MACROBIOFOULING ON OPEN-OCEAN SUBMERGED AQUACULTURE CAGES IN PUERTO RICO

By

Carolina Hincapié-Cárdenas

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Approved by:

_____	_____
Baqar Zaidi, Ph.D. Member, Graduate Committee	Date
_____	_____
Alexis Cabarcas, Ph.D. Member, Graduate Committee	Date
_____	_____
Dallas E. Alston, Ph.D. Chairman, Graduate Committee	Date
_____	_____
Giovanni Soto Torres, M.S. Representative of Graduate Studies	Date
_____	_____
Nilda E. Aponte, PhD Director of Department	Date

ABSTRACT

Biofouling in aquaculture cages is known as a significant problem in aquaculture cage farm operations. The purpose of this study was to assess biofouling composition and percentage of biofouling coverage over time on nets of two open-ocean submerged aquaculture cages. The cages were located 3 km south of Culebra Island, Puerto Rico. Sample nets, each measuring 1050 cm^2, using the same material of the cage netting, were fastened in four different locations on each cage (snapper cage-*Lutjanus analis* and cobia cage-*Rachycentron canadum*): above or below the cage rim; and upstream (predominant current) or downstream locations. The rim was located 16 m below the surface, at the middle of the cage. Biofouling growth was monitored from August 2002 to June 2003 by removing one net sample bimonthly from each location of each cage. In the laboratory phase, each sample net was photographed and Map Maker software (Version 1.0) was used to calculate the percent coverage. Individual organisms were identified to major groups (algae, sponges, hydroids, polychaetes, mollusks, crustaceans, ascidians, and bryozoans). There was no difference in biofouling coverage between snapper cage and cobia cage throughout the study (53% y 51% respectively). The sample nets attained 49% of biofouling coverage after two months of cage deployment. The percentage of biofouling coverage throughout the months analyzed was increased (71%) after 10-months of installation of the sample nets. This suggests biofouling growth after the two first months followed a classical succession process. Algae, hydroids, ascidians, bryozoans, and mobile organisms (polychaetes and crustaceans) were present in all locations. The above location had a higher abundance of algae (64%) and algal-hydroid assemblage (31%) than the below location which had abundances of algal-hydroid assemblage (46%), and algae (12%). The higher algal growth at the above location was probably stimulated by light availability. There were no differences in percent coverage and composition between upstream and downstream locations (51% and 54% respectively). Biofouling community growth in aquaculture operations in tropical areas could involve serious implications related to fish farming, but ecological benefits of biofouling must also be considered when developing appropriate prevention and control methods.

RESUMEN

El biofouling en jaulas de acuicultura es conocido como un problema significativo en operaciones de jaulas de acuicultura. El propósito de este estudio fue evaluar a través del tiempo la composición y porcentaje de cobertura del biofouling en dos jaulas de peces. Las jaulas de cultivo se encuentran sumergidas a 3 km al sur de la Isla Culebra, Puerto Rico. Muestras de red de 1050 cm^2, del mismo material usado en las jaulas, fueron atadas en cuatro posiciones diferentes de cada jaula (jaula de pargo-*Lutjanus analis* y jaula de cobia-*Rachycentron canadum*): arriba o abajo del "rim" de la jaula; y a favor (corriente predominante) o en contra de la corriente. El "rim" estaba localizado a 16 m debajo de la superficie, a mitad de la jaula. El crecimiento del biofouling fue monitoreado desde agosto del 2002 hasta junio del 2003, removiendo cada dos meses una muestra de red de cada posición de cada jaula. En el laboratorio, cada red fue fotografiada y se calculó el porcentaje de cobertura usando el programa Map Maker (Versión 1.0). Se identificaron los organismos en grupos principales (algas, esponjas, hidroides, poliquetos, moluscos, crustáceos, ascidias y briozoarios). No hubo diferencias significativas entre los porcentajes de cobertura de biofouling de las jaulas de pargo y cobia (53% y 51% respectivamente). Después de dos meses del inicio del estudio, la cobertura de biofouling en las redes fue de 49%. El porcentaje de cobertura a través del estudio fue mayor luego de 10 meses de la instalación de las redes (71%). Esto sugiere que el crecimiento del biofouling posterior a los dos primeros meses sigue un proceso clásico de sucesión. Algas, hidroides, ascideas, briozoos y organismos móviles (poliquetos y crustáceos) se observaron en todas las posiciones. La posición arriba del "rim" tuvo mayor abundancia de algas (64%) y ensamblaje alga-hidroides (31%) que abajo del "rim", con abundancias de 12% y 46% respectivamente. El alto crecimiento de algas arriba del "rim" probablemente fue estimulado por la mayor disponibilidad de luz. No hubo diferencias en el porcentaje de cobertura ni composición entre las posiciones a favor o en contra de la corriente (51% and 54% respectivamente). El crecimiento de la comunidad de biofouling en operaciones de acuicultura en áreas tropicales puede incluir serias consecuencias para el cultivo de peces, pero es importante también considerar los beneficios ecológicos del biofouling al desarrollar métodos apropiados para su prevención y control.

To my parents, my brothers, and my life's buddy,
Who always support me unconditionally

To ***the force*** for sustaining me in motion

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CHAPTER 1. INTRODUCTION

1.1 Definitions of Biofouling

There are many definitions concerning biofouling, depending on the context of where it is located. As described below, the definition may vary according to the perception of the author. Overall, biofouling is the attachment of an organism or organisms to a surface in contact with water for a period of time (Stanczak 2004). A brief compilation--from general to specific sources--of glossary biofouling definitions includes the following:

- Biofouling is the impairment or degradation of something, such as a ship's hull or mechanical equipment, as a result of the growth or activity of living organisms (Farlex 2007).
- Merriam-Webster Dictionary (2007) describes biofouling as the gradual accumulation of waterborne organisms (as bacteria and protozoa) on the surfaces of engineering structures in water that contributes to corrosion of the structures and to a decrease in the efficiency of moving parts.
- FAO (2007a) describes fouling as the assemblage of aquatic organisms attaching to and growing upon underwater objects, such as ship hulls, harbor structures, net cages, net pens and rafts (Figure 1). Extreme fouling of living organisms, such as mollusks or shrimp, can impede their normal body-functions leading to weakening and death.

Railkin (2004) explains that, since the earliest studies of hard substrates, there has been disagreement concerning the terms used to represent the communities of microorganisms and macroorganisms. Some authors described the fouling communities as special assemblage of organisms on artificial substrates and man-made structures rather than on natural objects. Others regard fouling as the process of colonization of any natural (living and non-living) substrate. But Railkin considered biofouling as the colonization of marine organisms on immersed surfaces.

Figure 1. Biofouling growing on a ship's hull and aquaculture net cage. (From AMBIO 2006, and FAO 2007a, respectively.)

1.2 Colonization Process

Similar communities may develop at the same stage of succession in the same region, only in the presence of similar properties of substrates and abiotic conditions. If al least one of those conditions is not met, the species composition and abundance of communities developing on different hard substrates in the same water area may be different (Railkin 2004).

Biofouling is composed of organisms having organic or mineral materials. The first phase of biofouling formation is known as microbial biofilm. Organic film accumulation composed of chemical compounds (mostly protein, proteoglycans and polysaccharides) make the surface suitable for bacterial colonization (Abarzua and Jakubowski 1995). Marine bacteria attached on submerged surfaces release substances important for the "conditioning" and subsequent attachment and growth of other organisms (Tosteson 1988), including diatoms, macroalgal spores, fungus, and protozoa. The process between primary colonizers (bacteria and diatoms) and secondary colonizers (spores of macroalgae, fungus, and protozoa) occurs within approximately one week (Figure 2). Tertiary colonizers, macrofoulers, are attached to the microfouling film and include larval sessile marine organism such as tunicates, coelenterates, bryozoans, barnacles, mussels (Abarzua and Jakubowski 1995), and free-living organisms as decapods. In the

later phase, macrofoulers need specific cues for attachment and metamorphosis to adult phase.

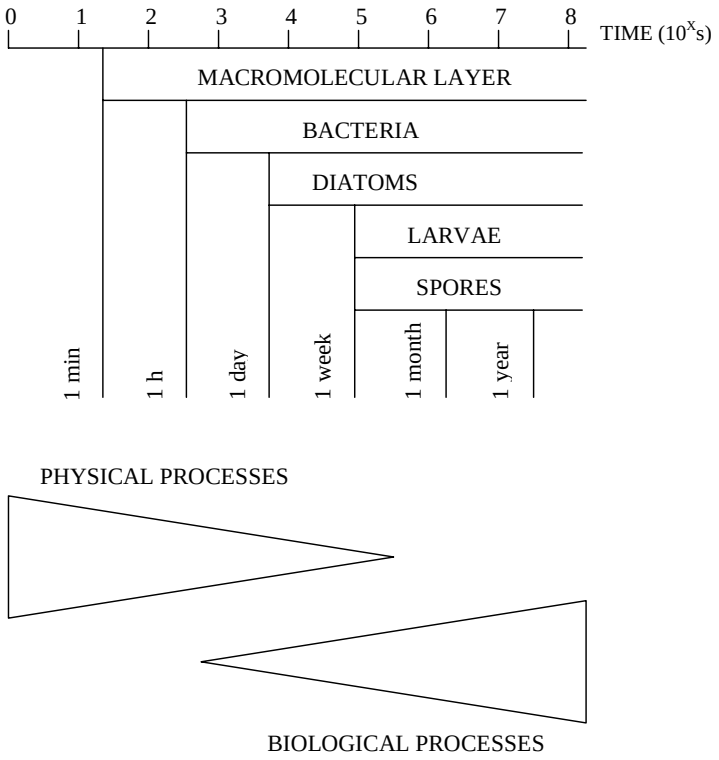

Figure 2. Chronology of colonization processes. (From Railkin 2004.)

The initial colonization process does not occur in a random fashion. Conditions must be favorable, including proper pH, humidity, and nutrient availability. Organisms appear to favor certain substrates or conditions; for example, bacteria creating biofilms on carbon and stainless steels and recirculating cooling systems are physiologically similar and are often the same species. Biochemistry may determine if and where biofilms attach, as in the case of *Vibrio alginolyticus*, a bacterium which produces organic compounds sensitive to changes in temperature and pH. Chemistry and biology also determine which organisms attach to the biofilm (Stanczak 2004).

In aquaculture cages the rate of fouling depends on the mesh size of the nets, temperature of the water, and productivity of the site. Smaller mesh (0.64–1.27 cm) can be easily fouled within 7–14 days while larger mesh (2.54–3.81 cm) is fouled within

about 1–2 months (Cheah and Chua 1979 in FAO 2007b). In tropical waters, the net-cage has to be cleaned at least once a month. So the biofouling growth rate and species composition is important to consider cages design.

Many other factors contribute to an organism's settlement on a substrate. For example, water velocity affects the settlement of the polychaete *Hydroides elegans* and the bryozoan *Bugula neritina* with the highest attachment at a flow rate of about 2 cm/sec. Barnacle larvae (*Balanus* spp.) settle in an range of water flow from 4 to 21 cm/sec (Qian et al. 2000).

1.3 Problems Related to Aquaculture

The effects and magnitude of fouling have been recognized worldwide from ancient times as early as the 5th century B.C. (WHOI 1952). However, the search for information to resolve biofouling problems first began with fouling control for private, commercial and naval ships, thereby ignoring nuisance biofouling in aquaculture.

The diversity and intensity of biofouling in aquaculture is site specific, depending on season, geographic location, and local environmental conditions. Biofouling is a major problem for aquatic culture systems with many related consequences (Hodson et al. 2000). Problem areas include fouling on infrastructure (immersed structures such as cages, netting, and pontoons) and stocked organisms (farmed species, particularly shellfish such as mussels, scallops and oysters). Biofouling reduces the efficiency of materials and equipment, often physically damaging equipment by abrading the material or increasing its brittleness, or by increasing the load or drag. This often results in damage to the netting with frequent loss of cultured organisms. Biofouling may eventually clog the mesh of the net walls, hence reducing the water exchange. This may cause unnecessary stress on the cultured fish due to oxygen deprivation and accumulated wastes. Biofouling communities can also directly compete for resources with cultured organisms and can include predators and harbor diseases (Phillippi et al. 2001, Tan et al. 2002, Willemsen 2006).

Multifilament netting on aquaculture cages offers an ideal substratum for the establishment of biofouling (Hodson et al. 1997). The increase in diameter and distances between threads facilitate the establishment of algae and animals within the netting strands (Dubost et al. 1996). Biofouling is exacerbated on aquaculture cages because of the increased nutrient enrichment from wastes released as uneaten feed, fish excretion, and fecal production which promote macroalgal growth (Ruokolahti 1988). Huang (2000) confirmed that biofouling of fish cages eventually may affect survival rates.

Algae grows rapidly on cage nets submerged in the water for extended periods, which occludes mesh, thereby requiring frequent, costly, and time-consuming cleaning procedures (Dubost et al. 1996, Hodson et al. 1997, Huang 2000). In tropical waters, the rate of fouling is much faster than in subtropical or temperate regions. In addition, hurricanes are more frequent in the tropics and sub-tropics and biofouled marine cages may increase risk of damage by currents which may reach 150 cm/s or more during hurricane events.

Fouling can also create health and safety concerns, i.e., fouling increases the weight and slipperiness of handled equipment, and in the tropics, the frequency of contact with stinging organisms and sharp surfaces is increased (Hasse 1974). The development of biofouling increases the use of combatant chemicals such as cypermethrin, azamethiphos, and emamectin benzoate for the treatment of parasites and diseases. But, their use could relate to detrimental environmental impacts, (Waddy et al. 2002).

1.4 Prevention and Control

Fouling causes huge material and economic costs in maintenance of mariculture, shipping industries, naval vessels, and seawater pipelines. Governments and industry spend more than US$5.7 billion annually to prevent and control marine biofouling.

One of the primary ways to prevent biofouling is to select the appropriate structure material. This may be accomplished by applying biological knowledge relating to the biofouling organisms. For example, zebra mussels find aluminum-bronze distasteful, so they tend to avoid such structures. Cupronickels (copper-nickel alloys) have good biofouling and corrosion resistance, and therefore are often used for surfaces or surface coatings (Stanczak 2004).

Preventative biofouling controls used in industrial applications typically attempt to inhibit or inactivate bacterial biofilms by use of oxidizing biocides or concentrated acids; displace the biomass by physical means; and/or destabilize the biofilm matrix using surfactants, dispersing agents, or chelating agents. Ideally, biofouling controls would prevent the formation of biofilms (ESTCP 2002).

A full spectrum of chemical treatments has been developed, and several are effective in the control of biofouling. Electrolytic chlorination of seawater is an effective biocide utilizing chlorine. Other chemical biocides are available; however, these materials are either metal-containing (tin, copper, zinc), non-biodegradable, or difficult/costly to use (peroxides, ozone). New materials are developed using silicone-based fouling-release coatings, generally in combination with mechanical cleaning (Hodson et al. 2000). Other coatings based on natural antifoulants also are used for netting and on cultch material for shellfish culture (De Nys et al. 2004).

Tributyltin (TBT) proved to be excellent for fouling prevention during the 1950s and early 1960s, but was later found to be toxic to marine organisms (Evans et al. 1995). Low concentrations of 20 ng/L of tributyltin (TBT) caused defective shell growth in the oyster *Crassostrea gigas* and concentrations of 1 ng/L caused development of male characteristics in female genitalia in the dog whelk *Nucella lapillus*.

Work involving the use of acoustics for control of biofouling was underway and showed some promise. The process, known as electro-hydraulic cavitation, uses an underwater plasma sparker to generate an acoustic shockwave causing physical damage to living

cellular matrix. However, there were concerns related to submarine applications because the effects dependent on frequency and species, without effectiveness over target organisms. For example, ultrasonic frequencies of about 20 kHz kill or inactivate bacteria and fungi, but not higher organisms, while lower frequency acoustic signals affect fishes (CETS 1996).

The most common method to control biofouling is by labor intensive and tedious mechanical cleaning involving brushing, scraping, or by using water jets (Hodson et al. 1997). Other methods include biological control using grazers (Hidu et al. 1981, Lodeiros and Garcia 2004) and fishes such as rabbitfish (*Siganus* sp.), pearl spots (*Etroplus*), and scat (*Scatophagus argus*), but their feasibility in large commercial farms have, however, yet to be demonstrated (FAO 2007b). Also, rotating net-cages have been suggested, but their applicability in tropical waters has yet to be fully tested.

1.5 Benefits from Biofouling

The vast abundance and biomass of organisms on hard natural substrates, including hard grounds, determine their important ecological role. Foulers are usually characterized by efficient detritus and grazing food chains. Communities of foulers function as biofilters by extracting pollutants and pathogenic microorganisms, precipitating suspended particles, and thereby purifying and clearing water. The ecological role of biofouling communities makes them an effective instrument of environmental protection, in particular, restoring perturbed ecosystems by means of artificial reefs which are colonized by foulers and accompanying organisms (Railkin 2004).

Braithwaite and McEvoy (2005) mentioned several positive attributes of biofouling that benefit aquaculturist. The most notable is the management of fouling for seeding mussel lines. Soft-bodied fouling organisms may reduce abrasion effects on caged fish and could provide supplemental foods for different cultured fishes. Macroalgal fouling in land-based aquaculture systems can increase dissolved oxygen concentrations, while

reducing ammonium levels. Lojen et al. (2005) considered that some biofouling organisms could function as an efficient biological sink for particulate organic matter released from fish cages, thus serving to transform or recycle waste nutrients into other forms.

1.6 Open-ocean Aquaculture

Open-ocean aquaculture (OOA) refers to aquaculture operations located in an exposed, open-ocean environment. OOA consists of floating or submerged cages moored to the ocean bottom. Few open-ocean aquaculture operations currently exist. Key federal policy makers envision OOA as the future of aquaculture in the United States (IATP 2004).

However, the fundamental principles of open-ocean aquaculture are similar to near-shore salmon aquaculture and carry environmental and health risks which include the following: fish meal and fish oil consumption; introduction of non-native species; use of drugs such as antibiotics, hormones, anesthetics, pigments, and vitamins; use of herbicides to control algae on cages; incubation of local diseases caused by high concentration of fishes; new diseases and parasites introduced by seed stock; fish sewage containing uneaten food, waste products, diseases, and pathogens; and non-native fish escapes competing with native species for food and habitat (Figure 3).

The National Oceanographic and Atmospheric Administration (NOAA) recently (March 12, 2007) submitted a bill to the US congress entitled “The Offshore Aquaculture Act 2007” to streamline permitting, establish long-term leases, and weaken fisheries management protection to accelerate open-ocean aquaculture (NOAA 2007). The bill crafted by NOAA seeks to support offshore aquaculture development within the federal waters of the EEZ; to establish a permitting process encouraging private investment in aquaculture operations, demonstrations, and research; and to promote research and development in marine aquaculture science and technology and related social, economic, legal, and environmental management disciplines (Naylor 2006). The

proposed bill was developed in consultation with industry, conservation groups, states, and researchers. It includes requirements to ensure that offshore aquaculture proceeds in an environmentally responsible manner to protect wild stocks, to maintain the quality of marine ecosystems, and to be compatible with other marine activities. NOAA provides funding for projects to demonstrate the technical and economic feasibility of offshore aquaculture. Offshore aquaculture includes all activities involved in the propagation and rearing of marine species in the exclusive economic zone (EEZ), which is the federal waters situated between 4.8 and 320 km offshore, thus beyond State or Territory jurisdiction.

Thus a number of Sea Grant Program universities are involved in research and development of largely carnivorous species, including genetically engineered fish for offshore cultivation. Cobia (*Rachycentron canadum*) is cultured in Puerto Rico, and amberjack (*Seriola dumerili*) and Pacific threadfin (*Polydactylus sexfilis*) are cultured in Hawaii. Other experimental operations are being deployed off the coast of California and planned for the Gulf of Mexico, and for the Pacific and Northeast Atlantic federal waters (IATP 2004).

Over the past decade, research funded by NOAA shows that offshore aquaculture can work well with proper location and the use of current best management practices for aquaculture. Currently, aquaculture demonstration projects in state waters, using submerged cages for finfish in New Hampshire, Puerto Rico, and Hawaii are yielding good production with little environmental impact. The University of New Hampshire had good results by culturing blue mussels (*Mytilus edulis*) in an exposed ocean environment by using submerged-rope culture techniques (NOAA 2007).

In relation to legal standards for environmental protection, the United States Environmental Protection Agency (EPA 2002) proposed a guide describing “best management practices” (BMPs) used by concentrated aquatic animal production facilities to minimize the discharge of pollutants and minimize potential adverse impacts pollutants on receiving waters. BMPs are intended to preserve environmental integrity

while eliminating cumbersome, identical, and unclear environmental permitting and licensing requirements. Aquaculturists following these practices meet the minimum standards necessary to protect and maintain water quality and wildlife habitat. These practices represent a mutually beneficial relationship between commercial aquaculture production and natural resource protection.

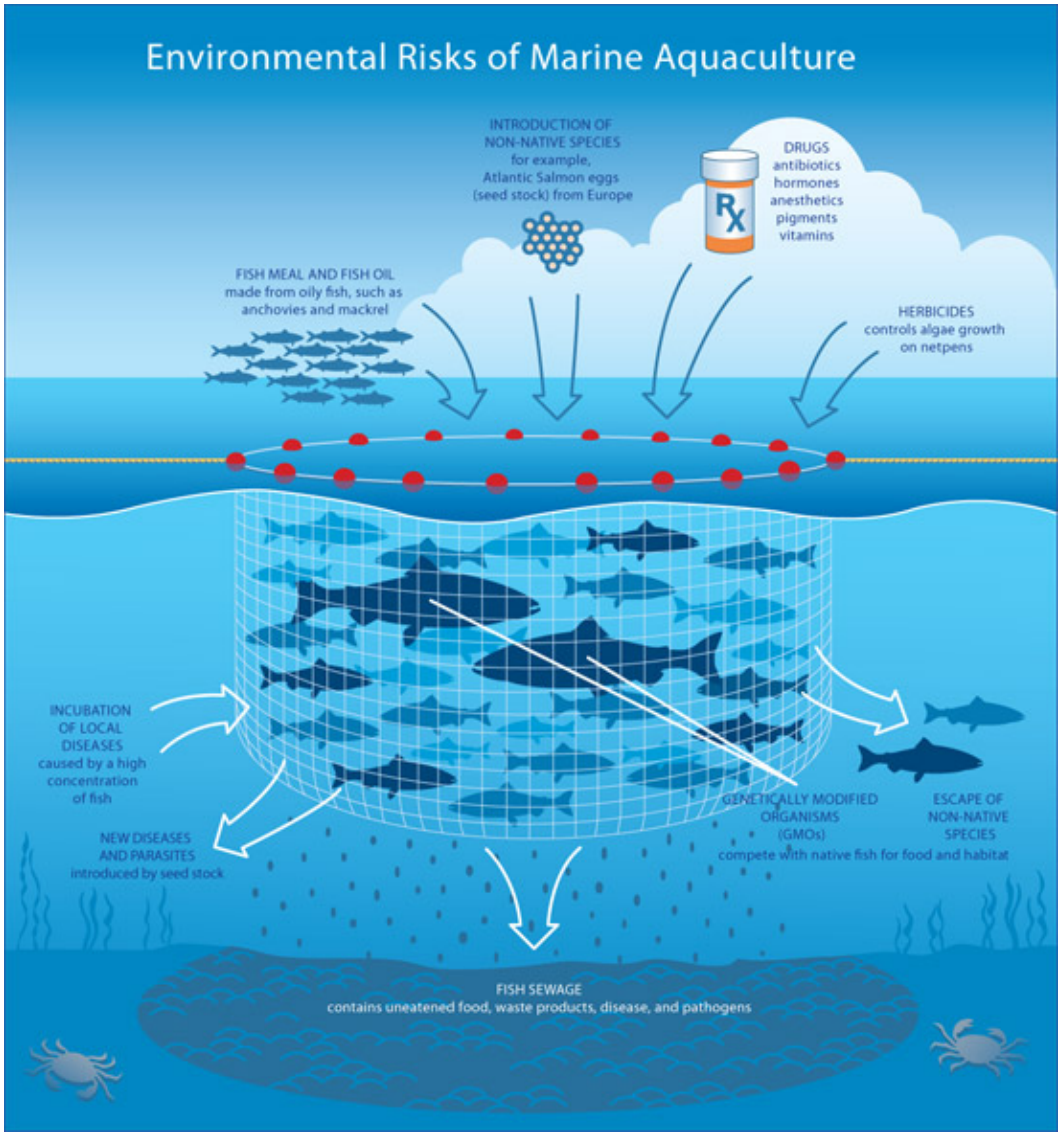

Figure 3. Environmental risk of marine aquaculture. (From Goldburg et al. 2001-art adapted from the David Suzuki Foundation 1996- in Pew Oceans 2003.)

EPA (2002) recommends to biofouling on net pens, regular cleaning of the production nets to ensure a constant flow of water through the production area. EPA suggests the following management practices for net-pen systems: minimize the concentration of net-fouling organisms released during changing or cleaning nets; remove fouled nets, transport ashore, air-dry, and clean with pressure washers, if necessary; avoid discharges of cleaning water or net-fouling organisms into open waters; avoid discharges of net-cleaning chemicals or other gear into open waters; and avoid using the antifoulant material tributylin.

CHAPTER 2. BIOFOULING GROWTH ON OPEN-OCEAN SUBMERGED AQUACULTURE CAGES IN PUERTO RICO

2.1 Abstract

Biofouling in aquaculture cages is a worldwide problem. This study assessed biofouling composition and percentage coverage from August 2002 to June 2003 on nets of two open-ocean submerged aquaculture cages located 3 km south of Culebra Island, Puerto Rico. Sample nets (1050 cm^2) were fastened in four different locations on each cage (*Lutjanus analis* and *Rachycentron canadum*): above or below the cage rim, either upstream (predominant current) or downstream. One net sample was removed bimonthly from each location of each cage. Algae, sponges, hydroids, polychaetes, mollusks, crustaceans, ascidians, and bryozoans were the major groups encountered. There was no significant difference in biofouling coverage between snapper cage and cobia cage (53% y 51% respectively). Biofouling coverage was 49% after two months of cage deployment. Percent coverage increased (71%) throughout the 10-month study, suggesting classical succession after the first two months. Algae, hydroids, ascidians, bryozoans, and mobile organisms (polychaetes and crustaceans) were present in all locations. The above location had more algae (64%) and algal-hydroid assemblage (31%) than the below location (algal-hydroid assemblage-46% and algae-12%); higher algal growth at the above location was probably stimulated by light availability. There were no differences in percent coverage and composition between upstream and downstream locations (51% and 54% respectively). Biofouling is detrimental to aquaculture cage operations; however, ecological benefits of biofouling must also be considered when developing appropriate prevention and control methods.

2.2 Introduction

Surfaces immersed in the marine environment become colonized by marine organisms a process known as biofouling (Railkin 2004). It occurs as a result of settlement, attachment, and growth of sedentary and semi-sedentary organisms on artificial structures placed in marine water (Venugopalan and Wagh 1990), such as on ship's hulls, seaside piers (Davis and Williamson 1995), and aquaculture cages. For the context of this investigation relating to open-ocean aquaculture, the WHOI (1952) definition is used which refers to biofouling as "the growth of unwanted organisms on the surfaces of man-made structures immersed in the sea, which has economic consequences". Thus, most of the biofouling literature is related to boats, pilings and intake structures.

The assemblage of organisms attached to aquaculture structures is different from those found on ship hulls than on fish cages, so the information related to biological communities attached to aquaculture structures is scarce (Braithwaite et. al 2004). Because of warmer temperatures and higher metabolic growth rates, biofouling is more serious in tropical waters.

Cost effective, sustainable solutions are essential to minimizing costs for aquaculture operations. However, biofouling persists as a significant economic barrier to the development of competitive aquaculture because management practices associated with controlling biofouling can be expensive (Beaz et al. 2005). For example, annual costs to replace nets and reapply antifouling for a medium-sized United Kingdom salmon farm are estimated to be about US$230,000 (Willemsen 2006).

Currently there are no sustainable and cost-effective solutions to the biofouling problem in aquaculture. In spite of several beneficial attributes of biofouling (Braithwaite and McEvoy 2005), biofouling in cages is still of concern for the cage aquaculturists (Hodson et al. 2000) because it may also reduce the light availability for cultivated organisms, accelerate corrosion of structures, reduce water flow passing through the cages,

increase drag (Phillippi et al. 2001, Swift et al. 2006), and should be considered a risk factor for diseases, all which affect fish survival rates (Huang 2000, Tan et al. 2002). Management techniques include costly diver-cleaning practices and bring the cages to the surface to expose a portion of the netting to sunlight.

Several international studies have examined biofouling community on aquaculture structures, factors affecting the colonization process, relation between fouling and cultured organisms, and other issues as biofouling control. However, many problems still exist and new methods of prevention and eradication of biofouling for submerged structures is becoming the primary target for many investigators.

In the Atlantic Ocean, only one study has been reported on open-ocean aquaculture cages. Greene and Grizzle (2006) studied the ecological succession of biofouling communities on the netting of fish cages in the western Gulf of Maine, USA. They found a potentially complex suite of biotic interactions tempered by physical factors to determine successional patterns. Their succesional model suggests that predation is a major factor affecting development of fouling communities on fish cages. The authors explore the idea that manipulation of predation as a natural mechanism of control of fouling could be an environmentally acceptable practice. In Atlantic Canadian waters in southwestern New Brunswick, Hall (1995) studied the biofouling attached to nets at three geographically distinct salmon farms and determined that the composition of communities was variously influenced by the time of year, location, and depth, but not by antifouling surface treatment. In tropical Atlantic Venezuelan waters in the Cariaco Gulf, Lodeiros (1996) studied seasonal changes of the growth and survival of the scallop *Euvola ziczac* in suspended culture. He found that fouling organisms, particularly organisms developing on the shells of scallops, was a major factor affecting the feeding rate of larger scallops, leading to a weakened physiological state. In later research in the Cariaco Gulf, Lodeiros and Garcia (2004) reported the usefulness of sea urchins in controlling fouling in tropical bivalve cultures. In the La Restinga Lagoon, Venezuela, Lodeiros et al. (2007) ran an experiment on mangrove oysters, *Crassostrea rhizophorae*, to evaluate the effects of adding different masses of artificial fouling to

different sites on the upper valve of the shell. They concluded that mortality could be affected by the position where artificial fouling was cemented, irrespective of the type of fouling mass. Whereas, the negative effects of fouling would only be exhibited if fouling on the upper valve attained a mass equivalent to three times the mass of the valve, but natural fouling is not likely to increase to the level that would affect growth or survival of the oysters.

In Australia, Hodson et al. (1997) determined the effectiveness of a prototype in situ cleaner and found that residual structures of fouling organisms led to rapid regrowth and recolonization. In a later study, Hodson et al. (2000) reported that commercial silicone coatings on fish-cage netting significantly reduced total biofouling mass and increased the effectiveness of in situ net cleaning. Tan et al. (2002) found that the biofouling surface may be a reservoir for the amoeba, *Neoparamoeba pemaquidensis*, resulting in amoebic gill disease of salmonids.

In Europe, Dubost et al. (1996) found that development of freshwater fouling on floating net cages depends on the submersion time, net surface, species present, and the physical and chemical variables. Mazouni et al. (2001) reported that oyster culture, including biofouling on oyster ropes, could influence nitrogen recycling in the water column with a potential annual dissolve inorganic nitrogen production of 2×10^7 mol/yr. Braithwaite et al. (2007) conducted experiments on a redundant finfish cage moored at a working salmon farm, using both antifoulant and untreated netting. They found that the copper-based antifoulant can provide at least 150 days of effective protection against biofouling conditions.

In Asia, Cheah and Chua (1983) found that the encrustation rate of biofouling on tropical marine cages varied with mesh size and net frame position. Qian et al. (1999) found that larval fouling organisms settling was species-specific in response to water flow, and suggested that this species-specificity is related to larval morphology, swimming ability, and behavior. Qian et al. (2000) found that larval settlement behavior is a complex interaction related to substratum characteristics and flow rate. Zongguo et al. (1999)

established that water quality explained the characteristics of the biofouling community in mariculture zones. Biofouling biomass was positively correlated with nitrite, nitrate, and silicate levels, and diversity indices were positively correlated with ammonium, phosphate, but negatively correlated with dissolved oxygen concentrations.

Little research on biofouling has been carried out in Puerto Rico. Research on evaporator tubes for a simulated Ocean Thermal Energy Conversion (OTEC) plant during 1980 indicated the long-term effects of microbiofouling on heat exchanger efficiency. They studied the nature of the biofilm and corrosion of metallic surfaces (Tosteson et al. 1980, Morgan et al. 1981, Sasscer et al. 1981, Sasscer et al. 1982, Sasscer et al. 1983, Zaidi et al. 1984). A recent study by Carbery (2006) determined distributions of the antifoulant Irgarol 1051® in marine systems outside temperate coastal regions. This study incorporated chemical assays to identify patterns of contamination in the Northeastern Caribbean, providing the basis for ecological risk assessment for resource managers.

Nevertheless, there are no reported studies of biofouling on fish cages in the Caribbean. Because characteristics may be significantly different in the Caribbean, biofouling studies will help the mariculture industry by providing additional information to be applied as management tools. The overall goal of the present study was to increase knowledge relating to biofouling attached to tropical marine aquaculture cages and contribute to future research concerning biofouling in mariculture operations. The specific objectives of this study included: determining the composition of tropical marine biofouling organisms attached to submerged open-ocean aquaculture cages; calculating the percentage coverage on netting samples placed on the cages during the culture period; determining effects of light versus shade on biofouling growth; and determining effects of the predominant current (upstream versus downstream) on biofouling growth.

2.3 Materials and Methods

Two Ocean Spar, Inc., Sea Station™ cages were purchased and assembled by Snapperfarm, Inc., about 3.0 km south of Culebra Island, Puerto Rico during June 2002 (Figure 4). Each 3000 m^3 cage was anchored over a sandy plain bottom at a depth of 28 m, and separated by about 30 m between them. One cage was stocked with snapper (*Lutjanus analis*) and another with cobia (*Rachycentron canadum*) during August 2002. A control site was located approximately 375 m south of the cages. The biofouling communities were studied on the netting of these cages since August 2002 to June 2003.

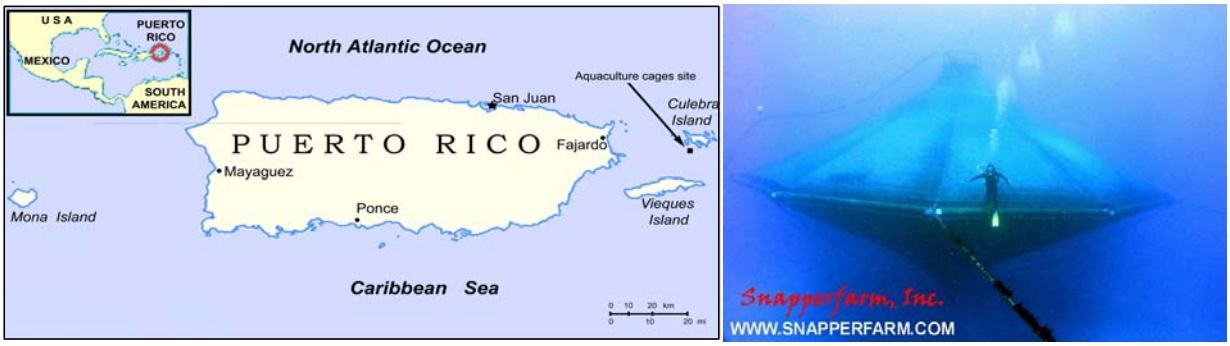

Figure 4. Fish culture cage site and lateral view of the Sea Station cage.

In this study, each Sea Station™ cage utilized Spectra netting, an ultra-high molecular-weight polyethylene material. Twenty sample nets of the same mesh were fastened to the netting of each cage. The dimensions of each sample net were 30 by 35 cm with an area of 1050 cm^2, representing 409 cm^2 of thread surface. At time zero (August 2002), five sample nets were placed in each of four different locations on each cage, 1 m above (lighted) or 1 m below (shaded) the rim on the north or south side of each cage (Figure 5). The “lighted” location received more sunlight from the surface than the “shaded” area underneath the rim because the cage was diamond shaped (Figure 4). The north and south locations were selected because they were upstream (north) or downstream (south) of the predominant current. The rim was located 16 m below the surface and consisted of a horizontal steel structure at the middle of the cage (Figure 4).

The biofouling growth on the cages was assessed bimonthly during ten months by removing one sample net from each location from each cage by scuba divers. Samples netting were preserved in 4% formalin, and transported to the laboratory for analysis and organism identification.

At the Marine Sciences Research Station on Magueyes Island of the University of Puerto Rico, the sample nets were analyzed. For biofouling coverage determination, each sample net (with accumulated biofouling) was photographed with a digital camera and analyzed with image-processing software, Map Maker (Version 1) to calculate areas. The major groups of organisms (algae, sponges, hydroids, polychaetes, mollusks, crustaceans, ascidians, and bryozoans) attached to each sample net were then identified with a dissection microscope. Taxonomic identifications were made mainly to major groups and several organisms to lowest possible taxa by using available bibliographic references.

The mean magnitude of the current at Culebra passage was 17 cm/sec with a maximum recorded speed of 60 cm/sec. Water temperature ranged from 27 to 29˚C throughout the sampling period. Turbidity was usually less that 1 NTU. Salinity remained almost constant throughout the sampling period with mean values of 34.6 ppt, and the mean dissolved oxygen saturation in water was 5.6 mg/L. Organic matter percentages fluctuated from 4.0 to 6.2% (Mejia 2005).

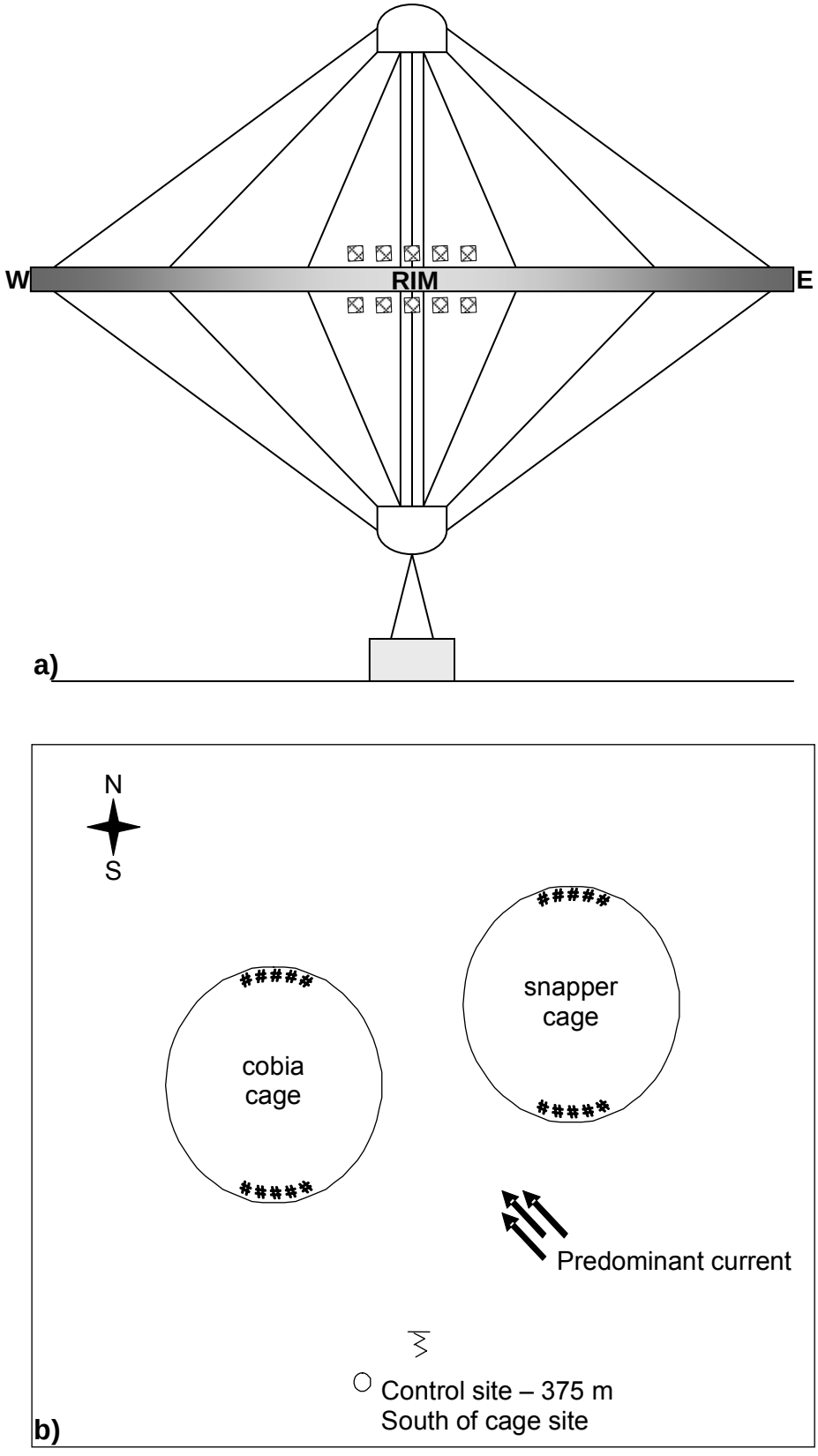

Figure 5. Locations of sample nets on Sea Station cages: a) lateral view, b) top view.

A second separate trial was initiated during October 2005, when only one of two cages contained cobias with netting treated. The antifouling coating used was Flexgard® waterbase preservative copper paint. The empty cage with untreated netting was considered as the control to compare the effects of biofouling growth under feeding and non-feeding conditions and differences encountered when sample net was attached to an antifoulant surface. The biofouling biomass was determined by weighing and labeling each of eight dry samples net. Four sample nets were placed 1 m above the rim on either the southwest of the cobia cage or the northwest side of the control cage. Each samples netting measured approximately 625 cm^2. After 28 days exposure, the sample nets were removed by diving and immediately preserved in 4% formalin solution. In the laboratory, each sample (biofouling and net) was dried at 60˚C until a constant weight was recorded.

Statistical analyses were performed by using Infostat Software (Version 3.0.2 2003). ANOVA analyses were utilized for all data and significant differences among means were analyzed using multivariate Tukey's Test. Contrast analysis tested differences between cages, among sampling net locations (above and below or upstream and downstream), and among months. The net locations of the cages were coded as downstream above (DA), downstream below (DB), upstream above (UA) and upstream below (UB) for the first trial. Contrast analysis was also used in the second trial to determine differences in biofouling biomass accumulation between cobia and control locations.

2.4 Results

The determination of biofouling coverage by each organism on each net was complicated because many organisms overlapped each other. Abarzua and Jakubowski (1995) affirmed that a clear separation of biofouling species is impossible. Percentages of biofouling coverage for each location, month, and cage are indicated in Table 1. Biofouling coverage on the snapper (53%) and cobia (51%) cages was not significantly different. After two months of cage deployment, the biofouling coverage on the cages

was 49% (Figure 6) and continued to accumulate, but at a slower rate. Contrast analysis (p-value < 0.05) indicated that biofouling coverage during the first 2-months of netting deployment was significantly lower (49%) than months 4, 8, and 10 (61%, 61%, and 71% respectively).

Biofouling net coverage during February (six months after the netting deployment) was highly significantly different from other months (p< 0.01) with only 19% of netting coverage, but was considered to be due to diver maintenance error. Coverage at three of the four locations (downstream above, downstream below, and upstream above the cages' rim) of each cage indicated more than 50% coverage during the culture period (Figure 7). There were no interactions between netting location and sampling date (p-value > 0.05). No significant differences in biofouling coverage were observed between downstream and upstream locations of each cage, nor for the locations above and below the cages' rim (p-value > 0.05).

Table 1. Biofouling coverage percents from October 2002 to June 2003 for each month, location, and cage. Net locations codified as downstream above (DA), downstream below (DB), upstream above (UA) and upstream below (UB).

	% coverage (snapper cage)				% coverage (cobia cage)			
	DA	UA	DB	UB	DA	UA	DB	UB
Months								
October	57	65	57	32	58	47	46	33
December	55	65	67	40	65	69	63	68
February	23	16	19	11	54	13	9	10
April	24	75	63	78	66	42	89	48
June	85	91	93	53	46	69	46	89

The major groups of organisms attached to the cages were algae, hydroids, sponges, ascidians, and bryozoans. Algae and hydroids were difficult to separate, so in some cases the assemblage of these two organisms was designated as an algal-hydroid assemblage. Polychaetes, mollusk and crustaceans were also present in low

abundance on the cages as associated fauna. Crustaceans are motile, so several probably escaped when the attached sample nettings were removed from the surface of the cage.

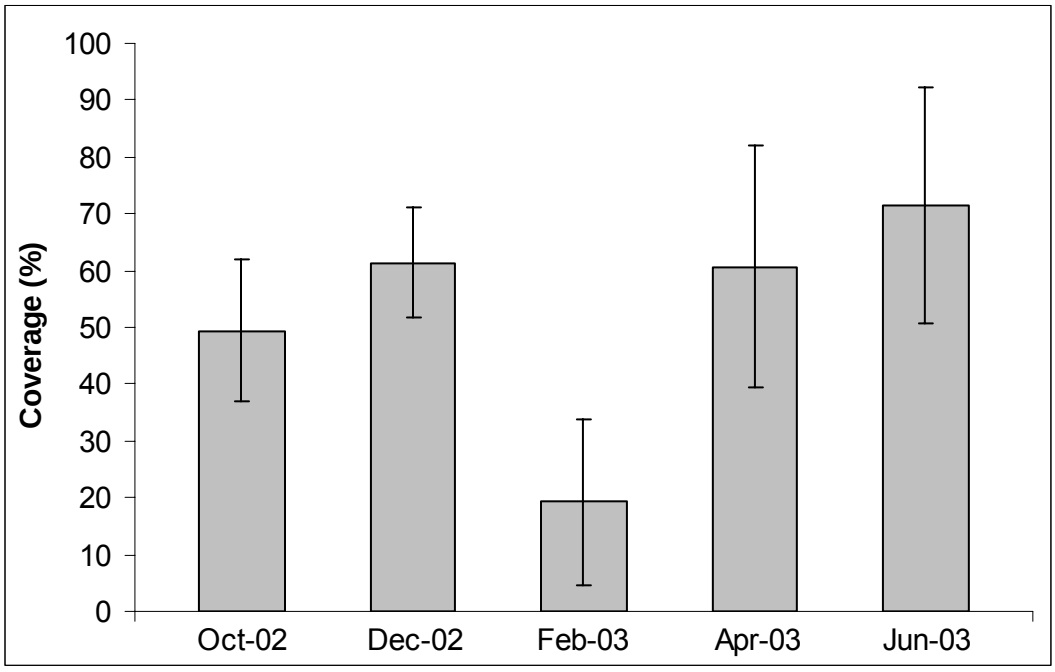

Figure 6. Biofouling accumulation in the cages from October-02 to June-03 (mean ± SD).

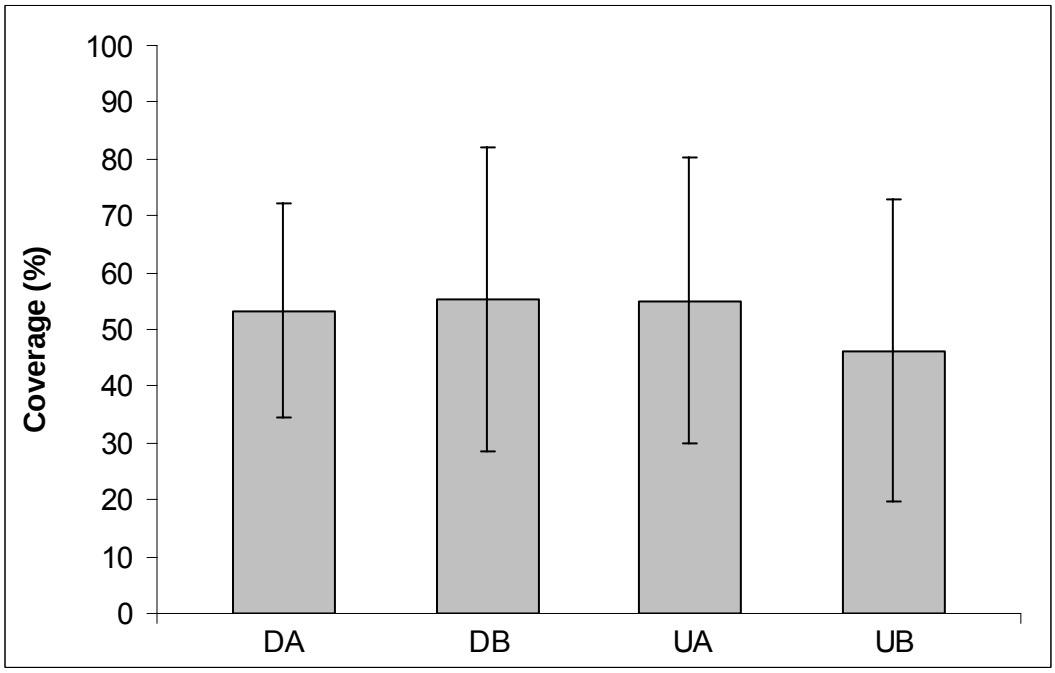

Figure 7. Biofouling coverage for each sampling location throughout the study (mean ± SD). Net locations codified as downstream above (DA), downstream below (DB), upstream above (UA) and upstream below (UB).

There was only one fish larvae (Tripterygiidae family) found in the February sample located on the snapper cage above the rim. Types of organisms on the sample nettings located above and below the rim were similar; however the proportion of each organism varied (Figure 8). The above location had a higher abundance of algae (64%) and algal-hydroid assemblage (31%) than the below location which had abundances of algal-hydroid assemblage (46%), and algae (12%). The higher algal growth at the above location was probably stimulated by the higher light availability. Polychaetes, crustaceans, and mollusks each represented less than 1% of the coverage at the above and below locations. Bryozoans had 1% above and 4% below; ascidians 3% above and 13% below; sponges 2% above and 5% below. Patches of hydroids (20%) observed were more abundant at the below location.

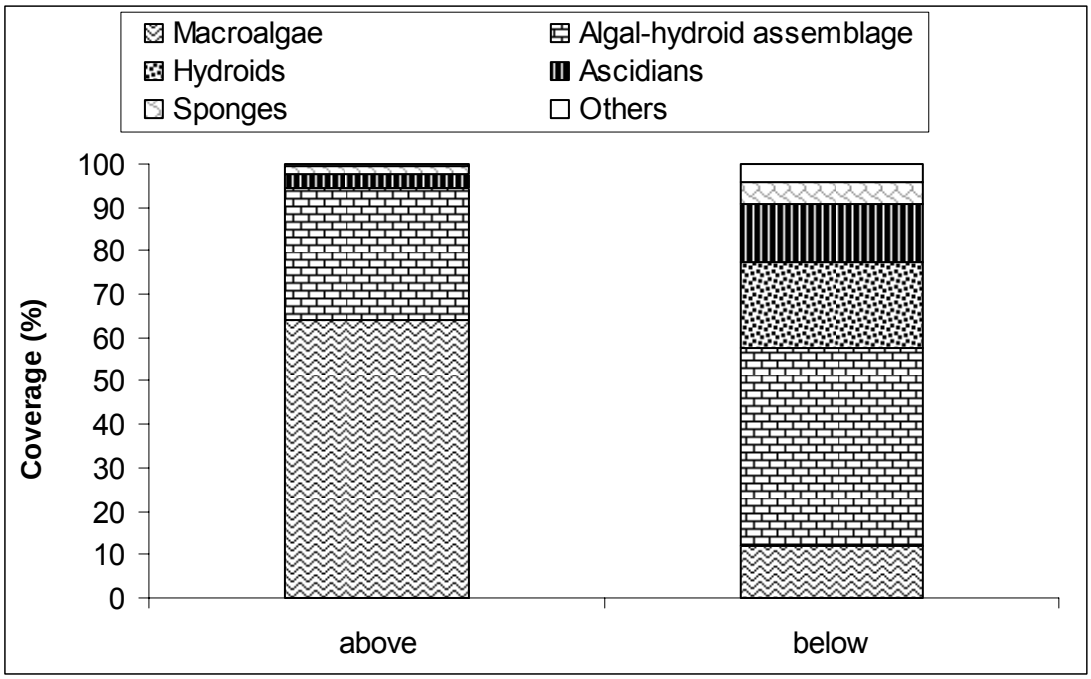

Figure 8. Biofouling predominant morphotype for sampling location above and below in the cages' rim throughout the study.

The dominance of each group changed over time with hydroids and algae predominating during the first two months (October 2002) while the last sampling date (June 2003) had more variety of groups (Figure 9). The October 2002 sampling indicated the following assemblage in order of abundance: hydroids (49%), algal-hydroid

assemblage (33%), macroalgae (16%) and others (2%). The June 2003 sampling indicated algal-hydroid assemblage (30%), macroalgae (22%), ascidians (21%), hydroids (13%), sponges (10%) and others (4%).

In composition, there were not differences in biofouling coverage between downstream and upstream of each cage (Figure 10). Biomass trial not shows differences between cage and control samples netting.

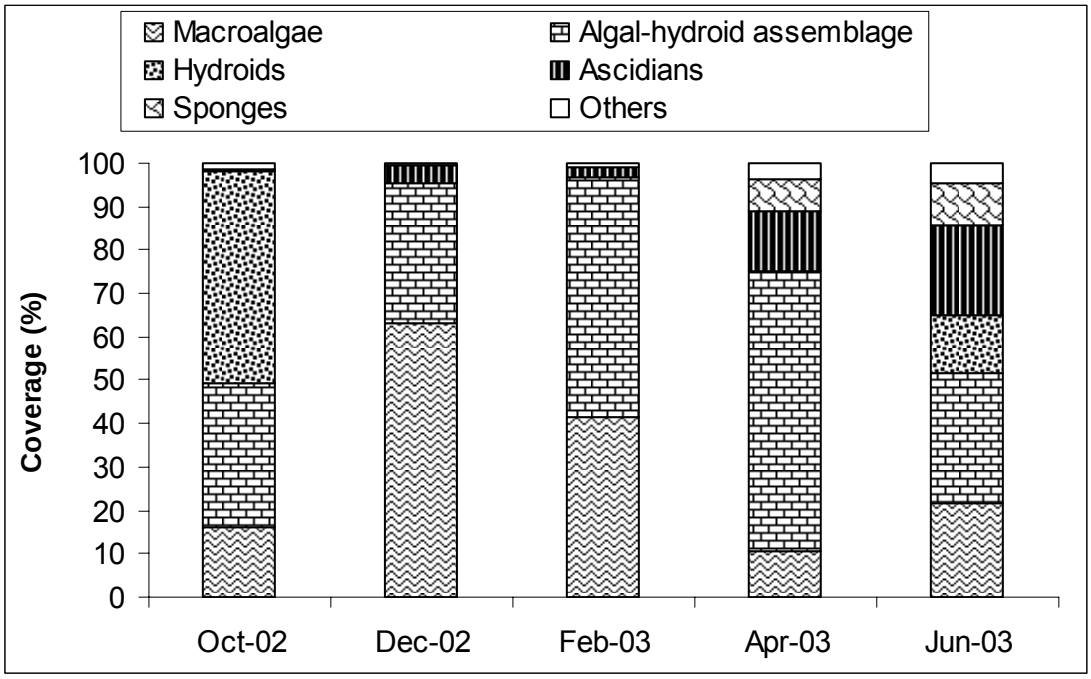

Figure 9. Biofouling predominant morphotype in the cages from October-02 to June-03.

2.5 Discussion

Each cage showed similar biofouling coverage in spite of differences in feed input for the snapper and cobia cages, suggesting biofouling occurrence may be more defined by factors other than nutrient loading in the water column. Dubost et al. (1996) found that most biofouling formation is dictated by chemical and physical characteristics of the water such as temperature, and current velocity. The maximum current rates for biofouling settling of different species may differ considerably; nevertheless, the literature suggests that the current rate from 10 to 50 cm/sec is optimal for the

development of hard-substrate communities of most species, and for many of them (Railkin 2004). So the Culebra cages systems were optimal to biofouling colonization processes with the mean magnitude of the current of 17 cm/sec.

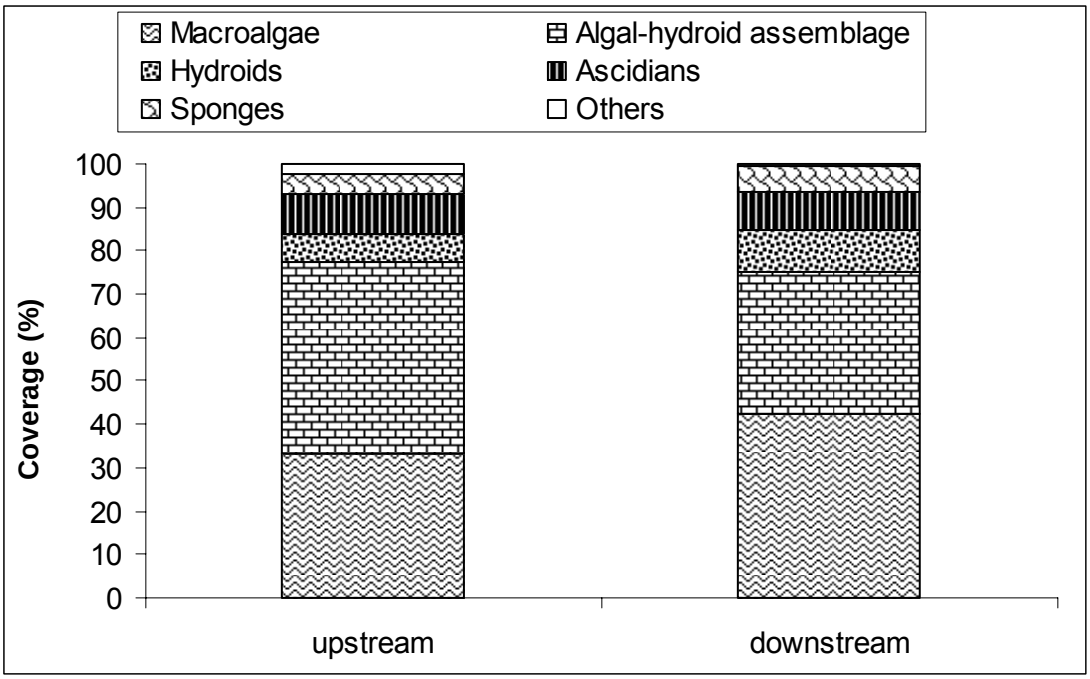

Figure 10. Biofouling predominant morphotype for sampling location upstream-downstream in the cages from October-02 to June-03.

While the biofouling attached to the Culebra cages showed no differences in composition between upstream or downstream locations (Figure 10), Railkin (2004) said that the “edge effect” can occur during the colonization process. The hypothesis suggests that, hydrodynamic factors may cause the margins of flat surfaces positioned parallel to strong flow to be fouled more that their middle areas. However in this study, the edge effect was not observed in the fouling coverage or composition at upstream or downstream locations.

The lower percentage of biofouling coverage (19%) during the fourth month of netting deployment (February) was due to accidental cleaning of the sample netting during routine maintenance of the cages. Large amounts of debris released during the mechanical cleaning process hinder diver visibility (Hodson et al. 1997), so the

accidental cleaning of the sampling net was not unexpected. Greene and Grizzle (2006) confirmed that routine cleaning of cages causes loss of organisms and initiation of ecological succession. Subsequently, biofouling growth probably occurred due to the survival of macroalgal remnants in crevices, ensuring rapid recolonization and regrowth of fouling. For that reason, two months later (April), the biofouling coverage on the cages increased by 42% from the February percent coverage (Figure 6).

Although the netting placed above and below the rim were only separated by approximately 2 m, the cage shape produced a shadow for the nettings below the rim. Consequently, the netting placed above the rim received more light from the sun, which probably explains higher algal growth at this position (Figure 8). Several researchers reported common fouling algae react to light intensity and indicate a preference for un-shaded conditions (Hodson et al. 2000, Greene and Grizzle 2006). Also, the difference in the percentage of composition below the rim included organism such as hydroids, ascidians, and sponges, which indicated different patterns of spatial distribution on surfaces based mainly on larval response to light. For example, Railkin (2004) suggested that responses to light of typical ascidian larvae allow them to select suitable light conditions, usually choosing to settle in a poorly illuminated place. Hydroid larvae prefer to settle in light-reducing sites, although light intensity does not affect growth (Huse et. al 1990, Nellis and Bourget 1996).

Even though light was a factor for differences in organism composition for the above and below locations, the percent coverage was not significantly different (Figure 7). Although depth is a factor affecting biofouling formation (Venugopalan and Wagh 1990, Dubost et al. 1996), apparently the biofouling organisms grew on the cage netting, regardless of the type of organism, covering the sample nets in a similar manner at the above and below locations. Cheah and Chua (1983) said that each organism had equal potential to foul netting, irrespective of where nets were placed, based on the optimal conditions required by each organism.

The accumulation of algal coverage was different over time (Figure 9) because, into the succession process, the algae as primary colonizer could have lost space to be covered by other organisms. This ability to overgrow organisms is referred as epibiotic potencial (Railkin 2004). One factor which could influence growth was the increasing amount of particulate and dissolved nutrients released from the cages as the cultured fish grew. However, in this study, the algal abundance was not associated with water pollution because there were no species identified as indicators of contamination. Algae are normal organisms present during the first stages of macrofouler colonization.

One fish larva was found in this study from the Tripterygiidae family. These triplefin blennies live primarily in tropical seas, near reef surfaces, rocky slopes, rubble, or algal-covered rocks. They feed on algae and tiny invertebrates (Allen and Robertson 1994). Alston et al. (2005) indicated that wild fishes frequent the cage site, probably due to the flow of organic matter into the water column. They also indicated the presence of juvenile fish, so the biofouling could possibly serve as protective habitat for larval fishes, including for the triplefin blenny larvae. Herbivorous fishes commonly inhabit hard substrates because biofouling communities can attract other organisms. Railkin (2004) confirmed that accumulation of hard substrates generates increased plankton productivity, which results in fish abundance.

Other organisms found such as hydroids, sponges, ascidians, bryozoans, mollusks, and crustaceans are typical organisms attached to aquaculture nets (Relini et al. 1994, Abarzua and Jakubowski 1995, and Dubost et al. 1996). In general, biofouling communities of hard substrates are dominated by suspension feeders which consume particulate organic matter from the water, whereas predatory forms are not abundant. Ascidians may clean and purify wastewater; Naranjo et al. (1996) suggested that species richness was enhanced when the percentage of organic matter increased (both in suspension and in the silt).

Zongguo et al. (1999) referred to “true foulers” as those sedentary organisms that remain attached for most of their life to a submerged substratum. The other organisms

are associated fauna, which search for either food or shelter among the growth of the major foulers. Zongguo et al. also reported that the associated fauna such as Decapods are unlikely to cause net blockage or an increase in weight because of their mobility and small size. In this study, juvenile spiny lobsters (*Panulirus argus*) were found among the associated biofouling fauna, but not in significant numbers because they escaped when the sample nets were removed from the surface of the cages. However, during a portion of this study, in the spring of 2003, thousands of spiny lobster larvae were observed settling on cages by the Snapperfarm personnel (Davis et al. 2006). A study related to these finding concluded that the collection of lobster pueruli and juveniles from cages for growout is technically feasible and has potential to be developed into a commercial business enterprise (Davis et al. 2006). This event could demonstrate an additional benefit of the biofouling community which was not included among the positive attributes reported by Braithwaite and McEvoy (2005). Thus, hard-substrate communities developed on man-made structures could be recruitment zones for macroorganisms with high aquaculture value, such as lobsters. However, Cruz et al. (2006) suggests that removing lobster juveniles from the wild for commercial growout to marketable sizes could have a negative impact on pre-recruits and subsequent catches. Nevertheless, any strategy requires accurate seasonal catch-and-effort data, and knowledge of population parameters to assist in preventing overfishing.

With increasing immersion time, benthic associations become complex, which is also demonstrated by the increased number of organisms present over time. At the first stage of macrofouling, the surface is colonized by fast-growing, frequently colonial foulers, such as hydroids, bryozoans, and serpulids (Figure 11). This stage is developed from 2-3 weeks to 1-2 years. The second stage of macrofouling is represented by slow-growing invertebrates (sponges and mussels).

Although the present research was an approach to biofouling attached to tropical marine aquaculture cages, when comparing the data with the specialized study by Green and Grizzle (2006), it is evident that the biofouling community would follow a classical scheme of settlement (succession). Figure 12 shows a schematic diagram based on this

study where algae and hydroids settled before ascidians, sponges, and other associated organisms. Also, Green and Grizzle (2006) found that hydroids lost their dominance during the early stages of the succession, to be replaced by other organisms. In the present study, as in their study, hydroid coverage on the cages dwindled from 49% through the first month to 13% by the end of the study (Figure 9).

There are no other published studies on biofouling relating specifically to open-ocean aquaculture in the Caribbean; however, other biofouling research indicates that the community follows colonization processes, independent of local conditions. Nevertheless, special environmental conditions may influence a fouling community to have particular characteristics. In tropical regions, such as the Caribbean Sea, seasonal changes are less pronounced and variations in temperature and food availability are mainly related to upwelling events associated with wind conditions. In contrast to coldwater regions, increased primary production is associated with low temperatures (Müller-Karger et al. 1989). Thus, in this study there was not a remarkable difference in percent coverage during seasons as in the Greene and Grizzle (2006) study.

In tropical areas, fouling develops on net cages within just a few weeks after initial immersion (Cheah and Chua 1983, Zongguo 1999, and Hodson 1997). However, biomass data from the present study was unexpected because there were two confounding variables, feeding and antifoulant coating that could have influenced the results. Braithwaite (2007) found that fouling was evident on control netting following approximately 50 days of immersion, but was not observed on antifoulant treated netting until approximately 150 days later. The exposure time in this study may have been too brief, consequently suggesting the reason for the weak data with no significant differences.

2.6 Conclusions

- Biofouling coverage on the snapper (53%) and cobia (51%) cages was not significantly different.

- Biofouling coverage on the cages accumulated throughout the study at a slow rate.
- No significant differences were found for percentage of coverage between downstream and upstream locations of each cage, nor for the locations above and below the cages' rim.
- Types of organisms attached to the sample nettings located above and below the rim were similar; however the proportions of each organism varied.
- The biofouling community on open-ocean aquaculture cages follows a typical colonization processes.
- Biofouling may provide protective habitat for larval fishes.
- Hard-substrate communities developed on man-made structures could serve as recruitment zones for macroorganisms with high aquaculture value, such as lobsters.
- Ecological benefits of biofouling should be considered when developing appropriate prevention and control methods.

2.7 Future Research Suggestions

- Evaluate physical conditions of the environment such as current and light effects before designing sampling methodology to facilitate comparisons of physical variables with the biofouling community and colonization process
- During the early phase, sample weekly during the first month of the biofouling colonization process; thereafter, sampling could occur monthly.
- Include detailed identification of macrobiofouling organisms to provide data to improve management practices using environmentally safe methods to prevent or eliminate detrimental macrofouling from aquaculture cages.
- Utilize the percentage net aperture occlusion (PNO) methodology to calculate fouling coverage with the aid of an image capture-and-analysis system (Braithwaite et al. 2004).

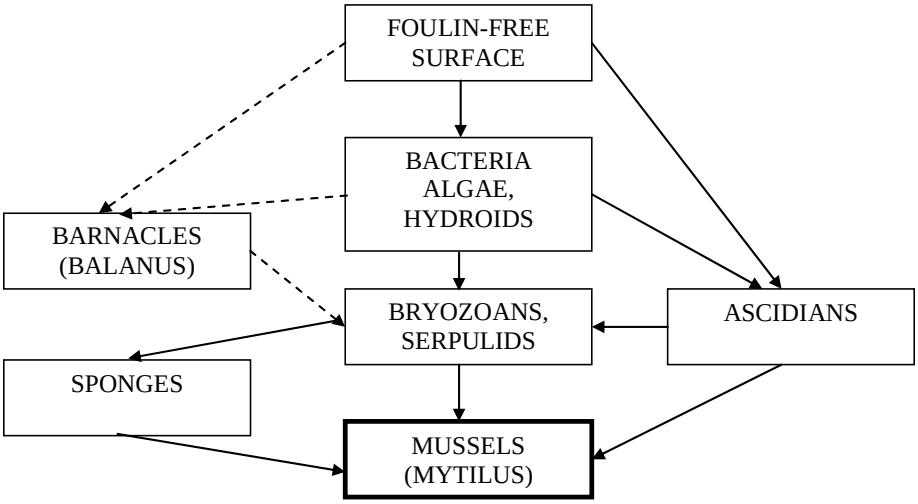

Figure 11. Classical scheme of succession of a biofouling community. Dashed lines show alternative paths of succession, solid lines indicate climax communities. (From Sheer 1945 in Railkin 2004.)

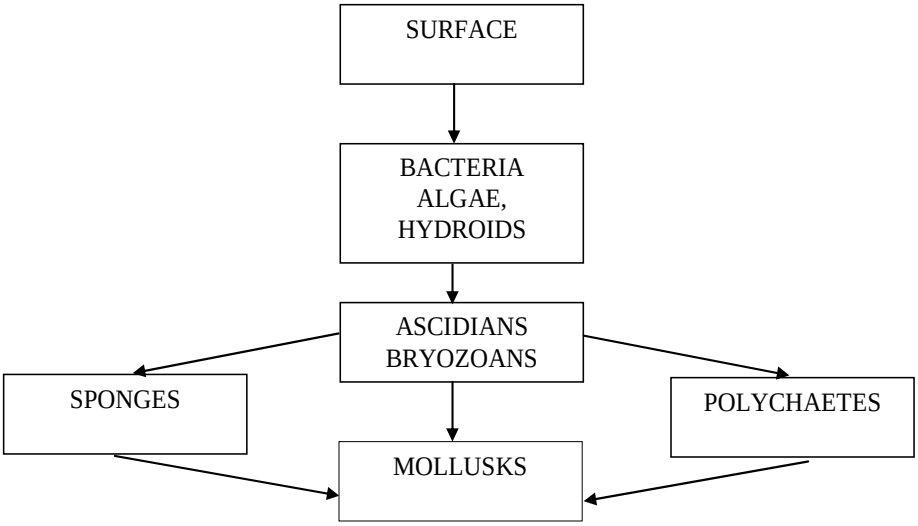

Figure 12. Scheme of organisms' settlement on aquaculture cages in Puerto Rico.

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