

ARTICLE

Composition of the biofouling community associated with oyster culture in an Amazon estuary, Pará State, North Brazil

Composición de la comunidad bioincrustante de un cultivo de ostras tropicales en un estuario del Amazonas, Estado de Pará, Norte de Brasil

Rafael A. das Chagas^{1*}, Mara Rúbia F. Barros¹, Wagner César R. dos Santos¹ and Marko Herrmann¹

¹Amazon Rural Federal University (UFRA), Institute of Social Environmental and Water Resources (ISARH), Av. Presidente Tancredo Neves 2501, Post Box n° 917, Bairro Montese, CEP: 66077-530, Belém, Pará, Brazil. *Corresponding author: rafael@benthos.eu

Resumen.- La contaminación biológica afecta a la acuicultura mundial con importantes impactos de productividad y rentabilidad, especialmente en el cultivo de moluscos, donde tanto la especie de cultivo objetivo como la infraestructura están expuestas a una diversidad de organismos contaminantes. En el cultivo de ostras, el impacto clave es la adherencia directa de organismos causando daño físico, interferencia mecánica, competencia biológica y modificación ambiental, mientras que también se efectúa la infraestructura. El presente estudio describe la composición de la bioincrustación en la superficie de la ostra de mangle *Crassostrea rhizophorae*, cultivada en un estuario Amazónico, ubicado en el estado de Pará, norte de Brasil. En total, 6.124 macroinvertebrados fueron muestreados en julio, agosto, octubre y diciembre de 2013. La epifauna recolectada estuvo representada por 5 grupos principales (Bivalvia, Gastropoda, Polychaeta, Crustacea y Anthozoa), 20 familias y 37 especies. Bivalvia fue la clase más abundante, presentando 5.183 mejillones de la especie *Mytella charruana*. El conocimiento sobre la composición de la incrustación biológica, así como la identificación de las principales especies que causan impactos directos, permite opciones de gestión más personalizadas y estratégicas, minimizando los costos, a menudo significativos, asociados con el control de la bioincrustación (antifouling).

Palabras clave: Macrobentos, epifauna, cultivo de ostras, acuicultura, cultivo de mariscos

Abstract.- Biofouling affects global aquaculture with significant impacts on productivity and profitability, especially in marine shellfish culture, where both the target culture species and/or infrastructure are exposed to a diverse array of fouling organisms. In oyster culture, fouling of stock causes physical damage, mechanical interference, biological competition and environmental modification, with infrastructure also colonized. The present study describes the composition of the biofouling community inhabiting the surface of the mangrove oyster *Crassostrea rhizophorae*, cultivated in an Amazon estuary, in the state of Pará, northern Brazil. In total, 6,124 macroinvertebrates were collected during July, August, October and December 2013. Collected epifauna was represented by 5 groups (Bivalvia, Gastropoda, Polychaeta, Crustacea and Anthozoa), 20 families and 37 species. Bivalvia was the most abundant class, with the mussel *Mytella charruana* by far the most dominant species with 5,183 individuals. Knowledge about the composition of biofouling as well as identifying the main species that cause direct impacts allows more tailored and strategic management options, minimizing the often-significant costs associated with biofouling control (antifouling).

Key words: Macrobenthos, epifauna, oyster farming, aquaculture, shellfish culture

INTRODUCTION

Biofouling or biological fouling is the accumulation of microorganisms, plants, algae, or animals on wetted surfaces, either artificial (e.g., hulls of ships and port facilities) or natural surfaces (e.g., bivalve shells) (Portella *et al.* 2009). The settlement and colonization of these organisms play an important ecological role in aquatic

ecosystems (Nybakken & Bertness 2004, Lacoste & Gaertner-Mazouni 2015). Because of the position they occupy within the food web, they contribute significantly to the decomposition of organic matter and the nutrient cycle (Nybakken & Bertness 2004). The type of substrate available defines the habitat structure, the macrozoobenthos community structure and, consequently, the composition

of the biofouling community, which can vary significantly between natural and artificial surfaces (Connell & Glasby 1999, Fitridge *et al.* 2012, Lacoste & Gaertner-Mazouni 2015).

Aquaculture, especially the malacoculture (mollusc culture), significantly influences ecosystem-level processes by providing additional or alternative surfaces for native macroepifauna (Marenghi *et al.* 2010). Cultured bivalves have similar functions than those living in natural environments (Shumway *et al.* 2003) and provide structure capable of maintaining other living organisms associated with the shells of the oyster at various levels of symbiosis (Kennedy 1996, Lacoste & Gaertner-Mazouni 2015). Biofouling impacts on shellfish culture can be extreme and occasionally devastating, often dependent on geographic location, the shellfish species, and the culture method used (Adams *et al.* 2011, Fitridge *et al.* 2012). Biofouling can reduce farm productivity by organisms competing for space and food with cultured shellfish (Sievers *et al.* 2013, Lacoste & Gaertner-Mazouni 2015). Locations where harmful organisms colonize oysters should be avoided or monitored (Sievers *et al.* 2014), since the mere presence of some of these organisms can impact shellfish culture, due to the consumption of resources and time in trying to combat them or prevent their occurrence, increasing production costs (Chellam 1991, Frigotto 2011).

In Brazil, studies investigating the composition of biofouling communities within bivalve hatcheries are conducted in the southern region, particularly Santa Catarina, the main producer of bivalve molluscs, and Paraná (Chagas 2016). Within these areas, studies have addressed biofouling associations with oysters of the genus *Crassostrea* (Pinto 2007, Frigotto 2011), the scallop *Nodipecten nodosus* (Carraro 2008, Macedo 2012) and the mussel *Perna perna* (Marenzi 2002, Marenzi & Branco 2006, Leite 2007, Macedo *et al.* 2012).

This study describes the composition of the biofouling community associated with the tropical mangrove oyster *Crassostrea rhizophorae*, cultured in the Amazon estuary of the Urindeua River, located in the state of Pará, northern Brazil. With this information, we aim to inform oyster farmers about the temporal occurrence of opportunistic or predatory species that can cause economic losses. Data such as this may improve our ability to predict the settlement particularly of the damaging biofoulers and allow husbandry practices to be strategically applied (*e.g.*, Sievers *et al.* 2014).

MATERIALS AND METHODS

STUDY AREA

The present study was carried out at the oyster hatchery of the Farmers, Fishermen and Aqua Farmers Association of the River Urindeua (Associação dos Agricultores, Pescadores e Aquicultores do Rio Urindeua - ASAPAQ), located in the estuarine area of the hydrographic basin of the Urindeua River (0°41'50.39"S, 47°22'12.45"W), placed at the village Santo Antônio of Urindeua (Fig. 1), municipality of Salinópolis, state of Pará, eastern Amazon, northern Brazil. At the ASAPAQ, *C. rhizophorae* is cultivated by seeds acquired at the community Nova Olinda, municipality of Augusto Corrêa, using suspended lantern cage and rack-and-bag off-bottom culture.

SAMPLING AND LABORATORY PROCEDURES

In total, the biofouling community attached to the surface of 93 oysters was sampled in July (25 oyster), August (21 oyster), October (22 oyster) and December (25 oyster) 2013. Biofouling organisms were carefully removed from the oyster's surfaces using a toothbrush with soft bristles and a low-pressure water jet. The retained biofouling community was sieved over a 1 mm mesh, using the international standard ISO 3310-1 (Rumohr 2009) and fixed afterwards in a 4% formaldehyde solution, buffered with sodium tetraborate decahydrate (borax), labeled, and transported to the laboratory of the Tropical Benthic Ecology research group at the Amazon Rural Federal University (UFRA) in Belém, Pará, Brazil.

In the laboratory, the biofouling community underwent an initial screening process, whereby organisms were separated into broad taxonomic groups, and preserved in 70% ethanol for further quantitative analysis and taxonomic identification under a stereomicroscope. Each organism was identified to the lower possible resolution using a variety of available sources, such as: crustaceans (Coelho & Ramos-Porto 1992, Poore 2004, Bezerra & Júnior 2006, Valencia & Campo 2007, Almeida *et al.* 2013, Soledade & Almeida 2013, Rosa 2014), molluscs (bivalves and gastropods) (Rios 1994, Leme 1995, Abbott & Dance 2000, Leal 2002a, b; Denadai *et al.* 2006, Matthews-Cascon & Lotufo 2006, Barroso *et al.* 2013, Amaral & Simone 2014), cnidaria (Dias *et al.* 2007) and polychaetes (Amaral & Nonato 1981, Uebelacker & Johnson 1984, Amaral & Nonato 1996, Santos & Lana 2001, Saiki 2010, Amaral *et al.* 2013).

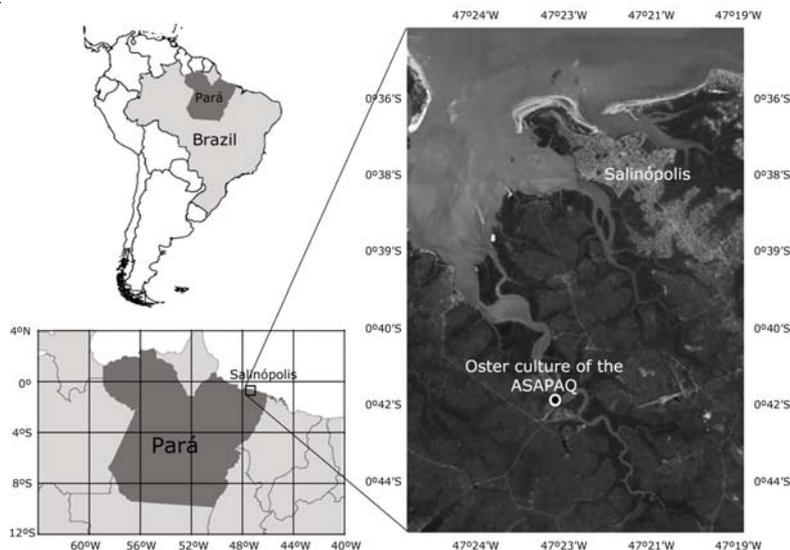


Figure 1. Oyster culture of the Farmers, Fishermen and Aqua Farmers Association (ASAPAQ), located in the Urindeua river, municipality of Salinópolis, Pará, Northern region of Brazil / Mapa de localización del cultivo de ostras de la Asociación de Agricultores, Pescadores y Acuicultores (ASAPAQ), situado en el río Urindeua, municipio de Salinópolis, Pará, norte de Brasil

Specimens of each species/genus were deposited as reference material at the Museum für Naturkunde - Leibniz Institute for Evolution and Biodiversity Science in Berlin¹, Raw data were published on the information system PANGAEA (Chagas & Herrmann 2016), with coloured images of each species/genus available in high-resolution (Tropical Benthic Ecology)².

RESULTS

In total, 6,124 individuals were collected, representing 34 species, 20 families and 5 broad taxonomic groups: Bivalvia, Gastropoda, Polychaeta, Crustacea and Anthozoa (Fig. 2). The highest abundance of fouling organisms on oyster shells was observed in July 2013 (1,624 individuals), and highest richness observed in December 2013 (30 species; Table 1).

The phylum Mollusca was the most abundant group (5,461 ind.) and the phylum Annelida the most diverse (16 spp.), while the phylum Cnidaria was both the least abundant and richness. Among molluscs, the class Bivalvia was responsible for the greatest abundance in the total study, with up to 88.5% of the total organisms (5,420 ind.), followed by the class Polychaeta with a total

of 9.2% (562 ind.). The subfamily Crustacea and the classes Gastropoda and Anthozoa were relatively less abundant, making up, 1.3% (78 ind.), 0.7% (41 ind.) and 0.4% (23 ind.) of the total number of organisms, respectively (Table 1). Only individuals from Bivalvia and Polychaeta were present in all 4 samples, with polychaetes the most diverse class in the study, represented by 16 species, followed by crustaceans and bivalves, with 7 and 5 species, respectively. Among the temporal relationships of biofouling biotic data, an increase in species richness was observed throughout the study, with a slight oscillation in the abundance of individuals (Table 1).

The subfamily Crustacea presented the highest number of families with 7 in total: Alpheidae, Balanidae, Diogenidae, Palaemonidae, Panopeidae, Porcellanidae and Portunidae, each represented by single species. The class Polychaeta is characterised by 6 families: Ampharetidae (three species), Nereididae (10 species), Polynoidae (one specie), Sabellariidae (one specie) and Terebellidae (one specie). The class Bivalvia is presented by 5 families: Corbulidae, Mytilidae, Ostreidae, Tellinidae and Veneridae, each represented by single species. The class Gastropoda presented 2 families: Columbidae (one species) and Muricidae (one species with four different morphotypes). Anthozoans were present with only one family (Actiniidae), represented by one species. Of these families, Mytilidae was most abundant, with 5,183 individuals, followed by the families Nereididae (280 ind.), Sabellariidae (229 ind.), Corbulidae (149 ind.) and Ostreidae (61 ind.).

¹<<http://zmb.sesam.senckenberg.de>>

²<<https://www.benthos.eu>>



Figure 2. Macroinvertebrates associated with surface of the mangrove oyster *C. rhizophorae*: A) *Crassostrea tulipa*, B) *Leukoma pectorina*, C) *Tellina diantha*, D) *Caryocorbula swiftiana*, E) *Mytella charruana*, F) *Stramonita haemastoma* morph. 1, G) *S. haemastoma* morph. 3, H) *S. haemastoma* morph. 4, I) *S. haemastoma* morph. 2, J) *Parvanachis obesa*, K) *Alpheus chacei*, L) *Macrobrachium surinamicum*, M) *Callinectes bocourti*, N) *Clibanarius vittatus*, O) *Sabellaria* sp., P) *Amphitrite* sp., Q) *Namalycastis abiuma*, R) *Alitta succinea*, S) *Perinereis ponteni*, T) *Bunodosoma cangicum*, U) *Amphiteis* sp., V) *Phyllocomus* sp. Scale bar: 1 mm (Q), 3 mm (J, and U), 5 mm (B, C and V), 8 mm (D), 10 mm (K, N, P, Q, R and S), 15 mm (F, G and L), 18 mm (H and I), 20 mm (E, M and T) and 22 mm (A) / Macroinvertebrados asociados con la superficie de la ostra de mangle *C. rhizophorae*. Barras graduadas: 1 mm (Q), 3 mm (J y U), 5 mm (B, C y V), 8 mm (D), 10 mm (K, N, P, Q, R y S), 15 mm (F, G y L), 18 mm (H y I), 20 mm (E, M y T) y 22 mm (A)

Table 1. Abundance of the biofouling community associated with the surface of mangrove oysters *Crassostrea rhizophorae* collected during July, August, October and December 2013. Superscript letters following species names correspond to the images of specimens shown in Figure 2 / Abundancia de los macroinvertebrados asociados a la superficie de la ostra de mangle *Crassostrea rhizophorae* colectado durante julio, agosto, octubre y diciembre de 2013. Las letras después de los nombres de las especies corresponden a las imágenes de los especímenes mostrados en la Figura 2

Taxa	Ref. Mat.	2013				
		Jul	Aug	Oct	Dec	
ANNELIDA						
Polychaeta						
Ampharetidae	<i>Amphicteis</i> sp. ^U	ZMB 11542		2	1	
	<i>Isolda</i> sp.	ZMB 11546			7	
	<i>Phyllocomus</i> sp. ^V	ZMB 11544		3	13	
Nereididae	<i>Alitta succinea</i> (Leuckart, 1847) ^R	ZMB 11532	6	18	5	9
	<i>Namalycastis abiuma</i> (Grube, 1872) ^Q	ZMB 11534	15	4		3
	<i>Neanthes bruaca</i> Lana & Sovierzovsky, 1987	ZMB 11535	1	16	10	2
	<i>Ceratonereis</i> sp.	ZMB 11533				3
	<i>Nereis</i> sp.	ZMB 11537		6	4	6
	<i>Nereis riisei</i> Grube, 1857	ZMB 11536		1		5
	<i>Perinereis anderssoni</i> Kinberg, 1866	ZMB 11538			4	1
	<i>Perinereis ponteni</i> Kinberg, 1866 ^S	ZMB 11539	15	53	54	27
	<i>Perinereis striolata</i> (Grube, 1878)	ZMB 11540			2	3
	<i>Pseudonereis</i> sp.	ZMB 11541			7	
Polynoidae	<i>Harmothoe imbricata</i> (Linnaeus, 1767)	ZMB 11545				1
Sabellariidae	<i>Sabellaria</i> sp. ^O	ZMB 11547		226		3
Terebellidae	<i>Amphitrite</i> sp. ^P	ZMB 11543				26
MOLLUSCA						
Bivalvia						
Corbulidae	<i>Caryocorbula swiftiana</i> (C. B. Adams, 1852) ^D	ZMB Moll 122209			1	148
Mytilidae	<i>Mytella charruana</i> (d'Orbigny, 1842) ^E	ZMB Moll 122200	1,587	2,074	744	778
Ostreidae	<i>Crassostrea tulipa</i> (Lamarck, 1819) (seeds) ^A	ZMB Moll 122205			15	46
Tellinidae	<i>Ameritella diantha</i> Boss, 1964 ^C	ZMB Moll 122207			2	2
Veneridae	<i>Leukoma pectorina</i> (Lamarck, 1818) ^B	ZMB Moll 122208			4	19
Gastropoda						
Columbellidae	<i>Parvanachis obesa</i> (C. B. Adams, 1845) ^J	ZMB Moll 122206				5
Muricidae	<i>Stramonita haemastoma</i> (Linnaeus, 1767) (morph. 1) ^F	ZMB Moll 122201				19
	<i>S. haemastoma</i> (morph. 2) ^I	ZMB Moll 122202		1		10
	<i>S. haemastoma</i> (morph. 3) ^G	ZMB Moll 122203		1		3
	<i>S. haemastoma</i> (morph. 4) ^H	ZMB Moll 122204		2		
ARTHROPODA						
Crustacea						
Alpheidae	<i>Alpheus chacei</i> Carvacho, 1979 ^K	ZMB Crust 29697		2		11
Balanoidae	<i>Amphibalanus amphitrite</i> (Darwin, 1854)	ZMB Crust 29696		2		
Diogenidae	<i>Clibanarius vittatus</i> (Bosc, 1802) ^N	ZMB Crust 29700		1	2	14
Palaemonidae	<i>Macrobrachium surinamicum</i> Holthuis, 1948 ^L	ZMB Crust 29698		1		
Panopeidae	<i>Acantholobulus bermudensis</i> (Benedict & Rathbun, 1891)	ZMB Crust 29695		6	8	14
Porcellanidae	<i>Petrolisthes armatus</i> (Gibbes, 1850)	ZMB Crust 29699		1	9	4
Portunidae	<i>Callinectes bocourti</i> A. Milne-Edwards, 1879 ^M	ZMB Crust 29694		1	2	
CNIDARIA						
Anthozoa						
Actiniidae	<i>Bunodosoma cangicum</i> Belém & Preslercravo, 1973 ^T	ZMB Cni 15978		16	6	1

In terms of individual species, the most abundant was the mussel *Mytella charruana* with 5,183 ind., followed by the polychaetes *Sabellaria* sp. and *Perinereis ponteni*, and the bivalves *Caryocorbula swiftiana* and *Crassostrea tulipa*, with 229 ind., 149 ind., 149 ind. and 61, respectively (Table 1).

Frequency analyses per sampled oyster showed that, *M. charruana* was present in all 4 samples and was found on all 93 collected oysters, followed by *P. ponteni*, *Alitta succinea*, *C. tulipa* and *C. swiftiana*, frequent on 57, 26, 21 and 19 oysters, respectively.

DISCUSSION

The settlement of biofouling in aquaculture occurs on a worldwide scale and is known to affect farm productivity and profitability (Fitridge *et al.* 2012). Determining the composition of biofouling communities within aquaculture regions is the first step before conducting manipulative studies on how these organisms may influence production (Frigotto 2011).

Globally, bivalve aquaculture is affected by biofouling via settlement on the shell surface of cultivated target species and/or on the culture systems, which can be submersed (*e.g.*, long line) or fixed in intertidal zones (*e.g.*, tables with pillows, lanterns and bags) (Fitridge *et al.* 2012, Sievers *et al.* 2013, 2014; Lacoste & Gaertner-Mazouni 2015, Chagas 2016).

The impact of biofouling on bivalve cultures is complex and may result in negative or positive outcomes. The negative impacts caused by the development of biofouling can represent up to 30% of operating costs (Lacoste *et al.* 2014, Lacoste & Gaertner-Mazouni 2015). Furthermore, biofouling control methods can be stressful and detrimental to cultivated species, leading to reduce stock fitness and likely economic implications to farmers (Sievers *et al.* 2017). Typical biofouling control strategies involve the periodic cleaning of stock shells, mainly because the presence of these influences the visual appearance of the bivalves, which makes the commercialization challenging (Fitridge *et al.* 2012, Lacoste *et al.* 2014, Lacoste & Gaertner-Mazouni 2015, Chagas 2016).

Given the challenges and costs of biofouling control, documenting fouling patterns may provide an alternate, less-intensive strategy for farmers to help deal with biofouling. Studies that address the quantitative aspects of biofouling development are, however, scarce, and published information on colonization over time is limited

(but see Woods *et al.* 2012, Sievers *et al.* 2014, Atalah *et al.* 2017).

The initial composition of biofouling in bivalve aquaculture generally includes sponges, barnacles, serpulid worms, ascidians, bryozoans, hydroids, algae and other bivalves (Dürr & Watson 2010, Fitridge *et al.* 2012, Sievers *et al.* 2014). Subsequently, this primary colonization allows the insertion of other organisms, such as, for example, polychaetes, crustaceans or echinoderms (Mazouni *et al.* 2001, Sá *et al.* 2007, Mallet *et al.* 2009). These authors comment that secondary colonization often occurs months after initial biofouling colonization. However, in the present study, polychaetes were collected from oyster surfaces within month, and crustaceans in the following month. This difference could be explained by the complexities of the recruitment and settlement of marine invertebrates (Keough 1984). Colonization patterns of biofouling communities can be influenced by the geographic zone, environmental factors, the characteristics and reproductive periods of the colonizers, as well as their intra or interspecific food relations, type of substrate, depth (Gribben *et al.* 2006, Underwood & Chapman 2006, Sievers *et al.* 2013, 2014). In addition, colonization and succession patterns of biofouling vary according to climatic zones. For example, in tropical areas, biofouling typically settles constantly throughout the year, whereas it is more periodic in temperate waters (Fitridge *et al.* 2012, Lacoste & Gaertner-Mazouni 2015). Therefore, patterns in the colonisation of biofouling communities are likely to vary greatly amongst farming locations, highlighting the necessity for local-scale surveys.

Although biofouling composition studies, as well as the impacts of biofouling are important, it is important to identify those species that cause direct impacts, as biofouling management strategies can be tailored to the removal of specific species, reducing the cost of execution (Sievers *et al.* 2013).

Polychaetes are often the most abundant taxonomic group observed in benthic macroinvertebrate communities within coastal regions around the world (Diener *et al.* 1995, Probert *et al.* 2001, Ellingsen 2002). Although the ecological importance of this group is generally known, there is a lack of knowledge on how individual species affect shellfish aquaculture operations (Chagas 2016). In their review, Fitridge *et al.* (2012) highlight two genera, *Polydora* and *Boccardia*, are common biofoulers that can cause impacts to bivalve culture by damaging the shells, reducing aesthetics and ultimately impacting the saleability of the product (Campbell & Kelly 2002).

High mussel abundance observed in the studied oyster culture has been observed elsewhere; Costa *et al.* (2007) observed high settlement of *Mytella* mussels within oyster culture, while Sievers *et al.* (2014) observed distinct temporal peaks in the settlement of *Mytilus* spat within mussel long-line culture. Significant fouling by mussels is detrimental for oyster farming, as mussels and oysters can compete for space and food, which can lead to reduced survival and growth of cultured oysters (Lacoste & Gaertner-Mazouni 2015, Chagas 2016). Competition for food may mean that the cultivated bivalve does not receive sufficient food resources, and requires a longer period to develop to commercial size (Fitridge *et al.* 2012).

In the same way the presence of the crab *Petrolisthes armatus* is harmful to bivalve farming, since the high densities of this crab cause an excess of movement on the oysters, providing a high stress on the culture, the survival rate is concerned. However, there are reports of oyster farmers using *P. armatus* to control the biofouling, extending ropes that facilitate the climbing of the crab (Hollebone & Hay 2007).

The presence of the gastropod family Muricidae in the biofouling composition is another unfavorable factor that can affect oyster farming. This family is a specialized predator of bivalves, able to drill into their shells and consume the visceral mass (Ruppert & Barnes 1996). During this study, we observed predation by the rock snail *Stramonita haemastoma* on cultured oysters. Documenting temporal patterns in the settlement of this family is therefore critical to farmers, since removal strategies should be implemented as soon as possible.

The knowledge of the species that compose biofouling is important, as well as the identification of dominant species and these that have a direct impact on the cultivated oyster, so that appropriate management measures can be implemented to mitigate the negative impact of these species on oyster farming. Such a negative impact may occur through competition for space and/or food and by predation that will lead to increased mortality rates of cultured bivalves. Moreover the abundance of biofouling on the surface of the cultivated oysters rush into a damaging structure of the shells which will influence in the final price marketed.

ACKNOWLEDGMENT

The authors thank to the members of the Farmers, Fishermen and Aqua Farmers Association of the River Urindeua (ASAPAQ) of St. Anthony Village Urindeua on

behalf of its former president Mr. Anthony and the current president D^a Maria, the National Counsel of Technological and Scientific Development - CNPq for partial funding, the Amazon Rural Federal University for supplying the transport for fieldwork and the members of the research group Tropical Benthic Ecology².

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Received 21 November 2016 and accepted 30 November 2017

Editor: Claudia Bustos D.