

The spread of the introduced ascidians *Ciona robusta* Hoshino & Tokioka, 1967 and *Rhodosoma turcicum* (Savigny, 1816) in the southwestern Atlantic

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ABSTRACT

New records of the introduced solitary ascidians, *Ciona robusta* and *Rhodosoma turcicum*, have been added to the southwestern Atlantic Ocean, from Cabo Frio to Ilha Grande Bay, Brazil, in surveys conducted since 2009. Both species occurred on natural and artificial substrates, in predator-protected habitats, and regions close to harbors or other maritime activities. The distribution of *Ciona robusta* was related to water temperature, influenced by upwelling intensity and temperatures below 25°C, while *R. turcicum* was not sensitive to the range of recorded water temperatures. The occurrence of both species on sites close to maritime terminals suggests vessels as potential vectors.

Descriptors: Ascidiacea; Marine fouling; Introduced species; Marine activities.

INTRODUCTION

Marine bioinvasions have been recorded globally and have increased in recent decades in terms of the numbers of recorded species and numbers of scientific papers published (Seebens et al., 2013; Dias et al., 2019). Invasive species (IS) and nonindigenous species (NIS) have been identified as being responsible for reducing diversity in invaded regions, leading to local extinctions, and causing impacts on economic activities (Creed et al., 2017; O'Brien et al., 2017; Blackburn et al., 2019). Academic discussions concerning those issues are continually increasing (Junqueira, 2013; Boltovskoy et al., 2018; Fowler et al., 2020). Awareness of the importance of ascidians as invasive species is growing worldwide (Carlton and

Eldredge, 2009; Zhan et al., 2015; Colarusso et al., 2016).

Eutrophication (Marins et al., 2010; Crooks et al., 2011), aquaculture facilities (McKindsey et al., 2007; Rocha et al., 2009), and harbor and shipping operations (Darbyson et al., 2009; Grey, 2010) have been indicated as important influences in increasing their spread. However, NIS introduction and spread are also subjected to limiting factors. Predation by fish (Marins et al., 2009; Dumont et al., 2011; Dias et al., 2013; Roth et al., 2017) and competition with the local fauna (Paetzold et al., 2012) are two biological factors that can regulate invasiveness, while environmental factors (such as temperature) can act as selective forces that prevent the establishment and persistence of invasive species. Therefore, environmental and biological factors will delimit species niches and are determinants for successful bioinvasions (Granot et al., 2017).

Ascidians can be found in many different habitats, and the photonegative behavior of their larvae

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favors their occurrence in crevices or under rocks, more protected from predation and sedimentation (Millar, 1971; Lambert, 2005). Communities that settle on artificial substrates have often been found to be different from those encountered on natural surfaces (Perkol-Finkel et al., 2006). Artificial substrates are common in ports and marinas and serve as gateways for species introductions (Lambert, 2005; Ignacio et al., 2010). Surveys of invasive species must, therefore, use specific methodologies for data collection (Kakkonen, 2019).

The species *Ciona robusta* Hoshino & Tokioka, 1967 and *Rhodosoma turcicum* (Savigny, 1816) are considered invasive in many parts of the world. Both species have been recorded in Brazil but studies indicated that they are susceptible to native predators (Marins et al., 2009; Skinner et al., 2013). At least 14 other non-indigenous ascidian species have likewise been recorded in Rio de Janeiro State (Rocha and Costa, 2005; Marins et al., 2010; Granthom-Costa et al., 2016; Skinner et al., 2016; Oricchio et al., 2019).

Ciona robusta (formely identified on Brazil as *C. intestinalis*) was reported by Millar (1958) in São Sebastião (São Paulo state), and later in Guanabara Bay (Rio de Janeiro state), by Costa (1969) in 1958 and 1961, and elsewhere in Rio de Janeiro (Marins et al.,

2009) and São Paulo (Rocha, 1995; Rocha and Bonnet, 2009; Dias et al., 2013; Vieira et al., 2012) coasts in the following decades. *Rhodosoma turcicum* has been recorded from the states of Bahia (Rocha et al., 2012a) and Rio de Janeiro (Skinner et al., 2013).

We describe here the current distribution of *Ciona robusta* and *Rhodosoma turcicum* in coastal areas of Rio de Janeiro (~250 km in total), highlighting the importance of sea temperature on their distributions. We also discuss the current knowledge on possible transport vectors and their spreading along the southeast Brazilian coast.

METHODS

This study was performed with research authorization from the Instituto Estadual do Ambiente - INEA (Auth. #057/2011 and 025/2017), RESEX Arraial do Cabo (ICMBio Auth. #25024), and Estação Ecológica Tamoios (ICMBio - Auth. #36194).

STUDY AREA

The present study was conducted within the coastal transition zone between Tropical and Warm Temperate regions (Spalding et al., 2007) in Rio de Janeiro State (Figure 1A and 1B). The area has intense maritime activities like shipping, harbor, oil industry

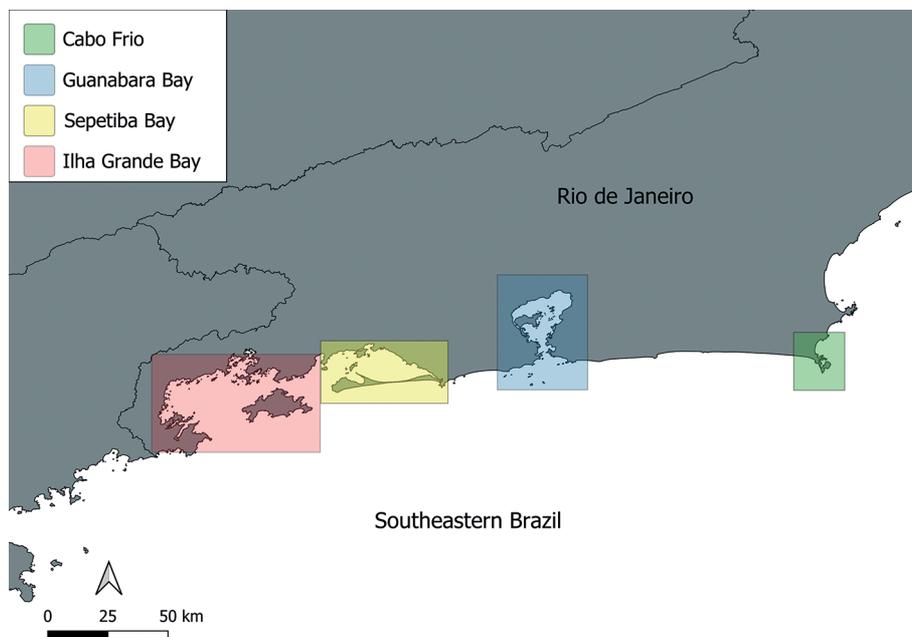


Figure 1. Surveyed areas on the coast of Rio de Janeiro State, Brazil: Cabo Frio (green), Guanabara Bay (blue), Sepetiba Bay (yellow), and Ilha Grande Bay (red).

and other nautical activities, such as tourism, fishing, and coastal development (Silva et al., 2018; INEA, 2015; Ignacio et al., 2010). The climate is considered tropical humid, with a rainy season from October to April, and a dry season from May to September. Despite that general pattern, rainfall can vary greatly along the coast, mainly due to the proximity of the Serra do Mar Mountains, with average annual rainfall rates varying from 800 to 2,100mm (INEA 2015; Coe et al., 2007). There is a seasonal upwelling, which is stronger close to Cabo Frio, becoming weaker close to Ilha Grande Bay (Valentin, 2001; Creed et al., 2007).

WATER MONITORING - SEA SURFACE TEMPERATURES

Sea surface temperatures (SST) were measured using IButton[®] sensors programmed to record every hour at Cabo Frio and Ilha Grande Bay (at two sites in the latter: Dois Rios and Ponta Leste). The sensors were accommodated inside falcon tubes (to protect them from direct contact with the seawater) and placed at depths of 1-2 m, depending on the site. The shallow positions of the sensors ensured that no effects of water stratification were measured. SST data were aggregated monthly, and mean, and standard deviations were calculated. SST measurements were initiated in 2012 and extended until January 2014 in Cabo Frio and December 2019 in Ilha Grande Bay. Additionally, we extracted monthly SST data from satellite imagery during the period between January 2009 and December 2019 (data provided by NCEP, OISST Version 2, available from https://iridl.ldeo.columbia.edu/maproom/Global/Ocean_Temp/), from the Brazilian Navy Oceanographic Buoys Program (PIRATA), and from the National Oceanographic Data Center (BNDO, 2020) for Cabo Frio, covering 2009-2010, 2012-2013, and 2016-2018.

SAMPLING

We employed two sampling strategies: on artificial experimental structures (Marins et al., 2009; Kremer and Rocha, 2011; Skinner et al., 2013) and by active SCUBA or free diving searches. Our approach was similar to Kakkonen et al. (2019), which emphasize habitats favoring ascidian recruitment.

ARTIFICIAL STRUCTURES

Granite plates (21 x 12cm) and sandwich black polyethylene plates (15x15cm), *sensu* Kremer and

Rocha (2011) were used to collect ascidians within protected cages, needed to prevent fish predation on ascidian recruits (Marins et al., 2009; Skinner et al., 2013). The experimental units were immersed 1 m below the surface at 11 sites positioned in the study areas: Cabo Frio (at Itajuru Inlet, Forno Harbor, and Cabo Frio Island); Guanabara Bay (Urca and Praia Vermelha); Sepetiba Bay (Junqueira and Guaíba Island); and Ilha Grande Bay (Piraquara de Fora, Praia do Anil, Ponta Leste, Abraão, and Dois Rios). Polyethylene plates were installed only at sites off Ilha Grande (Table 1).

SCUBA AND FREE DIVING

Exploratory scuba diving was undertaken for periods of 50 minutes each, actively searching for solitary ascidians on potentially predator-protected substrates such as holes, caves, or under boulders (Rocha, 1995). Those searches were performed at 26 sites at Ilha Grande Bay and two sites at Cabo Frio (Table 1). Free diving was used at some sites with submerged artificial structures; no scuba diving was undertaken in Guanabara Bay or Sepetiba Bay due to low water visibility.

DATA ANALYSIS

Mean monthly SST was calculated from daily data for Cabo Frio and Ilha Grande Bay collected by Ibutton[®] sensors and buoys. Satellite SST was retrieved to gain a wide temporal and regional perspective. Mean SST values (< 25.0°C and > 25.1°C) were used in combination with records of *Ciona robusta* and *Rhodossoma turcicum* in the selected temperature ranges to perform a square Fisher analysis (Underwood 1997) testing the hypothesis that temperature influences *Ciona robusta* distribution, but not *Rhodossoma turcicum* occurrences. The SST value of 25°C was chosen as the upper physiological limit of survival for *C. robusta* (Caputi et al., 2015; Rocha et al., 2017; Kim et al., 2019). The physiological temperature limits for *R. turcicum* are unknown, but due to its tropical distribution the species may thrive in water temperatures higher than 22°C, as suggested by Shenkar and Loya (2009).

RESULTS

WATER TEMPERATURE MONITORING

SST data from Ibutton[®] sensors, buoys, and satellites indicated large SST variations between seasons, years, and monitoring methods (Figure 2). Satellite

Table 1. Survey sites, including geographical coordinates, survey methods, and types of substrates sampled.

Surveyed site	Region	Latitude	Longitude	Survey methods	substrate
Meros Island	Ilha Grande Bay	23°11'S	44°34'W	Scuba	Natural
Ratos Island	Ilha Grande Bay	23°11'S	44°36'W	Scuba	Natural
Ponta do Arpoá	Ilha Grande Bay	23°12'S	44°36'W	Scuba	Natural
Deserta Island	Ilha Grande Bay	23°13'S	44°33'W	Scuba	Natural
Mantimento Island	Ilha Grande Bay	23°11'S	44°39'W	Scuba	Natural
Paraty Mirim	Ilha Grande Bay	23°14'S	44°37'W	Scuba, granite plate	Natural, Artificial
Ponta Leste	Ilha Grande Bay	23°3'S	44°14'W	Scuba, Granite and polyethylene plate	Natural and Artificial
Anil beach	Ilha Grande Bay	23°0.5'S	44°18'W	Granite plate	Artificial
Bonfim beach	Ilha Grande Bay	23°1'S	44°19'W	Scuba	Natural
Piraquara de Fora	Ilha Grande Bay	23°1'S	44°26'W	Granite plate	Artificial
Laje Grande	Ilha Grande Bay	23°0.8'S	44°15'W	Scuba	Natural
Sororoca Island	Ilha Grande Bay	23°2'S	44°9'W	Scuba	Artificial
Longa Island	Ilha Grande Bay	23°8'S	44°19'W	Scuba, polyethylene plates	Natural, Artificial
Sítio Forte Point	Ilha Grande Bay	23°6'S	44°17'W	Scuba, polyethylene plates	Natural, Artificial
Bananal Point	Ilha Grande Bay	23°6'S	44°15'W	Scuba	Natural
Macacos Island	Ilha Grande Bay	23°4'S	44°13'W	Scuba	Natural
Aventureiro	Ilha Grande Bay	23°11'S	44°18'W	Scuba	Natural
Tacunduba	Ilha Grande Bay	23°11'S	44°16'W	Scuba	Natural
Parnaioca	Ilha Grande Bay	23°11'S	44°15'W	Scuba	Natural
Sardinhas Cove	Ilha Grande Bay	23°11'S	44°12'W	Scuba	Natural
Amarração Island	Ilha Grande Bay	23°10'S	44°10'W	Scuba, polyethylene and granite plates	Natural, Artificial
Palmeiras Point	Ilha Grande Bay	23°10'S	44°10'W	Scuba	Natural
Cavalinho Point	Ilha Grande Bay	23°11'S	44°10'W	Scuba	Natural
Jorge Grego Island	Ilha Grande Bay	23°12'S	44°9'W	Scuba, polyethylene plates	Natural, Artificial
Santo Antônio rocks	Ilha Grande Bay	23°10'S	44°8'W	Scuba	Natural
Lopes Mendes	Ilha Grande Bay	23°11'S	44°7'W	Scuba, polyethylene plates	Natural
Caxadaço Point	Ilha Grande Bay	23°10'S	44°9'W	Scuba	Natural
Castelhanos	Ilha Grande Bay	23°9'S	44°5'W	Scuba, polyethylene plates	Natural, Artificial
Palmas Island	Ilha Grande Bay	23°8'S	44°6'W	Scuba	Natural
Abraão	Ilha Grande Bay	23°8'S	44°10'W	Granite plate	Artificial
Junqueira	Sepetiba Bay	23°58'S	44°2'W	Granite plate	Artificial
Guáíba Island	Sepetiba Bay	23°59'S	44°7'W	Granite plate	Artificial
Praia Vermelha	Guanabara Bay	23°57'S	43°9'W	Granite plate	Artificial
Urca	Guanabara Bay	23°57'S	43°10'W	Granite plate	Artificial
Cabo Frio Island	Cabo Frio	23°0.1'S	42°0.3'W	Scuba, Granite plate	Artificial
Forno Harbor	Cabo Frio	22°58'S	42°0.8'W	Scuba, granite plate	Artificial
Forno Cove	Cabo Frio	22°58'S	42°0.3'W	Snorkeling, granite plate	Artificial
Itajuru Inlet	Cabo Frio	22°52'S	42°1'W	Granite plate	Artificial

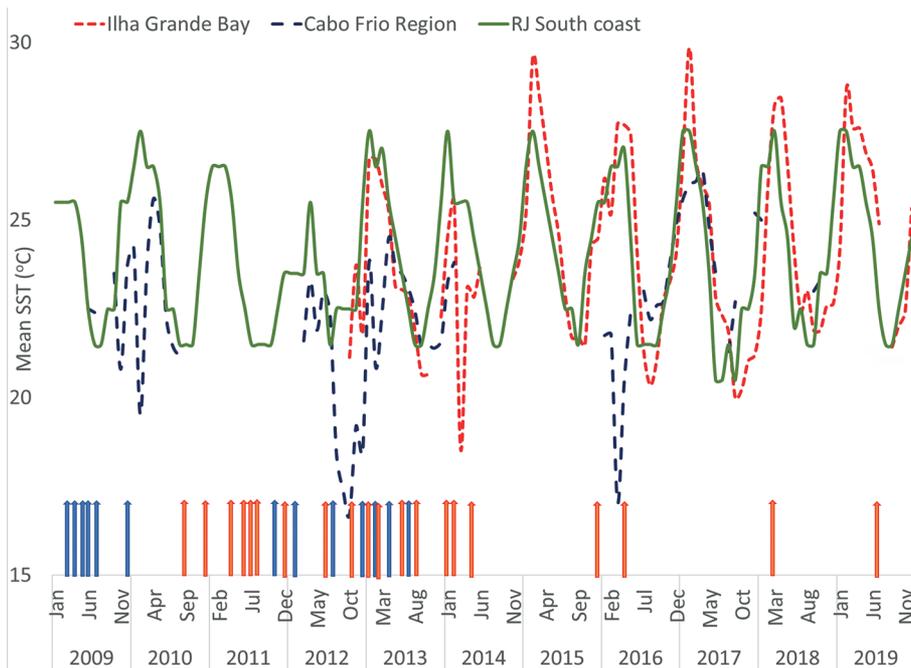


Figure 2. Mean sea surface temperatures (SST) at Ilha Grande Bay (IGB, red line), Cabo Frio (CF, blue line), and the southern coast of Rio de Janeiro State (green line) from January/2009 to December/2019. Data were obtained from Ibutton® sensors or oceanographic buoys for IGB and CF, and from satellite data for the southern coast of RJ (https://iridl.ldeo.columbia.edu/maproom/Global/Ocean_Temp). Arrows indicate the samples of *Rhodosoma turcicum* (red arrow) or *Ciona robusta* (blue arrow). Gaps in plots represent absence of data due to malfunctioning or lost sensors.

data from 2009 to 2019 indicated seasonal oscillations of SST, with higher values (circa 27°C) during the austral Spring-Summer, except during the 2011-2012 season (mean 23.5°C). The mean SST estimated by this method ranged from 20.5 to 27.5°C (mean 24°C). Data from Ibutton® and buoys showed more extreme SST values (both high and low).

At Ilha Grande Bay, the warmest summers occurred in 2014/2015 and 2016/2017, with 63.2% of approximately 3,300 SST records from November through March 2014/2015 higher than 27.1°C. In the 2016/2017 season, 84.2% of the SST records were higher than 24.1, and 40.8% higher than 27.1°C. SST values lower than 18.5°C were associated to upwelling, as occurred in February/2014 and October/2017.

The SST at Cabo Frio (Figure 2) from June/2009 to October/2018 reveals the influence of local upwelling, usually occurring from September through March. Mean SST ranged from 16.7°C in October/2012 to 26.4°C in April/2017. Two seasons draw attention: October-December/2012 and March-April/2016, with very contrasting low temperatures in Cabo Frio compared to Ilha Grande Bay and the remaining study sites.

Combining the SST data from all sources, we estimated the mean monthly SST values for 99 months, of which the means of 53 months (54%) were lower than or equal to 25°C, while those of 43 months (46%) were higher than or equal to 25.1°C. Those data were used to test the influence of temperature on the presence of the two species.

SPECIES OCCURRENCES

CIONA ROBUSTA HOSHINO AND TOKIOKA, 1967

Ciona individuals found along the coast of Rio de Janeiro agreed with the description of *C. robusta* by Hoshino and Tokioka (1967), as well as the revisions conducted by Sato et al. (2012) and Brunetti et al. (2015). The tunic of *C. robusta* is cartilaginous, yellowish, with the siphons having almost equal sizes at the anterior end of the body. The tunic has many tubercular protuberances, although they are sometimes restricted to the siphon or anterior regions. The body is covered by six strong longitudinal bands, from the posterior end of the body up to the siphons and red spots were observed at the margin between adjacent siphon lobes. The circular muscles of the siphons are

not as strong as the longitudinal muscles, and do not form bands.

We analyzed the material collected between 2008 and 2013 from all regions of Rio de Janeiro State (Figure 3), and those deposited at the Zoological Collection of the Universidade do Estado do Rio de Janeiro (CZFFP), as listed in Table 2.

Ciona robusta was recorded on artificial plates in all surveyed regions (Figure 3). Ilha Grande Bay was the only site with occurrences on natural substrates, specifically at Jorge Grego Island (depth 18m), where eight individuals were found under large boulders, which are habitats resembling those simulated by experimental cages.

The lengths of the 33 individuals collected ranged from 40 to 150 mm, with the largest specimens being those collected on natural substrates at Jorge Grego Island, at depths of 18m. The maximum number of individuals collected was 8, at Jorge Grego Island (natural substrate, August/2013), and six at Guaíba Island (artificial substrate, May/2009) (Table 3).

RHODOSOMA TURCICUM (SAVIGNY, 1816)

The material analyzed comprised specimens collected between 2009 and 2019 at Cabo Frio and Ilha Grande Bay (Figure 3). Most individuals were encountered within protected cages, while those found on

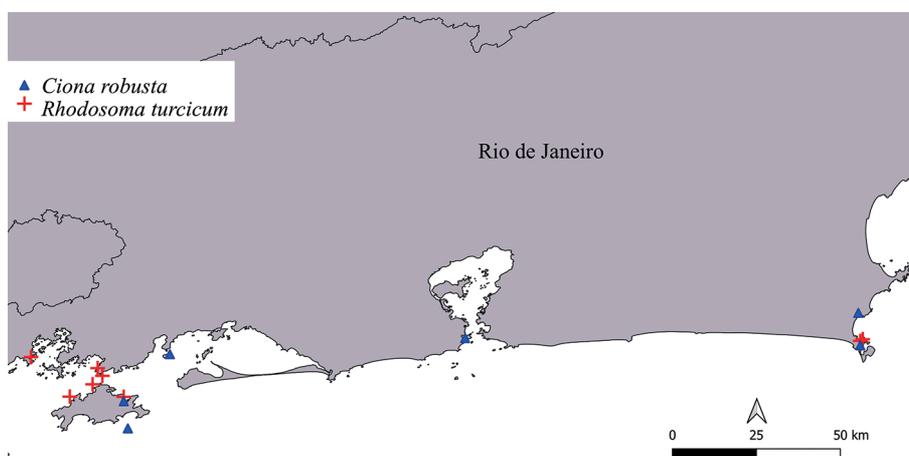


Figure 3. Occurrences of *Ciona robusta* (blue triangles) and *Rhodosoma turcicum* (red cross) in this study along the coast of Rio de Janeiro State, Brazil.

Table 2. Voucher numbers of *Ciona robusta* individuals identified and deposited in the Zoological Collection of the Universidade do Estado do Rio de Janeiro (CZFFP).

Voucher	Site	Date	Collector
CZFFP-ASC 25	Urca - Rio de Janeiro	11/Nov./2008	L.F. Skinner and F.O. Marins
CZFFP-ASC 26	Sepetiba Bay	May/2009	L.F. Skinner and F.O. Marins
CZFFP-ASC 27	Urca - Rio de Janeiro	09/Apr/2009	L.F. Skinner and F.O. Marins
CZFFP-ASC 28	Sepetiba bay	Mar/2009	L.F. Skinner and F.O. Marins
CZFFP-ASC 31	Forno Harbor – Arraial do Cabo	02/Dec/2011	L.F. Skinner and D.F. Barboza
CZFFP-ASC 33	Abraão – Ilha Grande Bay	Aug/2012	L.F. Skinner and D.F. Barboza
CZFFP-ASC 34	Abraão – Ilha Grande Bay	21/Jan/2013	L.F. Skinner and D.F. Barboza
CZFFP-ASC 35	Itajuru Inlet - Cabo Frio	Feb/2013	L.F. Skinner and D.F. Barboza
CZFFP-ASC 207	Jorge Grego Island - Ilha Grande Bay	11/Apr/2013	L.F. Skinner

Table 3. *Ciona robusta* records for the coast of Rio de Janeiro State, Brazil, from 2009 to 2013, in the regions of Cabo Frio, Guanabara Bay, Sepetiba Bay, and Ilha Grande Bay, including sites, years and months of records, and the total number of individuals collected (** - information not available).

Region	Site	Year	Month	Number of individuals
Guanabara Bay	Urca	2008	November	1
Sepetiba Bay	Guaíba Island	2009	May	9
Guanabara Bay	Urca	2009	June	1
Sepetiba Bay	Guaíba Island	2009	**	2
Guanabara Bay	Urca	2009	**	1
Sepetiba Bay	Guaíba Island	2010	January	1
Cabo Frio	Forno Harbor	2011	December	1
Ilha Grande Bay	Abraão	2012	March	1
Ilha Grande Bay	Abraão	2012	August	3
Ilha Grande Bay	Abraão	2013	January	1
Cabo Frio	Itajuru Inlet	2013	March	1
Cabo Frio	Forno Harbor	2013	April	1
Ilha Grande Bay	Jorge Grego Island	2013	April	2
Ilha Grande Bay	Jorge Grego Island	2013	August	8

natural substrates were under boulders - and so protected from predators. The specimens are deposited in the Zoological Collection of the Universidade do Estado do Rio de Janeiro (CZFFP), as listed in Table 4.

We have identified 114 individuals obtained on 30 sampling dates. The maximum numbers of individuals collected were 13 (July/2011) and 11 (October/2010 and June/2014) found at Forno Harbor, in the Cabo Frio Region, all on artificial substrates (Table 5). Most individuals were collected during 2010 ($n=31$) and 2011 ($n=32$), the most intensively studied years at Cabo Frio.

The entire specimens examined (with tunic) varied from 9 to 41 mm in length and 3 to 23 mm in width. Their tunics were thick with epibionts and had greenish colorations that were lost after fixation. They were attached to the substrate by the right sides of their bodies. We found small papillae on the tunics, mainly close to the anterior region of the body. A lid in that region can be displaced by internal muscles to cover both oral and atrial siphons. It is easier to spot individuals in the field when the lid is open. Dissected individuals showed short and simple oral tentacles. Muscles were visible close to the siphons and extended onto the hinge of the lid. The dorsal tubercles were horseshoe-shaped. The pharynx was plain, without folds, and had straight stigmata and papillae

sustaining the vessels. Our specimens conformed well with the descriptions provided by Van Name (1945), Kott (1985, 2005), and Rocha et al. (2012a, b).

THE INFLUENCE OF TEMPERATURE ON SPECIES OCCURRENCES

Ciona robusta was recorded 15 times within the temporal window considered. Among those records, 86.7% occurred with SST between 21–25°C, and 53.3% occurred with SST between 23.1 to 25.0°C. Only two records (13.3%) occurred with SST higher than 25.1°C. The Fisher exact test was significant ($p=0.023$; 1 d.f.), indicating this environmental variable as a driver of the presence of *C. robusta*.

Among the 27 records of *R. turcicum*, 11.1% corresponded to SST below 21.0°C, 59.3% between 21.1–25.0°C, 25.9% between 25.1–29°C and 3.7% with SST higher than 29.1°C. The Fisher exact test was not significant ($p=0.1160$; 1 d.f.), indicating that temperature did not influence the presence of *R. turcicum*.

DISCUSSION

We detected a wider distribution range of *Ciona robusta* than previously recorded for the studied area (Millar, 1958; Costa, 1969; Marins et al., 2009). The only previously collected specimens that we have

Table 4. Vouchers of *Rhodosoma turcicum* individuals analyzed and deposited in the Zoological Collection of the Universidade do Estado do Rio de Janeiro (CZFFP).

Voucher	Site	Date	Collector
CZFFP-ASC 14	Forno Harbor – Arraial do Cabo	23/Sep/2011	L.F. Skinner
CZFFP-ASC 16	Abraão – Ilha Grande Bay	Aug/2012	L.F. Skinner and D.F. Barboza
CZFFP-ASC 19	Forno Harbor – Arraial do Cabo	08/Apr/2013	L.F. Skinner and D.F. Barboza
CZFFP-ASC 21	Forno Beach – Arraial do Cabo	14/May/2013	L.F. Skinner and D.F. Barboza
CZFFP-ASC 22	Ponta Leste – Angra dos Reis	27/Aug/2015	L.F. Skinner and D.F. Barboza
CZFFP-ASC 23	Bananal Point – Ilha Grande Bay	12/May/2016	L.F. Skinner and D.F. Barboza
CZFFP-ASC 24	Macacos Island – Ilha Grande Bay	12/May/2016	L.F. Skinner and D.F. Barboza
CZFFP-ASC346	Piraquara de Fora – Angra dos Reis	15/Aug/2019	L.F. Skinner

Table 5. List of *Rhodosoma turcicum* records for the coast of Rio de Janeiro State, Brazil, from 2010 to 2019, in the regions near Cabo Frio region and Ilha Grande Bay, including sites, years and months of records, and the total number of individuals collected (** - information not available).

Region	Site	Year	Month	Number of individuals
Cabo Frio	Forno Harbor	2010	January	9
Cabo Frio	Forno Harbor	2010	February	1
Cabo Frio	Forno Harbor	2010	May	3
Cabo Frio	Forno Harbor	2010	September	6
Cabo Frio	Forno Harbor	2010	October	11
Cabo Frio	Forno Harbor	2010	**	1
Cabo Frio	Forno Harbor	2011	July	13
Cabo Frio	Forno Harbor	2011	August	4
Cabo Frio	Forno cove	2011	August	3
Cabo Frio	Forno Harbor	2011	September	6
Cabo Frio	Forno Harbor	2011	**	6
Cabo Frio	Forno Harbor	2012	February	1
Ilha Grande Bay	Abraão	2012	August	7
Cabo Frio	Forno Harbor	2012	December	1
Cabo Frio	Forno Harbor	2013	January	1
Cabo Frio	Forno Harbor	2013	February	1
Cabo Frio	Forno Harbor	2013	April	4
Cabo Frio	Forno cove	2013	May	2
Cabo Frio	Forno Harbor	2013	August	1
Cabo Frio	Forno Harbor	2014	February	8
Ilha Grande Bay	Longa Island	2014	June	11
Ilha Grande Bay	Ponta Leste	2015	August	1
Ilha Grande Bay	Ponta Leste	2016	January	1
Ilha Grande Bay	Bananal point	2016	May	1
Ilha Grande Bay	Macacos Island	2016	May	1
Ilha Grande Bay	Piraquara de Fora	2018	April	3
Ilha Grande Bay	Piraquara de Fora	2019	August	5
Cabo Frio	Forno Harbor	**	**	1
Cabo Frio	Forno Harbor	**	**	1

examined were those of Marins et al. (2009) but considering the confirmation of recent specimens in Rio de Janeiro as *C. robusta*, and the widespread occurrence of that species in the Southern Hemisphere, we believe that earlier records in Brazil belongs to this species and not to *C. intestinalis*, and should be corrected (Sato et al., 2012; Brunetti et al., 2015).

Ciona robusta was detected at Ilha Grande Bay during an early phase of our monitoring, which appears to be related to intermediate water temperatures. This is because we have not recorded *C. robusta* at Ilha Grande Bay since 2013, when higher than normal SSTs (sometimes reaching 33°C) have occurred (Skinner, 2018a, b). The temperature tolerance range of *C. robusta* was experimentally tested by Kim et al. (2019) and showed that egg development and larval settlement is greater at temperatures between 16 and 20°C. Caputi et al. (2019) indicated that *C. robusta* density is associated with SST, with low densities from May to September, the time period with the highest SSTs (24.3 to 27.5°C). Rocha et al. (2017) reported that a temperature range of 20 to 25°C is best for egg, larval, and adult development and recruitment. Our data, in a multiyear comparison, indicate predominance of *C. robusta* records during periods with mean SST lower than 25.0°C, corroborating the findings of Caputi et al. (2015).

Previous records of *C. robusta* in Brazil were made from Cabo Frio (RJ) to São Paulo (Millar, 1958; Costa, 1969; Marins et al., 2009; Rocha and Bonnet, 2009; Dias et al., 2013), covering the Warm Temperate Southwestern Atlantic (W TSA) Marine Ecoregion (Spalding et al., 2007; Bouchemousse et al., 2016), influenced by upwelling events, mainly during spring and summer months. SST values in the Cabo Frio region may drop below 20°C (Skinner et al., 2011; Batista et al., 2017; Boltovskoy and Valentin, 2018). Extreme high SST, however, can prevent reproduction or even kill adults growing in shallow waters in the region (Caputi et al., 2015; Rocha et al., 2017), which would contribute to the irregular historical records of the species along the coast of Rio de Janeiro. Those records, and successful introductions of *C. robusta* along the southeastern coast of Brazil, could be related to *El Niño/La Niña* events that increase or reduce SSTs in the Atlantic Ocean. The 2007-2008-2009, 2010-2011-2012 seasons, when some of our records were made, were characterized

by strong to neutral *La Niña* seasons with negative average anomalies (NOAA, 2019). Data from sensors and buoys confirmed lower SSTs during those years. Records of *C. robusta* at Ilha Grande Bay ceased after the strong *El Niño* events of 2014-2015-2016, when SST reached 33°C (at depths down to 10m) during summer months. There is no evidence of the presence of *C. robusta* at Cabo Frio in summer 2016 when SST presumably reached favorable conditions for the species (17 to 22°C).

Shenkar et al. (2018) argues that *C. robusta* is acclimated to both temperate and tropical areas, matching the environmental conditions of the southwestern Brazilian Coast. Bouchemousse et al. (2016) published the global distribution of *C. robusta*, which included the South Brazilian coast (after introductions in the mid-20th century). According to the SST patterns recorded along the coast of Rio de Janeiro State, the most suitable region for the continuous occupation of *C. robusta* is the region near Cabo Frio, where SST usually range from 12.5 to 29.0°C (Skinner et al., 2011; Batista et al., 2017).

Ciona robusta is an invasive species, often fouling substrates in aquaculture farms (Clancey and Hinton, 2003; Daley and Scavia, 2008; Ramsay et al., 2008; Fofonoff et al., 2019; Global Invasive Species Database, 2019; Kim et al., 2019), and their establishment in bivalve farms along the southern Brazilian coast is a threat to that industry. The socking or pearl net methods (Baylon, 1990) usually employed by farmers help to prevent predation events but could favor *C. robusta* recruitment, growth, and spread. Some fouling prevention procedures can also deter *C. robusta* development, but certain suspended structures can serve as refuge for the species.

Rhodossoma turcicum was first recorded at Cabo Frio from 2009 to 2012 (Skinner et al., 2013), at Sepetiba Bay in 2012, and at Ilha Grande Bay from 2012 to 2019. There are other reports about this species on northeast Brazil, at Bahia and Ceará, but those records remain unpublished on thesis and reports. The species was recorded on both artificial and natural substrates at Ilha Grande and Cabo Frio. The first records of *R. turcicum* at Ilha Grande Bay were on eastern sites (2012); records from the most western site refer to 2018 and 2019 samples. Thus, our records and those from Granthom-Costa et al. (2016) indicate that this species is spreading in Rio de Janeiro

State, mainly at Cabo Frio and Ilha Grande Bay. The alternative hypothesis of variable sampling effort is not supported because even at sites where sampling was performed along an extensive period, from early 2012 to 2019, records of this species were scarce.

SST lower than 25°C and up to 31°C do not appear to be limiting for *R. turcicum*, as our surveys both at the warmest (Piraquara de Fora) and at the coldest sites (Arraial do Cabo Bay) recorded the species. Based on the global distribution of *R. turcicum*, its temperature range is from 20 to 30°C, with most records within the 25 - 30°C range (OBIS, 2019). Shenkar and Loya (2009) suggested 22°C as the lower limit for this species. It is a tropical species recorded from the Caribbean, Mediterranean, and the Red Sea, and is tolerant to high temperatures.

Some aspects are relevant to the detection of *C. robusta* and *R. turcicum*. The tunics of both species are reasonably soft as compared to other solitary ascidians recorded on natural or artificial substrates in Rio de Janeiro State, such as *Phallusia nigra* Savigny, 1816, *Styela plicata* (Lesueur, 1823), *Herdmania pallida* (Heller, 1878), and *Microcosmus exasperatus* Heller, 1878 (Marins et al., 2010; Granthom-Costa et al., 2016; Skinner et al., 2016). Those differences in tunic thicknesses and chemical/physical defenses will be determinant for ascidian palatability to predators such as fish (Monniot et al., 1991; Tarallo et al., 2016). The recently described *Pyura beta* Skinner, Rocha & Counts (2019), recognized as introduced into the SW Atlantic, also have a hard tunic that reduces predation. The effects of predator control on *Ciona* spp. (Marins et al., 2009; Dumont et al., 2011) and other ascidian species are frequently cited in the literature (Epelbaum et al., 2009; Freestone et al., 2011).

Another common and important factor potentially affecting species introduction and spreading is the proximity of harbors and marinas. The region from Cabo Frio to São Sebastião in the SW Atlantic experiences intense marine ship traffic, potentially connecting those coastal areas to several tropical and subtropical biogeographical realms throughout the world (Seebens et al., 2013; Brasil, 2019; Sardain et al., 2019). Transoceanic cruise ships, passenger vessels, and many fishing boats and yachts cross those waters daily, and could locally spread introduced species (Zabin et al., 2014; Skinner et al., 2016; Kauano et al., 2017).

It will be necessary to establish continuous monitoring and experimental procedures considering predator exclusion near Cabo Frio to test the hypothesis that the continuous records of *C. robusta* there, in contrast to the discontinuous sightings at Ilha Grande and other regions, can be attributed to the lower SST related to upwelling. It would also represent a good opportunity to test the effects of upwelling strength and other climatic and oceanographic variables such as wind intensity and direction on *C. robusta* establishment.

AUTHOR CONTRIBUTIONS

D.F.B.: Conceptualization; Investigation; Methodology; Formal analysis; Writing - original draft; Writing - review & editing.

L.F.S.: Conceptualization; Investigation; Methodology; Funding acquisition; Project administration; Supervision; Writing - original draft; Writing - review & editing.

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