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IDENTIFICAÇÃO DE ESPÉCIES, BIOLOGIA REPRODUTIVA E DINÂMICA POPULACIONAL DE CORAIS DO GÊNERO *TUBASTRAEA* NO SUDESTE DO BRASIL

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Tese apresentada ao Programa de Pós-Graduação em Dinâmica dos Oceanos e da Terra – DOT da Universidade Federal Fluminense - UFF, como parte dos requisitos para a obtenção do grau de Doutor em Ecologia Marinha, sob orientação do Professor Doutor Ricardo Coutinho.

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RESUMO

O presente estudo tem como propósito gerar informações sobre o atual cenário da invasão dos corais do gênero *Tubastraea* no Oceano Atlântico Ocidental. Os processos que envolvem o estabelecimento e aumento da distribuição foram abordados neste estudo. A estratégias de história de vida dos invasores foram verificadas por meio da biologia reprodutiva, taxas de crescimento, dados de assentamento e recrutamento, que são os principais mecanismos regulatórios no processo de invasão. Três enfoques foram dados no presente estudo: (1) esclarecer sobre a diversidade morfológica e genética de corais do gênero de *Tubastraea* spp. a partir de populações do hemisfério sul e norte. Uma abordagem morfológica de macro e microestruturas complementada com a genética molecular (gene ITS) para caracterizar a diversidade dos corais *Tubastraea*; (2) investigar as estratégias de história de vida a partir das taxas de crescimento, de fecundidade das três espécies dos corais, além de avaliar períodos de maior assentamento e porcentagem de cobertura em campo relacionando-os com dados de temperatura e luminosidade local e (3) avaliar aspectos da biologia reprodutiva avaliando a produção de gametas, períodos de picos reprodutivos, fecundidade e autonomia larval para esclarecimento da atividade reprodutiva numa região de ressurgência. Foram delimitadas e identificadas três espécies distintas: *Tubastraea aurea*, *Tubastraea coccinea* e *Tubastraea* sp. Morfologicamente, *T. aurea* é diferente de *T. coccinea* devido a fortes indícios macro e micro morfológicos, principalmente devido ao quinto ciclo de septos, não existente em *T. coccinea*. *Tubastraea* sp. de Arraial do Cabo, anteriormente reconhecido como *Tubastraea tagusensis*, exibiu características morfológicas distintas em comparação ao holótipo de Wells (1982) e como ainda não foi identificada chamou-se de *Tubastraea* sp. Análises moleculares mostraram que os corais do gênero no Brasil caíram em dois clados monofiléticos bem suportados e amostras coletadas nos Estados Unidos se sobrepuseram em ambos os clados, além de apresentar maior diversidade genética. Os corais demonstraram um processo de colonização desenvolvido na Baía de Arraial do Cabo, ocupando margens rochosas, inclusive na região entremarés. *Tubastraea coccinea* aumentou sua cobertura e cresceu mais do que o relatado em estudos anteriores na região. O crescimento entre espécies foi inversamente proporcional à fecundidade. Temperaturas mais baixas favorecem o crescimento e podemos ver um padrão diretamente proporcional de taxa de assentamento e aumento da amplitude térmica. Em diferentes ensaios, encontramos altas taxas de fecundidade, ocorrência de pelo menos dois ciclos gametogênicos por ano, produção contínua de gametas, incubação de larvas e vários eventos de planulação. O sucesso na introdução e estabelecimento dos corais do gênero *Tubastraea* é resultado das estratégias oportunistas identificadas nesses organismos, como altas taxas de crescimento e incremento de pólipos, elevada produção de gametas; vários eventos de planulação, longos períodos de assentamento e ampla tolerância às variações ambientais. Nossos resultados demonstraram a ocorrência de uma terceira espécie do gênero invasor no litoral brasileiro e esclareceu sobre os processos da invasão bem-sucedida dos corais *T. aurea, T. coccinea* e *Tubastraea* sp. na região de Arraial do Cabo, RJ.

Palavras-chave: Bioinvasão; Taxonomia Integrativa; Dinâmica Populacional; Reprodução; Coral-sol.

ABSTRACT

The present study aims to generate information about the current scenario of the invasion of corals of the genus *Tubastraea* in the Western Atlantic Ocean. The processes that involve establishing and increasing distribution were addressed in this study. The invader's life-history strategies were verified through reproductive biology, growth rates, settlement and recruitment data, which are the main regulatory mechanisms in the invasion process. Three approaches were given in the present study: (1) clarifying the morphological and genetic diversity of corals of the genus *Tubastraea* spp. from populations in the southern and northern hemispheres. A morphological approach of macro and microstructures complemented with molecular genetics (ITS gene) to characterize the diversity of *Tubastraea* corals; (2) investigate life-history strategies based on growth rates, the fecundity of the three coral species, in addition to assessing periods of greater settlement and percentage of coverage in the field, relating them to local temperature and luminosity data and (3) evaluate aspects of reproductive biology by assessing the production of gametes, periods of reproductive peaks, fecundity and larval autonomy to clarify reproductive activity in a upwelling region. Three distinct species were delimited and identified: *Tubastraea aurea*, *Tubastraea coccinea*, and *Tubastraea* sp. Morphologically, *T. aurea* is different from *T. coccinea* due to strong macro and micromorphological evidence, mainly due to the fifth cycle of septa, which does not exist in *T. coccinea*. *Tubastraea* sp. from Arraial do Cabo, formerly recognized as Tubastraea tagusensis, exhibited distinct morphological characteristics compared to the holotype of Wells (1982) and as it has not yet been identified it was called *Tubastraea* sp. Molecular analyzes showed that corals of the genus in Brazil fell into two well-supported monophyletic clades and samples collected in the United States overlapped in both clades, in addition to presenting greater genetic diversity. The corals demonstrated a colonization process developed in Arraial do Cabo Bay, occupying rocky margins, including in the intertidal region. *Tubastraea coccinea* increased its coverage and grew more than reported in previous studies in the region. The growth between species was inversely proportional to fecundity. Lower temperatures favor growth and we can see a directly proportional pattern of settlement rate and increase in thermal amplitude. In different trials, we found high fecundity rates, the occurrence of at least two gametogenic cycles per year, continuous production of gametes, incubation of larvae and various planulation events. The success in introducing and establishing corals of the genus *Tubastraea* is the result of the opportunistic strategies identified in these organisms, such as high growth rates and increase in polyps, high production of gametes; various planulation events, long settlement periods and wide tolerance to environmental variations. Our results demonstrated the occurrence of a third species of the invasive genus on the Brazilian coast and clarified the processes of the successful invasion of the corals *T. aurea*, *T. coccinea* and *Tubastraea* sp. in the region of Arraial do Cabo, RJ.

Keywords: Bioinvasion; Integrative Taxonomy; Population Dynamics; Reproduction; Sun-Coral.

SUMÁRIO

INTRODUÇÃO GERAL

Invasão biológica é um conceito que compreende a chegada e proliferação de uma espécie não-nativa capaz de gerar desequilíbrio ecológico. A espécie invasora passa a competir com as espécies nativas afetando o meio ambiente, a economia e até mesmo gerando riscos à saúde humana (Bax, et al. 2003, Ruiz e Carlton, 2003). Conhecer a origem dos vetores é imprescindível para desenvolver estratégias de prevenção e manejo. Além disso, para entender os processos de invasões biológicas devemos avaliar a história de vida das espécies e investir no conhecimento sobre a distribuição geográfica, mecanismos reprodutivos, genética e sistemática auxiliando a descobrir a ancestralidade e a origem dos organismos (Avise, 2000, Kolar e Lodge, 2002, Lodge, et al. 2006, Bernardi, et al 2010).

Espécies exóticas podem afetar as espécies nativas e a comunidade local através da hibridização, competição por recursos, predação, além de mudanças na estrutura da comunidade através do aumento quantitativo do invasor (Maida, et al. 1995, Lages, et al. 2006). Os corais do gênero *Tubastraea* vêm promovendo uma série de impactos ecológicos já registrados. Miranda e colaboradores (2016), descreveram a morte do tecido dos corais nativo *Siderastrea stellata*, *Mussismilia hispida* e *Madracis decactis* quando competem por espaço com corais do gênero *Tubastraea* spp. Estudos anteriores também mostraram o efeito negativo de corais *Tubastraea* spp. sendo capazes de alterar comunidades bentônicas e ameaçar espécies nativas (Lages, et al. 2011, Riul, et al. 2013).

Há uma grande preocupação devido a rápida dispersão, facilidade de colonização de novos habitats, estratégias de defesa e reprodução desses corais. A espécie *T. coccinea* nos dias atuais encontra-se aumentando sua distribuição através da costa do México e leste do Pacífico (Reyes-Bonilla, et al. 1997), em Fiji (localização tipo; Wells, 1982), Costa Rica, Colômbia, Mar Vermelho (Prahl, 1987, Cairns, 1991), México costa do

Pacífico (Reyes-Bonilla, et al. 1997), Belize e Cozumel (Fenner, 1999), Golfo do México e Flórida Keys (Fenner e Banks, 2004, Sammarco, et al. 2004). A espécie é reconhecida por Sammarco e colaboradores (2010) como o coral mais abundante em substratos artificiais no Golfo do México e tem sido observado o aumento de sua cobertura e densidade na costa brasileira (Ferreira, 2003, De Paula e Creed, 2004, Kitahara, 2006, Mantelatto, et al. 2011, Costa, et al. 2014, , Silva, et al. 2014, Batista, et al. 2017). No Brasil, a espécie foi avistada pela primeira vez no final da década de 1980 em uma plataforma de petróleo situada na Bacia de Campos, no norte do Estado do Rio de Janeiro (Castro e Pires, 2001). As primeiras observações em substratos naturais no Brasil, especificamente nos costões rochosos costeiros, foram feitas no final da década de 1990 nas regiões da Baía de Ilha Grande e Arraial do Cabo, Rio de Janeiro (De Paula e Creed, 2004, Ferreira, 2003) após este período a espécie já foi registrada nos estados da Bahia, Santa Catarina e São Paulo e novas áreas no estado do Rio de Janeiro (Creed, et al. 2016, Costa, et al. 2014, Silva, et al. 2011, Mantellato, et al. 2011, Sampaio, et al. 2012, Capel, 2012). A espécie *Tubastraea tagusensis* Wells (1982), tem registro original no Arquipélago de Galápagos, ocorrendo entre 3- 43 m de profundidade (Wells 1982). No Brasil, *T. tagusensis* foi registrado até 15 metros em Ilhabela (Mantelatto, et al. 2011) e até 22 m em Salvador (Sampaio, et al. 2012). Recentemente a espécie foi registrada no Golfo do México por Figueroa, et al. (2019).

Devido a habilidade para dispersão e facilidade para colonizar novos ambientes (Glynn, et al. 2008) os corais *Tubastraea* spp. vêm aumentando sua distribuição nos costões rochosos de Arraial do Cabo. O registro e a expansão inicial da distribuição de *Tubastraea coccinea* na região foi documentado por Ferreira (2003) e recentemente atualizado por Batista e colaboradores (2017). Acredita-se que os corais *Tubastraea coccinea* Lesson, 1829 e *Tubastraea tagusensis* Wells, 1982 foram introduzidos no Brasil

através de plataformas de petróleo e representam as primeiras introduções de escleractíneos no Atlântico Sul (De Paula e Creed, 2004).

Os corais utilizam um conjunto de recursos energéticos para serem compartilhados entre uma variedade de funções vitais incluindo reprodução sexual e assexuada, crescimento, manutenção e reparo. Existe uma dificuldade na identificação de qual e como estes recursos são alocados para cada função. A reprodução e o crescimento são funções particularmente importantes e competem potencialmente por recursos remanescentes após o requerimento para manutenção e reparo ter sido realizado (Harrison e Wallace, 1990). Taxas de crescimento em muitas espécies de corais diminuem com o aumento do tamanho e idade e tem sido proposto que isto pode ser causado pelo início da reprodução sexual e aumento da fecundidade. O desenvolvimento de gametas ou plânulas pode competir por espaço com o requerimento de alimento no interior do pólipo (Harrison e Wallace, 1990). Espécies que liberam gametas para fertilização e desenvolvimento externos habitualmente sofrem um ciclo único de gametogênese a cada ano, visto que a maioria das espécies incubadoras de larvas possui múltiplos ciclos gametogênicos (Harrison e Wallace, 1990). Em muitos corais a ovogênese é iniciada primeiro do que a espermatogênese dentro de cada ciclo reprodutivo, com uma diferença de poucos meses, para que subsequentemente os dois gametas estejam maduros no mesmo momento. A gametogênese é frequentemente sincronizada com cada colônia ou coral solitário e parcialmente sincronizado com os membros de populações reprodutoras (Harrison e Wallace, 1990). O estudo da reprodução sexual e biologia larvar é importante para um melhor entendimento da ecologia e história de vida dos escleractíneos (Fadlallah, 1983).

Apesar dos inúmeros registros de ocorrência de *Tubastraea* no Brasil e no mundo esses corais ainda têm uma taxonomia e história sistemática confusa. Por este motivo, uma melhor avaliação das características taxonômicas do gênero, bem como as

características macro e microestruturais, foi importante para a caracterização e demarcação das espécies locais. No atual estudo, foi realizada uma abordagem molecular integrativa com a relação entre populações do Atlântico ocidental caracterizando o gene ITS em amostras da Flórida que foram correlacionadas com amostras de Arraial do Cabo e demais sequências do Genbank para esclarecer sobre a relação entre as populações.

Nos últimos anos aumentou-se o interesse por questões sobre a diversidade genética dos corais do gênero *Tubastraea*, mas por outro lado, estudos morfológicos, reprodutivos ou sobre os processos conducentes ao recrutamento têm sido menos explorados, principalmente relacionado à *Tubastraea tagusensis*. Na abordagem sobre estratégias de história de vida, taxas de crescimento e de fecundidade, características diretamente relacionadas com o potencial de invasão, três espécies dos corais do gênero *Tubastraea* foram exploradas. As taxas de assentamento e porcentagem de cobertura em substrato manipulado em campo foram correlacionadas com dados de temperatura e luminosidade do local para avaliar a dinâmica do estabelecimento. A avaliação do padrão de comportamento da larva, assentamento e recrutamento são de extrema importância para a compreensão dos mecanismos regulatórios das populações e das relações ecológicas existentes. Portanto, um melhor entendimento desses eventos é imprescindível para uma melhor compreensão da dinâmica do processo de invasão. Os resultados ajudaram a esclarecer os processos relacionados ao estabelecimento e sobre a amplitude da expansão desses corais nos costões rochosos de Arraial do Cabo, local considerado *hotspot* de biodiversidade e influenciado pela ressurgência.

O estudo dos aspectos da biologia reprodutiva dos corais *Tubastraea* spp. dos costões rochosos de Arraial do Cabo foi realizado para esclarecer sobre as habilidades no investimento de energia para a produção de gametas, períodos de picos reprodutivos, épocas de liberação de larvas e tempo de sobrevivência da larva das três espécies. Essas

informações comparadas com os estudos anteriores sobre o comportamento e aptidão dos corais quanto ao potencial de invasão fornece um entendimento do cenário atual e futuro dos processos de colonização e potencial de dispersão desses escleractíneos invasores do oeste do Atlântico. Este estudo teve a finalidade de gerar conhecimento sobre a diversidade dos corais *Tubastraea* e sobre aspectos biológicos e ecológicos que definem sua história de vida a fim de gerenciar a invasão adequadamente. Possivelmente, temos outra espécie do gênero *Tubastraea* na região que ainda não foi descrita. É essencial delimitar corretamente as espécies de corais invasores encontradas na baía de Arraial do Cabo para monitorar o processo de bioinvasão. As médias de temperatura mais baixa dentro da baía, comparadas aos outros locais onde os corais do sol ocorrem na costa brasileira, podem afetar os processos fisiológicos dos corais que demonstram atividade reprodutiva e colonização mais restritas na região.

Lista de artigos

Esta tese é baseada nos seguintes artigos, referenciados no texto, através de capítulos em numeração romana:

I. Nathália D. Bastos, Sávio H. Calazans, Luciana Altvater, Elisabeth G. Neves, Vinícius Padula, Alexa L. Trujillo, Willian C. Sharp, Eric A. Hoffman, Ricardo Coutinho. (2019). A western Atlantic invasion of sun corals: Using morphology and genetics to refine species identification. Submissão no periódico Plos One.

II. Nathália D. Bastos, Layla T. Poubel, Ricardo Coutinho. Life history strategy of *Tubastraea* spp. corals in an upwelling area on Southwest Atlantic: *Growth Rate, Fecundity, Settlement and Recruitment.* Submissão no periódico Aquatic Invasions.

III. Nathália D. Bastos, Carolina Terra, Ricardo Coutinho. Reproductive biology aspects of three species of invasive corals in an area affected by the upwelling in southeastern Brazil. Submissão no periódico Biological invasions.

A colaboração dos co-autores incluiu o auxílio durante aquisição de dados no campo, coleta de indivíduos, atividades laboratoriais, análise de dados e escrita.

CHAPTER 1

A western Atlantic invasion of sun corals: Using morphology and genetics to refine species identification

Nathália D. Bastos [1], Sávio H. Calazans [1], Luciana Altvater [1], Elisabeth G. Neves [2], Vinícius Padula [3], Alexa L. Trujillo [4], Willian C. Sharp [5], Eric A. Hoffman [4], Ricardo Coutinho [1]

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Running Title: Morphology and genetics of *Tubastraea* corals

Keywords: Bioinvasion; *Tubastraea aurea*; *Tubastraea coccinea; Tubastraea tagusensis*; Integrative taxonomy; Ribosomal DNA; Orange cup corals.

ABSTRACT

Scleractinian corals in the genus *Tubastraea* historically occurred throughout the Indo-Pacific but are currently globally dispersed owing to anthropogenic spread. Although morphologically diverse, *Tubastraea* corals have a confusing taxonomy because overlapping morphological characters occur across species. Currently, two species of *Tubastraea* are known along the Brazilian coast: *T. coccinea* (registered in Arraial do Cabo) and *T. tagusensis* (not registered to occur in Arraial do Cabo). Additionally, many morphotypes have been observed but not characterized along the southeast coast of the United States. Here we sought to investigate the taxonomy of *Tubastraea* species found throughout the western Atlantic based on both morphological and molecular characters (ITS gene). In this study, we observed three morphotypes in Arraial do Cabo – Brazil, that were delimitated and identified as belonging to three distinct species: *T. aurea*, *T. coccinea* and *Tubastraea* sp. Morphologically, *T. aurea* is different from *T. coccinea* due to larger corallites, presenting one more cycle of septa, S_5, that is the same size or larger than the forth cycle S_4. *Tubastraea* sp., previously known as *Tubastraea tagusensis*, exhibited distinct morphological characteristics compared to the other two species. Molecular analyses showed that Brazilian *Tubastraea* fell into two well supported monophyletic clades and samples collected from the United States overlapped in both clades. Moreover, United States samples showed greater diversity, presenting haplotypes in at least four other internal clades. This study highlights the need for an integrative approach to further examine species delimitation in *Tubastraea*, which is essential for the clarification and management of bioinvasions events by sun coral species.

1. INTRODUCTION

In the marine environment the main facilitators of exotic species introduction are, among others, are the ship traffic and oil rigs [1, 2]. Indeed, several studies have confirmed the transportation of hundreds of non-native species on the ship hulls and in ballast water tanks [3 - 6]. The establishment of numerous oil platforms and associated boats near coastal areas has allowed fouling organisms to use these gigantic hard substrates as new areas for colonization [7] and possible stepping-stone pathways for range expansion, especially when the rigs move from port to port [8]. These exotic organisms generally have competitive advantages and benefit from the absence of predators, allowing them to threaten the survival of native species, especially in fragile and degraded environments [9, 10]. Furthermore, exotic species can cause ecological changes in the long-term, reducing the natural ability of ecosystems to recover to pre-invasion conditions [11].

Scleractinian corals are considered sensitive to environmental changes [12] and this is believed to limit the distribution of these organisms into non-native habitats [13]. Despite this, over the past few decades, some species of azooxanthellate scleractinian corals of the genus *Tubastraea* have become globally widespread, overcoming important dispersal barriers and revealing themselves as organisms of great invasive ability. Corals of this genus have come to epitomize a successful invasion in the Atlantic. *Tubastraea* corals are naturally distributed throughout the Indo-Pacific, mostly observed in tropical shallow-water environments [14]. They are popularly known as orange cup corals and/or sun corals, due to the brightly colored tissue of the dendroid polyps, particularly when tentacles are expanded during feeding. According to Cairns (2000) [14], the earliest record *Tubastraea* in the western Atlantic was *Tubastraea coccinea* Lesson, 1829 in 1930's, in the Caribbean (Puerto Rico and Curacao), verified by Boschma (1953) [15]

through unpublished material collected by Vaughan and Wells (1943) [16]. In Brazil, Castro and Pires (2001) [17] recorded *Tubastraea* in offshore oil rigs in Campos basin in the late 80s. Further studies have confirmed that *Tubastraea coccinea* has also continued expanded its distribution into both the Caribbean [18], including Flower Garden Bank Marine Sanctuary [19], South Florida artificial structures [20] and in Brazil [17, 21] during the early 2000s. Currently, *Tubastraea coccinea* and *Tubastraea tagusensis* Wells, 1982 are reported as exotic organisms from northeastern to southern Brazil [22]. *T. coccinea* was recorded initially from coastal rocky shores at Ilha Grande Bay and Arraial do Cabo, both in the State of Rio de Janeiro [21, 23]. Later, *T. coccinea* was recorded from different regions along the Brazilian coast, from Ceará to Santa Catarina State, on artificial and natural substrates [24 - 29]. Additionally, *Tubastraea micranthus* Ehrenberg, 1834, another exotic species in the western Atlantic, was originally described from the Philippines, and later recorded in the Gulf of Mexico [14, 30]. The continuously expanding distribution for *Tubastraea* in the western Atlantic has become a concern due to possible impacts to the native benthic community and interference with ecosystem balance [31, 32].

Despite the records of different species and occurrences of *Tubastraea* in the western Atlantic, there is still the demand for a better evaluation on the taxonomic characteristics of the genus. Usually, the identity of the colonies remained mostly based on their external shape and color. However, *Tubastraea* corals have a confusing taxonomy and systematic history, with different studies questioning the validity of some species (i.e. morphological convergence and/or phenotypic plasticity) [15, 33, 34]. On the other hand, previous phylogenetic analysis based on mitochondrial DNA (COI sequence data) pointed to the monophyly of the family Dendrophylliidae, to which *Tubastraea* belongs and include a specimen of *Tubastraea aurea* and *Tubastraea coccinea* in the

phylogenetic analysis [35]. A recent integrative phylogenetic study of the Dendrophylliidae, based on both nuclear and mitochondrial molecular markers as well as microstructural features (e.g., skeleton fiber arrangement, characteristics of septal teeth and granules) also pointed to monophyly of the family and, of the genus *Tubastraea* and listing *T. aurea* and *T. coccinea* as distinct species [36].

The primary aim of this study was to characterize both the morphology and genetics of *Tubastraea* species found in the western Atlantic, from collections made in one location in the southern hemisphere and another one in the northern hemisphere. Previous publications have listed only two species of *Tubastraea* in southeastern Brazil: *Tubastraea coccinea* and *T. tagusensis* [21]. However, we observed three morphotypes in Arraial do Cabo in southwestern Brazil. Two morphotypes, which exhibit plocoid arrangement, are commonly considered as variations of *T. coccinea* [23] and a third morphotype, with phaceloid arrangement and spaced and extended corallites, has been referred to as *T. tagusensis*. At the northern end of the *Tubastraea* species invasion, two species have been previously characterized as having invaded the Gulf of Mexico, *T. coccinea* and *T. micranthus* [20, 37, 38]. Specifically, this study sought to clarify the identity of *Tubastraea* spp. from both southern (i.e. Brazilian) and northern (i.e. southeast United States) populations. We combined morphological characters of macro and microstructures and molecular genetics (ITS gene) to characterize the diversity of *Tubastraea*. We address two questions: 1) how well do morphology and genetics align to demarcate species in the southern region, and 2) how does genetic diversity of western Atlantic *Tubastraea* compare between Brazil and the southeast United States?

2. MATERIAL AND METHODS

2.1. Study area

In Brazil we sampled sun corals in Arraial do Cabo Marine Extractive Reserve, Rio de Janeiro State (22° 57’ S - 42° 1’ W), a sheltered bay area that supports a highly diversified subtidal benthic community. Arraial do Cabo is a transitional zone between the meridional tropical and the warm temperate Southwestern Atlantic Ocean [39], with 22 °C in average water temperature inside the bay [40], a limit for many species, including corals [17, 41]. Arraial do Cabo region is influenced by an intermittent upwelling system, a deep, cold, and nutrient-rich South-Atlantic Central Water (SACW) that rises towards the sea surface as a result of the coastal morphology and constant northeasterly trade winds ($10 m.s^{-1}$) that pushes Coastal Water offshore and favor the Ekman transport and the rise of SACW [42, 43]. Due to increasing offshore oil exploitation, the region has seen an increase in oil platforms and associated boat traffic, thus promoting the arrival and establishment of non-native species [40]. Associated with the influx of alien species, three morphotypes of *Tubastraea* occur in sympatry at Arraial do Cabo making it an ideal location to evaluate species that possibly represent the southwest Atlantic invaders corals. The sites sampled in the southeastern United States are located along the coast of Florida, USA. Florida forms a peninsula that projects southward into tropical marine waters, generating transitions of faunal compositions between hot temperate Atlantic and Mexico Gulf faunas in the Carolingian zoogeographic province [44 - 46]. In this region, *Tubastraea* spp. is recorded throughout the Gulf and Atlantic coast to several artificial reef sites in the Florida Keys [38].

2.2. Sampling

For preliminary identification in the field, the main characteristics used to differentiate among *Tubastraea* species was polyp size and coenosarc color. The three

morphotypes of *Tubastraea* from Brazil were preliminarily identified as two morphotypes of *T. coccinea*, and one of *T. tagusensis.* Colonies of these three morphotypes were collected at two neighboring sites, Porcos Island (22º96'S 41º98'W) and Saco do Anequim (22º98'S 41º98'W), by scuba diving (SISBIO authorization #51.094). A total of 41 specimens were collected: 15 specimens of *T. coccinea* - morphotype I (Porcos Is. = 5, Saco do Anequim = 10), 16 specimens of *T. coccinea* - morphotype II (Porcos Is. = 6, Saco do Anequim = 10) and 10 specimens of *T. tagusensis* morphotype III (all from Porcos Is.) at a maximum depth of 10m. All samples were carefully removed from the substrate avoiding skeletal fragmentation. Samples from 152 colonies were collected in Florida from four locations, three in the Florida Keys [Upper Keys, n=32, Duane Wreck, sunk 1987, (24°59'22.7"N 80°22'55.2"W) / Middle Keys n=60, (A) - Artificial Reef, sunk 1986-87, 1994, 1996 (24°43'34.5"N 80°49'50.7"W) and (B) - Thunderbolt Wreck, sunk 1986 (24°39'28.9"N 80°57'54.0"W) / Lower Keys, n=30, Vandenberg Wreck, sunk 2009 - 24°33'35.7"N 81°48'17.1"W) and one from the Northern Gulf of Mexico n=30, near the Florida Panhandle, Oriskany Wreck, sunk 2006 (30°02′33″N 87°00′23″W). Samples in Florida were authorized under permit FKNMS-2016-016 issued by the Florida Keys National Marine Sanctuary. Tissue fragments were obtained from all colonies of *Tubastraea* collected in Brazil and Florida populations for DNA analysis.

2.3. Morphological characterization

Skeletons were cleaned and preserved by bleaching of the 41 colonies in a 4% sodium hypochlorite (NaOCl) solution for tissue removal and up to six corallites were randomly selected from each corallum for morphological analysis. The characters used in the morphological assessment were: largest (CorDma) and shortest (CorDmi) diameter corallum; number of polyps (PN); largest (CollDma) and shortest (CollDmi) diameter corallite; number of septa (SN); corallite projection (CP); depth of columellar fossa

(FCD); largest (ColmDma) and shortest (ColmDmi) columella diameter; space between columellar centers (CS); fusion of septa (SF); coenosarc color (CC) and tentacle color (TC). Colonies were photographed, and structures measured with a caliper. Micromorphology was examined only in the samples of Brazil with Scanning Electron Microscope (*SEM*) micrographs. The samples were bathed in xylol to clean the residues and covered with Au/Pd on a Quorum metallizer (model Q150T ES - Nanjing Tansi Technology Co.Ltd.). The photographs were taken with a SEM-ZEISS EVO-40 Scanning Electron Microscope in the research center CENPES/PETROBRAS (Centro de Pesquisas Leopoldo Américo Miguez de Mello/Petroleo Brasileiro SA.). To verify species identification, taxonomic observations were compared with previous descriptions [14, 15, 34, 36, 47, 48]. All specimens were deposited in the scientific collection of the Instituto de Estudos do Mar Almirante Paulo Moreira, Brazilian Navy (IEAPM).

2.4. Statistical analysis of morphological structures

A cluster analysis covering all specimens was applied based on the measurements of the following characters: CS, CollDma, CollDmi, CP, FCD, ColmDma, ColDmi. The similarity matrix was created using the Bray-Curtis coefficient. Similarity percentages analysis (SIMPER) was performed to determine which characters were the most important in comparisons between and within specimen groups. The analyses were carried out using the software Primer V.6 [49].

2.5. Molecular analysis

We extracted genomic DNA using the Wizard® extraction kit (Promega blood and tissue extraction kit) following the manufacturer's instructions. A portion of ribosomal DNA, including the 18S rDNA, ITS-1, 5.8S rDNA, ITS-2, and 28S of the rDNA genes, was amplified using primers A18S (5'-GATC-GAACGGTTTAGTGAGG-3') [50] and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [50], following the protocol in Benzoni et

al. [52]. Polymerase chain reactions (PCR) were performed in a 20 µl reaction containing 2mM $MgCl_2$, 0.8mM dNTPs, 1x PCR buffer, 0.5 µM forward and reverse primers, 1 unit Taq DNA polymerase and 50 - 100 ng DNA template. The cycling conditions for amplification consisted of an initial denaturation for 4 min at 94 °C; followed by 36 cycles of denaturation at 94 °C for 45s, annealing at 45 °C for 30s, and elongation at 72 °C for 30s; followed by a final extension period at 72 °C for 7 min. Successful PCR products were then purified using exonuclease I and shrimp alkaline phosphatase (ExoSAP; USB, Cleveland, OH, USA) and were submitted for sequencing in both directions to the University of Arizona Genetics Core (Tucson, AZ, USA).

The sequences were edited and consensus sequences were generated in Geneious R6 (version 6.1.5) [53]. All nucleotide sequences were blasted against NCBI database. We used Muscle [54] in MEGA7 [55] to align all sequences and those downloaded from GenBank (Table 1). The best-fit evolutionary models were selected with Modeltest version 3.7 [56]. Poorly aligned positions and divergent regions of the ITS dataset were eliminated using GBlocks [57, 58] with all less stringent selection options selected. To estimate evolutionary relationships, we constructed a Bayesian phylogeny with MrBayes v3.2.2 [59], using each unique haplotype only once and also using *Leptopsammia pruvoti* (MD02) and *Rhizopsammia verrilli* (HS2888) as outgroups. Parameters for MrBayes included two independent runs of 5×10^6 generations with the first 10,000 trees discarded as burn-in. Upon completion, we analyzed the MrBayes output data in Tracer v1.5 [60, 61] to confirm stationarity and sufficient sampling of the posterior. Node support was represented with number of substitutions per nucleotide. The trees were visualized in FigTree version 1.2 [62] and edited for publication in Corel Photo-Paint X7. All sequences generated for this study were deposited in GenBank (Table 1).

Table 1. Samples of the Tubastraea, Haplotype codes, locality and source of sequences of coral species included in the molecular analysis.

Haplo code (samples size)	Species/Morphotypes	Locality	GenBank number	Source of information
MD02	*Leptopsammia pruvoti*	Mediterranean Sea	HG965397.1	Arrigoni et al. (2014)
HS2888	*Rhizopsammia verrilli*	New Caledonia	HG965402	Arrigoni et al. (2014)
MY070	*Tubastraea* cf. *aurea*	Mayotte Island	HG965408	Arrigoni et al. (2014)
H01 (n=1)	*Tubastraea coccinea* (Morph -II)	Middle Keys (MidK)	MK716384	present study
AY97	*Tubastraea aurea*	Taiwan: Penghu Island	AY722796.1	Chen, C.A. Et al. 2004
H02 (n=2)	*Tubastraea coccinea* (Morph -II)	Lower keys (LowK)	MK716385	present study
H03 (n=7)	*Tubastraea coccinea* (Morph -II)	Arraial do Cabo, Brazil	MK716386	present study
H04 (n=2)	*Tubastraea aurea* (Morph -I)	Arraial do Cabo, Brazil	MK716387	present study
H05 (n=2)	*Tubastraea aurea* (Morph -I)	Arraial do Cabo, Brazil	MK716388	present study
H06 (n=3)	*	Gulf of Mexico (GoM) Lower keys (LowK)	MK716389	present study
H07 (n=2)	*	Gulf of Mexico (GoM)	MK716398	present study
H08 (n=1)	*	Gulf of Mexico (GoM)	MK716390	present study
H09 (n=2)	*	Lower keys (LowK)	MK716397	present study
H10 (n=35)	*	Upper Keys (UppK) / Middle Keys (MidK)	MK716391	present study
SO119	*Tubastraea* cf. *aurea*	Socotra Island, Yemen	HG965409	Arrigoni et al. (2014)
AO101	*Tubastraea diaphana*	Japan, Amami-Oshima	HG965413.1	Arrigoni et al. (2014)
H11 (n=2)	*	Gulf of Mexico (GoM)	MK716392	present study
AF110	*Tubastraea coccinea*	-	AF180110.1	Hunter, C.L.
H12 (n=1)	*	Middle Keys (MidK)	MK716393	present study
AO100	*Tubastraea micranthus*	Japan	HG965414	Arrigoni et al. (2014)
M768	*Tubastraea micranthus*	Maldives	HG965416	Arrigoni et al. (2014)
MY072	*Tubastraea micranthus*	-	HG965417	Arrigoni et al. (2014)
HS2883	*Tubastraea* sp.2	New Caledonia	HG965420	Arrigoni et al. (2014)
HS2884	*Tubastraea* sp.2	New Caledonia	HG965421	Arrigoni et al. (2014)
HS2890	*Tubastraea* sp.2	New Caledonia	HG965422	Arrigoni et al. (2014)
AQ2	*Tubastraea coccinea*	isolate="AQ2"	HG965410.1	Arrigoni et al. (2014)
T11	*Tubastraea sp*	-	DQ533621.1	Hizi-Degany et al. 2007
H13 (n=6)	*Tubastraea* sp. (Morph -III)	Gulf of Mexico (GoM); Arraial do Cabo, Brazil	MK716394	present study
H14 (n=2)	*Tubastraea* sp. (Morph -III)	Arraial do Cabo, Brazil	MK716395	present study
H15 (n=1)	*Tubastraea* sp. (Morph -III)	Arraial do Cabo, Brazil	MK716396	present study
Y756	*Tubastraea micranthus*	Yemen	HG965418	Arrigoni et al. (2014)
K12	*Tubastraea* sp.3	Japan	HG965423	Arrigoni et al. (2014)
MY105	*Tubastraea* sp.1	Mayotte Island	HG965419	Arrigoni et al. (2014)

*Florida samples were not used to determine the morphotype.

3. RESULTS

3.1. Morphological characterization

The *Tubastraea coccinea* morphotype I corresponded to previous descriptions of *Tubastraea aurea* in Boschman (1953) [15], while *Tubastraea coccinea* morphotype II corresponded to *T. coccinea sensu stricto* [14, 47, 48]. The third morphotype corresponded to an undetermined species, hereafter referred to as *Tubastraea* sp., and does not morphologically correspond to the species previously named as *Tubastraea tagusensis* [16, 47]. From here, we will use *T. aurea*, *T. coccinea*, and *Tubastraea* sp. to

refer to the morphotypes described in this study. A descriptive summary of the present morphotypes compared to published data is presented in Table 2.

T. aurea is characterized by regular hemispherical colonies, with dark yellow tentacles, generally the tips are orange in color and projecting more conspicuously over the coenosteum (Fig 1). *T. aurea* corallitte diameter on average reached almost 14 mm compared to 9 mm in *T. coccinea*. The columellar fossa is deeper and the septal edges highly granulated which was not observed for *T. coccinea* (Fig 1). On the other hand, *T. coccinea* is characterized by an irregular growing, in which the colonies extend laterally, forming flat clusters of polyps that do not exsert too much above the coenosteum. The corallites are distributed near one another, allowing a greater number of polyps per colony when compared to *T. aurea* (Fig 1). *T. aurea* is different from *T. coccinea* due a fifth cycle of septa, S_5, that were the same size or larger than S_4. *Tubastraea coccinea* has four cycles of septa, with first and second cycles larger than others reaching the columella (Table 2). The third cycle of septa in *T. coccinea*, is large but not well formed, whereas the fourth cycle often appeared incomplete and often equal in size to third cycle.

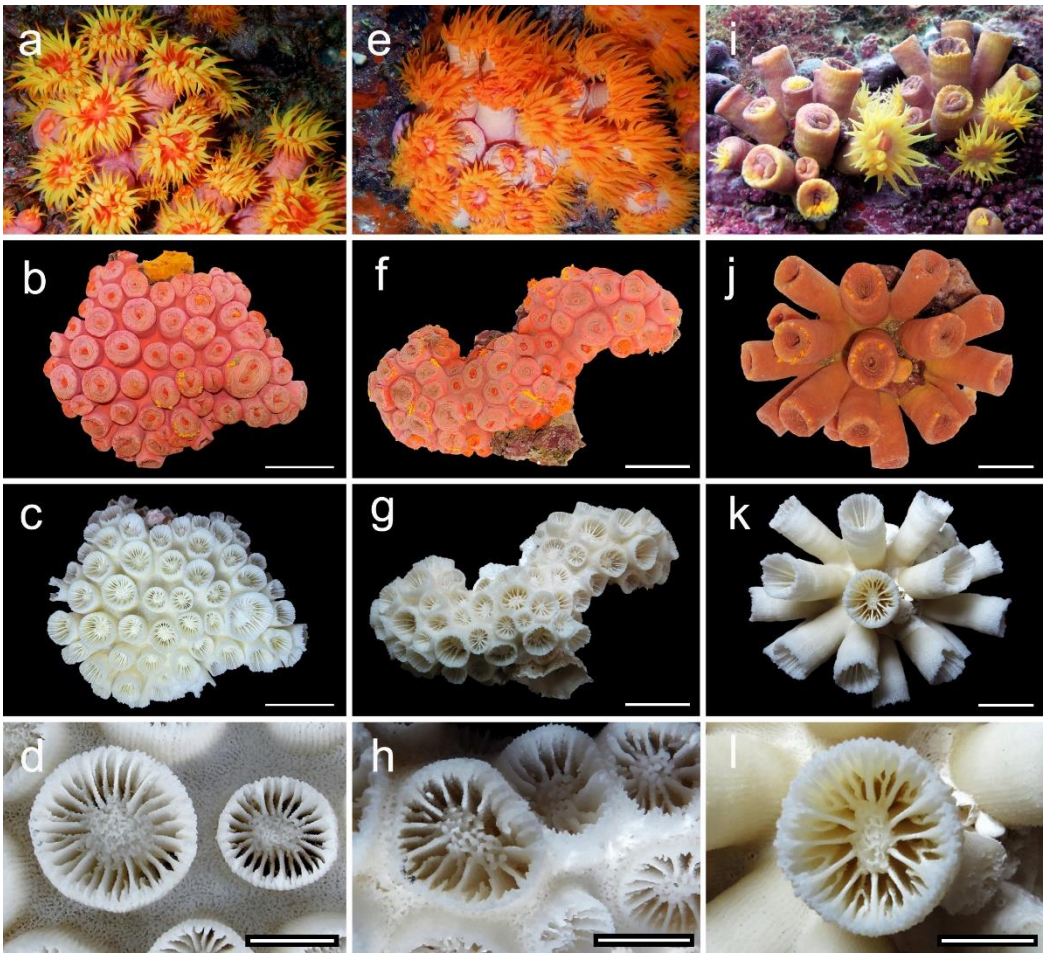
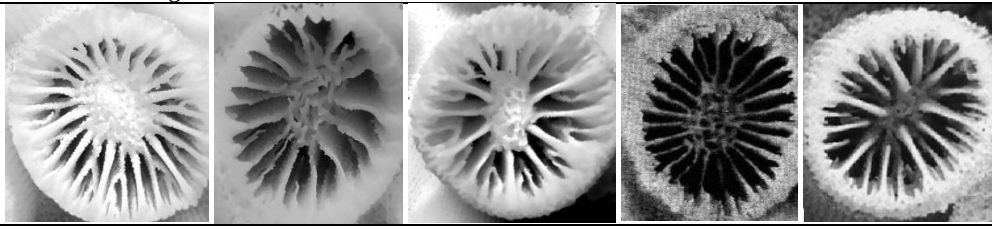


Figure 1. Morphology of the three *Tubastraea* species from Arraial do Cabo, RJ. a-d) Morphotype I (*Tubastraea aurea*); e-h) Morphotype II (*Tubastraea coccinea*) and; i-l) Morphotype III (*Tubastraea* sp.) Scale: b. 3cm; c. 2cm; d. 1 cm; f. 3 cm; g. 2 cm; h. 1 cm; j. 0,75 cm; k. 0,5; l. 0,5 cm.

Regarding the differences between *Tubastraea* sp. and *Tubastraea tagusensis*, the columella of *Tubastraea* sp. is distinctly bigger and robust, and they have a large septum of third cycle. In contrast, the columella of *T. tagusensis* is usually rudimentary, and the S_3 septa short and weakly developed. In *Tubastraea* sp. the fusion between septa were very common in the larger corallites that were not observed in *T. tagusensis*. Morphologically, *Tubastraea* sp. is very distinct from *T. aurea* and *T. coccinea*, mainly due to greater polyp projection, deeper columella fossa, and tissue and tentacle coloration (see Figure 1 and Table 2). The *Tubastraea* sp. polyps are widely spaced from each other and project very high above the coenosteum. In some colonies, *Tubastraea* sp. polyps form small clusters connected basally by a reduced coenosteum tissue. Polyps (oral disc,

tentacles and column) are usually light yellow in color (Fig 1). Morphological data of the three species is summarized in Table 2 and detailed morphological descriptions are provided in the species descriptions section below.

Table 2. Morphological data of *Tubastraea* recorded from the western Atlantic.

Caracteres	*T. aurea*	*T. coccinea*	*Tubastraea* sp.	*T. tagusensis*ǂ	*T. micranthus**
Maximum corallum diameter (mm)	140	135	110	145	Dendroid attain 1 m in height
Septa cycles	S_{1-2}> S_3> $S_4 \leq S_5$	S_{1-2}>$S_3 \geq S_4$	S_{1-2}> $S_3 \geq S_4$	S_{1-2}>S_3>S_4	S_1>S_2>S_3
Septa characteristics and number	S_{1-2} equal in size and attaining the columella. S_3 laciniate, rudimentary or reaching the columella. S_4 is incomplete or rudimentary. S5 is incomplete and may connect to the third and fourth cycles. Maximum number of septa is 92.	S_1 and S_2 equal in size and attaining the columella. S_3 very laciniate, usually incomplete and rudimentary, in some cases can reach the columella. S_4 most often incomplete or rudimentary, sometimes equal in size to S_3. Number of septa variable, maximum 36.	S_1 and S_2 are straight and direct, S_3 and S_4 are laciniate. S_3 could reach the columella. Fusion of S_1 or S_2 can occur, fusion between S_3 and S_4 is very common. Number of septa variable, maximum 48.	S_1 and S_2 vertical and straight equal in size. S_3 very short, weakly developed. S_4 sometimes rudimentary or absent. All straight. S_1 paliform lobes. S_{1-4} having laciniate axial edges in larger corallites.	S_1 with straight inner edges attaining the columella. S_2 a little less wide and straight inner edges attaining the columella too. S_3 usually rudimentary, represented by a very narrow laciniate lamela.
Septa fusion	S_3 fusing of the first, fourth and fifth cycles of septa.	Fusion between septa rarely observed.	Fusion of S_1 or S_2 can occur, fusion between S_3 and S_4 is very common.	Absent or S3 rarely uniting with the first group	-
Largest corallite diameter (mm)	26	11	17	12,8	12
Corallite projection (mm)	17	10	36	35	7
Spacing of corallites	Close	Very close	Sparse	Closely spaced	Sparse
Columella diameter maximum (mm)	2.5-8.5	1.4-6.4	0.8-4.9	0-3.5	-
Description of columella	Large spongy trabeculae mass irregular and compact form	Small, slender and spongy trabeculae mass irregular	Slender trabeculae mass irregular	Trabecular tangle half the diameter of the calice or rudimentary	Rudimentary, composed of solid, elongate fusion of lower, inner edges of the S_{1-2}
Maximum depth of columellar fossa (mm)	14	7	11	-	Deep fossa
Colour of coenosarc	Light red	Dark red	Lemon-yellow	Yellow	Striking dark green or brown-black
Colour of tentacles	Bright yellow with tip and base sometimes orange	Orange	Light yellow	-	-
Septa View					

ǂ = Wells 1982 [34], De Paula and Creed 2004 [21]; ***** = Sammarco et al. 2004 [7].

3.2. Statistical analysis

Three major groups of samples were formed in the cluster analysis (Fig 2). The first, named '*T. aurea* group' (84.72 of similarity), was composed only by samples of *T. aurea* (100% morphotype I) (N = 64 polyps); the second, '*T. coccinea* group' (83.32 of similarity), was composed of all samples of *T. coccinea* (85% morphotype II) (N = 85 polyps), two samples of *Tubastraea* sp. (2% morphotype III) and a few samples of *T. aurea* (13% morphotype III) (N = 13 polyps); and the third group, the '*Tubastraea* sp. group' (86.68 of similarity), was composed of samples of *Tubastraea* sp. (98% morphotype III) (N = 50 polyps) and one sample of *T. aurea* (1.9% morphotype I). SIMPER analysis indicated that the largest diameter of corallites (CollDma), the shortest diameter of corallites (CollDmi), and the spacing between columellar centers (CS) were the characters that contributed the most to similarity within the '*T. aurea* group' as well as within the '*T. coccinea* group' (Table 3). The most relevant characters for the similarity within the '*Tubastraea* sp. group' were the corallite projection (CP) and the space between columellar centers (CS). Correspondingly, the characters that contributed to the dissimilarity between the '*T. aurea* group' versus the '*T. coccinea* group' (27.73 dissimilarity) and also between the '*T. aurea* group' versus the '*Tubastraea* sp. group' (24.12 of dissimilarity) were the largest diameter of corallites (CollDma), the spacing between the columellar centers (CS), and the corallite projection (CP) (Table 4). Dissimilarity between the '*T. coccinea* group' and the '*Tubastraea* sp. group' (29.45 of dissimilarity) was influenced mostly by corallites projection (CP), spacing between columellar centers (CS), and the depth of columellar fossa (FCD).

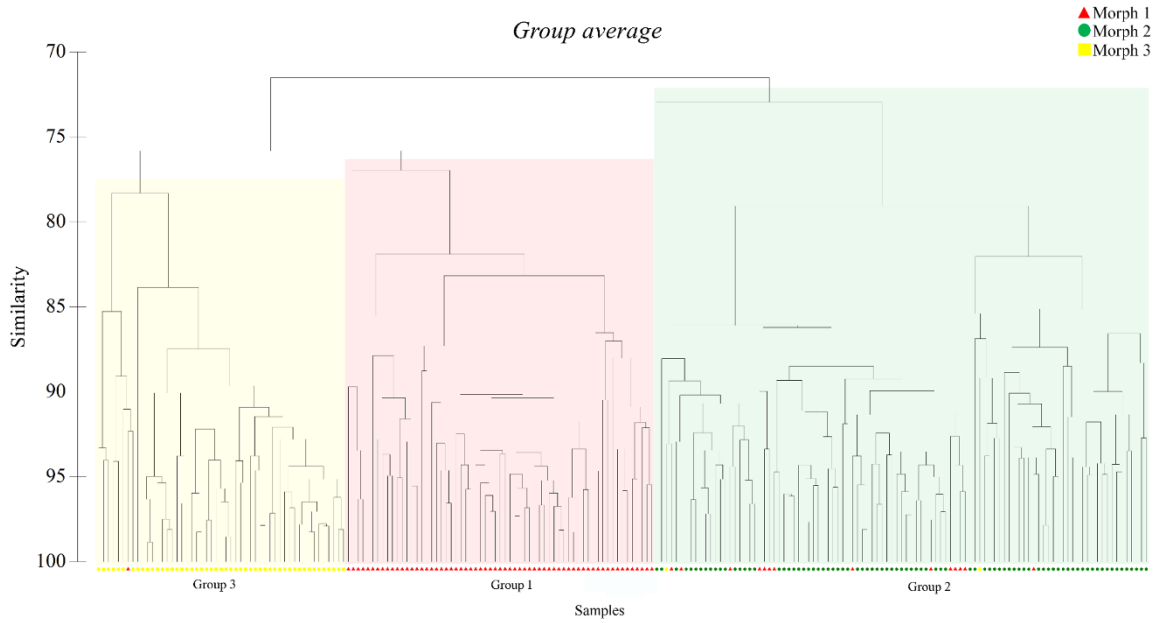


Figure 2 - Cluster analysis of three morphotypes of *Tubastraea* only from Brazil, Arraial do Cabo, based on measurements of the morphological characters. Morphotype markers: (Red): *Tubastraea aurea* (Morph I); (Green): *Tubastraea coccinea* (Morph II) and (Yellow): *Tubastraea* sp. (Morph III).

Table 3. Morphological characters that contributed to the similarity within groups in SIMPER analysis using Bray-Curtis coefficient.

Groups	**Average similarity**	**Characters**	**Average Abundance**	**Average Similarity**	**Contribution %**	**Cumulative contribution %**
Group 1 (Morph I) *T. aurea*	84.72	CollDma	1.46	19.49	23.01	23.01
		CollDmi	1.32	17.68	20.87	43.88
		CS	1.32	17.13	20.22	64.1
		CP	0.9	10.03	11.84	75.95
		ColmDma	0.62	7.66	9.05	84.99
		FCD	0.64	6.86	8.1	93.09
Group 2 (Morph II) *T. coccinea*	83.32	CollDma	0.9	20.08	24.1	24.1
		CS	0.81	18.09	21.71	45.81
		CollDmi	0.81	17.5	21	66.82
		FCD	0.45	8.78	10.54	77.36
		CP	0.48	7.61	9.14	86.5
		ColmDma	0.34	6.87	8.25	94.74
Group 3 (Morph III) *Tubastraea* sp.	86.68	CP	1.93	24.34	28.08	28.08
		CS	1.6	20.71	23.9	51.97
		CollDma	1.02	13.88	16.01	67.99
		CollDmi	0.93	12.51	14.44	82.42
		FCD	0.68	8.81	10.17	92.59

CollDma = corallite largest diameter; CollDmi = corallite shortest diameter; CS = space between columellar centers; CP= corallite projection; ColmDma= largest columella diameter; FCD= depth of columellar fossa.

Table 4. Characters that contributed to the dissimilarity between groups of samples in SIMPER analysis using Bray-Curtis coefficient.

			Average abundance				
	Average dissimilarity	Characters	G1	G2	Average Dissimilarity	Contribution %	Cumulative contribution %
Groups G1 and G2	27.73	CollDma	1.46	0.9	5.21	18.77	18.77
		CS	1.32	0.81	5.02	18.09	36.86
		CP	0.9	0.48	4.87	17.57	54.43
		CollDmi	1.32	0.81	4.8	17.32	71.76
		FCD	0.64	0.45	2.81	10.15	81.9
		ColmDma	0.62	0.34	2.63	9.49	91.39
Groups G1 and G3	24.12	CP	0.9	1.93	7.88	32.65	32.65
		CS	1.32	1.6	3.39	14.06	46.71
		CollDma	1.46	1.02	3.34	13.86	60.58
		CollDmi	1.32	0.93	3.03	12.55	73.13
		FCD	0.64	0.68	2.21	9.16	82.28
		ColmDmi	0.47	0.18	2.16	8.94	91.22
Groups G2 and G3	29.45	CP	0.48	1.93	13.49	45.81	45.81
		CS	0.81	1.6	7.41	25.16	70.97
		FCD	0.45	0.68	2.59	8.78	79.75
		CollDma	0.9	1.02	2.05	1.34	6.95
		CollDmi	0.81	0.93	2.02	1.29	6.87

CollDma = corallite largest diameter; CollDmi = corallite shortest diameter; CS = space between columellar centers; CP= corallite projection; ColmDma= largest columella diameter; FCD= depth of columellar fossa. G1 (Morph I - *T. aurea*); G2 (Morph II - *T. coccinea*)*;* G3 (Morph III - *Tubastraea* sp.).

T. aurea has a higher mean polyp diameter than *T. coccinea* and, therefore, was the main character that differentiated these two species. *T. aurea* polyps present greater distances between the columellar centers, moreover, the arrangement of polyps in the colony is more spaced compared to *T. coccinea* where the polyps are smaller and very close to each other. Projection of corallite also contributed to the dissimilarity between both where the wall of the *T. aurea* polyp is usually more protuberant than that of *T. coccinea*. The characters, CollDmai, CS and CP, distanced the species *T. aurea* and *Tubastraea* sp., due to the greater diameter of *T. aurea* polyps compared to *Tubastraea* sp. and the disparity between the projection of the corallites of *Tubastraea* sp. and the distance between the columellar centers were also much bigger. The characters CP, CS and FCD were responsible for the separation of the species *T. coccinea* and *Tubastraea* sp. due to the biggest differences between them.

3.3. Molecular analyses

Among the samples collected, a total of 68 ITS fragments were successfully amplified, 17 from Brazil (*T. aurea* – Morph I, n=4; *T. coccinea* – Morph II, n=7; *Tubastraea* sp. – Morph III, n=6) and 51 sequences from Florida (Upper Keys n=19; Middle Keys n=18; Lower Keys n=6 and North Gulf of Mexico n=8) (Table 1). Overall, the genetic data revealed that ITS genetic variation was present in the genus *Tubastraea*; however, the ITS sequence variation did not uncover clearly distinct monophyletic clades that might delineate each species. All in-group samples fell into a highly supported clade (Fig 3) comprised of two clades with high support one related to *T. coccinea* (Clade 1; Fig. 3), another related to *T. micranthus* (Clade 2; Fig. 3), and two samples (K12 and MY105) which fell outside of these clades (Fig. 3). Both K12 and MYN05 were GenBank samples and may indicate an occurrence of two unique, unidentified species of *Tubastraea* that were not found in either Brazil or Florida. Regarding our phylogeny, we found that Florida samples comprised 10 haplotypes, whereas Brazil samples were comprised of 6 haplotypes. Furthermore, Florida samples exhibited much greater genetic variation among haplotypes than Brazilian samples (Fig. 3). Despite the genetic variation found in Florida samples, there is fairly little overlap among regions. Some sequences with species names were incorporated into our tree from GenBank and did not form monophyletic clades (Fig. 3). For example, samples AQ2, AF110, were identified as *T. coccinea*, but occur in different clades on the phylogenetic tree (Fig 3). Our morphological identification suggests that H03 would be *T. coccinea sensu stricto.* Similarly, samples SO119, AY96, MY070, H04, and H05 are all named *T. aurea*, but they did not group together either. Moreover, our phylogenetic analysis suggests that haplotypes H10, H11 and H12 are likely additional species invading Florida that were not detected in Brazil. Regarding Clade 2, one haplotype (H13) was found in both Brazil and

Florida. This haplotype belonged to samples of *Tubastraea* sp., whereas most named GenBank samples within the clade were labelled as *T. micranthus*.

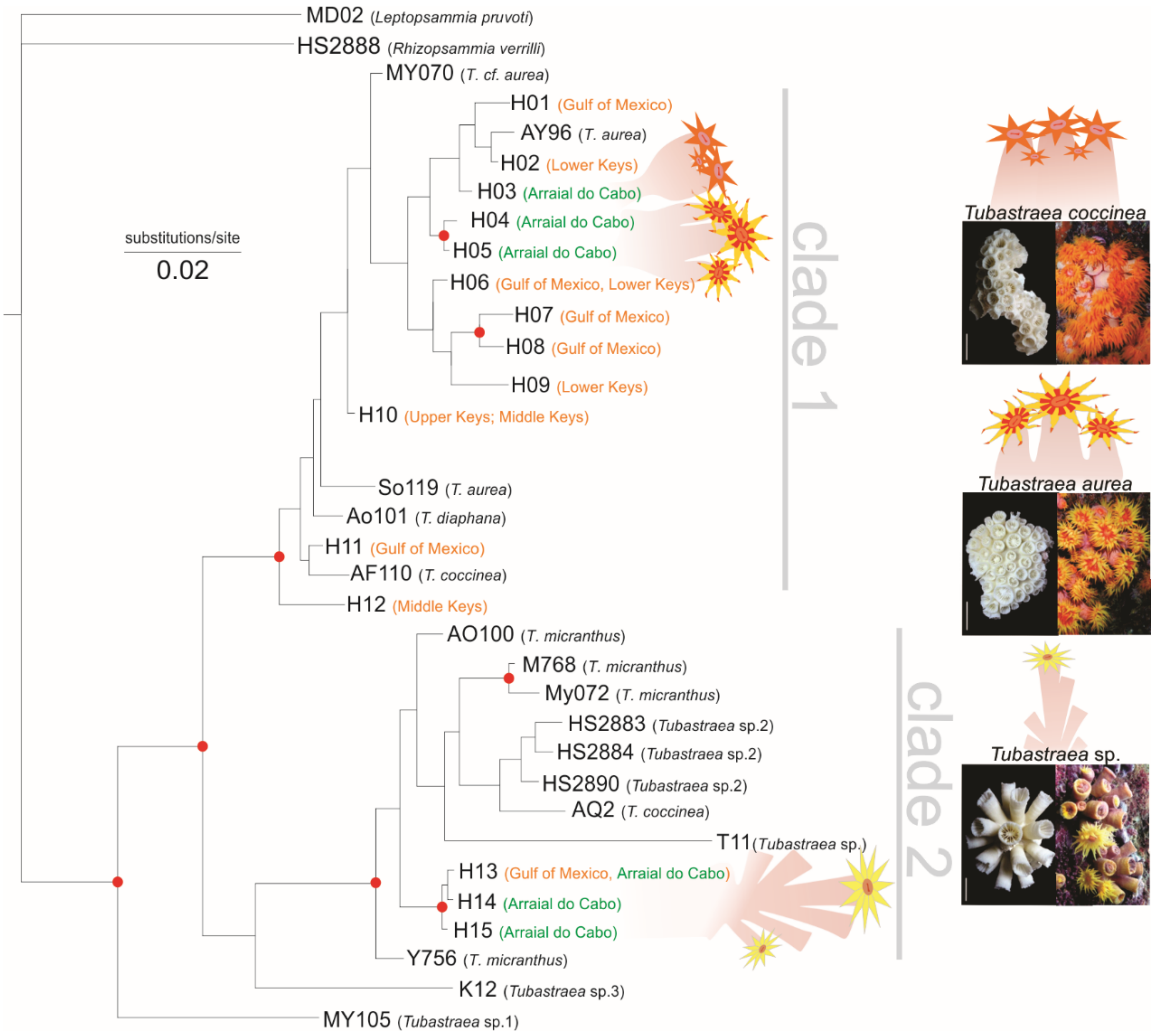


Figure 3 - Bayesian estimation of *Tubastraea* phylogeny tree based on the ITS gene. Specific values for those nodes that were strongly supported in the analyses (substitutions per site > 0.95) are reported in the tree as a red dot. Scale bar represents substitutions per nucleotide site. Images generated with Brazilian Samples and GenBank data.

3.4. Species descriptions

The three species of *Tubastraea* from Brazil delimited through the integration of morphological and molecular analyses are presented below. For detailed morphometric data, see Table 2.

Order Scleractinia Bourne 1900

Suborder Dendrophylliina Vaughan and Wells, 1943

Family Dendrophylliidae Gray, 1847

Genus *Tubastraea* Lesson, 1829

Tubastraea aurea (Quoy and Gaimard, 1833)

Figure 1a-d

Material examined - Fifteen colonies: Ilha dos Porcos, Arraial do Cabo, Brazil (22º 96' S, 41º 98' W) (Five colonies); Saco do Anequim, Arraial do Cabo, Brazil (22º 98' S,41º 98' W) (Ten colonies). Samples used for SEM (Fig 4) and skeletons for morphological studies were deposited in IEAPM Scientific Collection (IEAPM 002068, IEAPM 002069, IEAPM 002072).

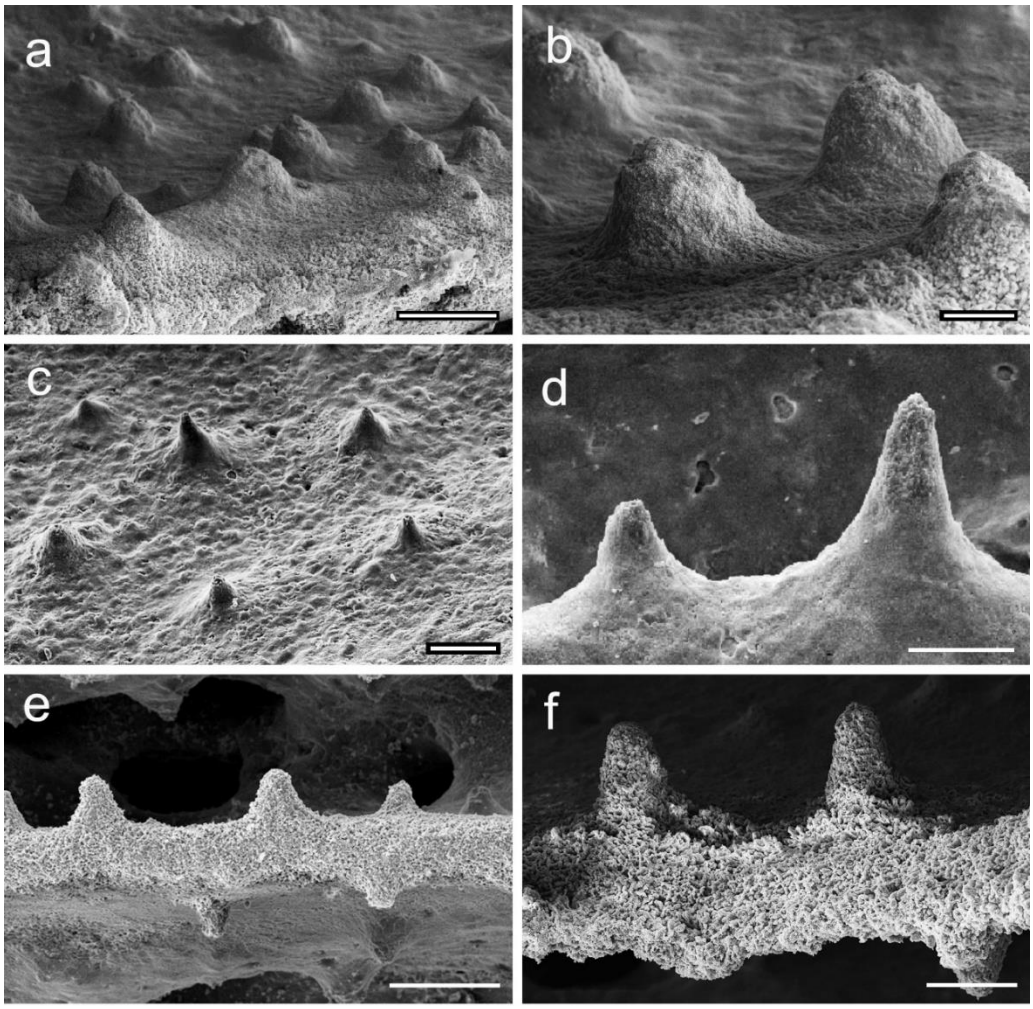


Figure 4 – Scanning Electron Microscope (*SEM*) micrographs. Details of the morphology of septals wall spines. a, b - *Tubastraea aurea*; 200 μm (a); 60 μm (b); c, d – *Tubastraea coccinea*, 100 μm (c); 100 μm (d); and e, f – *Tubastraea* sp.; 200 μm (e); 100 μm (f).

Morphological description - Colonies slightly spherical, coenosarc pink, oral disc orange and tentacles dark yellow, frequently with orange tips (Fig 1). Corallum white and porous, measuring up to 140 mm in diameter. Corallites closely spaced in a plocoid arrangement, sometimes sub-plocoid. Calices slightly elliptical or spherical, reaching a maximum diameter of 19 mm and, when fused, reaching up to 30 mm. Corallites exert up to 17 mm from the base of coenosteum, or do not form corallites wall and occur at baseline. Extramural budding (increase) occurs outside of the wall of the parent corallite from the base of the founder. Polyp growth continues from the edge of the colony or, less common, between old corallites. Fission of polyps is common. Space between corallites (from one columella center to another) is 13 mm on average, the biggest was 19 mm. Corallite walls have intercostal furrows and were highly porous. Deep columellar fossa reached 5 mm on average. Columella were rather large, irregular and compressed (or compact), consisting of a spongy trabecular mass. Columella diameter presented averages of 4.3 mm of minimum diameters to 5.3 mm averages of maximum diameter. Septa hexamerrally arranged in four cycles. A fifth cycle of septa (S_5) may appear with the same size or larger than S_4 (Fig 5). Septa and size arrangement: $S_{1\text{-}2} > S_3 > S_4 \leq S_5$. $S_{1\text{-}2}$ virtually equal in size (Fig 5), but S_1 slightly thicker, both reaching the columella. Third cycle S_3 rudimentary or, in some cases, reaching the columella, with a laciniate axial board. It was observed S_3 fusing of the first, fourth and fifth cycles of septa. Fourth cycle S_4 is in the most cases, incomplete or rudimentary. Fifth cycle S_5 is incomplete and may connect to the third and fourth cycles. Septal faces of all cycles have slightly rounded spines (Fig 4). Number of septa varied with the diameter of the corallite, from 28 to 92 (average of 42 septa).

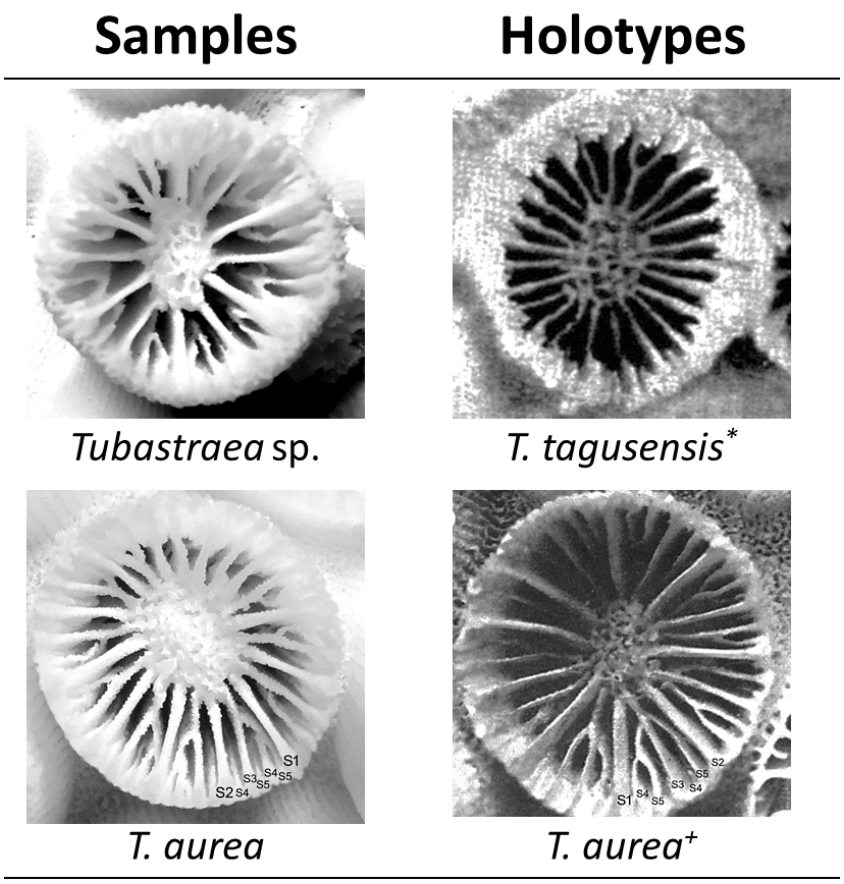


Figure 5 – Septa patterns comparison between samples and holotypes of *T. tagusensis* and *T. aurea*. * Wells, 1982 [34]; + Boschman, 1953 [15].

Tubastraea coccinea Lesson, 1829

Figure 1 e-h

Synonyms

Astropsammia pedersenii Verrill, 1869
Dendrophyllia aurantiaca Quoy and Gaimard, 1833
Lobopsammia aurea Quoy and Gaimard, 1833
Placopsammia darwini Duncan, 1876
Tubastraea tenuilamellosa (Milne Edwards and Haime, 1848)

Material examined - Sixteen colonies: Ilha dos Porcos, Arraial do Cabo, Brazil (22º 96' S, 41º 98' W) (six colonies); Saco do Anequim, Arraial do Cabo, Brazil (22º 98' S, 41º 98' W) (ten colonies). Samples used for SEM and skeletons for morphological

studies were deposited in IEAPM Scientific Collection (IEAPM 002066, IEAPM 002067, IEAPM 002071).

Morphological description - Colonies slightly spherical or irregular, coenosarcs soft red, tentacles orange in color (Fig 1). Corallum white, reaching up to 135 mm. Corallites very closely spaced in plocoid arrangement, in some cases sub-plocoid. Space between corallites measured 8 mm on average, with the largest being 12 mm. Calices elliptical or spherical, maximum diameter 11 mm, no fission of polyps was observed. Corallites project 4.6 mm on average and with a maximum of 10.8 mm from the base of coenosteum. Extramural budding occurs outside of the wall of the parent corallite usually between older corallites (Fig 1). Intercostal furrows and highly porous wall. Fossa moderately deep (4 mm in average). Columella slender, consisting of a spongy trabeculae irregular mass with diameter of 3.4 mm. Septa and size arrangement following the scheme: $S_{1\text{-}2}>S_3\geq S_4$. First ($S_1$) and second, ($S_2$) cycles of septa equal in size and reaching the columella. Third cycle of septa (S_3) very laciniate and smaller than S_1 and S_2, usually incomplete and rudimentary, but in some cases reaching the columella. Fourth cycle (S_4) most often incomplete or rudimentary, sometimes equal in size to S_3. Fusion between septa rarely observed. Septal faces of all the cycles covered with sharp spines (Fig 4). Number of septa varied from 18 to 36, according to the diameter of corallite (average of 27 septa).

Tubastraea sp.

Figure 1 i-l

Material examined - Ten colonies from Ilha dos Porcos, Arraial do Cabo, Brazil (22º 96’ S, 41º 98’ W). Samples used for SEM and skeletons for morphological studies were deposited in IEAPM Scientific Collection (IEAPM 002065, IEAPM 002070).

Morphological description – Colonies spherical, measuring up to 110 mm in diameter. Corallum white, phaceloid and porous. Coenosarc varies from light yellow to light green, tentacles light yellow (Fig 1). Calices slightly compressed or spherical, reaching up to 13 mm in diameter. Coralittes protruding more than 36 mm from coenosteum. Budding extra tentacular generally appear on the wall of corallites and between corallites. Increase through intramural bud or fission is very rare. Corallites sparsely spaced, the largest measurement between them reaching 24 mm. Septa hexamerrally arranged in four cycles $S_{1-2} > S_3 > S_4$, sometimes S_3 and S_4 have the same size ($S_{1-2} > S_{3-4}$) (Fig 5). S_3 and S_4 are incomplete most of time. S_1 and S_2 are straight and direct, S_3 and S_4 are laciniate. S_3 could reach the columella. Fusion of S_1 or S_2 can occur, fusion between S_3 and S_4 is very common. Spines, not very sharp, occur in all septal faces (Fig 4). Columella spongy, always present, composed by a mass of slender trabeculae, with a diameter of 3.3 mm in average. Coralittes have intercoastal grooves and septal costae. Fossa is very deep, reaching averages up to 11 mm. The number of septa varied from 22 to 39, according to the diameter of corallites (average of 29 septa).

4. DISCUSSION

In this study, we identified three species of *Tubastraea* which invaded southeastern Brazil (samples taken from Arraial do Cabo, state of Rio de Janeiro) as supported by statistical inferences of fourteen traditional taxonomic characters measured as basic classification criteria. It was possible to distinguish two species (*T. aurea* and *T. coccinea*) from what previously has been considered polymorphic variations of *Tubastraea coccinea* [23]. Due to the morphological disimilarities with a *T. coccinea* synonym, we suggest to identify the morphotype I as *Tubastraea aurea* living in co-occurrence with *T. coccinea* on the rocky shores of southeastern Brazil. Due to the previous record of the species *T. tagusensis* to the south of the study site (De Paula and

Creed 2004), we also believed that our morphotype 3 was the same species. However, our morphotype 3 is not *T. tagusensis* and it has not been possible to identify it so far, being therefore called *Tubastraea* sp. Our genetic data clearly supported each of the two general morphological groups (*T. coccinea/aurea* versus *Tubastraea* sp.) as being differentiated from each other (i.e. Clade 1 and 2), despite the finer scale level of differentiation not as divergent. Overall, our morphological and genetic data indicated three different species invading the southeastern Brazilian coast (*T. aurea*; *T. coccinea*; *Tubastraea* sp.) and six different genetic units invading the Southeastern US.

Many species of *Tubastraea* corals have been described and later synonymized, creating doubt about the naming of valid species due to the interspecific overlap of diagnostic characters [33, 34]. Several *Tubastraea* species have been described to be ecovariants but the fact that they occupy the same habitat sheds doubt on this as a mechanism creating morphological divergence as discussed by Wells (1982) [34] with regard to the synonymization of *T. faulkneri* and *T. coccinea* occurring in the same site. One well debated species contrast is whether *T. coccinea* and *T. aurea* comprise a single species. Comparing *T. coccinea* and *T. aurea*, Boschma (1953) [15] proposed the authenticity of *T. aurea* described by Quoy and Gaimard (1833), which was later refuted by Cairns (1994) [48] who proposed that *T. coccinea* and *T. aurea* were actually synonymous. However, the present observations of conspicuous morphological features, already described here and reported by Boschma [15] do not support this synonymization. Additionally, Boschma (1953) [15] compared morphological traits between samples of *T. tenuillamellosa* and *T. aurea*, and determined that clear morphological differentiation also occurred between these species. In our study, we observed different forms of new budding and larger and more developed calyces in *T. aurea* and one more cycle of septa differentiating it from *T. coccinea*. Comparing *T. coccinea* and *T. tagusensis*, De Paula

and Creed (2004) [21] have distinguished them based on differences in calicular diameter, protuberance of polyps and coenosarc color, based on previous description by Cairns (1991) [47]. It was reported by De Paula and Creed (2004) [21] that colonies of *T. coccinea* and *T. tagusensis* from Brazil present a wider variation of corallite morphology and corallite exsert when compared to descriptions of specimens from the Pacific Ocean, raising speculations that it could be the result of adaptive responses to different environmental conditions [21]. But the present results suggest that the morphology provides evidence in support of species differentiation, where it may be corroborated by a broader integrative study. With regard to the undefined species we describe in this study (i.e. *Tubastraea* sp.) found in both Brazil and the USA, structural features provided evidence of differentiation from *Tubastraea tagusensis*. According to Cairns's description [47], the *T. tagusensis* columella is usually rudimentary and occasionally robust. In Ilha Grande, Brazil colonies of *Tubastraea tagusensis* present very small columella size [21] compared with *Tubastraea* sp. from Arraial do Cabo. In addition, *Tubastraea* sp. columella, reaches up to 4.2 mm and average of 3.3 mm, quite different from *T. tagusensis* from Ilha Grande, in which columella varies from absent to 3.5 mm in diameter, according to De Paula and Creed (2004) [21] based on descriptions of Wells (1982) [34]. Comparing our samples of *T. aurea* and *Tubastraea* sp. with its holotypes we can visualize the differences between a *T. tagusensis* polyp with direct septa cycles connected to columella and almost no septa fusion and, on the other hand, fewer septa cycles reaching columella and more laciniate and fused septa in *Tubastraea* sp. (Fig. 5). In the same image, the specimen of *T. aurea* and the holotype of the species are similar in the arrangement of the septa and mainly the fifth cycle of septa.

Despite the uncertainty that has arisen from past studies with regard to naming species within the genus *Tubastraea*, our study found that morphological diagnostic

characters were still useful for differentiation at the species level. Ocanã and collaborators [63] used initial colonial development as well as colony and budding macromorphology to provide taxonomic clarification on *Tubastraea* species. One problem is that, in corals, morphological characteristics that define species may vary within species. However, Budd (1990) [64] argues that the architecture of the corallites can still be used to distinguish species of colonial reef corals, especially for features related to size. Microstructures are important characters for a more accurate evaluation of coral differentiation according to Budd [65]. In the present study, we observed that each species had different shapes of teeth and granules along the faces of septa, visible under SEM (Fig 4), which were good characters for differentiation according to Arrigoni et al. (2014) [36] and Budd et al. (2010) [65]. Arrigoni *et al*. (2014) [36] found and discussed the relevance of the study of microstructures for the systematics of the Dendrophyllidae, which were congruent with our study. Additionally, Storlarski (2003) [66] reinforced that the microstructural approach of the coral skeleton was a useful tool to elucidate the evolutionary relationships in Scleractinia order. Despite historical conflicts on the reliance of inconspicuous morphological characters among closely related scleractinian species, our study was congruent with other studies that found variation in certain morphological features such as corallite size, septa number, and septa arrangement, which are sufficient criteria to distinguish *Tubastraea* congeners [15, 34, 48, 67].

Since the first discovery of *Tubastraea* in the Campos Basin, north of the state of Rio de Janeiro, the spread of this genus has been associated with oil and gas exploration in Brazil [7, 17, 22]. According to Creed et al (2016) [22] and Sammarco et al. (2004) [7], oil platforms have provided environments for stepping stone dispersal of these invasive species of *Tubastraea* and they could have arrived in Brazil from different locations using different routes, redeployed from either Africa, Gulf of Mexico or the

Indo-pacific [17, 22]. Also, *T. coccinea* was frequently found on platforms and vessels monitored in Arraial do Cabo in the 1990's, further supporting the arrival of *Tubastraea* through these structures (Gonçalves JE, personal communications). Moreover, the similarity of samples found in both Brazil and southeastern US provide evidence of a possible relationship between Brazil and US populations. It is possible that both regions were colonized in the same way (i.e. transport via oil rigs) or that one region is a descendent of the other. The similarity by other species of the benthic encrustation community that are shared, but not native to either the southeast US coast and/or the Brazilian coast such as *Perna perna*, *Megabalanus* barnacles, and *Mytella charruana* [68, 69, 70] further supports the intertwined invasion history of these two regions.

In general, genetic data has been complementarily used with morphology for identification of corals. Different genes have been used for species delimitation, including common barcoding genes such as ITS and cytochrome oxidase subunit I (COI) [36, 71]. However, COI has been discouraged because it does not typically exhibit the differentiation necessary to demarcate species or for intraspecific comparisons owing to the slow mutation rate exhibited by corals at this gene [72]. Therefore, the ITS has been primarily used to evaluate coral specific differentiation. The ITS region has been found to be highly variable and thus suitable for studies of closely related species and populations and has been used in different groups of corals for general relationships [73] and also for species identification in different groups of corals [36, 74]. Our molecular analyses contributed to our understanding of the species that are invading both Brazil and the Southeastern US in four ways. First, our genetic data indicated that all three morphotypes found in Brazil were genetically distinct (i.e. they did not share haplotypes). Second, our data revealed that two of the species we found were invading both Brazil and the Southeastern US (i.e. *T. coccinea* and *Tubastraea* sp.). Third, the genetic diversity

found among Southeastern US haplotypes suggests that this region is likely being invaded by additional genetic variants, not identified morphologically in this study. Finally, when our data were compared to samples that had been uploaded to GenBank, the genetic data revealed that many samples could be either misnamed species, composed of wide variety of morphotypes that did not match our samples, or the ITS gene failed in its ability to identify species. Regardless, the fact that we found many haplotypes that did not group within the morphologically defined clades studied here the present data shows the need for additional studies combining morphological and genetic data.

In summary, this study found that there were three species invading the Brazilian coast (*T. coccinea, T. aurea,* and an undefined species *Tubastraea* sp.), and specimens with similar genetic composition also seem to be invading the southeastern US coast. In addition to these three morphotypes recorded in Brazil, other genetic variants appear to be invading Florida. This indicates that both South American coast as well as the Florida Keys and the North Gulf of Mexico are currently being invaded by multiple species of *Tubastraea*. Comparisons of our genetic data with GenBank failed to clarify species identification owing to the plethora of species that were either unnamed or misnamed in GenBank, according to our phylogeny. In this study morphology and genetics did not align with specifying species-level morphotypes found. In order to accurately resolve issues with species identification within the genus of *Tubastraea*, we need to use other genetic markers and continued evaluation of both genetic and morphological data to create a clear key matching name, morphology, and genetic identification.

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CHAPTER 2

Life history strategy of *Tubastraea* spp. corals in an upwelling area on Southwest Atlantic: *Growth Rate, Fecundity, Settlement and Recruitment*

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ABSTRACT

Tubastrea corals expansion in Arraial do Cabo rocky shores, Rio de Janeiro, Brazil, occupying physical space of native benthic species is an alert to the need for biological and ecological studies to monitor the invasion process. The relationship between growth, reproduction and survival of three *Tubastraea* species (*Tubastraea coccinea*, *Tubastraea aurea* and *Tubastraea* sp.) was investigated indicating characteristics of their life history strategy and population dynamics. We look up to understand the growth and reproductive activity such as fecundity, planulation events and settlement responses to temperature variation and luminosity. In addition, we have detected in a period which larvae that have successfully recruited produced small recruits and colonies in delimited substrates. *Tubastraea* corals demonstrated a developed colonization process in Arraial do Cabo Bay. Sampled sites are directly affected by upwelling phenomenon that is responsible for decrease in average temperature inside and mainly outside the bay. *T. coccinea* have increased their coverage and have grown more in the region, from 3.31 cm^2/year to 4.84 cm^2/year in this study, whereas *T. aurea* and *Tubastraea* sp. grew 4.35 and 5 cm^2/year, respectively. Even so, it is still smaller than reported for Ilha Grande in southeastern Brazil region, with 5.85 cm^2/year in *T. coccinea* and 5.11 cm^2/year in *T. tagusensis*. The largest increase in polyps was *T. coccinea*, 8.1 polyps/year, while for *T. aurea* was 6.03 polyps/year and *Tubastraea* sp. was 5.07 polyps/year. The growth among species was inversely proportional to the fecundity. Also, below-average temperatures favored the growth and in months with higher temperatures, corals showed a high fecundity rate. The highest total fecundity rates have been recorded during April, May, June and July with average temperature almost 1 °C above the lowest fecundity months and the species with the highest rates were *T. aurea* and *Tubastraea* sp., with 43.50 ± 5.23 and 43.60 ± 4.04 oocytes/cm^2. *T. coccinea* presented the lowest fecundity rate with 34.64 ± 4.72 oocytes/cm^2. The present study demonstrated a directly proportional pattern of settlement rate and thermal amplitude, in addition to high rates of coverage area in the field. The results found in this study can contribute to the creation of measures to control invasion and mitigate impacts on the ecosystem.

1. INTRODUCTION

Exotic species introduction can promote a range of ecological impacts into an environment. Some examples of these potential impacts are, among others, biodiversity reduction, generation of hybrids, interference in ecological relations and alteration of food chain and nutrient cycle (Lodge 1993, Huxel 1999, Crooks 1998, Vitousek 1990, Ruiz et al. 1997). Scleractinian corals of *Tubastraea* genus became globally widespread living in natural and artificial substrates of shallow tropical waters (Cairns 1994). In the last three decades, corals of *Tubastraea* genus are expanding their distribution in the southwest Atlantic and becoming increasingly common in the Brazilian underwater landscapes. Currently, *Tubastraea* corals are found discontinuously throughout 3000 km area over Brazilian coastline (Creed et al. 2016) and it is also the most abundant scleractinian coral in the northern Gulf of Mexico on artificial substrata (Sammarco et al. 2017).

T. coccinea presents a wide tolerance to environmental stress. It is resistant to temperature, desiccation and pressure, where has been found 108 m depth (Reyes-Bonilla et al. 2005) and between tides (Creed 2006). Strategies such as the detachment of an adult non-skeletonized polyp from the colony and fix it elsewhere, known as polyp ‘‘bail-out’’, has also been documented (Capel et al. 2014). In addition, sun corals, which is how organisms of *Tubastraea* genus are known, may also facilitate invasion of other exotic species, as described by Rosa (2015), where *T. coccinea* and *T. tagusensis* corals species have become a consolidated substrate for species of invasive exotic bivalves like *Myoforceps aristatus* and *Isognomon bicolor*.

Tubastraea spp. expanded their distributions through incrustation in oil and gas rigs and transported to various globe areas (Creed et al. 2016). Today these corals represent the first introduction of scleractinian in South Atlantic (De Paula and Creed 2004). They also have high recruitment and settlement rates, which have potential to

occupy empty substrates (Paula and Creed 2005, Mizrahi 2008, Mizrahi et al. 2014), and it have rapid growth (Vermeij 2005, Lages et al. 2011, De Paula 2007). *T. aurea* species, for instance, have large polyps and massive colonies and are distributed throughout Korea, Japan, West Atlantic and Indo Pacific (Song 1982), and currently in southeastern Brazil.

Despite being a tropical region, Arraial do Cabo, on Brazilian Coast, has already recorded many occurrences of low temperatures (Batista 2017). According to Valentin (1984), coastal geographical and prevailing winds from east and northeast results in a rise of cold waters and full of nutrients that enrich primary production. A more restricted development of sun corals has already been suggested in Arraial do Cabo compared to other locations such as Baía da Ilha Grande (De Paula and Creed 2014), Gulf of Mexico and Florida coast where *T. coccinea* have a successful colonization (Fenner and Banks 2004). Some studies considered that the causes of this restricted coral expansion and high diversity of benthic communities are related to lower average temperatures at Arraial do Cabo (Ferreira 2003).

Sun coral larvae are known to have efficient swimming activity, rapid metamorphosis and settlement, and have gregarious behavior (Paula and Creed 2005). Larvae pelagic metamorphosis and polyps clustering allow greater potential dispersion in *Tubastraea coccinea*, reported by Mizhari et al. (2014) which is able to survive for 6 months in water column. In Fenner (2001), the author suggested that *Tubastraea* larvae remained competent for up to 100 days. According to Connell (1985), three processes have direct participation in settlement rates for marine invertebrates: larval supply, specific local hydrodynamic conditions and behavioral factors of suitable settlement substrate choice. This process is related to reproductive biology of organisms, but mainly

to behavioral factors of adults and the larva itself, together with specific oceanographic processes.

Studies of reproduction and larval biology are important for a better understanding of life history and ecology of scleractinians populations (Fadlallah 1983; Harrison and Wallace 1990). Also, changes in the reproductive effort are important indicators of environments changes and can help to understand species invasion patterns. In this way, reproductive studies help to develop control measures against invasive populations (Wotton et al. 2004). The knowledge of ecological and biological aspects of these corals are extremely important for environment and biota management.

Species success in invasion process depends on many factors and colonies fecundity and growth are the main characteristics that determine the ability of invasive species (Harrison and Wallace 1990). Growth rate may indicate coral potential expansion along with the settlement rate and gives us a better understanding of population dynamics and behavior of invaders in the studied area. Number or frequency of individuals, eggs or larvae and larval dispersion are predominantly related to success in the colonization process (Gaines and Bertness 1993) and fecundity is a fundamental demographic process for population dynamics studies (Alvarez-Noriega et al. 2016).

Lower average temperatures over the year at the study site can affect the physiological processes of corals and may reflect a slower invasion process than in other invaded areas. In this study, we investigated life history strategies of three co-occurring species of *Tubastraea* in Arraial do Cabo based on physiological parameters like growth rates, fecundity, settlement and coverage area, relating them to external environmental factors – temperature and local radiance. The biological and ecological parameters evaluated helped us to understand the processes related to the establishment of corals in

a nonnatural environment and understanding reproductive aspects that contributed to the successful colonization of sun coral species.

2. MATERIALS AND METHODS

2.1. Study area

Arraial do Cabo, at Rio de Janeiro State (22° 57’ S - 42° 1’ W), is a transitional zone between tropical and warm temperate Southwestern Atlantic Ocean (Spalding et al. 2007) and is influenced by an upwelling system. Deep, cold and nutrient-rich waters rise towards the sea surface as the result of coastal morphology and predominant east and northeast winds (Valentin 1984). Arraial do Cabo Bay is a sheltered area protected from direct action of upwelling system and from oceanic waves (Fig. 1). Inside the bay, water average temperature is around 22 °C (Ferreira et al. 2001). Due to these peculiar characteristics, Arraial do Cabo has a unique environment, with rocky coasts supporting a highly diversified subtidal benthic community, being also considered the southern tropical limit for many species, including corals (Laborel 1969, Castro and Pires 2001, Ferreira 2001). Due to increasing in offshore oil exploration, the region began to shelter platforms and supplier’s activities, thus promoting the arrival, and establishment of non-native species (Ferreira et al. 2006).

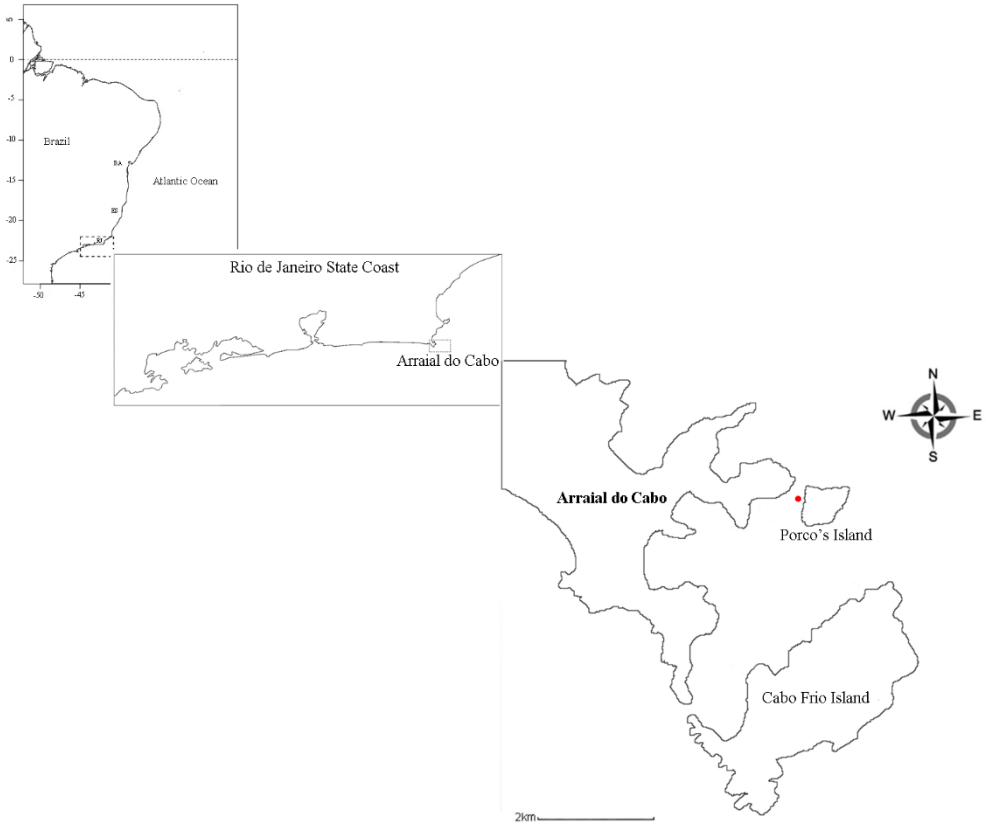

Figure 1. Map of Arraial do Cabo in southwest of Brazil. Scale: 2 Km.

Porcos Island, – sampling site of this study – delimits Arraial do Cabo Bay from the North (Candella 2009). Protected from the predominant NE winds, the study site was subject to little wave action (Carriére et al. 2009). Few records of cold water under 15 °C and high temperatures ranging from 25 to 27 °C near the study area were found in Batista et al. (2017), which creates environment condition for a fitting calcification rate of azooxanthellate and zooxanthellate corals (Marshall and Clode 2004). This region features a tropical coralline oasis (Laborel 1969) with substrate exhibiting rocks of various diameters and heights that extends from intertidal zone to a sandy plain 5 to 13 meters deep. Subtidal benthic community consists of anthozoans, ascidians, polychaetes, algaes, sponges, bryozoans and barnacles and cirripedians (Ferreira 2003, Araújo 2016).

According to Batista et al. (2017) the study site has the highest density of colonies of sun coral within the bay with 98 colonies per 15 m^2 and also exhibited the highest density of recruits with 44 by 15 m^2 and probably constitute the first populations of Arraial do Cabo.

2.2. Growth

Growth data of three species were collected during a one-year sampling. Colonies were chosen randomly, constituting all sizes of natural occurrence from initial and adult diameters. Total sampling was divided into two groups of 20 colonies each (N=40), for each species (N_{total} = 120), according the positions in areas of high or low light incidence (HL and LL, respectively). Definition about areas of highest and lowest light incidence was accomplished by visual observation according to substrate slope, but previously information for the same location and depths about irradiance rates were provided by Tunala et al (2019). Places defined as LL were small caves and stones with negative slopes. Sites of HL were fewer sloping areas or boulders exposed to light directly. In general, 40 individuals of each species had their largest and smallest diameter measured through a caliper rule during scuba diving at depths between 3 and 5 meters, in places with high density of *Tubastraea* species. Measurements were made every two months, from November 2015 until October 2016, (where T0 = first measurement and T5 = last measurement). Colony area (A), i.e. coverage area, was calculated through: $A = (d_{major}/2) \times (d_{minor}/2) \times 3.14$, where d_{major} is the largest diameter measure and d_{minor} is the smallest diameter of colony (e.g., Connell 1973; Hughes and Connell 1987). Growth rate was achieved through: Annual = Ct = Af - Ai, where Ct is the total growth; Ai = Initial area; Af = Final area (Af = area at T5 and Ai = area at T0). Bi-monthly growth between times was defined as $Cx = Ax – A_{(x-1)}$, when x = time and C = growth. Increase in coverage percentage, calculated as: $\%C = (Ct \times 100) / Ai$. Increase in polyp number was also verified. Total increase in polyp numbers (I_t) = $P_f – P_i$; P_f = polyp numbers at T5; P_i =

polyp numbers at T0. And bi-monthly polyp increases between times (I_x) = $P_x - P_{(x-1)}$. A total of 1440 measurements and 720 counts of polyps were performed over the study. All data were analyzed by ANOVA repeated measures in R language and the environment for statistical computing (R Core Team, 2015) in R Studio (R Studio Team, 2015).

2.3. Temperature

Water temperature was recorded every 1 hour by temperature sensors - Data logger DS 1921 L-F5, i-Button, Maxim Inc. (Thermochron® iButtons®) between November 2015 and October 2016. Average temperatures for sampled times were calculated.

2.4. Settlement and recruitment

Ten squares of 20 by 20 cm at least 40 cm away from each other on vertical rocks were scraped for observation of larvae attachment in the period between Nov/2015 to Oct/2016. Scraped areas were randomly chosen from a location of sun coral occurrence, including patches or coral aggregations of the genus. Every 15 days a dive was performed to observe the occurrence and count of newly settled larvae. After counting, these larvae were scraped with a wire brush for removal of individuals and any other organisms that were there. Substrates were scraped to avoid competition with another biofouling. Twenty-four dives were performed in 1 year to acquire data and gather information from planulation periods.

After this observation period of larval settlement, the same areas were maintained without manual intervention and photographed after two years to estimate the percentage of coverage of organisms and identification of species or groups within the square. Field pictures were analyzed using the Coral Point Count with Excel extensions 4.1 software (CPCe 4.1) (Kohler and Gill 2006). To estimate the percentage coverage of benthic

organisms in squares, a grid with 92 evenly distributed points was superimposed on the image.

2.5. Fecundity

Three colonies of each species were collected monthly between November 2015 and October 2016 at depths of 3 to 6 m depth and fixed in 10% formaldehyde for subsequent polyp dissection. Colonies were decalcified in 10% formic acid solution and 5% formalin solution. The largest diameter of oral disc and the distance between oral disc and the base of the polyp were measured with a caliper to calculate polyp area and to correlate with number of oocytes by polyp area (cm²), that is, the fecundity (Hall and Hughes 1996). In order to verify periods of greater species reproductive activity in sampled site, we collect monthly samples throughout the year. Gametes number found in two polyps from each colony in each sampling was verified. Polyps were observed in a stereo microscope for visualization of oocytes, embryos and/or larvae.

3. RESULTS

3.1. Growth

3.1.1. Colony area

Average areas of colonies of three species considered throughout all sampling period were 18.65 ± (SD 9.39) cm², 18.31 ± (SD 10.54) cm² and 17.26 ± (SD 8.39) cm² for *T. aurea*, *T. coccinea* and *Tubastraea* sp., respectively. Mean initial colony sizes in *T. aurea* were 17.9 ± (SD 5.11) cm^2 at low light incidence and 15.1 ± (SD 4.49) cm^2 at high light incidence. In *T. coccinea* the average area of individuals in low light was 16.66 ± (SD 7.36) cm^2 and in high light 15.31 ± (SD 7.71) cm^2. In *Tubastraea* sp. the averages were 14.38 ± (SD 6.33) cm^2 and 16.1 ± (SD 7.54) cm^2 in low and high light incidence, respectively. There was no significant difference ($p > 0.05$) when we analyzed all sampling time together.

When we analyzed coverage area of colonies in relation to time, we noticed highly significant differences. These differences are presented from the T3 (18.63 ± 9.60cm²) in relation to the beginning of samplings (T0, 15.83 ± 9.31cm²), ANOVA F = 5,267, p = 0.0225. For T4 and T5 the differences were even greater (19.55 ± 9.47 cm², ANOVA F = 9,372, p = 0.002, 20.07 ± 9.171cm², ANOVA F = 16,382, $p = 7.0 \times 10^{-5}$, for T4 and T5, respectively), in relation to T0. It is important to note that the high variance of mean occurs due to sampling colonies of varying size classes. For colonies individually, these significant differences in size increase over time are maintained (Tab. 1).

Table 1. Average coverage area of three species of *Tubastraea* over a year (Nov/2015 to Oct/2016). *Ta* = *T. aurea*; *Tc* = *T. coccinea*; *Ts* = *Tubastraea* sp.

Specie	**Colony area/cm² (mean ± sd)**					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
Ta	16.56±9.30	17.10±9.44	18.12±9.62	19.24±9.40	19.93±9.36	20.91±9.02
Tc	15.99±10.34	16.77±10.47	17.76±10.68	18.77±10.69	19.75±10.62	20.83±10.26
Ts	15.24±8.07	14.84±7.88	16.71±8.13	17.89±8.78	18.94±8.53	20.24±8.33
Total	15.93±9.22	16.24±9.30	17.53±9.47	18.63±9.59	19.55±9.47	20.07±9.17

*T0= November; T1 = January; T2 = April; T3 = June; T4 = August and T5 = October.

No significant difference was shown when we analyzed colony area values between species within each time period each time ($p > 0.05$). However, we observed significant differences between sample times within each species group. These differences were observed in *T. aurea* (T0 versus T5, ANOVA F = 4.507, p = 0.037), *T. coccinea* (T0 versus T5, ANOVA F = 4.420, p = 0.038) and *Tubastraea* sp. (T0 versus T5, ANOVA F = 0.005, T0 versus T4, ANOVA F = 4.482, p = 0.037). *Tubastraea* sp. showed the greatest difference in coverage area from the beginning in relation to the final sampling period, with the greatest increase in final coverage area (Fig. 2).

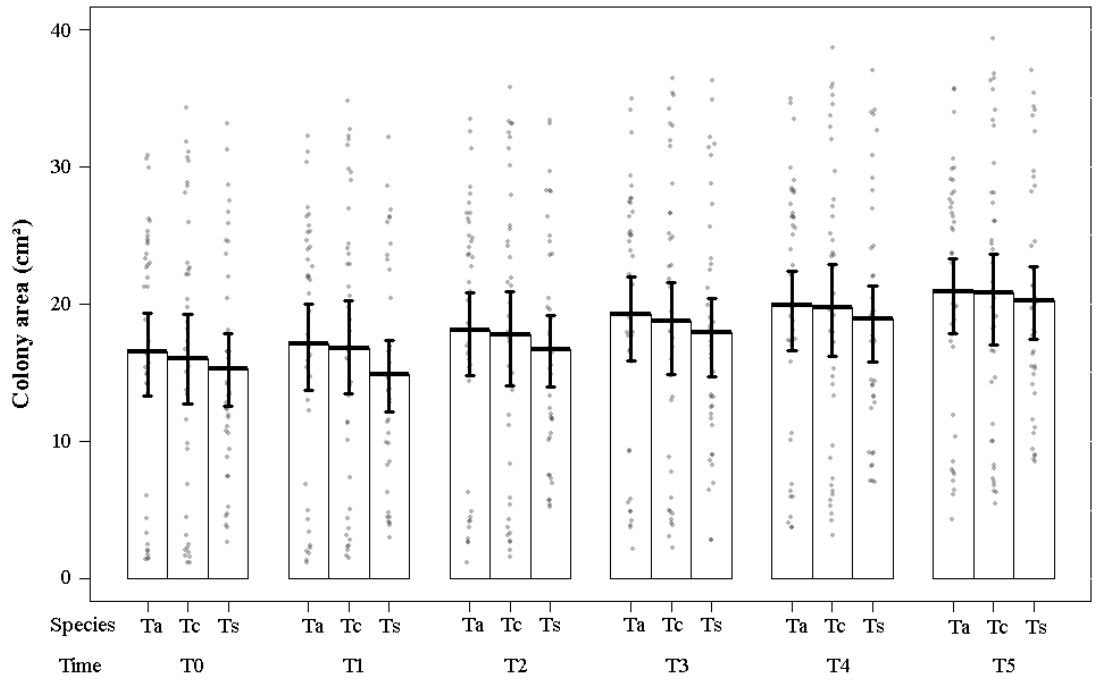

Figure 2. Average coverage area of 3 species of *Tubastraea* colonies from November/2015 to October/2016. Ta= *T. aurea*; Tc= *T. coccinea*; Ts= *Tubastraea* sp.

No significant difference was observed for the whole sample in relation to light levels (LL and HL). Over time, significant differences were observed for LL and HL in both T5 and T4 when compared to T0 (LL, ANOVA $F = 7.552$, $p = 0.0069$, $F = 4.148$, $p = 0.043$; HL, ANOVA $F = 8.857$, $p = 0.0035$, $F = 5.261$, $p = 0.0235$). Significant differences occurred within the *T. aurea* sample only, comparing the light levels LL × HL (ANOVA $F = 5.034$, $p = 0.025$), with mean values of 19.99 ± 10.40 and 17.29 ± 8.08, respectively.

However, when we observed light levels between species, we note a significant difference between *T. aurea* × *Tubastraea* sp. = 8.490, $p = 0.0039$) and *Tubastraea* sp. × *T. coccinea* (ANOVA $F = 0.301$, $p = 0.039$) for LL, while HL values were homogeneous. It is important to note that *Tubastraea* sp. presented significative differences between both species group in LL.

3.1.2. Growth rate

The annual total growth of all species, was equivalent to a coverage area of 567.74 cm² (Tab. 2). Greatest growth in time was in the interval between T1 and T2, that is, C_2 = 155.06 cm², equivalent to an increase in size of 15.36% in total for this period, presenting a highly significant difference of initial growth (C1 = 49.24cm², ANOVA F = 20.238, $p = 1.06 \times 10^{-5}$).

Light levels (LL × HL) did not show significant differences for C_t (total growth) or between times, but they showed a highly significant difference within the LL group and also HL between $C_1 \times C_2$ (ANOVA F = 12.177, p = 0.0006; ANOVA F = 8.162, p = 0.005, respectively).

Table 2. Growth area in cm² along the times, in each species of *Tubastraea* during a period of one year, in the different light levels and the total in each of them.

Times	**Growth area (cm²)**					
	T. aurea	***T. coccinea***	***Tubastraea* sp.**	**All species**	**LL**	**HL**
C1	22.00	31.23	-15,99	37.25	17.69	19.55
C2	40.67	39.71	74.67	155.06	83.82	71.24
C3	44.83	40.01	47.16	132.01	53.14	78.86
C4	27.57	39.67	42.16	109.41	68.15	41.25
C5	39.02	43.15	51.82	134.00	61.26	72.73
Ct	174.09	193.77	199.82	567.74	284.07	283.67

Among species the highest C_t was in *Tubastraea* sp., 199.83 cm², with an average growth of 5 cm² per colony per year. *T. coccinea* presented average growth per colony of 4.84 cm^2/year, while *T. aurea* grew 4.35 cm^2/year which presented the lowest growth when compared to other two species. After showing growth in initial measurement period (C1) equal to zero, *Tubastraea* sp. showed the largest general increase in area occurring in T2, responsible for the largest total increase in coverage in all period. From C2 we can observe that all growth rate of *Tubastraea* sp. represented the highest values between all species. The average growth rate of *T. aurea* in LL was 4.46 cm^2/year while in HL it was 4.24 cm^2/year. In *T. coccinea*, growth rate was 4.92 cm^2/year in LL and 4.77 cm^2/year in

HL. For *Tubastraea* sp. species the means were 4.83 cm^2/year and 5.17 cm^2/year in LL and HL, respectively.

About C_t among species there was a significant difference in *T. aurea* × *T. coccinea* (ANOVA $F = 6.229$, $p = 0.014$) and highly significant among *T. aurea* × *Tubastraea* sp. (ANOVA $F = 12.424$, $p = 0.0007$), i.e., *T. aurea* had significantly lower increase than *T. coccinea* and *Tubastraea* sp. Within groups of species, *T. aurea* presented significant differences for $C_1 \times C_2$ (ANOVA $F = 5.537$, $p = 0.021$) and for $C_3 \times C_4$ (ANOVA $F = 5.135$, $p = 0.026$), *T. coccinea* did not present significant differences between growth intervals and *Tubastraea* sp. showed a highly significant difference for $C_1 \times C_2$ (ANOVA $F = 14.261$, $p = 0.0003$).

Analyzing light levels, a significant difference was also observed for LL between $C_1 \times C_2$ in both *T. aurea* and *Tubastraea* sp. (ANOVA $F = 7.149$, $p = 0.011$, ANOVA $F = 6.707$, $p = 0.013$, respectively). In HL all species presented significant differences, being for *T. aurea* in $C_3 \times C_4$ (ANOVA $F = 9.397$, $p = 0.004$), for *T. coccinea* in $C_3 \times C_4$ (ANOVA $F = 4.103$, $p = 0.049$) and in $C_4 \times C_5$ (ANOVA $F = 4.185$, $p = 0.047$) and for *Tubastraea* sp. in $C_1 \times C_2$ (ANOVA $F = 8.500$, $p = 0.005$) (Fig. 3).

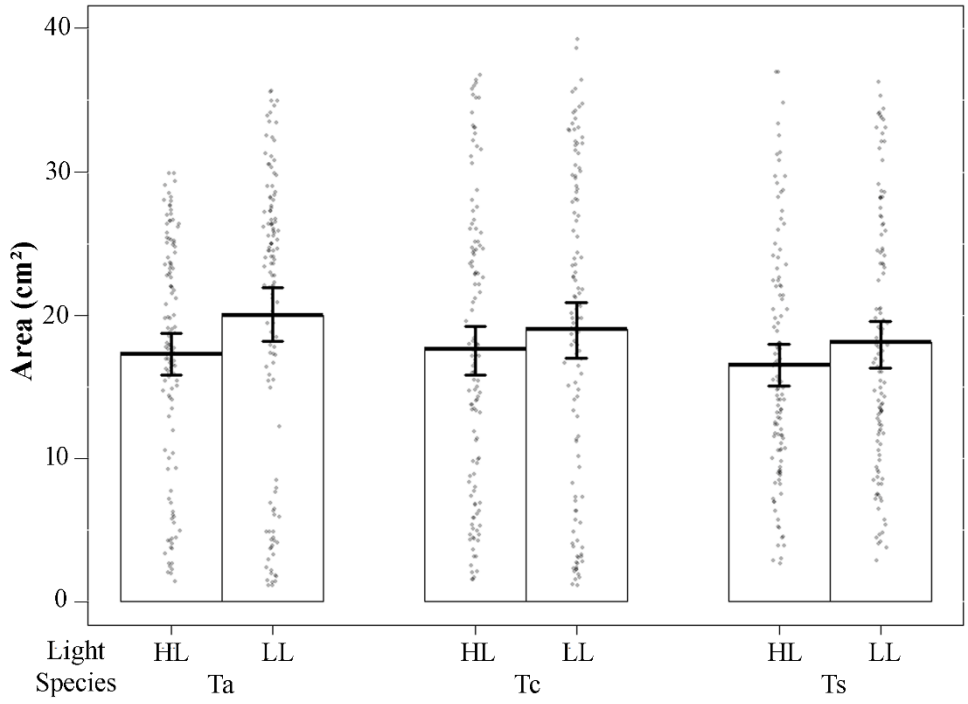

Figure 3. Growth in different light levels by species. A. Low Light (LL) and B. Growth in High Light (HL). Ta= *T. aurea*; Tc= *T. coccinea*; Ts= *Tubastraea* sp.

3.1.3. Polyps

A total average increment of 6.42 ± 1.70 polyps/year per colony was observed in *T. aurea* in the low light incidence (LL) samples, while in the high light incidence (HL) samples the average was 5.65 ± 1.77 polyps/year. *T. coccinea* presented an average increment of 8.4 ± 4.01 polyps/year in LL and 7.8 ± 4.07 polyps/year in HL. In *Tubastraea* sp. increment averages were 4.95 ± 1.93 polyps/year in LL levels and 5.2 ± 2.01 polyps/year in HL levels.

Total polyp increment (I_t) in a year were 253, 392 and 230 polyps for *T. aurea*, *T. coccinea* and *Tubastraea* sp., respectively. Significant difference in total increase occurred only between *T. aurea* and *T. coccinea* (ANOVA $F = 7.820$, $p = 0.0065$). Each specie group presents significative difference between polyp increments in time intervals, all of which presented at least between $I_1 \times I_2$. For *T. aurea*, all incremental intervals

presented significant difference in relation to the previous one (ANOVA F = 10.594, p =0.0016; ANOVA F = 9.758, p = 0.0025; ANOVA F = 7.531, p = 0.0075; ANOVA F = 4.608, p = 0.034), for $I_1 \times I_2$, $I_2 \times I_3$, $I_3 \times I_4$ and $I_4 \times I_5$, respectively. For *T. coccinea* there was only difference in $I_1 \times I_2$ (ANOVA F = 8.492, p = 0.0046) and the same for *Tubastraea* sp. (ANOVA F = 7.732, p = 0.0068). Significant differences were detected between polyp increments of whole sample (Tab. 3). These differences were strongly verified between times as we can see below. Also, we can observe a strong contribution of *T. coccinea* at all intervals, except in I_2, being the largest difference occurred in *Tubastraea* sp. compared to other species.

Table 3. Polyp increment between times for each three species of *Tubastraea* sp. and the total between the times, where intervals (I) represent the difference in the number of polyps between time T and time T (-1).

Specie	**I_1**	**I_2**	**I_3**	**I_4**	**I_5**
Ta	41	61	26	57	68
Tc	86	35	67	91	113
Ts	20	66	51	21	72
Total	**147**	**162**	**144**	**169**	**253**

Ta= T. aurea; *Tc= T. coccinea*; *Ts= Tubastraea* sp.

Regarding the levels of light exposure, increase in polyps do not presented significant differences when we considered all species together. Although, differences occurred between species, where ANOVA results for *T. coccinea* at LL were $p < 0.05$ for all species × light interaction. Analyzing groups of light levels between them (LL x HL), within each species, no significant difference was detected ($p > 0.05$), except for *T. coccinea* when p = 0.04.

Between times differences were evident, occurring from the T2 in relation to the beginning of sampling, being T0 × T5 (ANOVA F = 48.159, $p = 3.7 \times 10^{-11}$), T0 × T4 (ANOVA F = 17.883, $p = 3.35 \times 10^{-5}$) and T0 × T2 (ANOVA F = 6.491, p = 0.011). Within each time period there were significant differences only in T1, among *T. coccinea* × *Tubastraea* sp. species (ANOVA F = 8.111, p = 0.0056) (Tab. 4).

Table 4. Average polyp numbers for 3 species of *Tubastraea* sampled over a year at Arraial do Cabo Bay.

Specie	**Polyps number (mean ± sd)**					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
Ta	9.21±5.60	10.20±5.48	11.70±5.57	12.10±5.56	13.50±5.82	15.22±5.50
Tc	10.27±4.75	11.70±5.37	12.45±5.46	14.07±5.70	16.31±4.38	18.37±5.31
Ts	8.60±4.73	8.52±3.81	10.17±3.75	11.45±4.13	11.87±4.23	13.67±4.11
Total	9.20±5.08	10.20±5.09	10.85±5.00	12.01±5.15	12.63±4.85	14.20±5.18

Ta = T. aurea; *Tc = T. coccinea*; *Ts = Tubastraea* sp.

Within each species, all *Tubastraea* species showed differences between the times. For *T. aurea* the differences occurred in T0 × T5 (ANOVA $F = 19.537$, $p = 3.15 \times 10^{-5}$), T0 × T4 (ANOVA $F = 10.928$, $p = 0.0014$) and T0 × T3 (ANOVA $F = 7.537$, $p = 0.0074$). For *T. coccinea* the differences occurred in T0 × T5 (ANOVA $F = 11.303$, $p = 0.001$) and T0 × T4 (ANOVA $F = 7.617$, $p = 0.007$) and for *Tubastraea* sp. occurred in T0 × T5 (ANOVA $F = 17.839$, $p = 6.46 \times 10^{-5}$), T0 x T4 (ANOVA $F = 10.009$, $p = 0.022$) and in T0 × T3 (ANOVA $F = 7.489$, $p = 0.0076$).

For light factor, there was no significance for the total number of polyps between LL and HL, nor within the species ($p > 0.05$). However, comparing species among different levels of light there was a significant difference in LL for *T. coccinea* × *Tubastraea* sp. and *Tubastraea* sp. × *T. aurea* (ANOVA $F = 12.324$, $p = 0.0005$; ANOVA $F = 4.146$, $p = 0.042$). Over time, the differences appeared for both LL and HL. In LL there were significant differences from T2 in relation to T0, being: T0 × T5 (ANOVA $F = 29.537$, $p = 3 \times 10^{-7}$), T0 × T4 (ANOVA $F = 18.385$, $p = 3.71 \times 10^{-5}$), T0 × T3 (ANOVA $F = 10.227$, $p = 0.001$) and T0 × T2 (ANOVA $F = 5.941$, $p = 0.016$). For HL the differences occurred between T0 × T5 (ANOVA $F = 21.765$, $p = 8.2 \times 10^{-6}$), T0 × T4 (ANOVA $F = 12.874$, $p = 0.0004$) and T0 × T3 (ANOVA $F = 9.640$, $p = 0.002$). Comparing species within light levels in time periods only *T. coccinea* × *Tubastraea* sp. showed a significant difference for LL (ANOVA $F = 5.317$, $p = 0.026$). Both species had

a large increase in polyp numbers by extra-tentacle budding through the coenosarc (Fig. 4).

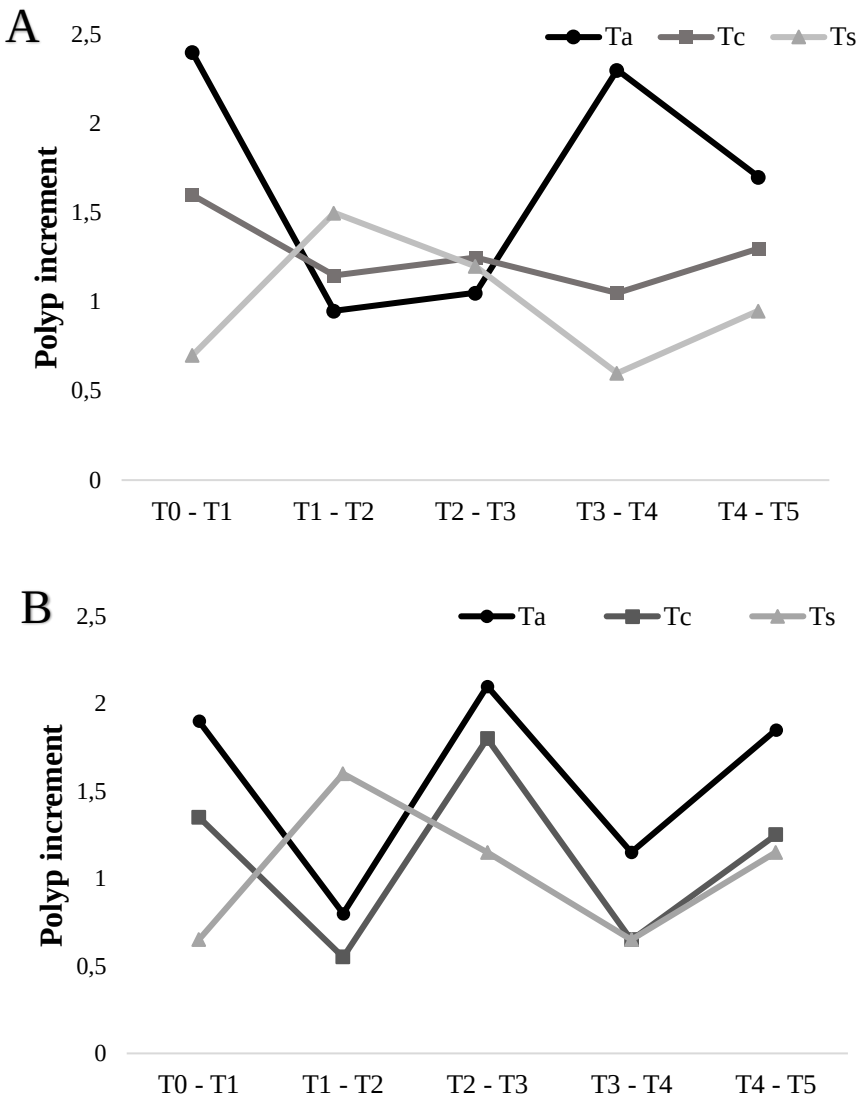

Figure 4. Increase of the number of polyps at different light levels by species. A. Low Light LL) and B. High Light (HL). Ta= *T. aurea*; Tc= *T. coccinea*; Ts= *Tubastraea* sp.

3.2. Temperature

Temperature values sampled during a year were analyzed. Below is a graph with all data recorded by I-button temperature sensors and their daily moving average (Fig. 5). Annual average was 21.84 ± 1.73 °C, with maximum temperature of 26.25 °C and minimum of 14.5 °C. March and April/2016 were the months that reached the maximum temperature and January/2016 reaching the minimum.

We considered that values below 20 °C refer to the influence of upwelling waters – in May to September/2016 no upwelling event was detected, i.e. in months after end of summer and early spring (in which event is commonly observed).

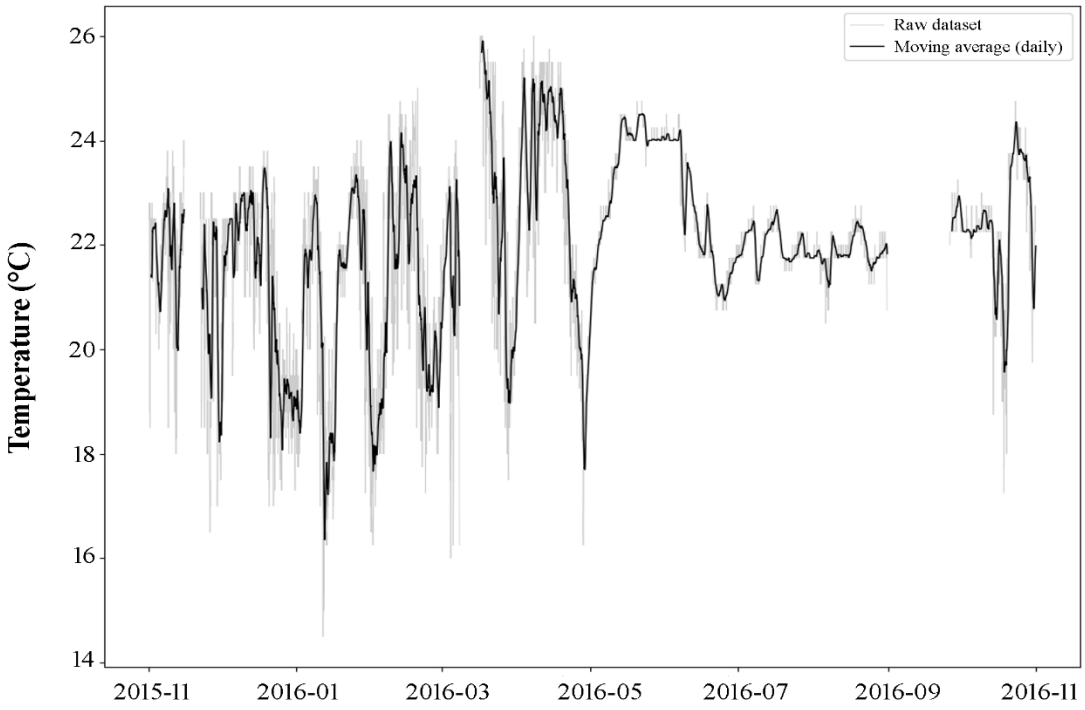

Figure 5. Temperatures recorded hourly over a year at Arraial do Cabo Bay. The gray line shows the raw data and the black line represents the average daily temperature (°C).

When we observe these data, we can notice certain seasonality in the thermal variation (Fig. 6). Plotting the data more broadly was observed wide and differentiated thermal amplitude (Δ°C) for the months of late spring until late summer. Meanwhile the data referring to autumn and winter months have a much lower variability than months in which there was detection of upwelling influence.

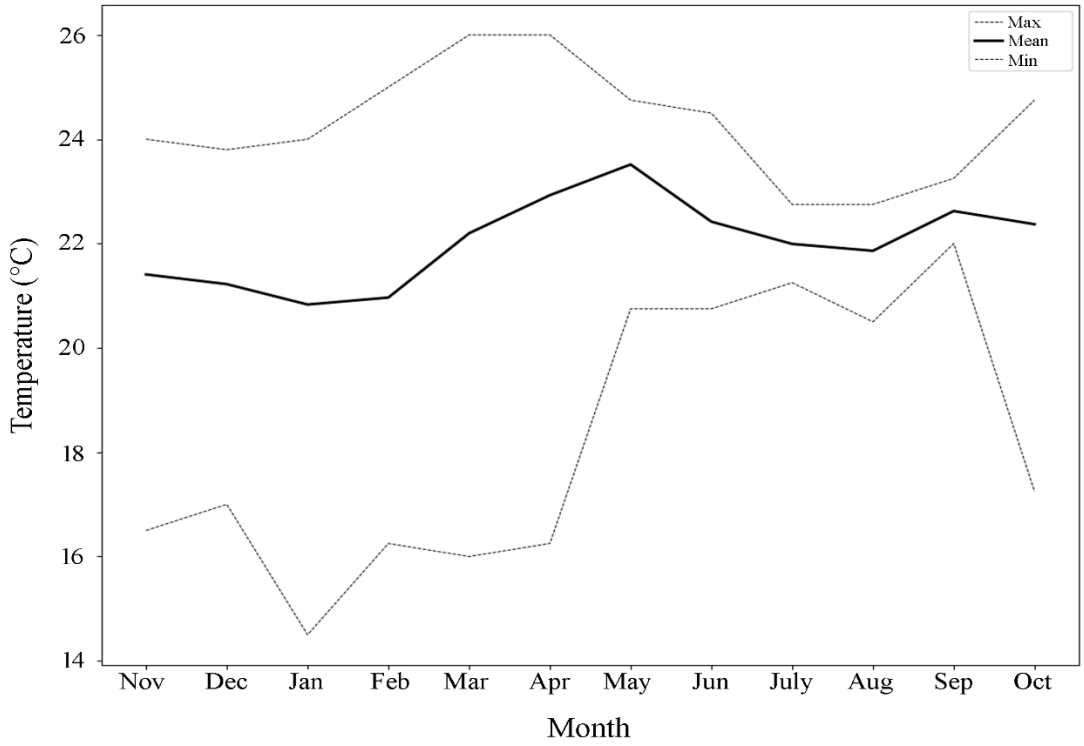

Figure 6. Annual thermal amplitude (Δ°C) over a year of sampling at Arraial do Cabo Bay, RJ – Brazil. Upper dotted line represents the maximum temperatures while the dotted line below the minimum temperatures. Bold line represents the averages by time.

From the information in the graph above, we can plot the data to view only the thermal amplitude data for the same sampling period (Fig. 7). Graph shows us a pattern of temperature variation throughout the year, with high variations in the summer period, reaching 10 °C in March.

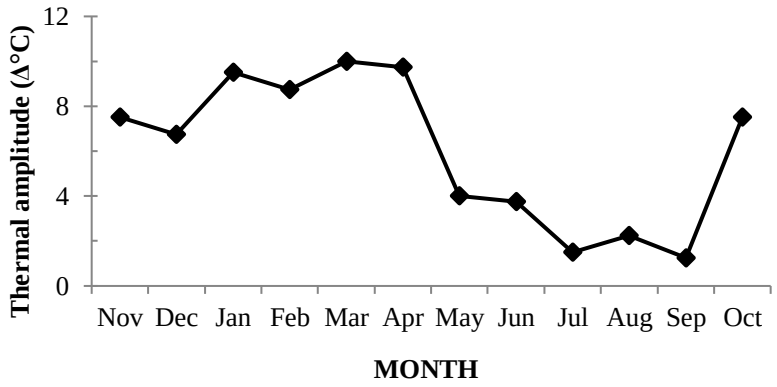

Figure 7. Thermal amplitude at Arraial do Cabo Bay over a year (November/2015 to October/2016). Months with the greatest variations of minimum and maximum temperature were considered under the influence of upwelling, showing also the highest growth rates of *Tubastraea* colonies, in some cases.

3.3. Settlement and Recruitment

In graph below (Fig. 8) we present the fortnightly settlement values, where T1 represents the first sampling of November 2015 and T2 the second, T3 and T4 refer to the December samplings, and so on. The fortnightly average settlement rate was 4.04 ± 2.88 larvae/quadrat, with maximum values for October (T23 and T24) followed by April (T11 and T12). Minimum rates occurred in February (T7 and T8), with no settlement occurring and then in July (T17 and T18), and later from late August to early September (T20 and T21).

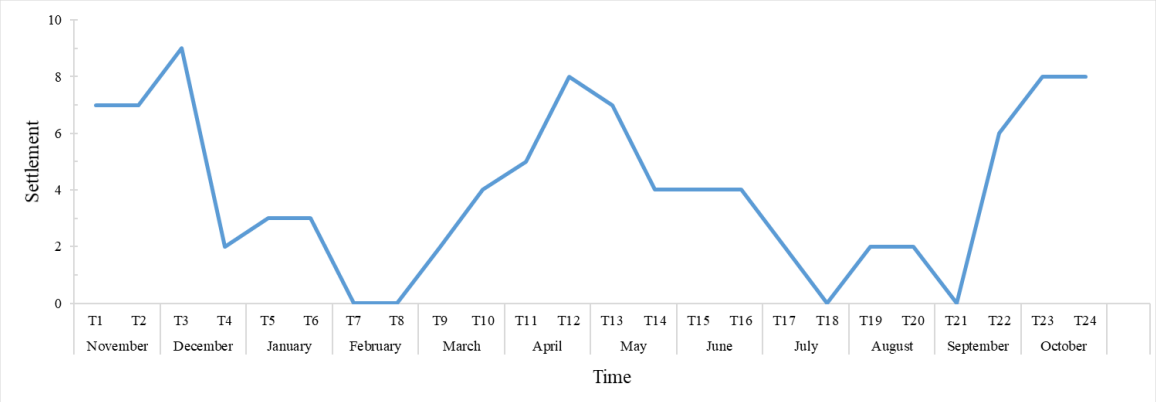

Figure 8. Biweekly average settlement rate of *Tubastraea* spp. larvae during the period from Nov/2015 to Oct/2016 in 10 scraped areas on the rocky shore.

We can see a directly proportional pattern of settlement rate and thermal amplitude, from April. However, in previous period we observed minimum settlement rates, from January to March (Fig. 9), which also corresponds to the lowest temperatures.

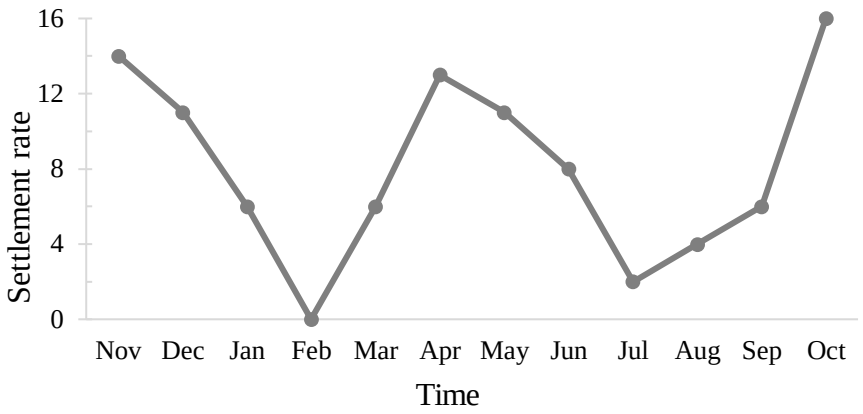

Figure 9. Average monthly settlement rate of *Tubastraea* larvae in ten scraped areas on the rocky shore over a one-year period. The settlement rate is directly related to the thermal amplitude and the minimum temperatures during periods of greatest temperature variation.

In scraped areas on rocky shore, two years later, newly fixed larvae of *Tubastraea* corals covered an area percentage of 2.85%, while recruits and colonies represented 2.8 and 3.3% of coverage area, respectively. Among fixed larvae, recruits and sun coral colonies, 8.9% of total coverage area sampled was obtained. Calcareous algae were predominant occurring in 20% of studied areas. (Fig. 10). Nine of 10 shaved areas contained freshly settlement larvae and/or recruits and/or sun coral colonies. Together, all sponges viewed at intersection points totaled 13.8%, algae covered 37.1%, bryozoans 8.6%, cnidaria (*Palythoa caribaeorum*) 9.9% and ascidians 1.7%.

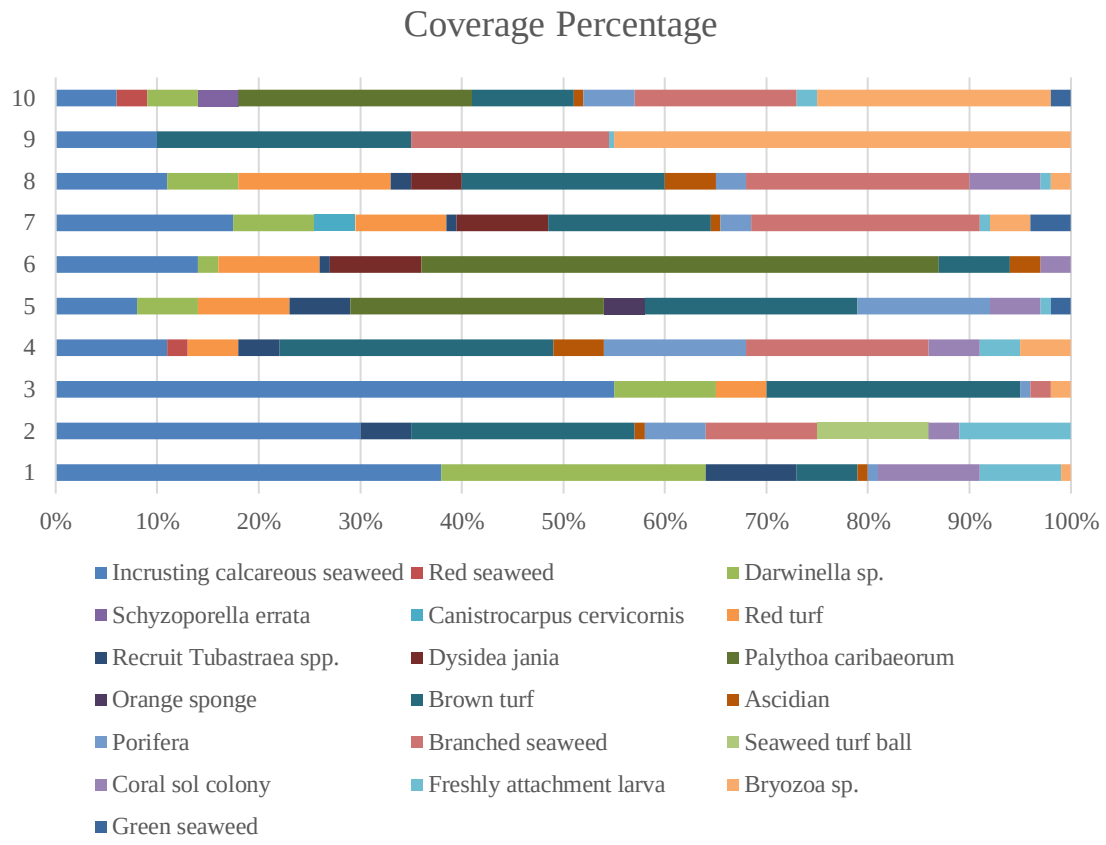

Figure 10. Percentage of coverage of organisms found in the scraped areas numbered from 1 to 10 in the place of occurrence of sun coral. Not all individuals were identified, so they were given names regarding their shape or color.

3.4. Fecundity

During the same growth rate sampling period (Nov/2015 to Oct/2016), colonies were monitored monthly for fecundity rates (oocytes/cm²). Total average oocyte/cm² for the entire sampling period was 40.58 ± 3.22. The highest average fecundity rates were for *T. aurea* and *Tubastraea* sp., with 43.50 ± 5.23 and 43.60 ± 4.04 oocytes/cm² for each species, respectively, without significant differences between them. Tc presented the lowest rate among species, 34.64 ± 4.72 oocytes/cm².The lowest total fecundity rates, summing the three species observed throughout the sampling period occurred in January, November and December, with 65.18, 94.24 and 97.88 oocytes/cm², while the highest fecundity rates occurred in June and July, with averages of 64.11 ± 4.46 and 53.80 ± 0.08 oocytes/cm² (Fig 11).

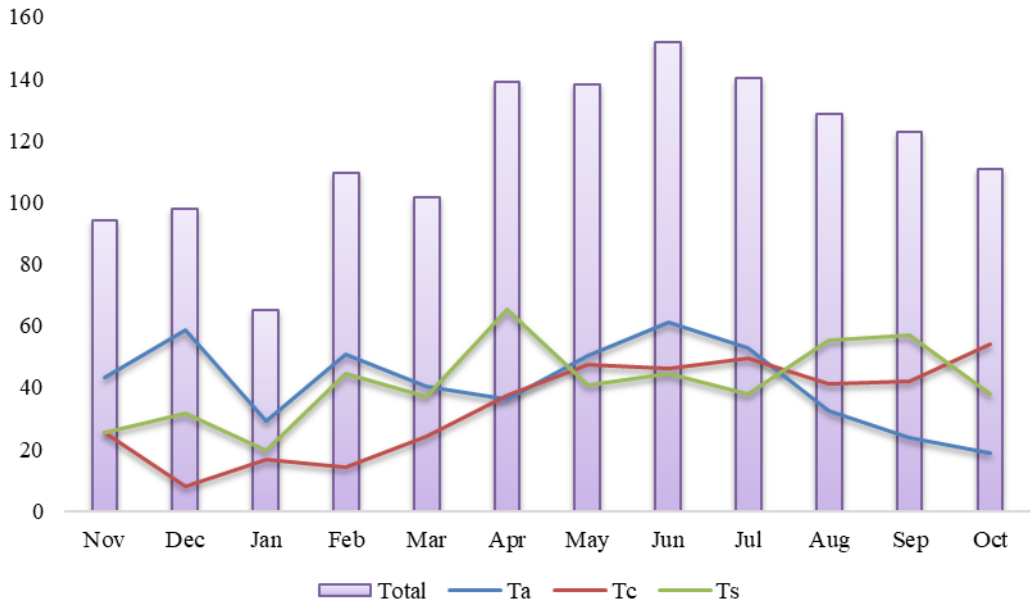

Figure 11. Fecundity rate (oocytes/mm^2) of three *Tubastraea* species sampled between November/2015 and October/2016. The highest total fecundity rates occurred in June and July and the species with the highest rates for Ta and Ts, with the month of June being Ta species. Ta= *T. aurea*; Tc= *T. coccinea*; Ts= *Tubastraea* sp.

3.5. Growth versus Fecundity and Thermal amplitude

When we related temperature data to growth rates (i.e. growth in area) we can observe that occurred the highest growth of all species in period between January and March, a period also presented the highest temperature variation throughout the year sampled (Fig. 6). During this period there was also the greatest increase in area in *Ts* and the increase in area of the colonies when compared to the previous period was higher in March/May (T2-T3) than January/March (T1-T2) for all species together. Highest increases in different light level groups were also related to the same period mentioned above both for LL (January – March), and in HL especially for *Ts*. When we contrast growth with fecundity rates throughout the year, we can see something approaching a behavior pattern: when fecundity rates are higher, from June to July, growth declines, increasing again when the number of oocytes per area begins to decrease. Also, when we have the lowest fecundity rates over the summer months, we can see an abrupt growth

from February (Fig. 12). Still, when plotting thermal amplitude line, we observe that high temperature variation does not favor the fecundity of *Tubastraea* colonies and the lower temperatures favored growth. However, temperatures below 16 °C may be acting as a barrier to growth (see the graph with maximum and minimum temperatures, Fig. 5). Above this value, growth rates increase again even with higher temperatures, i.e.> 24 °C. More constant temperatures seem to favor higher fecundity rates or high temperatures that precede high fecundity months may be acting as a trigger, or as a catalyzer for the next fecundity period (Fig. 12).

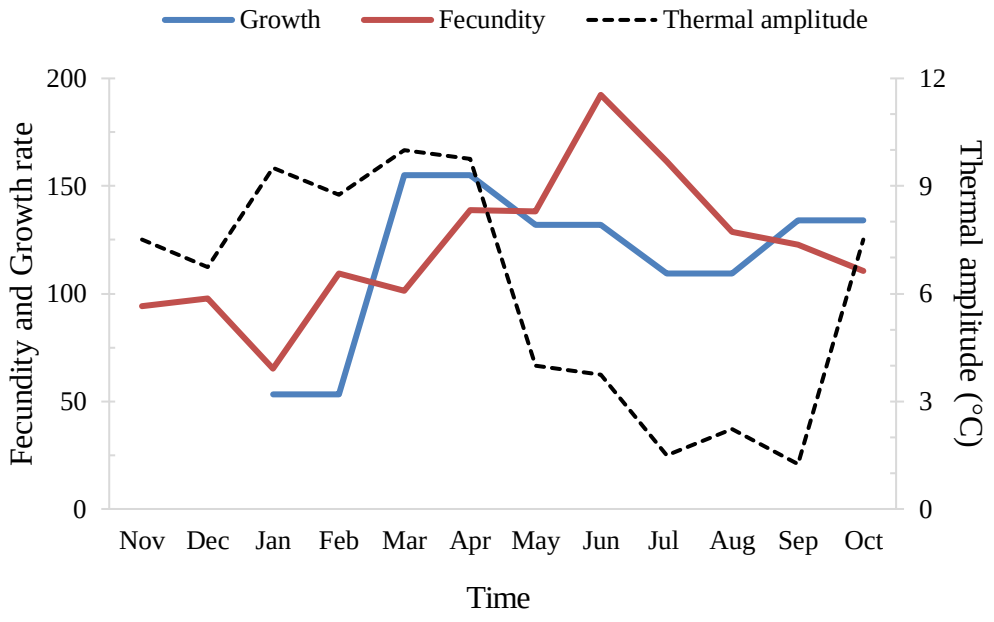

Figure 12. Fecundity and growth rates of three species of *Tubastraea* over the period of one year. The right axis represents the thermal amplitude over the same period (dashed line). In periods of more constant temperature, growth and fecundity rates show a clear pattern. Fecundity in oocyte/cm^2 and Growth rate in cm^2.

4. DISCUSSION

In this study, we observed the highest total growth in the period of greatest temperature variation, between January and March, where the lowest annual temperature reached 14.5 °C and the highest 26.2 °C. Species that more contributed to this growth was *Tubastraea* sp. which achieved the highest growth rate during this period. *T. aurea*

showed greater growth during April and May, which had an average temperature of 22.86 °C, higher than the annual average of 21.98 °C. *T. coccinea* colonies grew more in the period of lower thermal amplitude, when temperatures varied between 20 and 23 °C (August and September) with an average of 22.3 °C. Average temperature between months of greatest growth of *Tubastraea* sp. was 21.27 °C, showing greater growth affinity at lower temperatures. The same did not happen for the other species that grew at the highest temperatures. If we consider that an annual temperature cycle is repeated over the years, we can suggest that the input of nutrients in upwelling periods related to higher densities of zooplankton in Arraial do Cabo (Valentin 1984) contribute to area growth of *Tubastraea* sp. Smaller increases in area occurred in the months later the upwelling events (June and July) and in the spring months (November and December). Expenditure in energy for individual maintenance and survival are also directly related to growth (Odum, 1988).

A positive relationship between increase in temperature and growth in *T. coccinea* was recorded by Mizhari (2008) where the coral increased 4.59 cm^2 $year^{-1}$ in a place with 21.6 °C, while in the place with 20.8 °C growth was 1.14 cm^2 $year^{-1}$. *T. aurea* and *T. coccinea* colonies showed a greater affinity to increase in temperature and increased growth, showing greater area increase in warmer periods. Also, according to Lough and Barnes (2000), increase of 1°C in sea surface increased annual calcification rate and the annual average extension of massive corals of *Porites* genus. Average growth of all *Tubastraea* colonies analyzed, in the same area, was 4.73 cm^2/year, much higher than that recorded for the massive coral of the genus *Siderastrea*, with 2.5 mm per year of linear growth rate, according to Lins de Barros (2006). Vermeij (2005) considered that *Tubastraea* have accelerated growth, the author described the growth of 3.02 cm^2 $year^{-1}$ in Curaçao.

Table 5. Difference in area increase between bi-monthly data acquisition periods.

Times	*T. aurea* (cm^2)	*T. coccinea* (cm^2)	*Tubastraea* sp. (cm^2)	Total	Temperature average (°C)
T0-T1	0.54	0.78	-0.11	1.32	21.07
T1-T2	1.02	0.99	1.87	3.88	21.33
T2-T3	1.12	1.01	1.18	3.31	22.95
T3-T4	0.69	0.98	1.05	2.72	22.09
T4-T5	0.98	1.08	1.3	3.36	22.28

T. aurea showed to be more influenced by light regime in relation to growth. These corals preferentially inhabit shaded areas because they do not have symbiosis with photosynthetic algae, on the other hand these algae help increase the capacity of corals to synthesize calcium carbonate (Muscatine 1990). Mizrahi (2008) reported that in places with higher temperatures and light there was a higher growth rate in *T. coccinea* and where there was lower temperature and light there was less growth. In this study, we found no significant differences in growth at different levels of light. In a biological reflection of the data, the species *T. aurea* and *T. coccinea* had greater growth in areas with less incidence of light, demonstrating that they are more adapted to shady places than to light ones. In the species *Tubastraea* sp. the opposite was observed, colonies of higher incidence of direct light areas grew more, and at the study site, this species is more common in these areas than in the shaded areas.

Polyp multiplication followed growth pattern, more polyps grew in low light regime in *T. aurea* and *T. coccinea*, while in *Tubastraea* sp. more polyps appeared during high light regime, showing a total average increase of 6.42 in LL and 5.65 polyps/year in HL; 8.4 in LL and 7.8 polyps/year in HL; 4.95 in LL and 5.2 polyps/year in LL, respectively. *Tubastraea coccinea* showed the highest number of polyps increment over one year, with 8.1 polyps/year average. Morphological studies pointed out as a characteristic of species the greater number of polyps per area of colony compared to *T. aurea* and *Tubastraea* sp. (Bastos et al. Submitted). *T. coccinea* has polyps with calyx

diameters smaller than the others and its skeleton is not robust like *T. aurea*. To expand the colony, corals can reproduce asexually. Budding is a form of colony enlargement that is very common among sun corals where new polyps grow from an old polyp or from oral disc of a parental polyp. Fragmentation can also occur through formation of two new colonies (Campbell 1983) but this has not been observed in the field.

Continuous and simultaneous recording, throughout the year, of temperatures and reproductive aspects such as fecundity and settlement, allowed to verify the relationship between them. Two spawning periods occurred, one in spring (October, November and December) and another in autumn (April, May and June). It should be considered that average temperature between seasons was similar, showing an average of 22.06 °C in spring, 22.17 °C in summer, 21.27 °C in autumn and 22.17 °C in winter. Months of greatest settlement showed almost 1 °C warmer than months of little or no larval settlement. In autumn, there were high fecundity and settlement rates and average temperature exhibited 22.44 °C. Fecundity rates remained high in winter (July, August and September) with an average temperature of 22.17 °C. We can observe an affinity between higher averages of water temperature and greater reproductive activity of *Tubastraea* corals. Invading corals produce gametes and larvae continuously, due to occurrence of larvae in at least one of samples in all months of the year. Reproductive peaks related to fecundity occurred between April and July and related to establishment of larvae between April and June and October and December. This leads us to suggest that the *Tubastraea* corals can release larvae constantly, but there were two main reproductive peak periods considered spawning periods. In summer, there was low fecundity and little or no settlement was verified, inversely proportional to growth that was greater in this period. Some benthic animals were seen inside the shaved square, they could be feeding on newly fixed larvae. Grazing fish removes algae and, in most cases,

scrape juvenile corals (Sebens, 1983), which could justify the absence of established larvae, among other variables, in months with fertile colonies.

In this study, growth among species of *Tubastraea* corals was inversely proportional to fecundity (see Figure 11). In the eastern Pacific (Panama), species *Pocillopora damicornis* did not release larva over a period of 2 years and during this time, exhibited a higher linear growth rate of 3.6 to 6 cm per year. Data on fecundity, growth rates and the energetic content of tissue allow an estimation of the relative caloric investment in both colony growth and reproduction processes via planulation (Richmond 1987). According to the author, colonies of *P. damicornis* in two different areas allocate similar amounts of colony caloric content to biomass production, however, while in one region most of this energy is represented by planulation, other is allocated to colonies growth and subsequent fragmentation. Fecundity rate in Arraial do Cabo was much larger than in Ilha Grande Bay, Rio de Janeiro (*T. aurea* 43.50 ± 5.23, *T. coccinea* 34.64 ± 4.72 and *Tubastraea* sp. 43.60 ± 4.04 oocytes/cm², against 10 oocytes/cm^2 in *T. coccinea* and 2.68 oocytes/cm^2 in *T. tagusensis*) (De Paula 2007). This difference can be explained because, in this study, number of oocytes and larvae was counted through histological slides and not through the polyp dissection that impairs the full reproductive propagules count.

Increase in temperature averages from March until May was positively related to increase in growth rate in *T. aurea,* highlighting that April reached the highest average of the year with 24.13 °C. In this same period, we observed peaks of settlement larvae in the field. A second increase in average temperature occurs in September when *T. coccinea* grew, so the relationship between temperature increase and higher growth rates is observed. Larvae settlement has already been positively related to temperature increase by Harriott and Fisk (1988) although, in Airi et al. (2014), they reported a decrease in

reproductive efficiency of endemic zooxanthellate corals with excessive increasing water temperatures, up to 32 °C, in the Mediterranean Sea. Fecundity was also related to temperature increase in *T. coccinea* and *Tubastraea* sp. during this period of temperature rise. According to Airi et al. (2014), changes in water temperature can alter the physiological function, reproductive output and demography of marine organisms. Babcock et al. (1986), Oliver et al. (1988) and Harrison and Wallace (1990) suggest that reproductive cycle of corals can be regulated by variations in water temperature and photoperiod. Therefore, distinct environmental pressures may act on the reproductive strategy of corals at different locations of their geographical distribution (Castro et al. 2006). We suggest that low temperatures may be acting as a limiting factor for larval settlement.

Incrusting calcareous macroalgae, brown turf algae, branched algae, *Palythoa caribaeorum*, *Bryozoa* sp., *Darwinella* sp. and red turf were the most frequent space-occupying organisms found at all square. Area covered by *Tubastraea* larvae, recruits or small colonies was observed among 19 other identified organisms. According to Lages et al. (2011), sites where *Tubastraea* spp. was present and more abundant had greater diversity, uniformity and species richness when compared to the same places without these exotic corals. Since the substitution of species composition is outside the scope of this research, a large time scale was used for collection of biotic data, causing variations in species abundances to be lost and temporal patterns in substrate occupation by different dominant species could not be observed. Great occurrence of richness and diversity found in available areas demonstrate that *Tubastraea* spp. corals co-occur with native organisms and may not be negatively affecting the development of benthic assemblies. Highly competitive potential of these species in colonization of new substrates reflected in covered area found of 8.9%. In contrast, in Arraial do Cabo, studies have shown low

abundance and a restricted distribution of corals of the genus *Tubastraea*, when compared with other regions (Batista et al. 2017). In addition, low percentages of coverage (0.2 %) were found in Araújo (2016) and, according to the author, richness, diversity and equitability of benthic species did not differ between invaded and non-invaded communities.

Tubastraea coccinea is increasing its area extension capacity with higher growth rates than documented about 10 years ago in the region (Mizrahi 2008). Due to acquisition method, corals fecundity proved to be much higher when compared to studies of reproductive effort in the Ilha Grande Bay (De Paula 2007), approximately 300 km away. Also, species showed relevant coverage rates at the coast region. With information generated in this research, we found that these invading corals are well established and adapted to studied site. It is still possible to see that reproductive strategies are even more efficient. Therefore, it becomes increasingly important and urgent to monitor the invasion of the rocky shores of Arraial do Cabo to investigate and monitor the mechanisms of colonization, dispersion and demographic growth of these species.

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CHAPTER 3

Reproductive biology aspects of three species of invasive corals in an area affected by the upwelling in southeastern Brazil

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ABSTRACT

In the present study we adressed the follow question: would *Tubastraea* corals have limited reproduction activity due to the low average water temperature? To answer this question, we evaluated the reproductive biology aspects of three invasive coral species in an area influenced by the cold waters of the Cabo Frio upwelling region in southeastern Brazil. The results showed that there was no decrease in reproductive activities when compared with upwelling and non-upwelling sites. Throughout different trials, we found: high fecundity rates; occurrence of at least two gametogenic cycles per year; continuous gamete production; incubation; gametogenesis overlap; and various planulation events. These characteristics do not appear to be negatively influenced by lower temperature averages in the region. Larvae survived more days in vitro assays than has been reported in Brazil. Average fecundity rates were similar between species - 293.8, 196.7 and 230.2 oocytes/cm^3 for *T. aurea*, *T. coccinea* and *Tubastraea* sp., respectively. Species were characterized as simultaneous hermaphrodites and brooding their larvae. In this study, we found three reproduction peaks and two gametogenic cycles. The highest average oocyte diameter III, the highest occurrence of the mature oocyte stage and mainly fecundity were considered reproductive peak for all species. The largest oocytes III occurred in *Tubastraea* sp. with 2.25 mm. There was interspecific synchrony of the oogenesis. Reproduction processes keep coral populations and to succeed in controlling and managing invasive populations, we need to be acquainted with elements of their biology especially in regards to reproductive traits. To clarify inter- and intra-population reproductive patterns, gametogenesis, embryogenesis and planulation are of great importance for possible methodologies of species management and control.

1. INTRODUCTION

First reported as non-indigenous organisms in the Caribbean Sea (Vaughan and Wells 1943), coral species of the genus *Tubastraea* are currently widely distributed in the Caribbean (Cairns 2000), Gulf of Mexico (Sammarco 2004), Florida (Fenner and Banks 2004) and Brazil (De Paula and Creed 2004). The specie *Tubastraea coccinea* Lesson, 1829 was originally described in Bora Bora, French Polynesia while *Tubastraea tagusensis* (Wells, 1982) was originally described in Tagus Cove, Galapagos Islands. Currently, both species are recorded from northeast to south of Brazil (Creed et al. 2016) the first sighting having been in the 80s (Castro and Pires 2001). Corals of *Tubastraea* genus have a confusing taxonomy and systematic history due to interspecific overlap of morphological characters. A recent study described morphologically three species in southwest Brazilian coast, recognizing *T. aurea*, *T. coccinea* and *Tubastraea* sp. (Bastos et al., Submitted data). *T. aurea* is considered synonymous with *T. coccinea* and its distribution is between Korea, Japan, West Atlantic and Indo Pacific (Song 1982). These three species were the focus of this study, two morphotypes were commonly considered as variations of *T. coccinea* and the third, previously considered *T. tagusensis*, was called *Tubastraea* sp.

The success of *Tubastraea* invasions are mainly due to its highly competitive potential that allows rapid expansion and colonization of new areas (Cairns 2000; Sammarco et al. 2004). This coral is a clear example of an invasive species, presenting efficient reproductive strategies, ability to settle on different substrates and high growth rate, which allows rapid species establishment allowing it to quickly dominate new areas (Vermeij, 2005; Creed and De Paula 2007). In addition, *Tubastraea coccinea* has hermaphroditism, external fertilization (Ayre and Resing 1986) or self-fertilization, brooder and release lecithotrophic larva and has continuous reproduction (Fenner and

Banks 2004; Glynn et al. 2008; De Paula et al. 2014). *T. coccinea* was recorded producing asexual planulae in Australia (Ayre and Resing 1986), here in Brazil, was described performing self-fertilization by De Paula (2007). *T. aurea* (synonym of *T. coccinea*) has been reported as a gametes-releasing coral in the Great Barrier Reef, Australia (Harrison 1985). According to Vermeij (2006) the beginning of reproductive age occurs at 1.5 years, but according to Glynn et al. (2008) the coral begins its reproductive activity within months of life, for example colonies with only two polyps.

The species *T. coccinea* was described in Arraial do Cabo region, Rio de Janeiro state, in the year 1999 (Ferreira 2003) and by 2008 *T. tagusensis* already occupied mono-buoys near Forno Harbor (R. Coutinho, per. comunication). Nowadays, both species were recorded as established in the rocky shores of Arraial do Cabo bay, indirectly influenced by the upwelling phenomenon with an annual average of 22 °C. In this region, these corals do not occur in places directly influenced by temperatures lower than 12.5 °C, as registered in Batista et al. (2017). Some studies have already reported that sun corals in Arraial do Cabo would present a restricted expansion compared to other parts of the coast of Rio de Janeiro state and pointed out high benthic communities diversity, hence upwelling cold waters are a possible cause for sun coral restricted expansion (Ferreira 2003, Mizrahi 2008). Studies of reproduction aspects and biology of larvae are important for better understanding life history and ecology of scleractinian populations (Fadlallah 1983; Harrison and Wallace 1990).

Tubastraea corals incubate their embryos and larvae, resulting from internal fertilization and the embryogenesis and planula development occur in the gastrovascular cavity (Fan et al. 2006). Both are considered simultaneous hermaphrodites (De Paula et al. 2014). The gametogenesis of the three species was studied to find the temporal pattern of reproduction. Reproductive characteristics such as number and duration of

gametogenic cycles, number of gametes produced and larvae characteristics can clarify on their reproductive strategies and also on the dispersal capacity of gametes or larvae.

Reproductive activity is considerably reduced when water temperature is seasonally low. Glynn et al. (2008) reported greater presence of *T. coccinea* larvae when water temperature was higher. Yonge (1940) suggested that temperature is an important factor that determines geographic distribution of reef corals by controlling spawn timing and reproductive behavior. Moreover, it is known that reproductive traits as a reproductive mode (gametes release for external fertilization or incubation of larvae) and sexual pattern may be variable among different populations within the same species (Harrison and Wallace 1990, Soong 1991, Ward 1992). Therefore, different environmental pressures may act on the reproductive strategy presented by corals at different locations of their geographical distribution (Castro et al. 2006).

This study aimed to verify the reproductive features of three species of *Tubastraea* corals from Arraial do Cabo, evaluating the inter- and intraspecific synchronicity of gametogenesis and fecundity. And furthermore, find out if *Tubastrea* spp. is less efficient in areas with lower temperature regime. This study is also the first investigation of *T. aurea* reproductive aspects and larval behavior in southeastern Brazil. The gametogenesis, timing of gamete maturation, reproductive peaks, fecundity and larval behavior were evaluated and can help in choosing suitable species management and control methodologies in a region influenced by upwelling.

2. MATERIAL AND METHODS

2.1. Study area

Collections were carried out in Arraial do Cabo (Fig. 1), a region influenced by upwelling phenomenon (Valentin 1994, Kampel et al. 1997) that due to meteorological

and topographic factors, deep, cold and nutrient-rich waters reach the surface enriching water column and local marine community (Valentin 1988, Yoneshigue 1985, Castro et al. 1995). The coastal upwelling is seasonal and occurs in greater intensity in the months of September to March, in spring and summer, being less frequent in winter and fall (Calil 2009). Upwelling consequences involves decrease in temperature (between 15 and 18 °C) and water column eutrophication outside of the bay (Valentin 1994). Arraial do Cabo Bay is a protected area that supports a highly diverse subtidal benthic community influenced by anthropogenic activities where predominant water temperature is 20 °C (Guimaraens and Coutinho 1996). The coldest recorded average temperature was 9.8 °C in 1998 and the hottest was 28.7 °C in 1975 (Calil 2009).

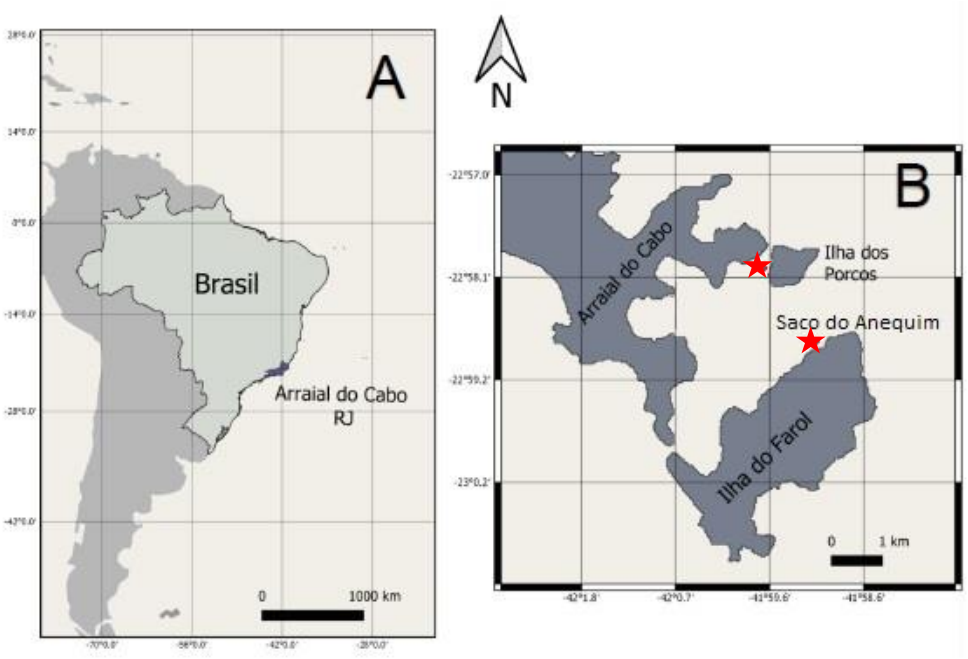

Figure 1. Map of Arraial do Cabo – RJ, red stars identify collection points.

2.1.1. Field collection and Histological procedures

Six colonies of each species, *T. aurea*, *T. coccinea* and *Tubastraea* sp. were collected monthly from November 2016 to November 2017 from rocky shores of two nearby areas within the bay of Arraial do Cabo, Ilha dos Porcos (22º 96’ S - 41º 98’ W)

and Saco do Anequim (22º 98’ S - 41º 98’ W). Specimens were collected through SCUBA diving at 4 to 7 m depth.

Specimens were fixed in 10% formaldehyde solution in the field and brought to the laboratory. Samples were decalcified in a solution of 10% formic acid and 5% formaldehyde. Colony central polyps were selected for histological procedures to avoid risk of being recent polyps and not having reproductive age (Rinkevick and Loya 1979; Wallace 1985, Chornesky and Peters 1987, Sakai 1998). Polyps were dehydrated in alcohol series, diaphanized in xylol and embedded in paraffin. Longitudinal polyp sessions of 7.0 μm were stained with Mallory's trichrome stain, according to Pires et al. (1999). The largest axes of oocytes (with apparent nucleus) was measured with a micrometer eyepiece, using an optical microscope. Photographs were obtained with a digital camera coupled to the Zeiss Axio Cam microscope. Studied material was deposited in IEAPM's scientific biofouling collection. Table of species registered in the collection in Annex (*Supplementary Table I*).

2.2. Gametogenesis and reproduction temporal pattern

Colonies of *Tubastraea aurea* (n=37), *Tubastraea coccinea* (n=38) and *Tubastraea* sp. (n=37) were analyzed and two central polyps from each colony were used for these analyzes, a total of 112 colonies and 224 polyps. Approximately twenty slides of each polyp were produced. Oogenesis and spermatogenesis were classified into three development stages, in order that male and female gametes were established according to size, color and morphology of the cells, as adapted from Szmant-Fröelich et al. (1980) and Pires et al. (1999). Stage I represents the beginning of development, stage II is an intermediate stage of development and Stage III represents mature cells. Embryogenesis has also been classified into stages of development. Early-stage embryos were called blastulas, with poorly defined peripheral cell densities covering an indistinct calf mass

dispersed in the cytoplasm. The study of gametogenesis and embryogenesis allowed evaluation of inter- and intra-specific synchronicity throughout the reproductive cycle, within a sample year. The same numbers of samples and slides were used for all species. Twenty slides were prepared for each polyp.

2.3. Fecundity

Fecundity rates were verified in July, August and September 2017. Three central polyps of the decalcified colonies were chosen, measured (major and minor diameter) and dissected under a stereomicroscope to count oocytes. Distance between the oral disk and the base of the polyp and larger diameter of the oral disk were measured with a caliper to estimate polyp volume. Fecundity rate was estimated from the relation between the number of oocytes and volume of the polyp according to De Paula et al. (2014).

2.4. Larval behavior

Some colonies of the three *Tubastraea* species were collected in September 2016, month of reproductive activity according to previous studies, and brought to the laboratory until larvae release. The day after the collection, spontaneously the aquarium was full of larvae and three larvae were placed in each well with 10 ml of sea water. Six replicates were made for two treatments in all three species. One treatment was with seawater collected in the area where it is known or considered for not having sun coral colonies, identified as PW (Pure Water); another treatment with seawater collected from an area with sun coral colonies, identified as CW (Colony Water). A total of 36 larvae of each species were observed until they died. Three conditions were used to determine the state of the larvae: alive, metamorphosed or dead. A total of 108 larvae of three species of *Tubastraea* spp. (*T. coccinea*, *T. aurea* and *Tubastraea* sp.) were analyzed in two treatment over time. Larvae were observed in a Stereomicroscope and were identified as "Alive" when they had swimming activity. They were considered "Dead" when there was

no movement, if it was deformed. And finally, they were considered “Metamorphosed” when flattened on water surface or fixed to the bottom and side of the well. The observation period varied between species since each species had a different period until the last larvae died or metamorphosed. In each species, 36 larvae were observed, 18 of each treatment (54 PW + 54 CW in total). The group “dead” and “metamorphosed” larvae were termed "Unviable larvae".

2.5. Data analysis

Data were analyzed by ANOVA repeated measures in R language and the environment for statistical computing (R Core Team, 2015) in R Studio (R Studio Team, 2015). The graphs were plotted with Microsoft Excel® software.

3. RESULTS

3.1. Reproductive pattern and sexual reproduction mode

The species *T. aurea*, *T. coccinea* and *Tubastraea* sp. presented simultaneous hermaphrodite reproductive pattern, due to observations of the production of gametes of both sexes occurring simultaneously in the same polyp from all three species at different periods throughout the reproductive period (Fig. 2). Most of the polyps that produced cysts and oocytes at the same time were *T. coccinea*. Collected colonies measured from 5.8 to 45 cm^2 of area with 6 to 66 polyps for gametogenesis investigation.

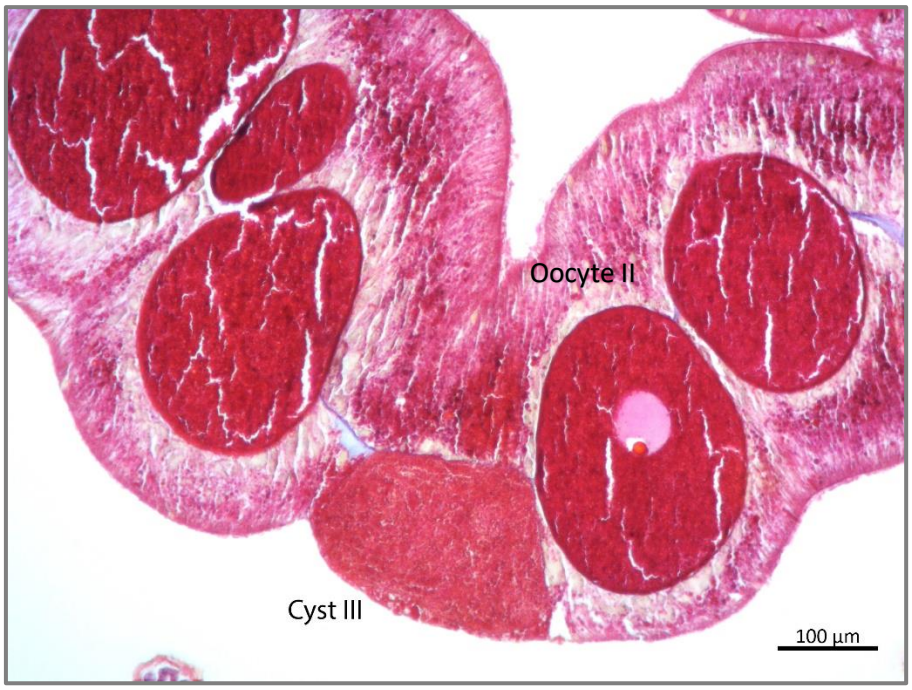

Figure 2. Photomicrograph of longitudinal sections by digital camera AxioCam ER c5s Zeiss. Fertile mesentery with cyst and oocytes together in a *T. coccinea* polyp. IEAPM 00896.

Due to the observations of embryos in early stages and larvae in histological slides, in addition to observations of larval release, Arraial do Cabo species have brooding reproduction mode.

3.2. Gametogenesis

Polyps presented gametes in almost all apparent mesenteries on the slides. In each cut, generally from five to eight complete and incomplete mesenteries appeared (Fig. 3). Female gametes and some spermatic cysts appeared enveloped in a thin layer of mesogloea, stained blue. Oogenesis and spermatogenesis were divided into three development stages and the diameters average of oocytes I, II and III were similar among the species. The total number of oocytes found was 10.560, it was not possible to count the cysts, because mostly they are mixed together making it seem a unique thing, without clear delimitations.

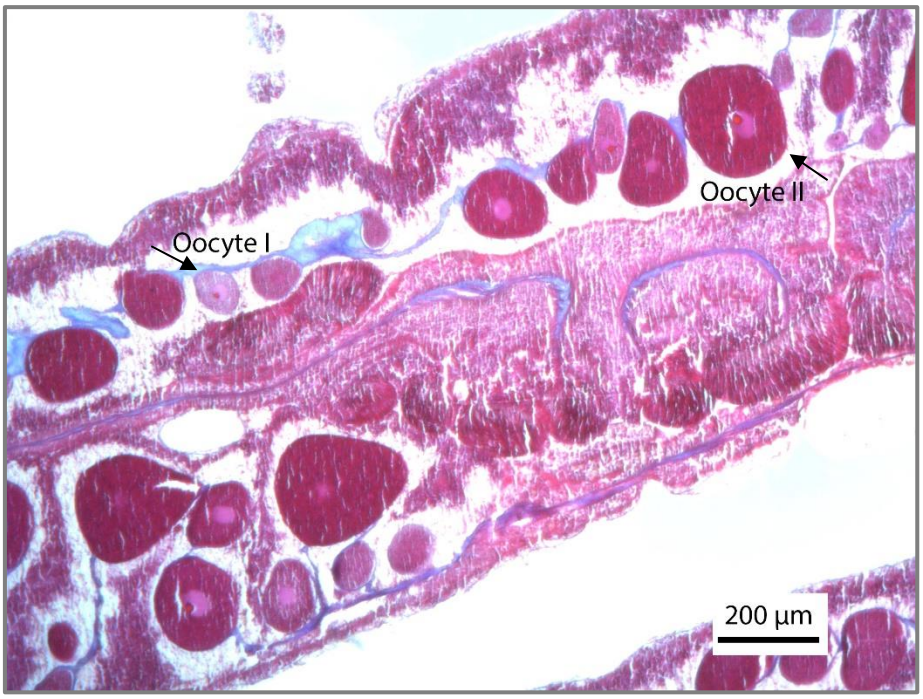

Figure 3. *Tubastraea coccinea* polyp longitudinal cut showing fertile mesentery with oocytes in stages I and II.

The mean sizes of oocytes I were 0.118, 0.115 and 0.116 mm for *T. aurea*, *T. coccinea* and *Tubastraea* sp., respectively. The total mean size of oocyte I was 0.106 ± 0.052 mm. Developmental stage I oocytes, total, ranged in diameter from 0.02 mm to 1.1 mm (Fig. 4). Oocytes II had averages of 0.24 in *T. coccinea*, 0.25 in *Tubastraea* sp. and 0.23 in *T. aurea* (Fig. 9). The total mean size of oocyte II was 0.258 ± 0.167 mm. The diameter ranged from 0.04 mm to 2.2 mm. Averages of oocytes III were 0.65 mm in *Tubastraea* sp., 0.52 mm in *T. coccinea* and 0.59 mm in *T. aurea*. The largest oocytes III occurred in *Tubastraea* sp. with 2.25 mm, the species presented the second largest polyp size mean, after *T. aurea*. The total mean size of oocyte III was 0.619 ± 0.202 mm. The diameter ranged from 0.1mm to 2.25 mm (Fig. 4).

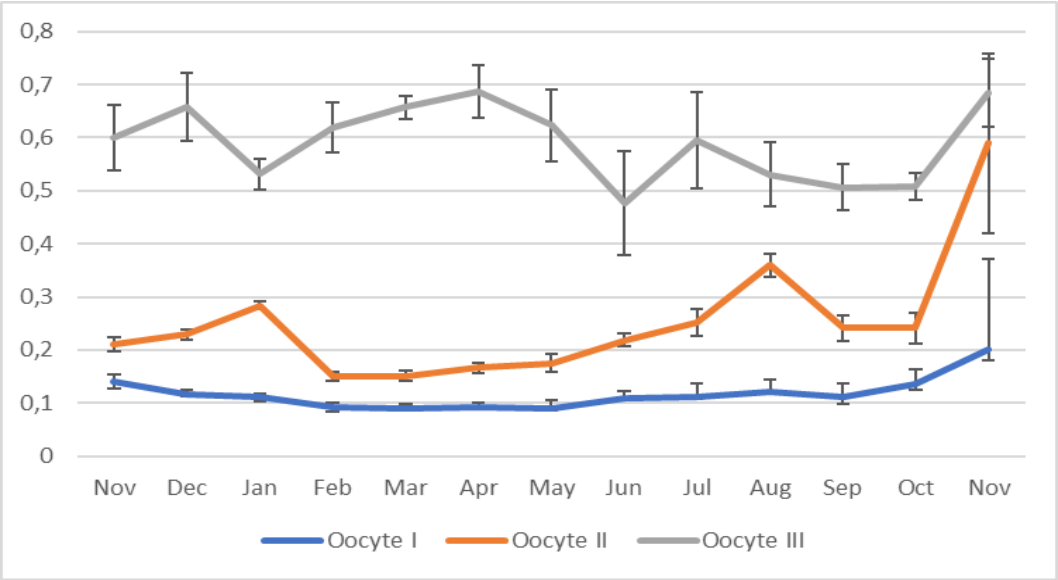

Figure 4. Size in mm of three stages of development of oocytes in each species during one year.

Gametogenic processes in corals are generally cyclical and it is common for species that incubate larvae to have multiple cycles. For this reason, there was overlap between all the stages (Fig. 5).

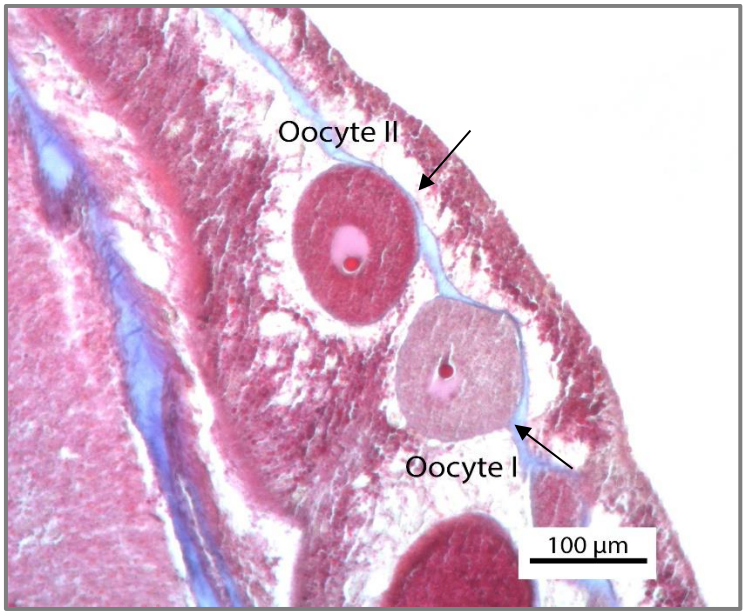

Figure 5. *Tubastraea aurea* oocyte I and II from with same approximate size.

3.2.1. Oogenesis

Stage I

The first oocyte development stage was characterized by a homogeneous cytoplasm, which varied from light blue and light pink or gray (Fig. 6). Nucleus occupied

much of cytoplasmic area and most often was observed in a central position. Nucleolus had an intense red color and, most of the time, was peripheral.

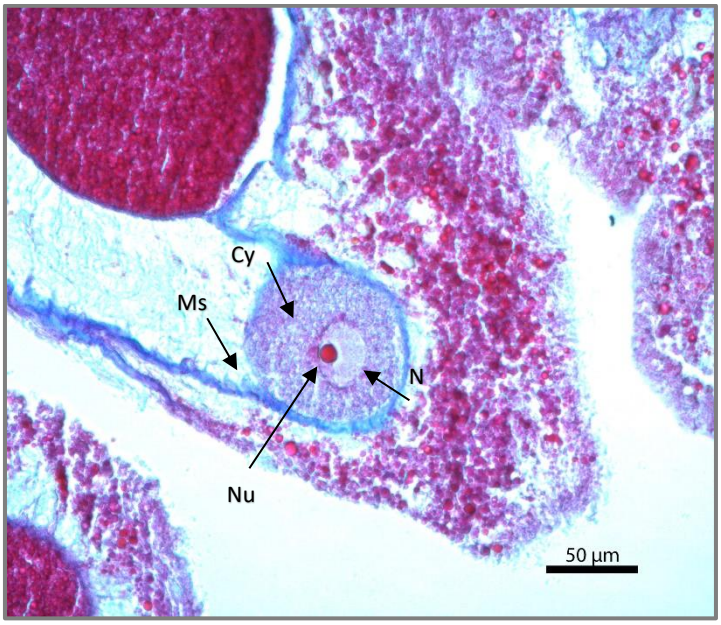

Figure 6. *Tubastraea coccinea* oocyte I involved by mesogloea stained in blue (Ms), homogeneous cytoplasm in lilac (Cy), nucleus (N) and red nucleolus (Nu).

Stage II

Oocytes showed a progressive cytoplasmic growth and proliferation of lipid vesicles (LV) (vitellogenesis), stained with rosy or orange (Fig. 7). Nucleus (N) was sometimes centralized, but usually in an intermediate position, close to the periphery.

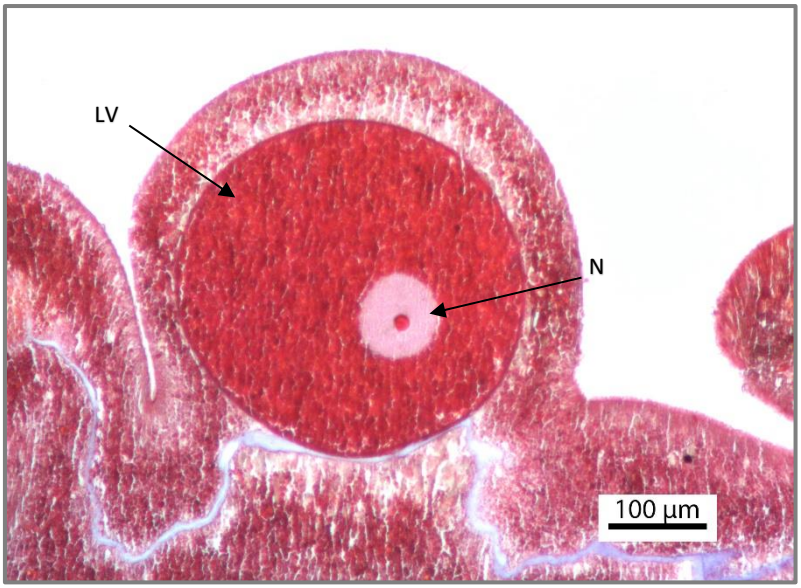

Figure 7. *Tubastraea* sp. oocyte at development stage II, almost III.

Stage III

Development stage III comprises mature oocytes filled with yolk. Oocytes III presented a completely vacuolated cytoplasm (Cy), full of lipid vesicles, orange or red (Fig. 8). Nucleus (N) showed to be close to the periphery or totally peripheral, near the cellular membrane. Nucleolus (Nu) was always peripheral, with an intense red coloration. Cells presented varied forms according to accommodation of the same in the space available in the mesentery. No oocyte reabsorption process or phagocytic elements close to the cells were observed.

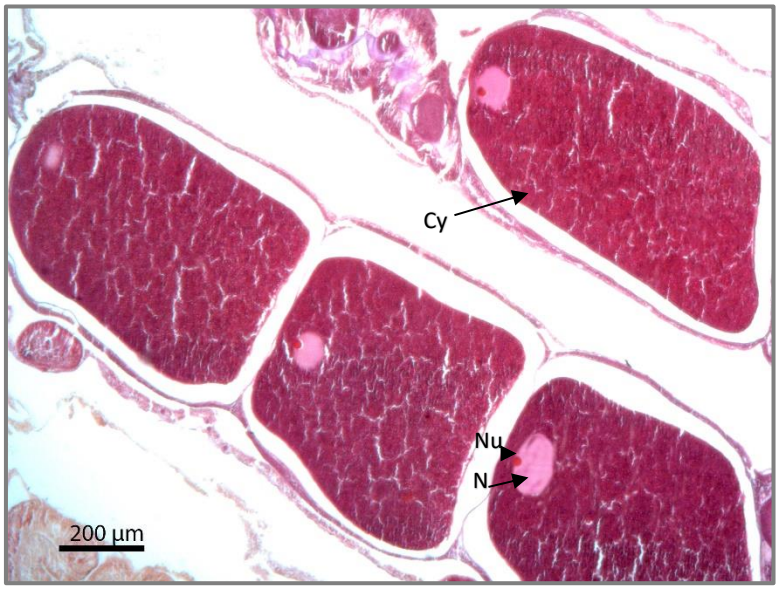

Figure 8. *Tubastraea aurea* oocytes at development stage III.

3.2.2. Spermatogenesis

Male sex cells aggregate to form a spermatic cyst involved by mesogloea (Ms). The first development stage or cyst I was characterized by clusters of cells surrounded by a thin mesogloeal layer and stained blue or light lilac, with 0.11 mm major diameter. Stage II spermatic cysts stained of red. There was formation of spacing (Lumen) within the cysts with 0.19 mm in diameter. Sperm cysts in stage III development had a large number of mature spermatozoa and their tails are easily visible, present 0.44 mm in

diameter. Spermatozoa were ordered with peripheral heads and flagella facing the center of the cyst. Tails stained bright orange or blue (Fig. 9).

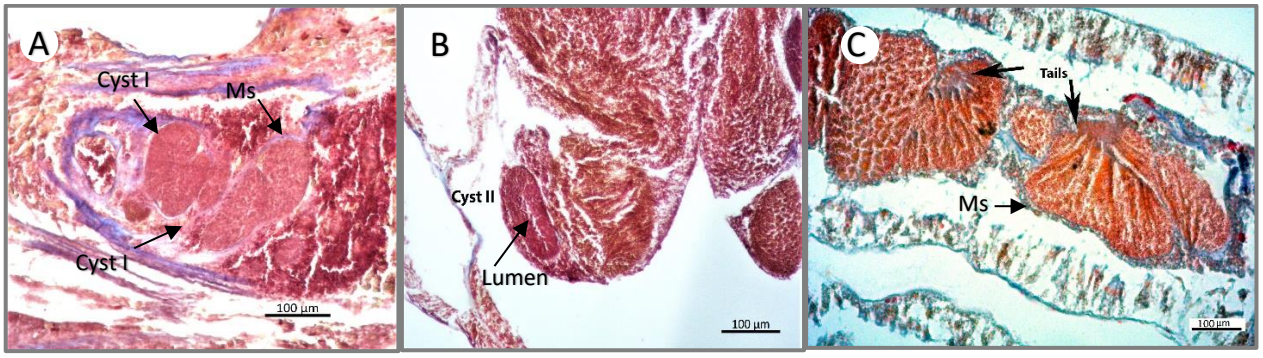

Figure 9. Different development stages of spermatic cysts. A. *T. coccinea* cysts I; B. *T. coccinea* Cysts II end, C. *T. aurea* Cysts III.

3.2.3. Embryogenesis

Visualization of the embryos was uncommon and only what appears to be an early developing embryo known as a blastula was observed. These embryos had the same staining properties as mature oocytes in the histological preparations. Larvae incubated in the gastrovascular cavity also were viewed (Fig. 10).

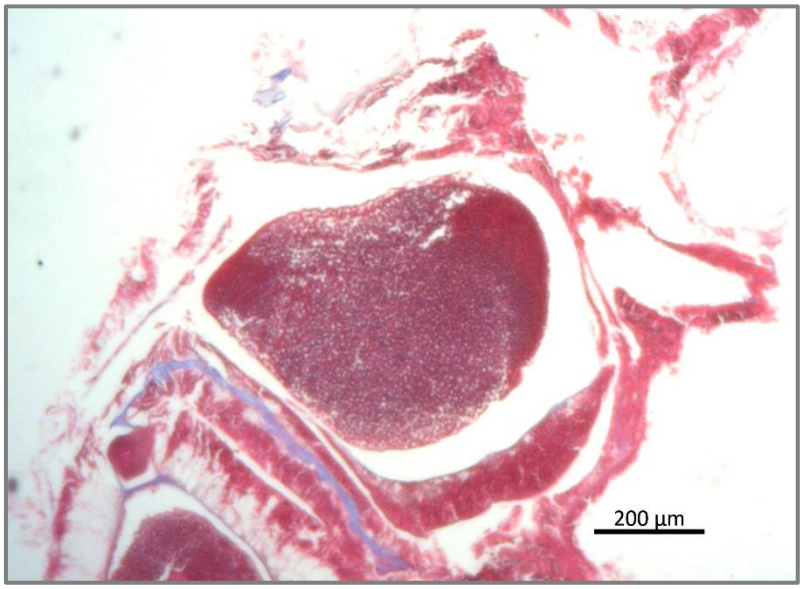

Figure 10. *Tubastraea coccinea* photomicrograph of embryo in initial formation. Stage initial called Blastula.

In histological slides *T. aurea* species presented more oocytes in May and June and fewer oocytes in January 2017, while *T. coccinea*, showed more oocytes in October and November 2018, and very few in December 2017, January and February 2018. *Tubastraea* sp. had a high number of oocytes in June and August, September and October 2017 and in November and December 2017 and January 2018 showed a smaller number (Table 1). The three species produce gametes continuously suggested by observing gametes in all the colonies examined.

Table 1. Table comparing ratio of oocyte number to number of analyzed polyps.

	T. aurea	*T. coccinea*	*T.* sp.
November/17	39	28	27.2
December/17	47.3	8.2	29.8
January/18	23.5	9.5	27.0
February/18	33.5	10.5	55.0
March/18	39.5	23.2	45.7
April/18	36.8	41.7	61.2
May/18	64	46.5	43.2
June/18	76.8	47.2	81.0
July/18	49.3	52.3	58.8
August/18	50.5	48.3	66.2
September/18	50.8	33.5	65.7
October/18	50	71.7	63.7
November/18	46	72	52.2
Total	46.69	37.9	52

3.3. Sex ratio

Almost 23% of *T. coccinea* polyps contained cysts, *T. aurea* e *Tubastraea* sp. showed lower rates, 13.51% and 16.21%, respectively. Of a total of 224 analyzed polyps, only 27 polyps (12%) of the samples found gametes of both sexes, while the others 88% presented only oocytes. Almost all polyps containing spermatic cysts (male gametes) also

contained oocytes (female gametes), in only three polyps, two of the same colony, of *T. coccinea* were only spermatic cysts observed.

3.4. Colonies

T. aurea has a robust skeleton, polyps larger than the other species studied (see Bastos et al., Submitted data) and presents higher colony area average. *T. coccinea*, according to the description of the species in Arraial do Cabo has smaller and closer polyps than others (Fig. 11). Colony area were calculated from the ellipse area formula: $A = \pi \times r(a) \times r(b)$. *Tubastraea* sp. had fewer polyps due to the colony shape where polyps are very spaced apart. A table of colony diameter and area measurements is given in Annex 1.

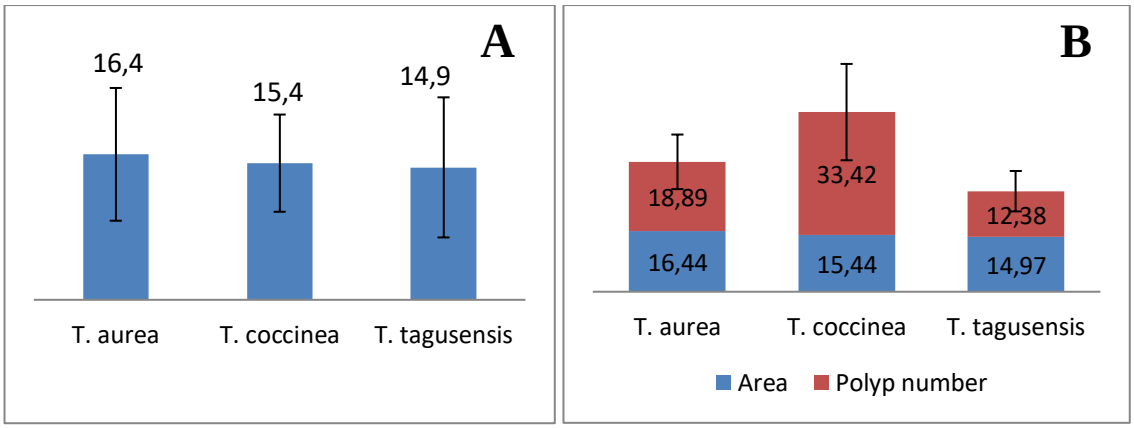

Figure 11. A. Colony area average for the three species; B. Colony area and polyp number averages.

3.5. Polyps

Polyps were calculated from cone volume formula: $V = (\pi \times r^2 \times h)/3$, due to the cone shape they feature. *Tubastraea aurea* had the highest average volume among other species. *Tubastraea* sp. presented the second highest average diameter among species and *T. coccinea* the lowest mean of diameter and polyp volume (Fig. 12).

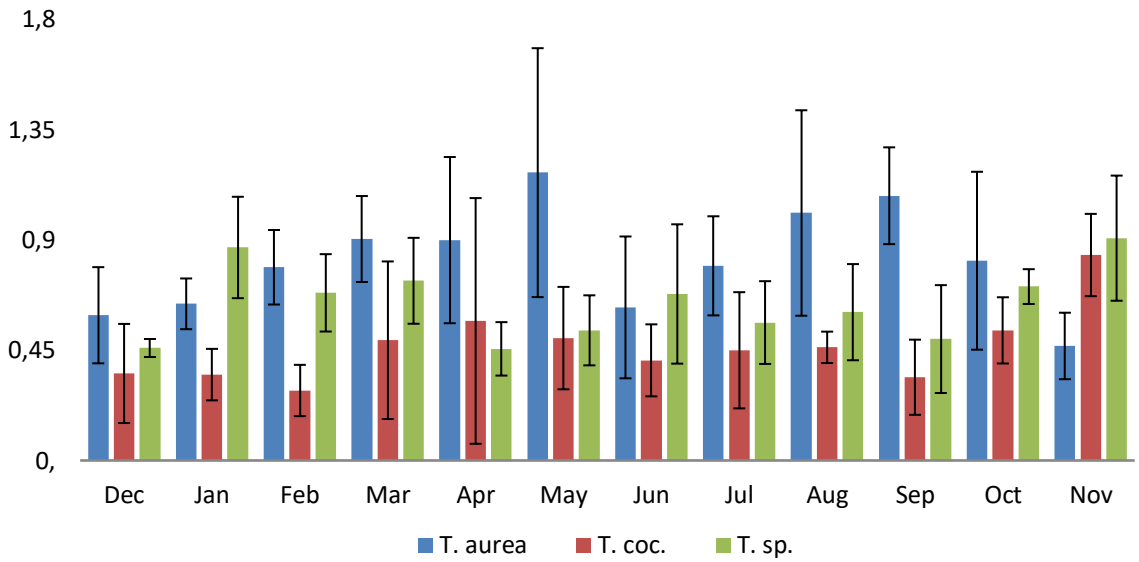

Figure 12. Polyp area average (mm^3) over a year.

3.7. Gametogenic cycle

Late stage embryos were not observed, only the onset of cell division was observed by cell density in the blastocele. Blastula was observed in *Tubastraea* sp. in July 2018 and in *T. coccinea* in November 2017. The months of occurrence of larvae varied between species.

Spermatic cysts in *T. coccinea* lasted two to three months long. Spermatic cysts in *T. aurea* and *Tubastraea* sp. appear only in November 2018 and May and October and November 2018, respectively. Cysts III occurred in periods when oocytes III were also observed but nothing related to possible internal cross-fertilization was seen. Spermatogenesis has a shorter duration than oogenesis, as it has been shown to be periodic and not continuous like oocytes.

In *T. aurea*, larvae were observed in histological slides in two periods throughout the year sampled: May and June; September, October and November of 2018. It was observed that the proportion of oocytes III was higher over a year, followed by oocytes I and II. In March there was a large occurrence of oocytes III and then, in May, the occurrence of cyst III and larvae (until June). In August, month of great proportional

occurrence of oocytes III, no spermatic cysts were observed before or after this month, only larvae occurred between September and December. As there was no counting of spermatic cysts we only refer on their occurrence. (Table 2).

Table 2. Oocytes percentage and occurrence of spermatic cysts, larvae and/or embryos on histological slides of *Tubastraea aurea.*

***T. aurea* (%)**	**Nov/ 17**	**Dec/ 17**	**Jan/ 18**	**Feb/ 18**	**Mar /18**	**Apr /18**	**May /18**	**Jun/ 18**	**Jul/ 18**	**Aug /18**	**Sep/ 18**	**Oct/ 18**	**Nov /18**
♀ I	26	21	12	40	28	43	44	59	39	26	16	17	1
♀ II	44	37	50	20	11	9	19	35	48	3	54	35	4
♀ III	30	42	38	40	61	48	37	6	13	71	30	48	95
♂ I	X	-	-	-	-	-	X	-	-	-	-	-	-
♂ II	X	-	-	-	-	-	X	-	-	-	-	-	-
♂ III	X	-	-	-	-	-	X	-	-	-	-	-	-
Larva	-	-	-	-	-	-	X	X	-	-	X	X	X
Embryo	-	-	-	-	-	-	-	-	-	-	-	-	-

"X" = occurs; "- "= Not occur.

Cysts were observed in *T. coccinea* in February; May and June; and September and October. The species presented larvae in January, April and May. Only in January no oocyte I occurred in the colonies examined. In all other sampled months, oocytes of all stages occurred and there was also a predominance of oocytes III, followed by oocytes II and I, respectively. There was no synchrony in the occurrence between cysts III and the larvae, only one concomitant occurrence in May (Table 3).

Table 3. Oocytes percentage and occurrence of sperm cysts, larvae and/or embryos on histological slides of *Tubastraea coccinea*.

***T. coccinea* (%)**	**Nov /17**	**Dec /17**	**Jan /18**	**Feb /18**	**Mar /18**	**Apr /18**	**May/ 18**	**Jun /18**	**Jul/ 18**	**Aug /18**	**Sep/ 18**	**Oct/ 18**	**Nov /18**
♀ I	23	2	0	36	28	41	44	47	33	9	6	1	4
♀ II	43	20	50	13	23	36	41	50	48	52	27	1	45
♀ III	34	78	50	51	49	23	15	3	19	39	67	98	51
♂ I	-	-	-	X	-	-	-	X	-	-	X	-	-
♂ II	-	-	-	X	-	-	X	X	-	-	-	X	-
♂ III	-	-	-	-	-	-	X	X	-	-	X	X	-
Larva	-	-	X	-	-	X	X	-	-	-	-	-	-
Embryo	X	-	-	-		-	-	-	-	-	-		-

X = occurs; - = Not occur.

Tubastraea sp. presented spermatic cysts only in November and October. Polyps of *Tubastraea* sp. presented larvae in December, May, July and October. Cysts III and larvae were observed in October. There was a higher relative occurrence of oocytes III throughout the year (Table 4).

Table 4. Oocytes percentage and occurrence of sperm cysts, larvae and/or embryos on histological slides of *Tubastraea* sp.

***T.* sp.** (%)	**Nov/ 17**	**Dec/ 17**	**Jan/ 18**	**Feb/ 18**	**Mar/ 18**	**Apr/ 18**	**May /18**	**Jun/ 18**	**Jul/ 18**	**Aug/ 18**	**Sep/ 18**	**Oct/ 18**	**Nov /18**
♀ I	13	16	17	48	30	48	54	51	54	19	9	7	9
♀ II	31	25	43	22	9	19	14	43	34	59	6	28	17
♀ III	56	59	40	30	61	33	32	6	12	22	85	65	74
♂ I	-	-	-	-	-	-	-	-	-	-	-	X	X
♂ II	-	-	-	-	-	-	-	-	-	-	-	X	X
♂ III	-	-	-	-	-	-	-	-	-	-	-	X	X
Larva	-	X	-	-	-	-	X	-	X	-	-	X	-
Embryo	-	-	-	-	-	-	-	-	X	-	-	-	-

X = occurs; - = Not occur.

Two gametogenic cycles were observed. We consider that an oocyte I takes two months to develop in a stage II that takes two months to develop in a stage III that takes one to two months to become a larva, that is, the period of gametogenesis revolves around six months. Spermatogenesis is periodic and faster than oogenesis and no synchrony was observed in spermatic cysts production between species. Only in May spermatic cysts was observed in *T. aurea* and also in *T. coccinea* and in October in *T. coccinea* and *Tubastraea* sp. also.

Tubastraea spp. larvae release was also observed through collection and/or manipulation of colonies in laboratory and field in April, May, September and November in all three species.

3.8. Frequency of oocyte stages

T. aurea had a higher occurrence of oocyte I in February, May and June and the highest proportional occurrence was in June. Oocytes II occurred in greater proportion in November 2017, January, July and September. On the other hand, oocytes III occurred in December 2017, March, April, August, October and November 2018 (Fig. 13). It is noted that two oocyte III occurrence peaks, one at the beginning of the year between February and April culminating in March, and another period in the end of the year that begins in August falling in September, recovering in October and culminates in November.

Overall, from November 2017 to May 2018 oocytes III did not vary much in frequency, with higher occurrences in March and April. There was a decrease in occurrence in June and July, in June larvae were observed in histological slides. This period may be related to a larval spawning period, where mature oocytes developed, formed larvae and were released. There was an increase in August, oscillating to a peak in November.

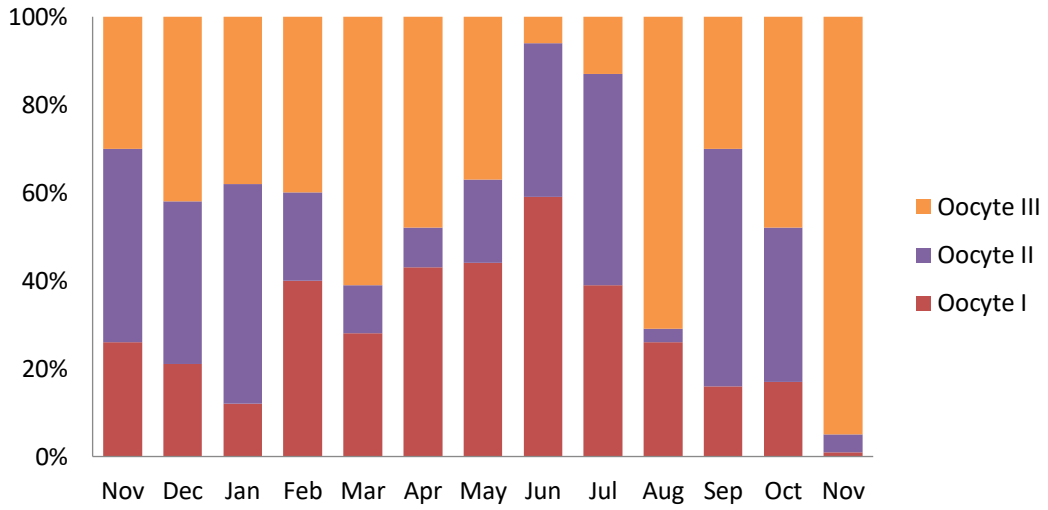

Figure 13. Frequency of oocyte stages occurrence in the months sampled in *Tubastraea aurea*.

Oocytes III seem to be more present in the period between September and December (Fig 14). April, May, June and July there was a fall in the occurrence of mature oocytes and gradual increase from August, culminating in October 2018. Embryos were sighted in November and two months later larvae were and two months later larvae were spotted on histological slides. The end of the year between September and December there is a reproductive peak and spawning period.

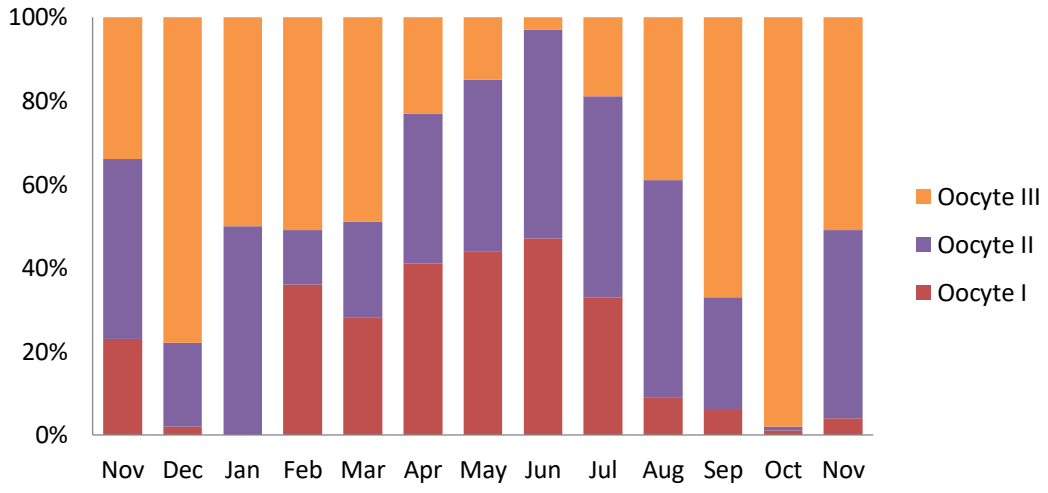

Figure 14. Frequency of oocyte stages occurrence in the months sampled in *Tubastraea coccinea*.

In *Tubastraea* sp. the highest occurrence of oocytes I was observed from April to July. Oocytes II occurred with varied and predominantly in June, July and August.

Oocytes III occurred in greater proportion than other oocytes in September, October, and November 2018 (Fig. 15). March was also a month with more oocytes III than others. Months with the lowest register of oocytes III were June, July and August. Larvae occurred in December 2017. Two spawning periods were configured with observation of larvae (May, June and July) and oocytes III (September, October and November).

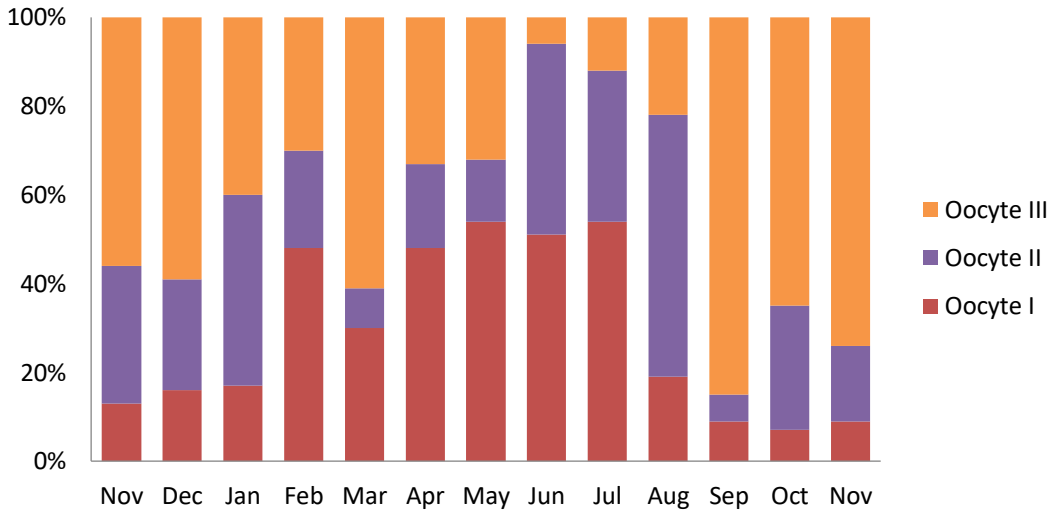

Figure 15. Frequency of oocyte stages occurrence in the months sampled for *Tubastraea* sp.

Colonies of *Tubastraea* presented continuous gametogenesis. Gametes occurred in the three stages of development throughout the year studied. In some samples the three stages of oocyte development were observed occurring in the same mesentery (Fig. 16). During the year it was possible to observe a similar frequency of occurrence of oocytes production and maturation among the species, indicating an interspecific synchrony.

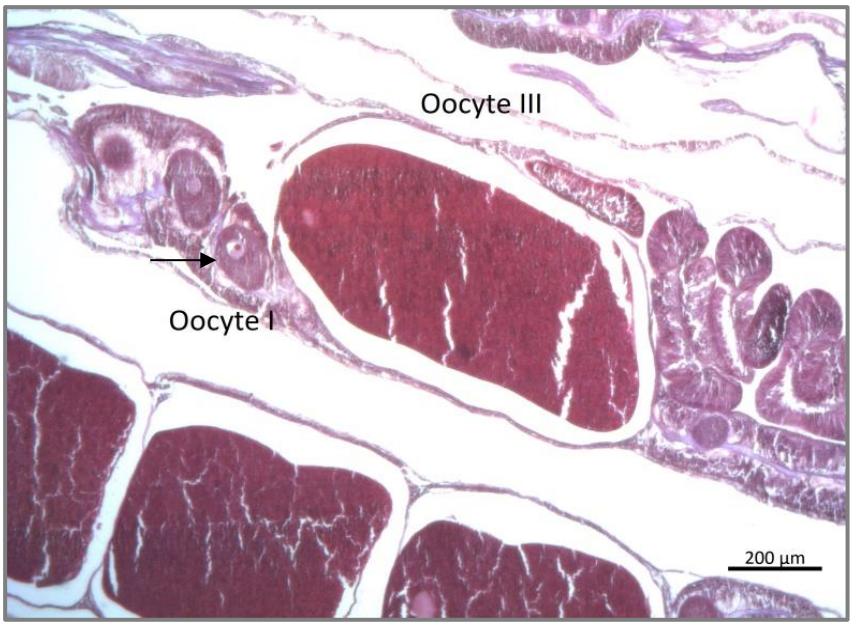

Figure 16. Oocytes of different stages occurring in the same mesentery. Oocyte III with no apparent nucleus and cytoplasm filled with lipid visicles and oocytes I less colored due to cytoplasm content.

3.9. Fecundity

The average fecundity rates between species were 293.8, 196.7 and 230.2 oocytes/cm^3 for *T. aurea*, *T. coccinea* and *Tubastraea* sp., respectively. The fecundity rate in *T. aurea* was 249, 293 and 339 oocytes/cm^3 in July, August and September. For *T. coccinea* the fecundity rate was 186.6 oocytes/cm^3 in July, followed by August with 232.3 and September with 171 oocytes/cm^3, while *Tubastraea* sp. demonstrated fecundity rate of 287, 215 and 188.3 oocytes/cm^3, for the respective months (Fig. 17).

The graph below suggests that *T. aurea* reached reproductive peak through the highest reproductive activity, in September 2018. *T. coccinea* had a higher reproductive propagules occurrence in August, while for *Tubastraea* sp. it was in July, declining in the following months.

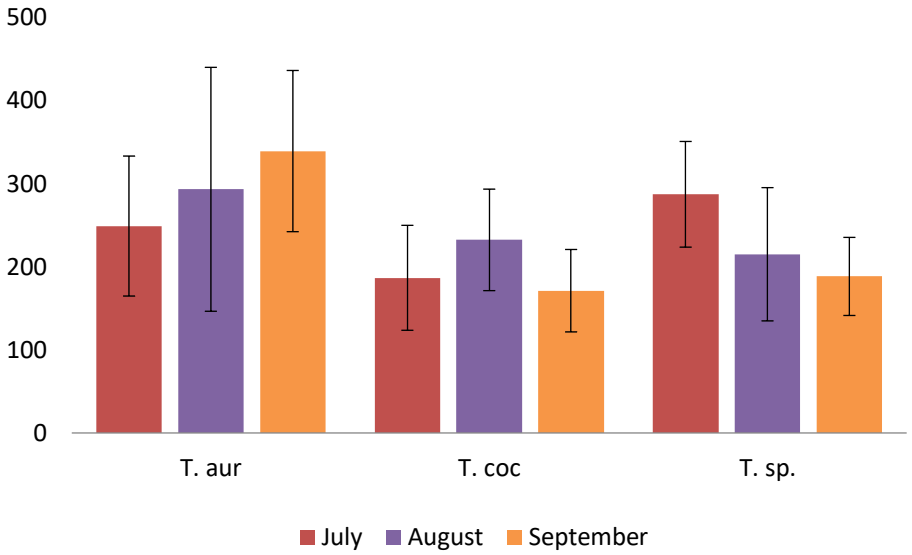

Figure 17. Average fecundity rates per species (oocytes/cm^3).

July and August showed a higher occurrence of oocytes with a rate of 246.8 and 240.9 oocytes/cm^3 and 232.9 oocytes/cm^3 in September, considering the three species. There was no differentiation of the cited because there was difficulty in visual differentiation of them. Reproductive structures or reproductive propagules were counted under a stereomicroscope (Zeiss).

3.10. Reproductive peaks

The average diameters among oocytes III measured in *T. aurea* were very similar between months (0.59 ± 0.08 mm annual average) but the month with the highest average was December with 0.69 ± 0.19 mm. Averages followed highs spread throughout the year as in February, April, May and August (0.64 ± 0.16, 0.64 ± 0.17, 0.66 ± 0.1 and 0.64± 0.12 mm). As of September, averages fell to 0.44 ± 0.12, 0.45 ± 0.14, 0.59 ± 0.13 mm until they reached their peak in December. In the graph below, we can see size oscillation and it looks like that early stage III oocyte takes 1 month to reach the final stage III (Fig 19). The species also showed a high predominance of oocytes III in November, August and March 2018 (95, 71 and 61%, respectively). In the previous chapter, the highest fertility rate of *T. aurea* was found in June and the second highest in December, with

61.18 and 58.41 oocytes/cm^2. Fertility between the sampled months (July, August and September) was higher in September with 339 oocytes/cm^3. Despite the inconsistency in the combination of the characteristics of the reproductive activity of *T. aurea*, it was possible to observe that the most representative months during the year were between February and June, where there were higher average size of oocytes (February, April and May), there were higher rates of fertility (June) and greater predominance of oocytes III (March). We can also consider August with large oocytes III and predominance of occurrence in the colonies and November that also included the three reproductive parameters. It is concluded that two reproductive peaks were formed in *Tubastraea aurea* during the year.

T. coccinea presented, on average, larger diameters of oocytes III in March, April and November with 0.63 ± 0.11, 0.63 ± 0.09, 0.69 ± 0.34. October was the month of highest fertility, along with July, and it was also a month where the highest prevalence of oocytes III occurred. December and September also had higher relative occurrences of oocytes III of the species. In accordance with the species *T. aurea*, there were also three reproductive peaks relative to the months when there was greater activity of reproduction based on three parameters: oocyte size, fertility rate and predominance of oocytes III. The first would be in March and April, where oocytes III obtained the highest size averages, along with November. The second in July, was the period with the highest fertility rate with 49.4 oocytes/cm^2, observed in the studies in the previous chapter. And the third was between September and December, covering all reproductive parameters considered.

In *Tubastraea* sp. oocyte III averages were similar (annual mean of 0.65 ± 0.09 mm). The largest diameters occurred in December, April, July and May, 0.74 ± 0.32, 0.78 ± 0.12, 0.73 ± 0.12 and 0.71± 0.16, respectively. January, August and October were the months with smallest oocytes III with 049 ± 0.11, 0.48 ± 0.14 and 0.51 ± 0.2. Largest

oocytes III occurred during the period of the highest occurrence of oocytes I (Fig. 16) which demonstrates continuous production of gametes. The species also has high reproductive activity and two peaks of activity have become visible. Abril stood out as the month where oocytes III reached their largest size and also where there was a higher fertility rate, being the first reproductive peak. In July, oocytes III showed an increase in size and in September, when the second peak begins, there was a predominance of oocytes III and the second highest fertility rate for the species, which ends in December, where there was high average size of oocytes (Fig. 18).

Few larvae were seen on histological slides and due to this, the highest average oocyte diameter III, the highest occurrence of the mature oocyte stage and mainly fecundity were considered reproductive peak for all species.

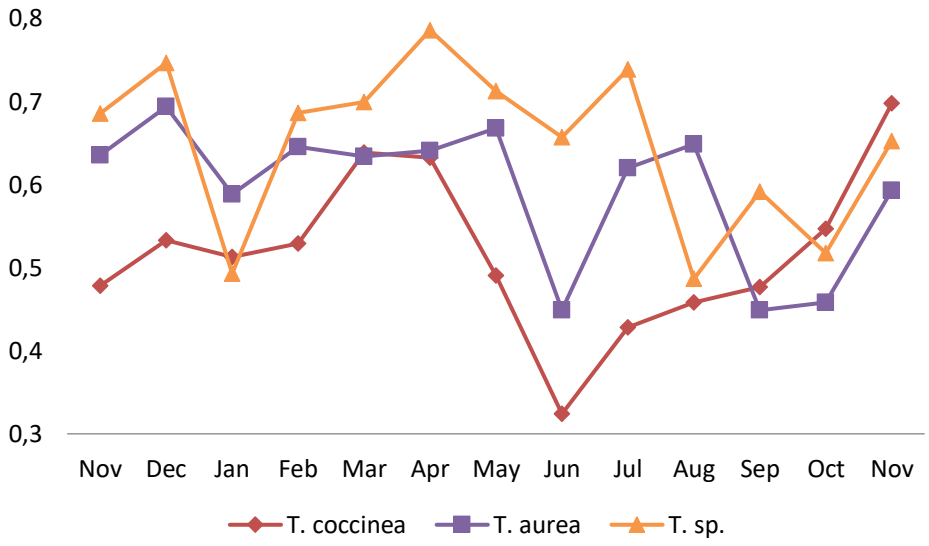

Figure 18**.** Reproductive peaks represented by oocyte III enlargement.

From November to February, the average size of oocytes III maintained similar behavior in the three species. Oocytes from *T. coccinea* and *Tubastraea* sp. start to drop in size from April to June, *T. aurea* also dropped from May to June followed by an increase for all three species. *T. coccinea* mateve increased from July to November. The sizes varied in *T. aurea* and from September there was an increase until November and

in *Tubastraea* sp. oocytes decreased and increased in size each month, from July, until the end of the observations. Throughout the year, there was synchronization of the increase and decrease of oocytes between the three species in various periods.

3.11. Larvae survival, death and metamorphosis

All larvae were placed in the same controlled environment condition without any interference throughout the experiment. The survival time, that is, the period in which live larvae were observed, was 19 days for *T. aurea*. After 120 (T5) hours the first larva in water collected near sun coral colonies (CW) died while in water collected in an area without colonies of sun coral (PW) one larva died in the first 24 hours. An abrupt fall was observed between T5 and T6 for CW, with 4 deaths and 1 metamorphosis. In T15 and T16 there was 3 metamorphosis in CW while for PW, there was between T11 and T12, two dead larvae and two metamorphosed (Fig. 19). Nevertheless, for larvae subjected to PW, we observed that the live larvae rate gradually decreased over time.

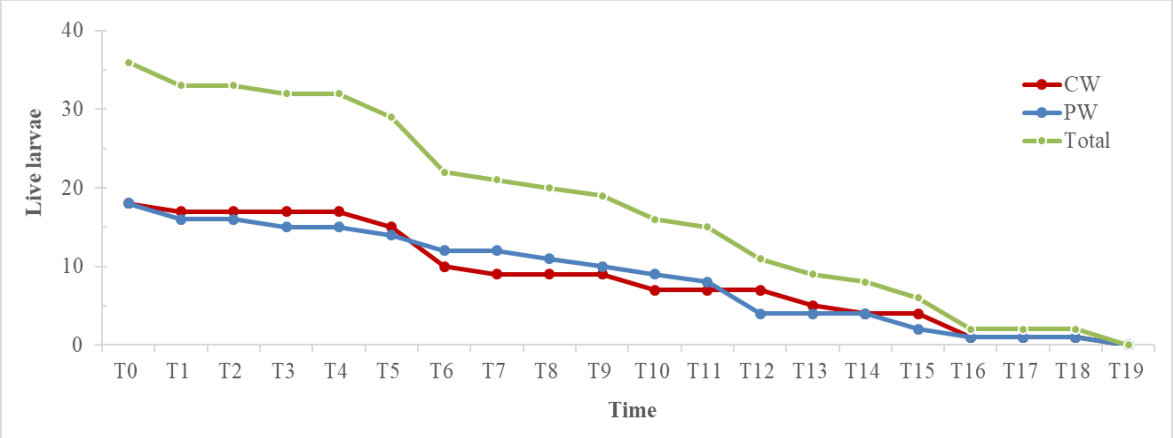

Figure 19. *Tubastraea aurea* live larvae in both water quality treatments (PW and CW) and the total live larvae over time, 19 days of viable survival. Larvae in PW showed a more constant drop in viability than those in CW. CW= water collected near sun coral colonies; PW= water collected in an area without colonies of sun coral.

No significant differences ($p > 0.05$) were observed for the entire sample. Similarly, no significant differences were observed when we statistically analyzed the

two treatments between each time. Our samples were statistically homogeneous throughout the experiment in *T. aurea*. Although statistical differences were not presented, we could observe some details that can be presented as biological differences. In the first 24 hours, the average number of larvae in the CW treatment was higher than in PW, 2.83 ± 0.40 and 2.66 ± 0.51, respectively. In CW this number remained until the T4 period but in T6 the number of live larvae was higher in PW due to some larvae that metamorphosed from the CW treatment. At T13 in each CW well, there was at least one metamorphosed larva (Fig. 20). T15 six larvae were metamorphosed in the PW and at the end of the experiment (T19), of the 18 larvae observed 10 were dead in the PW versus 8 in the CW and 8 were metamorphosed in PW and 10 metamorphosed in CW.

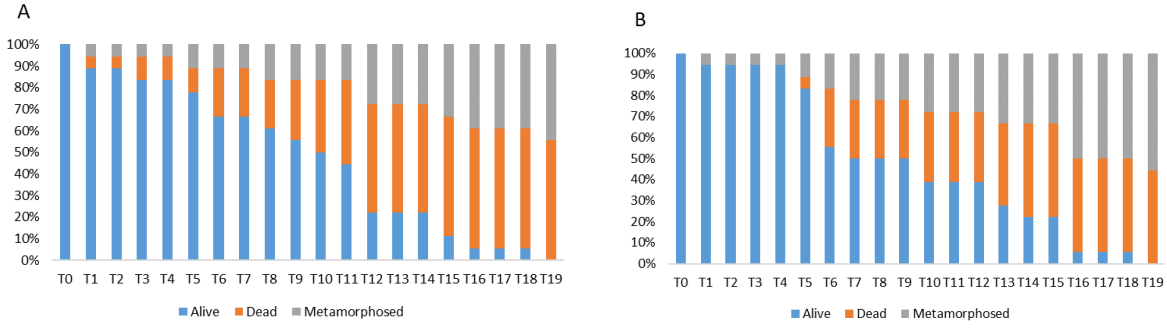

Figure 20. *Tubastraea aurea* larval behavior. A) Larvae in water collected in an area without colonies of sun coral (PW); B) Larvae in water collected near sun coral colonies (CW). The larvae showed a similar metamorphosis behavior between both treatments, which begins on the second day. In CW treatment the first larva died on the fifth day, while in PW on the second day.

T. coccinea larvae survived for 18 days. The first group where larval mortality was seen was in the PW, with 2 dead larvae in the T1. The first larva to metamorphose in PW was in the T4 period while in the CW it was in the T1. In CW treatment, one larva died within the first 24 hours. In the T13 period, while 8 larvae in the PW were dead, only 4 in the CW had died and eight larvae were metamorphosed in this treatment while 5 metamorphosed in the PW. In general, PW larvae died faster but at the end of the experiment the balance was just one deader larva compared to CW. As for the larval metamorphosis, nine larvae metamorphosed in the PW and 10 in the CW treatment. Two

PW larvae survived until T17 and metamorphosed from T17 to the end of the T18 experiment, while there was only one live larva until this period in CW that died in T18 (Fig. 21).

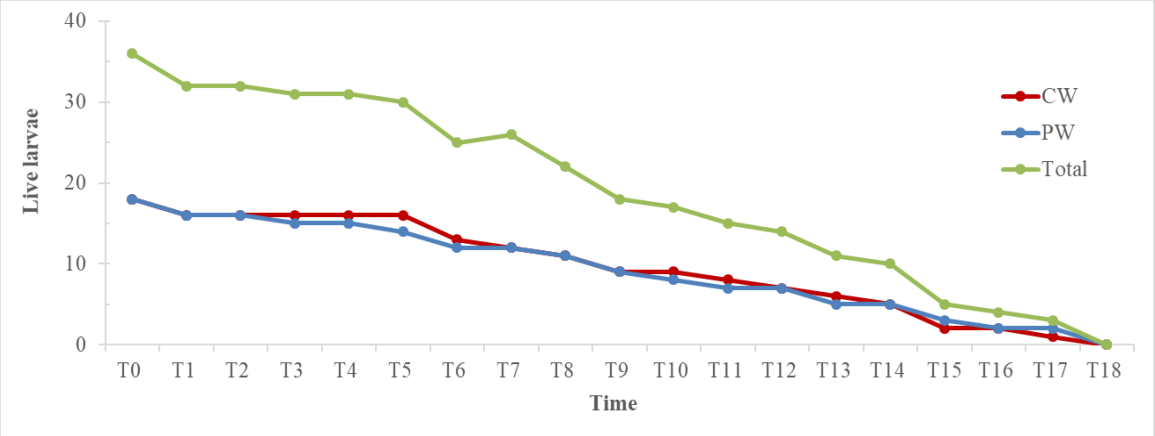

Figure 21. *Tubastraea coccinea* live larvae in both water quality treatments (PW and CW) and the total live larvae over time, 18 days of viable survival. CW= water collected near sun coral colonies; PW= water collected in an area without colonies of sun coral.

Further details on the percentage of occurrence of alive, metamorphosed or dead larvae over the 18 days of larval activity are shown below (Fig. 22). The water quality treatments showed no statistically significant difference between them. Again, our sampling during treatment was very constant for statistical relationships. Differences in larval behavior between species may be related to biological factors related to the species.

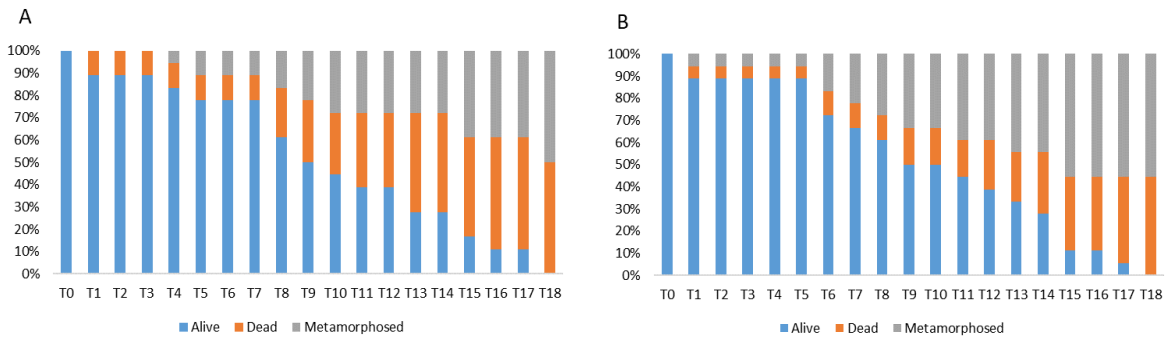

Figure 22. *Tubastraea coccinea* larval behavior. A) Larvae in water collected in an area without colonies of sun coral (PW); B) Larvae in water collected near sun coral colonies (CW). Metamorphosis started earlier in the CW treatment, from the second day, and at the end of the experiment, fewer larvae died in the CW treatment.

However, only statistically, we can suggest that both treatments did not influence the resilience of the larvae, i.e. there was no effect of the treatments.

Tubastraea sp. survived for 20 days, demonstrating to be the most resistant larvae among the three species of *Tubastraea* studied. The first larva to metamorphose was in PW in T2 and at the end of the experiment 10 larvae metamorphosed and 8 died. At the T16 period, no other larva metamorphosed until the end of the experiment and one larva that remained alive died in the T21. The last live larva in the CW died in the T21 period too and at the end of the experiment 11 larvae metamorphosed and 7 died. In this treatment, one larva died in T1 and another in T5, T6 and T7 and only in T16 two more larvae died. There were no significant differences between each treatment (Fig. 23).

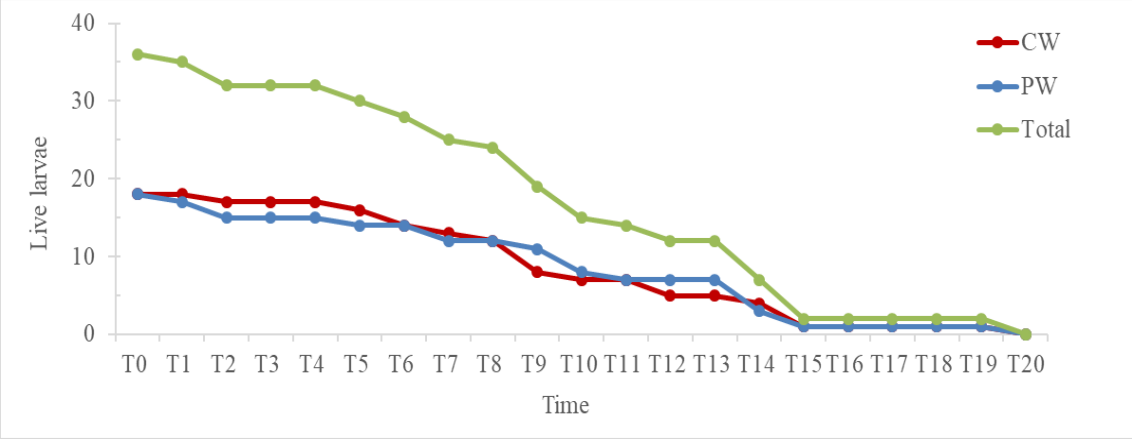

Figure 23. *Tubastraea* sp. live larvae in both water quality treatments (PW and CW) and the total live larvae over time, 20 days of viable survival. CW= water collected near sun coral colonies; PW= water collected in an area without colonies of sun coral.

In the wells where the experiments were performed there was no water current and the larvae that kept swimming were through active displacement. Overall average of live larvae throughout the experiment was 1.49 ± 0.19. Averages tend to have values around 1.5 because the experiment is a time to death and/or unviability of all larvae, the values did not vary strongly, considering that initial value per replica was 3 live larvae and the end for all zero live larvae. In general, each species had their highest mean live

larvae in the CW treatment, except for *T. aurea*, where the mean values in both treatments were very similar.

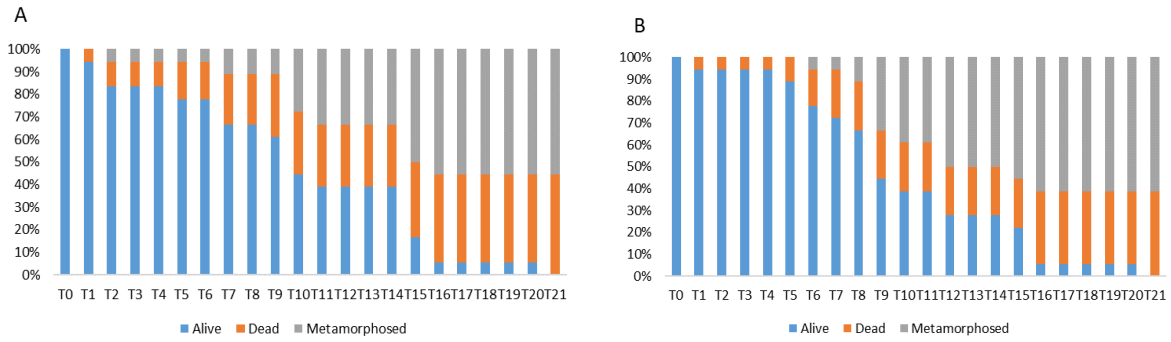

Figure 24. *Tubastraea* sp. larval behavior. A) Larvae in water collected in an area without colonies of sun coral (PW); B) Larvae in water collected near sun coral colonies (CW). Metamorphosis started earlier in the PW treatment, in the CW, more larvae remained alive for a longer time. In both treatments, the larvae survived until the twentieth day.

Although the three species of *Tubastraea* did not show significant differences either between them or between the water treatment groups in which they were submitted ($p > 0.05$) – either within the species groups or among the species – *T. coccinea* presented the highest live larvae averages than the other species, either in PW treatment or in CW. Thus, averages for *T. coccinea* were higher, in general, with 1.59 ± 0.188 and having as values for CW 0.61 ± 0.259 and PW with 1.57 ± 0.275. Lowest values were for *T. aurea*.

Table 5. Live larvae average values in each of the 3 *Tubastraea* species in each treatment and overall total in each of them, with their standard errors.

Species/Treatment	PW	CW	Total
T. aurea	1.43 ± 0.286	1.43 ± 0.251	1.44 ± 0.190
T. coccinea	1.57 ± 0.275	1.61 ± 0.259	1.59 ± 0.188
T. sp	1.42 ± 0.298	1.45 ± 0.294	1.44 ± 0.209
Total	1.47 ± 0.159	1.5 ± 0.28	1.49 ± 0.196

4. DISCUSSION

The corals *T. aurea*, *T. coccinea* and *Tubastraea* sp. demonstrated to be simultaneous hermaphrodites reinforcing the record of Fadlallah (1983), Harrison and

Wallace (1990) and Richmond and Hunter (1990) who stated that hermaphroditism is a common pattern among shallow scleractinians. The occurrence of spermatic cysts suggests a possible internal fertilization and subsequent larval production. However, low sex ratio between male and female polyps may indicate asexual (ameiotic) larval production, since cysts were present within a few months while larval release occurred almost the entire year. The study of De Paula and collaborators (2014) concluded the occurrence of asexual reproduction for the same reason observed in Arraial do Cabo. Production of asexual larvae ware aldo recorded in other scleractinious corals like *Pocillopora damicornis, T. coccinea* and *T. diaphana* by Ayre and Resing (1986). According to Capel et al. (2017) *Tubastraea spp.* did not present population structure across the Southwest Atlantic and results indicated that asexual reproduction is dominant in invaded area. As clones, these corals should have the same reproductive behavior. Indeed, many similarities were observed in a previous study in the Bay of Ilha Grande by De Paula et al. (2014) for the species *T. coccinea* and *T. tagusensis*, which are also simultaneous hermaphrodites and brooders, maintaining the reproductive strategies of opportunistic invading organisms.

Brooding reproduction mode is a characteristic of the members of Dendrophylliidae Family (Fadlallah 1983) and species that incubate planulae, usually, have multiple reproductive cycles (Harrison and Wallace 1990). Brooding hermaphroditic is a good strategy for occupation of less favorable habitats because of their high recruitment rates and small colonies (Szmant 1986), as observed for corals of *Tubastraea* genus. Other brooding species in Brazil, *Favia gravida*, *Porites astreoides* and *Scolymia wellsii*, also present several events of larval release during the reproductive cycle (Calderon *et al.* 2000, Pires and Caparelli 2002, Pires *et al.* 2002). According De Paula et al. (2014) at Ilha Grande Bay, *T. tagusensis* and *T. coccinea* release larvae

together in almost all periods of the year and there was a synchrony of spawning in January, April, May, July, September, October and November between our data and those of Ilha Grande. In June, the species that presented larvae in histological slides was *T. aurea*, not explored in Ilha Grande study, and in December, larvae of *Tubastraea* sp. were observed on histological slides. No larvae were observed in the months of March and August, where larvae were recorded at Ilha Grande (De Paula et al. 2014). Mizhari (2008) observed larvae in March and May and between September and January 2008 in Arraial do Cabo. It is possible to conclude that the species planning throughout the year, due to the occurrence of larvae almost every month, among our records and previous records. Corals are capable of producing gametes and larvae continuously depending only on some stimulus to release them.

Colonies of *T. coccinea* and *T. tagusensis* - Ilha Grande Bay/Brazil, presented two reproductive peaks related to oocyte size increase, suggesting the occurrence of at least two gametogenic cycles per year. From September to December and from February to May, continuous production of gametes, gametogenesis overlapss and duration of three to four months of embryogenesis and spawning events, was also recorded (De Paula et al., 2014). De Paula observed larvae floating after release, mainly on the water surface, and after swimming along the water column, planula explored the bottom and settled. Larval development and metamorphosis were completed in three to seven days, on average. In a study on timing of larval release, *T. aurea* planulation occurred throughout the day without a consistent peak in southern Taiwan according to Fan et al. (2006). On the field, Paz-García et al. (2007) described, in the Gulf of California, Mexico, newly released planula attached to a thread of mucus that descended from the mouth of the polyp to the bottom. Although we did not observe the larval behavior in the field, the larvae

presented behavioral differences between the species, related to the time of living, metamorphosis and death.

Polyps with wide chambers enables the genus *Tubastraea* incubate a large number of embryos and planulae until advanced stages of development possibly related to skeletal morphology particularly in Dendrophylliidae, as observed in *Balanophyllia elegans* (Fadlallah and Pearse 1982). *T. aurea* has an average of larger polyps and, therefore, more mesentery, and was the species that produced the most oocytes and obtained the highest fecundity rate among the others, probably because it has more storage space for gametes. The species *Tubastraea* sp. exhibited the second highest fecundity rate and second largest polyp size, followed by *T. coccinea* with smaller polyps as well as number of oocytes. Gamete production also tends to increase with colony size as more polyps produce more gametes (Kojis and Quinn 1981, Sakai 1998, Zakai et al. 2000), especially in invasive species.

The three reproduction peaks and the two gametogenic cycles observed in the year through increased oocyte size, continuous gamete production, gametogenesis overlap and occurrence of various planulation events are part of the establishment strategy suggested by Szmant (1986) as facilitators of the invasion process. Ovogenesis overlap and embryo incubation were also recorded for Dendrophylliidae *B. elegans* (Fadlallah and Pearse 1982) and *B. europaea* with temporary overlap (Goffredo and Telò 1998), and also for species of the *Madracis* spp. (Vermeij et al. 2004). For Harrison and Wallace (1990) and Smith and Buddemeir (1992), reproduction exhibits less stress tolerance than other vital functions and thus, changes in reproductive effort are important indicators of changes in coralline environments. Fecundity (number of oocytes per polyp size) is a good technique to estimate corals reproductive effort (Harrison and Wallace 1990). All three species showed high fecundity rates, demonstrating the possibility of a constant increase in the

population since the species produce many gametes and larvae throughout the year. Peak size of oocytes diameters in November may be related to the fact that it is the month with the highest average polyp size for *Tubastraea coccinea* species. Possibly the larger the space, the larger the diameter that the oocyte can reach, since it deforms according to the number of oocytes and space, as observed in histological slides.

Reproductive traits such as sexual mode and pattern may be variable among different coral populations of the same species (Harrison and Wallace 1990, Soong 1991, Ward 1992). As an example of this *Tubastraea aurea* was reported as a gametes-releasing coral in the Great Barrier Reef, Australia (Harrison 1985) but in Brazil this species are larval incubators. In this study it was evident that these invading corals have highly advantageous reproductive strategies such as high production of oocytes, embryos and nutrient-rich larvae (lecithotrophic larvae) that develop rapidly. The poor parental care for these organisms (Loya 1976, Fadlallah and Pearse 1982, Sinervo and McEdward 1988) increases the success of colonization in new areas also documented by De Paula et al. (2014) in the south of the state.

There was synchrony between species related to oocyte I, II and III production in different periods of the year. Synchronization between colonies of the same species was also described in De Paula et al. (2014). We observed cysts occurring simultaneously for *T. coccinea*, *T. aurea* and *Tubastraea* sp. in just one month. According to Harrison and Wallace (1990) gametogenesis is usually synchronized in the same colony and partially between colonies of the same population. Gametes from each cycle tend to mature together within each coral, but in relation to the population, less synchrony is apparent (Harrison and Wallace 1990). Gametogenesis can be synchronized in reproductively isolated groups as in the case of *Pocillopora damicornis* and its two morphologically different forms reported by Muir (1984). Differently from incubators of the genus

Tubastraea here in Brazil, asynchronous development of gametes has been observed in other incubator species such as *Balanophyllia elegans*, *Cyphastrea ocellina* and *Porites porites* (Fadlallah and Pearse 1982, Wright 1986, Tomascik and Sander 1987). The synchronicity of sexual reproductive cycles may be related to the environmental regulation of sexual activities already observed in populations of scleractinious corals (Giese and Pearse 1974, Freiwald et al. 2004).

Observation of larval behavior was able to demonstrate differences in larvae performance between species. According to Vermeij (2006), coral larvae move both actively and passively, and more the larvae consume energy reserves, more they lack sufficient energy to perform the metamorphosis, this fact can influence future stages of development and survival, and may have influenced the performance of the larvae, since the larval swimming activity was observed in the experiment wells. *T. aurea* metamorphosed faster than other species in the CW treatment, and then it was *T. coccinea* in the same treatment. Due to the recognized pattern of aggregation in the spatial distribution of these corals (Paula and Creed 2005), the larvae of *T. aurea* and *T. coccinea* that metamorphosed faster in the CW treatment demonstrate a facilitation in the process of larval development and subsequent recruitment. Further studies are needed to understand the factors that influence this process in the presence of parental colonies. *Tubastraea* sp. was the species that took the most time for metamorphosis and survived longer. The longer period of life of *Tubastraea* sp. larvae that can demonstrate higher stored energy related to the larger size of oocytes also found in the species. A study by Mizhari et al. (2014) reported that planulae produced by *Tubastraea coccinea* can metamorphose and aggregate into groups of up to eight polyps in the water column without settling on a benthic substrate this was not observed throughout the study.

Mizhari (2008) reported that the frequency and intensity of minimum temperatures in the internal area of Arraial do Cabo bay probably limited the distribution and growth of *T. coccinea*, and could also influence directly the reproductive and different life cycle phases. This study provided a new current scenario on the reproduction of three azooxanthellate invading corals in a region influenced by the upwelling phenomenon. Although the opposite was believed, high fecundity rates, occurrence of two gametogenic cycles per year, continuous gamete production, larvae brooder, gametogenesis overlaps and various larva release events are examples of reproductive success that do not appear to be negatively influenced by the lower temperature averages inside the Bay. It is important to note that the temperature averages inside the bay of Arraial do Cabo are not low as in the area outside it, where the resurgence occurs, in these areas there is no occurrence of corals of the genus *Tubastraea*. The waters inside the bay are influenced by the cold and nutrient-filled waters that enter and leave the bay during periods of the phenomenon.

As an invading organism, the sun coral is an opportunistic animal, with high production of gametes, several periods of planning, larvae with great dispersion capacity and fast settlement and great potential for occupation of free substrates (Vermeij 2005, Creed and De Paula 2007, Mizrahi et al. 2014). Information on reproductive biology is essential to encompass knowledge of physiology and invasion power of these organisms, including characteristics such as gametogenesis, sexual pattern (hermaphroditism and gonocorism), reproductive peaks, fecundity, and larval behavior that should be monitored. Studies such as the present one should be accompanied by the implementation of policies aimed at minimizing environmental risks.

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CONCLUSÕES GERAIS

- Identificamos três morfotipos no Brasil que foram delimitados e identificados como três espécies distintas: *T. aurea*, *T. coccinea* e *Tubastraea* sp. Todos os três corais são morfologicamente diferentes entre si. *Tubastraea* sp. (ainda não identificada) é morfologicamente diferente de *Tubastraea tagusensis*, que acreditava-se habitar a região;
- Encontramos uma semelhança genética entre amostras do Brasil e do sudeste dos EUA fornecendo evidências de uma possível relação entre as populações. É

provável que as regiões tenham sido colonizadas da mesma maneira ou que as populações dessas regiões sejam descendentes uma da outra;

- Faz-se necessária uma abordagem integrativa para examinar melhor a delimitação de espécies em *Tubastraea*, essencial para o esclarecimento e gerenciamento de eventos de bioinvasões por espécies de coral sol. Outros marcadores também deverão ser utilizados para uma investigação mais acurada e correta identificação de espécies;
- *Tubastraea coccinea* vem aumentando sua cobertura nos costões rochosos de Arraial do Cabo e cresceu mais do que o relatado em um estudo de 11 anos atrás na região, de 3,31 cm^2/ano para atuais 4,84 cm^2/ano, enquanto *T. aurea* e *Tubastraea* sp. cresceram 4,35 e 5 cm^2/ano, respectivamente. Apesar de registrarmos esse crescimento mais acelerado, a espécie ainda demonstra um crescimento menor comparado ao local onde foram descritas pela primeira vez, Baía de Ilha Grande, com 5,85 cm^2/ano para *T. coccinea* e 5,11 cm^2/ano para *T. tagusensis*;
- O crescimento entre espécies foi inversamente proporcional à fecundidade. Temperaturas mais baixas favoreceram o crescimento e podemos ver um padrão proporcional entre a taxa de assentamento e a maior amplitude térmica;
- Verificou-se ocorrência de pelo menos dois ciclos gametogênicos por ano, três picos reprodutivos ao ano, produção contínua de gametas, sobreposição da gametogênese, incubação de larvas e vários eventos de planulação. As larvas sobreviveram por 20 dias em poços de 10 ml, dois a mais do que o relatado em cultivo de aquário no Brasil;
- Podemos dizer que as espécies de corais do gênero *Tubastraea* estão bem adaptadas à região através da confirmação da expansão de sua área de cobertura e

suas eficientes estratégias reprodutivas. Torna-se cada vez mais importante monitorar e agir de forma apropriada para controlar esses invasores;

- Os corais do gênero *Tubastraea* demonstraram manter e até aperfeiçoar suas estratégias de história de vida possibilitando a manutenção e preservação de suas populações. Nossos estudos devem ser acompanhados de implementação de políticas que visem minimizar os riscos ambientais.

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ANNEX

Supplementary table I. Diameters and area calculation (A = π x r (a) x r (b)) of colonies used in histological procedures for reproductive activity analysis and their respective tipping numbers in the IEAPM scientific collection.

IEAPM ID	Species	Colony ID	Month	Polyp number	Area (cm^2)
001125	*T. aurea*	Sp 1 Nov col 1	November	11	11.68
001126	*T. aurea*	Sp 1 Nov col 2	November	35	26.28
001127	*T. aurea*	Sp 1 Nov col 3	November	16	21.4
001144	*T. aurea*	Sp 1 Dec col 1	December	18	26.28
00200	*T. aurea*	Sp 1 Dec col 2	December	15	21.2
00201	*T. aurea*	Sp 1 Jan col 1	January	14	12.23
00204	*T. aurea*	Sp 1 Jan col 2	January	11	11.02
00205	*T. aurea*	Sp 1 Jan col 3	January	15	13.66
00214	*T. aurea*	Sp 1 Feb col 1	February	11	16.06
00216	*T. aurea*	Sp 1 Feb col 2	February	12	19.16
00226	*T. aurea*	Sp 1 Feb col 3	February	11	12.15
00227	*T. aurea*	Sp 1 Mar col 1	March	13	10.83
00228	*T. aurea*	Sp 1 Mar col 2	March	22	14.08
00238	*T. aurea*	Sp 1 Apr col 1	April	17	20.44
00239	*T. aurea*	Sp 1 Apr col 2	April	15	10.8
00240	*T. aurea*	Sp 1 Apr col 3	April	14	12.31
00251	*T. aurea*	Sp 1 May col 1	May	20	25.62
00252	*T. aurea*	Sp 1 May col 2	May	32	28.93
00254	*T. aurea*	Sp 1 May col 3	May	18	15.92
00868	*T. aurea*	Sp 1 Jun col 1	June	25	18.42
00869	*T. aurea*	Sp 1 Jun col 2	June	35	28.61
00871	*T. aurea*	Sp 1 Jun col 3	June	14	14.14
00879	*T. aurea*	Sp 1 Jul col 1	July	34	29.8
00880	*T. aurea*	Sp 1 Jul col 2	July	28	15.97
00881	*T. aurea*	Sp 1 Jul col 3	July	12	19.78
00892	*T. aurea*	Sp 1 Aug col 1	August	21	19.46
00893	*T. aurea*	Sp 1 Aug col 2	August	26	24.82
00894	*T. aurea*	Sp 1 Aug col 3	August	17	10.36
00904	*T. aurea*	Sp 1 Sep col 1	September	28	18.21
00906	*T. aurea*	Sp 1 Sep col 2	September	19	18.37
00907	*T. aurea*	Sp 1 Sep col 3	September	20	20.11
00914	*T. aurea*	Sp 1 Oct col 1	October	25	24.49
00915	*T. aurea*	Sp 1 Oct col 2	October	18	12.01
001117	*T. aurea*	Sp 1 Oct col 3	October	24	11.74
00924	*T. aurea*	Sp 1 Nov col 1	November	20	28.26
00926	*T. aurea*	Sp 1 Nov col 2	November	18	15.1
00927	*T. aurea*	Sp 1 Nov col 3	November	11	18.98
001132	*T. coccinea*	Sp 2 Nov col 1	November	45	24.82
001135	*T. coccinea*	Sp 2 Nov col 2	November	40	20.14
001136	*T. coccinea*	Sp 2 Dec col 1	December	50	20.41

001137	*T. coccinea*	Sp 2 Dec col 2	December	65	17.9
001139	*T. coccinea*	Sp 2 Dec col 3	December	40	17.96
00206	*T. coccinea*	Sp 2 Jan col 1	January	15	15.54
00208	*T. coccinea*	Sp 2 Jan col 2	January	20	14.5
00210	*T. coccinea*	Sp 2 Jan col 3	January	22	15.58
00217	*T. coccinea*	Sp 2 Feb col 1	February	36	16.81
00219	*T. coccinea*	Sp 2 Feb col 2	February	40	16.81
00220	*T. coccinea*	Sp 2 Feb col 3	February	28	14.44
00231	*T. coccinea*	Sp 2 Mar col 1	March	29	16.84
00232	*T. coccinea*	Sp 2 Mar col 2	March	24	10.88
00234	*T. coccinea*	Sp 2 Mar col 3	March	20	15.02
00241	*T. coccinea*	Sp 2 Apr col 1	April	66	28.29
00242	*T. coccinea*	Sp 2 Apr col 2	April	18	11.38
00243	*T. coccinea*	Sp 2 Apr col 3	April	30	10.82
00255	*T. coccinea*	Sp 2 May col 1	May	39	18.57
00258	*T. coccinea*	Sp 2 May col 2	May	55	28.26
00882	*T. coccinea*	Sp 2 May col 3	May	21	12.78
00872	*T. coccinea*	Sp 2 Jun col 1	June	33	13.65
00873	*T. coccinea*	Sp 2 Jun col 2	June	20	11.54
00875	*T. coccinea*	Sp 2 Jun col 3	June	33	11.48
00883	*T. coccinea*	Sp 2 Jul col 1	July	29	17.72
00884	*T. coccinea*	Sp 2 Jul col 2	July	34	12.83
00887	*T. coccinea*	Sp 2 Jul col 3	July	39	19.46
00896	*T. coccinea*	Sp 2 Aug col 1	August	26	15.82
00898	*T. coccinea*	Sp 2 Aug col 2	August	43	14.98
00899	*T. coccinea*	Sp 2 Aug col 3	August	54	21.89
00908	*T. coccinea*	Sp 2 Sep col 1	September	32	15.51
00910	*T. coccinea*	Sp 2 Sep col 2	September	35	12.72
00916	*T. coccinea*	Sp 2 Sep col 3	September	24	22.51
00918	*T. coccinea*	Sp 2 Oct col 1	October	37	16.33
00919	*T. coccinea*	Sp 2 Oct col 2	October	42	16.09
001118	*T. coccinea*	Sp 2 Oct col 3	October	16	18.15
001119	*T. coccinea*	Sp 2 Nov col 1	November	14	15.02
00911	*T. coccinea*	Sp 2 Nov col 2	November	33	11.48
00928	*T. coccinea*	Sp 2 Nov col 3	November	29	17.72
001128	*Tubastraea* sp.	Sp 3 Nov col 1	November	24	37.18
001130	*Tubastraea* sp.	Sp 3 Nov col 2	November	10	22.45
001131	*Tubastraea* sp.	Sp 3 Nov col 3	November	21	28.06
001140	*Tubastraea* sp.	Sp 3 Dec col 1	December	16	21.18
001141	*Tubastraea* sp.	Sp 3 Dec col 2	December	15	15.72
001143	*Tubastraea* sp.	Sp 3 Dec col 3	December	25	28.08
00211	*Tubastraea* sp.	Sp 3 Jan col 1	January	18	28.68
00213	*Tubastraea* sp.	Sp 3 Jan col 2	January	10	17.56
00222	*Tubastraea* sp.	Sp 3 Feb col 1	February	15	11.61
00223	*Tubastraea* sp.	Sp 3 Feb col 2	February	14	19.78

00225	*Tubastraea* sp.	Sp 3 Feb col 3	February	18	32.42
00235	*Tubastraea* sp.	Sp 3 Mar col 1	March	13	9.74
00236	*Tubastraea* sp.	Sp 3 Mar col 2	March	19	13.35
00237	*Tubastraea* sp.	Sp 3 Mar col 3	March	13	10.33
00244	*Tubastraea* sp.	Sp 3 Apr col 1	April	10	18.66
00248	*Tubastraea* sp.	Sp 3 Apr col 2	April	14	12.65
00249	*Tubastraea* sp.	Sp 3 Apr col 3	April	15	16.48
00864	*Tubastraea* sp.	Sp 3 May col 1	May	14	19.34
00865	*Tubastraea* sp.	Sp 3 May col 2	May	10	12.95
00867	*Tubastraea* sp.	Sp 3 May col 3	May	11	13.14
00876	*Tubastraea* sp.	Sp 3 Jun col 1	June	24	18.02
00877	*Tubastraea* sp.	Sp 3 Jun col 2	June	22	19.08
00888	*Tubastraea* sp.	Sp 3 Jul col 1	July	20	22.03
00889	*Tubastraea* sp.	Sp 3 Jul col 2	July	16	14.28
00891	*Tubastraea* sp.	Sp 3 Jul col 3	July	19	13.88
00900	*Tubastraea* sp.	Sp 3 Aug col 1	August	10	12.56
00902	*Tubastraea* sp.	Sp 3 Aug col 2	August	11	17.27
00903	*Tubastraea* sp.	Sp 3 Aug col 3	August	10	12.41
00912	*Tubastraea* sp.	Sp 3 Sep col 1	September	11	17.66
001115	*Tubastraea* sp.	Sp 3 Sep col 2	September	12	14.13
00116	*Tubastraea* sp.	Sp 3 Sep col 3	September	8	10.05
00920	*Tubastraea* sp.	Sp 3 Oct col 1	October	12	18.37
00922	*Tubastraea* sp.	Sp 3 Oct col 2	October	11	10.8
00923	*Tubastraea* sp.	Sp 3 Oct col 3	October	12	15.54
001120	*Tubastraea* sp.	Sp 3 Nov col 1	November	10	22.45
001123	*Tubastraea* sp.	Sp 3 Nov col 2	November	10	17.56
001124	*Tubastraea* sp.	Sp 3 Nov col 3	November	13	16.48