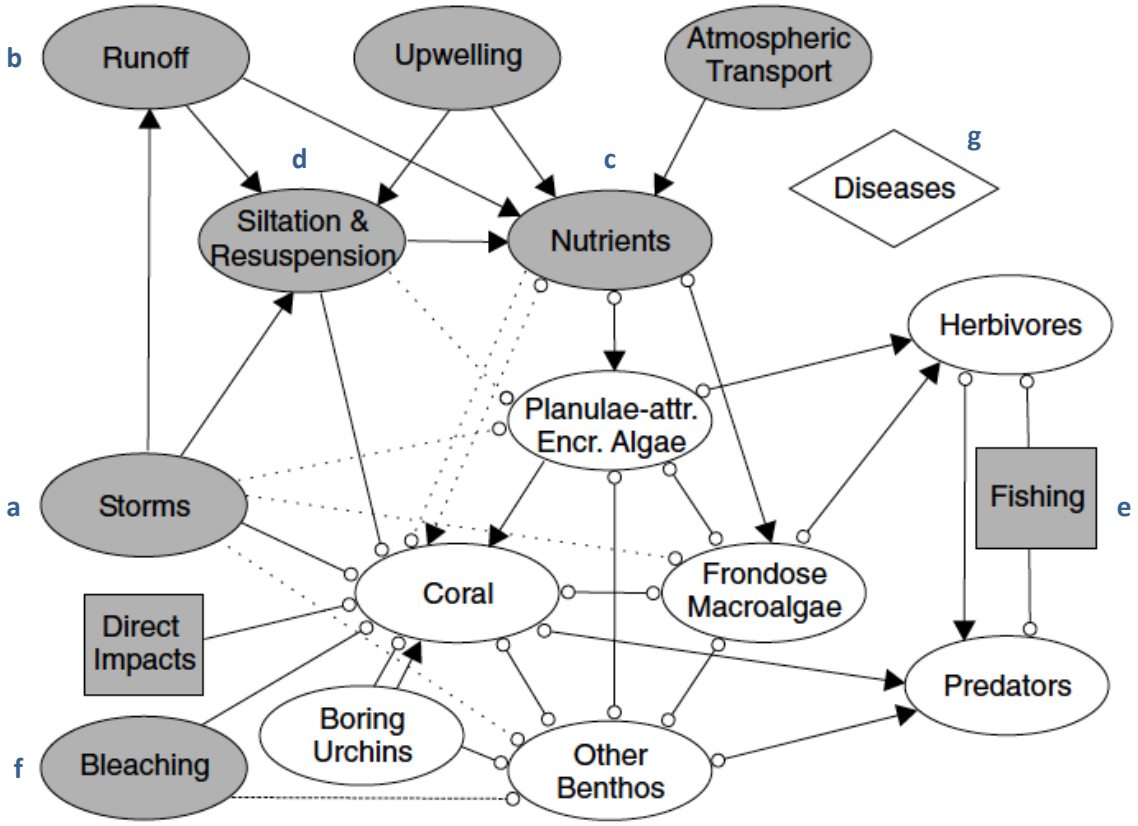


Figure 3: Factors involved in the coral–algal phase shift. Exogenous factors are in shaded enclosure. Specific anthropogenic effects are in rectangles. Disease affects all the living components, and the links have been omitted for simplicity. Arrows represent a gain to the component they touch, circles represent a loss. Dashed lines represent weak links or those with impacts under extreme conditions (from McManus and Polsenberg, 2004).

Hurricanes and storms, due to heavy rains, strong waves, increased turbidity, cause physical damage to scleractinian species thus creating free spaces allowing for the development of fast colonisers such as turf algae, macroalgae (Hughes, 1994, Littler and Littler, 1999) (Figure 3a). However, in some cases, storms have a positive impact on coral reefs by removing algal species temporarily (Lapointe, 1997, Lapointe *et al.,* 2006). In September 2004, Hurricanes Frances and Jeanne removed *Caulerpa brachypus* (Chlorophyceae) from some reefs of Florida (Lapointe, *et al.,* 2006). The effects of

hurricanes on reefs depend on their intensity, frequency, their geographical location as well as environmental anomalies (Gardner *et al.,* 2005). Besides, other catastrophic events, such as volcanic eruption, floods and earthquakes can degrade coral reefs. Anthropogenic influences combined with natural factors significantly compromise coral reef survival and recovery (Nystrom *et al.,* 2000).

I.1.2.1 Anthropogenic disturbances

Numerous anthropogenic disturbances, such as terrestrial run-offs, over fishing, diseases due to bad water treatments (waste-water and run-off waters), shipping and tourism, global warming can be responsible for coral reef degradation:

- Terrestrial run-offs (Figure 3b): industry, agriculture and urbanism are responsible for the production of nutrients (phosphate and nitrate) pollutants and contaminants. The use of nitrogen fertilizer has increased from 10 $Tg.year^{-1}$ to 80 $Tg.year^{-1}$ (1 Tg = 10^{12} g) between 1960 and 1990 (Matson *et al.,* 1997). Despite the improvement of water treatment systems and changes in policies some nutrients, such as nitrates and phosphates, are discharged in the coastal environment directly through pipes or through rivers. The discharge of nutrients into coastal waters and the expansion of human populations are responsible for changes in the marine coastal water quality and are a major cause of eutrophication (Lapointe, 1997). This phenomenon can have numerous direct and indirect effects on coral reef communities (Figure 3c) and will often lead to increases in algal turf, fleshy macroalgae and/or coralline algae (Lapointe, 1997, Lapointe *et al.,* 2005a, Lapointe *et al.,* 2005b, McClanahan *et al.,* 2005, Littler *et al.,* 2006a). Studies conducted in Glovers Reef, Belize, demonstrated a complexe interaction between herbivory, nutrients and organic matter on communities; inorganic nutrient increased the growth of turf forming species while organic matter reduced the abundance of small herbivores and increased (when added alone) the number of subdominant brown frondose algae (McClanahan *et al.,* 2005)**.**

In addition to nutrients, toxic compounds (heavy metals, Polychlorobiphenyls (PCB), etc.) are present in sewage, agricultural and industrial run-offs. Contamination by copper, zinc and some hydrocarbons was demonstrated to have impacts on the fertilization rate, fecundity and growth of both corals and zooxanthellae (Goh and Chou, 1997, Negri and Heyward, 2000, Reichelt-Brushett and Harrison, 2000, Reichelt-Brushett and Harrison, 2005). In addition, terrestrial run-off induces discharge of particles and sediments into the

seawater (Figure 3d). Land clearing for construction and agriculture is continuously affecting coastal ecosystem functioning (Fabricius, 2005). Deforestation in numerous tropical islands is responsible for water filtration and run-off modifications, which inevitably affect the coastal environment. Sedimentation can alter physical, chemical and biological processes on reefs. Coral species are negatively affected by increased levels of sediments (Rogers, 1990, Fabricius, 2005). The increase of sediments, which induces a decrease in the light penetration, was showed to alter the morphology of some coral species (Crabbe and Smith, 2005). Depending on the study, macroalgae and sponges seemed to be either favoured or damaged by sediment additions (Fabricius *et al.,* 2005, Golbuu *et al.,* 2008). Even if this phenomenon is chronic, Nugues and Roberts (2003), suggest that coral species are mainly affected by sedimentation during specific events such as tropical storms.

- Decrease in herbivores: overharvesting of marine species is known to cause major changes on the community structure of reefs (Figure 3e). Herbivory pressure is an important natural factor that can maintain a low algal biomass on the benthic substratum, especially in tropical areas where 60 to 97% of the total algal production can sometimes be removed by herbivores (Hay and Fenical, 1988). On many algal reefs, high herbivory pressure maintains algal turfs with low biomass and high productivity. Uncontrolled overfishing has led to a decrease in the amount and the diversity of both predators and herbivores. These modifications can be responsible for both the development and the colonisation of the reef by macroalgae (Hughes, 1994, Aronson and Precht, 2000, Aronson *et al.,* 2003). Strong negative correlations were found between sea urchins/algae and fishes/algae (McCook, 1996, McCook, 1997, Aronson and Precht, 2000, Williams and Polunin, 2001, Mumby *et al.,* 2007a). In addition to overfishing in coastal waters, the mass mortality of the black spined urchin *Diadema antillarum* in 1983 in the Caribbean has induced profound changes on coral reefs (Lessios *et al.,* 1983, Lessios *et al.,* 1984b). Within a year, populations of this species were reduced to at least 7% (Table 1) of their former numbers (Knowlton, 2001). This sea urchin is considered as one of the key stone species on Caribbean coral reefs (Knowlton, 2001). It is primarily a herbivore (Randall *et al.,* 1964) and was responsible for grazing on macroalgae thus limiting their proliferation. Its massive die off is possibly playing a major role in the phase shift phenomenon presently observed in the Caribbean (Hughes, 1994). Hay (1997) suggested that the disappearance of sea urchins alone, or fishes alone could not be responsible for the macroalgal colonisation

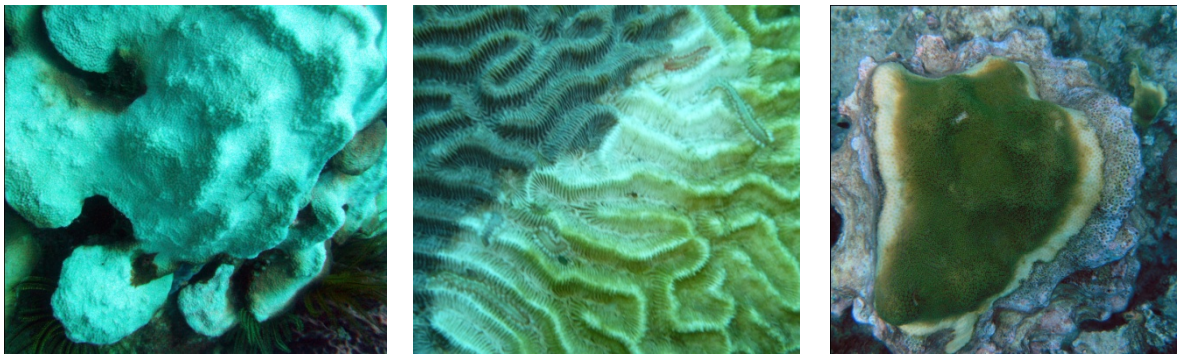
on the reef. However, Mumby *et al.,* (2007b) showed, using a simulation model, that Caribbean coral reefs did not demonstrate an algal dominated state when *Diadema antillarum* was present.

- Global warming: There is increasing concern about climate change and its possible effects on coral reefs (Eakin *et al.,* 2010). During the 20th century, sea temperatures increased by 0.7°C, pH decreased by 0.1 units and carbonate ion concentrations diminished by 30 µmol.kg^{-1} (Hoegh-Guldberg *et al.,* 2007). These modifications can damage coral species through two main pathways. i) Ocean acidification caused by rises in atmospheric CO_2 induces a reduction in seawater carbonate ion concentrations and pH which in turn may decrease coral calcification and growth (Kleypas *et al.,* 2006) (Figure 3f). ii) Environmental extremes, such as unusually high sea temperature and irradiance may damage the symbiotic interaction between coral species and the zooxanthellae. The loss of zooxanthellae (and / or reduction of pigment concentration) is refered to as "bleaching" (Figure 4a). Hoegh-Guldberg (1999) showed that an increase of 1 to 2°C for 3 to 4 weeks was responsible for bleaching events. Several stressors are known to result in bleaching, but this phenomenon has most commonly been associated with high sea temperature and irradiance (Baker *et al.,* 2008). Temperature also indirectly affects coral species through the enhancement of diseases (Harvell *et al.,* 2002, Bruno *et al.,* 2007). In September 2005, 90% of coral cover bleached on the reefs in the US Virgin Islands following anomalies in temperature from April to September 2005 (Miller *et al.,* 2006). Mass coral bleaching has increased in intensity over the past decades and in the face of global warming, it is likely that the frequency of mass-bleaching events will increase (Hoegh-Guldberg, 1999). Coral species responses to belaching events are variable and depend on the frequency and the extent of this process. Coral can exhibit lethal nonlethal or sublethal bleaching (Suggett and Smith, 2010). If coral death occurs, it will result in freeing space that could be colonised by fast growing organisms. Among all the scenarios described by Hoegh-Guldberg *et al.,* (2007), global warming will inevitably lead to a domination of algae on coral reefs.

Table 1: Non-exhaustive list of *D. antillarum* densities before and after 1983 at several localities in the Caribbean.

Year of survey	*D. antillarum* density (m^{-2})		Site	Country	Author
	Before 1983	After 1983			
Oct. 1984-Dec 1985		0.8 0.5 3.3		St Lucia Dominica Barbados	Hunte and Younglao, 1988
1983	17		North Bellairs reefs	Barbados	Hunte *et al.,* 1986
1975 1979 1983	12 7 4		Southeast	Curacao	Bak *et al.,* 1984
May/June 1983 oct-83	3.97	0.01	Holiday beach (3m depth)		
	0.39	0	Holiday beach (12m)		
	0.26	0	Holiday Beach (9-30m)		
	2.93	0.01	Carambi Buoy I (3 m)		
	2.49	0	Carambi Buoy I (12m)		
	0.73	0.02	Carambi Buoy I (9-36m)		
July-August 1983 Oct-83	4.16	0.05	Carambi Buoy II (3-16m)		
1990 – 2000		0.05 (Max.)	Tennessee Reef	Florida	Chiappone *et al.,* 2002
1960	1.2		Florida Keys	Florida	Randall *et al.,* 1964
	1.4 à 4.5 1		Molasses Reef Elbow Reef	Florida	Bauer, 1980
2000		5 (Urchin zone) 0 (Algal zone)	North coast of Jamaica	Jamaica	Edmunds and Carpenter, 2001
June-82 May-83/Feb-84	40000 in 2.9 ha = 1.38	5/458 in 12 ha	Punta Galita	Panama	Lessios *et al.,* 1984b
May-82 Die off to 2003	1.3	<0.5	Aguadargana	Panama San Blas Archipelago	Lessios, 1995, Lessios, 2005
Apr-82 Die off to 2003	0.88	0	Mamitupu		
Apr-82 Die off to 2003	0.5	0	Makerel West		
Apr-82 Die off to 2003	0.81	0	Mosquito		
Sept-82 Die off to 2003	10.98	<1	House Reef		
May-82 Die off to 2003	1.06	0	Korbiski East		
Dec-80 Die off to 2003	1.64	<0.5	Korbiski West		
	0.49	<0.2	Pinacles		
	2.8	<0.5	Tiantupu		
Nov-82 Die off to 2003	0.05	<0.02	Ulaksukan		
2000-2001		<0.6	9 sites investigated	St Croix	Miller *et al.,* 2003
1980	71		Discovery Bay	Jamaica	Sammarco, 1980
2001 to 2004		0.81 à 2.98	West coast	Dominica	Steiner and Williams, 2006
Before 1983 1983 to 1986	0.7 à 71	Around 0		Jamaica	Hughes *et al.,* 1987
June 2003- May2004		Around 0 Around 2 0 between 2 and 4 <2 around 4 around 0 between 6 and 8 around 0 between 6 and 8 around 0 around 4	Bonaire (algal zone) Bonaire (urchins zone) Barbados (algal zone) Barbados (urchin zone) Belize (algal zone) Belize (urchin zone) St Croix (algal zone) St Croix (urchin zone) Jamaica (algal zone) Jamaica (urchin zone) Grenada (algal zone) Grenada (urchin zone)	Caribbean	Carpenter and Edmunds, 2006

- Diseases (Figure 3g): The frequency of occurrence and geographic range of coral diseases have increased in the past decade (Harvell *et al.,* 1999, Garzon-Ferreira *et al.,* 2001, Rogers *et al.,* 2004). In the 1980's, *Acropora palmata* and *A. cervicornis* (coral) both suffered from an epizootic throughout the Caribbean (Aronson and Precht, 2001) and other coral species have been more recently affected (Gardner *et al.,* 2003). Garzon-Ferreira *et al.,* (2001) identified that six types of coral diseases (The black band disease, the white band disease (Figure 4b), the red band disease, the yellow band disease (Figure 4c) the white plague and the dark spot disease) were affecting 24 species of stony corals in the Colombian Caribbean. The increase of coral diseases observed over the past years could possibly be due in part to human activities (Harvell *et al.,* 1999, Bruno *et al.,* 2003).



a b c

Figure 4: Specimen of coral affected by diseases a) Bleached *Montastrea* sp. (in 2010), b) White band disease on *Diploria strigosa* and c) Yellow band disease on *Montastrea faveolata*.

- Shipping: even if it is a relatively less important factor, shipping can have several direct and indirect impacts on reefs. It can be responsible for direct physical damage by grounding and anchoring and it can cause less obvious damage by introducing new species which can become invasive through the ballast waters or attachment on the hull (Gollasch, 2006). Invasive species can cause many adverse effects to the ecosystems they invade (competition for space, food) and can sometimes dominate the new environment. Caribbean islands, among which Martinique, are currently concerned by the introduction of 2 invasive species: the seagrass *Halophila stipulacea* and the lion fish *Pterois* sp. Moreover, shipping through the use of antifouling paints can be responsible for the death of living organisms. Paints containing organotin such as tributyltin (TBT) were used worldwide until recently. Various studies have demonstrated TBT to cause many adverse ecotoxicological effects. As a result, its use has progressively been restricted in numerous countries and has been prohibited worldwide on any surface since 2008 (van Wezel and van Vlaardingen, 2004). TBT can prevent or reduce coral fertilization, larval

settlement and cause coral bleaching and mortality at low concentrations (Negri and Heyward, 2001, Smith *et al.,* 2003)**.** Antifouling paints can also contain metals such as copper and zinc. Experiments conducted by Negri *et al.,* (2002) showed sediments (collected from a ship grounding site) to cause sub-lethal effects on *Acropora microphthalma* (Coral) larvae and inhibit their settlement and metamorphosis. Small invertebrates such as sponges, bryozoans and sipunculid worms also suffered and often died from the toxic effect of the contaminated sediments.

- Marine tourism: Tourism through cruising, sailing and aquatic activities (jet skiing, scuba diving, snorkelling…) physically damages coral reefs. Scuba divers and snorkellers can be responsible for breaking coral colonies as well as re-suspending sediment, which is highly damaging corals (Barker and Roberts, 2004). Damage will depend on coral species present, the appreciation shown by divers to coral reef environments and their various activities underwater (taking pictures or not) (Barker and Roberts, 2004). Moreover people can trample on coral species and break them. In addition, tourism indirectly impact coral reefs through land management policies (the construction of facilities…).

The relative importance of these factors on the degradation of coral reefs is, however, unknown and debatable (Lapointe, 1997, Hughes *et al.,* 1999a), and the macroalgal dynamics on coral reefs remains poorly understood. Depending on the studies carried out, researchers have concluded that coastal pollution, decrease in herbivores and/or global warming were the main factors responsible for the phase shift phenomenon observed (Hughes, 1994, Lapointe, 1997, Hughes *et al.,* 2003, Pandolfi *et al.,* 2005). Regardless the difficulties of classifying these factors according to their importance in impacting coral reefs, all the damage seems to be responsible for reef degradation, sometimes inducing a shift from coral to algal communities. Renken and Mumby, 2009 developped a Bayesian belief network model (Figure 5) in order to predict the macroalgal growth on coral reefs. They demonstrated that top-down processes (rather than bottom-up) primarily drive macroalgal dynamics. Grazing by *Diadema antillarum* showed the greatest influence on macroalgal cover followed by parrotfish (Renken and Mumby, 2009).

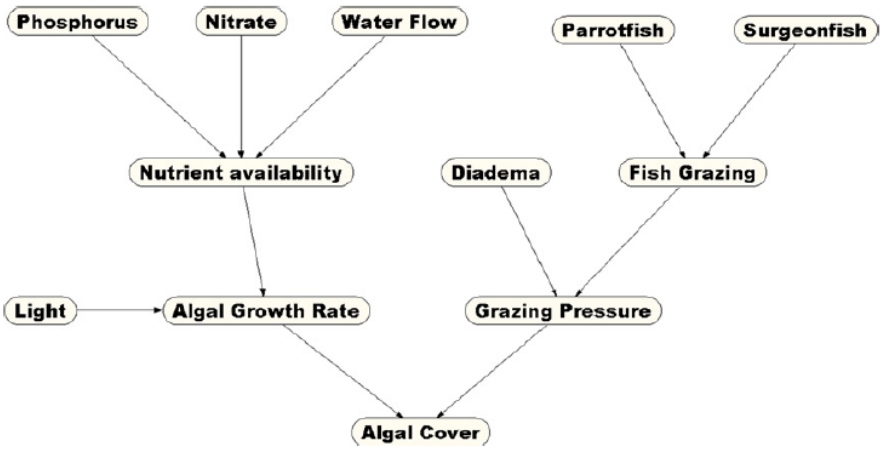


Figure 5: Graphical representation of the Bayesian belief network Algalnet (Renken and Mumby, 2009).

I.1.3. Phase shift phenomenon: Relationships between algal colonisation/presence and benthic species

Anthropogenic and natural disturbances have induced changes in the Caribbean benthic reef communities over the last decades and are likely to induce increases in macroalgal cover (Figure 6). However, recent publication suggesting the phase shift is an extreme phenomenon and that coral reef degradation leads mainly to intermediate states (Bruno *et al.,* 2009).

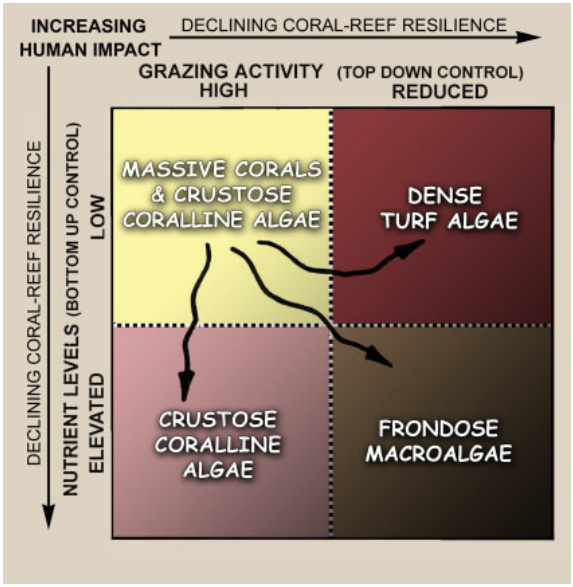


Figure 6: Competition-based relative dominance model (Littler *et al.*, 2006a).

It is known that the proliferation of algae can greatly modify the physical, chemical and biological properties of an ecosystem (Figure 7). These organisms are fast colonisers and

will compete for space with other benthic species. They are known to produce a wide range of secondary metabolites that have many effects (both positive and negative) on other species, their recruitment and their feeding (Hay, 1996, Steinberg and de Nys, 2002, Pereira *et al.,* 2003, Paul and Puglisi, 2004). The fast colonisation capacity can form harmful macroalgal blooms that can be responsible for depletion in oxygen and thus the death or migration of numerous benthic organisms (Lapointe, 1997). They can provide new habitats for epibiontes and refuges for larvae. Their growth can modify physical parameters by reducing the light penetration, decreasing the small scale currents and increasing the substratum abrasion by sweeping surfaces (Kennelly, 1989)**.** Modifications of hydrodynamics can have a great impact on the understorey assemblages and can modify larval supply and recruitment as well as the growth rate of some invertebrates (Eckman, 1983, Eckman and Duggins, 1991).

A few publications have addressed the issue of macroalgal effects on scleractinian coral and the competition mechanism between the two groups is still not fully understood (Reviewed by McCook *et al.,*(2001)). Competition depends on several criteria such as morphological and physiological traits of macroalgae and coral as well as environmental parameters (McCook *et al.,* 2001, Jompa and McCook, 2002b, Nugues and Roberts, 2003). The encrusting coral colonies were demonstrated to be more affected by algae than the branching or massive ones (Tanner, 1995, Lirman, 2001), except for some species such as the massive colony of *Montastrea faveolata* (Lirman, 2001). The different effects that macroalgae can have on coral, with exception of the allelochemichal effects, are correlated with seaweed morphology (Jompa and McCook, 2003).

Both direct and indirect impacts were described regarding the effects of algae on coral species:

- Competition mechanism for space (Figure 7a) was described between coral and macroalgae (Jompa and McCook, 2002b, Jompa and McCook, 2003). Space necessary for coral larval settlement can be unavailable if occupied by algae (Birrell *et al.,* 2005, Mumby *et al.,* 2005, Titlyanov *et al.,* 2005). In most case, macroalgae do not seem to colonise living corals, instead, they overgrow skeletons after their death (Lirman, 2001). However, some algae such as *Lobophora variegata* (Phaeophyta) were demonstrated to directly overgrow coral species, killing the surrounding tissues by encroachment (Jompa and McCook, 2002a).

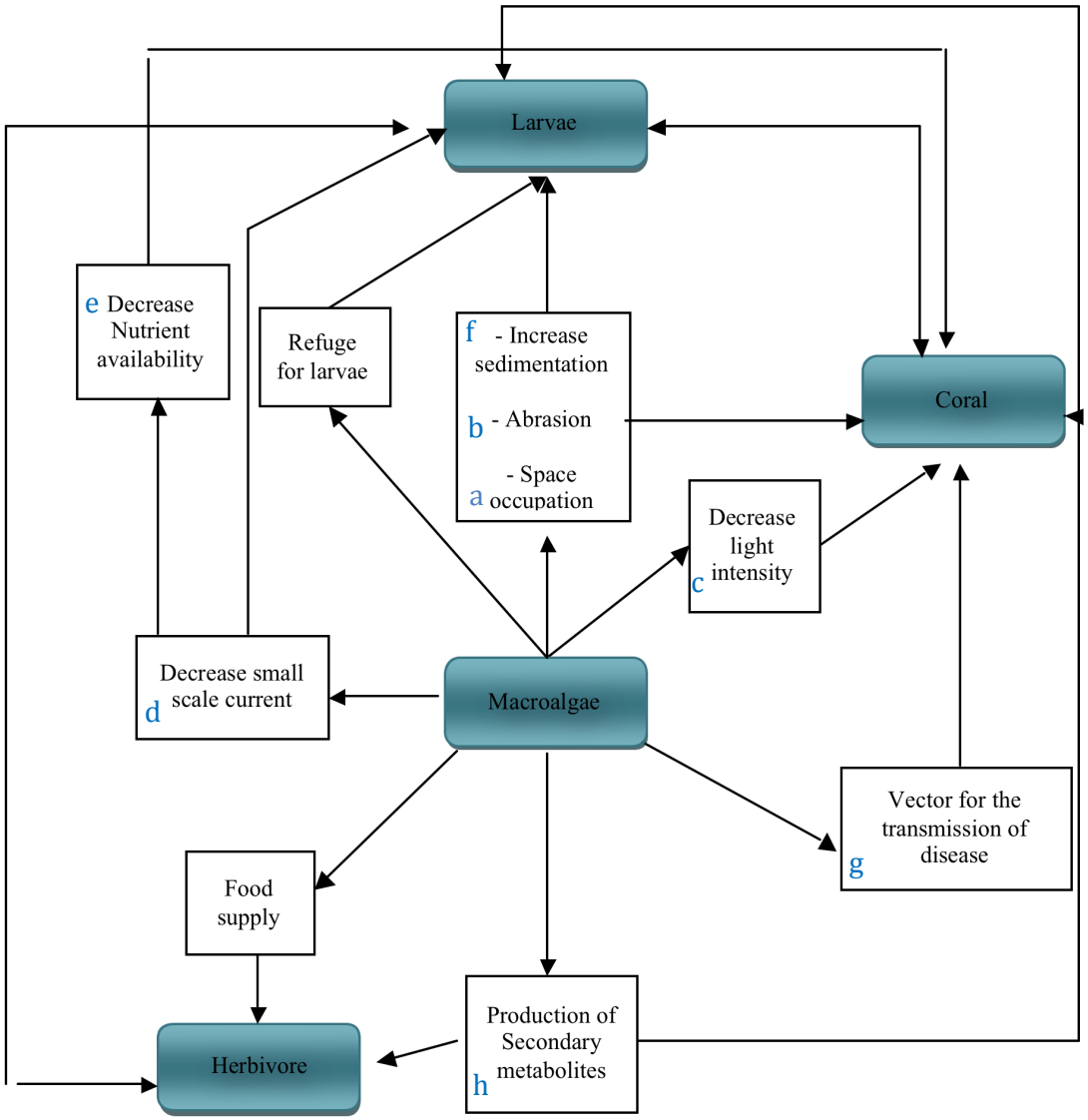


Figure 7: Conceptual model of the effects of macroalgae on some other benthic organisms. In order to simplify the diagram, the effects of other benthic organisms on macroalgae (e.g.: herbivory pressure) have been deliberately omitted. All the arrows correspond to a direct or indirect (positive or negative) impact of seaweeds on the other components.

- Whilst sweeping coral colonies under small-scale currents algae can also cause damage to coral colonies through several pathways. They can be responsible for the retraction of polyps, thus decreasing the light catchment by zooxanthelle, tissue degradation, and damage to the calcareous structure protecting the polyps (Lirman, 2001, McCook *et al.,* 2001, River and Edmunds, 2001, Jompa and McCook, 2003, Titlyanov *et al.,* 2005, Titlyanov *et al.,* 2007). Both *L. variegata* and *Dictyota pulchella* (Phaeophyta) were suggested to reduce juvenile *Agaricia* spp. (coral) growth by 60% and 31% respectively due to an abrasion effect (Box and Mumby, 2007). Seaweeds can also

jeopardise the number of recruits by sweeping the substrata and preventing larval fixation (Figure 7b).

- Algal canopies, (depending on their life stage, species composition etc.), may decrease the light intensity received by coral species (Figure 7c). This diminution may modify coral growth rate by decreasing zooxanthellae photosynthesis. Moreover, seaweed canopies induce changes in the small scale currents (Figure 7d), thus modifying the supply of nutrients to the organisms (Nugues and Roberts, 2003) (Figure 7e). Algal morphology plays an important role regarding the shading effect on coral species. As an example, shading by *Lobophora variegata* (likely to shade coral species) significantly increased *Agarcia* spp. mortality rates from 0 to 50% in 6 months, while shading by *Dictyota pulchella* resulted in 99% growth inhibition (Box and Mumby, 2007). Some macroalgae have positive and negative interactions with coral species. *Sargassum* cover was demonstrated to protect coral species from bleaching during exceptional weather conditions and flooding (Jompa and McCook, 1998). The author hypothesised the protection could be through a reduction of the effect of high temperatures and/or UV or maybe a reduction of low salinity waters mixing**.** The same genus was however demonstrated to have a negative impact (reduction of growth and abrasion) and competitive advantage over corals (River and Edmunds, 2001).
- The presence of algae can retain particles and increase sedimentation phenomenon (Figure 7f). Algal sediment mats affect *Colpophyllia natans* growth (Nugues and Roberts, 2003).
- Hyperspectral imagery combined with dissolved oxygen measurements demonstrated coral-algal interaction to vary with species (Barott *et al.,* 2010). Coral-fleshy algal interaction zones were characterised by disrupted coral tissues and hypoxia (suggesting microbial activity), while coral-CCA interaction zones were not hypoxic and coral tissues not damaged and coral-turf interaction zones were intermediate as no cleared coral skeletons were observed but turf remained disruptive (Barott *et al.,* 2010). Fleshy algae such as *Gracilaria* sp. (Rhodophyceae) appeared to overgrow coral species while cyanobacteria seemed to benefit from clear space and be opportunistic colonisers (Barott *et al.,* 2010).
- Some coral diseases are positively correlated with increases in algal cover (Harvell *et al.,* 1999) (Figure 7g). Algae are supposed to be vectors for the transmission of diseases (Nugues *et al.,* 2004, Smith *et al.,* 2006). Coral colonies of *Montastrea faveolata* in contact

with *Halimeda opuntia* (Chlorophyta) transplant developed white plague type II, whereas unexposed colonies did not (Nugues *et al.,* 2004). Algae were described as the reservoir for the causative agent (presence of the bacterium responsible for the disease on its surface). Not all the coral species are equally affected by this phenomenon (Smith *et al.,* 2006).

- The production of secondary metabolites may interfere with reproduction, settlement of coral larvae and health of coral tissues and thus be implicated in the phase shift phenomenon (Figure 7h). Secondary metabolites are organic compounds that are not directly involved in vital functions of an organism (growth, development, reproduction). Nevertheless, they may play important roles in defensive strategies against predators, competitors, fouling organisms and microorganisms. Types of natural products (secondary metabolites) and the way they mediate interactions between herbivore were discussed (Paul *et al.,* 2001). As an exemple of macroalgal-coral interactions, *Lyngbya majuscula* and *Lyngbya bouilloni* (Cyanophyta), *Dasyopsis spinuligera* (Rhodophyta) and *Corallophila huysmansii* (Rhodophyta) were suggested to poison healthy coral tissues producing toxic secondary metabolites (Littler and Littler, 1997, Jompa and McCook, 2003, Titlyanov *et al.,* 2005, Titlyanov *et al.,* 2007). Chemical effects appear to be lethal to tissue while physical effects seem to be less damaging (Jompa and McCook, 2003). Only a few studies directly assessed the role of secondary metabolites on coral health; thus, de Nys *et al.,* (1991), demonstrated the secondary metabolite chloromertensene from the alga *Plocamium hamatum* (Rhodophyta) to induce tissue necrosis in the octocoral *Sinularia cruciata* when in physical contact with the alga.

Alternatively, it has been proven that some secondary metabolites can have a positive impact on the recruitment of coral larvae. The alga *Halimeda opuntia* (Chlorophyceae) attracted *Favia fragrum* (coral) larvae even when more suitable substrata were present (Nugues and Szmant, 2006). The production of secondary metabolites by crustose coralline algae (CCA) such as *Amphiroa anceps, Hydrolithon onkodes, Lithophyllum insispidum, Lithophyllum kotschyanum, Mesophyllum* sp., *Neogoniolithon brassica-florida* and *Peyssonnelia* sp., seem to have a positive effect on coral species and induce coral larval metamorphosis (Heyward and Negri, 1999).

These mechanisms of interaction suggest that competition between scleractinians and algae have a key role in structuring the benthic communities on the reef ecosystems. Mumby and Steneck, (2008) described the possible positive and negative feedback in case of a phase shift phenomenon. They suggest that in case of a shift from coral to algal dominated state, macroalgal levels can become high enough to limit coral recruitment, thus creating a

bottleneck in the coral population. Recruitment processes are the first step in maintaining ecosystems for many marine invertebrates, and the effects of macroalgae on these processes must be addressed.

I.2. The case of *Sargassum* species

Sargassum Agardh species are Phaeophyceae belonging to the Order Fucales. The first name to be given to this macroalga was *sargazo* (gulfweed or seaweed) when discovered in tropical Atlantic waters by Portuguese navigators in the 15th century. Later, the genus *Sargassum* was described by C. Agardh (1820) from a specimen described by Tuner (1802) under the name *Fucus baccifer* Turner. Since then a significant number of *Sargassum* species have been described, and about 1400 different epithets (including varieties and forms) have been applied to *Sargassum* specimens throughout the world (data issued from *Index Nominum Algarum* available at http://ucjeps.berkeley.edu).

I.2.1. Classification and morphology

Sargassum belongs to the Domain: Eukaryota (Chatton, 27519 species accepted taxonomically), Kingdom: Chromista (T. Cavallier-Smith, 11066), Subkingdom, Chromobiota (T. Cavallier-Smith, 10927), Phyllum Heterokontophyla (3101), Class, Phaeophyceae (Kjellman, 1760), Order: Fucale (Bory de Saint-Vincent, 521), Family: Sargassaceae (Kützing, 480), Genera: *Sargassum* (C. Agardh, 338) (algaebase).

Sargassum is one of the most complex genera in the Phaeophyta. It is composed of a discoid holdfast strongly anchoring the seaweed to the substratum, and main axis(es) from which one to several laterals bearing leaf-like structures arise (Figure 8). Air bladders, or gas vesicles, maintain the laterals in upright position and are involved in the reproduction and colonisation processes. Both inter- and intra-specific morphological variations are described. *Sargassum* spp. are highly polymorphic; Kilar and Hanisak, (1989), described 47 morphotypes of *Sargassum polyceratium* in a single population of Florida. Several causes, among which feature environmental adaptation (De Ruyter van Stevenick and Breeman, 1987; Kilar and Hanisak, 1989; Mattio *et al*. 2008a), ontogenetic forms (Kilar and Hanisak, 1988), and hybridization (De Paula and Oliveira, 1982), have been described as responsible for these differences.

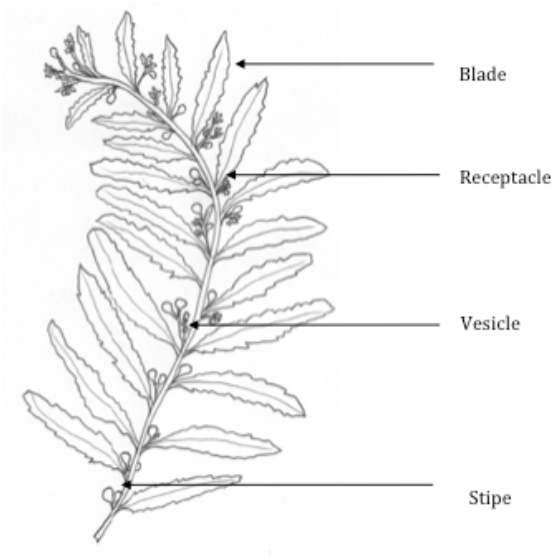


Figure 8: Diagram of one *Sargassum* frond bearing blades, receptacles and vesicles (Mattio, personal communication).

Sargassum taxonomy relies on a large variety of morphological traits including the holdfast, the receptacles, the main axis, the blades, the midrib and the air bladder (Figure 9). Even though morphological traits are useful, they usually are not sufficient to make a safe distinction between the species due to the high polymorphism rate, and recent studies have focused on genetic identifications (Mattio and Payri, 2009, Mattio *et al.,* 2009a), Mattio, 2011). In order to obtain a more practical taxonomy, numerous genetic studies have focused on phylogenetic relationships within the genus *Sargassum* in the Pacific and Caribbean regions (Stiger *et al.,* 2000, Stiger *et al.,* 2003, Mattio *et al.,* 2008b, Mattio and Payri, 2009, Mattio *et al.,* 2009a, Mattio *et al.,* 2009b, Mattio and Payri, 2011).

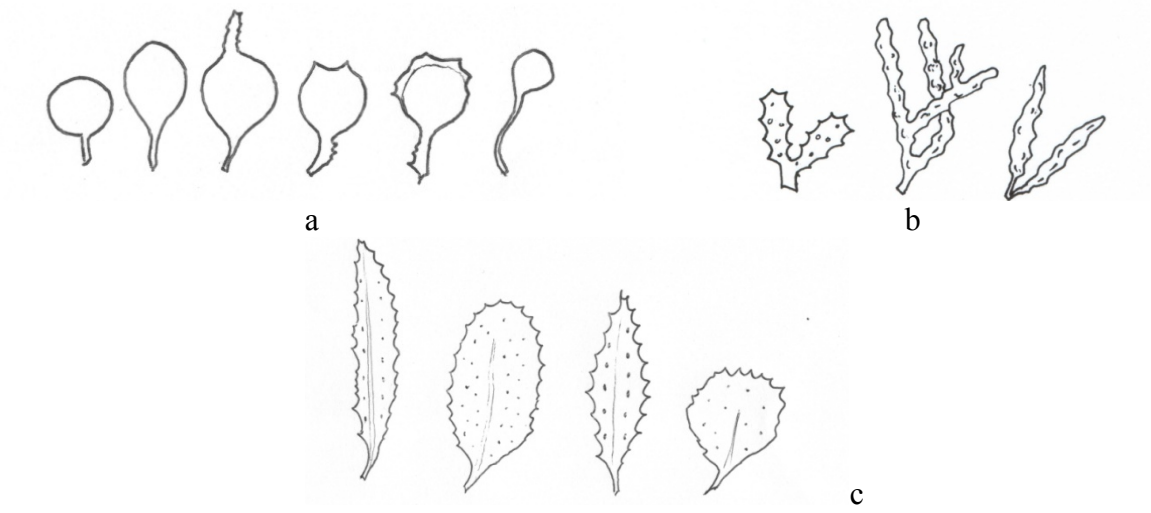


Figure 9: Variability of *Sargassum* morphological parameters a) vesicles, b) receptacles and c) blades (Mattio, personal communication).

I.2.2. Geographical distribution

Sargassum is a common benthic genus, which occurs naturally from temperate to tropical regions, where it often forms large coastal marine forests, providing food, habitat and nurseries to numerous marine organisms (Martin-Smith, 1992, Ornellas and Coutinho, 1998). Even though *Sargassum* was described as an important species in coastal ecosystems regulation, it is problematic on coral reefs where species started to become dominant at the expense of reef-building corals. As an example, *Sargassum polyceratium* and *Sargassum hystrix* have replaced the hermatypic coral species (eg: *Montastrea annularis*) on the North Coast of Jamaica (Lapointe, 1997, Lapointe and Thacker, 2002). Although *Sargassum* colonises reefs once coral species are already dead (McCook, 1997), they are also thought to play a role in reef degradation.

Sargassum species are present all around the world from tropical to temperate waters (De Ruyter van Stevenick and Breeman, 1987, Littler *et al.,* 1993, McCook *et al.,* 1997, McClanahan *et al.,* 1999, Stiger and Payri, 1999a, Engelen *et al.,* 2001, Mattio *et al.,* 2008a). It is one of the most conspicuous genera in the Philippines (Ang, 1986), French Polynesia (Stiger and Payri, 1999a), in the south west lagoons of New Caledonia (Mattio *et al.,* 2008a, Mattio *et al.,* 2008b), in the southern region of Taiwan (Dai, 1997), on reef flats of the Great Barrier Reef (Price, 1989, Vuki and Price, 1994, McCook, 1997) as well as in the Caribbean (De Ruyter van Stevenick and Breeman, 1987, Littler *et al.,* 1993, Lapointe, 1997, Engelen *et al.,* 2001). Increases in Pacific and Caribbean proliferation by *Sargassum* spp. occurred at the end of the 20th century, *Sargassum* began overgrowing Caribbean Martinique reefs in the late 1980s (Littler *et al.,* 1993) and since the late 70's on the west coast of the island (Battistini, 1978). By 1992, *S. polyceratium* colonised from the fore-reef to the back reef of Discovery Bay in Jamaica (Lapointe, 1997). *S. pacificum,* proliferation in French Polynesia began in the early 80's (Stiger and Payri, 1999a).

The highest diversity of *Sargassum* spp. is found in Southwest Asia ie: Indonesia, Philippines, Vietnam and Malaysia (Figure 10). Some species are endemic while others, such as *S. muticum*, have spread all around the world demonstrating the high colonizing capabilities of some species of this genus.

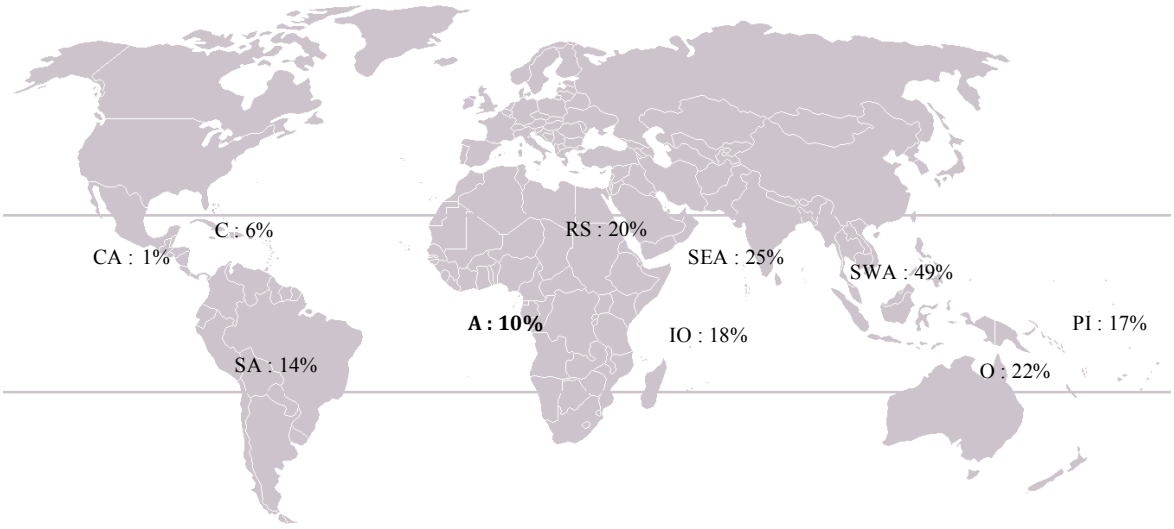


Figure 10: Tropical *Sargassum* species distribution. The proportion of species described in a region over the total number of *Sargassum* sp. known worldwide are indicated per areas: CA: Central America, SA: South America, C: Caribbean, A: Africa, RS: Red Sea, IO: Indian Ocean, SEA: South West Asia, SWA: South West Asia, O: Oceania, PI: Pacific Islands.

I.2.3. Biology

I.2.3.1 Life cycle flexibility

Proliferation success of *Sargassum* species also relies on its life cycle and reproductive strategies. *Sargassum*, like any Fucale, has a monophasic life cycle (de Reviers, 2003), the algae being meiosporophyte (2n chromosome, producing gametes). Diploid thalli produce haploid sperm and eggs by meiosis in antheridia and oogonia respectively, which are contained in conceptacles developing in specialized reproductive structures called receptacles (Figure 11). Plants are monoecious or dioecious depending on the species. The extended reproductive period for *Sargassum* populations (Stiger and Payri, 1999a, Engelen, 2004) could give this alga a competitive advantage over other species with limited reproductive periods such as broadcast spawning coral species. These algae are perennial or pseudo-perennial and subsist from one year to another, regenerating from their holdfast. Canopy percentage cover thus changes seasonally and according to species composition (Mattio *et al.,* 2008a). It may become very low if *Sargassum* forests are monospecific. The settlement and consequent development of other organisms might be hampered by the presence of *Sargassum* (thalli or holdfast) as they continuously occupy space.

The importance of recruitment versus regeneration was modelled for *S. polyceratium* (Figure 12) and was shown to vary with population, year and disturbance (Engelen *et al.,* 2005b). This highlights the ability to adapt to many situations.

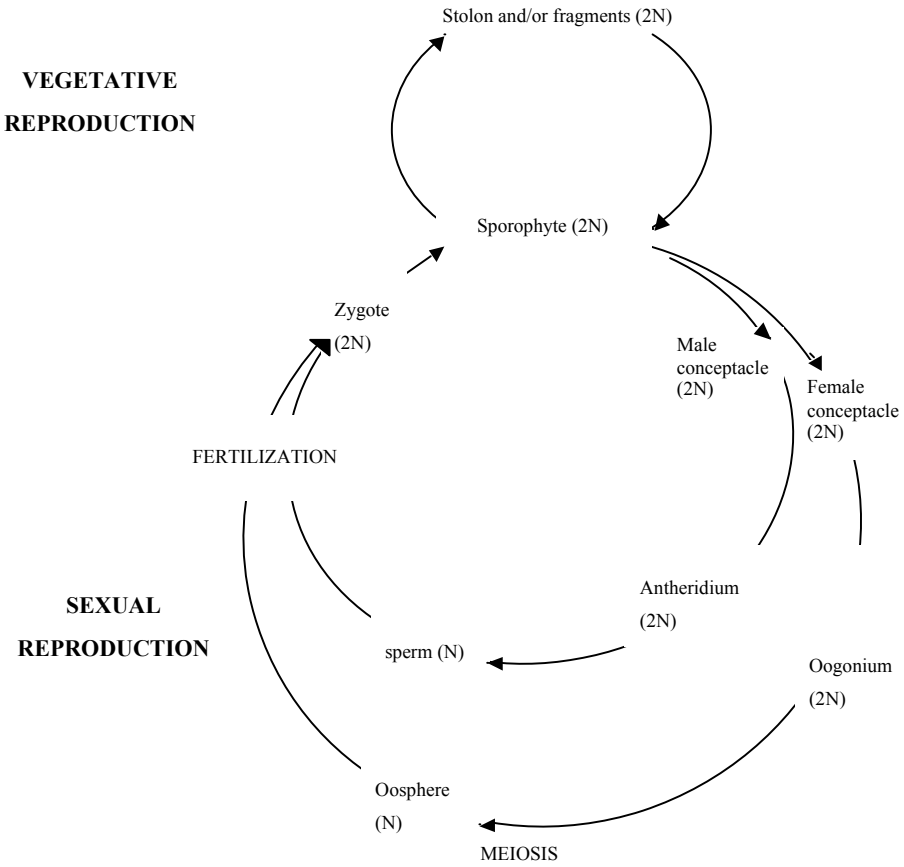


Figure 11: Graphical life cycle of *Sargassum* sp.

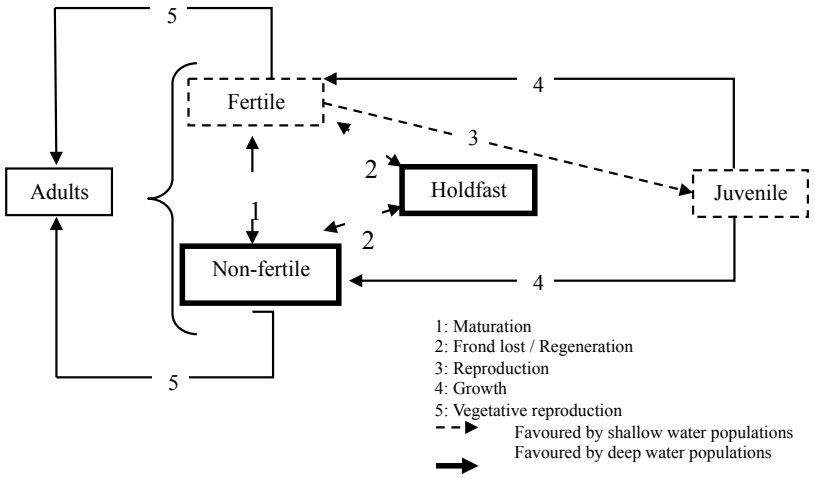


Figure 12: Schematic representation of *S. polyceratium* life cycle showing the different stages of development and life cycle strategies according to Engelen *et al.,* (2005b).

I.2.3.2 Dispersal

Dispersal of *Sargassum* species is often short distance as zygotes settle mainly near the adult plant (Stiger and Payri, 1999b). Long distance dispersal may happen due to the breakage of reproductive axis, which may float over long distance and lead to the colonization of new regions (Deysher and Norton, 1982, Norton, 1992). Fertilization is external but the young zygotes will remain attached to the parental thallus until they have reached a few cells stage (May and Clayton, 1991): this permits protection of the zygote and could maximize their survival rates.

Short distance dispersal

Short distance dispersal maintains populations in already colonised areas. Settlement and development of new recruits usually take place within 1m of the parent plants (Kendrick and Walker, 1991, Kendrick and Walker, 1995, Stiger and Payri, 1999a). Due to these characteristics, success of new habitat colonisation and species viability could be reduced, especially because recruit-recruit interactions and adult-recruit interactions (which increase in the case of recruitment in the parent vicinity) were demonstrated to be responsible for decreases in the recruitment success of *Sargassum* spp. (Kendrick, 1994).

Long distance dispersal

Sargassum reproductive axes or individuals may be pulled off the reef (wave action, storms, natural breakage) and drift over distances up to 600-900 km away from their reef of origin (Norton, 1992). The work of Stiger and Payri (2001) demonstrated that floating fragments of *Tubinaria* (also in the Sargassaceae family) may remain fertile for several weeks. A hypothesis is that *Saragssum* could behave the same way and upon their eventual arrival to a new place, fertile axes can potentially liberate their zygotes and thus colonize new areas. According to Van den Hoek (1987), because of their buoyancy, survival potential, once detached from substrata, rapid growth and the monoic reproductive strategy of a lot of species of *Sargassum* gives them high long distance dispersal potential.

Vegetative dispersal

A few cases of vegetative reproduction have been documented. *Sargassum stolonifolium* produces axes equivalent to stolons/runners that allow for the development of new thallus

(Phang and Yoshida, 1997). *Sargassum natans* has also been shown to achieve vegetative reproduction through fragmentation (Kilar *et al.,* 1992).

I.2.3.3 Seasonality

Sargassum growth rates, fertility rates and abundances are diriven by several variables such as light intensity, photoperiod, depth, geographical distribution, intra/inter specific competition, temperature, nutrient levels, wave action, herbivory pressure and the tide (McCourt, 1984, De Ruyter van Stevenick and Breeman, 1987, Hwang *et al.,* 2004).

Sargassum populations show marked seasonal changes in abundance, plant size, growth rates and reproductive patterns (Table 2). Tropical and subtropical *Sargassum* abundance peaks have been observed in both cool (McCourt, 1984, Stiger and Payri, 1999a) and hot seasons (De Ruyter van Stevenick and Breeman, 1987, Martin-Smith, 1992, Vuki and Price, 1994, Rogers, 1997) depending on the location and the species studied.

Table 2: Seasonal variations of *Sargassum* species demographical and physiological traits (Abundance, algal length, growth rates and fertility) based on their location.

Species	Region	Abundance (Max)	Biomass (Max)	Thallus length (Max)	Growth rates (Max)	Fertility rates (Max)	Reference
S. berberifolium	Taiwan		April-May				Hwang *et al.,* 2004
S. ilicifolium	Australia	Summer		Cold			Rogers, 1997
	Eritree					Cold	Ateweberhan *et al.,* 2005
S. mangarevence	French polynesia	Cool					Stiger and Payri, 1999a, Stiger and Payri, 1999b
S. oligocystum	Australia			Summer			Martin-Smith, 1992
	Hawaii			Cold			De Wreede, 1976
	Philippines			Cold			Trono and Lluisma, 1990
S. oligophyllum	Australia	Summer					Rogers, 1997
S. polyceratium	Curacao				Hot	Hot	De Ruyter van Stevenick and Breeman, 1987, Engelen *et al.,* 2005a
S. polycystum	Taiwan		January-April	Cold			Hwang *et al.,* 2004
	India			max number of main axes in winter		Cold	Srinivasa Rao and Umamaheswara Rao, 2002
	Australia	Hot					Rogers, 1997
S. sandei	Taiwan		Cold				Hwang *et al.,* 2004
S. siliquosum	Taiwan		May				Hwang *et al.,* 2004
Sargassum sp.	Australia					Hot	Diaz-Pulido and McCook, 2005
	Australia	Hot		Cold			Vuki and Price, 1994
	Philippines			Cold		Cold	Ortiz and Trono, 2000

Variations in thallus length can be observed for the same species according to its location: *S. oligocystum* reaches its maximum length in summer in Australia (Martin-Smith, 1992) and in the cool season in Hawaii (De Wreede, 1976) and the Philippines (Trono and

Lluisma, 1990). In a same area, maturity index varied with the benthic location, being higher in the outer ridge and fringing reef populations (Stiger and Payri, 1999b). This could be an adaptation mechanism to extreme environmental conditions (Stiger and Payri, 1999b). Population descriptive parameters such as biomass, density, stage distribution, allocation of energy to the holdfast and the timing of reproduction differ for *S. polyceratium* with wave exposure and depth (De Ruyter van Stevenick and Breeman, 1987, Engelen *et al.,* 2005a). Factors such as competition, predation and water column nutrient contents also regulate the algal population dynamics and are discussed later (Chapter I, paragraphs 2.3.4 and 2.4). Seasonal changes, such as abundance patterns, also depend on the species investigated, thus *S. berberifolium* (synonymous of *S. ilicifolium*) and *S. polycystum* have an abundance peak from late November to June while *S. sandei* (synonymous of *S. ilicifolium*) has one from December to February, and *S. siliquosum* from mid-April to August on Taiwan reefs (Hwang *et al.,* 2004).

In addition to morphological plasticity and life cycle flexibility, *Sargassum* seems to be adapted to various conditions and give this species good competition capabilities.

I.2.3.4 Effects of nutrients on *Sargassum's* growth

Oligotrophic waters in tropical reef areas are known to limit both macroalgal growth and reproduction (Lapointe *et al.,* 1987, Lapointe, 1997, Schaffelke and Klumpp, 1998, Lapointe, 1999, Lapointe *et al.,* 2004). In many tropical ecosystems, there is increasing concern about anthropogenic nutrient input in the coastal marine waters and the impacts this could have on macroalgal growth, recruitment, fertility rates and therefore the "phase shift phenomenon" (Barile and Lapointe, 2005, Lapointe *et al.,* 2005b, Lapointe and Bedford, 2010). As an example, higher Nitrogen (N) and Phoshorus (P) availability in neritic compared to oceanic waters of the western North Atlantic Ocean significantly enhance *S. natans* productivity (Lapointe, 1995). Increases in nutrients could augment reproductive productivity and thus enhance colonisation. Nonetheless *S. siliquosum* reproductive structure biomass was showed to decrease concomitantly with nutrient additions (Diaz-Pulido and McCook, 2005). In laboratory conditions, germling growth of *Sargassum baccularia* was stimulated by nutrient increases (Schaffelke and Klumpp, 1997), but very high nutrient concentrations were demonstrated to inhibit *Sargassum* growth (McCook, 1997, Schaffelke and Klumpp, 1998).

N is the primary nutrient limiting algal growth in temperate waters, while P is more important in carbonate rich waters (Hanisak, 1979, Lapointe *et al.,* 1987, Lapointe, 1989) where it was demonstrated to be the most limiting nutrient to *S. pteropleuron* and *S. polyceratium* growth (Lapointe, 1989). However, the limitation patterns are not always clear and can vary with the stage of development. Juvenile explants of *S. sandei*, *S. berberifolium*, and *S. polycystum* cultivated in laboratory under different nutrient conditions were P limited while the adults were N limited (Hwang *et al.,* 2004). *Sargassum* has demonstrated capabilities to overcome P limitation. It has high alkaline phosphatase activity (APA), which hydrolyses Dissolved Organic Phosphorus (DOP) as a source of P under conditions of limited Soluble Reactive Phosphorus (SRP), and, therefore, favour the algal development in limited SRP waters (Lapointe, 1997, Schaffelke, 2001) (Figure 13). Eutrophication threshold concentrations of nutrients were estimated at 1.0 mM dissolved inorganic nitrogen (DIN) and 0.1 mM SRP in tropical reef waters (Bell, 1992, Lapointe, 1997). *Sargassum* spp. also demonstrated an ability to utilise particulate organic sources of nutrients, suggesting remineralisation process could occur at the algal surface through bacterial activity and high APA (Schaffelke, 1999). Experimental addition of Nitrogen (N) increases APA while addition of Phosphorus (P) decreases APA in tropical macroalgae (Lapointe and O'Connell, 1989, Delgado and Lapointe, 1994). Over short time scales, *Sargassum* APA is controlled by P tissue content rather than by SRP water column concentrations (Schaffelke, 2001) while on longer time scales, tissue nutrients are controlled by water column nutrients which in turn will control APA (Schaffelke and Klumpp, 1998). Higher APA is likely to be beneficial to fast growing macroalgae in coral reef systems that are subject to significant N inputs (Schaffelke, 2001).

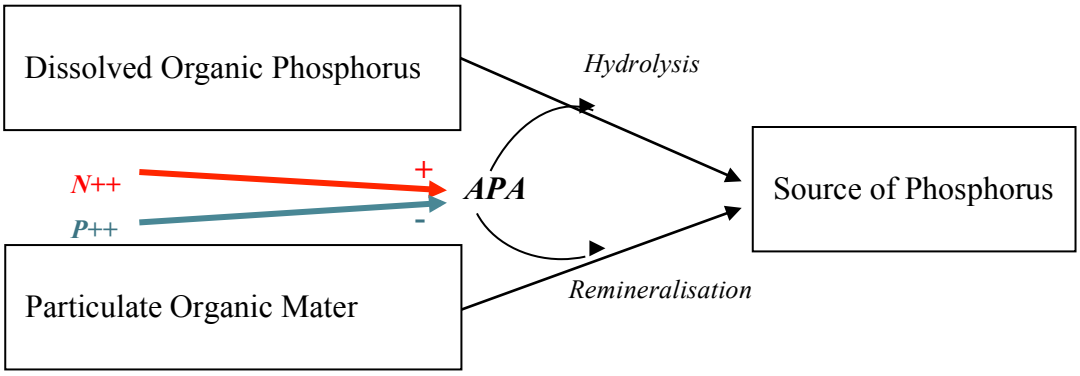


Figure 13: Alkaline phosphatase activity (APA) in the absorption of phosphorus (P) by macroalgae and the contol of nutrients (nitrogen, N and phosphorus, P) on this cycle. + means increase and - decrease.

Nutrient mechanisms of action on the life cycle of *Sargassum* spp. are complex and more information is required on the topic to understand the implications of nutrient concentration increases in tropical coastal waters on macroalgal development. Nevertheless, *Sargassum* seems to have developed strategies in order to adapt to nutrient limited environment which include inorganic nutrient pulses (Schaffelke and Klumpp, 1998, Schaffelke, 1999b), remineralisation of particulate nutrients (Schaffelke, 1999a) and DOP (Schaffelke, 2001).

I.2.4. Ecology

I.2.4.1 Ecological importance of *Sargassum* forests

Sargassum is considered as an ecosystem engineer ie: *"organisms that directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials. In so doing, they modify, maintain or create habitats"* (Jones *et al.,* 1994). *Sargassum* represents an equivalent to *Fucus* and *Cystoseira* beds of temperate northern hemisphere regions (Nizamuddin, 1962, Phillips, 1995, Thibaut *et al.,* 2005). Tropical *Sargassum* forests play an important ecological role for many benthic organisms they provide: i) a substratum to epiphytic, epibiontic and endobiontic species, ii) food to many grazers and iii) protection against predation and unfavorable environmental conditions (McClanahan *et al.,* 1994, Rossier and Kulbicki, 2000, Godoy and Coutinho, 2002, Leite and Turra, 2003). *Sargassum* forest mats constitute nurseries (Mukai, 1971, Ornellas and Coutinho, 1998, Barrabe, 2003, Tanaka and Leite, 2003, Abruto-Oropeza *et al.,* 2007) and host many invertebrates. As an example, in New Caledonia, for equivalent surfaces there would be four times more molluscs and crustaceans in *Sargassum* forests than in seagrass beds (Barrabe, 2003).

I.2.4.2 Proliferation

In the context of reef degradation, the relative importance of both nutrients and herbivores on algal proliferation has been studied (Littler *et al.,* 2007). Bottom up (ie: regulation of populations by sources of nutrients) and top down (ie: regulation of populations by higher trophic level pressures) are partly responsible for the colonization success of macroalgae in tropical reefs (Aronson *et al.,* 2003, Hughes *et al.,* 2003, McManus and Polsenberg, 2004). As an example, the development of *S. filipendula* along the coasts of Martinique in the late 80's was attributed to increased fishing pressure and sewage release (Littler *et al.,* 1993).

Cross-shelf transplant experiments demonstrated herbivory pressure to play an important role in the regulation of *Sargassum* spp. survival on mid-shelf reefs of the Great Barrier Reef (McCook, 1997). These findings suggested both low algal dispersal and herbivory pressure prevented *Sargassum* development on most mid-shelf reefs. However, as discussed by Lapointe *et al.,* (2005) nutrient concentrations were not considered at experimental sites despite the fact that reported CNP ratios suggested a nitrogen and phosphorus limitation. Herbivory/nutrient manipulative experiments are often too short in time to draw any safe conclusions; moreover, ambient nutrients background levels often exceed limiting levels to macroalgal growth and make it impossible to determine the importance of herbivory or nutrient pressure (Littler *et al.,* 2006b). Both phenomena may result in algal proliferation.

In addition to coping with anthropogenic and natural disturbances *Sargassum* has intrinsic characteristics which give it good proliferating abilities: i) *Sargassum* spp. are perennial or pseudo-perennial and have an extended reproduction period, ii) they may disperse at short or long distances, iii) their life cycle is relatively simple (monophasic), flexible and can adapt to several environments: shallow water populations (more impacted by swell) persist by survival of reproductive individuals and fertility, whereas deepwater populations do so by survival of non-reproductive individuals and vegetative growth (Engelen *et al.,* 2005a). *Sargassum polyceratium* has developed a "longer-lived" algal strategy (ie: elasticity values for survival are, generally, an order of magnitude higher than those for fertility). However, in case of a strong disturbance (such as hurricanes), it can switch to characteristics representative of "short-lived" plants (Engelen *et al.,* 2005a). This flexible, depth-dependent strategy that is adjusted to disturbance events (e.g. hurricanes) might confer on this seaweed the ability to develop, colonize and dominate numerous areas subjected to stochastic events. Other species such as *S. pacificum* were classified as r-strategy species, which enables them to colonize and spread very quickly in various types of environment (Stiger and Payri, 1999a). R-strategy organisms are characterised by a fast reproduction and a high production of propagules, allocating the maximum energy to reproduction.

Its polymorphy could also give this genus advantages over others to proliferate in areas with different environmental conditions. *S. polyceratium* adult intertidal plants, subjected to strong wave action are more compact and possess more axes on the holdfast than deeper plants (De Ruyter van Stevenick and Breeman, 1987). Juvenile length is shorter and the holdfasts are bigger in wave impacted areas (Engelen *et al.,* 2005a). *Sargassum* plants from

Philippines have longer thallus lengths in the subtidal rather than in the intertidal (Ortiz and Trono, 2000). Morphological differences could be due to hydrodynamic conditions, light exposure or desiccation phenomenon for algae subjected to tidal regimes (De Ruyter van Stevenick and Breeman, 1987, Ortiz and Trono, 2000, Engelen *et al.,* 2005a). An hypothesis is that morphological plasticity could give this alga advantages in order to develop in various habitats.

I.2.4.3 Interaction with other species

Many studies have focused on the relationships between reef benthic species and macroalgae. Among them, the herbivory pressure and competition mechanisms have received attention.

Herbivores

Herbivory pressure is an important factor that can maintain low algal biomass on benthic substrata, especially in tropical areas (Hay and Fenical, 1988). Fish belonging to the genera *Acanthurus, Naso, Kyphosus,* and *Siganus* are the main herbivores ingesting *Sargassum* species (Fox and Bellowood, 2008, Tolentino-Pablico *et al.,* 2008). However, some species such as *Siganus canaliculatus* were responsible for algal removal while others such as *Siganus doliatus* were suggested to graze only on epiphytes (Figure 14).

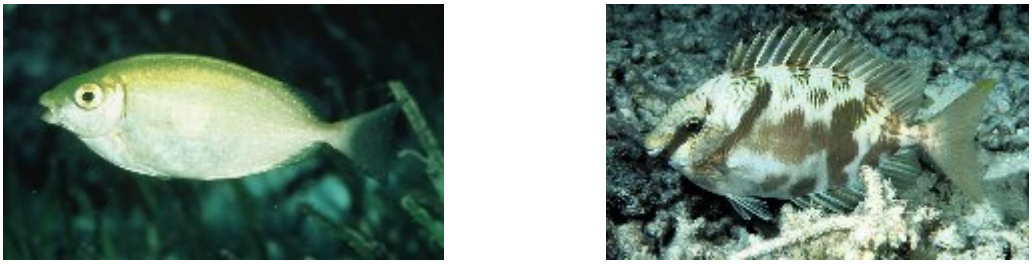


Figure 14: *Siganus canaliculatus* (left) and *Siganus doliatus* (right) (Randall fishbase).

Remote video assays conducted on the Great Barrier Reef (Australia) demonstrated *Sargassum* consumption by herbivores to vary with reef location, being higher on reef crests, lower on reef slopes and bases, and almost nonexistent on mid reef flats and inner reef flats (Fox and Bellowood, 2008, Hoey and Bellwood, 2009). In their study, the reef displayed five distinct areas depending on benthic composition ranging from algal zone (close to the shore) to coral dominated areas (reef crest and slope). Videos demonstrated juvenile Scarids, Siganids and Acanthurids and Pomacentrids to be the main grazers on

shoreward zones. The fact that *Sargassum* abundance is very low on shoreward zones suggests these fish feed preferentially on epiphytes (Fox and Bellwood, 2008). Thus S*argassum* consumption depends on fish genus, species and age, the algal location but also the algal species. *Sargassum cristaefolium* was eaten less than *Sargassum swartzii*, suggesting a differential response of herbivores due to variations in morphological, chemical defenses and/or quality of algae (Hoey and Bellwood, 2009). To resist herbivory pressure, macroalgae have developed several strategies such as: occupying refuges; growing rapidly to counter the loss caused by grazing; close association with unpalatable organisms and defense mechanisms (toxins, digestion inhibitors, secondary metabolites, reduced calorific contents, morphological shapes, texture, nutritional value) (Littler *et al.,* 1983a). Morphological traits were already implicated with thick leathery species mechanisms of defense (Littler and Littler, 1983b). Similarly Pennings and Paul, (1992) showed the toughness differences in *S. cristaefolium* and *S. polycystum* to be directly related to grazing pressure by an opistobranch gastropod.

The effect of secondary metabolites on herbivores has been investigated in laboratory-based experiments by various authors. Thus *S. furcatum* was demonstrated to be palatable to the amphipod *Parhyale hawaiensis* (Pereira and Yoneshigue-Valentin, 1999) and the crab *Pachygrapsus transversus* (Pereira *et al.,* 2000), however, it was less palatable to the gastropod *A. latispina* as compared to other algal species (Pereira *et al.,* 2002). Polyphenol production by Phaeophyceae is known to protect them against herbivores in temperate regions, but it is not an important protection mechanism in tropical regions where low levels of polyphenols were recorded in tropical species (Steinberg, 1986, Steinberg and Paul, 1990, Pereira and Yoneshigue-Valentin, 1999). It remains unknown why these metabolites, which could be active against herbivores at higher concentrations (Pereira and Yoneshigue-Valentin, 1999), are not produced in higher quantities by tropical brown algae. Phlorotanins produced by *S. filipendula* do not affect its susceptibility to herbivores (Cronin and Hay, 1996) and this species does not seem to produce unusual secondary metabolites and is fairly palatable to herbivores (Paul and Hay, 1986).

Actual conditions, marked by losses of important reef herbivores, such as the sea urchin *Diadema antillarum* in the Caribbean (Lessios *et al.,* 1983, Lessios *et al.,* 1984,Hawkins and Roberts, 2004) may result in algal proliferation. Even though *Sargassum* seems to be fairly palatable to some fishes, (up to 90% of its length can sometimes be removed (Hoey and Bellwood, 2009), their percentage cover remains very high on degraded reefs. This

suggests that either the number of herbivores is too low to remove efficiently the algal biomass and thus prevent or slow down the fast colonisation mechanism observed, or that the algal mechanisms of proliferation and/or defense are too good.

Interaction with benthic organisms

In addition to top down and bottom up processes previously discussed, competition is an important phenomenon structuring benthic communities especially in tropical environments harbouring high biodiversity. Following the coral-algal phase shift, impacts of macroalgae on reef species and their possible resilience were studied (Reviewed by (McCook *et al.,* 2001). The large dominance of *Sargassum* species has profoundly modified the landscape of many ecosystems. Studies focusing on *Sargassum* demonstrated this thick leathery species to induce strong shading or abrasion effects on coral colonies (River and Edmunds, 2001, McCook *et al.,* 2001). Contact of *S. hystrix* with adult *Porites porites* (coral) decreases coral growth rate by 80% (River and Edmunds, 2001). *Sargassum muricatum* and *Sargassum tenerrimum* induced modifications of swimming activity of 2-day-old *Platygyra daedalea* (Coral) larvae and decreased their percentage settlement (Diaz-Pulido *et al.,* 2010).

Sargassum presence has not always been correlated to reef degradation. Coral cover can remain high in the presence of *Sargassum* mats (McCook *et al.,* 1997) and *Sargassum* shading can be beneficial to coral (without species restrictions) in the case of a bleaching event (Jompa and McCook, 1998). The algae were considered to reduce the damage caused to coral species by decreasing UV, temperature or reducing the mixing of low salinity waters (Jompa and McCook, 1998). *Sargassum* lipid-soluble extracts from both Pacific and Caribbean areas did not affect bleaching and mortality of *Porites cylindrical* and *P. porites* (Rasher and Hay, 2010). These results contrast with those of River and Edmunds, (2001), and may be due to algal abundance as they differed in both studies.

Both physical and chemical mechanisms of defense are thought to play a role in competition mechanisms. A few studies have been carried out on tropical *Sargassum* sp. secondary metabolites and their potential biological activities (Table 3). Antibacterial activity was noticed in the species *S. wightii* and *S. johnshonii* (Sastry and Rao, 1994), phlorotannins obtained from *S. vestitum* and *S. natans* displayed an antifouling activity (Sieburth and Conover, 1965, Jennings and Steinberg, 1997) and *S. horneri* had antialgal

activity (Tanaka and Asakawa, 1988). *Sargassum vulgare* from Brazil possesses antifouling properties, the hexane extracts having the highest activity towards microalgae and the methanolic ones being more efficient against mussel settlement, suggesting both polar and non-polar molecules play a role in antifouling (Plouguerné *et al.,* 2010a).

Table 3: Biological activity of some *Sargassum* species extracts. The extraction procedures as well as the active concentrations are given in the table. MIC= Minimum Inhibitory Concentration; LC_{50} = Median lethal dose and EC_{50} = half maximal effective concentration.

Activity	Species	Organisms	Solvent used for extraction	Active concentrations	Author
Chemical effects					
Antialgal					
	S. vulgare (Brazil)	*Cylindrotheca closterium* *Chlorarachnion globosum* *Pleurochrysis roscoffensis* *Rhodella cyanea* *Scenedesmus armatus*	Acetone/water, dichloromethane, hexane, methanol,	MIC=0.2µg.mL	Plouguerné *et al.,* 2010a
Antibacterial					
	S. vulgare (Brazil)	*Vibrio aestuarianus* *Pseudoalteromonas elyakovii* *Staphylococcus aureus,*	Acetone/water, dichloromethane, hexane, methanol,	MIC=0.2µg.mL	Plouguerné *et al.,* 2010a
	Sargassum sp., (Venuzuela)		Hexane	-	Rios *et al.,* 2009
	*S. johnstonii (*India)		Diethyl ether and lipid extracts		Padmini Sreenivasa Rao *et al.,* 1986
	S. tenerrimum *S. wightii and S. Johnshonii*	*4 strains unknown*	Phlorotannins Chloroform	1mg per 6mm disc	Lau and Qian, 1997 Sastry and Rao, 1994
		Halomonas marina P. elyakovii Polaribacter irgensii *V. aestuarianus* *Bacillus subtilis* *Enterobacter aerogenes* *Escherichia coli* *Pseudomonas aeruginosa S. Aureus*		From 150µg.mL-1	Thabard *et al.,* 2011
Inhibition of embryonic development					

Activity	Species	Organisms	Solvent used for extraction	Active concentrations	Author
Inhibition of larval metamorphosis	*S. polyceratium* (Martinique)	*Diadema antillarum* *Pseudonereis sp* *Codakia orbicularis*	Hexane dipping extracts	From 5µg.mL-1	Thabard *et al.,* 2011
inhibitory towards byssal attachment	*S. tenerrimum*	*Hydroides elegans*	Phlorotannins	LC_{50}=13.984ppm EC_{50}=0.526ppm	Lau and Qian, 1997
Modify larval behaviour	*S. vulgare* (Brazil)	*Perna Perna*	Acetone/water, dichloromethane, hexane, methanol,	Natural concentrations	Plouguerné *et al.,* 2010a
Nematicidal	*S. thunbergii, S. confusum, S. tortile*	*Coryne uchidai*			Nishihira, 1968
Settlement inducer	*S. tenerrium* (Pakistan)	*Meloidogyne javanica*			Rizvi, 2006
Physical effect	*S. tortile*	*C. uchidai*			Kato *et al.,* 1975
	S. hystrix *Sargassum sp.* (GBR)				River and Edmunds, 2001 Jompa and McCook, 1998

Summary: Tropical Sargassum species seem to have developed several strategies and have optimised their biology, physiology and morphological traits to adapt to numerous or changing environmental conditions. They possess all the required characteristics to make them a good invasive and dominant species. This genus i) maximises its chances of colonisation through several dispersal strategies, long fertility periods and the protection of zygotes on parent plantss. ii) It can adapt to various environmental conditions as it is highly polymorphic, possesses a flexible life cycle and utilises several nutrient sources. iii) Once established, it increases its chances of competition. It is perennial or pseudo perennial, has a thick leathery morphology and produces active substances. Grazing could potentially reduce this algal abundance and allow for the recovery of degraded reefs, but the number of herbivores (fish, urchins) and/or Sargassum biology, make this possibility unlikely. Current conditions (nutrient increases coupled with decreased herbivore numbers) further increase its good colonising capabilities. Studies investigating the potential effect of this genus on other reef invertebrates its competition mechanisms with other reef could give a better insight into how it can colonise.

I.3. The present study

I.3.1. General information about Martinique

I.3.1.1 Localisation and economy

Martinique is a volcanic island located in the eastern Caribbean Sea (Lesser Antilles), between the latitudes 14˚50'N and 14˚23'N and at the longitude of 62˚12'W (Figure 15, Figure 16). This overseas region of France, with a land area of 1,128 km², is surrounded by the Caribbean Sea on the west side and the Atlantic Ocean on the east side. The north of the island is mountainous and highly forested due to a wet climate. The Mount Pelée volcano (1396 m) has created grey and black sand beaches in the north, contrasting markedly from the white sands in the south.

Figure 15: Location of Martinique in the Caribbean region (http://www.geographicguide.com/caribbean.htm).

Two main seasons are observed in this area: the dry season from December to July and the wet season from August to November. The population (401 000 inhabitants) has increased by 10% over the past 15 years (INSEE; www.insee.fr).

Figure 16: Map of Martinique (i-google-map.com).

Tourism is one of the key economic sectors of the island. The industries are settled in 2 main areas: the bay of Fort de France (Middle West of the Isle) and the Bay of Le Marin (South). Martinique agricultural production has decreased from 105.4 to 91.8 (Agricultural production index) while the total fertilizer use has increased by 3980 MT and the pesticides imports by $ 15 269 000 between 1961 and 1995 (Rawlins 1998). Importation of fertilizers and pesticides were recorded over the past years and are presented in the Table 4.

Table 4: Fertilizers and pesticides imports in Martinique from 2005 to 2007 (DIREN, 2009a).

In Tonnes	2005	2006	2007
FERTILIZER			
From animal origin	873	253	478
Nitrogenous	7207	7703	7663
Phosphorous	5	0	0
Potassic	11807	9043	9789
Binary and ternary	7203	5129	5027
Total	27096	22128	22957
PESTICIDES			
Insecticides	740	548	626
Fungicides	116	120	90
Herbicides	380	334	222
Total	1236	1002	938

Fishing in Martinique is mainly traditional and occurs near the coast. Overfishing is a known phenomenon in the island where the demographic pressure is important and fish consumption high (around 50 kg per habitant per year, Ramdine, 2004). The fishery pressure is 10 to 20 times higher in Martinique than in the neighbouring islands (Gobert, 2000). The local fishery committee and the regional council thus decided to creat nine non-permanent no-take zones between 1999 and 2002. Only eight of them still exist (Figure 17).

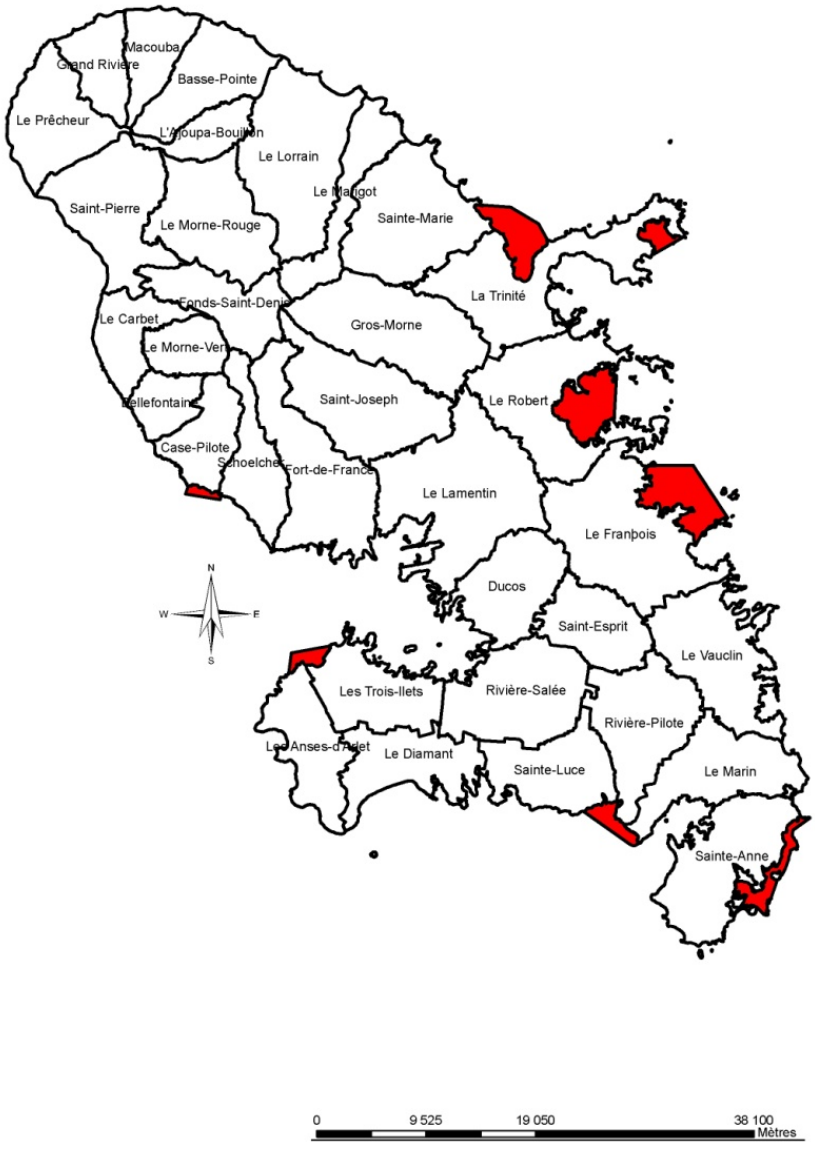


Figure 17: Location of the 8 no-take zones in Martinique, FWI.

Fishermen can decide on the closure and reopening dates, through administration decrees proposed by the Regional Fishery Committee to the Prefet Administration. Since their

creation, some no-take zones have been reopened for a few months, for experimental fishing campaigns (Table 5).

Table 5 : No-take zone details for the Martinique island until 2007 (Maréchal, 2008).

No-Take Zone	Date	Area protected	Status
Baie du Trésor	01/08/1999	228 Ha / 653 acres	Experimental fishing campaign 04/01/2006 to 09/30/2006 (Decree n°06-1049 03/27/2006) 07/01/2007 to 09/30/2007 (Decree n°07-2041)
Ilet à Ramiers	06/27/1999	184 Ha / 455 acres	Experimental fishing campaign 07/01/2007 to 10/31/2007 (Decree n°07-2042 06/29/2007)
Sainte Luce	12/29/1999	290 Ha / 717 acres	Experimental fishing campaign 04/01/2006 to 09/30/2006 (Decree n°06-1048 03/27/2006) 07/01/2007 to 10/31/2007 (Decree n°07-2043 06/27/2007)
Baie du Robert	03/23/2000	953 Ha / 2.355 acres	Closed since beginning
Trinité/Sainte Marie	02/01/2002	799 Ha / 1.974 acres	Experimental fishing campaign 07/01/2007 to 10/31/2007 (Decree n°07-2043 06/27/2007)
Petite Anse	03/12/2002	46 Ha / 114 acres	End March 2005
Case Pilote	09/12/2002		Closed since beginning
Sainte-Anne/Cap Chevalier	10/22/2002	447 Ha / 1.105 acres	Experimental fishing campaign 04/01/2006 to 09/30/2006 (Decree n°06-1047 03/27/2006) 07/01/2007 to 10/31/2007 (Decree n°07-2043 06/27/2007)
Baie du François	10/24/2005	91 Ha / 225 acres	Closed since beginning
TOTAL		3.038 Ha / 7.507 acres	

I.3.1.2 Reef construction and health status

Three types of coral reef formations can be observed in Martinique (Bouchon and Laborel, 1986, Legrand *et al.,* 2008) (Figure 18, Figure 19).

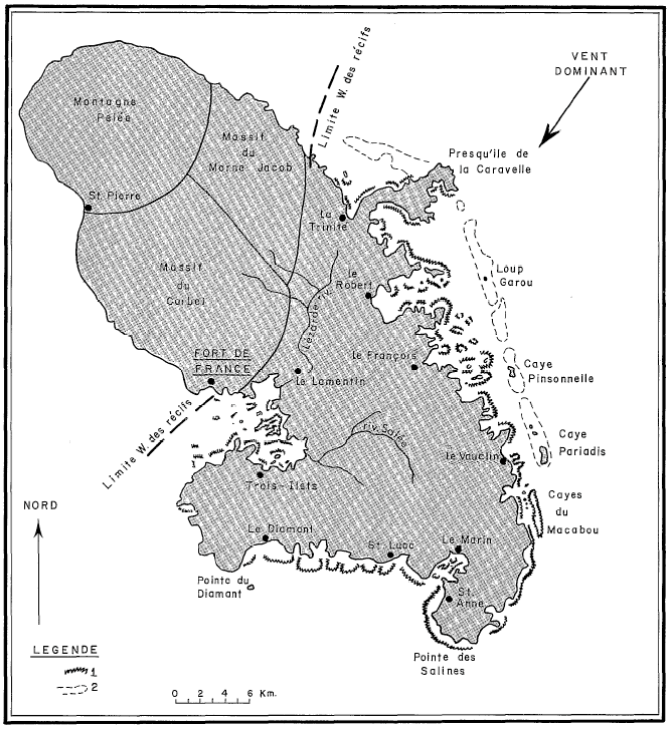
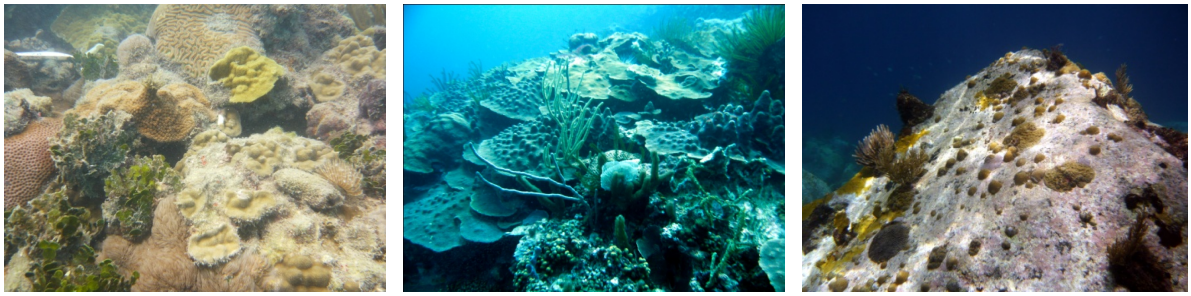


Figure 18 : Distribution of Martinique reefs 1) Fringing reefs, 2) Barrier reefs (from Battistini, 1978)



a b c

Figure 19: Reef geomorphology in Martinique. a) Barrier reef at Le François (14°37'57''N 60°51'23''W), b) Fringing Reef at Anse Mabouya (14°27'37''N 60°57'45''W), and c) Non constructive coral, at Cap Salomon (14°30'37''N 61°06'14''W).

- The barrier reef: the Atlantic coast (from the Caravelle to the south point of the island) comprises a subtidal platform ended by a fringing coral reef barrier (Figure 19a). The water running off from the land does not flush very well in this kind of conformation and thus can be responsible for the accumulation of toxicant in the coastal area. The reef is highly degraded and almost entirely covered by macroalgae and CCA.

- The fringing reef is present in the south of the island and extends from a few meters up to 1 km (Figure 19b). The reef health state varies with the site and goes from highly degraded to good.
- Non-constructive coral are found on the Caribbean coast, mainly in the north where the corals develop on rock substrata (Figure 19c).

Martinique coral reef composition and health vary widely depending on the area studied.

Studies conducted on Martinique reefs are recent and very few publications were published (Battistini in 1978, Bouchon and Laborel, 1986, Littler *et al.,* 1993, Maréchal, 2008, Legrand et al, 2008 and Rousseau *et al.,* 2010, Legrand, 2010, Rousseau, 2010). A few surveys were conducted in the 1980s' but reef monitoring only started with the program IFRECOR (Initiative FRançaise pour les REcifs CORalliens) in 2001 (4 stations monitored twice a year). Other monitoring programs have now been developed, among which the EU Water Framework Directive (15 stations monitored since 2007 but not every years), and the Reef Check program, (1 station monitored since 2009). Even though these programs are recent, some tendencies were already drawn. As an example, the results obtained through the IFRECOR program tend to show reef degradation at all sites (Figure 20).

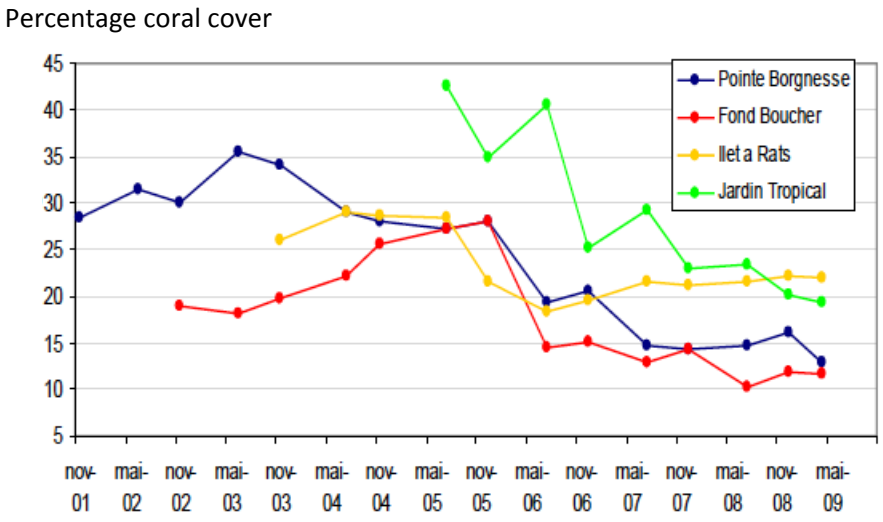


Figure 20: Coral percentage cover at the 4 stations monitored for the IFRECOR program from 2001 until 2009 (OMMM, personal communication).

Among the reasons responsible for this degradation, bleaching events of 2005 seems to have been responsible for an important coral loss, about 14% of the coral colonies in Martinique (Legrand *et al.,* 2008). More chronic events such as overfishing and terrestrial run-offs may also be responsible for the observed changes.

I.3.2. Thesis outline

Many coral reefs are threatened worldwide, sometimes inducing a shift from coral to macroalgal-dominated reef. The new state will inevitably have many implications for benthic reef species health and integrity as well as biodiversity. Gaps of knowledge still remain regarding the impacts that macroalgae can have on other benthic species' development, survival and recruitment. The resilience of coral reefs requires the colonisation of the substratum by reef building species, such as coral larvae, and the recovery of key regulatory species such as the herbivore *Diadema antillarum*. Such recovery is based on recruitment of these species on favourable substrata, which is one of the most precarious and crucial phases in the life cycle of marine species. Larvae are subjected to numerous physical, chemical or biological signals and their chances of metamorphosing into juveniles are scarce. Understanding the effect of algae on the recruitment of these species is of great importance for reef management.

In the present work, it was hypothsised that the presence of macroalgae (with emphasis on *Sargassum* species) may interfer with benthic coral reef species' recruitment through physical and / or chemical means. To address the overarching research goal, experiments were conducted from the field to the laboratory through three main steps: i) field observations (Chapter II), ii) manipulative experiments conducted in the field (Chapter III) and iii) laboratory experiments (Chapter IV).

Chapter II: Habitat types and juvenile presence

To determine wether the presence of coral juveniles was habitat dependent, south reefs of Martinique were investigated. It was hypothesised that the presence of coral juvenile would be reef habitat-dependent; their biodiversity and density being lower in macroalgal fields, intermediate in *Diadema antillarum* areas and the highest in coral dominated reefs, where most of the parent colonies are. In order to test this hypothesis, several habitats were investigated: *algal,* *coral* and *urchin,* dominated areas of Martinique reefs. This study aimed at i) giving information about reef habitat types in Martinique, ii) providing insight to the relationships between the habitat types and coral juvenile presence iii) and highlighting the potential impacts of the presence of sea urchins and algae on coral recruitment.

Chapter III: Impacts of algal canopies with emphasis on *Sargassum* species on field recruitment

Giving the conclusions drawn in Chapter II, impact of macroalgae (with emphasis on *Sargassum* species) was investigated towards recruitment. Two main hypotheses were tested in this chapter: i) macroalgae may have a negative impact on recruitment processes ii) *Sargassum* species may impact the recruitment through physical and / or chemical mechanisms. Field experiments, involving artificial substrates, were conducted on the reef to determine the potential effect of algae on the recruitment of benthic larvae. Particular attention was given to both the physical and chemical effects of *Sargassum* on the marine benthic invertebrate larvae. To test this, a manipulative experiment involving recruitment tiles was carried out on the south reefs of Martinique.

Chapter IV: *Sargassum sp.* surface molecules and waterborne cues effects on the development of benthic invertebrate embryos

Finaly, the conclusions of the manipulative experiment lead to laboratory based studies in order to further investigate the effects of molecules produced by *Sargassum* sp. on marine invertebrate embryos. The hypothesis was that marine natural products (MNP) produced by the alga could have a negative impact on benthic embryonic development and/or settlement. This study focused on molecules that could potentially interact with surrounding organisms ie: produced "outside of the algae". Thus, i) the effect of the molecules produced in the water column by *Sargassum* and ii) the effect of MNP produced at its surface on embryonic development of tropical invertebrates were evaluated.

The overall conclusions are summarized and discussed in **Chapter V.**

Chapter II: Reefs of Martinique: Biodiversity and Recruitment.



II.1. Introduction

Profound modifications of coral reef ecosystems have occurred over the last decades, sometimes leading to a "phase shift phenomenon", a dynamic process resulting in a shift from a coral dominated reef to an algal dominated reef. Coral reef degradation in Martinique has been reported for the past decades. Important algal growth occurred in the mid 1980's on the southern coast and near Fort de France, while reef overgrowth by *Sargassum* on the west coast has been noticed since the 1970's (Battistini, 1978, Bouchon and Laborel, 1986, Littler *et al.,* 1993). Seaweeds rapidly colonised coral reefs and are nowadays present all around the island (Chauvaud, 1997, Legrand, 2010). Even if the phase shift phenomenon from algae to coral does not seem to be a worldwide phenomenon (Bruno *et al.,* 2009), it is widely spread in Martinique (Figure 21), where the sever coral shift thresholds defined by Bruno *et al.,* (2009), (coral cover inferior to 10% and macroalgal cover superior to 60%) are observed on many reefs.

Reef communities monitoring is recent in Martinique, it could thus be possible that degraded sites around Martinque have always been of relatively poor quality. However, observations and bioassays carried out in 1987 supported the hypothesis of a recent shift between algal and coral communities near Fort de France (Littler *et al.,* 1993). During this survey Littler *et al.,* (1993), found that the percentage cover of *Sargassum* represented between 55.9% (at Cap Salomon) and 0.1% (at Pointe Borgnesse). Moreover, some sites of the Northern Caribbean coast have recently been colonised by *Sargassum* species (personnal observations), suggesting that this conforms to the phase shift phenomenon as described elsewhere in the Caribbean.

Numerous causes are responsible for this ecological change (Chapter I), including eutrophication, overfishing, climate change and diseases (McManus and Polsenberg, 2004). In the Caribbean, the mass extinction of the sea urchin *Diadema antillarum* is often described as a major cause of reef degradation (Lessios *et al.,* 1983, Lessios *et al.,* 1984). The pathogen responsible for its extinction is unknown but is thought to be a bacterium (Harvell *et al.,* 1999). While twenty-eight major epidemics have been reported in the Caribbean since 1980 (Harvell *et al.,* 1999), the massive extinction of *D. antillarum* is considered to be the most severe mortality recorded so far in marine animals (Lessios *et al.,* 2001).

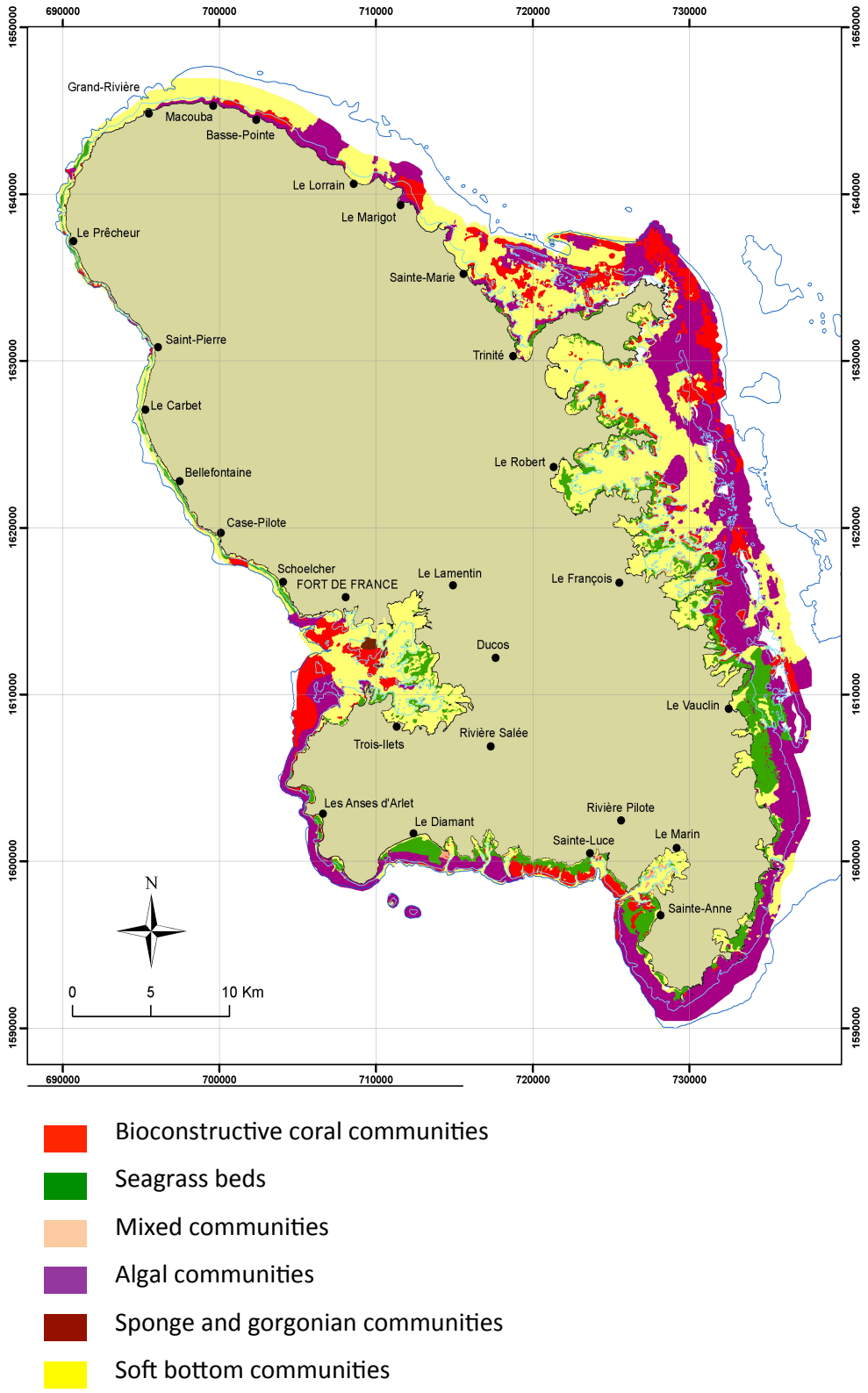


Figure 21: Distribution of benthic communities along the coast of Martinique (Legrand, 2010).

D. antillarum is described by many authors as a key stone species which can erode coral reefs, eat live coral, compete with other herbivores and influence algal cover and diversity (Lessios *et al.,* 1984a, Hughes *et al.,* 1999a, Knowlton, 2001). Its recovery is a very slow process, which is surprising considering its life history (Lessios, 2005). Recent observations attest for its possible recovery, which could be associated with a decrease in macroalgal cover and an increase in coral recruitment (Edmunds and Carpenter, 2001, Carpenter and Edmunds, 2006, Idjadi *et al.,* 2010).

Macroalgal development has led to important modifications of reef ecosystems (Chapter I). Seaweeds, directly or indirectly, may influence the ecology of coral reef benthic communities by modifying physical (current motion, light penetration), chemical (production of secondary metabolites, nutrient and oxygen uptake) and biological (competition, symbiosis) tutors. They are believed to decrease coral recruitment and kill live coral by both physical (such as sweeping), and chemical (production of toxic compounds) mechanisms, (Tanner, 1995, Lirman, 2001, McCook *et al.,* 2001, River and Edmunds, 2001, Jompa and McCook, 2002b, Jompa and McCook, 2003, Nugues and Roberts, 2003a, Birrell *et al.,* 2005, Mumby *et al.,* 2005, Titlyanov *et al.,* 2005, Box and Mumby, 2007). Scleractinian corals possess a bentho-pelagic life cycle, thus the resilience of coral populations depends upon their recruitment (Caley *et al.,* 1996). The whole ecology of coral reefs could be modified by the presence of macroalgae. As reef resilience is partly controlled by larval supply and development, it is interesting to observe which coral recruit, if any, can settle and develop in these areas.

This study sought to describe the biodiversity of the Southern Martinique reefs, and to determine i) the differences in benthic composition between the ecological areas that can be distinguished on south coral reefs in Martinique ii) the coral juvenile occurence and diversity in these areas and iii) the association between coral juveniles and other benthic species. In this chapter, it was hypothesised that juvenile coral densities (and maybe diversity) are dependent upon habitat, being lower in *algal* beds, higher in *coral* areas and intermediate in *urchin* areas where the sea urchin could promote coral recruitment (Carpenter and Edmunds, 2006).

II.2. Material and methods

The present study was based on Carpenter and Edmunds (2006) protocols. In their experiment they selected adjacent reef sites with high densities of *Diadema antillarum* (*urchin* zone) or low densities of this urchin and high percentage cover of macroalgae (*algal* zone). According to their study *Diadema antillarum* recovery may promote coral recruitment.

Based on their experiment and preliminary surveys conducted on southern reefs of Martinique, 3 areas were selected to carry out the present survey:

i) Dead coral reefs dominated by macroalgae (*algal* zones),

ii) Coral reefs in good health state with high percentage coral cover (*coral* zones),

iii) Intermediate reefs with low coral and algal cover. These zones presented high urchin densities and were thus described as *urchin* zone.

II.2.1. Study sites

In order to decrease the variability due to environmental conditions and reef configurations the present experiment focused on 5 sites located along the South coast of Martinique with the same geological and coral conformation i.e.: fringing reefs, constituted of a reef flat (to about 12 m depth) and a reef crest ended by an external slope (See Chapter I for a description of Martinique Reef geomorphology). Because the main objective was to analyse the relationships between the habitat types (coral dominated, seaweed dominated or intermediate with high urchin densities) and coral juvenile occurrence, sites where one of the 3 areas were observed during preliminary surveys were selected.

When possible, sites presenting the 3 ecological areas within a few 100 m^2 were choosen in order to attribute the observed effects to benthic community presence and not to environmental conditions. When the 3 ecological types were not present within the same site, adjacent reef habitats (present in the same water body and on the same reef) were selected to decrease the variation linked to environmental conditions (Figure 22).

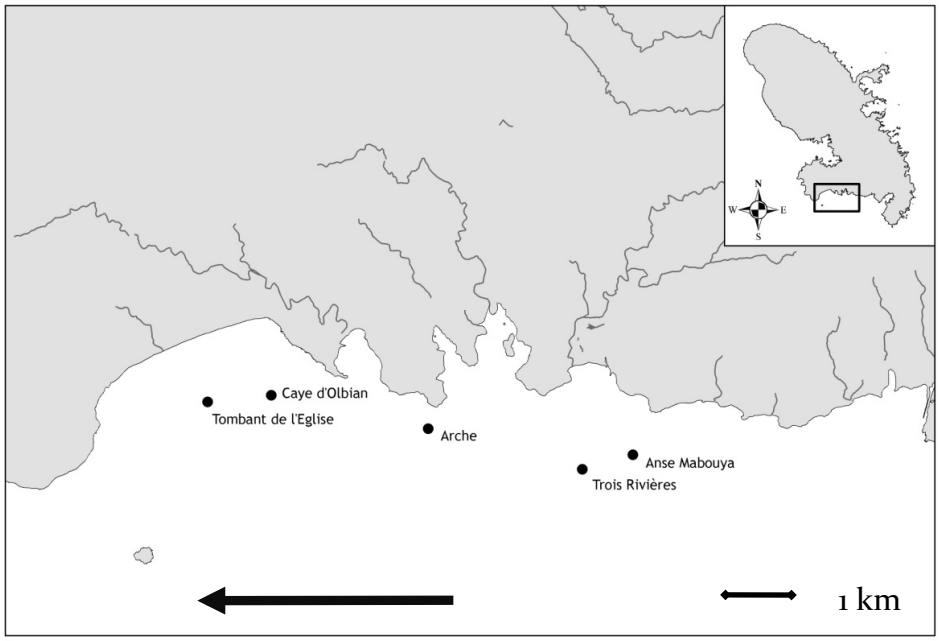


Figure 22: Map of Martinique showing the location of the study sites: Anse Mabouya, Arche, Caye d'Olbian, Tombant de l'Eglise and Trois Rivières along the south coast of Martinique. The arrow represents the main direction of currents.

Five sites located along the south reefs flat (near the reef crest) between 10-12 m depth were thus investigated (Table 7, Figure 23). It was essential for this study to focus on areas of the same depth as this parameter influences coral recruitment pattern (Penin, 2007).

Caye d'Olbian (14°28'06"N, 61°1'05"W) is located in the vicinity of the town le Diamant and presents all of the 3 typical areas on the reef flat within 10-12 m depth. The *algae* area is located on the west side of the site, followed by the *urchin* area and then the *coral* one. All these areas represent a few 100 m².

Trois Rivières (14°27'22"N, 60°58'03"W) and Anse Mabouya (14°27'43"N, 60°57'32"W) are located on the same reef near the river mouth of Rivière Oman, but present distinct characteristics. This river is surveyed biologically and physico-chemically (Table 6) for the Water Framework Directive (more formally the Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy).

Table 6: Physico-chemical characteristics of the River Oman in 2008 (DIREN, 2009b).

Measurement	Oman river	healthy rivers
Dissolved oxygen ($mg.l^{-1}$)	7.87	8-6
Saturation in dissolved O2 %	98.3	90-70
Organic Carbon ($mg.l^{-1}$)	2.8	5-7
PO43- ($mg.l^{-1}$)	0.05	0.1-0.5
Total P ($mg.l^{-1}$)	0.03	0.005-0.2
NH_4^+ ($mg.l^{-1}$)	0.05	0.1-0.5
NO_2^- ($mg.l^{-1}$)	0.05	0.1-0.3
NO_3^- ($mg.l^{-1}$)	2.49	10-50
pH min	7.3	6.5-6
pH max	7.6	8.2-9
Conductivity	417	
Chlorure	83	
Sulfate	8	
Particulate matter ($mg.l^{-1}$)	7.4	25-50
Turbidity (NTU)	10	

Trois Rivières has an algal and an urchin population on the reef flat between 10 and 12 m depth (Figure 24). The slope (not under investigation here) is also densely covered by macroalgae. The *urchin* and *algal* areas investigated form two distinc belts parallel to the reef crest, the urchin population being present closer to the crest. Anse Mabouya reef flat is a reef in good healthy state located between 10 and 12 m depths.

Tombant de L'Eglise (14°28'03"N, 61°1'42"W), located near le Diamant at 12-13 m depth, presents both a *macroalgal* and a *coral* zone. The algal populations are mainly present on the reef flat while the coral one develops from the reef flat until the reef slope. A sandy belt of 1 m width separates the two areas.

The last urchin zone was located further down the coast on the reef flat at Arche (14°27'46"N, 60°59'33"W) at 10 m depth.

Table 7: Characteristics of the investigated sites.

Site	Geographic Coordinate	Wave and current exposure	Depth of reef flat (near reef crest)	Geographic orientation of the slope	Source of disturbance	Substratum	Rate of sedimentation	Benthic characteristics
Caye d'Olbian	14°28'06"N 61°1'05"W	Weak (E→ W)	10-12 m	E→ W	Fishing, Diving Run-off	Coral built	Weak	Coral Algal Intermediate (Urchin area)
Trois Rivière	14°27'22"N 60°58'03"W	Weak (E→ W)	10-12 m	E→ W	Fishing Run off	Coral built	Weak	Algal Intermediate (Urchin area)
Anse Mabouya	14°27'43"N 60°57'32"W	Weak (E→ W)	10-12 m	E→ W	Fishing Run off	Coral built	Weak	Coral
Tombant de L'eglise	14°28'03"N 61°1'42"W	Weak (E→ W)	12-13 m	E→ W	Fishing, Diving Run-off	Coral built	Weak	Coral Algal
Arche	14°27'46"N 60°59'33"W	Weak (E→ W)	10-12 m	E→ W	Fishing, Diving Run-off	Coral built	Weak	Intermediate (Urchin area)

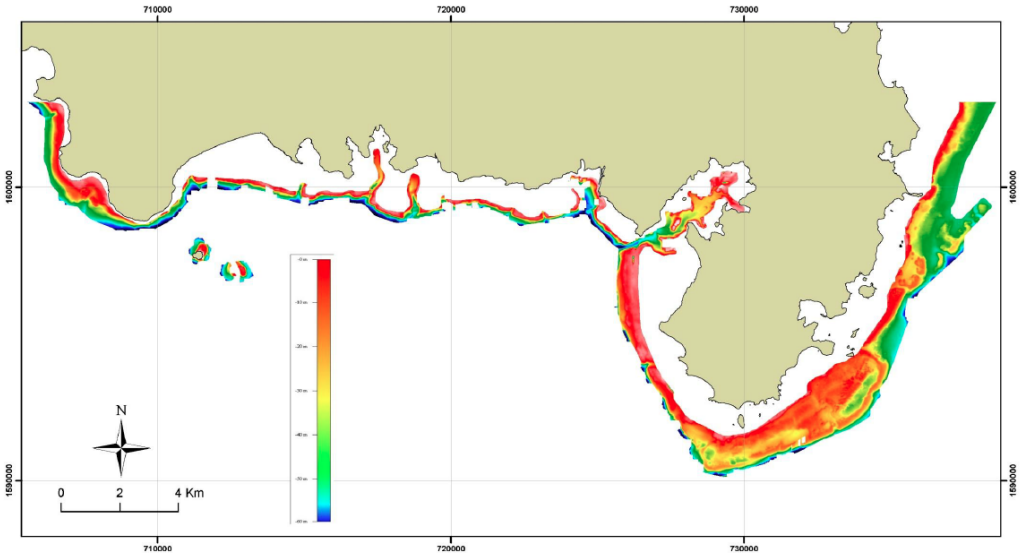


Figure 23: Bathymetric profile of the southern region of Martinique (Legrand, 2010).

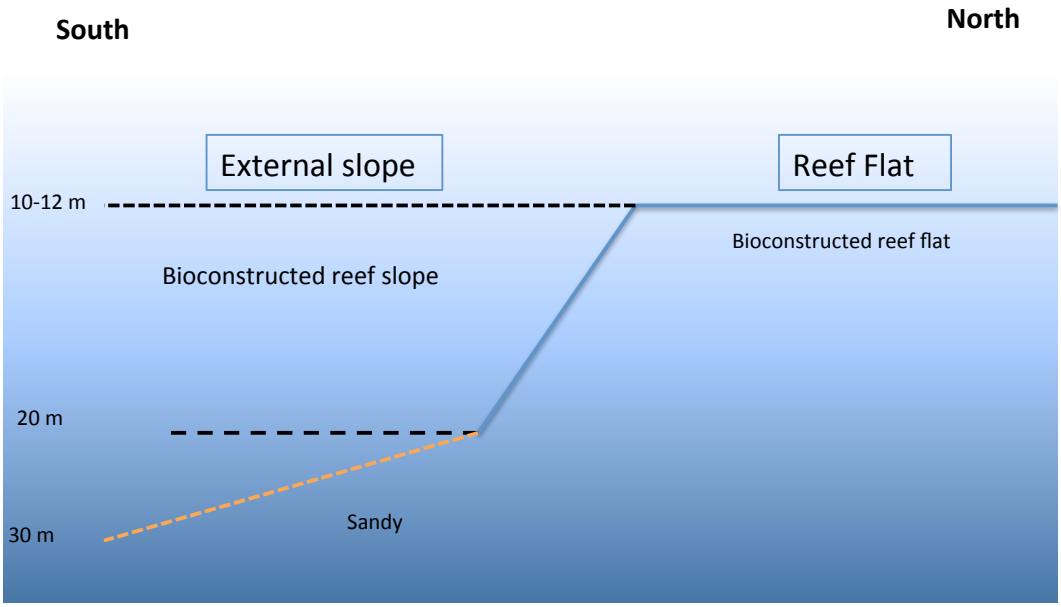


Figure 24: Reef configuration at Trois Rivière (from OMMM, 2005).

II.2.2. Survey methodology

The surveys were conducted at the 5 sites in November 2010. Observations were carried out by SCUBA diving at depths ranging between 10-13m. Two SCUBA divers carried out the work, each having one precise task to avoid bias of observation (Figure 27).

Leujak and Ormond, (2007), compared 6 commonly used coral reef survey methods and concluded regarding the accuracy, the precision, the time taken to record and analyse the data that VIDEO (video sampling) was the most efficient, followed by PHOTP (photo-quadrat analysed by point-sampling), followed by PHOTS (photo-quadrat analysed by outlining coral colonies), followed by LPT (line-point transect) that was most efficient than MAP (mapping of quadrat), followed by LIT (line intercept transect). As the device to perform VIDEO method was not available, the second most efficient method (PHOTP) was selected in the present study. Thus, at each site, 45 quadrat pictures were taken randomly by SCUBA diver 1 to characterise the benthic composition. A standard dispositive was employed to photograph quadrats of 50*50 cm with a camera Konica Minolta Dimage A2 (Figure 25).

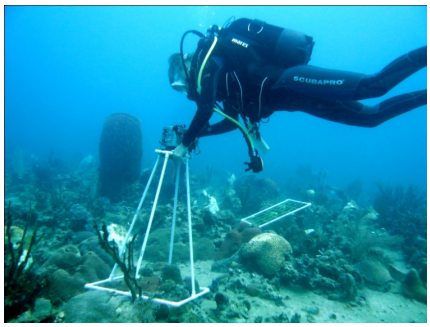


Figure 25: Experimental dispositive employed to photograph the 50*50 cm quadrats.

This protocol was adjusted for *algal* areas where most of the benthic species are hidden by the algal canopy inducing a false estimation of species richness. Thus 2 pictures were taken, the first one allowed for the determination of algal percentage cover, while the second (after removal of canopy forming algae) permitted to describe the understorey species richness (Figure 26).

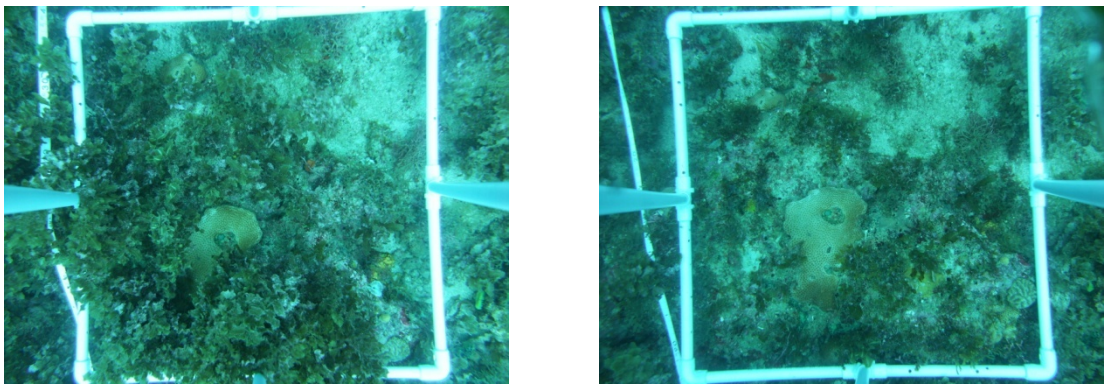


Figure 26: Pictures of *algal* zone 1) before and 2) after removal of seaweeds for determination of understorey benthic sessile organism percentage cover.

Simultaneously, SCUBA diver 2 was counting the number of coral spats in 30 quadrats of 50*50 cm randomly localised. Coral colonies ranging between 2-40 mm diameter, with circular outlines (attesting for colonies originated from larval settlement and not asexual proliferation) were considered as juveniles (Carpenter and Edmunds, 2006).

Finally the density of *Diadema antillarum* was determined using a 0.5 m measuring pole for scale. All the sea urchins were counted in a corridor of 0.5 m on both sides of three 15 m transects. The observation was made with caution as *Diadema antillarum* is cryptic during day time (Randall *et al.,* 1964).

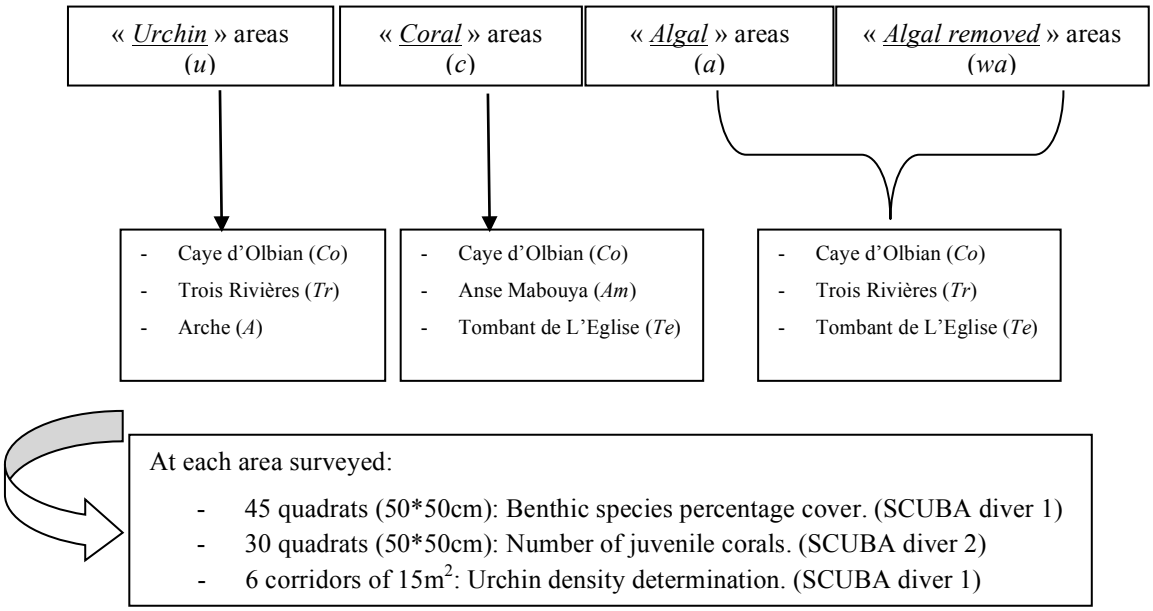


Figure 27: Schematic representation of the experimental design and the measures carried out in the field at the 9 areas surveyed. Indications are given regarding the task distribution between scuba divers 1 and 2. Abbreviations further used in the text are given.

II.2.3. Percentage cover determination

The pictures of each quadrat were cropped and the light corrected using Adobe Photoshop CS4. Then the percentage cover of benthic organisms was determined with the software CPCE 4.0 (Kholer and Gill, 2006). The method, consisting of overlaying points, was preferred to determine the percentage of each organism (Leujak and Ormond, 2007). The organisms present under each of the 200 points overlaid on the picture were determined to the species level when possible, allowing for the determination of both percentage cover and diversity index (Figure 28). Because diversity was an important factor in the study, the number of points overlaid on the picture was maximised in order not to omit small organisms.

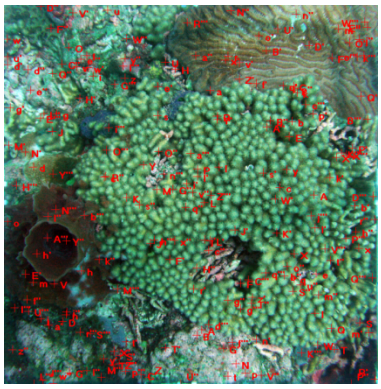


Figure 28: Estimation of benthic percentage cover using CPCE4. 200 points were overlayed.

II.2.4. Diversity index

The specific diversity, H', calculated from the Shannon Weaver Index, was employed to evaluate the benthic diversity in this study (Pielou, 1966).

$$H' = - \sum (pi \ lnpi)$$

pi= The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: (ni/N)
ni= The number of individuals in species, N= The total number of individuals.

II.2.5. Statistical treatment

All the data were statistically analysed with the software R (R, 2011).

First the benthic percentage data were tested for normality with a Shapiro-wilk test. As it is often the case with environmental data, they were not normaly distributed even after transformation. A pool of nonparametric tests was thus applied to the data (Table 8).

Table 8: Statistical procedure applied to the benthic organism percentage cover data (R packages employed for these analyses are indicated).

Statistical test	R package
Reduction of heteroscedasticity	Asin√p and log(x+1)
PCA + Hierarchical cluster	FactoMineR
Permanova (sites and groups)	Vegan
MRT (sites and groups)	Npmc

Prior the statistical analyses, the percentage data were Asin√p transformed following Legendre and Legendre's (1998) recommendation and the urchin and juvenile data were

log(x+1) transformed, in order to reduce the heteroscedasticity (which occurs when the variance of the errors varies across observations.).

A "principal component analysis" (PCA) followed by a hierarchical cluster were performed on the transformed benthic data with the package FactoMineR and Ade4 to highlight similarities and differences between data and to distinguish groups of data. PCA is a tool in exploratory statistics. This ordination method proceeds to orthogonal transformation to convert possibly correlated quantitative variables to new non-correlated variables called principal components. The graphic representation obtained in 2 dimensions highlights similarities and differences between data. The *"hierarchical clustering on principal component"* (HCPC) is a method of cluster analysis which performs a clustering on individuals. This function combines principal component methods, hierarchical clustering and partitioning to highlight the similarities between individuals.

To confirm the differences visually observed with the PCA and HCPC, "*permutation based non-parametric analysis of variance*" (PERMANOVAs) were realised for both zones and groups of zones (*algae, coral* and *urchin*). This technique, performed with the package vegan, tests the response of one or more variable to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods. A "*multiple range test*" (MRT) was then conducted with the package npmc to test all pairwise comparison between factors (sites or groups) and variables and thus highlight the variables responsible for the differences observed. These two tests, which can be applied to non-parametric data, were performed on transformed data.

The coral recruit and urchin densities were analysed for their differences between the study zones and groups of zones using a Kruskal-Wallis test followed by a non-parametric multiple range test (vegan and npmc packages). Kruskal-Wallis is a non-parametric method for testing equality of population medians among groups. This test could be applied as only one variable (juvenile or urchin) were tested here.

Then a Spearman rank correlation coefficient, which is a non-parametric measure of statistical dependence between two variables, was calculated to assess the relationship between the benthic species and the presence of juveniles. This measure is used to calculate the strength of the relationship between 2 variables.

II.3. Results

II.3.1. Benthic characterization of sites

II.3.1.1 General descriptions

The benthic species and substrates observed in the quadrat pictures were classified into 8 categories: coral (C), crustose coralline algae (CCA), gorgonian (G), macroalgae (Ma), sponges (S), substratum (SPR), turf (Tu) and zoanthid (Z) (Figure 29).

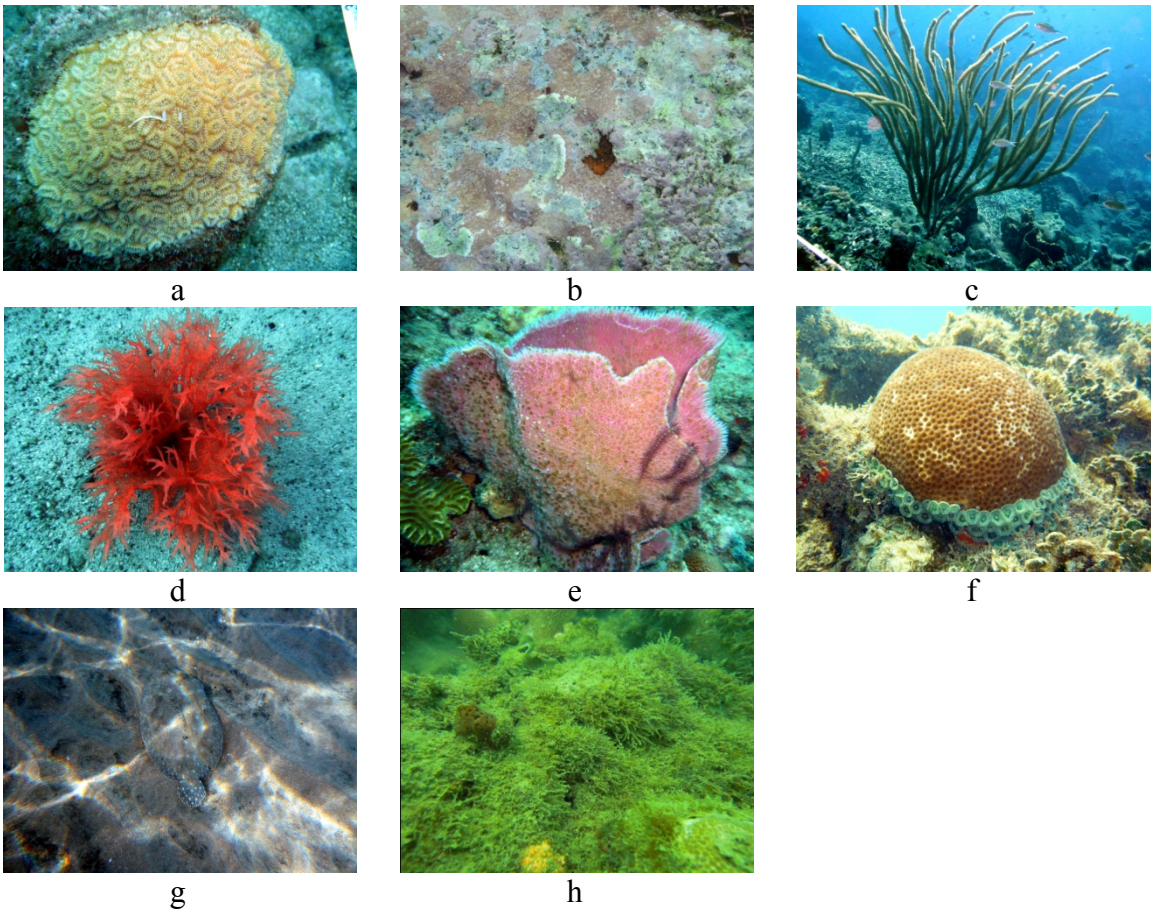


Figure 29: Some representative species of the 8 benthic groups defined: a) Coral (C): *Dichoceonia stokesii*, b) Rock covered by crustose coralline algae (CCA), c) Gorgonian (G): *Pseudoplexaura* sp., d) Macroalgae (Ma): *Kallymenia* sp., e) Sponge (Sp): *Niphates digitalis*, f) Zoanthid (Z), *Zoanthus pulchellus* surrounding a coral species g) Substratum (SPR) and h) Turf (Tu).

Based on the Shannon Weaver Index (for each species), a greater biological diversity was observed in *coral* (between 1.54 and 1.61) followed by *urchin* (1.14-1.26) and *algal* areas (0.88-1.03) (Table 9). The areas where the tall canopy forming algae were removed (*wa*) presented a higher biodiversity index (1.03-1.155) compared to the *algal* zones (0.88-1.03), almost as high as the *urchin* ones (1.14-1.26) demonstrating that some coral species could develop under the algal canopies.

Table 9: Species diversity index calculated from Shannon weaver indexes at the study sites.

Site	Shannon Weaver Index	Site	Shannon Weaver Index
Coral		***Algae***	
Anse Mabouya	1.61	**Caye d'Olbian**	1.03
Caye d'Olbian	1.57	**Tombant de l'Eglise**	0.88
Tombant de l'Eglise	1.54	**Trois Rivières**	0.89
Urchin		***Without algae***	
Arche	1.26	**Caye d'Olbian**	1.15
Caye d'Olbian	1.145	**Tombant de L'Eglise**	1.03
Trois Rivières	1.2	**Trois Rivières**	1.17

At the species level some coral, such as *Diploria stokesi*, *Madracis mirabilis*, *Millepora* sp., *Montastrea annularis*, *Montastrea faveolata, Mussa angulosa* and *Porites porites,* were absent from *algal areas* (Table 10).

Fifteen species of coral as well as 4 genuses were identified at the investigated sites (Table 10). *Montastrea* sp. *Porites* sp. and *Madracis* sp. were among the most important in terms of percentage cover at the coral sites. Most of the species presented similar percentages cover at the 3 *coral* zones (e.g: percentages cover of *Madracis sp.* were comprised between 8.45 ± 3.3 and 10.2 ± 2.7) except *Montastrea faveolata* which presented higher percentage cover at Tombant de l'Eglise.

Table 10: Abundance of coral species expressed as percentage cover (n=45 ± SE) in the 4 treatments (c= coral, u=urchin, a= algal and wa= without algae) of each site (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière).

Groups	Species/Site	Te (c)	Co (c)	Am (c)	Tr (u)	A (u)	Co (u)	Tr (wa)	Te (wa)	Co (wa)
Corals	*Agaricia sp*	0.42 ± 0.16	**0.95** ± 0.25	0.42 ± 0.14	0.24 ± 0.11	0.15 ± 0.08	0.33 ± 0.19	0.00 ± 0.00	0.14 ± 0.11	0.00 ± 0.00
	Colpophyllia natans	**1.61** ± 0.85	0.35 ± 0.17	**1.20** ± 0.59	0.07 ± 0.06	0.15 ± 0.07	0.37 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.27
	Dichocoenia stokesii	0.00 ± 0.00	0.01 ± 0.01	0.19 ± 0.10	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Diploria labyrinthiformis	0.00 ± 0.00	0.35 ± 0.24	0.84 ± 0.35	0.10 ± 0.10	0.00 ± 0.00	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Diploria strigosa	0.05 ± 0.05	0.22 ± 0.15	0.35 ± 0.14	0.04 ± 0.03	0.15 ± 0.07	0.06 ± 0.05	0.02 ± 0.02	0.00 ± 0.00	0.19 ± 0.11
	Eusmilia fastigiata	0.17 ± 0.15	0.09 ± 0.07	0.17 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Isophyllia sinuosa	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Madracis sp. (M. decactis+M. mirabillis)	**8.45** ± 3.33	**10.23** ± 2.78	**9.30** ± 2.77	0.00 ± 0.00	0.02 ± 0.02	0.14 ± 0.14	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	Meandrina meandrites	0.00 ± 0.00	**1.50** ± 0.40	**1.17** ± 0.32	0.18 ± 0.09	0.29 ± 0.21	0.65 ± 0.25	0.07 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
	Millipora sp (M. complanata + M. alcicornis)	0.07 ± 0.07	0.40 ± 0.22	0.10 ± 0.07	0.00 ± 0.00	0.04 ± 0.04	0.05 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Montastraea annularis	0.76 ± 0.58	0.48 ± 0.48	0.87 ± 0.85	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Montastraea cavernosa	**1.89** ± 0.91	**4.37** ± 0.94	**3.97** ± 0.99	0.47 ± 0.20	0.22 ± 0.11	**0.99** ± 0.32	0.13 ± 0.08	0.01 ± 0.01	0.14 ± 0.08
	Montastrea faveolata	**18.61** ± 4.04	**9.45** ± 2.18	**9.03** ± 1.61	0.00 ± 0.00	0.00 ± 0.00	**1.26** ± 0.71	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Mussa angulosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Porites astreoides	**2.54** ± 0.64	**5.28** ± 1.02	**4.08** ± 0.57	0.63 ± 0.26	0.52 ± 0.17	1.10 ± 0.32	0.22 ± 0.14	0.59 ± 0.22	0.18 ± 0.10
	Porites porites	**1.91** ± 0.66	**1.40** ± 0.42	0.22 ± 0.20	0.00 ± 0.00	0.01 ± 0.01	0.35 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Siderastrea sp. (S. radians + S. siderea)	**1.08** ± 0.55	**0.94** ± 0.53	**1.29** ± 0.52	0.72 ± 0.27	1.82 ± 0.61	0.34 ± 0.14	0.74 ± 0.29	0.59 ± 0.38	0.39 ± 0.17
	Stephanocoenia michelinii	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.03	0.01 ± 0.01	0.11 ± 0.05	0.01 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00

Percentage cover of benthic organisms varied with the study site (Figure 30, Appendix 1). Total percentage cover of sessile benthic organisms was higher in *coral* zones, where only 11-14% of substratum was bare. Coral percentage cover varied between 35-38% in *coral* zones, 3.6-4.01% in *urchin* zones and 0.7-1.3% in *algal* zones. Algal cover also varied greatly from one area to the other, between 7-14% in the *coral* zones, 24-32% in the *urchin* one and 53-64% in the *algal* zones. CCA were more abundant in the *coral* and *urchin* zones. No clear pattern could be distinguished for sponges. Two categories (gorgonians and zoanthids) presented very low percentages cover (< 2%) and were mainly observed in the *coral* zones.

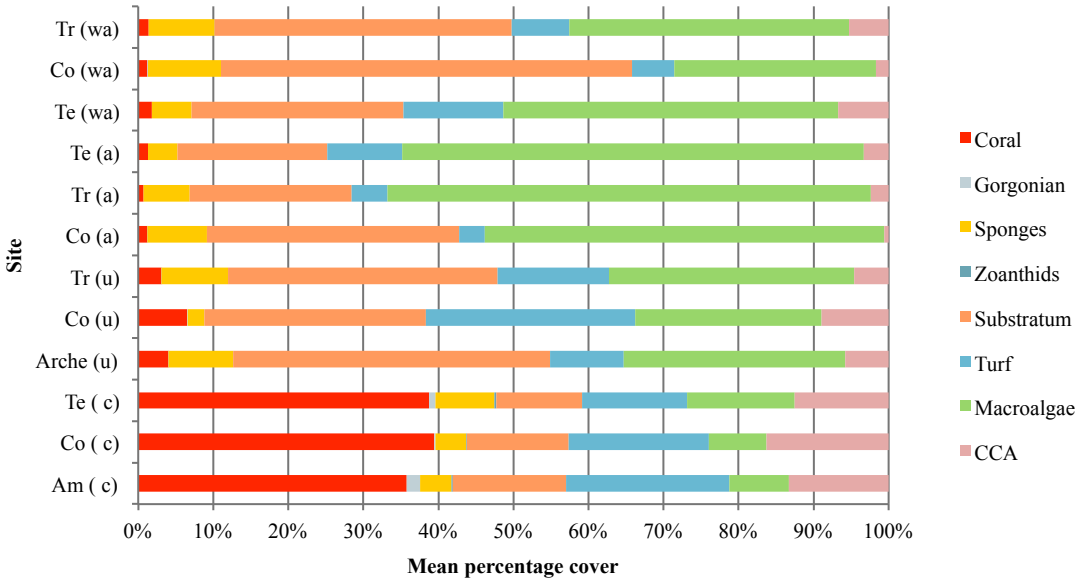


Figure 30: Mean percentage cover of benthic species groups at the sites investigated (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae).

The sites showed significant differences in their benthic composition (PERMANOVA, $p<0.01$). The MRT performed on zoanthids and gorgonians (data not shown) did not demonstrate any significant differences (except for Anse Mabouya). There were no intra-group (*coral (Am, Co, Te), urchin (Arch, Co, Tr), aglal (Co, Te, Tr)*) significant differences for coral species.

However, the PERMANOVA performed on data grouped by ecological areas (*a, c, u* and *wa*) was significant ($p<0.01$). The following MRT (Table 11) demonstrated the 4 groups to present significant differences in benthic percentage cover. The coral cover varied

significantly (except between *a* and *wa*), as well as the turf cover (except between *c* and *u*), substratum and CCA (except between *u* and *wa*). From the results it could be seen that algae were still present in the *wa* zone. Indeed, the species hidden by the first canopy layer (ie: having a smaller thallus) were not removed.

Table 11: Statistical differences (MRT) in the benthic percentage cover of organisms between the 4 zones (A=algae, C=coral, U=urchin and WA= without algae). Results are significantly different when p<0.05.

	Benthic groups	Algal zone	Urchin zone	Without algae zone
Urchin zone	Coral	<0.001	-	-
	Macroalgae	<0.001	-	-
	Turf	<0.001	-	-
	CCA	<0.001	-	-
	Sponges	0.18	-	-
	Substratum	<0.001	-	-
Without algae zone	Coral	0.43	<0.001	-
	Macroalgae	<0.001	<0.001	-
	Turf	<0.001	<0.001	-
	CCA	<0.001	0.20	-
	Sponges	<0.05	<0.001	-
	Substratum	<0.001	0.11	-
Coral zone	Coral	<0.001	<0.001	<0.001
	Macroalgae	<0.001	<0.001	<0.001
	Turf	<0.001	0.93	<0.001
	CCA	<0.001	<0.001	<0.001
	Sponges	<0.05	0.95	<0.001
	Substratum	<0.001	<0.001	<0.001

II.3.1.2 Habitat composition

In order to study the habitat variability between the zones and identify the affinities of the benthic structures, PCAs and HCPCS were performed on the benthic categories previously described without the zoanthid values, as they were present in very few quadrats (10 over the pool of quadrat analysed). The first PCA was performed on the data without considering the data of the *algal* areas after removal of algae (*wa* data) to take into account the presence of all macroalgae present in the field. The first axis explained 72.79% of the observed variability, while the second only accounted for 12.99%. The variables macroalgae (Ma) and substrate (SPR) were negatively correlated with the variables coral (C), gorgonians (G) and crustose coralline algae (CCA) (Figure 31). This ordination showed heterogeneity of the habitats and sites could be organised following a gradient from healthy to degraded. The *urchin* areas were mainly correlated with the second axis,

which is partly explained by turf and substratum, the *coral* zones were mainly correlated with the first axis and the presence of coral, gorgonians and CCA, while the *algal* areas were mainly explained by the first axis and the presence of macroalgae.

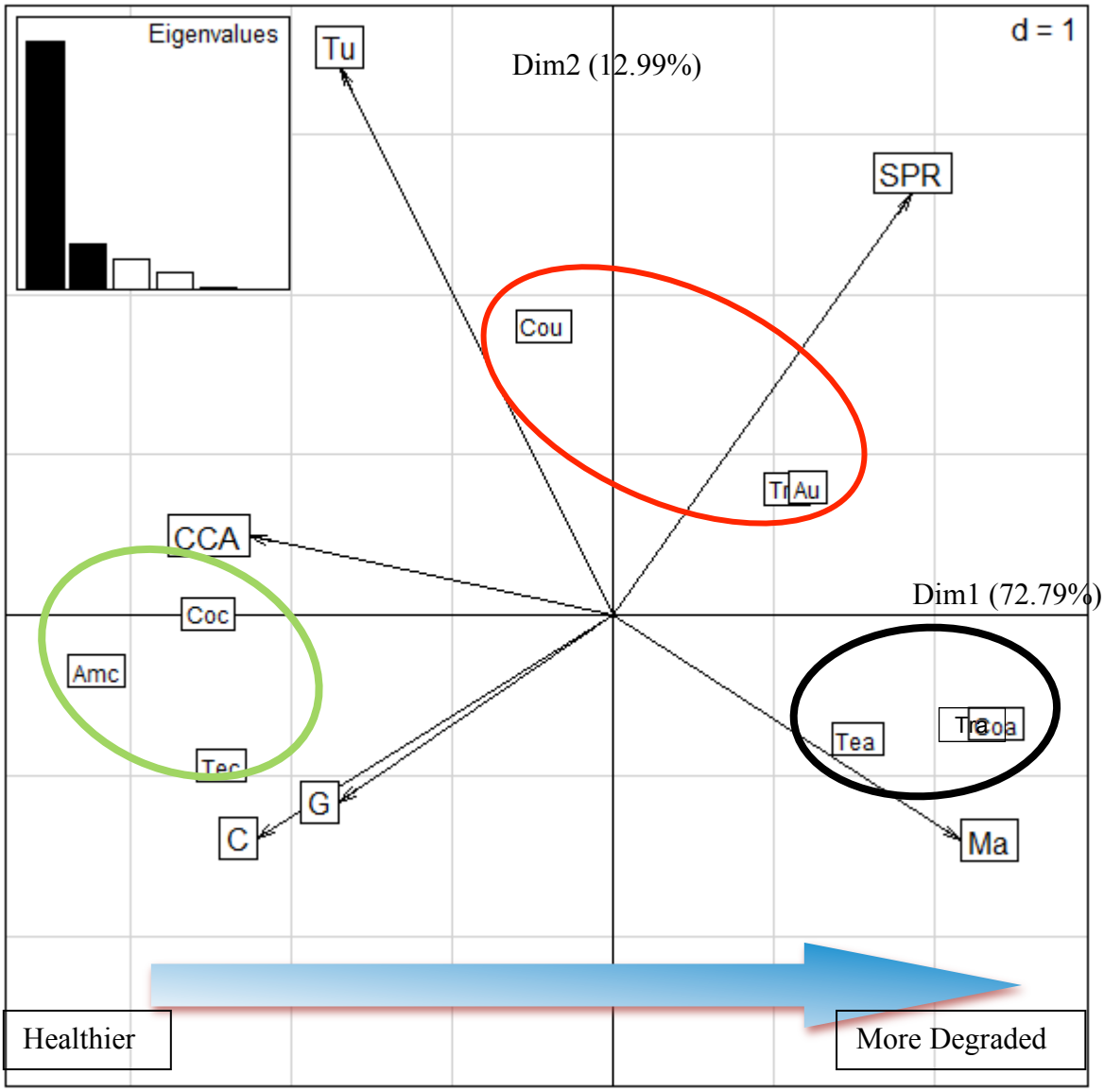
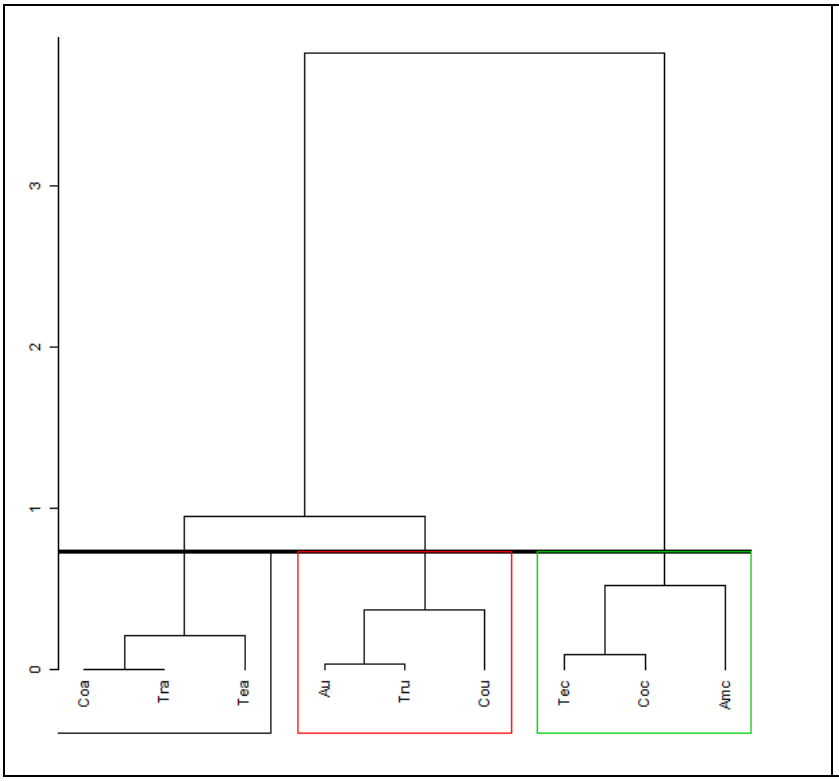


Figure 31: Habitat characterisation. Principal component analysis (R, Ade4) on the benthic percentage cover data transformed (Asin, squared rooted) for the sites investigated (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae). C=Coral, CCA=Crustose Coralline Algae, G= Gorgonian, Ma=Macroalgae, SPR=Substratum, Tu=Turf. The groups obtained after HCPC are circled. Healthier = high percentage coral cover, low macroalgae (less damaged by anthropogenic and natural impact) Degraded = important human and natural impacts. The groups obtained after HCPC are circled.

The hierarchical classification performed on the transformed data grouped the sites into 3 categories according to their benthic composition affinity: the *coral*, the *urchin* and *algal* groups the last two belonging to the same branch (Figure 32).

These groups followed the PCA organisation, the *coral* group being on the left of the PCA, the *urchin* one in the center and the *algal* one on the right side, following a degradation axis from healthy areas (left side) to more degraded ones (right side). The variables mainly responsible for this classification were "CCA", "Coral", "Macroalgae", and "Turf" as demonstrated by the correlation values (table of the Figure 32).



Species	Correlation	p-value
CCA	0.95	6.6e-05
Coral	0.90	8.7e-04
Turf	0.67	4.40e-02
Macroalgae	-0.98	2.63e-06

Figure 32: Hierarchical clustering classifying the sites after PCA (R, FactoMineR). Correlation indexes are given in the table with their p-values. (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae).

A second PCA was performed with the *wa* data in order to integrate the organisms present under the algal canopy in the study. The first axis explained 73.15% of the species distribution, while the second accounted for 11%. The variables had the same distribution than for the first PCA (substratum mainly correlated to the second axis and coral, CCA and gorgonians negatively correlated to macroalgae). Four groups could be distinguished according to PCA and Cluster analyses, the first one (in blue) correlated with the coral factor, the second ones (red and green) being *u* and *wa* categories, and finally, the macroalgae (black) zones (Figure 33).

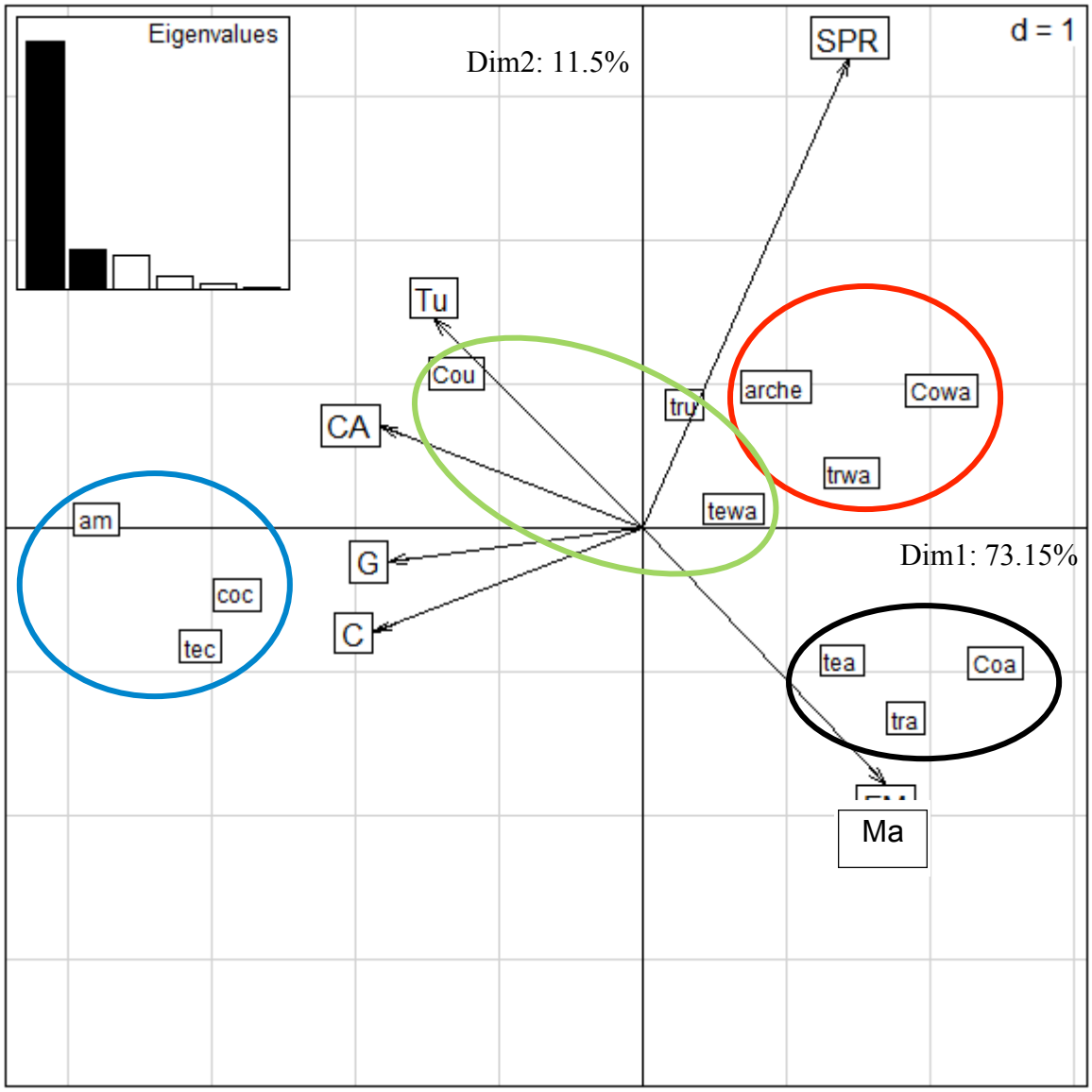
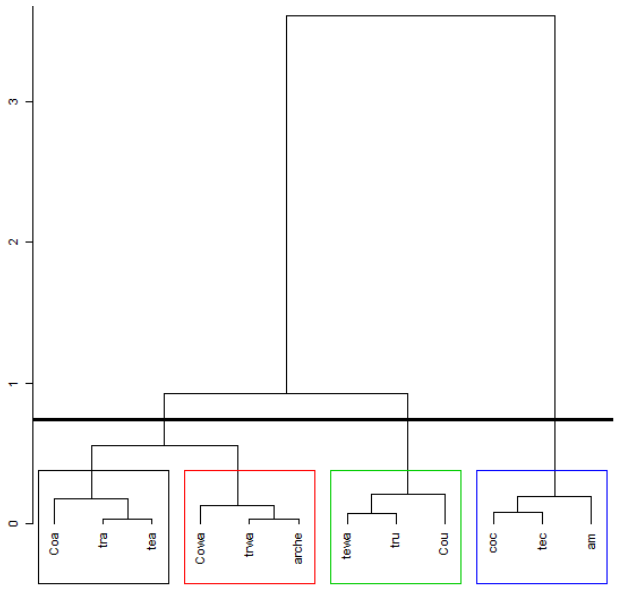


Figure 33: Principal component analysis (R, Ade4) on the benthic percentage cover data transformed (Asin, squared rooted) for the sites investigated (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae). C=Coral, CCA=Crustose Coralline Algae, G= Gorgonian, Ma=Macroalgae, SPR=Substratum, Tu=Turf. The groups obtained after HCPC are circled.

The *urchin* and *without algae* areas were not well separated as *Arche*, (an urchin site) belonged to the *without algae* group and *Te(wa)* (a without algae site) belonged to the *urchin* group. Benthic cover under algal canopies seemed thus to be equivalent to some urchin areas. Macroalgae, gorgonian and turf were the variables mainly responsible for the classification observed (correlation values are given in the table, Figure 34).



Species	Correlation	p-value
CCA	0.87	1.6e-04
Coral	0.84	5.6e-04
Gorgonian	0.75	4.4e-03
Turf	0.73	6.8e-03
Macroalgae	-0.89	7.0e-05

Figure 34: Hierarchical clustering (R, FactoMineR) classifying the sites after PCA (with wa data). The responsible factors for this classification are given in the table with their p-values. (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae).

The 3 zones can be considered as different following the results of the statistical analyses. The urchin area seems to be an intermediate state between heavily degraded areas (algal ones) and healthy zones (coral ones). However, some heterogeneity exists between the sites of a same habitat.

II.3.1.3 *Diadema antillarum* densities

Diadema antillarum densities varied between the 3 areas with averages ranging from absent to 2.2 urchin.m^{-2} (Figure 35). *Coral* and *urchin* zones had equivalent urchin populations, except for Tombant de L'Eglise (*c*), which exhibited a lower density than the others (1 urchin.m^{-2}). Differences were observed between zones (Kruskal-Wallis, $p<0.001$) and between groups of zones *coral*, *algal* and *urchin* (Kruskal-Wallis, $p<0.001$). The multiple range test performed between zones did not show any significant difference among the *urchin*, *algal* and *coral* zones (Table 12). The *urchin* and *coral* areas were not significantly different, except for *Tec* (different from all the sites except *Coc* and *Tru*), and *Tru* (different from *Am*). The *algal* zones were not statistically different the one from the other, but they differed from all the other zones. When testing for groups, the *urchin* and *coral* groups were not significantly different while the *algal* one was different from the two others.

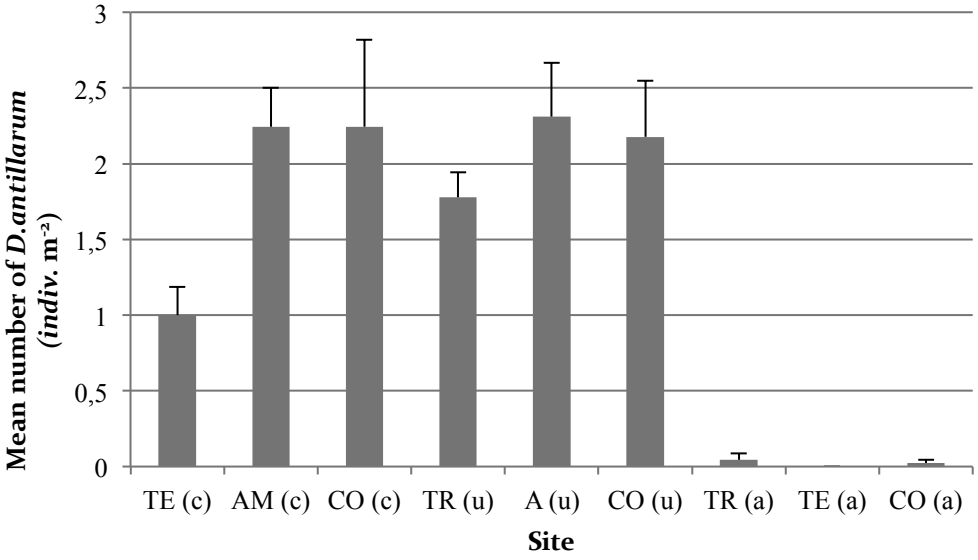


Figure 35: Densities of *Diadema antillarum* in the 3 treatments (c= coral, u=urchin and a= algal) at each of the study location (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière). Values shown are means per m^{-2} (n=6 ±SE).

Table 12: Summary of the multiple range test results (R, Package npmc) applied on the urchin data. (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin).

	Statistical results MRT
Zones	• Am(c)=Co(c)=A(u)=Co(u) • Co(c)=Te(c)=Tr(u) • Te(a)=Co(a)=Tr(a) • Co(u)=Co(c)=Tr(u)=A(u)
Groups	• C different from A • U different from A • C=U

II.3.2. Juvenile corals

Ten species of juvenile corals were identified (Figure 36).

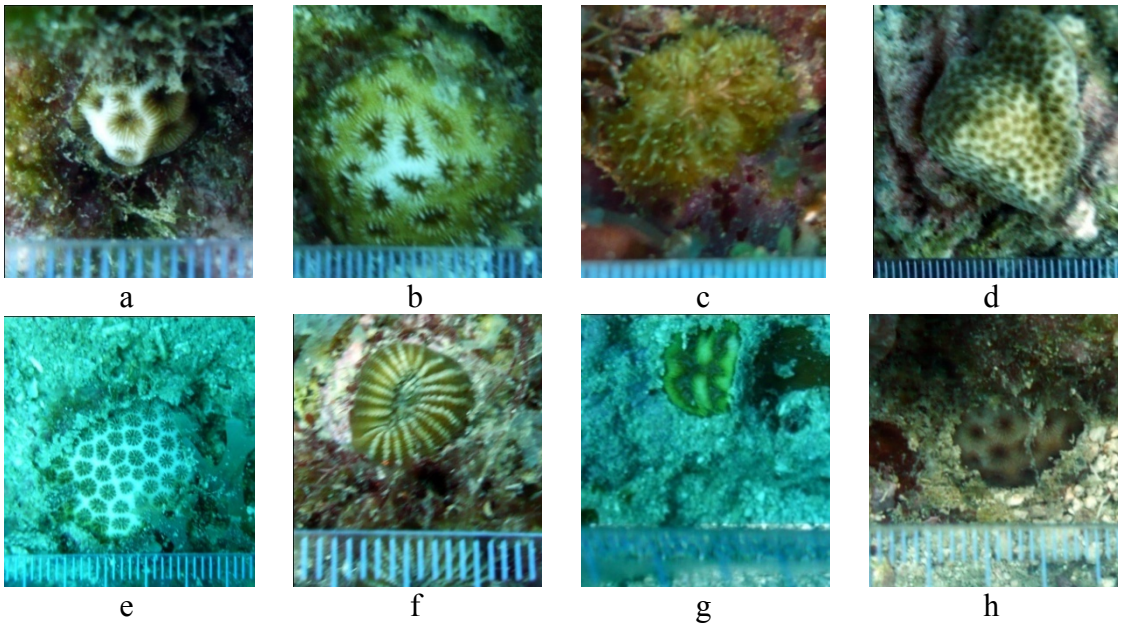


Figure 36: Coral spats observed in the field a) *Agaricia* sp. b) *Favia fragum* c) *Meandrina meandrites* d) *Porites* sp e) *Stephanoceonia mechelinii* f) Unknown g) unknown h) *Siderastrea siderea*.

The number of juveniles (total number=257) varied significantly between the sites studied (Kruskal-Wallis, $p<0.001$) (Figure 37). The *coral* area presented the highest number of juveniles (total number =174), the *urchin* area was intermediate (56) and the *algal* area had the lowest (27) dentsities. Mean densities ranged between 7 to 8 m^{-2} in *coral*, 2 to 3.2 m^{-2} in *urchin* and 0.9 to 1.4 m^{-2} in *algal* zones. The MRT did not show any significant difference among groups (Table 13). A significant difference was observed between the 3 areas, when grouping the data according to areas (*a, u* and *c*) (Kruskal-Wallis, $p<0.05$).

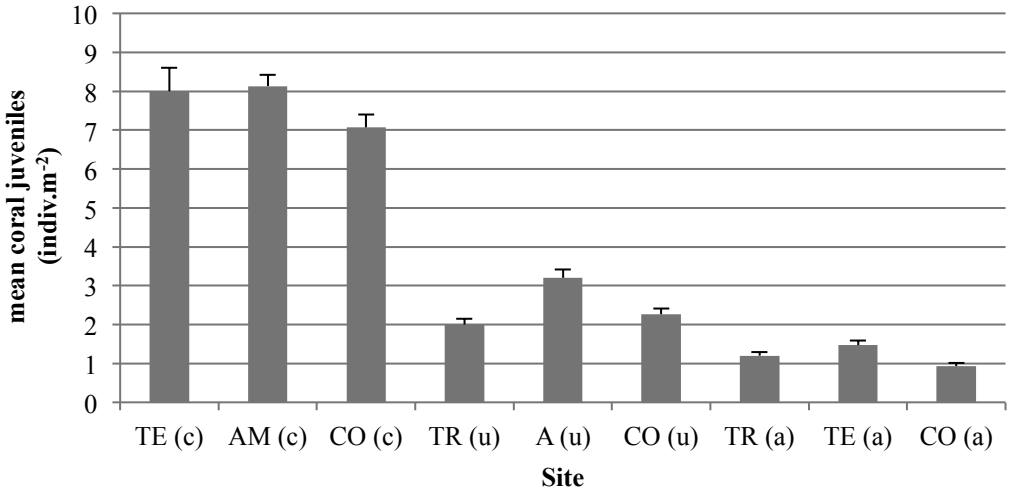


Figure 37: Densities of juvenile corals in the 3 treatments (c= coral, u=urchin and a= algal) of each of the study location (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière). Values shown are means per m^{-2} (quadrata 50*50 cm, n=30 ±SE).

Table 13: Summary of the multiple range test results (R, Package npmc) applied on the juvenile data. (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière) in the four conditions (a=algae, c=coral, u=urchin and wa= without algae).

	Statistical results MRT
Sites	• Am(c)=Co(c)=Te(c) • Co(u)=A(u)=Tr(u) • Te(a)=Tr(a)=Co(a)
Groups	• All the 3 groups are different the one from the other.

About the same number of juvenile coral genera were identified in the 3 zones (between 6 and 7; Table 14). However, the species recruiting differed according to the areas investigated. *Agaricia* sp. and *Porites* sp. recruited mainly in *coral* zones, accounting respectively for 47-75% and 19-33% of recruitment (relative abundance), while *Siderastrea* sp. and *Meandrina meandrites* recruited more in *urchin* and *algae areas* (respectively between 10-50% and 0-55%).

Table 14: Relative abundance of juvenile corals in the 3 treatments (c= coral, u=urchin and a= algal) of each of the study location (Am= Anse Mabouya, Te= Tombant Eglise, Co= Caye d'Olbian, Tr= Trois rivière). (quadrata 50*50 cm, n=30).

Site	Te (c)	Am (c)	Co (c)	Tr (u)	A (u)	Co (u)	Tr (a)	Te (a)	Co (a)	TOTAL
Species										
Agaricia sp.	45	29	33	1	3	8			1	120
Colpophyllia natans		1								1
Favia fragum		13								13
Meandrina meandrites	1		1	5			5	3	1	16
Montastrea sp.			1		2			1	1	5
Porites sp.	14	12	18		4	5		2		55
Scolymia sp.							1	1		2
Sidereastrea sp.		1		6	13	2	2	4	3	31
Stephanoceonia mechelini				2	1	1	1			5
Unknown		5		1	1	1			1	9
Total number of spats	60	61	53	15	24	17	9	11	7	257
Average per quadrat	2	2.03	1.77	0.50	0.80	0.57	0.30	0.37	0.23	
Average per m^{-2}	8	8.13	7.07	2.00	3.20	2.27	1.20	1.47	0.93	
Frequency of Brooders	1	0,98	0,99	0,4	0,3	0,75	0,5	0,45	0,5	
Frequency of Spawners	0	0,02	0,01	0,6	0,7	0,25	0,5	0,65	0,5	

The Sperman correlation performed on the data to observe the association strength between the young recruits and the other benthic components demonstrated a strong correlation between juveniles, CCA, coral, macroalgae and to a lesser extent urchin (Table 15). Juveniles were positively correlated to all the factors except macroalgae (negative correlation).

Table 15: Spearman test results for correlation between juveniles and benthic species.

	Coral	**Macroalgae**	**Urchin**	**CCA**
Rho	0.91	- 0. 88	0.7	0.93
p-value	0.001	0.003	0.03	0.0007

II.4. Discussion

Caribbean coral reefs are among the most degraded in the world (Pandolfi *et al.,* 2003, Bellwood *et al.,* 2004). Jamaican reefs, surveyed for years, give the best example of the phase shift phenomenon. In the 1970's, coral cover ranged from 40-70% and macroalgal cover was lower than 10% while in 1992, coral colonies represented less than 5% (except at the deepest sites) and macroalgal cover ranged between 44-79% (Andres and Witman, 1995).

Benthic community composition and health state were highly variable on the southern reefs of Martinique. Three main types of zones were defined: i) zones with erect macroalgae, lacking of sea urchins and exhibiting low percentage cover of sessile fauna (*algal* areas), ii) zones with healthy coral communities and very few macroalgae (*coral* zones) and iii) intermediate zones with low percentages cover of macroalgae and coral. Because these zones presented high urchin densities they were qualified as *urchin* zone. On the reefs investigated, urchins were restricted spatially to a belt of a few meters width, orientated parallel to the shore at the top of the external slope either in what is referred to as the *coral* or *urchin* areas. These patches of *Diadema antillarum* were surrounded by algae, which spreaded for a few tens of meters on the reef flat. It is the first time that this configuration is described in Martinique. However *urchin* and adjacent *algae* areas have already been described elsewhere in the Caribbean (Carpenter and Edmunds, 2006). It is important to point out that these areas are variable as many intermediate states are also present; however, this classification represents numerous south reef sites of Martinique (personal observations).

Percentage cover of scleractinian corals was high at some of the investigated sites on the south reefs of Martinique (the maximum observed being 38%) with 15 corals identified to the species level and 4 to the genera one. Bruno *et al.,* (2009) defined the threshold for severe phase shift to be coral cover <10% and macroalgal cover >60% which corresponds to the *algal* areas surveyed in this study. In the Caribbean, over the 530 sites analyzed by Bruno *et al.,* (2009), only 10% had macroalgal percentage cover superior to 50%, and 39% had macroalgal percentage cover > 25%. Here, the *urchin* areas benthic composition was inbetween *coral* and *algal* dominated areas. Coral percentage cover represented 4-6%, macroalgae 24-29%, turf 9-27% and CCA 4-8% in the *urchin* areas while they accounted respectively for 0.6-1.2%, 52-64%, 3.3-9.8% and 0.5-3.2% in the *algal* areas and 35-38%,

7-14%, 13-21% and 12-15% in the *coral* areas. These data were consistent with those of Rousseau (2010) who found 6.8% CCA, 44-53% coral and 10-12% macroalgae in *coral areas* of Martinique (Caye d'Olbian and Anse Mabouya), and 2.8% CCA, 13% coral and 39% macroalgae in *urchin area* (Trois Rivières). The differences noticed between these 2 studies could be related to the sampling date or the sampling methods. Here, picture analysis with point counts were preferred as they do not omit poorly represented species, but this method underestimates the percentage coral cover compared to line-point intercept method employed by Rousseau, (2010), (Leujak and Ormond, 2007). Carpenter and Edmunds, (2006), in their studies conducted at St Croix, Barbados, Jamaica, Grenada, Bonaire and Belize found *urchin* zones to be characterized by 4–37% of coral, 0–20% of macroalgae, 29–77% of algal turf and 7–37% of CCA and *algal* zones by 2–32% coral, 30–79% macroalgae, 13–53% algal turf and 0–7% CCA. The percentage cover found in the present study for scleractinians were lower in *algal* zones, however, they were consistent with the one found by Edmunds, (2000) (0.9-12.3% coral and 0.1-22.3% macroalgae in the Virgin Islands). The differences between percentage cover could be due to the geographical regions surveyed and/or the experimental method employed. Indeed, Carpenter and Edmunds, (2006), recorded the benthic component dominating each 25 subdivision of a 0.25 m^{-2} quadrats and reported this to 4% cover, which probably lead to an overestimation of the most abundant species and underestimation of the others. In addition, a distinction was made between what is described as *coral* areas, which have high percentage cover of coral and high densities of sea urchins and *urchin* areas, considered as transitory zones, with high densities of *Diadema antillarum* and low percentage cover of both algae and coral. Carpenter and Edmunds, (2006), did not make such a distinction and it is possible that what they consider as *urchin* zones actually corresponds to both *urchin* and *coral areas* of this study. Nevertheless, several zones were distinguished in these studies, and reduced macroalgal population could be observed in *urchin* and *coral* areas, being in concordance with their findings.

The zones observed could indicate competition between the benthic species present. Macroalgal populations could be regulated by *Diadema* grazing pressure, in turn, seaweeds could have a negative impact on sea urchins through the production of marine natural products, reducing their palatability, or influencing the urchin recruitment process (Paul and Fenical, 1986, Pereira *et al.,* 2003, Idjadi *et al.,* 2010). *Diadema antillarum* is an important grazer and areas where it is present are typically cleaned of erect algae but can

be colonised by crustose coralline algae or turf. In the present survey, *D. antillarum* densities were between 0 and 2.2 m^{-2}, which is comparable to recent observations in the Caribbean (Carpenter and Edmunds, 2006, Steiner and Williams, 2006). Surveys in Martinique are recent, since there is no information about reef health state before *Diadema antillarum* mass extinction; it is difficult to conclude about its possible recovery and impact on reef species. Other Caribbean studies, interpreted the patchy urchin configuration as evidence for an increase of *Diadema antillarum* population densities which is often associated with changes in both coral and algal communities, enhancing coral recruitment and limiting the algal growth (Edmunds and Carpenter, 2001, Carpenter and Edmunds, 2006, Idjadi *et al.,* 2010).

The 3 areas described presented statistically different coral juvenile densities. Numbers of coral juveniles varied between 7-8 indiv.m^{-2} in *coral* areas, 2-3.2 indiv.m^{-2} in *urchin* and 0.91-1.4 indiv.m^{-2} in *algal* areas. This was different from the findings of Carpenter and Edmunds, (2006) who found 2.5-12.9 indiv.m^{-2} in *algal* zones and 4.5-32.3 indiv.m^{-2} in *urchin* zones). Studies carried out in the Pacific showed juvenile densities to vary between 5.5 and 6.9 indiv.m^{-2} (Penin, 2007), which is more consistent with those reported here.

A strong positive correlation was observed between adult coral percentage cover and juvenile coral densities. Coral reproduction patterns vary with the species; they can either be brooders, or broadcasters. Brooders emit planulae already fertilized; they can reproduce several times a year and do not need other colonies, while broadcasters emit sperm and eggs in the water column, resulting in an external fertilization. Brooding is the most common reproductive mode in the Caribbean (Richmond and Hunter, 1990). *Agaricia* sp*., Porites* sp. and *Favia fragum,* the juvenile coral most represented in *coral* areas, are brooders while *Siderastrea siderea*, the species found in majority in *urchin* and *algal* areas is a spawner (Sammarco, 1996). Brooders have a minimum time of settlement of 4 hours, while the broadcasting species have a minimum time settlement of 6-72 hours (Sammarco, 1996). This could explain the fact that some species were more abundant at the vicinity of the parent colonies, ie: in *coral* areas and others can settle in *algal* areas.

A strong positive correlation was also observed between CCA and coral juveniles. This is consistent with chemical ecology studies that demonstrated the production of chemical cues by Crustose Coralline Algae to have a positive effect on coral species and induce coral larval metamorphosi*s* (Heyward and Negri, 1999). Urchins also had a positive

correlation with the number of juveniles. Previous studies suggested the recovery of *Diadema* might encourage settlement, increase growth rates and survival of corals (Carpenter and Edmunds, 2006, Idjadi *et al.,* 2010).

Alternatively, a strong negative correlation was noticed between juvenile corals and macroalgae (rho= -0.88). It is unlikely that this negative correlation was due to environmental parameters other than the presence of seaweeds as the study areas were selected in the same water bodies and reefs. Seaweeds could prevent the recruitment processes through several ways, including the production of marine natural products in the water column and abrasion phenomenon, with the fronds dislodging the new recruits (McCook, 2001). Mat forming and to a lesser extent canopy forming macroalgal assemblages, reduce dissolve oxygen and irradiance, increase dissolve organic carbon and soluble reactive phosphorus which directly affect the metabolism of corals (Hauri *et al.,* 2010). There is also evidence that water soluble chemicals produced by macroalgae can inhibit coral larval settlement (Miller *et al.,* 2009). Mumby *et al.,* (2007), found that macroalgal cover as low as 20-30% was negatively correlated with coral recruit density.

Sargassum and *Dictyota,* representing respectively 38-58% and 32-45% of macroalgae, were the most abundant seaweeds in the *algal* areas. *Sargassum spp.* was already described as one of the most conspicuous algae in the world (Chapter I). Coral reef recovery and phase shift reversal occurs through the replenishment of coral cover either by growth of partially dead colonies or settlement of new recruits and through the sustainability of key stone species populations (such as *D. antillarum*). Reef resilience and sustainability could thus be hampered by the presence of macroalgae as highlighted in the present study. The following experiments were designed to test if *Sargassum*, the alga the most represented on Martinique reefs affects recruitment processes on the reef and to determine the mechanisms involved in this phenomenon (physical, and/or chemical).

Conclusions

South Martinique reefs present algal and urchin belts as already described elsewhere in the Caribbean. Diadema antillarum populations in Martinique seem to be facing a recovery like in many other Caribbean Islands.

Some of the surveyed reef flats have high percentage coral cover, higher than most of the Caribbean stations surveyed. However, important algal beds, exhibiting coral and algal percentage covers over the phase shift thresholds, contrast this. Sargassum spp. and Dictyota spp. are the most abundant algae growing on Martinique reefs.

The juvenile coral presence is very low as compared to that reported in other studies. Brooders are the main juveniles observed in coral areas, while spawners are the main juvenile species in algal areas. CCA, urchins and coral all together have a positive impact on recruitment while algae have a negative one.

→How can algae impact reef recruitment process?

Chapter III: Benthic recruitment on reefs of Martinique: effects of algal canopy and *Sargassum* spp. presence on recruitment and early stages of development of benthic marine invertebrates.

III.1. Introduction

Numerous reefs of Martinique are colonised by macroalgae among which *Sargassum* is one of the most important in terms of percentage cover (Chapter II). Since the phase shift phenomenon can lead to the predominance of seaweeds over coral species, understanding their effect on existing communities is crucial. In the reef environment, macroalgae were shown to interact with coral species and interfer with their development and survival through 5 main mechanisms: sweeping, shading, increasing sedimentation, increasing diseases and producing secondary metabolites, (Miller and Hay, 1998, McCook, 1999, River and Edmunds, 2001, Chapter I). Coral recruitment declines when benthic algae are more abundant (Edmunds and Carpenter, 2001, Birrell *et al.,* 2005, Carpenter and Edmunds, 2006, Hughes *et al.,* 2007, Vermeij and Sandin, 2008, Chapter II) and numerous authors have suggested that coral planulae and early stages of development may be more sensitive to algae than adults (McCook *et al.,* 2001, Vermeij and Sandin, 2008). Small juvenile corals are considered to be more vulnerable because they cannot protect themselves against abrasion and because they have a smaller reservoir of energy to invest in competitive interactions (Zilberberg and Edmunds, 2001, Raymundo and Maypa, 2004).

Colonisation of a new substratum is a critical stage in the life history of sessile organisms (Pawlik, 1992a, Pawlik, 1992b). Most benthic marine invertebrates produce larvae that must settle onto a suitable substratum prior to metamorphosis. Settlement includes reversible contact with the substratum, exploratory behaviour, orientation and metamorphosis of the larvae, while recruitment combines settlement and post-settlement periods, including irreversible contact, until the organism is visible by an observer (Keough and Downes, 1982, Pawlik, 1992a). This definition, however, varies depending on the organisms studied and the authors. Concerning coral, recruitment was used to designate both processes occurring a short time after fixation and metamorphosis, (Babcock and Mundy, 1996, Gleason, 1996, Hughes *et al.,* 1999a, Mundy, 2000) and when recruits are visible (around 1cm diameter) on natural substrata (Edmunds, 2000, Miller *et al.,* 2000). Both settlement and post-settlement processes are impacted by biological, physical and chemical parameters (Figure 38) which determine the choice of a new substratum, and the survival and the development rates of new recruits (Pawlik, 1992a).

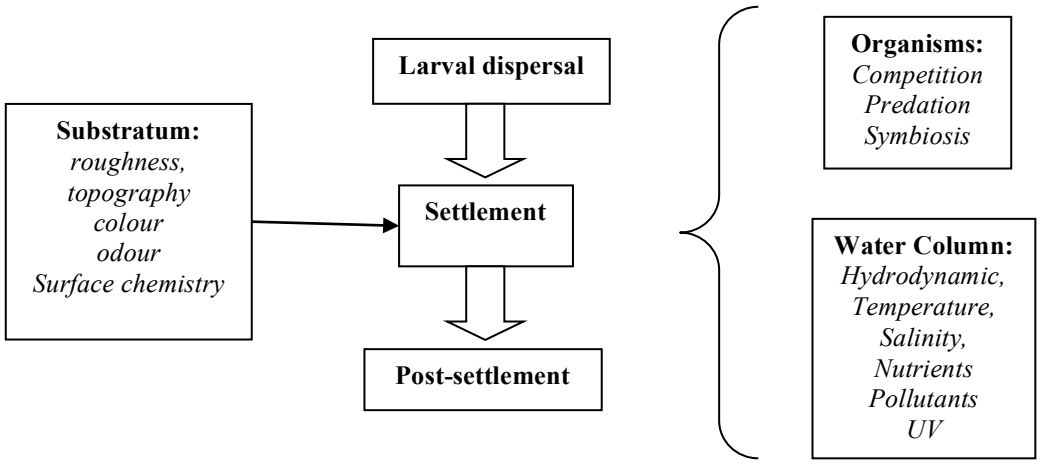


Figure 38: External factors influencing the recruitment process of marine invertebrates which display a pelagic phase. The arrow indicates the factor impacting only on settlement while the embrace indicates factors impacting the 3 phases.

The unusual presence of macroalgae on tropical reefs modifies environmental factors and could thus influence the recruitment process of benthic sessile organisms. Competition includes both direct physical mechanisms (McCook *et al.,* 2001, Birrell *et al.,* 2005, Box and Mumby, 2007, Mumby *et al.,* 2007) and indirect mechanisms through the production of allelochemicals and the stimulation of pathogens (Nugues *et al.,* 2004, Paul and Puglisi, 2004, Kuffner *et al.,* 2006, Smith *et al.,* 2006). Few studies have investigated the effect of macroalgae, and chemical cues on the early life stages and recruitment of coral larvae (Kuffner and Paul, 2004, Birrell *et al.,* 2005, Kuffner *et al.,* 2006, Diaz-Pulido *et al.,* 2010). Seaweed mechanisms of competition are in part driven by their morphology (Littler and Littler, 1980) and algal assemblages could influence larval fixation, metamorphosis and development through:

i. Light reduction; algal canopies, which induce a light decrease for understorey species could prevent the settlement and development of photosynthetic organisms. Some coral larvae contain symbiotic zooxanthellae (Richmond, 1997) and recruitment could be affected by light decreases.
ii. Modification of small scale hydrodynamic; seaweeds can form a barrier to currents and reduce their speed on the ground. Small scale hydrodynamic characteristics are known to play an important role in the choice of a substratum by larvae (Koehl, 2007).
iii. Reduction of oxygen in the water column; macroalgae perform photosynthesis and consume oxygen for their respiration. The unusual presence of macroalgae could

lead to hypoxia and anoxia phenomena, wich may cause adverse effects on invertebrate larvae.

iv. Abrasion of the substrata; macroalgal fronds can abrade the substratum and their presence and colonisation can lead to loss of available space preventing the settlement and development of larvae (Birrell *et al.,* 2005, Mumby *et al.,* 2005).

v. The production of secondary metabolites that can interfer with organisms surrounding them. Chemical cues are one of the most important factors in the settlement of larvae. They play an important role in determining the selection of habitats (Tamburri *et al.,* 1996). Numerous CCA induce coral larval settlement and enhance their metamorphosis (Morse and Morse, 1991, Morse *et al.,* 1996, Heyward and Negri, 1999, Harrington *et al.,* 2004, Kitamura *et al.,* 2007).

The present study investigated coral recruitment *in situ* without manipulating larvae during their settlement and early development. The purpose of this experiment was to analyse the recruitment of benthic species in *algal*, *urchin* and *coral* areas and to further identify the mechanisms employed by *Sargassum* spp. which interfere with recruitment processes. Three hypotheses were thus tested: i) the recruitment rates of benthic invertebrate larvae, among which coral larvae, would depend upon the presence/absence of macroalgae and *Diadema antillarum,* ii) a mechanical removal of macroalgae in algal fields would enhance benthic reef species recruitment and iii) *Sargassum* spp., the alga the most represented on reefs of Martinique, would hamper reef recruitment processes presumably through physical and/or chemical means.

Two complementary studies were carried out to test these hypothesis: i) benthic reef recruitment was assessed in the presence and absence of seaweeds and urchins at the sites of Trois Rivières and Anse Mabouya to determine if *Diadema antillarum* or algal presence had an effect on the recruitment process ii) physical and chemical effects of *Sargassum* spp. were tested on benthic reef species recruitment at the site Tombant de l'Eglise using a manipulative experiment.

III.2. Material and methods

III.2.1.Study sites

The 3 sites chosen for the study (Anse Mabouya, Tombant de L'Eglise and Trois Rivière) are located on the south coast of Martinique (Figure 39) and were selected for their benthic characteristics:

- Anse Mabouya is in a good healthy state with high percentage coral cover (*coral* zone)
- Trois rivière possesses both *urchin* and *algal* zones
- Tombant de l'Eglise is composed of *algal* and *coral* zones.

These 3 sites are located on the reef crest between 10-12 m depth. Sites characteristics (environmental and biological) were previously detailed in the chapter II.

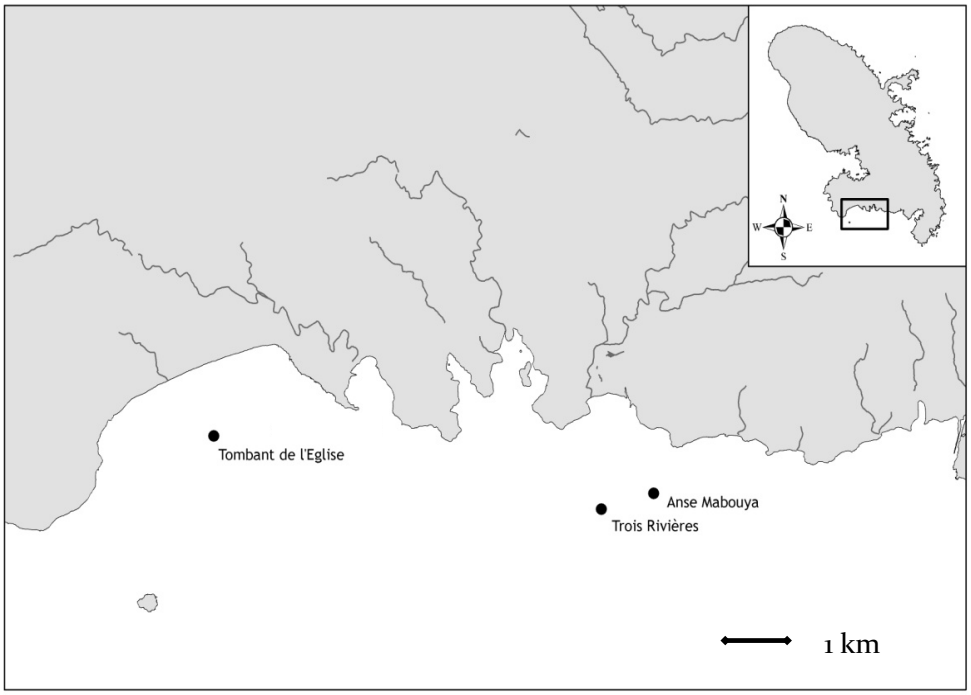


Figure 39: Location of the study sites Anse Mabouya, Tombant de l'Eglise and Trois Rivières along the south coast of Martinique.

III.2.2.Experimental designs

Two experimental designs were drawn in this chapter : i) the first one aimed at evaluating benthic reef recruitment in *coral, algal, urchin* areas and in zones where macroalgae were manually removed while ii) the second one aimed at testing the physical and chemical

effects of *Sargassum* spp. on benthic reef recruitment. Studying recruitment processes is a challenging task as larvae cannot be easily monitored in the field. Fixation of settlement plates, a method commonly employed to observe the variability of coral larvae settlement in marine environment was used in the following experiments (Mundy, 2000, Field *et al.,* 2007)

III.2.2.1 Impact of macroalgae on recruitment

For the first study (impact of macroalgae on recruitment), 4 distinct areas located at 2 sites were chosen. Anse Mabouya (*coral* zone), a zone naturally devoid of *Sargassum* spp. was selected as a reference area (Figure 40a) while Trois Rivières, was chosen for its important algal coverage and the presence of a *Diadema* population. Trois Rivières is composed of 2 distinct zones:

- One heavily colonised by algae (Figure 40b, Table 16)
- And another one almost devoid of seaweeds and coral but presenting high *Diadema antillarum* densities around 1.7 indiv.m^{-2} (Figure 40c; Chapter II).

Both sites are coral constructions located on the continental platform in the south of Martinique close to the estuary of the River Oman. They belong to the same reef and marine water body, which allows comparing them (Figure 39). These 2 sites were preferentially chosen for their geographical proximity and their differences in benthic composition (Figure 40).

a b c

Figure 40: Picture of the 3 study sites. a) Anse Mabouya (*Coral* zone) b) Trois Rivières (*Algal* zone) c) Trois Riviere (*Urchin zone*).

Table 16 : Algal species composition and weight at the site of Trois Rivières (*Algal* zone) in dry season 2008 (expressed as dry weight g.m^{-2}), (Thabard, unpub. data).

		Trois Rivières	
	Species	dry weight g.m^{-2}	Standard deviation
Chlorophyceae	***Verrucaria ventricosa* (C)**	0.07	0.16
	***Halimeda* spp. (C)**	1.13	1.81
	***Codium* sp. (C)**	3.60	5.64
Phaeophyceae	***Lobophora* sp. (P)**	1.60	1.55
	Other	4.92	2.99
	***Dictyopteris sp.* (P)**	5.49	5.10
	***Dictyota* sp. (P)**	11.99	6.54
	***Sargassum* spp.**	215.60	39.00
Rhodophyceae	***Botryocladia sp***	0.37	0.42
	Rhodymenia	0.99	1.28
	Amphiroa	7.90	4.38
	Galaxaura spp.	10.02	5.69
	***Bryothamnion* sp.**	34.64	19.32
	Total	298.29	28.80

The site of Trois Rivières was further subdivided into 3 areas: i) a zone with *algae* (*A zone*) was delimited to observe the recruitment under both physical and chemical effects of algae; ii) a zone where macroalgae were manually removed (*WA zone*) was created to evaluate recruitment in case of algal removal. This zone was cleaned every two weeks to ensure seaweeds would not re-grow during the experiment and iii) the last zone corresponded to the *urchin* zone (*U zone*) where the algae are naturally absent. This area was chosen to observe the differences of recruitment in the absence of seaweeds and the presence of *D. antillarum.* The presence of this sea urchin is suggested to creat favourable conditions to coral recruitment (Carpenter and Edmunds, 2006).

These 3 zones were located about 10 meters away the one from the others, ensuring the larval supply would be the same in the 3 treatments.

III.2.2.1.1. Time and duration of the experiment

Tiles were fixed in the field for 4 months twice a year (May-September and August-December) in 2008 and 2009 to compare recruitment seasonality (rainy and dry season) and inter annual variability (Table 17). The second season (from August-December) was of importance as, in the Western Atlantic, broadcast spawning corals typically release gametes several days after a full moon in August, September October, when water temperatures are maximal (Szmant, 1991, Wyers *et al.,* 1991, Acosta and Zea, 1997). As

an exemple, *Montastrea* sp. broadcast spawning typically occurs 7 days after full moon in Guadeloupe (FWI, close to Martinique Island) from August to October. In 2008, broadcast spawning observations were made on the 23rd and 24th of August as well as the 22nd of September while it was observed on the 11th of September in 2009 (Mazeas, personal communication). Coral larvae settle between 4 to 6 days after the spawning phase (Miller and Mundy, 2003). In order to maximize the chances of observing broadcast spawning coral species recruits on tiles, they were fixed in the field a few days before the first spawning pic i.e: around the 15th of August so that a biofilm could develop on them before. Erwin *et al.,* (2008), demonstrated that tile conditioning (in the reef environment) influenced *Acropora palmata* recruitment with low settlement (11%) on unconditioned tiles and high settlement (72-87%) on tiles conditioned for 2, 8 and 9 weeks. A three factor design analyses (Treatment (area of fixation), Season and Year) was conducted.

Table 17: Years and seasons at which experiments were conducted.

Year	Season
2008	May/September August/December
2009	May/September August/December

III.2.2.1.2. Tile characteristics and fixation

The surface type of plates is a crucial factor to take into account while studying the recruitment of coral species. Harriott and Fisk (1987), compared the recruitment on several surfaces and concluded that ceramic tiles attracted the largest number of spat (when attached in pairs). Good results were also obtained while using coral slices, but tiles are cheap, readily available, convenient to store, have a standard surface and are easy to look at.

Two main methods of plate fixation onto the substratum are described in the literature. The first one consists of racks (containing 2 to 6 plates) fixed to the substratum (Harriott and Fisk, 1987) while the second involves attaching the plates directly to the substratum using a small stainless steel base plate (Mundy, 2000, Field *et al.,* 2007). Both techniques are efficient but the direct method has the advantage of avoiding the problems associated with the violation of the assumption of independence required for most statistical analyses (Table 18).

Table 18: Comparison of the different methods used to analyse coral recruitment (Field *et al.*, 2007).

Method	Advantages	Disavantages
Attachment to wire racks (method 1)	More levels of variation in model	Each rack is a replicate. Therefore numbers of replicates is lower for any given number of tiles
	Attachment surface preparation on land	Racks create a change in the hydrodynamics of the site
	Easy removal of tiles	Under surface exposed to grazers
	Easy to locate	
	Racks can be deployed on any habitat type	
Attachment to substrata (method 2)	Individual tiles are independent replicates	Time consuming initial deployment of tiles in situ
	Tiles can be deployed to specific location	Relatively well shaded under surface
	Following first deployment changes of tiles in situ is easy	Deployment is limited to reef environment
	Cryptic surfaces protected from grazers	Tiles become cryptic with time making rediscovery difficult
	Little influence on hydrodynamics of the site	

Thus, in this study, terracotta tiles cut into equal squares of 100 cm^{-2} (10x10 cm) were fixed according to the second method, "attachment to substrata". The tiles drilled in their middle (diameter 6 mm), were thus directly nailed onto hard substratum (Figure 41).

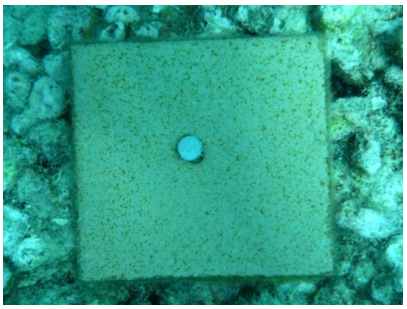


Figure 41: Fixation method of the tiles. Tiles were drilled in the middle and fixed by a nail on the substratum.

III.2.2.1.3. Tiles lay out

In each of the 4 zones (*coral, algal, without algae* and *urchin*), a surface of 100 m^2 (10 m x 10 m) was delimited where 20 tiles were attached (Figure 42). They were randomly nailed on hard substratum in the area. Although Mundy, (2000) demonstrated that small differences in depth, plate angle and topography had little measurable effects on coral recruitment (explaining less than 6% of the variability on coral recruitment on replicate settlement plates), the plates were attached to the substratum parallel to the reef flat to limit additional variability. The depth of fixation of the tiles was the same at each location (between 9-11m).

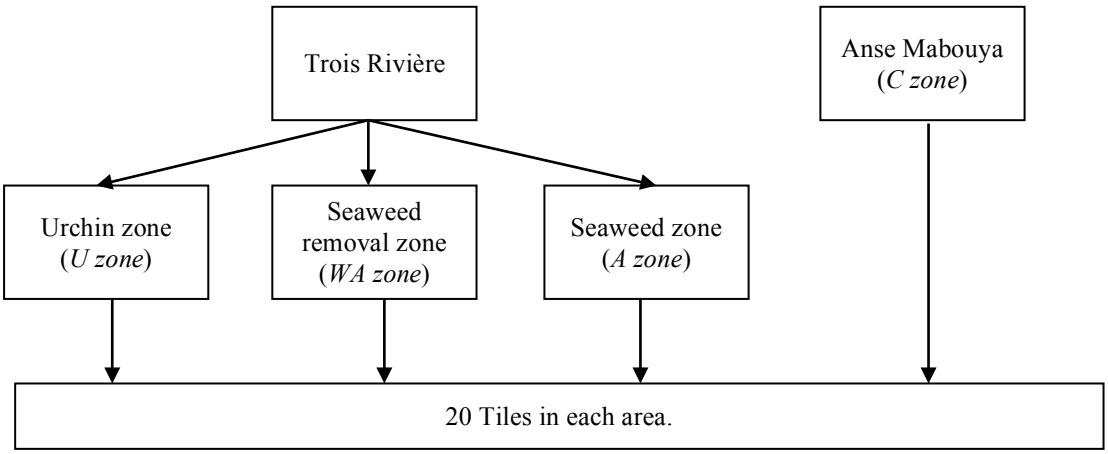


Figure 42: Schematic representation of the tile experiment conducted in dry and wet seasons 2008/2009 at the sites Anse Mabouya and Trois Rivières in the 4 areas: *A*: *Algal* Zone, *C*: *Coral* zone, *U*: *Urchin* zone and *WA*: *Without algal* zone.

III.2.2.2 Effect of *Sargassum* spp. on recruitment

The second study was conducted at Tombant de l'Eglise also located on the south coast of Martinique (Figure 39). This site was selected because it presented both a population of seaweeds (mainly *Sargassum* spp.) on the reef platform until 13 m depth and a healthy coral community from 13 m to 20 m depth. The site of Tombant de l'Eglise was selected in order not to introduce *Sargassum* spp. in a pristine area, and the experiment was conducted at the junction of the *algal* and *coral* zones. Manipulative experiments might result in the introduction of a species in an environment it is absent from and, if the environmental conditions are suitable, its colonisation.

III.2.2.2.1. Time and duration of the experiment

This second experiment was conducted for 5 months, from August to December 2010, as the objective was to study the impact of *Sargassum* spp. on recruitment and not coral larvae recruitment seasonality.

III.2.2.2.2. Tile characteristics and fixation

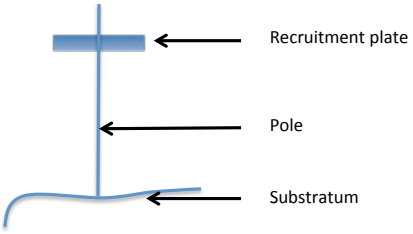
According to the results obtained during the first experiment (impact of macroalgae on recruitment), the organisms (except algae) settled preferentially on shaded areas i.e.: under the tiles (see p114). In the present experiment, this layer of the tile needed to be in direct contact with *Sargassum* spp. in order to observe the physical effects of macroalgae on coral recruitment. Therefore, tiles were drilled in their middle (diameter 6 mm) and inserted on poles in order to be elevated (and thus in contact with algae). The plates were maintained on the poles thanks to 2 pieces of plastic pipes (diameter 8 mm) so that they could not slip during the experiment (Figure 43).

Figure 43: Fixation method of the tiles. Tiles were fixed on a pole 15 cm above the substratum.

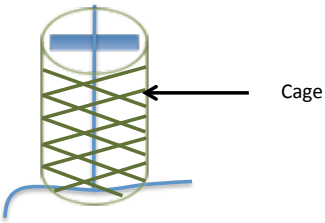
III.2.2.2.3. Tiles lay out

This experiment was conducted in the belt between the *algal* and *coral* areas in order to ensure that numerous coral parent colonies were present and to make sure that the chances of recruitment would be equal for the 5 treatments.

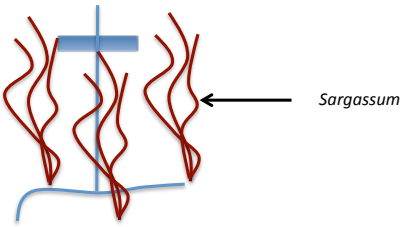
To test for the physical and chemical effects of *Sargassum* spp., 5 treatments were set-up (Figure 44, Figure 45):



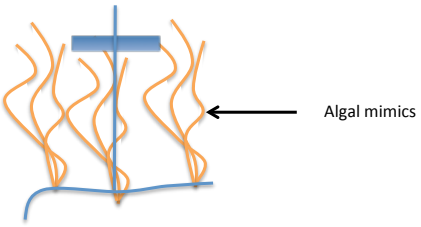
Control (C) i.e.: tiles were not in contact with *Sargassum* spp.. This consisted in 28 poles, each bearing one plate fixed randomly in the belt between algal and coral zones. Ropes were attached to the substratum to ensure this parameter would not influence recruitment (see P+Ch; P and Ch).



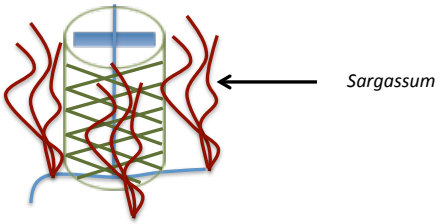
Cage control (Cc) was set-up in order to compare the results with the one of the chemical effect. Each pole was surrounded by one plastic cage (mesh size 2 cm, 20 cm height). Ropes were attached to the substratum to ensure this parameter would not influence recruitment (see P+Ch; P and Ch).



Physical and chemical effects (P+Ch) of *Sargassum* spp. This consisted in poles bearing one plate surrounded by *Sargassum* spp.. The algae were tangled in ropes attached to the substratum.



Physical effect (P) of algal mimics. Tiles were in contact with plastic algae to simulate abrasion from seaweeds. This treatment was the same as the (P+Ch) one except that algal plastic mimics made of ropes replaced *Sargassum*.



Chemical effect (Ch) of *Sargassum* spp. Tiles were in a cage isolated from algae. This treatment was the same as the one described for the cage control (Cc) except that *Sargassum* were tangled in ropes fixed on the substratum around the cages. (MNP could be in contact with tiles but not the algae).

The experimental devices (ropes, poles and cages) were cleaned every two weeks (algae that had gone were replaced and cages and plastic mimics were brushed).

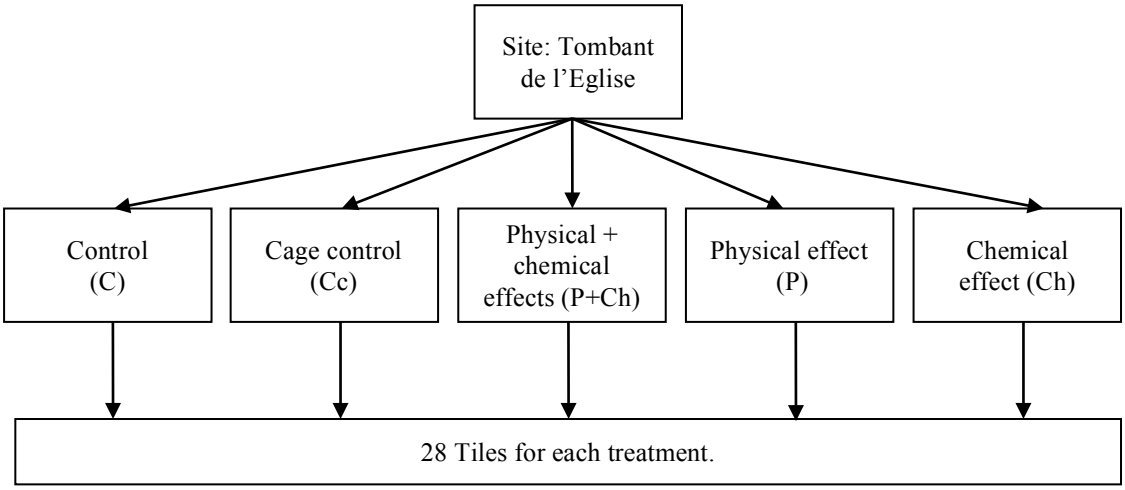


Figure 44: Schematic representation of the experiment on the effects of *Sargassum* spp. on benthic species recruitment and early development conducted in 2010. C= control, Cc= cage control, P+Ch= physical+chemical, P=physical and Ch=chemical effects.

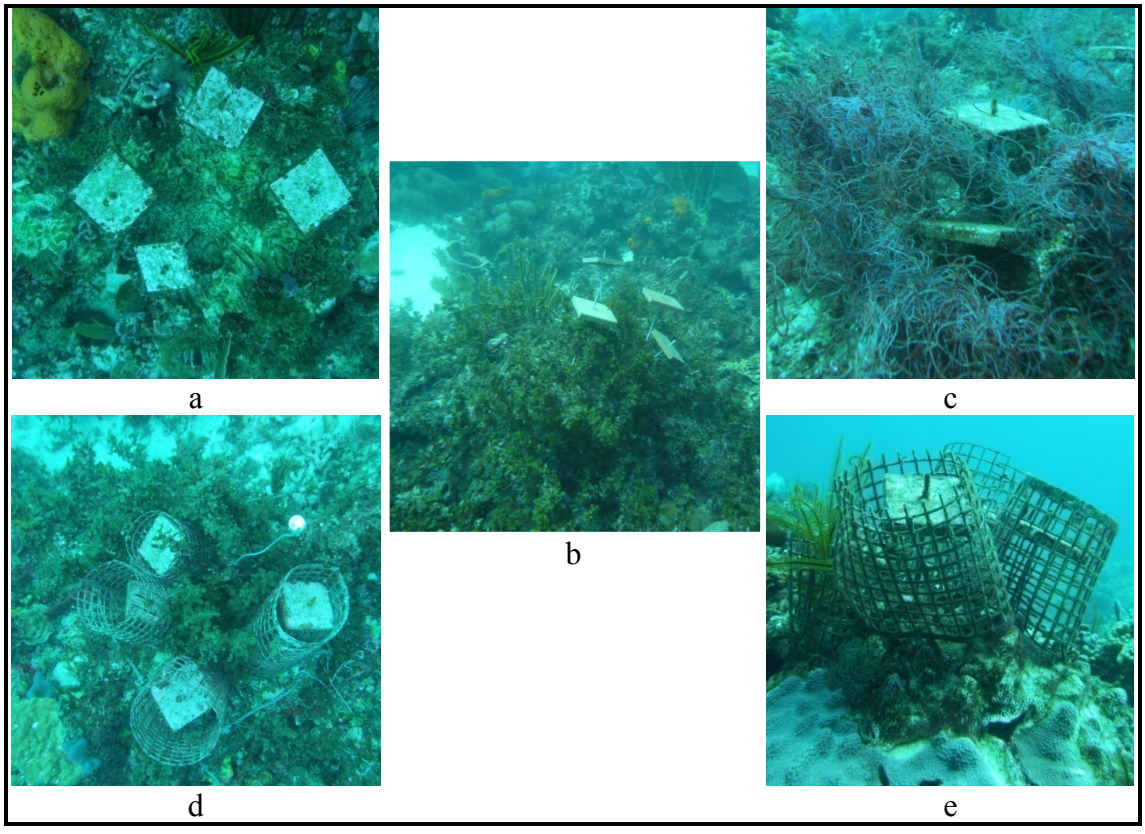


Figure 45: Pictures of the devices employed to test the effects of *Sargassum* spp. on coral recruitment : a) Control (C), b) Physical and chemical effect (P+Ch), c) Physical effect (P), d) Chemical effect (Ch) and e) Control cages (Cc).

III.2.3. Tile observations

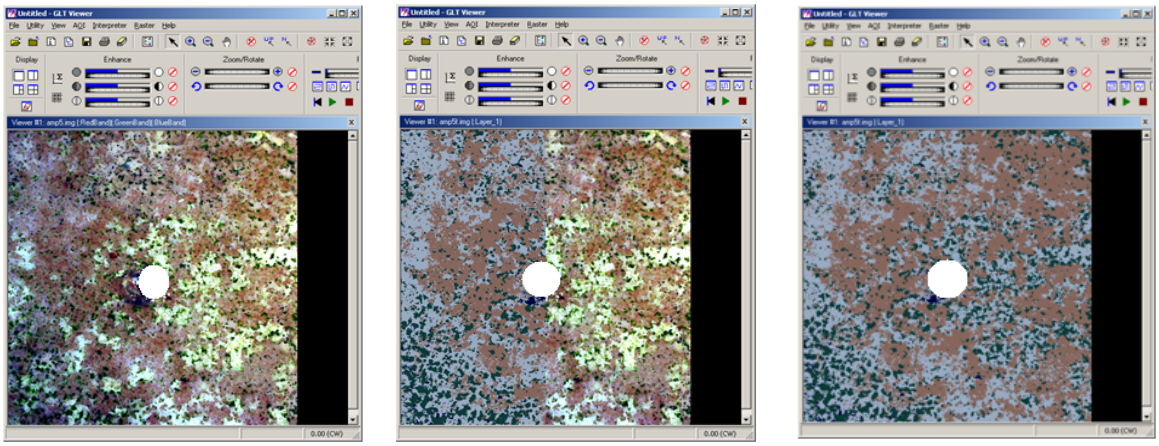
Once collected, the plates were transported to the laboratory in containers filled with seawater so that the organisms could remain alive. A picture of each side of the plates was taken and the tiles were fixed using a solution of ethanol (70%) before freezing.

III.2.3.1 The exposed layer

The exposed surface was colonised by macroalgae, CCA and turf. These organisms were characterised by different colourations, which were numerically treated to determine the percentage cover on the tiles. A remote sensing method (Supervised classification) was applied to the pictures using the software ERDAS IMAGINE 9.3 in order to determine the percentage cover of organisms present. Remote sensing is the science by which information about material objects is obtained without coming into physical contact with the object. Supervised Classification relies on the *a priori* knowledge of the identity of organisms cover types that are in the image. This knowledge allows the classification to be made and the set up of discrete and usually small classes called "*Training sites*". These areas are created by circumscribing them with polygonal boundaries (usually more than one) drawn using the computer mouse. These areas are used to "*train*" the classification algorithm to recognize organism cover classes based on their spectral signatures. This procedure thus identifies spectrally similar areas on an image by identifying '*training*' sites and then extrapolating those spectral signatures to other areas.

The classification method was used to organise the pixels of the picture into 6 categories: CCA, encrusting green, turf and sediment, macroalgae, brown algae and bare substratum (Figure 46). Classification techniques based on pixel colours are usually employed in geography and satellites image analyses to create classes (for examples, field, urban areas...) and thus determine the percentage cover of each area.

A signature was drawn for each tile, as the pictures luminosity (and consequently the pixel colour of each organism) was different from tile to tile. After obtaining the final picture with the different classes, the percentage cover of each class was determined as the ratio of pixel of the class divided by the total number of pixel of the tile area.



a b c

Figure 46: Image treatment using ERDAS software. a) original picture of the tile taken before performing analysis; b) is the picture once the treatment has been done (half treated-left/ half not treated-right) and c) is the results after treatment.

III.2.3.2 The lower layer

The lower surface of the tile was colonised by numerous invertebrates. Several analysis techniques were developed for use depending on the organisms observed.

- The tubeworm percentage cover was determined from the picture taken by adjusting the threshold of the picture (software Image J) and measuring the percentage of dark pixels present on the picture (Figure 47b).
- The tiles were then observed under a binocular microscope and organisms such as corals were counted (the number of coral spats presented in the results corresponds to coral settled on the whole tile).
- Encrusting organisms were observed using a binocular microscope and their percentage cover was determined by surrounding their shape using the software Image J. This allowed determining the number of pixels inside the area surrounded (Figure 47a). The ratio between this number of pixel and the total number of pixels in the photograph allowed the determination of the percentage cover.

a

b

Figure 47: Image treatment using Image J software. a) Measure of the percentage cover of organisms on the tile (the table: 1 represents the measures carried out on the entire tile and 2 the measures of the selected area). b) Threshold adjusting and measure of worm percentage cover on the tile.

III.2.4. Statistics

All the data were statistically analysed with the software R cran.

The percentage cover data were tested for normality using a Shapiro-wilk test. As they were not parametric even after transformation, a pool of nonparametric tests was applied to the data.

Prior to the statistical analyses, the percentage data were Asin√p transformed following Legendre and Legendre, (1998) and the coral and schizoporella data were log(x+1) transformed, in order to reduce the heteroscedasticity.

A PCA followed by a HCPC were performed on the transformed data obtained in 2008/2009 with the packages "Ade4" and "FactoMineR" to highlight similarities and differences between data and allow distinguishing groups of data.

To confirm the differences observed visually with the PCA and HCPC, PERMANOVAs were realised. This technique performed with the package "vegan" allows the comparison between the different variables (*algal*, *coral*, *urchin*, and *without algae* areas). A multiple range test (MRT) was then conducted with the package "npmc" to test all pairwise comparison between factors and variables and thus highlight the variables responsible for the differences observed. When only 2 groups were analysed (the case for years or seasons), a Mann Whitney test was performed (Table 19).

Table 19: Statistical treatments applied to the tiles (exposed layer) transformed data.

Factor	**Tests applied**		
Treatment	PERMANOVA	MRT	
Year, Season	PERMANOVA	Mann Whitney	
Year*Treatment, Year*Season, Treatment*Season	PERMANOVA	MRT	
Year*Treatment*Season	PERMANOVA	MRT	ACP/Cluster

For the 2010 results, focusing only on the hidden layer of tiles, PERMANOVA and MRT analyses were performed.

III.3. Results

Percentage cover of organisms colonising the settlement plates as well as the number of solitary organisms were determined on tiles collected from the field. This allowed the determination of the effects of algal canopies and, more particularly *Sargassum* spp., on recruitment.

III.3.1.Effects of algal canopy on tile colonisation

III.3.1.1 Trends for benthic biota groups on the upper layer of the tile

III.3.1.1.1. General observations

The exposed surface of tiles was mainly colonised by algae. The benthic species observed were classified into 5 categories: Crustose Coralline Algae (CCA), turf, macroalgae (macro), green and brown encrusting algae (Figure 48, Appendix 2).

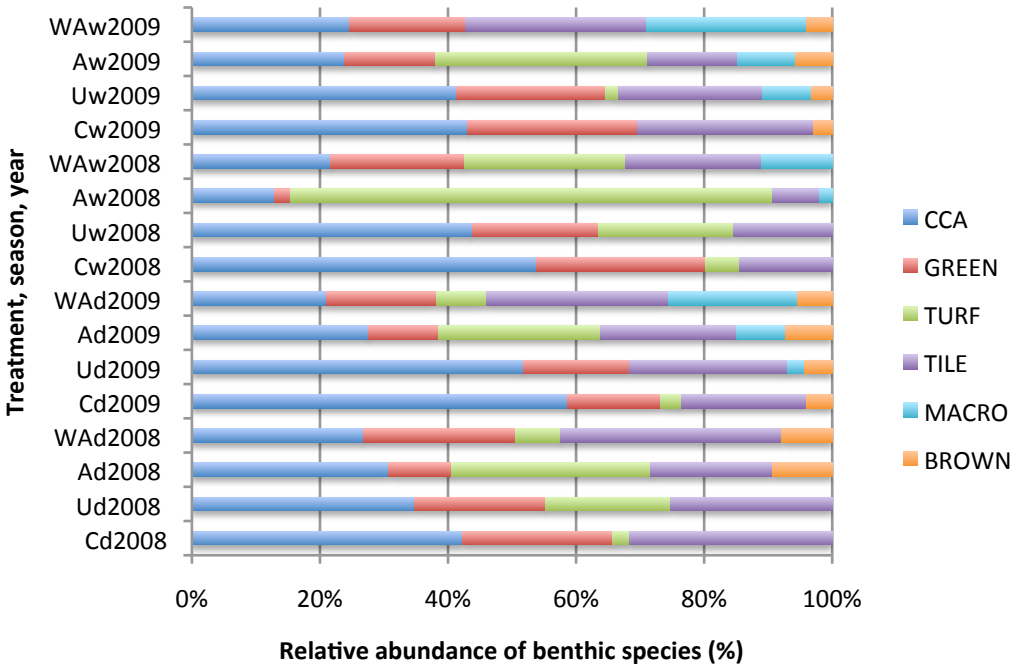


Figure 48 : Relative abundance (%) of benthic species groups on the upper layer of the tiles in the four treatments (C= *Coral area*, A= *Algal area*, U= *Urchin area* and WA= *Without Algae* area) for each season (d=dry and w=wet) over the 2 years of study, (2008 and 2009) (n=20).

Mean organism percentage cover on tiles was comprised between 64.4% and 92.6% (respectively for WAd2008 and Aw2008). The CCA were more abundant on the tiles fixed

in the *urchin* or *coral* zones (respectively between 35-53% and 42-59%) than on the tiles fixed in the *algal* or the *without algae* zones (respectively 13%-31% and 21%-27%). Turf was present at Trois Rivière, essentially in the *algal* area (between 27.5% and 75%) while it was almost absent from the *coral* area (between 0 and 5%). Macroalgae were mainly growing on tiles present in the *WA* area, where they had been manually removed.

In order to compare the tiles depending on treatments, season, years and identify their affinity, PCAs were performed. The first axis explained 43.9% of the species distribution, while the second accounted for 29.9% (Figure 49). From the variable graph it can be seen that turf was negatively correlated with green algae. The macroalgae and brown encrusting algae mainly explained the second axis. *U* and *C* were associated with the presence of CCA while *A* was partly associated with the presence of turf.

The cluster analyses separated the treatments into 3 categories (Figure 49): cluster 1: *A* area data; cluster 2: *WA* data, except the one from the wet season in 2008; cluster 3: The *C* and *U* areas with the *WA* tiles from the wet season 2008.

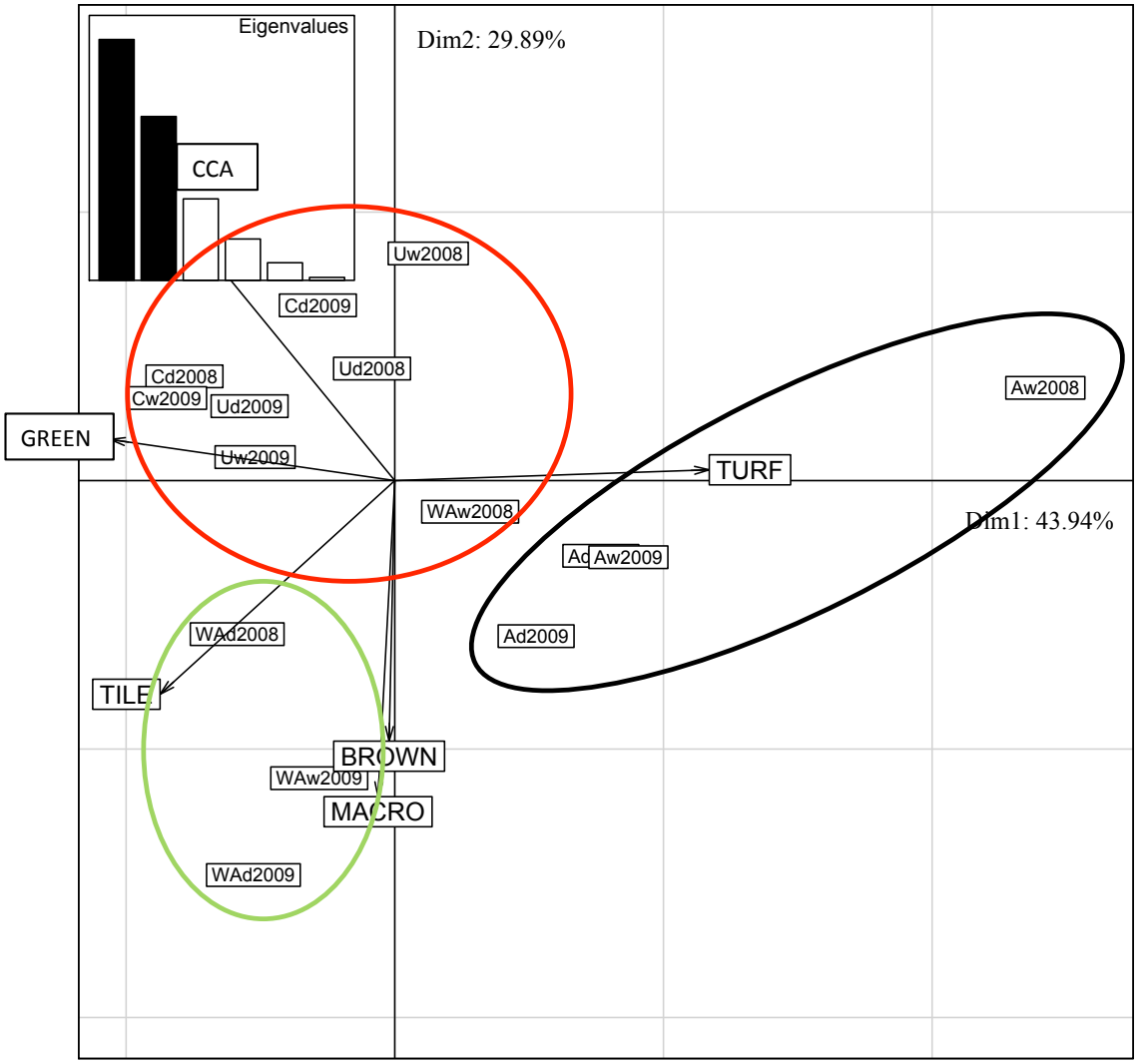


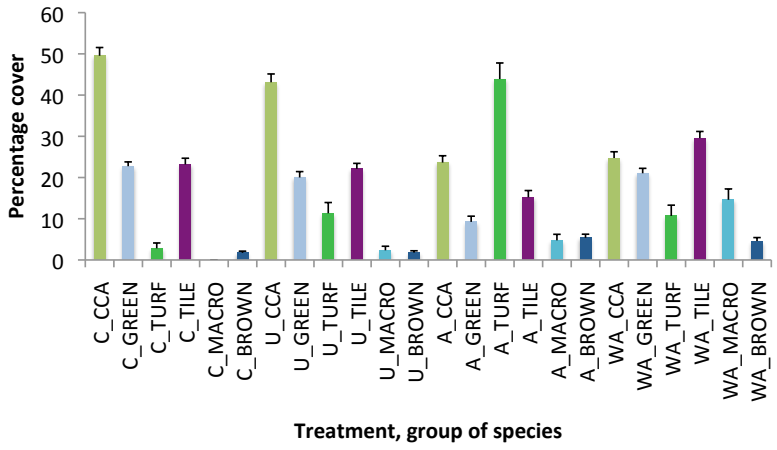
Figure 49: Principal component analysis (R, ade4 + FactoMineR) on the relative abundance of organisms and free substratum (Brown, CCA, Green, Macro, Tile and Turf) on the exposed layer of the tiles collected in the 4 areas (C= *Coral area*, A= *Algal area*, U= *Urchin area* and WA= *Without Algae* area), 2 seasons, (w=wet season and d= dry) in 2008/2009. Data were transformed prior analyses (Asin, squared rooted) and the results of the hierarchical clustering are reported (3 groups).

III.3.1.1.2. Differences according to treatments, seasons and years

Results were plotted according to treatments (*C, U, A* and *WA*) (Figure 50), then according to seasons (Figure 51) and finally to years (Figure 52). The PERMANOVA performed on data demonstrated the site, season, year, site x season, site x year, season x year and site x year x season to have a significant impact on the tile colonisation by organisms ($p<0.05$).

III.3.1.1.3. Differences according to treatment

Treatments had a crucial importance regarding the colonisation of organisms (Figure 50). CCA was higher in *urchin* (U = 43.1%) and *coral* (C = 49.5%) areas than in the others (respectively 23.6% and 27.7% in *A* and *WA* areas). The MRT results showed CCA cover not to be significantly different in *C / U* areas ($p>0.05$), and *algal (A)* / *without algae (WA)* areas ($p>0.05$). Turf was significantly more abundant in the *A* (43.8%) area than in *WA* (10.6%). The *WA* and *U* areas (11.31%) did not demonstrate any differences in turf cover while *U area* had significantly more turf than the *coral* area (2.81%) ($p<0.05$). For both brown and green algae, only *A* was statistically different from the others.

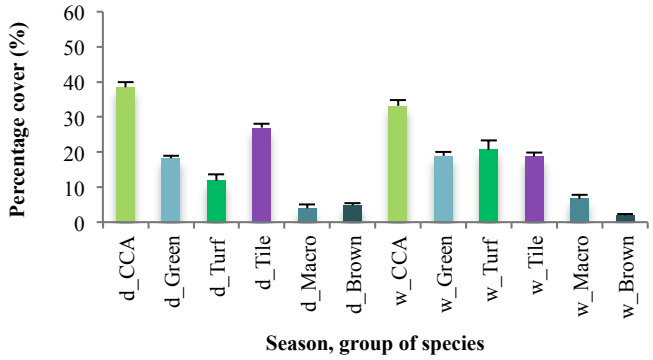


Class	Npmc Results
CCA	C = U > A= WA
Green	C = U = WA> A
Turf	A >WA = U > C
Tile	WA> C = U > A
Macro	WA> A = U > C
Brown	A > WA = U = C

Figure 50: Variation of the percentage cover of organisms on the upper layer of the tiles among treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area)*. (n=20 ±SE). The table summarizes the results of the statistical treatment.

III.3.1.1.4. Differences according to seasons

Brown and CCA percentage cover significantly decreased, respectively from 4.8% to 1.9% and 38.5% to 33% between the dry and the wet seasons ($p<0.05$) while the macroalgal percentage cover increased significantly from 4% to 6.6% (Figure 51). Both green and turf cover did not change significantly ($p>0.05$, Figure 51). The available space on the tile decreased significantly between the dry and wet season from 26.8% to 18.7%.



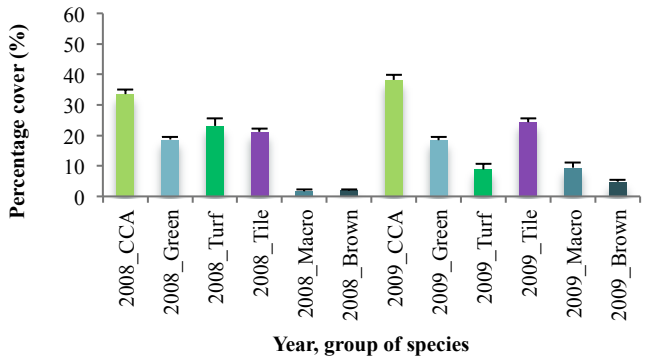
Class	Mann Whitney results
CCA	d > w
Green	d = w
Turf	d = w
Tile	d > w
Macro	w > d
Brown	d> w

Figure 51: Variation of the percentage cover of organisms on the upper layer of the tiles among seasons (w=wet and d=dry). (n=20 ±SE). The table summarizes the results of the statistical treatment.

III.3.1.1.5. Differences according to years

Only the Turf, Brown, Tile and Macro presented a significant difference in percentage cover according to years (Figure 52). Turf was significantly more abundant in 2008 (23.1%) than in 2009 (8.8%) while Macroalgae were more abundant in 2009 (4.7%) than in 2008 (1.6%).

Treatment (*C, U, A* and *WA)* was the factor having the most significant impact on the development of organisms on the exposed layer of the tile. CCA were more abundant in the *coral* and *urchin* areas, while turf and macroalgae were mainly present in the *algal* and w*ithout algae* ones.



Class	Mann Whitney results
CCA	2009 = 2008
Green	2009 = 2008
Turf	2008 > 2009
Tile	2009 > 2008
Macro	2009 > 2008
Brown	2009 > 2008

Figure 52: Variation of the percentage cover of organisms on the upper layer of the tiles among years (2008/2009). (n=20 ±SE). The table summarizes the results of the statistical treatment.

III.3.1.2 Trends for benthic biota groups on the lower layer of the tile

III.3.1.2.1. General observations

Other categories of organisms were observed on the lower part of the tiles, among which numerous invertebrates (bryozoans (bryo), ascidians (asc), corals, sponges and worms). Some CCA could also be observed on the lower part of the tiles, but were not considered in this part of the analysis as focus was only made on the invertebrates (Figure 53).

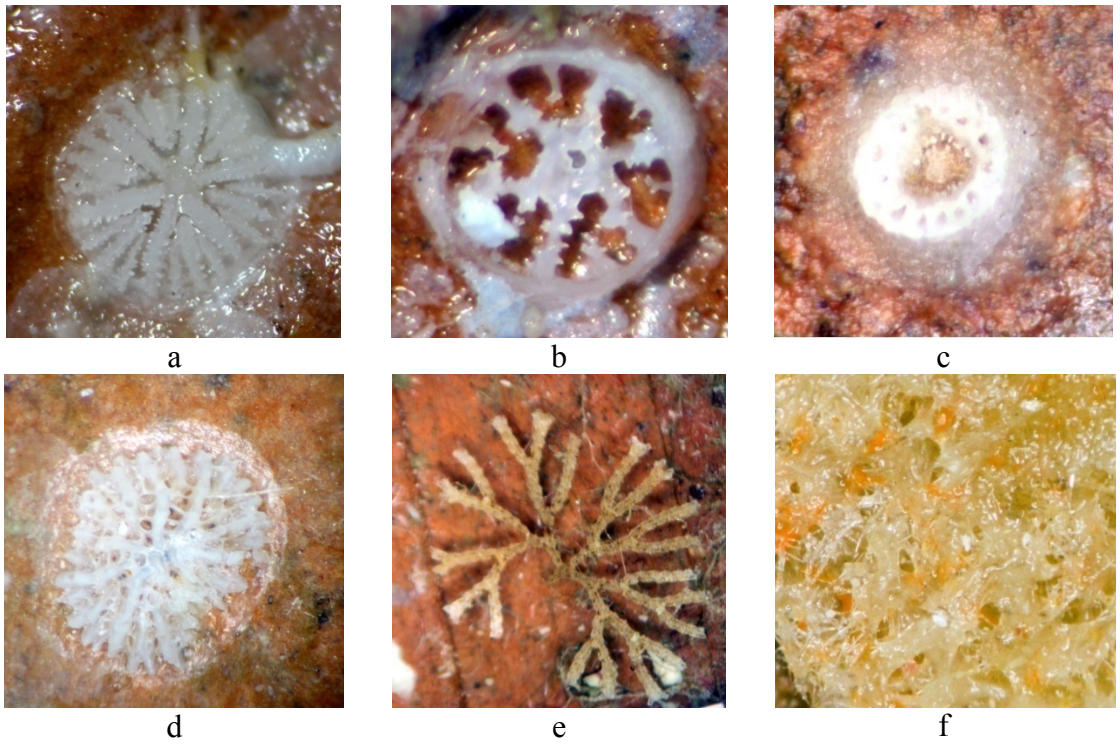


Figure 53: Picture of some benthic sessil organisms observed on the lower layer of tiles with a binocular microscope. a) Recruit of Agariciidae, b) Recruit of Poritidae, c) Unknown coral recruit d) *Schizoporella* e) Bryozoan, f) Sponge.

Percentage cover of organisms on tiles remained low and varied between 4.7% and 16.3% depending on the tile location. Recruitment was less abundant in *algal* areas and was also generally reduced in the wet season compared to the dry (Figure 54, Appendix 3).

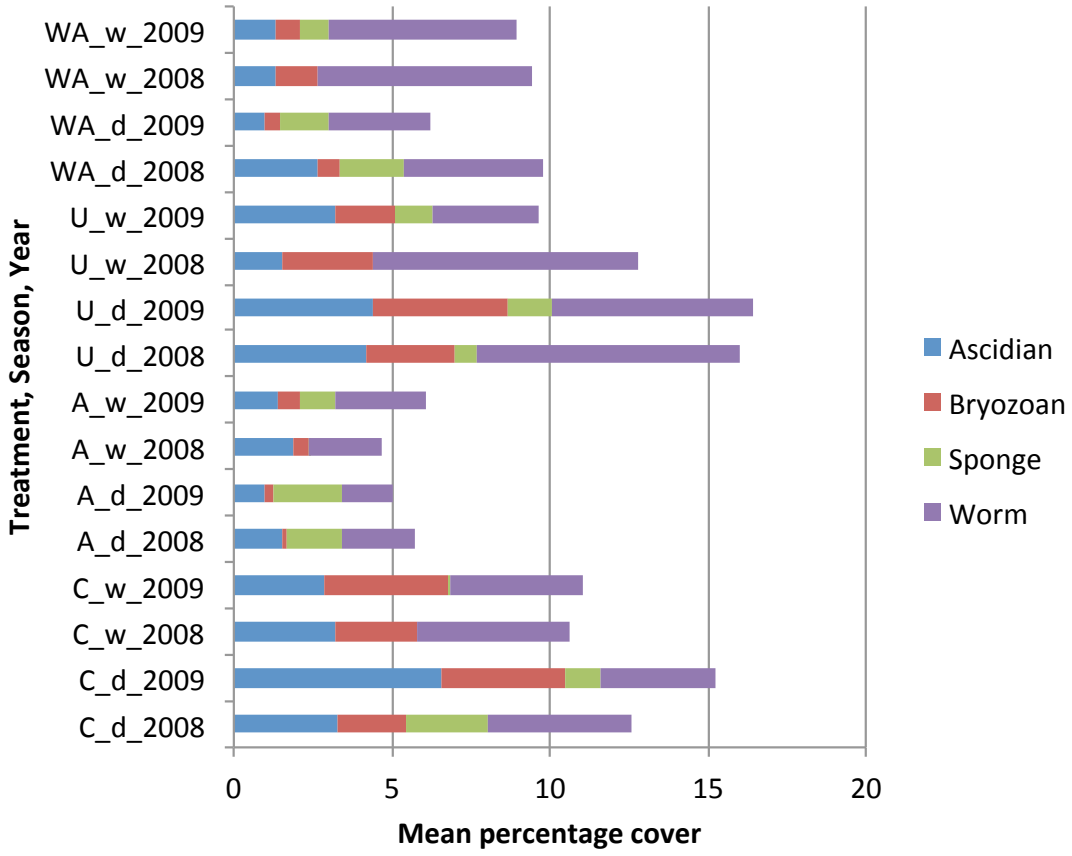


Figure 54: Variation of the percentage cover of organisms on the tiles (n=20) among treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area*), for each season (w=wet and d=dry) for each year of the study (2008/2009). Results are given for the 4 main groups defined (Ascidians, Bryozoans, Sponges and Worms).

In order to compare the tiles depending on treatments, seasons, years, and identify their affinity, a PCA was carried out on the variables previously described. The first axis explained 56% of the species distribution, while the second only accounted for 22.4% (Figure 55). From the variable graph it could be seen that sponges were negatively correlated with the other variables. The *C* and *U* tiles were associated with the presence of ascidians and bryozoans (factor map), while *A* was mainly associated with the presence of sponges (Figure 55).

The cluster allowed classification of the tiles into 3 categories, which slightly differed from the categories described on the exposed surface of the tiles (Figure 55). The first category

grouped the *algal* and *without algae* areas of 2009, the second one grouped the *without algae* data of 2008 and the wet *urchin* data from 2008 while the last category grouped the remaining data from the *coral* and *urchin* areas.

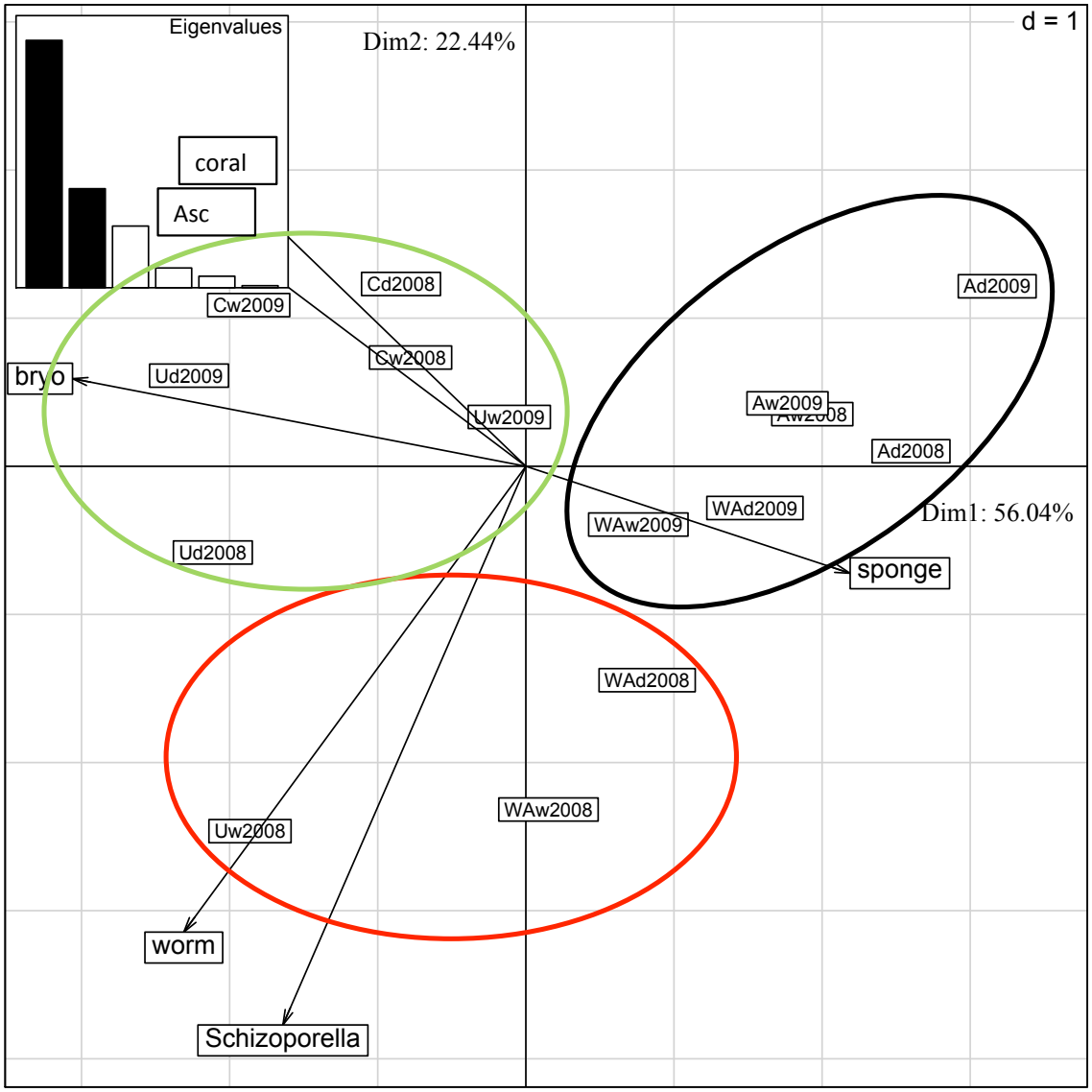


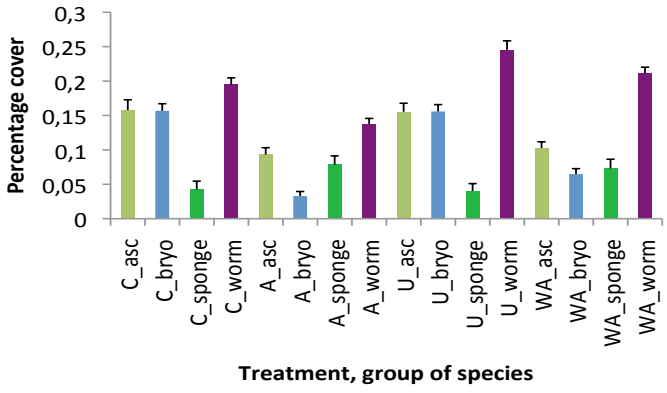
Figure 55: Principal component analysis (R, ade4 + FactoMineR) on the relative abundance of organisms (percentage cover or number of solitary organisms on the exposed layer of the tiles collected in the 4 treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area*), for each season (w=wet season and d= dry) for each year of the study (2008/2009). Data were transformed prior analyses (Asin, squared rooted or log(x+1)) and the results of the hierarchical clustering are reported (3 groups).

III.3.1.2.2. Differences according to treatments, year and season for encrusting organisms

The PERMANOVA analyses conducted for the different levels only demonstrated the treatment and year data to be significantly different ($p<0.05$).

III.3.1.2.3. Differences according to treatments

Percentage cover of organisms on plates fixed in the *coral (C)* and *urchin (U)* zones were not statistically different from each other (except for worm percentage cover which was respectively of 0.24% and 0.19%). *Algal (A)* and *without algae* (*WA*) zones differed for both bryozoans (respectively 0.03% and 0.06%) and worm percentage cover (0.13% and 0.21%). *WA* seemed to be in between *U-C* group and *A* one (Figure 56). Ascidians and sponges percentage covers were not statistically different between *A* and *Wa* and between *C-U* and *Wa*.

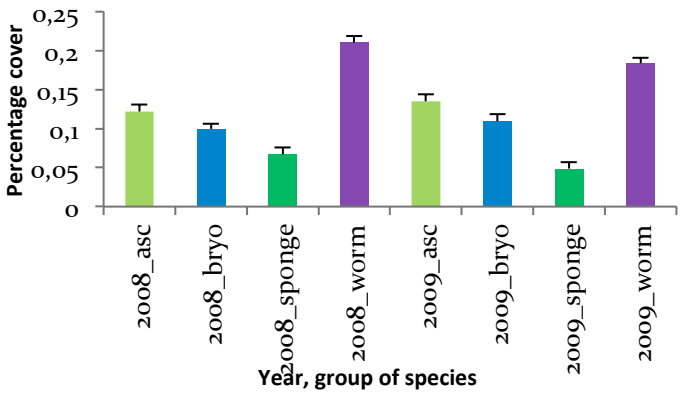


Class	Npmc Results
Ascidian	C = U = WA and WA=A
Bryozoan	C = U > WA> A
Sponge	C = U = WA and WA=A
Worm	U > C = WA > A

Figure 56: Variation of the percentage cover of organisms on the lower layer of the tiles among treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area)*. Asc= Ascidian and bryo=bryozoans. (n=20 ±SE). The table summarizes the results of the statistical treatment.

III.3.1.2.4. Differences according to years

The differences noticed between years were only significant for worms and sponges and were very weak (Figure 57). Worm percentage cover varied between (0.21% in 2008 and 0.18% in 2009) while sponge percentage cover varied from 0.06% in 2008 and 0.04% in 2009.



Class	Mann whitney results
Ascidian	2008 = 2009
Bryozoan	2008 = 2009
Sponge	2008 > 2009
Worm	2008 > 2009

Figure 57: Variation of the percentage cover of organisms on the lower layer of tiles among years (2008 and 2009). Asc= Ascidian and bryo=bryozoans. (n=20 ±SE). The table summarizes the results of the statistical treatment.

III.3.1.2.5. Differences according to treatment, year and season for coral spats and schizoporella

The species and numbers of coral spats varied according to treatments and years. They were more abundant in the *coral* zone (between 2.1 and 0.95 indiv. 100 cm^{-2}), followed by the *urchin* area (between 1.2 and 0.3 indiv. 100 cm^{-2}) and almost absent from the *algal* (between 0.1 and 0 indiv. 100 cm^{-2}) and *without algae* (between 0.15 and 0 indiv. 100 cm^{-2}) zones. Agariciidae and Poritidae families were more abundant in the *coral* area (respectively between 0.45-0.8 and 0.25-0.6 indiv. 100 cm^{-2}), but a few spat had settled in the *urchin* zone (respectively between 0.05-0.1 and 0-0.2 indiv. 100 cm^{-2}) (Figure 58).

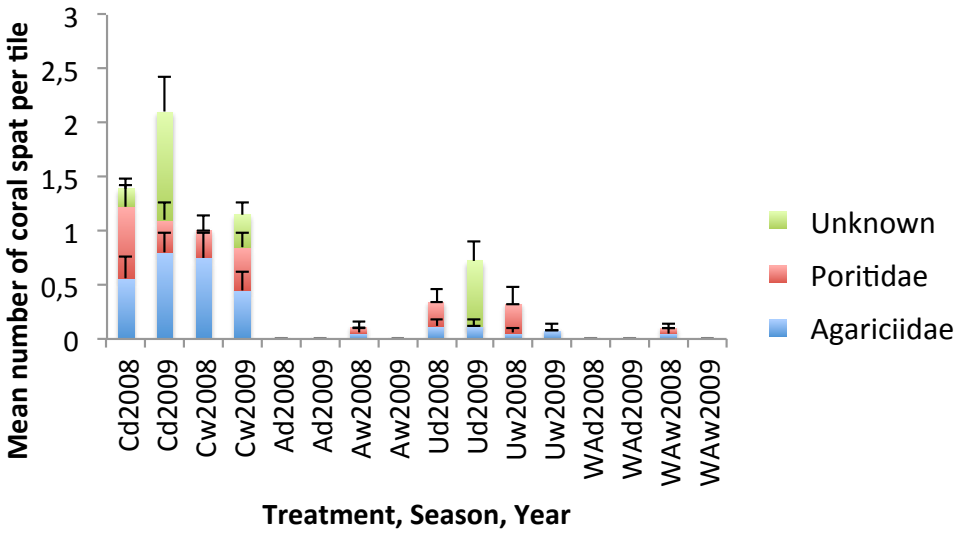


Figure 58: Variation in recruitment rates of coral spats (mean number of recruits per tiles, n=20±SE) among treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area*) for each season (w=wet and d=dry) over the 2 years of study (2008/2009). Results are given for the 3 families (Agariciidae, Portidae and unknown).

The number of schizoporella did not differ significantly between treatments, seasons or years (Figure 59).

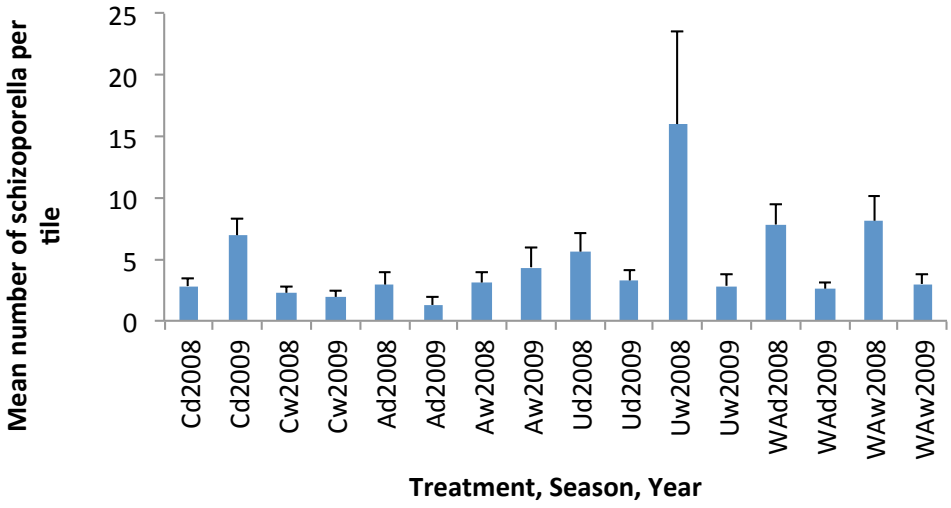


Figure 59: Variation in recruitment rates of schizoporella (mean number of recruits per tiles, n=20±SE) among treatments (C= *Coral* area, A= *Algal* area, U= *Urchin* area and WA= *Without Algae area*) for each season (w=wet and d=dry) over the 2 years of study (2008/2009).

III.3.2.Effects of *Sargassum* spp. on tile colonisation (2010 survey)

This experiment aimed at testing the physical and chemical impacts of *Sargassum* sp. on recruitment.

III.3.2.1 Trends for benthic biota groups on the lower layer of the tile

Very few ascidians or sponges were observed on the tiles (only one sponge on all the tiles analysed). These categories were thus not analysed statistically. The PERMANOVA conducted on the results demonstrated a significant difference between the 5 treatments (C, Cc, Ch, P and P+Ch). The MRT showed the differences to be significant ($p<0.05$) only between the control and the other categories for coral spats, bivalves and worms (Figure 60). The percentage cover of worm was a lot lower on the coral tiles (around 5% while it was around 15% on the other tiles). Bivalves and coral spats were more abundant in coral zones where they respectively accounted for 4 and 0.4 indiv. 100 cm^{-2} while they only accounted for about 0.1 and 0.05 indiv. 100 cm^{-2} on the other tiles. From the coral spats figure (Figure 60), it can be observed that there was some coral recruitment in the presence of *Sargassum* spp., however the standard error of the means is important and this number of coral spats settled occurred only over one tile.

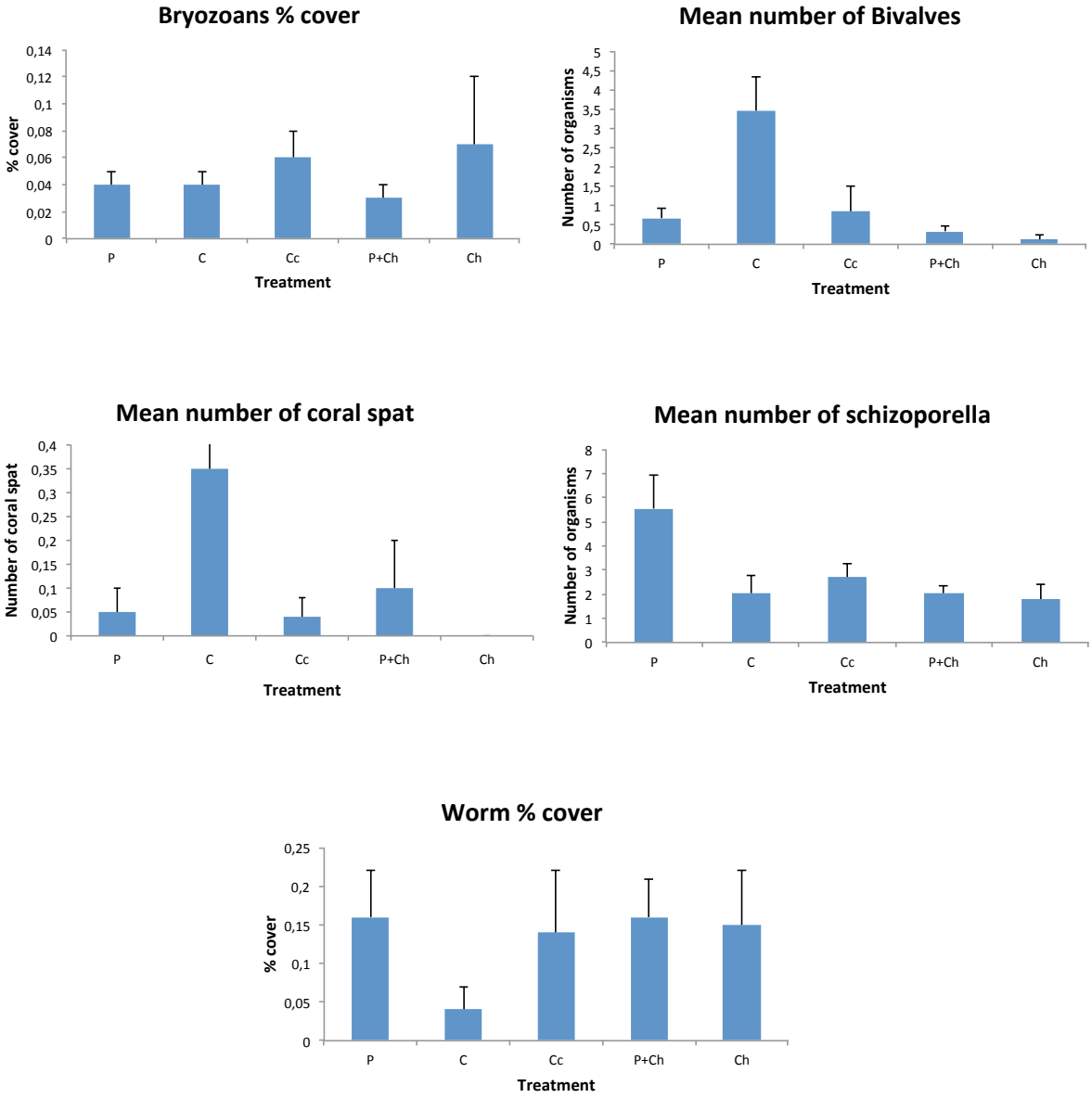


Figure 60: Variation in recruitment rates and percentage cover of organisms (n=28±SE) among treatments (P = Physical effects, C = control, Cc= cage control, P+Ch= Physical+chemical effects and Ch=Chemical effects). Results are expressed as mean percentage cover (for worms and bryozoans) and number of organisms per tile (for bivalvia, corals and schizoporella).

Experiment 1: Effects of algal canopy on recruitment

Treatment (i.e presence of algae, urchins or coral) was the factor having the most significant impact on the development of organisms on the lower part of the tiles. Coral spats were more abundant in the coral and urchin areas and percentage cover of encrusting organisms was very low in the algal beds. Three categories could be identified from the results: i) the tiles fixed in the algal and without algae (2009) areas, ii) the tiles fixed in the urchin (wet 2008) and WA (2008) area and iii) the tiles grouping in the urchin and coral areas. The without algae and urchin areas had characteristics in between the algae and coral areas regarding recruitment and early development stages of invertebrates.

Experiment 2: Effects of Sargassum spp. on recruitment

Differences were only observed between the control and the other treatments for coral, bivalves and worms. The number of coral spats settled on tiles during this study was a lot lower than at the sites of Anse Mabouya and Trois Rivières (urchin area). Benthic groups not observed in the first experiment, such as bivalves, were present on the tiles in the second experiment.

III.4. Discussion

The transition from pelagic to benthic life stage determines the environmental conditions in which sessile species will evolve. Some larvae, zygotes and algal spores actively choose the substratum to settle on (Fletcher *et al.,* 1992, Clare and Aldred, 2009, Hellio *et al.,* 2009a). As an example, coral pelagic larvae can detect and respond to acoustic cues and direct their swim towards reef sounds (Vermeij *et al.,* 2010b) and chemical cues partly drive settlement processes (Paul and Puglisi, 2004). Sessile organism recruitment process is hazardous and depends on biological, chemical and physical factors (Pawlik, 1992a, Fusetani, 2004).

Poritidae and Agariciidae*,* known as pioneer reef colonisers, were the main coral spats recruiting on tiles. However, more species were expected to recruit, especially because other juveniles such as *Siderastrea* sp. and *Meandrina meandrites* were noticed in the field (Chapter II). *Agaricia* sp. and *Porites* sp. (brooders) produce planulae which settle within a few hours and are thus less subjected to displacement, and water column constraints (Sammarco, 1996). Moreover, brooders, can reproduce several times a year thus favouring their chance of settling compared to broadcasters (Van Moorsel, 1983). *Madracis* sp. (brooder) reproducing from March to December with a peak release from September to November has already been observed in recruitment experiments (Birkeland *et al.,* 1981, Vermeij *et al.,* 2003). *Madracis* sp. spats, however, did not recruit in the present experiment while the presence of parent colonies at the investigated sites was important (Chapter II). The differences observed between the coral juveniles (Chapter II) and coral recruits (Chapter III), suggests that the experimental design could be unfavourable to some species: i) the substratum employed might not be suitable for some coral species, or some associated species producing chemical cues required for their settlement (Penin, 2007). Nozawa *et al.,* (2011) demonstrated the surface structure of settlement plates to be an important factor concerning coral recruitment. Crevices increase the number of settled spats and their diversity, suggesting crevices to attract more coral recruits and/or to provide them a refuge and reduce their post-settlement mortality rates (Nozawa *et al.,* 2011) and ii) The duration of the experiment (4 months) might not be suitable for some organisms but may favour the recruitment of pioneer reef colonizers.

The average number of coral spats settled in the present experiment varied between 0.95 and 2.1 indiv. 100 cm $^{-2}$ in *coral area* which corresponds respectively to 285 and 630 recruits.m^{-2}.year^{-1}. (these averages are extrapolation from the experiments conducted for 4 months, thus the results could be different if the tiles had been left for a year in the field). These levels were a lot lower than most of the one found in the Great Barrier Reef, but were higher to the levels already observed in the Caribbean (Table 20). Recruitment rate could thus be important in Martinique as compared to other Caribbean sites, however, results are hardly comparable as i) these studies were conducted in Bahamas, Barbados and Bermuda (very distant) ii) the methods for tiles deployment (orientation, attachment, depth, immersion time) and iii) the tile surfaces (type and size) were variable. Studies demonstrated these characteristics to be highly influencial in the coral spat settlement process (Harriott and Fisk, 1987, Mundy, 2000, Field *et al.,* 2007, Penin, 2007).

In the present experiment, treatment (*coral, urchin, algae, without algae*) was the main factor influencing organism's settlement and early development. Turf algae were more abundant in *algae* and *without algae* areas, while CCA were more abundant in *coral* and *urchin* ones. CCA are often abundant in zones characterised by high coral cover, strong herbivory pressure and low fleshy algal percentage covers, which was the case of *urchin* and *coral* areas (Steneck, 1997; Furman and Heck, 2009). Analysis of the data lead to the establishment of 3 groups i) the *WA* one (except wa2008) correlated to Tile, Brown and Macroalgae, ii) the *Urchin-Coral* one which was mainly correlated to CCA and Green and iii) the last one grouping the *algal areas* and correlated to the presence of Turf. This study demonstrated turf and sediments to be abundant in *algal* areas where they could, therefore, prevent benthic invertebrate larval recruitment. Certain algal turf assemblages, coupled with sediment or not, have already been demonstrated to interfere with coral recruitment process (Birrell *et al.,* 2005). Organisms present on the lower layer of the tile also demonstrated a variation according to treatments. *Urchin* zones that were previously suggested to provide good conditions for coral to recruit (Carpenter and Edmunds, 2006), favoured the settlement of coral species compared to *algal* areas. The *urchin, algal* and *without algal* areas were located at the same site, a few meters apart the one from the other, ensuring the larval stock would be the same and that the variation observed could only be the fact of algal or urchin presence/absence.

Diadema antillarum presence, as suggested by Carpenter and Edmunds, (2006), enhanced

coral spat recruitment. It is possible that the recovery of this sea urchin creates favourable conditions and participates to reef resilience. However, sites where the coral diversity was richer promoted better the coral spat recruitment. This is consistent with the results of the Chapter II which deonstrated that the density of juveniles was higher in *coral* areas as compared to *urchin* zones (intermediate zones).

Table 20: Scleractinian recruitment rate on artificial substrata at several localities. (Penin, 2007). Recruits abundance has been averaged between sites and years, except if experimental conditions prevented it. In that case, only controls were used. GBR: Great Barrier Reef, H: horizontal, V: vertical, O: Oblique.

Localisation	Substrates	Attachement	orientation	depth (m)	surface (cm²)	immersion (months)	recrutement (nb.m-².year-1)	references
PACIFIC								
Moorea	Terracota	Racks	H/V	-	225	4	38-125	(Gleason, 1996)
Guam	Plastic	Direct	H/V	6-36	75/225	1.5-6	57	(Birkeland *et al.,* 1981)
GBR North	Terracota	Racks	H	2	122	2	1840	(Baird and T.P., 1997)
	Terracota	Racks	H	2	122	2	526	(Baird and T.P., 1997)
	Terracota	Racks	-	4-5	225	4/9	1135	(Maida *et al.,* 1994)
GBR meridional zone	Terracota	Direct	H	1	122	2	4222	(Hughes *et al.,* 2000)
	Terracota	Direct	H	1	122	2	4590	(Hughes *et al.,* 1999b)
	dead coral	Racks	O	3-14-18	3-400	6/12	2092	(Sammarco, 1991)
	Terracota	Racks	H	7-10	144	1.5	633	(Babcock, 1988)
GBR south	Variable	Racks	-	4	-	4.5	2044	(Harriott and Fisk, 1988)
	Terracota	Variable	H/O/V	9	122	-	150	(Mumby, 1999)
	Terracota	Racks	H	9-12	400	5/12	307	(Dunstan and Johnson, 1998)
South East Asia	Terracota	Racks	-	9-19	225	0.5-1	173	(Banks and Harriott, 1996)
	Terracota	Racks	-	6-9	225	5	132	(Harriott and Banks, 1995)
Taiwan	Variable	Racks	H/V	-	225	1-2	0-133	(Soong *et al.,* 2003)
Hong-Kong			H/V	7	900	3-24	0-70	(Lam, 2003)
CARAIBBEAN								
Bahamas	calcareous	Racks	H/V	10-100	225	4-8	106	(Avery and Liddell, 1997)
Barbados	Terracota	Racks	H/V	-	225	17	79	(Hunte and Wittenberg, 1992)
Bermuda	Variable	Racks	-	-	-	-	37	(Smith, 1992)
RED SEA								
Eilat	Terracota	Racks	O	6	100	3-4	190	(Glassom *et al.,* 2004)

All the invertebrate categories, except sponges, recruited less in the *algal* area (mean percentage cover of organisms of 5.3% in *algal* area while it was of 8.5% in the *without algae* one, 12.6% in the *coral* one and 13.6% in the *urchin* one). Some organisms, such as sponges, seemed to have developed a capability to grow in these degraded zones. Indeed

their percentage cover was of 1.25% in *algal* areas while this was of 1.1%, 0.93% and 0.8% respectively in *WA*, *coral* and *urchin* areas. Reef degradation has already been associated with an increase in sponge percentage cover (Lapointe *et al.,* 2007).

The *without algal* area, created to observe the effect of recovery in case of algal mechanical removal on invertebrates recruitment and early stages of development, did not allow for a good coral development, but promoted other benthic organisms settlement. PCA analyses demonstrated this area to be associated either to the *algal* or *urchin* zones. Recovery could thus potentially be possible when removing seaweeds. It could be possible that the weak presence of coral spats in this area was due to the presence of algal beds at the vicinity (chemical cues still released in the water column).

Tiles were fixed in the field during the dry and the wet seasons 2 years in a row. Seasons only influenced organism settled on the exposed layer of the tile. Macroalgae were more abundant on tiles during the wet season while CCA colonised them more during the dry one and turf presented no difference between the 2 seasons. The higher presence of macroalgae during the wet season was in concordance with nutrient run-offs which were more frequent at this period (Lapointe *et al.,* 2004). Moreover, physicochemical studies conducted for the European Water Framework on the littoral of Martinique demonstrated nutrients (DIN and Orthophosphates) to be the most abundant in October, at the end of the wet season, and the lowest in January and March, during the dry season (Impact Mer, 2009). Organisms settling on the lower layer of the tile surprisingly did not demonstrate variations between the dry and the wet seasons (PERMANOVA, $p>0.05$). The present experimental design, except for coral species, was based on the identification of "group of species" (sponge, worms…). It is possible that seasonality exists for species (different reproduction and settlement period) but not for groups.

In the Chapter II, the coral juvenile presence was demonstrated to vary according to habitat types, being higher in *coral* areas followed by *urchin* and finally *algal* one. Several factors could have been responsible for these differences, among which environmental parameters, larval supply and effects of benthic species on recruitment and/or juvenile development. The tile experiment was designed to test for the effects of some benthic species on recruitment (no site effect, no differences in larval supply), some of these factors can be withdrawn: i) the sites are located on the same reef in the same water body, environmental factors are unlikely to be the cause for the differences observed ii) moreover, the proximity

of the sites suggests that larval supply is not the explaining factor for the differences observed.

Tiles fixed in the *urchin*, *algal* and *without algae* areas were distant from about 10 m. Consequently; the larval supply was the same within these 3 zones. The recruitment of a few Agariciidae spats on the tiles fixed in the *algal* area, where no parent colonies are present (0% *Agaricia* sp. Chapter II), further demonstrate that coral larvae were present in this area. It is thus likely that the differential recruitment patterns observed between the *algal, without algae* and *urchin* zones were due to benthic differences (presence or not of seaweeds and urchins) rather than supply differences resulting from proximity of sites.
The *coral area*, (Anse Mabouya), located a few 100 m away from the 3 others (Trois Rivières), presented more coral spat. Here, the benthic difference is the most likely factor. The brooding character of the observed recruits suggests the parent colonies to be located at their vicinity. The higher percentage cover of possible "parent" colonies encountered in this area as compared to the others could explain the differences in recruitment pattern.

The presence of macroalgae seems thus to have a significant impact on benthic invertebrate recruitment processes, decreasing the recruitment of invertebrates such as coral. Algal effect on invertebrates may occur through the production of marine natural compounds and/or through physical effects. *Lyngbya* spp., (Cyanobacteria), *Dictyota* spp., (Phaeophyceae) and *Lobophora variegata* (Phaeophyceae) affected *P. astreoides* larvae which either avoided algae, had a recruitment inhibition or increased mortality rates (Kuffner and Paul, 2004, Kuffner *et al.,* 2006). On the contrary, the alga *Halimeda opuntia* favours the recruitment of *Favia fragrum* even in the presence of other suitable substrata (Nugues and Szmant, 2006). Contact (physical and chemical) of seaweeds with coral is species dependent, soft non-abrasive thallus macroalgae caused bleaching of *Porites* sp. while tougher algae, such as *Sargassum* sp. did not (Rasher and Hay, 2010). However, other experiments demonstrated the abrasive effect of *Sargassum* species within 20 days (River and Edmunds, 2001). The differences between these 2 experiments were suggested to be due to the experimental design and the abundance of *Sargassum* (Rasher and Hay, 2010). Conclusions regarding *Sargassum* effects vary from one study to the other. The second part of the present study aimed thus at understanding how *Sargassum*, the most represented species on Martinique reefs, could affect invertebrate recruitment.

A difference in species composition was highlighted between the 2 studies while both of them were conducted from August/December. Numerous bivalves recruited in the second study (effects of *Sargassum* on recruitment) while they were absent in the first experiment. Inversely, no sponges were observed in the second study. These differences could be due to i) Catastrophic events: Martinique was impacted by two events in 2010: A bleaching period which lasted for more than a month, and a hurricane, Thomas which crossed St Lucia, (neighbouring island), and was responsible for a severe swell in Martinique. Coral bleaching, tropical storms and hurricanes significantly impact coral reef recruitment (Crabbe, 2009, Mallela and Crabbe, 2009). Reduced gametogenesis, lower fertilization success, increased larval mortality rates and lowered settlement survivorships were observed following bleaching events of exposure to elevated temperature (Omori *et al.,* 2001, Bassim and Sammarco, 2003, Negri *et al.,* 2007, Randall and Szmant, 2009). These events could partly explain the weak recruitment observed in 2010 as compared to 2008 or 2009. ii) The study site: the species present at both sites differed in their richness and percentage cover (Chapter II). Coral spat response to soft coral allelopathy was already demonstrated to be site and species dependent: studies showed coral growth rate to be higher in the presence of *Sarcophyton glaucum* at Lizard Island while coral spat survivorship was higher in the presence of *Sinularia flexibilis* at Orpheus Island and higher with *Sarcophyton* at Lizard Island (Maida *et al.,* 2001) and iii) The experimental design: as explained in the material and methods, the tiles were fixed on poles so that their lower surface could be in contact with the algae. This inevitably modified i) the light received by the lower side of the tile and ii) the distance between the reef substratum and tiles.

The second study unfortunately did not permit drawing conclusions about the physical and or chemical impacts of *Sargassum* on other benthic species. Recruitment of coral and bivalves was significantly different in the control treatment. However, no other significant differences could be noticed among the results. The cages or physical treatment had a similar effect to that of the *Sargassum* spp.. It is possible that the treatments hampered the fixation and development of some organisms because the larvae could not reach the substrata. Even if there was no difference between the control cages and *Sargassum* treatment, these treatments were different from the control, suggesting a physical impact (barrier) of algae on recruitment processes. Indeed, canopy-forming algae might, under backwash effects, prevent the larvae from accessing the substratum. From these results, it remains unknown if chemical cues, in addition to the barrier effect observed can play a role

in larval settlement and development of young recruits. To further assess the chemical effects of *Sargassum* spp. on recruitment, surface molecules and waterborne cues produced by *Sargassum* species were tested against marine larvae (Chapter IV).

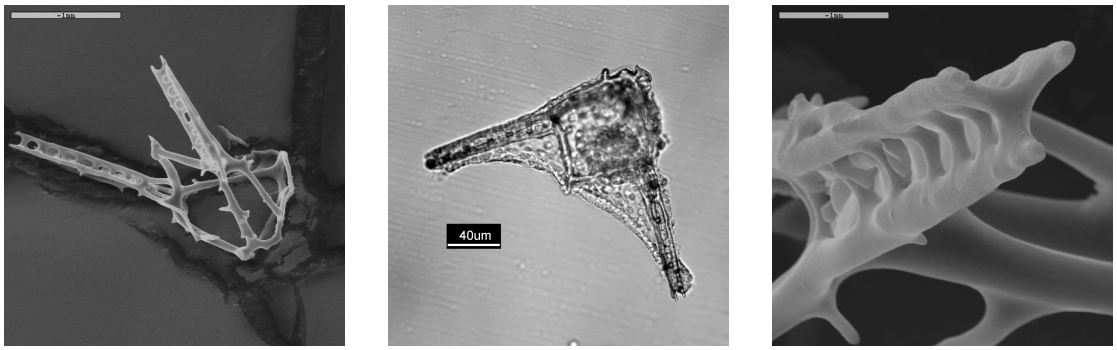
Colonisation depended upon sides of the tiles, their location and to a minor extent years and seasons (dry/wet). Coral spats (mainly brooders) only recruited on the lower side of tiles. CCA grew more in urchin and algal zones while turf developped more in algal zones. All invertebrates except sponges recruited less in algal areas.

The area where algae were manually removed seemed to promote invertebrates recruitment, but these results were moderated by coral spat recruitment which was still as low as in the algal zone.

Macroalgae interfered thus with recruitment processes. The second experiment only allowed concluding about the barrier role of Sargassum spp. and no information was acquired on the possible chemical effects of Sargassum spp. on reef recruitment. (Only the weak coral recruitment rates in cleaned areas (first experiment) could suggest a "toxic" effect of Sargassum spp. waterborne cues towards benthic reef recruitment).

→Does Sargassum spp. impact reef recruitment process by the production of allelochemicals?

Chapter IV: Evaluation of biological activities of *Sargassum polyceratium* surface molecules and waterborne cues towards embryonic development of tropical benthic species.



Light microscope and SEM pictures of *Diadema antillarum* pluteus stages, larvae were treated with bleach to reveal their skeleton.

IV.1. Introduction

Studies concerning marine natural product (MNP) production, activity and chemical ecology have increased over the past decades. Particular attention has been given to the production of secondary metabolites by macroalgae, microalgae, invertebrates, cyanobacteria, octocorals, sponges and ascidians (Hay and Fenical, 1996, Paul and Puglisi, 2004, Paul *et al.,* 2007, Hellio *et al.,* 2009b). Algae produce secondary metabolites with a wide range of biological activities, such as antifungal, antibacterial, antibiotic, antifouling (AF), UV radiation protection, feeding deterrence, inhibition of competitors, gamete attractant and inhibition of larval settlement and development (Hay and Fenical, 1988, Paul *et al.,* 1988, Hay, 1996, Hellio *et al.,* 2001, Hellio *et al.,* 2002, Steinberg and de Nys, 2002, Birrell, 2003, Paul and Puglisi, 2004, Hellio *et al.,* 2005, Paul *et al.,* 2007, Mokrini *et al.,* 2008, Plouguerné *et al.,* 2010a).

To survive the environmental pressures they are subjected to and to successfully colonise new areas, macroalgae have developed several strategies, including both specific morphological characteristics and the production of active compounds, to avoid epibiont overgrowth (Littler and Littler, 1980, Littler *et al.,* 1983a, Littler *et al.,* 1983b, Paul and Puglisi, 2004). Several examples demonstrate that specific anatomical structures localised at the surface of the algae release natural products into the environment (Dworjanyn *et al.,* 1999, De Nys and Steinberg, 2002). For example, vesicular physodes located at the surface of phaeophyceae contain phlorotannins (Ragan and Glombitza, 1986). The role of phlorotannins as a macroalgal defense mechanism was extensively described in the literature, but remains not fully understood. These compounds were described as UV screens, herbivore deterrents and antimicrobial agents, but are also known to play a role in primary metabolism (Paul and Puglisi, 2004). Moreover, fluorescence microscopy combined with chemical analyses, demonstrated that *Delisea pulchra* (Rhodophyceae) releases AF compounds (halogenated furanones) from gland cells located on its surface (Dworjanyn *et al.,* 1999, De Nys and Steinberg, 2002). The “corps en cerise” found on cortical cells of *Laurencia snyderae* (Rhodophyceae) could be a primary location of halogenated natural products (Young *et al.,* 1980). However, even though it has been stated that the “corps en cerise” are the main reserve for halogenated compounds in the alga *Laurencia obtusa* and store high concentrations of bromine and chlorine (Salgado *et*

al., 2008), they were not found at the surface of the algae, and neither was there any structure linking them to the algal surface (De Nys *et al.,* 1998).

Sargassum species have been extensively scrutinised for their allelochemical activities for several reasons: i) some of them are only lightly fouled in the field, ii) it is one of the most conspicuous alga genera in numerous regions, especially the tropics (Ang, 1986, De Ruyter van Stevenick and Breeman, 1987, Littler *et al.,* 1993, Lapointe, 1997, Engelen *et al.,* 2001), iii) some species are known to be invasive and have colonised marine habitats all around the world (Plouguerné, 2006). Antibacterial activity was found in the species *Sargassum muticum* (Hellio *et al.,* 2002, Plouguerné *et al.,* 2008, Plouguerné *et al.,* 2010b), *Sargassum wightii* and *Sargassum johnshonii* (Sastry and Rao, 1994). Sieburth and Conover (1965) observed antibiotic activity of phlorotannins extracted from *Sargassum vestitum* and *S. natans*. Tanaka and Asakawa (1988) found antialgal activity in extracts from *Sargassum horneri*. *Sargassum vulgare* extracts from Brazil demonstrated AF activity towards microalgae and *Mytilus edulis* (mussel) settlement (Plouguerné *et al.,* 2010a). Finally, *Sargassum muricatum* and *Sargassum tenerrimum* induced modifications of swimming activity of 2-day-old *Platygyra daedalea* (Coral) larvae and decreased their settlement percentage, which seemed to reflect chemical defenses (Diaz-Pulido *et al.,* 2010).

S. polyceratium, the macroalga the most represented on degraded tropical reefs of Martinique since the phase shift phenomenon, has never been investigated for MNP production. Coral reef colonisation by macroalgae has induced significant changes in the community structure and diversity. As an example, *S. polyceratium* and *S. hystrix* have supplanted the hermatypic coral species on the Jamaican North Coast (Lapointe, 1997, Lapointe and Thacker, 2002). Algal canopies are known to affect understorey species (Eckman and Duggins, 1991) and could interfere with invertebrate larval recruitment processes (Pawlik, 1992a, Birrell, 2003, Titlyanov *et al.,* 2005).

The sea urchin *Diadema antillarum* appears to be almost absent from the *Sargassum* areas, but populations have developed nearby, forming distinct belts (Chapter II). *Diadema* recovery is a very slow process in the Caribbean despite the fact that its biology should make it fast to recover (Lessios, 1995). Several hypotheses have attended to explain such phenomenon on the reef (Lessios, 2005). It is possible that the fast colonisation by *S.*

polyceratium on coral reefs in the 1980's is in part responsible for this slow recovery process by affecting or preventing larval recruitment.

Chemical ecology is an important approach to understanding organism interactions and community structures. Numerous studies have focused on a wide spectrum of secondary metabolites. But in the elucidation of the ecological function of MNP in larval development, it seems essential to concentrate on molecules produced at the surface of the algae (De Nys *et al.,* 1998) or directly released into the water column (Walters *et al.,* 1996). Indeed, only these molecules can directly be in contact with the surrounding organisms and, therefore, act on their physiology and settlement. It is known that many chemical cues have positive or negative impacts on marine benthic recruitment (Steinberg *et al.,* 2002).

This study focused on the potential antibacterial and deterrent activities of *S. polyceratium* and their possible interactions with other marine tropical species. In order to complete a broad-spectrum analysis of the bio-activity of MNP extracted from the surface of *S. polyceratium* surface, 4 marine tropical invertebrates were used, one urchin (*Diadema antillarum*), one bivalve (*Codakia orbicularis*) one annelid (*Arenicola brasiliensis*) and one coral (*Montastrea annularis*), thus representing organisms from different phyla and different tropical ecosystems (reef, seagrass bed and mangrove). *In vitro* effects of *S. polyceratium* extracts on embryos of marine invertebrates and bacteria (4 marine and 5 soil strains present in coastal waters) were investigated. In addition, effects of *S. polyceratium* waterborne cues (conditioned water) were evaluated towards embryos of the sea urchin *D. antillarum*.

It was hypothesised that the molecules produced by *Sargassum* species in the field could interfer with the embryonic development of invertebrate species. It might be possible that *Sargassum* slows down the development of the embryos, prevents the egg cleavage and / or kill the embryos.

IV.2. Material and methods

IV.2.1. Preparation of surface molecule extracts and conditioned water

Several protocols were established to test the biological activities of both surface molecules and waterborne cues (Figure 71 for summary).

IV.2.1.1 Surface molecules

IV.2.1.1.1. Algae collection site

The site Trois Rivières (Figure 61) was chosen for algal collection. This site is a fringing coral reef in the south of Martinique close to the river mouth of the Oman River. *Sargassum polyceratium* is a dominant algal species at this site (Chapter II).

Figure 61: Algal collection site at Trois Rivières, Maritnique (14°27'22''N, 60°58'03''W) for prepaartion of surface molecule extracts.

IV.2.1.1.2. Algal extractions

S. polyceratium samples were collected in October 2008, January 2009 and March 2010 (rainy and dry seasons). Thalli were removed with their holdfast (by breaking a piece of substratum with a chisel and mallet) to avoid causing any stress to the algae (which could be responsible for secondary metabolites production), by SCUBA diving at 18m depth. The algae were cleaned of epiphytes, by gently rinsing them with SW, and transported to

the laboratory in a container filled with clean SW. The fresh samples were soaked in hexane (Fisher, UK) (De Nys *et al.,* 1998) following the ratio 1L hexane/1Kg wet weight *S. polyceratium*. All the extractions were performed in the dark (some secondary metabolites such as the polyphenolics compounds are known to react with light). The hexane dipping method was employed to extract molecules produced both by the alga and by its associated biofilm at the algal surface in order to obtain all the natural products that could interact with eukaryotic and/or prokaryotic organisms present in the algal environment.

Three extractions were performed leading to 3 different extracts:

- Extract A, (October 2008 samples, Rainy season), *S. polyceratium* thalli were dipped for 30s in hexane for preliminary tests.

- Extract B, according to the results of the tests conducted on the extract A and the observations on the algal surface for breaks (see results), extract B was prepared, (January 2009 samples, Dry season) to test for the effect of dipping time on the extract efficiency. Thus, *Sargassum polyceratium* surface molecules were extracted in hexane for 2 different dipping times (10s and 30s).

- Extract C: *Sargassum polyceratium* thalli collected in March 2010 were extracted for 30s in hexane following the protocol previously described. According to the preliminary results and their potential meaning in the field, embryo test protocols employed for the extracts A and B were adapted (see embryo toxicity tests p 144).

IV.2.1.1.3. Observation of the algal surface

The objective was to isolate only those molecules present at the surface of *S. polyceratium*. The hexane dipping method was already demonstrated to break some algal surface cells when the algae were dipped for more than 30s, (De Nys *et al.,* 1998). Observations of the algal surface were thus carried out in order to determine the damages (cells breaks resulting in leaks of cell content) caused by hexane on algae dipped for 30 s, 1 min and 10 min to determine the best dipping time. Investigation was performed under an epifluorescent microscope (Nikon eclipse 80i microscope; Filter FITC 494 nm excitation / 514 nm emission; Nikon Dxm1200F camera) using a protocol adapted from Cerocic *et al.,* (1999). UV excitation of plant leaves is known to induce two distinct types of fluorescence (Cerocic *et al.,* 1999).

IV.2.1.2 Preparation of conditioned water

IV.2.1.2.1. Algae collection site

Algae present at the vicinity of the urchin populations were harvested by SCUBA diving at 5 m depth (Figure 62).

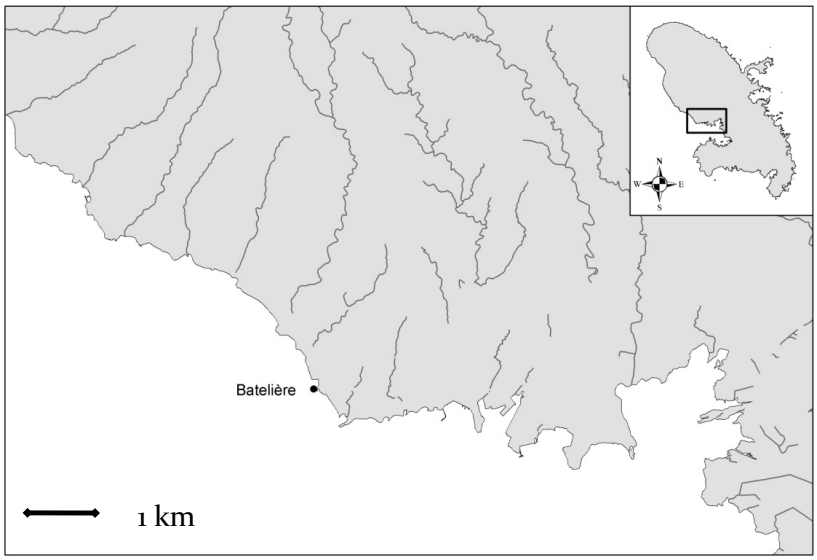
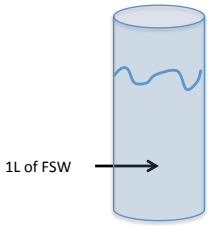


Figure 62: Algal collection site at Batelière, Martinique (14°36'13''N, 61°06'02''W) for the preparation of conditioned waters.

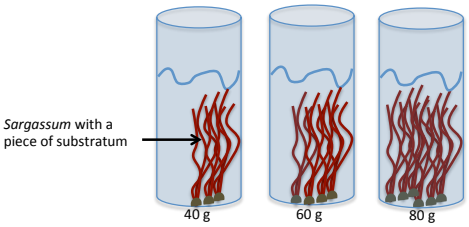
IV.2.1.2.2. Preparation of the conditioned water

Two methods (CWA and CWB) were employed for the preparation of conditioned waters:

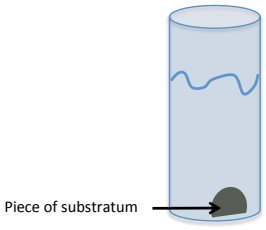
Conditionned water A (CWA): This protocol was adapted from Birrell, (2003). *Sargassum* samples were collected with a piece of substratum. They were cleaned from epiphytes and transported to the laboratory in fresh SW. Conditioned waters were prepared within 2 hours after the algal collection.



Control: UV filtered SW (0.22µm) was poured in a glass beaker and incubated for 90 min and 10 hours.



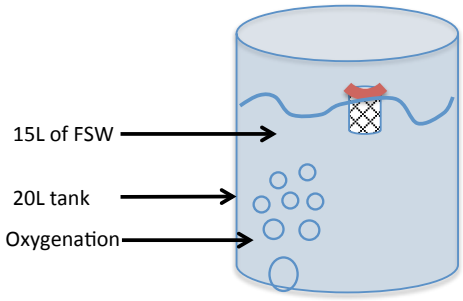
***Sargassum* treatment:** *Sargassum* was incubated in glass beakers at 3 concentrations: 40, 60 or 80 $g.l^{-1}$ at 25°C under natural light. Incubation lasted for 90 minutes and 10 hours in UV Filtered SW (0.22µm).



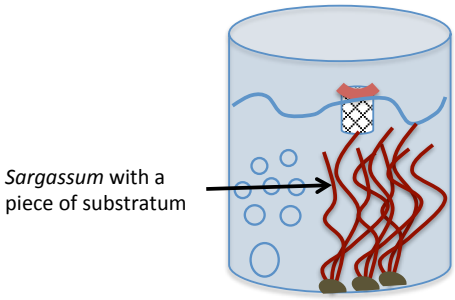
Second control: A piece of substratum ($25g.l^{-1}$) was incubated in UV filtered SW (0.22µm) for 90 and 10 hours (this because the *Sargassum* were collected with a piece of substratum).

All the solutions were filtered (0.22µm) to prevent bacterial contamination prior to the experiments.

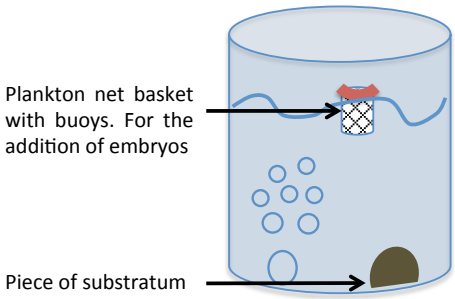
Conditionned water B (CWB): The second protocol aimed at testing directly the biological activity of molecules produced in the water column on embryonic development. Thus, algae and embryos were present in the same tank, (seep 144). Nine 20L transparent tanks were filled with 15L FSW (0.1µm, UV treated). Oxygenation was provided to the tanks by air pumps. These 9 tanks were divided into 3 treatments:



Control: 3 tanks were filled with FSW



***Sargassum* treatment:** 3 tanks were filled with FSW in which 150g of *S. polyceratium* attached to a pice of substratum were installed. The small amount of substratum was kept to minimize the stress caused to the algae.



Second control: 3 tanks were filled with FSW and a piece of substratum (50g) was added to ensure the activity observed in *Sargassum* treatment would be due to the algae and not to the organisms present on rocks.

All tanks were prepared 24 hours prior the experiments in order to condition the water.

IV.2.2. Bioassays

Several tests were conducted depending on the extracts (see Figure 71 for summary).

IV.2.2.1 Bacteria

IV.2.2.1.1. Culture of bacteria

Four marine bacterial strains were used *viz. Halomonas marina* (ATCC 25374), *Pseudoalteromonas elyakovii* (ATCC 700519), *Polaribacter irgensii* (ATCC 700398) and *Vibrio aestuarianus* (ATCC 35048). These bacteria were chosen as they are typical marine fouling bacteria (Plouguerné *et al.,* 2010a). *P. elyakovii* and *V. aestuarianus* are also known to cause infections in marine organisms such as molluscs, crustaceans, fishes or algae, the latter being thought to be responsible for the summer mortality of *Crassostrea gigas* (Labreuche *et al.,* 2005). Marine bacteria were cultivated with marine broth (5% Tryptone diluted in SW) and incubated at 30° C to allow for their development (Plouguerné *et al.,* 2008).

Five soil bacteria strains, known to be present in estuaries and coastal environment (Mokrini *et al.,* 2008), were used: *Bacillus subtilis* (NCIMB 1026), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (B 81), *Pseudomonas aeruginosa* (NCIMB 10390) and *Staphylococcus aureus* (NCIMB 8625). *B. subtilis* and *S. aureus* are Gram-positive bacteria. The others are Gram-negative. Soil bacteria were cultivated on a nutrient broth (CM0067, Nº2, Nutrient media Powder Oxoid, 25g/L) and incubated at 30° C.

IV.2.2.1.2. Antibacterial assays

Hexane extracts were recovered in hexane and diluted to obtain 3 concentrations 15, 150 and 300 $\mu g.mL^{-1}$. 100 µL of each hexane extract (Extracts A and B) were poured in 6 wells of 96 well plates (Fisher, UK) for each bacterial assay following the protocols described by Plouguerné *et al.,* (2010a). In addition 6 wells free from extracts and 6 wells containing the solvent used for extraction were used as controls. The plates were first dried under a flow cabinet to evaporate the solvent and then left for 15min in a UV cabinet for sterilization.

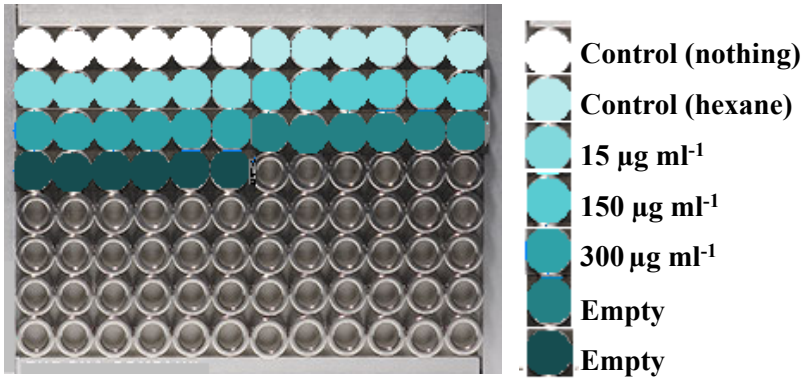


Figure 63: Schematic representation of the 96 well plates and filling method.

Biological activities of extracts were evaluated following the method of Amsterdam, (1996). The Optical Density (OD) of bacterial stock cultures was measured at 630nm for every sample to determine the quantity of suspension required to obtain 1 mOD (mili optical density) and 100µL of bacterial suspension were then added under aseptic conditions and the plates incubated for 48 hours at 30°C for bacterial growth. Activity was obtained comparing the controls and the wells containing the extracts. Solutions were considered to be active if bacteria did not grow in 4, 5 or 6 wells; bacterial growth was noted by the presence of a cloudy suspension. One plate was used for each strain to avoid the cross contamination risk (Thabard *et al.,* 2009). Results were expressed as Minimum inhibitory concentration (MIC), which is the lowest concentration of antimicrobial at which there is a complete inhibition of growth of organism.

IV.2.2.2 Invertebrates

IV.2.2.2.1. Organisms

The toxicity of the extracts was examined against larvae of *Arenicola brasiliensis, Codakia orbicularis, Diadema antillarum* and *Montastrea annularis*. These organisms are tropical species and represent typical organisms from the 3 marine ecosystems (seagrass bed, reef and mangrove). Their spawning and early larval development has been described previously (Gros *et al.,* 1997, Eckert, 1998). For both *C. orbicularis* and *D. antillarum,* spawns were induced in the laboratory under controlled conditions while *A. brasiliensis* embryos and *M. annularis* gametes were collected from the field.

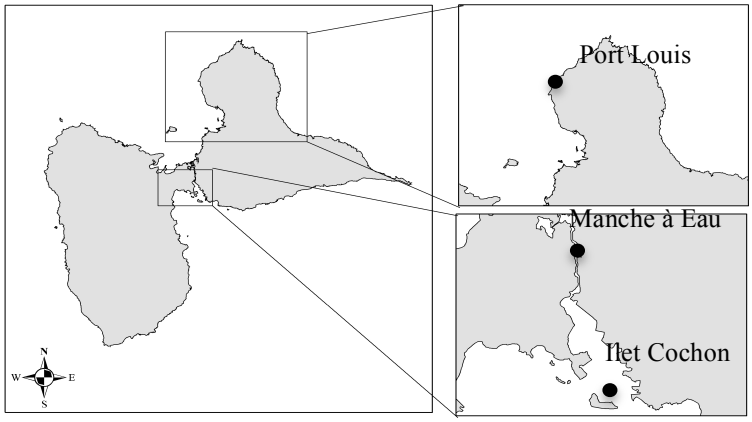


Figure 64: Map showing the invertebrate collection sites (Guadeloupe) at Ilet Cochon (16°12'56''N, 61°32'20''W), Manche à Eau (16°16'36''N, 61°31'24''W) and Port Louis (16°25'18''N, 61°32'02''W).

- *Codakia orbicularis* (Linné 1758) (Mollusca, Bivalvia, Veneroida, Lucinida) is a tropical bivalve that is found from Florida to Brazil (Abbott, 1974). Adult *C. orbicularis* (between 40 and 60mm in shell length) were collected by hand from seagrass beds in Guadeloupe, Ilet Cochon (Figure 64) in July 2009. The fertilization process was carried out following the method previously described by Gros *et al.* (1997). Adults were cleaned with a brush and spawning was induced by injection of 0.3 mL of a 4 mM serotonin solution in 0.22 µm FWS into the visceral mass. Sperm and oocytes were mixed in a 1 L cylinder until the appearance of 2 cell embryos.

Figure 65: Adult *Codakia orbicularis*, (O. Gros)

Fertilization was performed under constant aeration as the eggs are slightly negatively buoyant (Gros, personnal communication). *C. orbicularis* embryonic development follows the general development of bivalves (Gros *et al.,* 1997). Appearance of the first polar body

(attesting for fertilization) is not always visible under a dissecting microscope, thus the 2 cells embryos were chosen to ensure that fertilization had occurred and to conduct the toxicity experiments (Figure 66).

Stage of development	Time
4 cells (a)	**To+3h**
Morula (b)	**To+6h**
Gastrula (c)	**To+21h**
Young trochophore (d)	**To+24h**
Late trochophore (e)	**To+27h**

a b c d e

Figure 66: SEM pictures (Magnification=500) and time scale of early stage of *Codakia orbicularis* development (after Gros *et al.*, 1997). a) 4 cell stage, b) morula, c) gastrula, d) trochophore and e) late trochophore.

- *Diadema antillarum* (Philippi 1845)*:* The black spined sea urchin *D. antillarum* (Echinoidea, Diadematidae) was selected to conduct this experiment. For extracts A and B, adults were collected on the shore at Port-Louis, (Guadeloupe, Figure 64) during summer 2009. Urchins were acclimated in the laboratory (25°C) for a week and fed on agar pellets containing a mixture of algae (including *Ulva lactuca* and *Sargassum sp.*) following the protocol of Pereira *et al.,* (2003). After a week, the urchins were transposed to another tank containing 29°C SW. Thermal shocks (3-5 °C) induced spawning of *D. antillarum* within a few minutes (Moe, personal communication).

For extract C and conditioned waters A and B, samples of *D. antillarum* were collected from La Batelière (Martinique, Figure 62) and induced to spawn by injection of 1ml 0.5M KCl (VWR) solution into the peristome as described by Bielmyer *et al.,* (2005). This method was employed because the facilities were not present in Martinique to conduct the less invasive one (thermal shock).

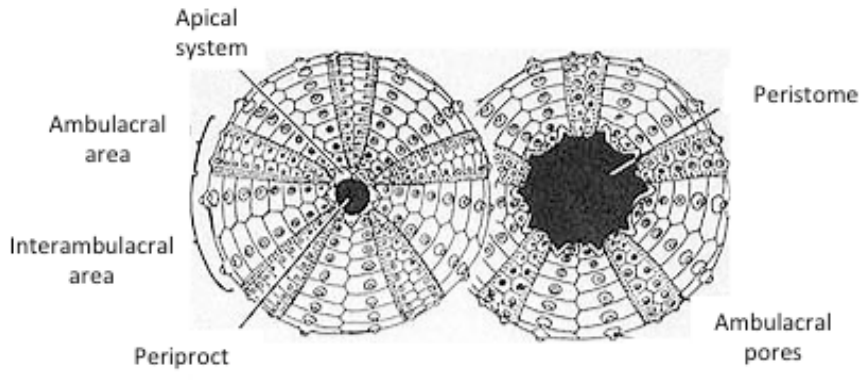
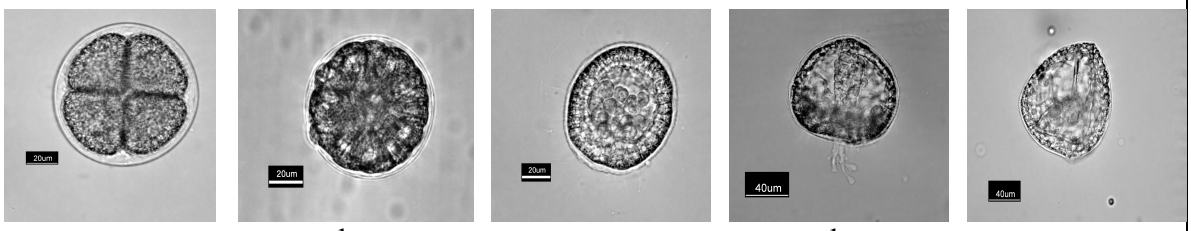


Figure 67: Upper surface (left) and lower surface (right) of a sea urchin (Benton and Harper, 1997).

The fertilisation technique remained the same for both tests. As soon as spawning occurred, both male and female gametes were pipeted and diluted in 10 L of 0.22 µm FSW (25°C) to induce fertilization. The eggs are slightly negatively buoyant so aeration was used to keep them suspended. The embryos at the 2 cells stage (To+1h) were chosen to conduct the experiment (Figure 68).

Stage of development	Time
4 cells (a)	**To+1h**
Morula (b)	**To+3h**
Blastula (c)	**To+7h**
Gastrula (d)	**To+19h**
Prism (e)	**To+30h**



a b c d e

Figure 68: Light microscopy images (magnification=40) and time scale of *Diadema antillarum* early stage of development. a) 4 cells stage, b) morula, c) gastrula, d) gastrula e) prism.

- *Arenicola brasiliensis* (Nonato, 1958): egg balloons of this worm (Annelida, Polycheta, Scolecida, Arenicolidae) were collected (while snorkelling) from the mangrove Manche à Eau (16°16'36"N, 61°31'24", Guadeloupe, FWI, Figure 64) in summer 2009 and 2010. The egg balloons containing young embryos of *A. brasiliensis* (i.e: at the stage blastula-early gastrula) were chosen to conduct the experiment. Egg balloons were

transported to the laboratory in containers filled with clean SW. They were broken and the embryos were pipetted to conduct the experiment.

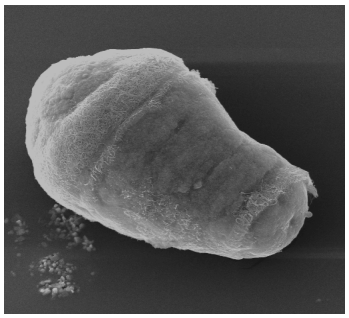


Figure 69: *A. brasiliensis* (magnification x500) trochophore (picture: Olivier Gros).

- *Montastrea annularis* (Ellis, 1786) (Cnidaria, Anthozoa, Scleractinia, Faviidae): this broadcast spawners typically releases its eggs and sperm 7 days after the full moon from August to October (Mazeas, personal communication). This species is well represented in Martinique (Chapter II). The experiment was conducted during the last week of September 2010. Nets for collecting gametes in a non-invasive way were designed. Ten plankton nets ended with a plastic jars were sets over corals by SCUBA divers in Guadeloupe (FWI). Unfortunately a bleaching event resulted in no spawning that year and the experiment coud not be conducted.

IV.2.2.2.2. Embryo toxicity tests

Several tests were carried out to elucidate the activity of molecules produced by *S. polyceratium* (see schematic representation, Figure 71):

a) Test the activity of surface molecules dispersed in SW.

b) Test the bioactivity of a surface molecule coating to mimic field conditions,

c) Test the effect of short-time waterborne cues produced by macroalgae on embryonic development.

d) Test the bioactivity of waterborne cues "continuously" produced by the seaweed on embryonic development.

a) Test of surface molecule recovered in DMSO (extracts A, B and C)

DMSO is a solvent that can be used as a toxicant vehicle in toxicity assays. Ura *et al.,* (2002) demonstrate that DMSO at concentrations below (or equal) 1% were not toxic towards the nematode *Caenorhabditis elegans*.

Preliminary tests demonstrated that this solvent was not toxic to embryos until the trochophore stage for both *C. orbicularis* and *A. brasiliensis* and prism stage for *D. antillarum* when used at the concentration 0.5% (Figure 70).

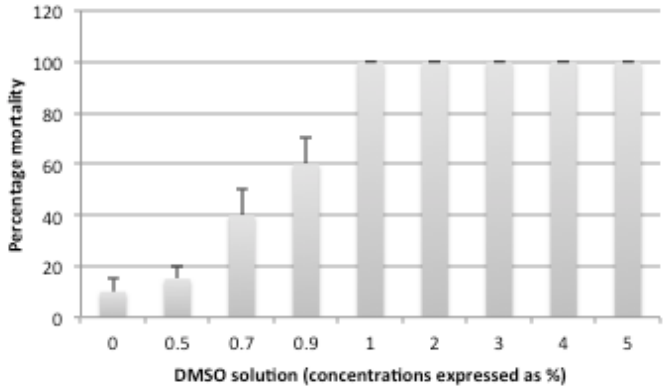


Figure 70: Viability of *Codakia orbicularis* embryos after 30h exposure to DMSO at 9 concentrations.

In order to disperse the extracted molecules in SW, the dried hexane dipping extracts were recovered in a DMSO solution (0.5 %) in 0.22µm FSW (extracts A, B and C).

Extracts A and B: The tests were conducted in 96 well plates (Fisher, UK). Extracts were tested at 7 concentrations: 1, 5, 10, 15, 50, 100 and 200 $\mu g.mL^{-1}$. 200 µL of each extract was added per well (6 replicates). In addition, 6 wells containing FSW, 6 containing a solution of 1% CuSO4 (known to kill larvae, (Bielmeyer *et al.,* 2005)) and 6 containing a solution of 0.5% DMSO were used as controls. Four larvae were added per well and allowed to develop for 24h to 30h at 25°C only in order to reduce bacterial development. The percentage of mortality and the embryonic development (stage reached) were recorded. All the assays were performed on 2 independent batches of embryos as described by Hellio *et al.,* (2004). Embryo toxicity assays results were analysed using Kruskal-Wallis. A multiple range test (MRT) was then conducted with the package npmc to test all pairwise comparison between factors and variables. The results of the 2 larval batches were pooled as no significant differences could be noticed.

Extract C: Each 96 well plates held 11 different concentrations of the extracts (1, 5, 10, 15, 20, 25, 37.5, 50, 75, 100, 200 $\mu g.mL^{-1}$) and 2 controls (FSW and DMSO 0.5 $\mu g.mL^{-1}$). The protocol previously described for the distribution of extracts and embryo tests was employed. More concentrations were selected as compared to the tests previously carried out for extracts A and B in order to determine the median lethal concentration i.e.: the

concentration of the toxicant at which 50% of sea urchins embryos died (LC_{50}). LC_{50} were calculated on the last set of results (extracts from March) with the Software R and the package doBy. *"This package calculates the LC_{50} for a model of the form logit(p)=beta1 x1 + ... + betap xp + gamma d where none of the explanatory variables x1 ... xp contains the dose d."*

b) Test of surface molecules forming a coating (only for extract C)

Sargassum polyceratium extracts were not recovered in DMSO, which disperses the molecules in the water column, but in hexane. Extracts diluted at 1, 5, 10, 15, 20, 25, 37.5, 50, 75, 100, 200 $\mu g.mL^{-1}$ in hexane were poured in propylene 96 well plates (only propylene plates are resistant to hexane that reacts with other plastics) and left for evaporation for 2 hours so that the molecules remained all around the well (all the surface molecules are not present as the volatile compounds will evaporate during the process). The molecules extracted by hexane are highly non-polar (hydrophobic) and it is possible that they are not emitted in the field but instead remain at the algal surface. Thus the effect of a molecule coating was used to mimic field conditions and to test for the activity of surface molecules towards organisms. As this test was performed on new extracts obtained in March 2010, tests performed with molecules recovered in DMSO were repeated to compare both the effects of the coating and molecules dispersed in the water column. Indeed, seasonality is known to occur for extract activity and, if comparing these results with the one obtained for the extracts A and B, it would have remained unknown wether the differences observed were due to seasonality or recovery method. The distribution of extracts and embryo tests were performed as previously described. The concentration of the toxicant at which 50% of the embryos died (LC_{50}) was evaluated using the method previously described.

For both recovery methods, embryo toxicity assays results were analysed using Kruskal-Wallis. A multiple range test (MRT) was then conducted with the package npmc to test all pairwise comparison between factors and variables. The results of the 2 larval batches were pooled as no significant differences could be noticed.

c) Test of waterborne cues produced in 90 min and 10 hours

Conditionned water A: 96 well plates (Fisher, UK) were filled in with the *Sargassum* conditioned water 40, 60 and 80 g L^{-1} and the 2 controls (6 wells for each treatment). Four *D. antillarum* embryos (2 cells) were added to each well and their development was followed as previously described.

d) Test of waterborne cues produced continuously

Conditionned water B: *Diadema antillarum* embryos (around 10 000) were added in each of the 9 tanks prepared with FSW, (see p 137). In order to recover the embryos easily and so that they could not be in direct contact with the seaweed, they were added in a plankton net basket floating in the tanks. The nets allowed the embryos to develop in the conditioned water, but to be isolated from the *Sargassum* thalli. After 30h, embryos were collected using pipettes and observed under a dissecting microscope (x 40) using a Sedgewick rafter counting cell. A hundred embryos from each tank were examined for stages of development (Blastula, gastrula, prism and pluteus) and abnormality.

Waterborne cue toxicity assays results were analysed using a chi square test. The numbers of pluteus *vs* other conditions (dead, or earlier stages) were compared for the 3 treatments.

IV.2.3. Elucidation of samples composition

In order to define the active molecules present in the hexane dipping sample and quantify them, GC-Ms was run. A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-1 (30 m x 0.32 mm) fused silica gel column was used for the quantification of the oils. Oven temperature was programmed as follows: 50° C for 5 min and then up to 250° C at 3° $C.min^{-1}$. Injector temperature: 200° C. Carrier gas: He with a flow rate of 2 $mL.min^{-1}$. Injector temperature: 250° C. Mass spectra were obtained from GC-MS analysis on a Hewlett-Packard HP5973/HP6890 equipped with a 30 m x 0.25 min DB-5 capillary column. The samples were also analysed on a SGE CydexB column. The mass spectrometer was operating (full scan mode) in the EI mode at 70 eV. The identification of the chemical constituents was based on comparisons of their relative retention times and mass spectra with those obtained from authentic standards and/or the NIST/NBS and Wiley libraries spectra. Evaluation of the enantiomeric purity of the constituents showed

that all major metabolites were present as a single isomer and did not reveal any obvious racemization during the process.

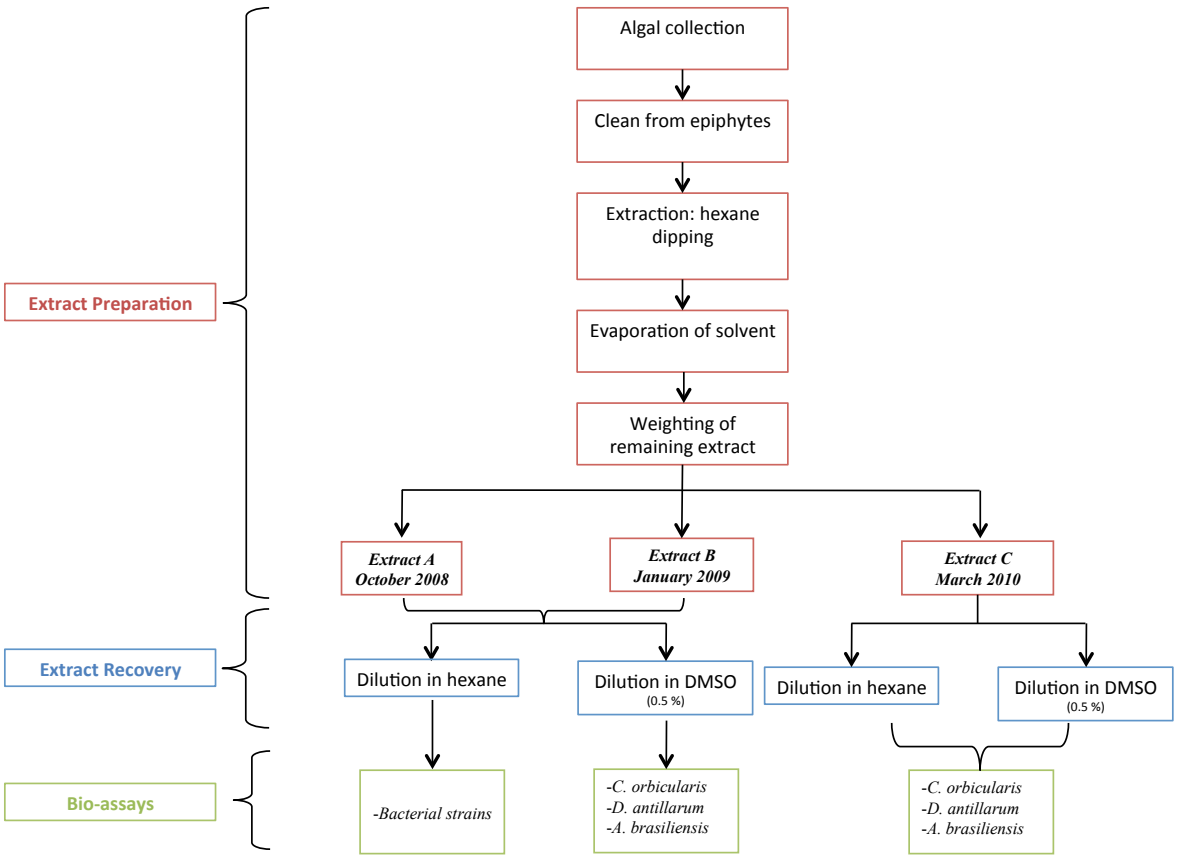


Figure 71: Diagram of the methodologies employed for algal extraction, molecules recovery and bioassays.

IV.3. Results

IV.3.1. Algal surface observation

Epifluorescence microscopy pictures demonstrated that the algal surface cells were broken from 1 min of dipping or more but were intact after up to 30 s dipping.

The cells were well formed and not broken (Figure 72 a-b) for 10 and 30s dipping periods while the presence of leaks, suggesting the lysis of the cells surface, was noticed for the other dipping times (Figure 72 c-d). The assays towards organisms were thus only conducted with the extracts prepared for the shortest times (10 and 30s) for protocols A and B.

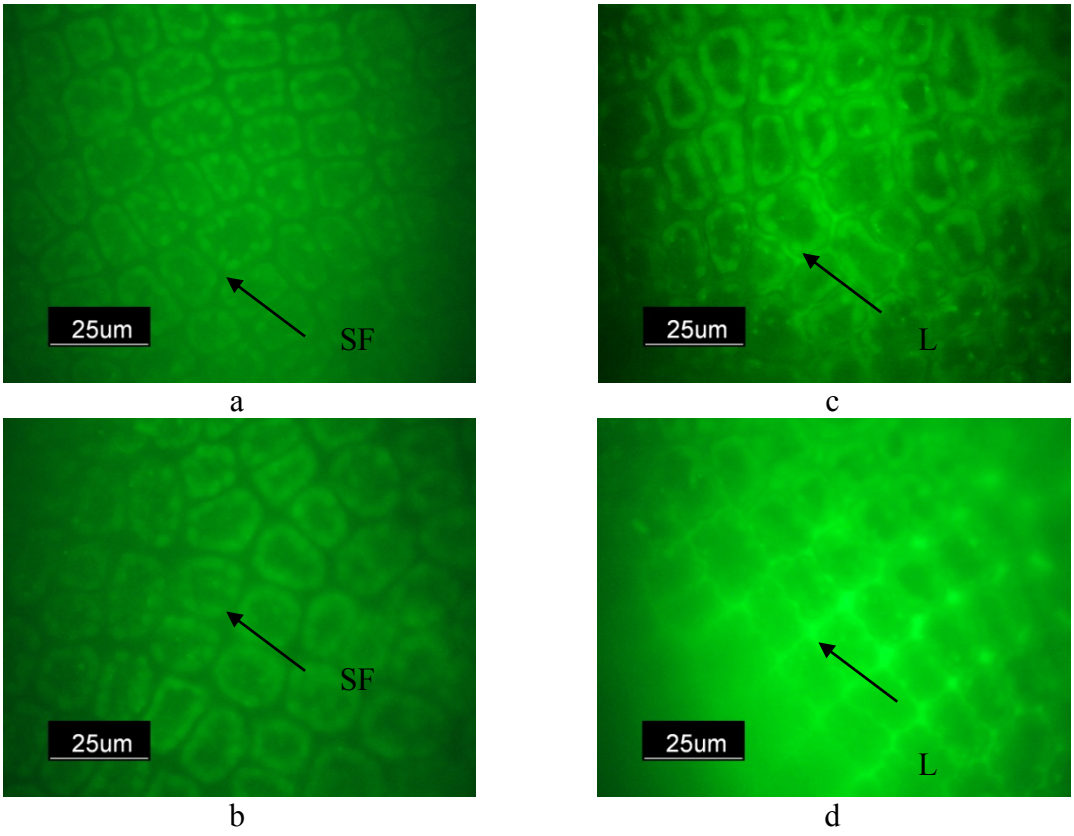


Figure 72: Epifluorescent microscopic observation of *S. polyceratium* frond surface: a) control, b) 30 s, c) 1 min and d) 10 min after being dipped in hexane. (Magnification=100). SF, surface cells and L, cellular content leaks.

Hexane dipping time less than or equal to 30s does not damage cells and can be used for extraction of surface molecules.

IV.3.2. Assessment of antibacterial activity

From the results (Table 21) it can be seen that bacterial growth was not inhibited at concentrations of 15 µg.mL^{-1} of *S. polyceratium* algal extracts. The lowest minimum inhibitory concentration was 150 µg.mL^{-1}. In general, marine bacteria were the most sensitive to the extracts and soil bacterial strains growth was only inhibited by the 30s October and the 10s January extracts.

Differences were observed between the various dipping times. The 10s extraction sample from January exhibited more activity against bacteria than the 30s one from the same month (except for *V. aestuarianus*).

Differences were also noticed according to the month of extraction. The 30s extraction samples prepared in October were the second most active.

Table 21: Antibacterial activity of the hexane dipping extracts expressed as Minimum Inhibitory Concentrations (µg.mL^{-1}). The months of algal collection and the dipping times (s) are indicated (Thabard *et al.*, 2011).

Antibacterial activity (Minimum Inhibitory Concentrations in µg.mL^{-1})				
Extract Tested	Date of extraction	October 2008	January 2009	
	Dipping time	30 s	10 s	30 s
Soil species	*Bacillus subtilis*	150	300	>300
	Enterobacter aerogenes	N	300	>300
	Escherichia coli	300	300	>300
	Pseudomonas aeruginosa	150	300	>300
	Staphylococcus aureus	N	300	>300
Marine species	*Halomonas marina*	300	150	300
	Polaribacter irgensii	150	150	300
	Pseudoalteromonas elyakovii	150	150	300
	Vibrio aestuarianus	150	150	150

Extracts were active from 150 µg.mL^{-1} and more activity was observed towards marine than terrestrial bacteria.

IV.3.3. Surface molecules activity towards embryos

IV.3.3.1 *Diadema antillarum*

IV.3.3.1.1. Hexane dipping extracts from October and January (Extracts A and B)

The algal extracts tested affected *Diadema antillarum* embryo development (Kruskal-Wallis: $p<0.0001$). From the results, it can be seen that 100% of the embryos died in the highest concentrations (from 10 to 200 $\mu g.mL^{-1}$) as well as in the copper (positive control) (Figure 73). Less than 70% embryos survived when exposed to the 5 $\mu g.mL^{-1}$ extract. Only the solutions 1 $\mu g.mL^{-1}$ and the 10s January extract at the concentration 5 $\mu g.mL^{-1}$ allowed a good survival of embryos and were not significantly different from the control.

Apart from the 10s January extract at the concentration 5 $\mu g.mL^{-1}$, no significant differences could be observed between the October and the January extracts (as it was the case for bacteria).

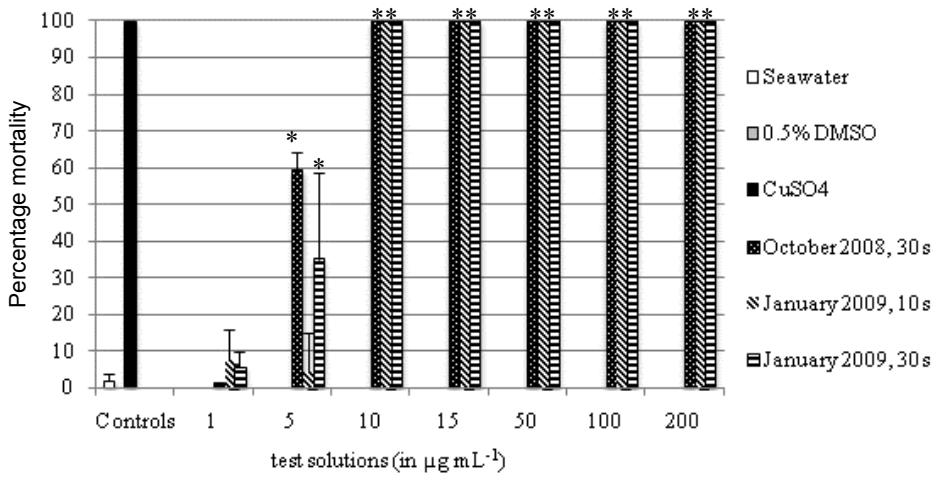


Figure 73: Mean percentage mortality of *Diadema antillarum* embryos after 30 h exposure to molecules extracted from *Sargassum polyceratium* in October 2008 or January 2009. Dates of algal collection and extraction times are indicated in the key. (n=12 + SE). * = Significant differences (multiple comparison test) between control (DMSO) and one of the solutions; ** = significant differences observed between the control (DMSO) and a group of solutions. (Thabard *et al.*, 2011).

Embryonic development stopped at different stages according to the solution tested (Table 22). Only the embryos developing in the controls (DMSO and FSW) or the extract at 1 $\mu g.mL^{-1}$ survived to the prism stage. The other embryos stopped their development progressively with decreasing concentrations of algal extracts, i.e: 2-4 cells stage at the concentrations 100-200 $\mu g.mL^{-1}$, morula at 15 and 50 $\mu g.mL^{-1}$, late blastula early gastrula at 10 $\mu g.mL^{-1}$, gastrula at 5 $\mu g.mL^{-1}$ and prism at 1 $\mu g.mL^{-1}$.

Table 22: Larval stages of development in *Diadema antillarum* embryos after 30 hours exposure to *Sargassum polyceratium* hexane surface extracts.

Concentration (µg.mL^{-1})	Stage of development
1	Prism
5	gastrula
10	Late blastula-early gastrula
15	Morula
50	Morula
100	2-4 cellules
200	2-4 cellules
CuSO4	2-4 cellules
DMSO (0.5%)	Prism
Seawater	Prism

IV.3.3.1.2. Hexane dipping extracts from March 2010 (Extract C)

According to these results tests were performed with surface molecules extracted for 30s with hexane and recovered either in a 0.5 % DMSO solution or in pure hexane.

Recovered in DMSO:

Molecules dispersed in SW demonstrated activity towards *D. antillarum* larval development. Mortality rates varied between 12.5% in the 1µg.mL^{-1} to a 100% for the highest concentrations (Figure 74, Appendix 4). A significant difference in the % of mortality was noticed between the treatments (Kruskal-Wallis, $p<0.05$). The Multiple range test conducted on the results demonstrated the mortality rates to be significantly different between the controls and the solutions at concentrations equal or higher than 25 µg.mL^{-1} ($p<0.05$).

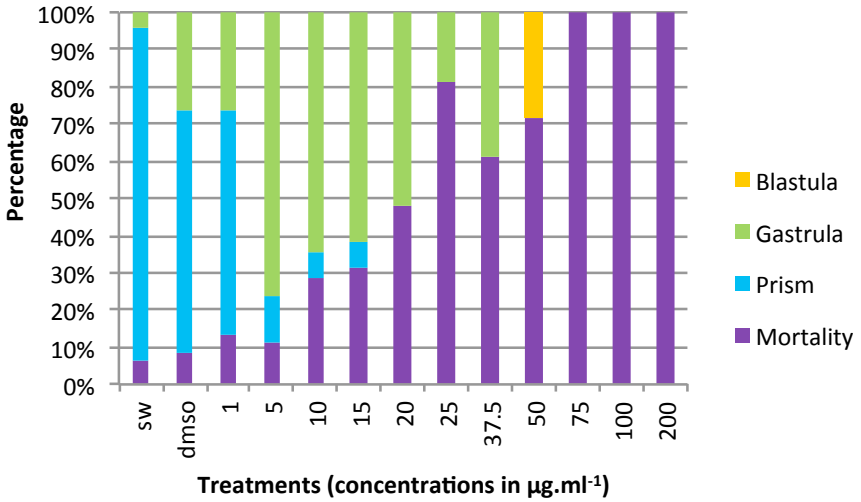


Figure 74: **Mean percentage mortality of *Diadema antillarum* embryos after 46 h exposure to molecules extracted from *Sargassum polyceratium* in march 2010 and recovered in DMSO. The stage of development of the organisms still living is given (Percentage of Blastula, Gastrula or Prism).**

Embryos developed to the prism stage in the controls (respectively 88% and 65% for the SW and DMSO, Figure 74). Only some embryos grown in the 1µg.mL^{-1} to 15µg.mL^{-1} reached the prism stage and significant differences between controls and solutions were observed starting from the 5 µg.mL^{-1} solution (MRT). Concentrations higher than 15 µg.mL^{-1} showed no development past late blastula/ early gastrula stage (Figure 74). LC_{50} was evaluated at 26.4 µg.mL^{-1} (Lower=21.4 µg.mL^{-1}, upper=31.4 µg.mL^{-1}). The extracts from March demonstrated less activity than those from October or January (Figure 73, Figure 74).

Recovered in hexane:

Mortality rates were low in controls (6.80% for the SW control and 10.29% for hexane) (Figure 75, Appendix 5). Mortality for the treatments of *Sargassum* extracts varied with concentrations from 20.13% in 1µg.mL^{-1} to 97.57% in 200µg.mL^{-1}(Figure 75). Significant mortality of *D. antillarum* embryos in comparison to the controls of FSW and hexane were seen from 37.5 µg.mL^{-1} with the value of 70% mortality of the population (Kruskal-Wallis and MRT, $p<0.05$).

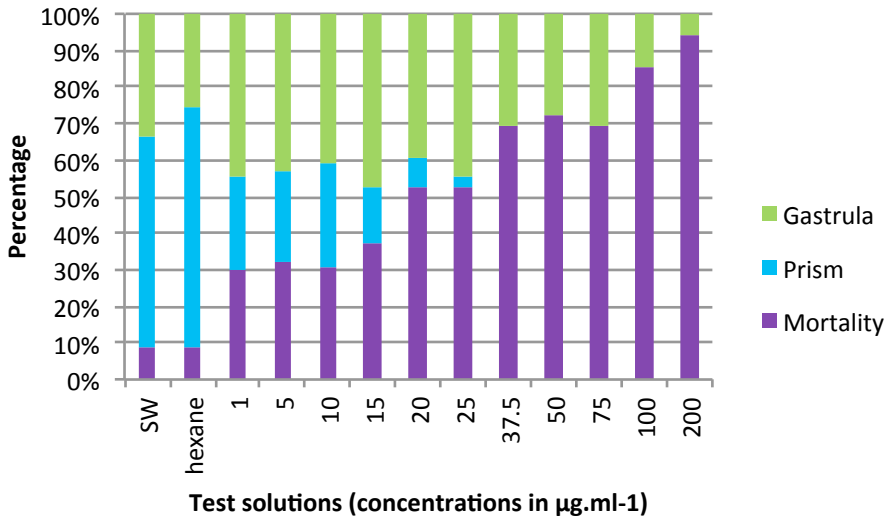


Figure 75: Mean percentage mortality of *Diadema antillarum* embryos after 46 h exposure to molecules extracted from *Sargassum polyceratium* in march 2010 and recovered in hexane. The stage of development of the organisms still living is given (Percentage of Gastrula or Prism).

The two controls (FSW and hexane) allowed good development of prisms (respectively 45.1 and 61.5%, Figure 75). Only 16.67% of embryos developed to the prism stage in the 1µg.mL^{-1} solution (MRT, $p<0.05$). Only some of the embryos developing in the 1µg.mL^{-1} to 25 µg.mL^{-1} solutions reached the prism stage, the other concentrations showed no development past late blastula/ early gastrula stage (Figure 75).

The LC_{50} was evaluated at 38.3 µg.mL^{-1} (Lower=33.2, upper=43.4). No significant difference could be observed when comparing the pairs of concentrations using the 2 recovery methods (hexane and DMSO), with a multiple range test, (MRT, $p<0.05$).

Surface molecules extracted from S. polyceratium affected D. antillarum embryo development.

Solutions for algae sampled in October and January demonstrated more activity than the one prepared in March.

Molecules dispersed in SW or in coatings demonstrated bioactivity. No significant differences could be noticed between DMSO and hexane extracts at the same concentrations.

LC_{50} was evaluated at 26.4 µg.mL^{-1} for extracts in DMSO and 38.3 µg.mL^{-1} for the extracts in hexane.

IV.3.3.2 *Codakia orbicularis (Extracts A and B)*

The results for *C. orbicularis* embryos were similar to those of *D. antillarum* (Figure 76) (Kruskall Wallis: $p<0.0001$). All embryos died in the highest concentrations of *S. polyceratium* extracts (50-200 µg.mL^{-1}) and copper. Percentage mortality decreases at 15 µg.mL^{-1} but the embryos did not developed to the trochophore stage. Only the embryos cultured in the 1 µg.mL^{-1} solution developed to the last stage and showed percentage mortality similar to the control.

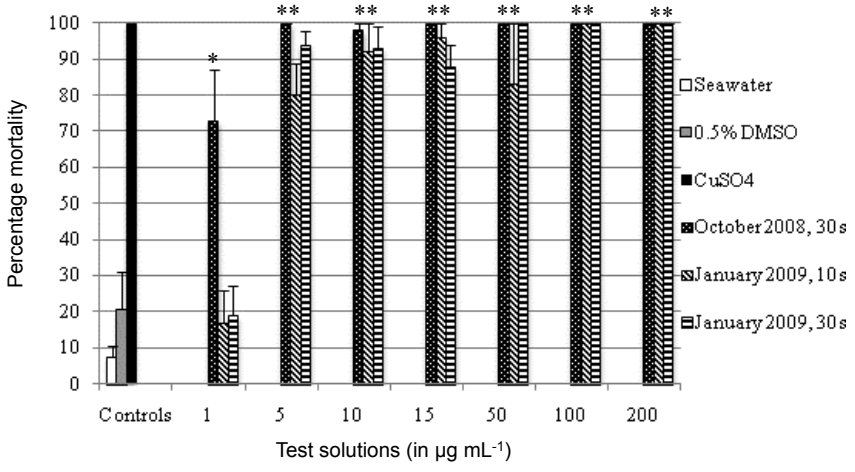


Figure 76: Mean percentage mortality of *Codakia orbicular*is embryos after 24 h exposure to molecules extracted from *Sargassum polyceratium* in October 2008 or January 2009. Dates of algal collection and extraction times are indicated in the key. (n=12±SE)*, significant differences (MRT) between control (DMSO) and one of the solutions; ** significant differences observed between the control (DMSO) and a group of solutions. (Thabard *et al.*, 2011).

The embryonic development was a function of the concentration of the solution tested, and the development stoped progressively with increasing concentrations (Table 23) i.e: 2-4 cells stage at the concentrations 100-200 µg.mL^{-1}, morula at 15 and 50 µg.mL^{-1}, late blastula early gastrula at 5 and 10 µg.mL^{-1} and trochophore at 1 µg.mL^{-1}.

Table 23: Larval stages of development in *Codakia orbicularis* embryos after 30 hours exposure to *Sargassum polyceratium* hexane surface extracts.

Concentration (µg.mL^{-1})	Stage of development
1	Trochophore
5	late blastula-early gastrula
10	late blastula-early gastrula
15	Morula
50	Morula
100	2-4 cells
200	2-4 cells
CuSO4	2-4 cells
DMSO (0.5%)	Trochophore
Seawater	Trochophore

IV.3.3.2.1. Hexane dipping extracts from March 2010 (Extract C)

Recovered in DMSO:

Mortality rates were relatively high in controls (between 16.1% and 15.7% respectively for SW and DMSO). The DMSO results did not show a mortality progression following concentration as for the *Diadema* ones, while the tests were carried out from the same solutions (Figure 77, Appendix 6). The percentage mortality varied, being highest in solutions more concentrated (from 37.5 µg.mL^{-1}), however, the lowest concentrations did not show a clear tendency in mortality rates (comprised between, 33.3% for 1 µg.mL^{-1} to 0% for 15 and 20 µg.mL^{-1}). There was a significant difference for the extracts recovered in DMSO in terms of mortality, abnormality and percentage of trochophores (Kruskal-Wallis, $p<0.05$). The Multiple range test results showed two distinct groups the controls and solutions from 1 to 50 µg.mL^{-1} and the solutions from 50 to 200 µg.mL^{-1}. The solution at 50 µg.mL^{-1} was not different from any of the solutions.

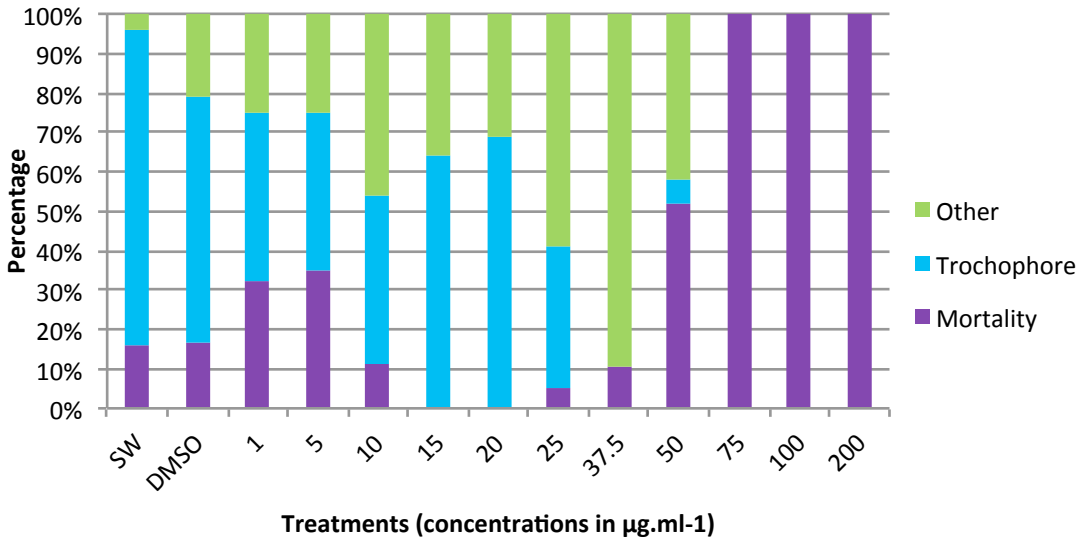


Figure 77: Mean percentage mortality of *Codakia orbicularis* embryos after 24 h exposure to molecules extracted from *Sargassum polyceratium* in march 2010 and recovered in DMSO. The stage of development of the organisms still living is given (Percentage of trochophor and others (abnormal ones + earlier stages)).

The number of trochophore varied in the solutions, being higher in controls (79.7% in SW and 60% in DMSO). Significant differences was seen from 37.5 $\mu g.mL^{-1}$. The extracts from March demonstrated less activity than those from October or January (Figure 76, Figure 77). LC_{50} dose were calculated for solutions recovered with DMSO and estimated at 51.2 $\mu g.mL^{-1}$ (Lower=47.7 $\mu g.mL^{-1}$, upper=54.7 $\mu g.mL^{-1}$).

Recovered in hexane:

Mortality rates were low in controls (2.4% for the SW control and 4.2% for the hexane one) (Figure 78). Mortality in *Sargassum* surface molecule extracts varied with concentrations from 0% in the 1$\mu g.mL^{-1}$ to 100% in the 200$\mu g.mL^{-1}$(Figure 78, Appendix 7). Significant mortality of *C. orbicularis* embryos in comparison to the controls of FSW and hexane were seen from 75 $\mu g.mL^{-1}$ with the value of 100% mortality of the population (Kruskal-wallis and MRT, $p<0.05$).

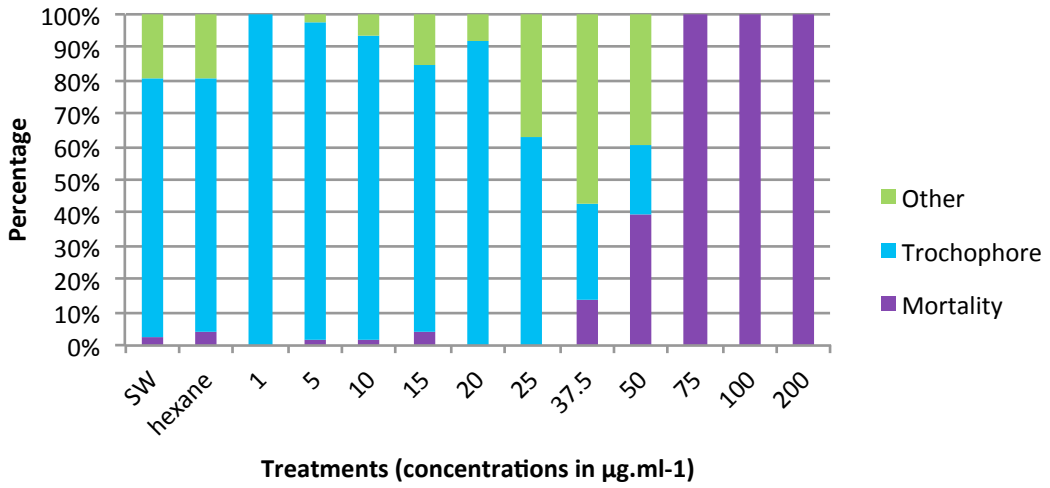


Figure 78: Mean percentage mortality of *Codakia orbicularis* embryos after 24 h exposure to molecules extracted from *Sargassum polyceratium* in march 2010 and recovered in hexane. The stage of development of the organisms still living is given (abnormal ones + earlier stages)).

Kruskal-Wallis test conducted on the percentage of trochophores and abnormal embryos was significant ($p<0.05$). MRT showed a significant difference from 37.5 $\mu g.mL^{-1}$. LC_{50} was evaluated at 49.0 $\mu g.mL^{-1}$ (lower=45.2 $\mu g.mL^{-1}$, upper=52.8 $\mu g.mL^{-1}$). No significant difference could be observed when comparing the pairs of concentrations using the 2 recovery methods (hexane and DMSO), with a multiple range test, (MRT, $p<0.05$).

Surface molecules extracted from S. polyceratium were active towards Codakia orbicularis embryos.

Extracts prepared with algae collected in October and January demonstrated more activity than the one prepared in March.

Molecules dispersed in SW or in coatings were bioactive. No significant differences were observed between hexane and DMSO results.

LC_{50} was evaluated at 51.2 $\mu g.mL^{-1}$ for extracts in DMSO and 49.0 $\mu g.mL^{-1}$ for the extracts in hexane. These values were about two times higher than the one obtained for Diadema antillarum.

IV.3.3.3 *Arenicola brasiliensis*

IV.3.3.3.1. Hexane dipping extracts from October and January (Extracts A and B)

Sargassum extracts were less efficient against *A. brasiliensis* embryos than the other species (Figure 79). Only the highest concentrations of extracts tested (100 and 200µg.mL-

^{1}) and the copper solution inhibited 100% of the larval development. *S. polyceratium* solution extracted for 30s (1 µg.mL^{-1}) in January had some activity against the larvae (15% mortality), although it was not significantly different from the control.

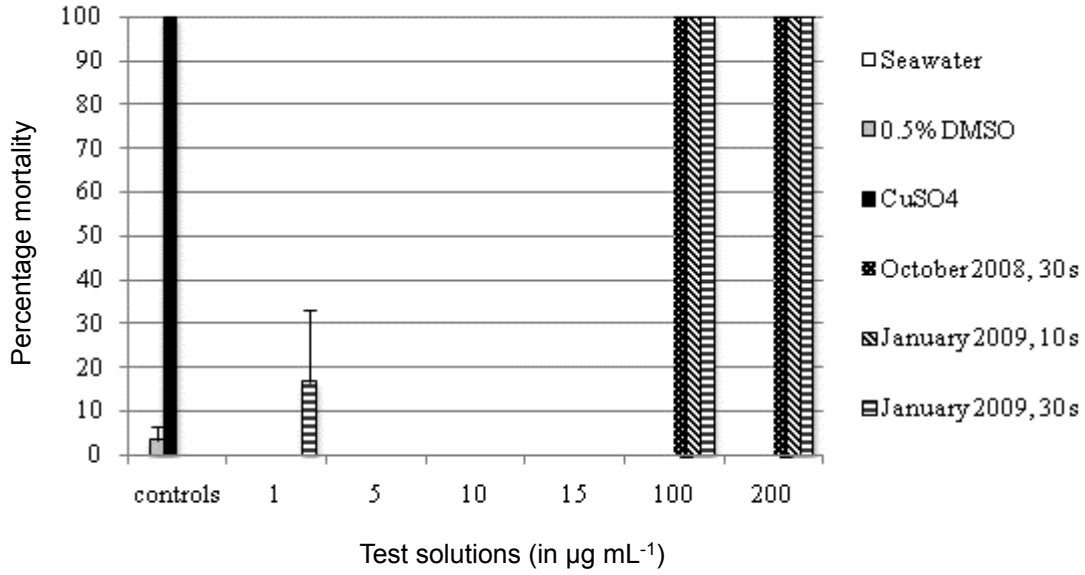


Figure 79: Mean percentage mortality of *A. brasiliensis* embryos after 48 hours exposure to molecules extracted from *Sargassum polyceratium* in October 2008 or January 2009. Dates of algal collection and extraction times are indicated in the key. (n=12 ±SE). (Thabard *et al.*, 2011).

Even though all the larvae survived the treatments, their swimming behaviour differed between the control and the lowest concentration (except 1 µg.mL^{-1}). The larvae were almost motionless in the lowest concentrations or were swimming very slowly (personal observation).

IV.3.3.3.2. Hexane dipping extracts from March 2010 (Extract C)

Both extracts recovered with DMSO (dispersed) or with hexane (coating) had effects on *A. brasiliensis* (Figure 80, Appendix 8). Kruskal Wallis test was significant for both DMSO and hexane tests ($p<0.05$). Multiple range test results demonstrated concentrations from 37.5 µg.mL^{-1} to 200 µg.mL^{-1} not to be different the one from the others. The test solutions from 1 µg.mL^{-1} to 25 µg.mL^{-1} as well as DMSO and SW were not different the one from the other. However, the highest concentrations were different from the lowest ones and controls (MRT, $p<0.05$). Similar results were observed for hexane but groups were from 50-200 µg.mL^{-1} and 1-37.5 µg.mL^{-1} plus controls (MRT, $p<0.05$). High mortality rates were observed for the concentrations from 37.5 µg.mL^{-1}. These results were concordant

with the results from October and January. The same trend was also observed for hexane results, except for the 1 µg.mL$^{-1,}$ which was not different from 37.5 µg.mL^{-1}.

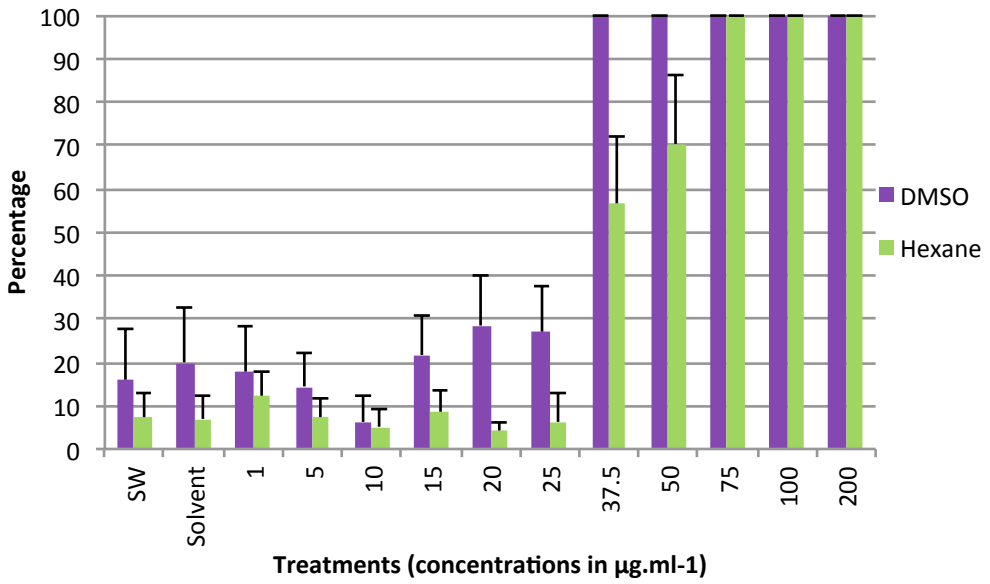


Figure 80: Mean percentage mortality of *A. brasiliensis* embryos after 48 hours exposure to molecules extracted from *Sargassum polyceratium* in march 2010 and recovered in DMSO or hexane. (n=12 ±SE).

When comparing the 2 pools of data with MRT analysis, the pairs of concentrations (1 µg.mL^{-1} DMSO, 1 µg.mL^{-1} hexane) were all equal demonstrating the effects of molecules either dispersed in water or in coating to be equivalent. LC_{50} values were calculated for both solutions recovered with hexane and DMSO and respectively estimated at 39.0 µg.mL^{-1} (Lower=35.8 µg.mL^{-1}, upper=42.3 µg.mL^{-1}) and 24.7 µg.mL^{-1} (Lower=22.3 µg.mL^{-1}, upper=27.0 µg.mL^{-1}).

Surface molecules were active towards Arenicola brasiliensis embryos.

Arenicola brasiliensis embryos were less sensitive to algal surface molecules than the other organisms for the January and October but not for the March extracts.

Dilution performed in March allowed for a better estimation of surface molecules activity towards this organism.

Molecules dispersed in SW were slightly more active than those in coatings. However, these results were not significant. LC_{50} values were evaluated at 24.7 µg.mL^{-1} for extracts in DMSO and 39.0 µg.mL^{-1} for the extracts in hexane, being in concordance with the results obtained towards Diadema antillarum embryos.

IV.3.4. Waterborne cues activity towards *Diadema antillarum* embryos

IV.3.4.1 Test of waterborne cues produced in 90 min and 10 hours

No significant differences could be noticed among all the treatments, *D. antillarum* embryos developed to the prism stage in all the test solutions.

IV.3.4.2 Test of waterborne cues produced continuously

Both controls of UV FSW and substratum showed no significant difference (X-squared=2.2; p=0.8) in values for early development, mortality, abnormal growth or normal population respectively demonstrating normal = 69.16%, abnormal = 5.83%, dead = 6.33%, early = 12.16% and normal = 79.16%, abnormal = 5.83%, dead = 5.83%, early = 10.33% (Figure 81). Even though a small difference between *Sargassum* tanks and the controls could be observed regarding the percentage of normal development, it was also not significant (X-squared= 9.3; p=0.09).

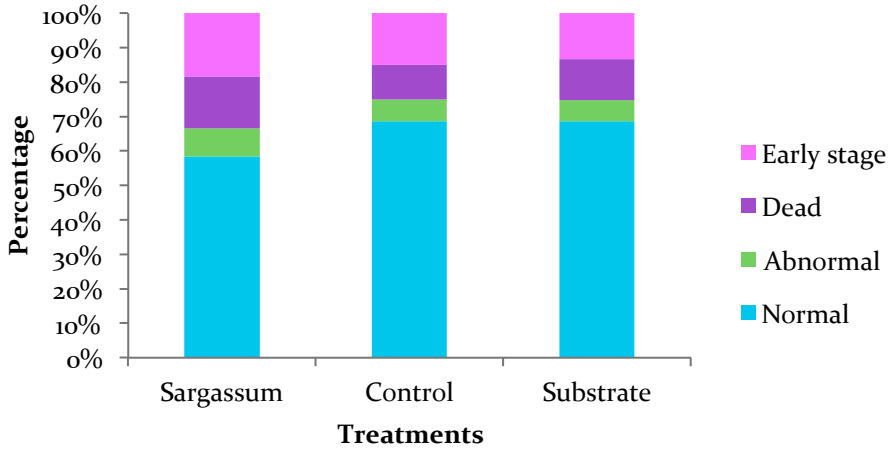


Figure 81: Effects of waterborne cues on the embryonic development of *Diadema antillarum*.

IV.3.5. GC-Ms results

The concentration of the samples in compounds that could be analysed by GC-MS was so low that it did not allow any safe identification of substituents (Figure 82). These results only showed the samples to contain fatty acids but even the size of the acids could not be determined and thus no quantification of the active compound could be done.

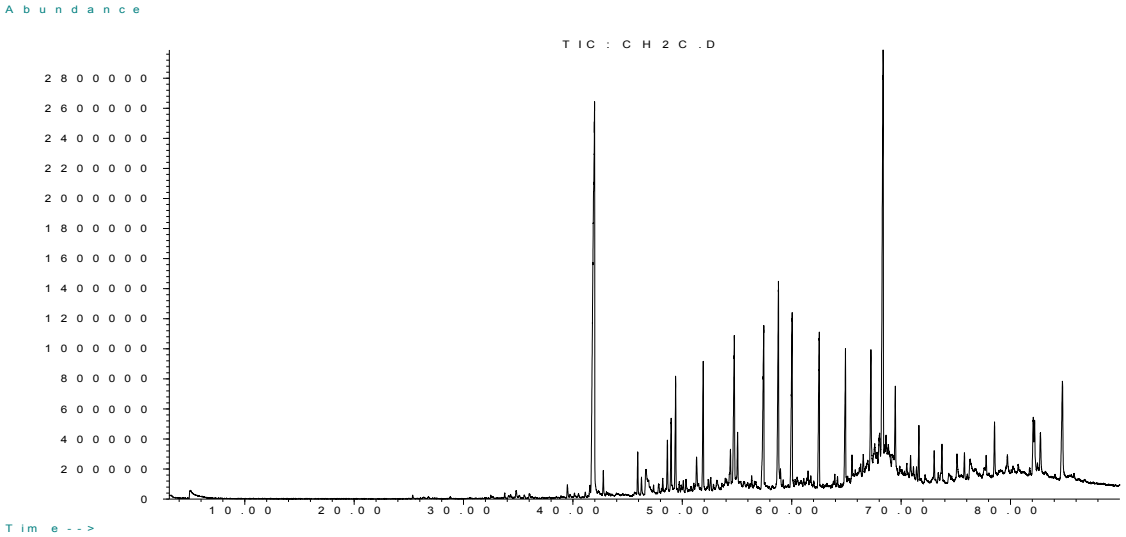


Figure 82: GC-MS profile obtained with the *Sargassum polyceratium* hexane dipping extract from October 2008.

Summary of main results :

	Hexane dipping Extract				***Conditionned water***	
	October 2008 **Extract A**	January 2009 **Extract B**	March 2010 **Extract C**		A	B
Organisms	Recovered in DMSO			Recovered in hexane		
Codakia orbicularis	No significant difference with control only for the solution 1 µg.mL^{-1}		LC_{50}= 51.2 µg.mL^{-1}	LC_{50}= 49.0 µg.mL^{-1}	X	X
Diadema antillarum			LC_{50}= 26.4 µg.mL^{-1}	LC_{50}= 38.3 µg.mL^{-1}	No significant activity	
Arenicola brasiliensis	No significant difference with control only for the solution 15 µg.mL^{-1}		LC_{50}= 24.7 µg.mL^{-1}	LC_{50}= 39.0 µg.mL^{-1}	X	X
Montastrea annularis	No spawning					

Table 24 : Summary of *Sargassum polyceratium* biological activities towards marine invertebrates' embryos (X means not tested).

IV.4. Discussion

Secondary metabolites produced by algae are known to affect the settlement, recruitment, development and behaviour of numerous organisms (Pawlik, 1992a, Steinberg and de Nys, 2002, Paul and Puglisi, 2004, Paul *et al.,* 2007). Compounds isolated from *Dictyota menstrualis* and *Dictyota ciliolata* caused significant mortality (*Bulga neritina* [Bryozoan]), abnormal development (*B. neritina*) and reduced settlement rates (*Amathia conoluta, B. neritina,* [bryozoans] and *Eudendrium carneum* [hydroids]) (Schmitt *et al.,* 1998). Algal waterborne cues affect *Acropora millepora* [coral] larval recruitment (Birrell, 2003) and are toxic towards the fouling polychaete *Hydroides elegans* at early stages of development (Dobretsov *et al.,* 2006). Algal natural products are species specific and can act on different larval development stages, thus *Dictyota spp.* and *Laurencia sp.* were toxic against larvae of *H. elegans* and *B. neritina*, *Padina sp.* and *Halimeda sp.* inhibited their larval settlement, *Hypnea sp., Ulva sp.* stimulated the larval settlement and *Sargassum spp*. had no effect (Walters *et al.,* 1996).

The results of the present experiment demonstrate that *S. polyceratium* surface molecules significantly affected the embryonic development of 3 tropical invertebrates and bacterial growth. These organisms are not all in direct contact with *S. polyceratium* except *D. antillarum*, but they represent species from 3 invertebrate phyla giving thus a hint of *Sargassum's* potential toxic activity towards several types of organisms. Regarding the embryo toxicity assays, the concentrations to which the extracts were active varied according to the species tested and season. Both *C. orbicularis* and *D. antillarum* demonstrated similar responses while *C. orbicularis* was expected to be more resistant because of the large envelope surrounding its embryos (Gros *et al.,* 1997). *A. brasiliensis* embryos were the most resistant to the extracts (for the October and January extracts). In the control *C. orbicularis* embryos developed in 24h to the trochophore stage and *D. antillarum* embryos developed in 30h to the prism stage, which was consistent with the values found in the literature (Gros *et al.,* 1997; Eckert 1998). However, in the treatments, *S. polyceratium* surface compounds had a progressive effect on the embryonic development (tests on extracts A and B), the highest concentrations (100-200 $\mu g.mL^{-1}$) blocking the development at 2-4 cells stage, the medium ones (15-50 $\mu g.mL^{-1}$) allowing the development until the morula stage and the lowest concentrations (1-10 $\mu g.mL^{-1}$) until

the blastula, gastrula and finally trochophore or prism stage (respectively for *C. orbicularis* and *D. antillarum*), suggesting the extracts block egg cleavage. Similarly, Paul and Fenical, (1986), demonstrated caulerpyne to block the cleavage of developing sea urchin eggs. The division of *Paracentrotus lividus* (urchin) embryos was inhibited by extracts of 20 species, among which included algal extracts (Martin and Uriz, 1993). Dihydrorhipocephalin, aldehyde, udoteal, petiodal, dihydroudoteal, rhipocephalin, halimedatrial, halimeda tetraacetate (4,9-diacetoxy-udoteal) isolated from caulerpal species (*Halimeda* spp., *Penicillus* spp., *Ripocephalus phoenix*, *Udotea* spp.) exhibited toxicity against developing sea urchin eggs, sperm and larvae (Paul and Fenical, 1986) with ED_{100} levels (lowest concentration leading to 100% inhibition of cell division) comprised between 0.2 and 16 $\mu g.mL^{-1}$. In the present study, the half maximal effective concentration (LC_{50}) was estimated to be around 26-38 $\mu g.mL^{-1}$ for *D. antillarum*, 49-51 $\mu g.mL^{-1}$ for *C. orbicularis* and 25-39 $\mu g.mL^{-1}$ for *A. brasiliensis* (depending on the solvent used for molecules recovery). Even though the indices are not the same (ED_{100} and LC_{50}), they suggest *S. polyceratium* had a lower activity than the Caulerpales solutions tested by Paul and Fenical, (1986). However, the species studied were not the same, and the tests performed by Paul and Fenical (1986) concerned isolated secondary metabolites and not crude extracts. It is possible that the active compounds tested in this study were present in the crude extracts at very low concentration. On the other hand, it has been demonstrated that some molecules act synergistically and that a pool of molecules is required to induce a biological effect (Hay and Fenical, 1996). The first GC-MS results obtained on the samples could not allow safe identification of the substituent and thus no quantification could be made. Further analytical chemistry tests involving molecule purification and chemistry analyses (GC-MS and NMR) would give a better idea on the chemical nature of the bioactive compounds as well as on their activity level.

The molecules extracted at the algal surface with hexane are probably non-polar as hexane is the most non-polar solvent. *Sargassum* species are known for their production of polyphenols (polar metabolites) but some non-polar extracts, such as *S. vulgare* hexane extracts were demonstrated to be highly active towards the development of microalgae, suggesting the production of active non-polar secondary metabolites by this alga (Plouguerné *et al.,* 2010a). So far, no methods allowing for the extraction of polar molecules located at the surface of the algae were developed and the activity observed here thus represents only one part of the possible MNP present at the surface of *S. polyceratium*.

No differences were observed for the toxic activity towards embryos between the 30s extracts from October and January. However, antibacterial activity was different between these 2 extracts, suggesting a possible seasonality in the surface molecule activities. Temperate algal extracts have a seasonal variation in molecular composition, antimicrobial activity and AF activity (Steinberg and Van Altena, 1992, Hellio *et al.,* 2004, Maréchal *et al.,* 2004, Plouguerné, 2006, Maréchal and Hellio, 2011). The October extracts were from the rainy season while the January ones corresponded to the dry season. Moreover, a difference could be observed between the tests carried out on extracts A and B (from rainy and early dry season) and C from late dry season, the activity towards embryos being higher for the first 2 assays. Hellio *et al.,* (2004), demonstrated *S. muticum* secondary metabolites activity to vary with seasons and to be higher during the summer months, when the fouling pressure from, for example, bacteria, is the most intense. It is thus possible that macroalgae develop specific mechanisms of protection in the rainy season when bacteria concentrations are higher in coastal environments (Futch *et al.,* 2010). In the present study, the levels to which extracts were active towards bacteria were high (150 to 300 $\mu g.mL^{-1}$) in comparison with other algal crude extracts. Crude extracts obtained from seaweeds collected in Brittany were active when concentrations were comprised between 24 and 96 $\mu g.mL^{-1}$ (Hellio *et al.,* 2001) and between 0.1 and 100 $\mu g.mL^{-1}$ (Plouguerné *et al.,* 2008). Marine bacteria were the most sensitive strains, suggesting defense strategies of *S. polyceratium* to be specific. Such orientated defense strategies were already described in the literature for other algal species (Paul and Puglisi, 2004).

Tests carried out either with molecules dispersed in the water or fixed as a coating demonstrated an equivalent activity towards embryo development. Studies conducted on reef species demonstrated allelopathic interactions to be mainly the result of direct contact rather than via transmission through the water (Thacker *et al.,* 1998, Kubanek *et al.,* 2002). This suggests that allelopathic metabolites are lipid rather than water-soluble and that their effects are generated through contact (Rasher and Hay, 2010). In the present experiment, results concerning embryos developing in contact with waterborne cues or in control tanks were not significantly different. Similarly, Walters *et al.,* (1996) did not observe differences in the mortality rates of both *Hydroides elegans* and *Bugula neritina* when cultured in conditioned water prepared from *Sargassum echinocarpum* or *Sargassum polyphyllum*.

Bioactivity of *S. polyceratium* extracts were highlighted; however it remains uncertain whether the active molecules are produced by *S. polyceratium* or by its associated biofilm (Kientz *et al.,* 2011). Secondary metabolite isolation from algae could be biased by its associated microorganisms as they can prevent the determination of the origin of metabolites production. As the study focused on surface molecules only, it was impossible to clean the macroalgal surface from microepiphytes using existing methods such as ethanol (Kientz *et al.,* 2011) without breaking the surface cells (de Nys *et al.,* 1998). The surface extracts, tested in the present study, include all the low polar molecules present at the *Sargassum* surface ie: the molecules produced by the alga as well as by its epiphytes. Numerous bacteria living in SW produce active secondary metabolites (Jensen and Fenical, 1994). Moreover, studies demonstrated host specific associations between algae and bacteria and suggested that algae may control the associated bacteria (Lachnit *et al.,* 2009). The bacterial biofilm may in turn confer protection to the host algae by the production of secondary metabolites.

This study demonstrated *S. polyceratium* surface extracts to inhibit bacterial growth and embryo development of 3 tropical marine invertebrates, including *D. antillarum,* a tropical herbivore keystone species thought to play a role in macroalgal populations' regulation. However, studies aiming at quantifying the active molecules concentration present at the *Sargassum* surface should be carried out to determine if the tested levels are equivalent to the environmental ones and therefore if the environmental levels are sufficient to prevent embryonic development. In order to test for the allelochemical effects of *Sargassum* on benthic species recruitment in the field, manipulative experiments based on Nugues and Smantz, (2006) protocols could be performed. They tested the effect of *Halimeda opuntia* (Chlorophyceae) on *Favia fragrum* (Coral) recruitment. Experimental design based on theirs ie: introduction of larvae in incubation chambers (located on the reef at the bottom of the sea) with a *Sargassum* plant and a piece of substratum for fixation could give answers about coral recruitment potential when at the vicinity of *Sargassum*. Such experiments could focus on larval species that are known to settle in such conditions and which are easily acquired in the field. Focusing on brooders might be easier as it does not involve manipulation and controlled fertilization, thus *Favia fragum* could be employed as it has already been investigated in the field (Nugues and Smantz, 2006). Broadcasting species could also be good test organisms, their reproduction patterns are well known and larvae of

these species (such as *Montastrea faveolata*) were already manipulated in the field (Vermeij *et al.,* 2010).

***Sargassum polyceratium* surface molecules demonstrated broad spectrum activity towards marine fouling organisms (bacteria) and tropical invertebrates (*Codakia orbicularis*, *Diadema antillarum* and *Arenicola brasiliensis*). LC_{50} were comprised between 22 and 55 µg.mL^{-1} demonstrating sensitivity to MNP to be species dependant.**

The extracted molecules could come from either the alga itself or its associated biofilm.

Waterborne cues did not prevent the good developpement of D. antillarum embryos.

The presence of macroalgae could thus impact the recruitment process of marine invertebrates by contact (especially because Diadema antillarum emits gametes in the field at the vicinity of algal populations).

Chapter V: Conclusions and perspectives.

Over the past 30 years many coral reefs, including those of Martinique, have faced profound modifications due to both anthropogenic and natural events (Wilkinson, 2008; Legrand, 2008; Legrand, 2010; Rousseau, 2010). Coral percentage cover sometimes decreased to the benefit of opportunistic organisms such as macroalgae which, when the environmental conditions are suitable, can sometimes form large beds over dead coral colonies (McManus and Polsenberg, 2004). This phenomenon is called *phase shift* (Knowlton, 1992). The new state, dominated by macroalgae, will inevitably have many implications for benthic reef species. This PhD work aimed at determining the effects of macroalgae (with an emphasis on *Sargassum* sp.) on benthic reef recruitment. This is the first work investigating early coral recruitment times in Martinique. Most importantly, it provides information regarding Martinique southern coral reef i) habitat types, ii) benthic composition and iii) recruitment, required by reef managers to maintain healthy coral reef communities and build coral reef survivability.

V.1.1. Reef habitat types and benthic composition

The efficient management of ecosystems requires knowledge about habitat composition. A 1:25 000 map of Martinique marine benthic ecosystem from 0-50 m depth was elaborated in 2010 (Legrand, 2010), however, the information provided by this map needs to be implemented by punctual surveys and monitoring to follow reef evolution. The following paragraph discusses the benthic composition and health states at 5 locations in Martinique.

Habitat types and reef health state: important coral coverage and high heterogeneity

In the present work, habitat characterisation as well as benthic organism percentage covers were determined at 5 locations of the southern reefs of Martinique in 2010. Sites were classified according to their benthic characteristics into 3 classes: the *coral* (high percentage cover of coral, weak algal presence and high *D. antillarum* occurrence), the *urchin* (intermediate state with low algal and coral cover but high *D. antillarum* occurrence), and the *algal* areas, dominated by *Sargassum spp.* (high percentage algal cover but no *D. antillarum* and weak coral occurrence). *Coral* areas were the most colonised by benthic organisms (between 11-14% bare substratum while it was compared to between 19-42% for *urchins* and *algal* areas) with coral, CCA, gorgonians and turf presenting higher percentage covers than in the other zones. Concerning the two other areas, *D. antillarum* and algal populations often formed two distinct belts parallel to each

other. While *urchin/algal* belts were already observed on reefs throughout the Caribbean (Carpenter and Edmunds, 2006), it is the first description in Martinique.

The coral percentage covers recorded were above 35% in three of the investigated stations, which was comparable to other surveys carried out in Martinique on the same reefs (Rousseau, 2010). Coral cover, an indicator of reef health state (Kaufman, 2011), was high compared to some values found in the Antilles (Wilkinson, 2008). Numerous surveys conducted in the Caribbean showed a decrease in coral cover with rates lower than 30 % in 2008 (maximum percentage covers: 29 % in Panama, 28 % in Mexico, 26.1 % in the Virgin Islands, 26 % in Costa Rica, 24.5 % in Anguilla, 24 % in Honduras, lower than 20% in Guatemala, Antigua, St Eustache and Trinidad and Tobago) (Wilkinson, 2008). Carpenter and Edmunds (2006), who conducted a study in several Caribbean islands, observed percentage coral covers were inferior to 40 % at all six locations investigated (Belize, St Croix, Barbados, Jamaica, Bonaire and Grenada).

Despite the high coral percentage cover at some stations surveyed, coral cover was highly variable and two stations studied at the same location (10 meters apart) could present major differences in their benthic composition, even though the environmental pressures were equivalent. It was the case for Trois Rivières, Caye d'Olbian and Tombant de l'Eglise. Some of the southern reefs of Martinique are heavily degraded and present algal cover above the phase shift thresholds determined by Bruno *et al.,* 2009 (coral cover <10% and macroalgal cover >60%).

Benthic communities' compositions are highly variable in Martinique, stations at the same location under the same anthropogenic pressures present very different health state. These findings raise interrogations about reef degradation occurrence. The high scleractinian coral cover (equivalent to the highest rates observed in the Caribbean), contrasted with the strong reef degradation observed nearby. The implementation of activities to conserve this richness must be undertaken in the island where only no fish take zones have been developed so far. Several monitoring programs are currently being conducted in Martinique, among which the IFRECOR program and the EU WFD. Indicators are being developed to assess the reefs and water bodies' health states. Knowing that coral cover (main indicator of reefs health state) can be highly variable even though reefs seem to face the same pressure (10 m away the one from the other) should be considered in the development of such indicators and the scale of investigation.

Variation in benthic composition according to the habitat types surveyed: possible macroalgal effects

Forty-five coral species have been identified in Martinique (Bouchon and Laborel, 1986; Bouchon *et al.,* 1987 – GCRMN protocol). Rousseau (2010) observed 30 species while surveying 14 stations of the southern and Caribbean coast of Martinique. In the present experiment, conducted between 9 and 13 m depth on the southern reefs of Martinique, 20 species as well as one genus have been identified. This lower richness could be due to the sampling design. Indeed, the methods employed: line intercept, point intercept and photoquadrat give different results (Leujakand Ormond, 2007). Moreover, the surveys were conducted at less stations and narrower depth ranges in the present study. Finally these surveys were conducted at different times and it is possible that some species have suffered from extinction in Martinique; it is the case of *Acropora cervicornis* that has not been observed since 2007 (Maréchal, personal communication). This underlines the importance of conducting regular surveys to follow benthic reef composition and evolution.

In the present survey, coral species and morphotype diversities varied between the three habitat types defined. *Dichocoenia stokesii, Diploria labyrinthiformis, Eusmilia fastigiata, Isophyllia sinuosa, Madracis mirabilis, Millipora sp., Montastrea annularis, Montastrea faveolata, Mussa angulosa and Porites porites*, were absent from all macroalgal fields.

When considering coral shapes as described by Humann, (1993) (brain coral, flower coral, fleshy coral…) some morphotypes appeared to be absent from *algal* and / or *urchin* areas (Figure 83).

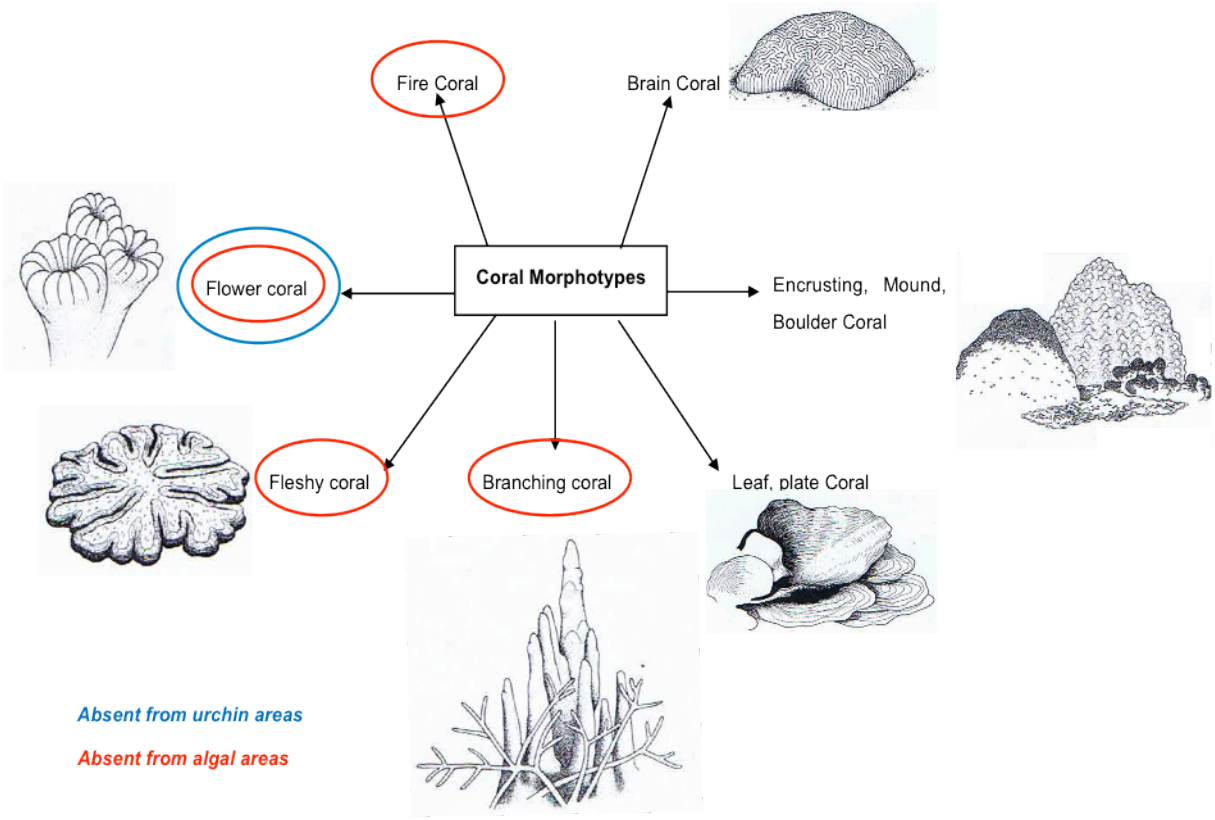


Figure 83: Coral morphotypes presence at the investigated areas (drawings are from Humann, 1993; pictures of representative of each morphotype are inAppendix 9).

A possibility is that some adult morphology could be more vulnerable than others to algal canopies. Indeed, fleshy coral is not protected by external skeleton and branching and flower corals form small entities, which could be more sensitive to the abrasion phenomenon than compact species such as encrusting or boulder species (Appendix 9 for pictures). The physical effects of *Sargassum* have already been highlighted on *Porites porites* development (River and Edmunds, 2001). Lirman, (2001), demonstrated that algal impacts on 3 encrusting, boulder coral morphotypes (*S. siderea*, *P. astreoides* and *M. faveolata*) were species dependant with both *P. astreoides* and *M. faveolata* being affected but not *S. siderea*. Interaction mechanisms appear to be complex and driven by coral morphotypes as well as species. Some differences in coral diversity were also noticed between coral sites, demonstrating that even though habitats and pressures were similar, high heterogeneity could be observed. Recruitment patterns and prevention of larval

settlement and / or recruitment by macroalgae could also explain in part the absence of some adult coral species in algal fields.

Some coral species and morphotypes seemed to be more vulnerable than others to algal stressors. Even though all coral species are threatened and belong to the IUCN red list, discriminating the most impacted species is of primary importance in order to preserve coral biodiversity. This work focused on the impact macroalgae could have on recruitment, but further tests aiming at determining the most vulnerable species should be conducted to help discriminating the most efficient conservation methods.

V.1.2. Invertebrates' recruitment in coral reefs

Larval settlement, one of the most precarious times in the life of a marine benthic sessile organism, determines the environment in which the future adult will evolve. In the case of coral scleractinian species it will also partly determine the reef structure and thus the development of its associated communities. Acquiring knowledge on reef recruitment process (that partly drives reef resilience) is of paramount importance for reef managers. This work allowed characterising the differences in juvenile coral biodiversity and the effects that macroalgae could have on their distribution.

Biodiversity of coral juveniles and recruits in the defined habitats: importance of coral life cycle

Juvenile coral presence in the 3 habitat types varied with the reproductive group they belonged to: spawners or brooders (Chapter II). The frequencies of juvenile brooders were around 1 in *coral* areas, while they were almost equivalent (around 0.5 brooders and 0.5 spawners) in *algal* fields. This could suggest that brooders planulae (e.g: *Porites* sp. and *Agaricia* sp.) settle preferentially in the vicinity of their parent colonies. This is consistent with previous studies which demonstrated that brooders settle within a few hours, therefore closer to their parent colonies (Sammarco 1996). In another study, the settlement of the brooder coral *Stylophora pistillata* was genetically demonstrated to be closer to the parent colonies than for the spawner *Acropora tenuis* (Nishikawa *et al.,* 2003). Recent research have demonstrated coral larvae dispersal not to be only driven by currents but also by larval swimming behaviour which can be adapted to either disperse further or settle closer to their parental colonies (Pizarro and Thomason, 2008). These first conclusions are crucial

regarding reef preservation and conservation methods. The investigation of Martinique reef connectivity and coral dispersal dynamics using genetic tools (Van Oppen and Gates, 2006) could be performed to give a better insight into how these genera disperse.

The highest recruitment value observed on tiles was 2.1 indiv.100 cm^{-2} (in a 5 month experiment). This is (if extrapolating to indiv.m^{-2}.yr^{-1}) higher than some values found elsewhere in the Caribbean (Chapter III). Poritidae and Agariciidae were the most common genuses settling on terracotta tiles suggesting that this substratum might probably not be suitable for some other species to recruit on. It should be pointed out that these two species are brooders and opportunistic. These characteristics may in part explain the fact that they were the species observed in majority on tiles.

These results give insight into the coral settlement capacities under several conditions. As stated previously, understanding coral reef recruitment processes is of major importance for reef managers, and many research program concerning coral larvae, there biology, physiology and settlement capacities are currently undertaken in the French West Indies (Maréchal, personal communication). The results of the present work could be considered by reef managers to develop preservative measures. Indeed, if management policies of Martinique reefs were focusing on the installation of artificial structures, these PhD findings ie: i) the settlement of some brooders at the vicinity of parent colonies; ii) the settlement of some species was restricted on terracotta tiles, should be taken into consideration to maximize the chances of success (provide parent colonies of brooders to favour recruitment on the structure, develop artificial reefs with structures and substrates favourable to the settlement of many organisms to promote reef complexity).

Effects of macroalgae on invertebrates' recruitment

Species interaction could also be a determining factor in juvenile coral settlement patterns and, therefore, the presence of adult colonies. CCA are known to be a planulae attractant and were more abundant in <u>*coral*</u> (12-15%) followed by <u>*urchin*</u> (5-9%) and finally <u>*macroalgal*</u> zones (0.5-3%) where coral juvenile presences were respectively between 7-8 indiv.m^{-2}, 2-3 indiv.m^{-2} and 0.9-1.4 indiv.m^{-2}. Moreover, canopy-forming macroalgae could have a direct impact on juvenile settlement and recruitment. Seaweeds, among which includes *Sargassum* spp. have already been demonstrated to interfere with coral

recruitment, development and survival (McCook *et al.,* 2001; Birrell, 2003; Diaz Pulido *et al.,* 2010).

In the present work, emphasis was put on the effects of macroalgae on recruitment processes, in part because this process is essential regarding reef resilience, but also because coral juveniles were described as more vulnerable than adults to macroalgal pressures (Zilberberg and Edmunds, 2001, Raymundo and Maypa, 2004). Benthic reef recruitment was lower in *algal* areas compared to the other investigated area types (Chapter III, see Table 25 for a summary of the results). Algal canopies are dense on the reefs of Martinique (53-64% cover at degraded sites) and they seem to have a barrier role, sweeping a large reef surface under the action of swell and probably preventing the larvae from reaching the substratum. Removing seaweeds (WA zone) did not enhance coral recruitment (in the short term) but promoted the development of other reef benthic species, suggesting that algal canopies are among the responsible factors for the weak benthic cover recruitment in algal colonised areas. WA zones needed to be cleaned every two weeks to prevent algae from recolonizing the area. In the light of these results, removing algae from reefs on a large scale in order to promote benthic recruitment is likely to be inefficient.

Table 25: Summary of the main findings regarding both field observations and recruitment experiments.

		Macroalgal	**Urchin**	**Coral**	**Algal removal zone**
Field observations	Coral % Presence	Weak under algal canopies	Weak	High	No field observations
	« prevalent » organisms	Algae,	Intermediate state	Coral, CCA, Gorgonian	
	Juveniles	Low Very few brooders	Intermediate Few brooders	High Many brooders	
	Diadema antillarum	None	Comparable to Caribbean averages		
Recruitment experiment	Comparison	Weak % cover of all organisms but sponges, turf and Macroalgae	% comparable except for coral (higher in coral area) and worms , turf, macroalgae (higher in urchin area)		Intermediate state (but almost none coral recruit)

Coral spats only recruited on control tiles, while investigating the physical and chemical effects of *Sargassum* on coral recruitment. Here the physical role (barrier) of algae was underlined as plant mimics or cages exhibited the same results. Surprisingly, no spats were fixed on tiles located in cage controls while other experiments, using cages to prevent fish grazing, demonstrated coral to settle on tiles (Penin, 2007). No satisfactory conclusions could be drawn regarding the effect of waterborne cues produced by *Sargassum* spp. on recruitment processes in the field experiment. In the future, manipulative experiments based on the introduction of coral larvae in incubation chambers (located at the bottom of

the sea) with a *Sargassum* plant and a piece of substratum for fixation (protocol of Nugues and Smantz 2006) could give answers about the coral recruitment potential when in contact with *Sargassum* waterborne cues. *Favia fragum* (Brooder) or *Montastrea faveolata* (spawner) larvae could be tested as they have already been investigated in the field (Nugues and Smantz 2006, Vermeij *et al.,* 2010).

Even though the allelochemical role of algae on coral could not be determined in the field, laboratory experiment demonstrated that *Sargassum polyceratium* impacted reef species embryonic development including that of *Diadema antillarum* (Chapter IV, Appendix 10). Surface molecules, which can be in contact with surrounding organisms, had a negative impact on the tropical embryos' development. The ecological role of these molecules should, however, be taken with caution as the quantification of their natural concentration was not possible. Further experiments aiming at testing those molecules against some coral reef species embryonic development need to be carried out. Such studies were planned; however, the bleaching event that occurred in 2010 resulted in no coral spawn in October 2010.

The recent recovery of Diadema antillarum in Martinique could partly explain coral recruitment rates

Herbivore richness and diversity have received particular attention over the past years as they partly explain the links between reef biodiversity and ecosystem functioning. *Diadema antillarum*, is described as a key stone species by many authors and is thought to play a major role in regulating macroalgal population overgrowth and thus reef ecosystem functioning.

In this survey, *D. antillarum* densities were comparable to recent observations in the Caribbean (between 0-2.2 m^{-2}). Even though *D. antillarum* populations were not monitored before and right after the mass mortality event in Martinique in the 1980s, the present results suggest a recovery of Martinique populations such as described elsewhere in the Caribbean (Carpenter and Edmunds, 2006, Steiner and Williams, 2006).

In the literature, the presence of this sea urchin at high densities is often associated with its recovery as well as an increase in coral recruitment rates (Carpenter and Edmunds, 2006, Idjaidi *et al.,* 2010). Recruitment in *urchin areas* seemed to be favoured compared to *algal* areas (Chapter II and III), supporting the hypothesis that *D. antillarum* populations'

recovery enhances coral recruitment (Edmunds and Carpenter, 2001; Carpenter and Edmunds, 2006).

In the tile experiment where seaweeds had been manually removed (Chapter III), urchins were observed to migrate little by little into the cleared area (personal observations), demonstrating the urchins to benefit from algal removal and to colonise newly available substrates. These preliminary observations raise questions about the dynamic processes existing between the two *algal*, *urchin* belts. It was proven that *Sargassum* could have an impact on the development of these urchin embryos under laboratory conditions, however *D. antillarum* impact (grazing) on this alga remains unknown in Martinique. Considering that *D. antillarum* seems to benefit from algal removal and that this species enhances coral recruitment, understanding the dynamics of these urchin patches is of primary importance for reef managers. Experiments conducted in the Canary islands demonstrated that a density of *Diadema antillarum* above 2 indiv. m^{-2} drastically reduces non-crustose macroalgal cover below 15% (Hernandez *et al.,* 2008). These densities are around the ones found on some Martinique reefs, suggesting these urchins could be enough to induce macroalgal decreases and maybe a reversal in the phase shift. Based on Hernandez *et al.,* (2008) 's work, experiments should be conducted to determine the grazing thresholds in the Caribbean and ensure 2 indiv.m^{-2} are enough. Laboratory based experiments could also be conducted to determine their grazing capabilities according to the algal species (Pereira and Yoneshigue-Valentin, 1999, Pereira *et al.,* 2002). Such feeding assays were conducted during this PhD, however, maintaining urchins in good conditions so that they could feed in the lab was not achieved. Hay *et al.,* (1987) had already highlighted problems while conducting feeding preference assays with *D. antillarum*. The adults did not want to feed in 3.8L jars and for this reason they focused on juveniles (Hay *et al.,* 1987). In the present trials, *D. antillarum* adults were cultured in 40L tanks but this attempt remained unsuccessful.

This work provides information about Martinique reef habitat types, species diversity and reef recruitment.

Tropical Sargassum species seem to possess numerous required characteristics to make them a good proliferative and dominant species (several dispersal strategies, long fertility period, highly polymorphic, a flexible life cycle, capable of utilising several nutrient sources, both perennial or pseudo perennial, thick leathery morphology and the production of active substances). Their presence on Martinique reefs seemed to be detrimental to coral and other benthic species recruitment.

This research project was in part funded by the French Government (DEAL services: Direction de l'Environnement de l'Aménagement et du logement) ***and aimed at understanding whether reef restoration should focus on removing algal populations to promote reef conservation. The results of this work suggest that other conservative methods should be investigated first. Coral recruitment enhancement is critical in order to maximize the chance of recovery of reef ecosystems. Artificial restoration plans should consider these PhD findings in order to focus on adapted restoration techniques that could be used to aid and speed the recovery of damaged reefs by enhancing or supplementing the natural processes of resilience. Technics such as: protection of areas where parent colonies are the most abundant; utilisation of coral species that are known to recruit better if coral transplant could be employed in order to promote the colonisation of damaged zones.***

In the light of these results the first step to be taken, would be to preserve the high biodiversity and high coral presence covers at some of the investigated sites. Preservative measures need to be taken rapidly to preserve Martinique reefs. They could focus on several methods such as i) limit pressure from catchment basin, ii) increase the herbivory pressure by reducing fisheries activities iii) work on restoration methods, coral transplant, artificial reefs.

Even though the creation of two marine protected areas are currently being discussed in Martinique, no MPAs (except the no-take zones that can be re-opened) exist so far around the island.

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APPENDIXES:

Appendix 1: Mean percentage cover of the 8 groups of organisms present at the different sites (n=45 ± SE).

Site	Coral	CCA	Gorgonian	Macroalgae	Sponge	Substratum	Turf	Zoanthids
Coral								
Anse Mabouya	34.98 ± 2.92	12.99 ± 1.02	1.77 ± 0.39	7.75 ± 1.1	4.05 ± 0.94	14.73 ± 1.7	21.30 ± 1.77	0.18 ± 0.09
Caye d'Olbian	37.83 ± 3.24	15.60 ± 1.26	0.15 ± 0.08	7.38 ± 0.97	3.94 ± 0.81	12.98 ± 2.06	17.91 ±2.1	0.05 ± 0.04
Tombant de l'Eglise	38.21 ± 4.23	12.32 ± 1.47	0.82 ± 0.37	14.16 ± 1.72	7.78 ± 2.73	11.24 ± 2.6	13.80 ± 1.64	0.26 ± 0.22
Urchin								
Arche	4.01 ± 0.73	5.78 ± 0.85	0.04 ± 0.04	29.59 ± 1.52	8.63 ± 1.65	42.22 ± 2.82	9.82 ± 1.31	0.00
Caye d'Olbian	6.40 ± 1.06	8.88 ± 1.4	0.15 ± 0.14	24.55 ± 2.07	2.16 ± 1.1	29.23 ± 3.59	27.56 ± 2.3	0.00
Trois Rivières	3.06 ± 0.53	4.60 ± 0.58	0.07 ± 0.04	32.56 ± 2.25	8.84 ± 2.19	35.74 ± 2.55	14.76 ± 1.66	0.00
Algae								
Caye d'Olbian	1.23 ± 0.47	0.55 ± 0.13	0.01 ± 0.01	52.81 ± 2.25	7.87 ± 1.63	33.28 ± 1.9	3.36 ± 0.58	0.00
Tombant de l'Eglise	1.27 ± 0.37	3.27 ± 0.41	0.00	61.23 ±1.2	3.92 ± 0.63	19.90 ± 1.33	9.89 ±0.73	0.00
Trois Rivières	0.69 ± 0.17	2.36 ± 0.32	0.00	64.28 ± 1.63	6.14 ± 1.14	21.56 ± 1.51	4.75 ± 0.63	0.00
Without algae								
Caye d'Olbian	1.23 ± 0.4	1.71 ± 0.24	0.03 ± 0.03	26.57 ±1.41	9.70 ± 1.63	54.21 ± 2.29	5.63 ± 0.82	0.00
Tombant de l'Eglise	1.77 ± 0.44	6.66 ± 0.61	0.00	44.36 ± 1.36	5.28 ± 0.79	28.08 ± 1.76	13.26 ± 0.93	0.00
Trois Rivières	1.40 ± 0.34	5.27 ± 0.61	0.00	37.18 ± 1.55	8.76 ± 1.45	39.49 ± 2.43	7.64 ± 0.64	0.00

Appendix 2: Mean percentage cover of the 5 groups of organisms and substratum (Brown, CCA, Green, Macro, Tile and Turf) of the benthic group of organisms present on the upper surface of the tile under the different treatments, seasons and years (n=20± SE). C= *Coral area*, A= *Algal area*, U= *Urchin area* and WA= *Without Algae area*.

Site	Season	Year	Brown	CCA	Green	Macro	Tile	Turf
C	**dry**	**2008**	0	42.3 ± 2.3	23.4 ± 1.5	0	31.7 ± 2.15	2.6 ± 1.8
		2009	4.0 ± 1.3	58.7 ± 5.0	14.5 ± 1.4	0	19.6 ± 3.5	3.1 ± 2.5
	wet	**2008**	0	53.8 ± 3.7	26.4 ± 2	0	14.4 ± 2.0	5.3 ± 4.1
		2009	2.97 ± 0.8	43.0 ± 3.5	26.5 ± 2.2	0	27.4 ± 2.9	0
U	**dry**	**2008**	0	34.8 ± 2.6	20.4 ± 1.8	0	25.2 ± 2.7	19.6 ± 4.7
		2009	4.4 ± 1.2	53.1 ± 3.4	17.1 ± 2.2	2.75 ± 2.0	25.3 ± 2.9	0
	wet	**2008**	0	43.86 ± 5.4	19.7 ± 2.6	0	15.4 ± 1.4	21.0 ± 7.6
		2009	3.2 ± 1.1	41.3 ± 3.0	23.2 ± 4.8	7.7 ± 3.7	22.5 ± 2.9	2.0 ± 2.0
A	**dry**	**2008**	9.5 ± 1.5	31.1 ± 2.4	10.0 ± 3.2	0	19.3 ± 3.8	31.4 ± 6.7
		2009	7.9 ± 2.7	29.9 ± 4.2	11.7 ± 2.7	8.3 ± 4.5	22.9 ± 3.6	27.5 ± 7.7
	wet	**2008**	0	12.9 ± 2.4	2.4 ± 0.7	1.9 ± 1.4	7.4 ± 1.2	75.4 ± 3.5
		2009	5.7 ± 1.4	23.8 ± 2.5	14.2 ± 3.3	9.2 ± 3.7	14.0 ± 3.5	33.2 ± 7.0
WA	**dry**	**2008**	7.8 ± 1.3	26.7 ± 2.4	23.7 ± 2.0	0	34.4 ± 3.0	7.0 ± 3.2
		2009	6.9 ± 2.9	26.3 ± 3.7	21.5 ± 3.0	25.1 ± 7.8	35.6 ± 2.8	9.7 ± 5.7
	wet	**2008**	0	21.6 ± 3.5	20.9 ± 3.0	11.1 ± 3.6	21.1 ± 3.8	25.2 ± 7.2
		2009	4.0 ± 1.5	24.7 ± 2.8	18.0 ± 1.4	25.0 ± 5.5	28.3 ± 2.0	0

Appendix 3: Mean percentage cover (Ascidians, Bryozoans, Sponges and worms) or organism numbers (Corals and Schizoporella) of the benthic group of organisms present on the lower surface of the tile under the different treatments, seasons and years (n=20± SE). C= *Coral area*, A= *Algal area*, U= *Urchin area* and WA= *Without Algae area*.

Treatment	Season	Year	Ascidian (%)	Bryozoan (%)	Coral Nb	Schizoporella Nb	Sponge (%)	Worm (%)
C	**dry**	**2008**	3.3 ± 0.7	2.1 ± 0.3	1.38 ± 0.6	2.8 ± 0.6	2.6 ± 1.6	4.5 ± 1.7
		2009	6.5 ± 1.5	3.9 ± 0.8	2.1 ± 0.4	6.9 ± 1.5	1.1 ± 0.6	2.7 ± 0.5
	wet	**2008**	3.1 ± 1.5	2.6 ± 0.5	0.95 ± 0.3	2.2 ± 0.6	0	4.8 ± 0.7
		2009	2.8 ± 1	3.9 ± 1.1	1.1 ± 0.3	2.85 ± 0.9	0.02 ± 0.02	4.2 ± 0.7
U	**dry**	**2008**	4.1 ± 0.7	2.8 ± 0.5	0.3 ± 0.1	5.5 ± 1.5	0.7 ± 0.5	8.3 ± 2.0
		2009	4.4 ± 0.8	4.3 ± 0.7	0.7 ± 0.2	3.2 ± 0.8	1.3 ± 1.0	6.3 ± 1.3
	wet	**2008**	1.5 ± 0.6	2.8 ± 0.5	0.4 ± 0.2	16.0 ± 7.6	0	8.4 ± 1.3
		2009	3.2 ± 1.0	1.9 ± 0.8	1.2 ± 1.2	4.3 ± 1.6	1.2 ± 0.8	3.3 ± 0.5
A	**dry**	**2008**	1.5 ± 0.6	0.1 ± 0.05	0	2.9 ± 1.0	1.8 ± 0.7	2.2 ± 0.5
		2009	1 ± 0.2	0.3 ± 0.1	0	1.2 ± 0.6	2.1 ± 1.4	1.6 ± 0.6
	wet	**2008**	1.9 ± 0.8	0.5 ± 0.3	0.1 ± 0.07	3.0 ± 1.0	0	2.3 ± 0.4
		2009	1.4 ± 0.4	0.7 ± 0.3	0	1.8 ± 0.6	1.1 ± 0.6	2.8 ± 0.4
WA	**dry**	**2008**	2.6 ± 0.7	0.7 ± 0.3	0	7.8 ± 1.7	2.0 ± 0.7	4.4 ± 0.6
		2009	1 ± 0.2	0.5 ± 0.2	0	2.5 ± 0.5	1.5 ± 1.0	3.2 ± 0.5
	wet	**2008**	1.3 ± 0.4	1.2 ± 0.3	0.15 ± 0.08	8.1 ± 2.0	0	6.8 ± 0.7
		2009	1.3 ± 0.4	0.8 ± 0.3	0.05 ± 0.05	3 ± 0.8	0.9 ± 0.5	5.9 ± 1.7

Appendix 4: Average percentage mortality and percentage of the different stages of development (Blastula, Gastrul and Prism) of *Diadema antillarum* embryos after 46h exposure to surface molecules recovered in DMSO. (n=12± SE)

µg.ml^{-1}	**SW**	**DMSO**	**1**	**5**	**10**	**15**	**20**	**25**	**37.5**	**50**	**75-200**
Mortality	6.6±6.6	8.3±5.2	13.0±6.0	11.1±5.1	28.3±7.8	31.6±8.1	48.3±10.8	81.2±9.7	60.8±10.1	71.6±12.6	100
Prism	89.1±7.1	65.2±8.1	60.5±12.0	12.5±8.5	7.5±4.7	6.9±4.5	0	0	0	0	0
Gastrula	4.1±4.1	26.3±6.6	26.3±12	76.3±9.2	64.1±8.2	61.3±10	51.6±10.8	18.75±9.7	39.1±10.1	0	0
Blastula	0	0	0	0	0	0	0	0	0	28.3±12.6	0

Appendix 5: Average percentage mortality and percentage of the different stages of development (Blastula, Gastrul and Prism) of *Diadema antillarum* embryos after 46h exposure to surface molecules either recovered hexane. (n=12± SE)

µg.ml^{-1}	SW	hexane	1	5	10	15	20
Mortality	6.8±3.9	7.9±3.6	20.1±9.7	13.0±4.25	16.6±5.3	14.7±4	27.6±6.6
Prism	45.1±6.31	61.5±8.8	16.6±6.48	10±4.7	15.1±4.5	6±3.1	4.1±6
Gastrula	26.4±7.6	24.2±7	29.6±8.5	17.3±4.7	21.8±6.3	18.4±5.31	20.9±6.
µg.ml^{-1}	**25**	**37.5**	**50**	**75**	**100**	**200**	
Mortality	38±7.9	65±8.5	76.8±8.5	81.25±7.4	90.6±4.5	97.5±0.8	
Prism	1.6±9.2	0	0	0	0	0	
Gastrula	32.2±9.2	28.6±8.3	29±8.4	35.5±10.2	15.2±4.4	6.1±1.7	

Appendix 6: Average percentage mortality of *Codakia orbicularis* and percentage of the different stages of development (trochophores (troch.) and other (abnormal and earlier stages) embryos after 24h exposure to surface molecules either recovered in DMSO. (n=12± SE)

µg.ml^{-1}	SW	DMSO	1	5	10	15	20	25	37.5	50	75-200
Mortality	16.1±3.45	15.7±6.3	33.3±2.0	33.3±4.7	10.9±5.8	0	0	5.4±2.8	10.7±4.9	52.0±14.5	100
Troch	79.7±5.3	60±10.1	44.4±5.4	37.7±10	43.2±11.8	65.9±12.0	68.7±10.7	35.4±11.3	0	6.2±4.5	0
other	4.1±3.6	20.1±7.7	25.9±9.9	25.9±9.9	45.9±12.5	36.8±11.6	31.2±10.7	59.1±11.3	89.3±4.9	41.6±13.1	0

Appendix 7: Average percentage mortality of *Codakia orbicularis* embryos and percentage of the different stages of development (trochophores (troch.) and other (abnormal and earlier stages) after 24h exposure to surface molecules either recovered in hexane. (n=12± SE)

µg.ml^{-1}	SW	hexane	1	5	10	15	20	25	37.5	50	75-200
Mortality	2.4±1.7	4.2±4.1	0.0	2.1±2.0	2±2.0	4.2±4	0.0	0.0	14.6±8.9	38.2±7.7	100.0
Troch.	78.8±4.3	77.8±6.9	100.0	95.8±4.1	91.7±4.7	81.9±7.1	90.3±5.8	63.3±5.6	30±8.2	20.1±9.8	0.0
Abnormal	19.8±4.1	19.7±6.6	0.0	2.3±2	6.8±3.2	15.2±6.6	8.3±5.8	37.7±5.6	60.5±10.3	38.6±10.8	0.0

Appendix 8: Average percentage mortality of *Pseudonereis* embryos after 48h exposure to surface molecules either recovered in DMSO or Hexane. (n=12± SE)

µg.ml^{-1}	SW	Solvent	1	5	10	15	20	25	37.5	50	75-200
DMSO	7.1±5.5	6.9±5.1	12.0±5.6	7.0±4.3	5.1±3.8	8.3±5.0	4.1±1.7	6.25±6.3	56.5±15.3	70.4±15.8	100
Hexane	15.9±11.7	20±12.6	17.7±10.7	14.4±7.7	6.2±6.3	21.7±9.2	28.5±11.6	27.0±10.6	100±0	100±0	100

Appendix 9 : Pictures of the main coral species observed in the study classified according to their morphology (Humann, 1993):

Hydrocoral:

➔ Fire Coral

Order: Milleporina
Millepora Alcicornis

Stony Corals:

➔ Brain corals

Suborder: Faviida
Family: Faviidae
Diploria strigosa

Suborder: Faviida
Family: Faviidae
Diploria Labyrinthiformis

Suborder: Faviidae
Family: Favidae
Colpophyllia natans

Suborder, Faviida
Family: Meandrinidae
Meandrina meandrites

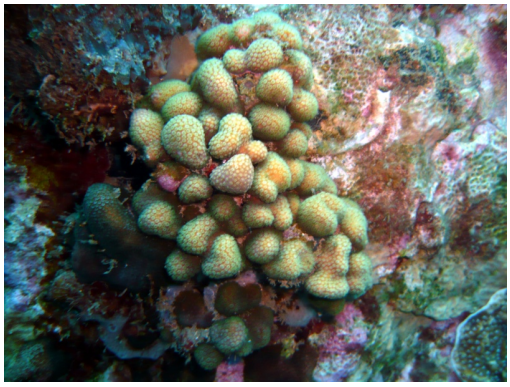
➔ **Fleshy corals**

Suborder: Faviida
Family: Mussidae
Mussa angulosa

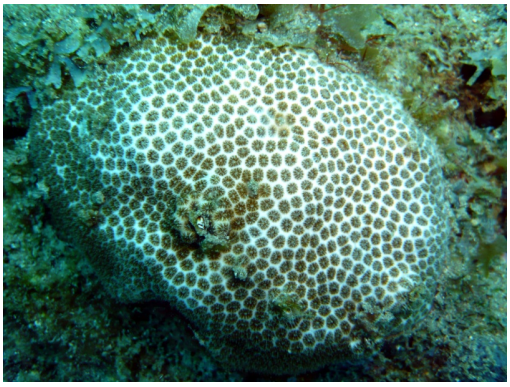
➔ **Flower and Cup corals :**

Suborder: Caryophyliida
Family: Caryophyliidae
Eusmilia fastigiata

➔ Encrusting, Mound and Boulder Corals :

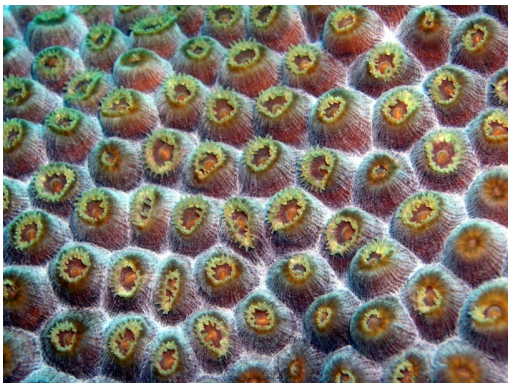


Suborder: Astrocoeniina
Family: Pocilloporidae
Madracis decactis

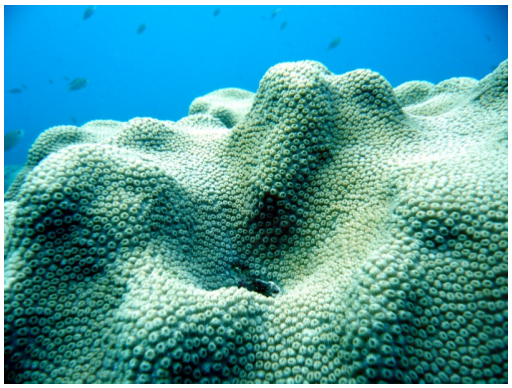


Suborder: Astrocoeniina
Family: Astrocoeniinae
Stephanocoenia mechelinii

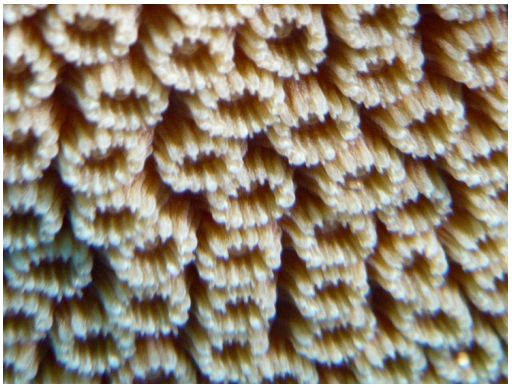
Suborder: Faviida
Family: Faviidae
Montastrea cavernosa



Detail of the polyps

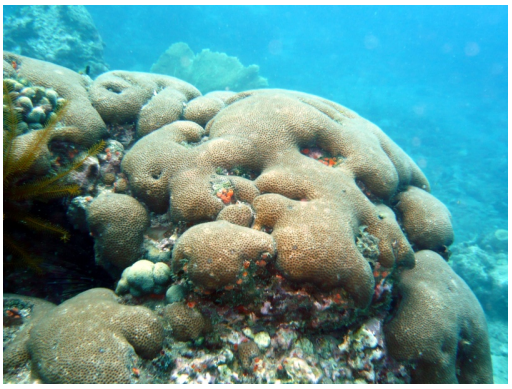


Suborder: Faviida
Family: Faviidae
Montastrea faveolata



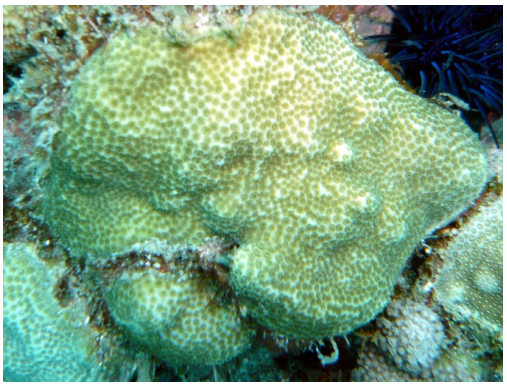
Detail of the polyps

Suborder: Faviida
Family: Faviidae
Montastrea annularis



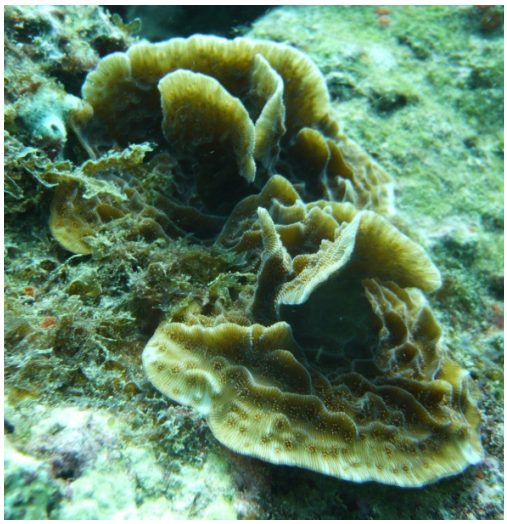
Suborder: Fungiida
Family: Sidereastreidae
Siderastrea siderea

Suborder: Faviida
Family: Meandrinidae
Dichocoenia stokesii



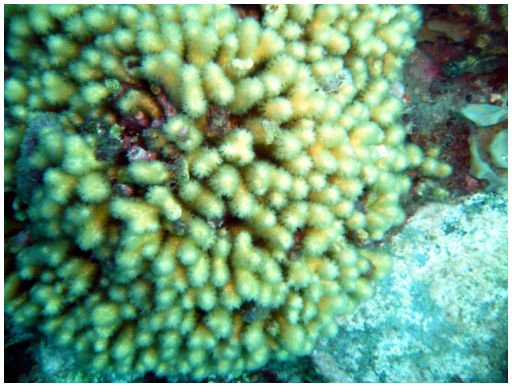
Suborder: Fungiida
Family: Poritidae
Porites astreoides

➔ **Leaf, Plate and Sheet Corals**

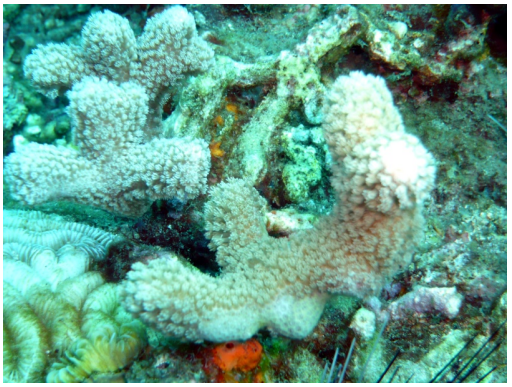


Suborder: Fungiida
Family: Agariciidae
Agaricia sp.

➔ **Branching and Pillar corals :**



Suborder: Astrocoeniina
Family: Pocilloporidae
Madracis mirabilis



Suborder: Fungiida
Family: Portidae
Porites porites

Appendix 10: Publication accepted by Botanica marina

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Sargassum polyceratium (Phaeophyceae, Fucaceae) surface molecule activity towards fouling organisms and embryonic development of benthic species

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Abstract

Coral reefs have undergone profound ecological changes over recent decades. Areas formerly covered by scleractinian coral species are now often overgrown by macroalgae. In Martinique (West Indies), this phenomenon has lead to the colonisation of numerous coral reefs by algae, amongst which *Sargassum* is one of the most prominent. This study focuses on potential defence molecules produced by *Sargassum polyceratium*. The hexane dipping method was employed to extract surface molecules on fresh material, and their bioactivities were assessed against bacteria (marine and estuarine), and marine tropical invertebrates [an annelid (*Pseudonereis* sp.), a bivalve (*Codakia orbicularis*) and a sea urchin (*Diadema antillarum*)]. Extracts were active against all microorganisms tested (MIC=150 or 300 μg ml^{-1}), early stages of development in *Pseudonereis* sp. (MIC=100 μg ml^{-1}) and embryos of *C. orbicularis* and *D. antillarum* (MIC=5 μg ml^{-1}), suggesting the production of defence compounds by *S. polyceratium*.

Keywords: bacteria; embryonic development; hexane dipping; *Sargassum polyceratium*; toxicity.

Introduction

Studies on production of marine natural products (MNPs) and their activities have increased over the past 20 years. Particular attention has been given to the production of secondary metabolites by macroalgae, microalgae, invertebrates, Cyanobacteria, octocorals, sponges and ascidians (Hay and Fenical 1996, Paul and Puglisi 2004, Paul et al. 2007, Hellio et al. 2009). Algae produce secondary metabolites with a wide range of biological activities including antifungal, antibacterial, antibiotic, antifouling (AF), UV radiation protection, feeding deterrence, inhibition of competitors, gamete attraction and inhibition of larval settlement and development (Hay and Fenical 1988, Paul et al. 1988, Hay and Fenical 1996, Hellio et al. 2001, 2002, 2005, Steinberg and de Nys 2002, Birrell 2003, Paul and Puglisi 2004, Paul et al. 2007, Plouguerné et al. 2010a).

Several examples demonstrate that specific structures localised on the surfaces of algae release active compounds into the environment (Dworjanyn et al. 1999, De Nys and Steinberg 2002); Vesicular physodes located on the surfaces of phaeophyceans contain phlorotannins (Ragan and Glombitza 1986). The role of phlorotannins as macroalgal defence mechanism has been extensively described in the literature, but yet remains not fully understood. These compounds have been described as UV screens, herbivore deterrents and antimicrobial agents, but are also known to play a role in primary metabolism (Paul and Puglisi 2004). Moreover, fluorescence microscopy, combined with chemical analyses, has demonstrated that *Delisea pulchra* (Grev.) Mont. (Rhodophyceae) releases AF compounds (halogenated furanones) from gland cells located on its surface (Dworjanyn et al. 1999, De Nys and Steinberg 2002).

The ''*corps en cerise*'' found on cortical cells of *Laurencia snyderae* E.Y. Dawon (Rhodophyceae) may be a primary location of halogenated MNPs (Young et al. 1980). However, even though it has been stated that the ''*corps en cerise*'' are main reserves for halogenated compounds in the alga *Laurencia obtusa* Lamour and store high concentrations of bromine and chlorine (Salgado et al. 2008), they are not found at the surface of the alga and no structure linking them to the algal surface have been found (De Nys et al. 1998).

Sargassum species have been extensively analysed for their allelochemical activities for several reasons: 1) some of them are lightly fouled in the field, 2) the genus is one of the most conspicuous algae in numerous areas, especially the tropics (Ang 1986, De Ruyter van Stevenick and Breeman 1987, Littler et al. 1993, Lapointe 1997, Engelen et al. 2001), 3) some species have invaded many parts of the world (Plouguerné et al. 2006). Antibacterial, anti-algal, anti-fungal and anti-invertebrate activities have been demonstrated in *S. muticum* (Yendo) Fensholt samples (Hellio et al. 2002, Plouguerné et al. 2008, 2010b, Maréchal and Hellio 2011), *S. wightii* Greville *ex* J. Agardh and *S. johnshonii* V.J. Chapman (Sastry and Rao 1994).

Sieburth and Conover (1965) found antibiotic activity in phlorotannins extracted from *S. vestitum* (R. Brown *ex* Turner) C. Agardh and *S. natans* (Linnaeus) Gaillon, and Tanaka and Asakawa (1988) found antialgal activity in extracts from *S. horneri* (Turner) C. Agardh. *S. vulgare* C. Agardh extracts from Brazil had AF activity against microalgae and mussel settlement (Plouguerné et al. 2010a). *S. muricatum* and *S. tenerrimum* J. Agardh induce modifications of swimming activity in two-day-old larvae of *Platygyra daedalea* Ellis *et* Solander (coral) and decrease their proportional settlement, indicating chemical defences (Diaz-Pulido et al. 2010).

Sargassum polyceratium W.R. Taylor, one of the most abundant macroalgae of Martinique tropical reefs (M. Thabard, unpublished data), has, however, never been investigated. *Sargassum* species have been recorded since the 1970s on the Atlantic coast of the island (Battistini 1978). Coral reef colonisation by macroalgae has induced significant changes in the structure and diversity of communities. As an example, *S. polyceratium* and *S. hystrix* J. Agardh have supplanted hermatypic coral species on the Jamaican north coast (Lapointe 1997, Lapointe and Thacker 2002). Algal canopies are known to affect understorey species (Eckman and Duggins 1991) and may interfere with invertebrate larval recruitment processes (Pawlik 1992, Birrell 2003, Titlyanov et al. 2005). To survive the environmental pressures they are subjected to, and to successfully colonise new areas, macroalgae have developed several strategic mechanisms, including both specific morphological characteristics and production of deterrents to avoid epibiont overgrowth (Littler and Littler 1980, Littler et al. 1983a,b, Paul and Puglisi 2004).

In a survey conducted in Martinique in 2007 (M. Thabard unpublished data), the sea urchin *Diadema antillarum* Philippi was found to be almost absent from the *Sargassum* area, but a sea urchin population had developed nearby, forming distinct belts. *D. antillarum*, which is considered as a keystone herbivore species on Caribbean coral reefs (Knowlton 2001), suffered mass mortality in 1983 in this region (Lessios et al. 1983, 1984). This induced profound changes on coral reefs, and is thought to have played a major role in the community composition shift observed nowadays in the Caribbean due to reduced grazing pressure on macroalgae (Hughes 1994). *Diadema* recovery is proving to be a very slow process in the Caribbean although its reproductive biology seems to indicate that the species should recover rapidly (Lessios 1995). There are several hypotheses (too few adults, pathogens remaining in water, etc.) for this phenomenon (Lessios 2005). It may be that fast colonisation by *Sargassum* sp. on coral reefs in the 1980s (Littler et al. 1993) is in part responsible for this slow recovery process as it is possible that the seaweed prevents the larval recruitment process.

The present study focuses on potential antibacterial and deterrent activities of *Sargassum polyceratium* and their possible interactions with other tropical species. In order to perform a broad spectrum analysis of the toxic activity of MNPs extracted from *S. polyceratium* surface, three marine tropical invertebrates were tested, one sea urchin, one bivalve and one worm, representing organisms from different phyla. *In vitro* effects of *S. polyceratium* extracts towards bacteria and embryos of marine invertebrates were investigated.

Materials and methods

Algal collection site

Martinique is a volcanic island located in the eastern Caribbean Sea (Lesser Antilles) between latitudes 14°50′N and 14°23′N and at mean longitude of 62°12′W. It has a land area of 1128 km^2 and is bordered by the Caribbean Sea to the west and the Atlantic Ocean to the east. The climate is defined by distinct dry and wet periods, the dry season lasting from December to July and the wet season from August to early December.

The site chosen for this study is a fringing coral reef to the south of Martinique, close to the mouth of *Trois Rivières* river. This site was chosen for the dominant presence of *Sargassum polyceratium*.

Study organism

Sargassum polyceratium is commonly found in moderately turbulent habitats from the lower intertidal zone to depths over 50 m (Littler and Littler 2000, Engelen 2004). It has a tough crowded thallus and dense branches reaching to 100 cm. The main axes roughened with small spines may be numerous. The blades measure 3–8 mm in width and 1.5–2.0 cm in length. The holdfast is strong and disc like. The importance of recruitment vs. regeneration was modeled and demonstrated to vary with population, year and disturbance (Engelen et al. 2005).

Algal extractions

Sargassum polyceratium samples were collected in October 2008 and January 2009 (wet and dry seasons). Thalli were removed with their holdfasts by breaking away pieces of substratum, thus reducing stress on the algae; stress may be responsible for secondary metabolite production. The samples were collected by SCUBA diving at 18 m depth. The algae were cleaned of epiphytes, rinsed with seawater (SW) and transported to the laboratory in a container filled with clean SW. The fresh samples were soaked in hexane (Fisher, Loughborough, UK) in the ratio 1 l hexane kg^{-1} wet weight *S. polyceratium* (De Nys et al. 1998). All the extractions were performed in the dark as some secondary metabolites, such as the polyphenolics are known to react to light. The goal of using the hexane dipping method was to extract molecules produced both by the alga at its surface and by its associated biofilm in order to obtain the whole range of MNPs that might interact with eukaryotic and/or prokaryotic organisms present in the algal environment.

Two protocols were used:

- Protocol A (October 2008 samples, rainy season), *Sargassum polyceratium* thalli were dipped for 30 s in hexane for preliminary tests.
- Protocol B (January 2009 samples, dry season) was developed based on results of both tests conducted on 30 s extracts (October) and the observations of algal surfaces for breaks (see results). This amended protocol was used to test for the effect of dipping time on extract efficiency.

This was done by dipping algae in hexane for two different periods (10 s and 30 s), with the algal surface remaining intact.

Observation of algal surface

The objective of our experiments was to select the molecules present at the surface of *Sargassum polyceratium* only. The hexane dipping method was shown to break some algal surface cells when thalli were dipped for more than 30 s (De Nys et al. 1998). The algal surface was therefore checked for breaks that would result in leaks of cell contents in thalli dipped for 30 s, 10 min and 30 min to determine the best dipping time. As UV excitation of plant leaves is known to induce two distinct types of fluorescence, damage caused to the surface cells of *S. polyceratium* by hexane dipping was investigated by epifluorescent microscopy (Nikon, Tokyo, Japan, Eclipse 80i microscope; Filter FITC 494 nm excitation/514 nm emission; Nikon Dxm1200F camera) using a protocol adapted from Cerocic et al. (1999).

Bioassays

Bacteria Culture of bacteria: Four marine bacterial strains were used: *Halomonas marina* (Cobet et al.) Dobson *et* Franzmann (ATCC 25374), *Pseudoalteromonas elyakovii* (Ivanova et al.) Sawabe et al. (ATCC 700519), *Polaribacter irgensii* Gosink et al. (ATCC 700398) and *Vibrio aestuarianus* Tilson *et* Seidler (ATCC 35048). These bacteria were chosen because they are typical marine fouling bacteria (Plouguerné et al. 2010b). *Pseudoalteromonas elyakovii* and *Vibrio aestuarianus* are also known to cause infections in marine organisms, such as molluscs, crustacean, fishes or algae, the latter being thought responsible for the summer mortality of *Crassostrea gigas* Thunberg (Labreuche et al. 2005). Marine bacteria were cultivated with marine broth (5% tryptone, Oxoid, Basingstoke, UK, diluted in SW) and incubated at 30°C to allow development (Plouguerné et al. 2008).

Five terrestrial bacterial strains known to be present in estuaries and coastal environment (Mokrini et al. 2008) were used: *Bacillus subtilis* (Ehrenberg) Cohn (NCIMB 1026), *Enterobacter aerogenes* Hormaeche *et* Edwards (ATCC 13048), *Escherichia coli* (Migula) Castellani *et* Chalmers (B 81), *Pseudomonas aeruginosa* (Schroeter) Migula (NCIMB 10390) and *Staphylococcus aureus* Rosenbach (NCIMB 8625). *B. subtilis* and *S. aureus* are Gram-positive bacteria. The remainder are Gram-negative. Terrestrial bacteria were cultivated on a nutrient broth (CM0067, No. 2, Nutrient media Powder Oxoid 25 g l^{-1}) and incubated at 30°C. Biological activities of extracts were evaluated following the method of Amsterdam (1996).

Antibacterial assays: Aliquots of 100 µl of each hexane extract were poured in six wells of 96 well plates (Fisher) for each bacterial assay following the protocols of Plouguerné et al. (2010b). The solutions were tested at three concentrations: 15, 150 and 300 µg ml^{-1}. In addition, six wells free of extracts and six wells containing hexane were used as controls. The plates were first dried in a flow cabinet to evaporate the solvent and then left for 15 min in a UV cabinet for sterilization.

The optical densities (OD) of bacterial stock cultures were measured at 630 nm for every sample to determine the quantity of solution required to obtain 1 mOD (mili optical density). Then, 100 µl of bacterial solutions were added under aseptic conditions and the plates were incubated for 48 h at 30°C for bacterial growth. Activity was obtained comparing the controls and the wells containing the extracts. Solutions were considered to be active when bacteria did not grow in four, five or six wells. Bacterial growth was noted by the presence of a cloudy solution. One plate was used for each strain to limit the cross-contamination risk.

Invertebrates Organisms: The toxicity of the extracts was tested against larvae of *Codakia orbicularis* L., *Diadema antillarum* and *Pseudonereis* sp. These organisms are tropical and represent typical species from three marine ecosystems (seagrass bed, reef and mangrove). Their spawning and early larval development has been described previously (Gros et al. 1997, Eckert 1998). For both *C. orbicularis* and *D. antillarum*, spawns were induced in the laboratory under controlled conditions, while *Pseudonereis* sp. embryos were collected from the wild.

Codakia orbicularis is a tropical bivalve mollusc distributed from Florida to Brazil (Abbott 1974). Adult *C. orbicularis* (between 40 and 60 mm shell length) were collected by hand from seagrass beds in Ilet Cochon (Guadeloupe, Figure 1B) in July 2009. Fertilization was induced following the method described by Gros et al. (1997). Adults were cleaned with a brush and spawning was induced by injection of 0.3 ml of a 4 mM serotonin solution in 0.22 µm filtered SW into the visceral mass. Sperm and oocytes were mixed in a 1 l cylinder until the appearance of two-cell embryos. Fertilization occurred under constant aeration to hold eggs in suspension as they are slightly negatively buoyant. *C. orbicularis* embryonic development follows the general development of bivalves (Gros et al. 1997). Appearance of the first polar body (indicating fertilization) is not always visible under a dissecting microscope, thus the two cells embryos were chosen to ensure the fertilization had occurred, and these were used in toxicity experiments.

Diadema antillarum: The black spined sea urchin *D. antillarum* was selected for experiments. Adults were collected on the shore at Port-Louis (Guadeloupe, Figure 1B) during summer 2009. Urchins were acclimated in the laboratory (25°C) for a week and fed on agar pellets containing a mixture of algae (including *Ulva lactuca* L. and *Sargassum* sp.) following the protocol of Pereira et al. (2003). After a week, the urchins were transferred to another tank containing 29°C filtered SW. Thermal shocks (3–5°C) that induce spawning of *D. antillarum* (M. Moe, personal communication) were applied over a few minutes. Both male and female gametes were pipetted from this tank and diluted in 10 l of 0.22 µm filtered SW (25°C) to induce the fertilization. The eggs are slightly negatively buoyant, thus aeration was used to keep them suspended. Embryos at the two cells stage [fertilization (To)+1h] were chosen for experiments.

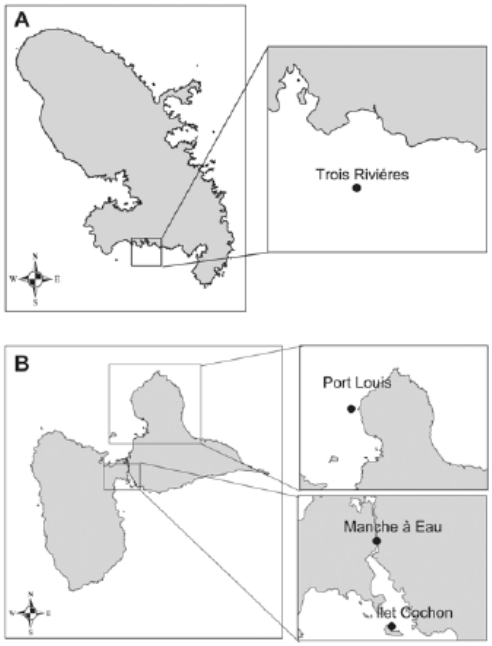

Figure 1 (A) Map showing the algal collection site (Martinique) at *Trois Rivières* (14°27′22″N, 60°58′03″W). (B) Map showing the invertebrates collection sites (Guadeloupe) at *Ilet Cochon* (16°12′56″N, 61°32′20″W), *Manche à Eau* (16°16′36″N, 61°31′24″W) and *Port Louis* (16°25′18″N, 61°32′02″W).

Pseudonereis sp.: Egg balloons were collected from the mangrove Manche à Eau (Guadeloupe, Figure 1B) in summer 2009. Egg balloons containing young embryos of *Pseudonereis* sp. (i.e., at the blastula-early gastrula stage) were chosen to conduct the experiment.

Embryo toxicity tests: Dimethyl sulphoxide (DMSO) (Fisher) was used as a solvent carrier in order to dilute molecules extracted with hexane in 0.22 µm filtered SW. Preliminary tests demonstrated that this solvent was not toxic for embryos until the trochophore stage for both *Codakia orbicularis* and *Pseudonereis* sp. and prism stage for *Diadema antillarum* when used at a concentration 0.5 µg ml^{-1} (data not shown).

Tests were conducted in 96-well plates (Fisher). Extracts were tested at seven concentrations: 1, 5, 10, 15, 50, 100 and 200 µg ml^{-1}. Two hundred microlitres of each extract were added to each well (six replicates). In addition, six wells filled with SW, six with 1% $CuSO_4$ (known to kill larvae, Bielmeyer et al. 2005) and six with 0.5% DMSO were used as controls. Four larvae were added to each well and allowed to develop for 24 h at 25°C (to reduce bacterial development). Percentage mortality and embryonic development (stage reached) were recorded. All the assays were performed on two independent batches of embryos (Hellio et al. 2004).

Statistical analyses

Embryo toxicity assays results were analysed using non-parametric tests (Kruskal-Wallis) and comparison between treatments was performed using a multiple comparisons test with the software R and the package npmc. The results of the two larval batches were pooled as no significant differences were apparent. These statistical tests were selected as the data (percentages) did not fit a normal distribution (Kolmogorov-Smirnov) even after transformation.

Results

Algal surface observation

Epifluorescence microscopy images showed cells to be well formed and not broken (Figure 2A, B) in the control and after 30 s of dipping in solvent, while there were leaks, suggesting lyses of the cell surfaces, after longer dipping times (Figure 2C, D). The assays tests were thus conducted only with the extracts prepared for the shortest times (10 and 30 s), protocols A and B.

Bacterial bioassay

Sargassum polyceratium extracts at a concentration of 15 µg ml^{-1} did not inhibit bacterial strains (Table 1). The January 10 s extraction sample was more more active against bacteria than the January 30 s sample (except in the case of *Vibrio aestuarianus*). The 30 s extraction samples prepared in October were the second most active. Marine bacteria were most sensitive to extracts; growth of terrestrial bacterial strains was inhibited by only the 30 s October and 10 s January extracts.

Activity towards larvae

Diadema antillarum Algal extracts affected *Diadema antillarum* embryo development (Kruskal-Wallis: $p<0.0001$). One hundred percent of embryos died in the highest concentrations (from 10 to 200 µg ml^{-1}) and in the copper solution (positive control) (Figure 3). Less than 70% of embryos survived when exposed to 5 µg ml^{-1}. Only solutions at concentrations of 1 µg ml^{-1} and the 10 s January extract concentration of 5 µg ml^{-1} permitted good survival of embryos (not significantly different from the control; $p=0.9$ for comparison between DMSO and the January 10 s at a concentration of 5 µg ml^{-1} and $p=1.0$ for the others; multiple comparisons tests).

Apart from the 10 s January extract at a concentration of 5 µg ml^{-1}, there were no significant differences between the October and the January extracts.

Embryonic development stopped at different stages depending on the solution tested (Table 2, Figure 5). Only embryos developing in the controls (DMSO and SW) or the extract at 1 µg ml^{-1} survived to the prism stage. The development of the other embryos was stopped progressively with decreasing concentrations of algal extracts, i.e., 2–4 cell stage at concentrations 100–200 µg ml^{-1}, morula at 15 and

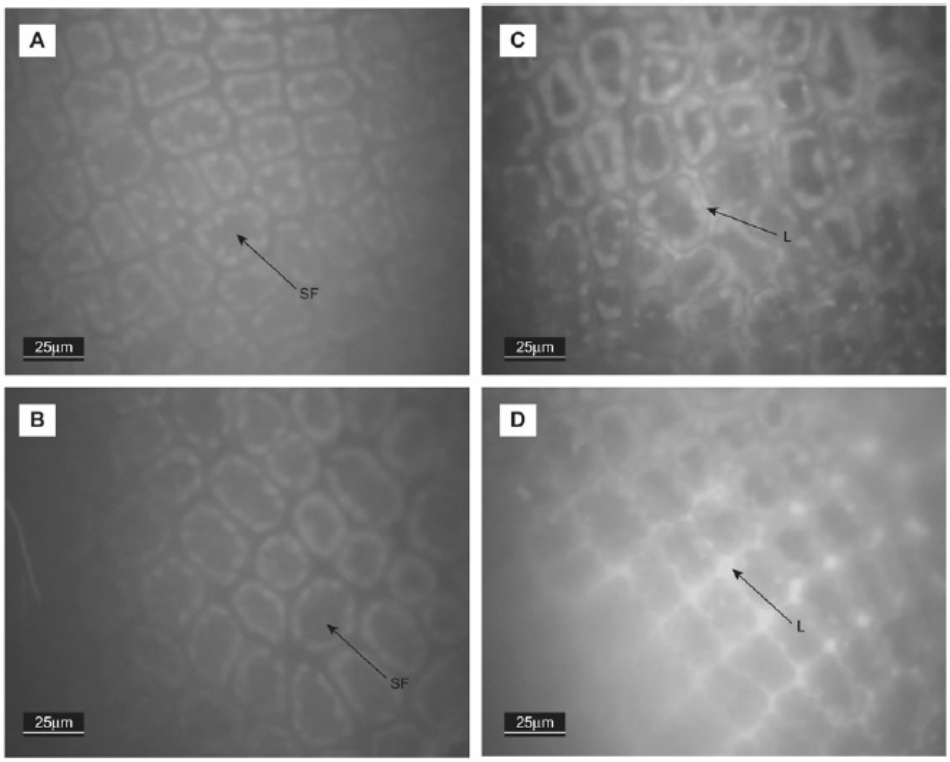

Figure 2 Epifluorescent microscopic images of *Sargassum polyceratium* ''leaf'' surfaces: (A) control, (B) 30 s, (C) 10 min and (D) 30 min after being dipped in hexane. (magnification=×100). L, cellular content leaks; SF, surface cells.

50 µg ml^{-1}, late blastula early gastrula at 10 µg ml^{-1}, gastrula at 5 µg ml^{-1} and prism at 1 µg ml^{-1}.

Codakia orbicularis The results for *Codakia orbicularis* embryos were similar to those for *Diadema antillarum* (Figure 4) (Kruskal-Wallis: $p<0.0001$). All embryos died in the highest concentrations assayed (50–200 µg ml^{-1}) and in the copper control. Percentage mortality decreased at 15 µg ml^{-1} but the embryos did not develop to the trochophore stage. Only embryos cultured in the 1 µg ml^{-1} solution devel-

Table 1 Antibacterial activity of hexane dipping extracts expressed as minimum inhibitory concentrations (µg ml^{-1}).

Bacterial species	October 2008	January 2009	
	30 s	10 s	30 s
Terrestrial			
Bacillus subtilis	150	300	N
Enterobacter aerogenes	N	300	N
Escherichia coli	300	300	N
Pseudomonas aeruginosa	150	300	N
Staphylococcus aureus	N	300	N
Marine			
Halomonas marina	300	150	300
Polaribacter irgensii	150	150	300
Pseudoalteromonas elyakovii	150	150	300
Vibrio aestuarianus	150	150	150

N, no active extracts.
Months of algal collection and dipping times are indicated.

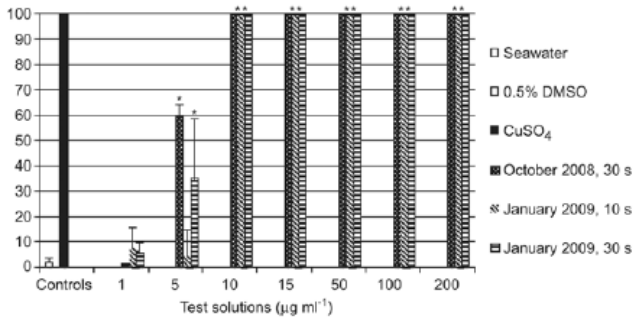

Figure 3 Mean percentage mortality of *Diadema antillarum* embryos after 30 h exposure to molecules extracted from *Sargassum polyceratium*.
Dates of algal collection and extraction times are indicated in the key. Values are means+SE, n=12. *Significant differences ($p<0.05$, multiple comparisons test) between control (DMSO) and one of the solutions; **significant differences ($p<0.05$) observed between control (DMSO) and a group of extract solutions.

Table 2 Stages of development in *Codakia orbicularis* and *Diadema antillarum* embryos after 30 h exposure to *Sargassum polyceratium* hexane surface extracts.

Concentration	Stage of development	
	Codakia orbicularis	*Diadema antillarum*
1 µg ml^{-1}	Trochophore	Prism
5 µg ml^{-1}	Late blastula-early gastrula	Gastrula
10 µg ml^{-1}	Late blastula-early gastrula	Late blastula-early gastrula
15 µg ml^{-1}	Morula	Morula
50 µg ml^{-1}	Morula	Morula
100 µg ml^{-1}	2–4 cells	2–4 cellules
200 µg ml^{-1}	2–4 cells	2–4 cellules
$CuSO_4$	2–4 cells	2–4 cellules
DMSO (0.5%)	Trochophore	Prism
Seawater	Trochophore	Prism

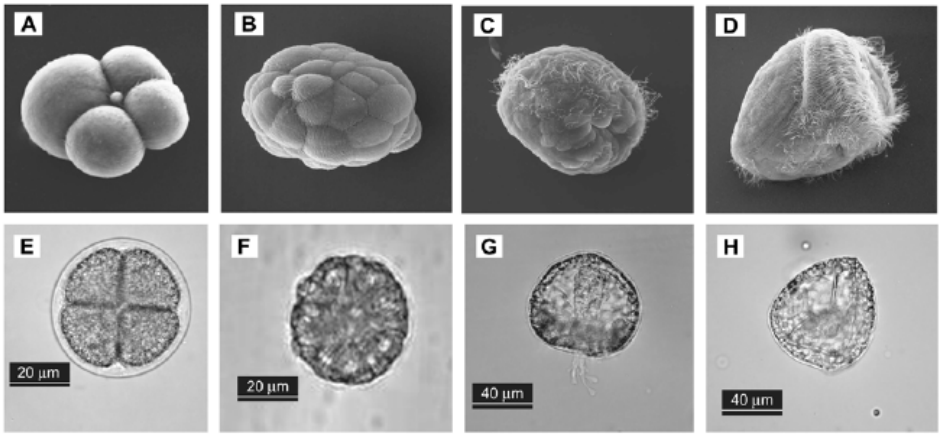

Figure 4 (A–D) SEM images (magnification=×500) of early stage of *Codakia orbicularis* development: (A) 4 cell stage, (B) morula (t+6 h), (C) gastrula (t+21 h), and (D) trochophore (27 h). (E–H) Light microscopy images of *Diadema antillarum* early stage of development: (E) 4 cell stage, (F) morula, (G) gastrula, (H) prism.

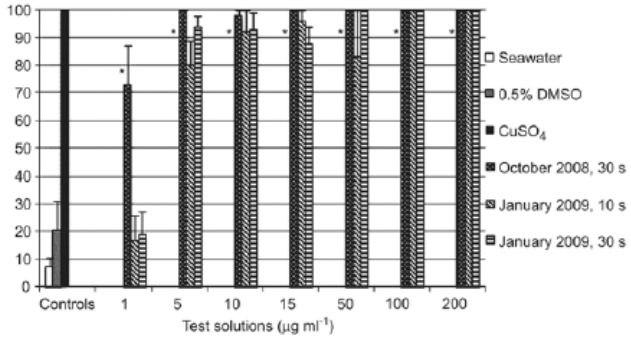

Figure 5 Mean percentage mortality of *Codakia orbicularis* embryos after 24 h exposure to molecules extracted from *Sargassum polyceratium*.
Dates of algal collection and extraction times are indicated in the key. Values are means+SE, n=12. *Significant differences ($p<0.05$, multiple comparisons test) between control (DMSO) and one of the solutions; **significant differences ($p<0.05$) observed between control (DMSO) and a group of extract solutions.

oped to the last stage and had percentage mortalities similar to the control ($p=1.00$ for the January extracts and $p=0.55$ for the October extracts).

Embryonic development was a function of the concentration of the solution tested, and stopped progressively with decreasing extract concentrations (Table 2, Figure 4), i.e., 2–4 cell stage at concentrations 100–200 µg ml^{-1}, morula at 15 and 50 µg ml^{-1}, late blastula early gastrula at 5 and 10 µg ml^{-1} and trochophore at 1 µg ml^{-1}.

***Pseudonereis* sp.** *Sargassum* extracts were less effective against *Pseudonereis* embryos than against other species (Figure 6). Only the highest concentrations of extracts tested (100 and 200 µg ml^{-1}) and the copper solution completely inhibited larval development. The *Sargassum polyceratium* solution extracted for 30 s (1 µg ml^{-1}) in January had some activity against the larvae (15% mortality), although it was not significantly different from the control. Even though all larvae survived the treatments, their swimming behaviour differed between the control and the lowest concentrations (except 1 µg ml^{-1}); affected larvae were almost still or swam very slowly.

Discussion

Secondary metabolites produced by algae affect settlement, recruitment and development of numerous organisms (Pawlik 1992, Steinberg and de Nys 2002, Paul and Puglisi 2004, Paul et al. 2007). Compounds isolated from *Dictyota menstrualis* (Hoyt) Schnetter, Hörning and Weber-Peukert and *D. ciliolata* Sonder *ex* Kützing caused significant mortality [*Bugula neritina* Linnaeus (bryozoan)], abnormal development (*B. neritina*) and reduced settlement rates [*Amathia convoluta* Lamarck, (bryozoan), *B. neritina, Eudendrium carneum* Clarke, (hydroid)] (Schmitt et al. 1998). Algal waterborne cues affect larval recruitment in *Acropora millepora* Ehrenberg, (coral) (Birrell 2003) and are toxic towards early developmental stages of the fouling polychaete *Hydroides elegans* Haswell (Dobretsov et al. 2006). Algal

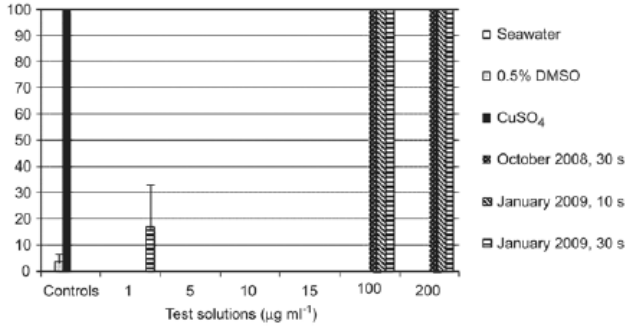

Figure 6 Mean percentage mortality of *Pseudonereis* sp. embryos after 48 h exposure to molecules extracted from *Sargassum polyceratium*.
Dates of algal collection and extraction times are indicated in the key. Values are means+SE, n=12.

MNPs are often species-specific and can have an effect on different larval development stages. *Dictyota* spp. and *Laurencia* sp. are toxic against larvae of *H. elegans* and *B. neritina*; *Padina* sp. and *Halimeda* sp. inhibit their larval settlement; *Hypnea* sp. and *Ulva* sp. stimulate larval settlement and *Sargassum* spp. have no effect (Walters et al. 1996).

Our results demonstrate that *Sargassum polyceratium* surface molecules significantly affect embryonic development of three tropical invertebrates and bacterial growth. These organisms are not all in direct contact with *S. polyceratium* (except *Diadema antillarum*), but they represent species from three invertebrate phyla and thus give some indication of potential toxic activity of *Sargassum* towards several types of organisms. In the embryo toxicity assays, the active extracts concentrations varied by species tested. Both *Codakia orbicularis* and *D. antillarum* had similar responses although *C. orbicularis* was expected to be more resistant because of the large envelope surrounding its embryos (Gros et al. 1997). *Pseudonereis* embryos were the most resistant to the extracts. In the control, *C. orbicularis* embryos developed in 24 h to the trochophore stage and *D. antillarum* embryos developed in 30 h to the prism stage, consistent with previously reported values (Gros et al. 1997, Eckert 1998). However, *S. polyceratium* surface compound treatments had progressive effects (according to increasing concentration) on embryonic development, suggesting the extracts block egg cleavage. The highest concentrations blocked development at the 2–4 cells stage, the medium concentrations allowed development until the morula stage and the lowest concentrations until the blastula, gastrula and finally trochophore or prism stages (respectively for *C. orbicularis* and *D. antillarum*). Similarly, Paul and Fenical (1986), showed that caulerpyne blocks the cleavage of developing sea urchin eggs. The division of *Paracentrotus lividus* Lamarck (urchin) embryos was inhibited by algal extracts (Martin and Uriz 1993). Dihydrorhipocephalin, aldehyde, udoteal, petiodal, dihydroudoteal, rhipocephalin, halimedatrial, halimeda tetraacetate (4,9-diacetoxy-udoteal) isolated from siphonous green algal species (*Halimeda* spp., *Penicillus* spp., *Rhipocephalus phoenix* Ellis *et* Solander, *Udotea* spp.) are toxic against developing sea urchin eggs, sperm and larvae (Paul and Fenical 1986), with ED_{100} levels (lowest concentration leading to 100% inhibition of cell division) ranging from 0.2 to 16 $\mu g\ ml^{-1}$. Concentrations of MNPs extracted from siphonous algae have higher activity against both *Codakia* and *Diadema* than the solutions tested in this study (ED_{100} ranging between 50 and 100 $\mu g\ ml^{-1}$). However, the tests performed by Paul and Fenical (1986) were performed with isolated secondary metabolites and not crude extracts. It is possible that the active compounds tested in this study were present in the crude extract at very low concentration ($\mu g\ ml^{-1}$). On the other hand, some molecules act synergistically such that a pool of molecules is required to induce a biological effect (Hay 1996). Further analytical chemistry tests involving molecule purification and analytical chemistry analyses (GC-MS and NMR) will give further insights into the chemical nature of the bioactive compounds and their activity levels.

Molecules extracted at the algal surface with hexane are non-polar. *Sargassum* species produce polyphenols (polar metabolites), but some non-polar extracts, such as *S. vulgare* hexane extracts, are highly active towards the development of microalgae, suggesting production of active non-polar secondary metabolites by this alga (Plouguerné et al. 2010b). So far, no methods allowing the extraction of polar molecules located at the surface of the algae have been developed and the activity observed here thus represents only a portion of the possible MNPs present at the surface of *S. polyceratium*.

There were no differences in toxic activity towards embryos between the 30 s extracts in October and January. However, antibacterial activity was different between these two extracts, suggesting a possible seasonality in surface molecule activities. Temperate algal extracts have a seasonal variation in molecular composition, antimicrobial activity, and AF activity (Steinberg and Van Altena 1992, Hellio et al. 2004, Maréchal et al. 2004, Plouguerné 2006). The October extracts were from the rainy season, while those from January were collected in the dry season. Hellio et al. (2004) demonstrated that *S. muticum* secondary metabolite activity varies with seasons and is higher during the summer months when fouling pressure (including fouling by bacteria) is most intense. It is thus possible that macroalgae develop specific mechanisms for protection in the rainy season when bacterial concentrations are high in coastal environments (Futch et al. 2010). In the present study, the levels at which extracts were active towards bacteria were high (150–300 $\mu g\ ml^{-1}$) in comparison with other algal crude extracts. Crude extracts obtained from seaweeds collected in Brittany were active when concentrations ranged between 24 and 96 $\mu g\ ml^{-1}$ (Hellio et al. 2001) and between 0.1 and 100 $\mu g\ ml^{-1}$ (Plouguerné et al. 2008). Marine bacteria were the most sensitive strains, suggesting that defence strategies of *S. polyceratium* are specific. Such targeted defence strategies have been described for other algal species (Paul and Puglisi 2004).

Bioactivity of *Sargassum polyceratium* extracts was marked; however, we do not know whether the active molecules were produced by *Sargassum polyceratium* or by its associated biofilm. Secondary metabolite isolation from algae can be confounded by associated microorganisms. As this study focused on surface molecules only, it was impossible to clean the macroalgal surface from microepiphytes using existing methods, such as ethanol, without breaking the surface cells (De Nys et al. 1998). The extract obtained therefore corresponds to *Sargassum* and/or its associated biofilm. Numerous bacteria living in SW produce active secondary metabolites (Jensen and Fenical 1994). Moreover, there are host-specific associations between algae and bacteria, and algae may control associated bacteria (Lachnit et al. 2009). The bacterial biofilm may in turn confer a protection to the host alga through production of secondary metabolites.

Sargassum polyceratium surface extracts inhibited bacterial growth and embryo development of three tropical marine invertebrates one of which was *Diadema antillarum*, a tropical herbivorous keystone species that controls macroalgal

populations. Testing surface molecules was a first investigation step and further work will be carried out to relate the natural compounds to their possible ecological role. These results require more work focused on 1) concentrations produced on the algal surface, 2) the source of production (alga or biofilm, or both) and 3) the release of compounds into the water column, a process that might interact with embryonic development of organisms surrounding *Sargassum*. We are currently performing tests on molecules present in the waters in which algae are immersed (conditioned water) in order to assess the activity of these cues against tropical invertebrate marine larvae in the same environment.

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