

Research Article

Depth and density distribution of the invasive vermetid *Eualetes tulipa* (Rousseau in Chenu, 1843) in the southwestern tropical Atlantic

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Citation: Skinner LF, de Albuquerque Tenório A, Penha FL, Soares DC (2019) Depth and density distribution of the invasive vermetid *Eualetes tulipa* (Rousseau in Chenu, 1843) in the southwestern tropical Atlantic. *BioInvasions Records* 8(1): 65–79, <https://doi.org/10.3391/bir.2019.8.1.07>

Received: 25 July 2018

Accepted: 30 September 2018

Published: 6 November 2018

Handling editor: Fred Wells

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Abstract

Vermetid gastropods are geologically useful as sea-level and palaeoclimatic indicators, and are known as bioengineering species that contribute to biological diversity. Vermetids are adapted to a sessile lifestyle but can sometimes be transported to distant regions and become non-indigenous and/or invasive species. *Eualetes tulipa* is considered invasive in Hawaii and Venezuela, and we detected it in 2009, inside Forno Harbour (22°58'22.39"S; 42°00'49.44"W) at Arraial do Cabo, Brazil in the southwestern Atlantic. Site surveys were performed at four depths to determine their occurrence, depth distribution, and size structures, including natural and artificial substrates. A total of 494 individuals of *E. tulipa* were recorded in the three sites investigated, with the highest population densities found on artificial substrates (breakwater) inside the harbour (1.5 ind m⁻²) and at the greatest depth (4.0 ind m⁻²). Their size structures indicated recent introduction, with the species spreading in the Arraial do Cabo region over natural and artificial substrates.

Key words: early detection, Cabo Frio, marine protected areas, harbour activities, invasive gastropods, depth distribution

Introduction

Biological invasions are one of the principal drivers of global biodiversity losses, stimulating countries and international organizations to promote continuous monitoring, surveillance, and eradication programs. The importance of invasive species was included and discussed at the Convention on Biological Diversity (Convention on Biological Diversity 2015), as the Aichi target 9.

Monitoring programs and research for the early detection of potentially invasive marine species is now an important task due to the recognition of the high impacts of invasive species on local biodiversity and national economies (Lehtiniemi et al. 2015; Hoffman et al. 2016; O'Brien et al. 2017; Tait et al. 2018). Early detection of an introduced species is essential to its control, to understanding its adaptation to local conditions and the local biota, its probable source, and its potential for spreading (Ferreira 2003; Batista et al. 2017; Bieler et al. 2017; Mantelatto et al. 2018). That

information, in turn, is important for decisions concerning the removal of the introduced species locally or over wider geographic areas (Creed et al. 2017; Trebitz et al. 2017).

Among numerous invasive marine species recognized worldwide (Carlton and Ruiz 2003), vermetid gastropods are probably among the most poorly known – as they are frequently confused with serpulid polychaetes, their taxonomy is complex (Simone 2001; Carlton and Eldredge 2009; Bieler and Petit 2011), and their removal from marine substrates or from their shells is quite difficult. All of these difficulties arise from the low number of experts in its taxonomy. Vermetids have been reported as invasive species in regions such as Hawaii and Venezuela (Coles et al. 2002; Strathmann and Strathmann 2006; Weinberger et al. 2010).

The Vermetidae are specialized gastropods with tube-shaped shells that are adapted to a sessile life (as opposed to free-living gastropods) and are found from tropical to warm-temperate seas. Vermetids are adapted to marine or slightly brackish waters, although some Red Sea populations can live in a salinity range of from 40 to 46 (Vescogni et al. 2008). There are approximately 144 species described for the group (Bieler and Petit 2011).

Some reef building vermetids (such as those of the genera *Petalconchus* and *Dendropoma*) form very dense aggregate populations comprising thousands of individuals per square meter (Hadfield et al. 1972; Eston et al. 1986; Antonioli et al. 1999; Martin et al. 2007; Breves et al. 2016). These complex structures are important to species diversity (Schiaparelli and Cattaneo-Vietti 1999; Goren and Galil 2001; Lindeman et al. 2009). Vermetid reefs in the Mediterranean Sea are considered warm-temperate homologues of coral reefs (Antonioli et al. 1999; Abdulla et al. 2008) as well as sea-level and temperature indicators (Spotorno-Oliveira et al. 2016). Increases in the sizes of their aggregations in certain regions have been associated with power plant activities (Miloslavich et al. 2009; Teixeira et al. 2012; Breves et al. 2016).

Sixteen species representing four genera were recorded in Brazilian waters: two *Dendropoma* Mörch, 1861, ten *Petalconchus* Lea, 1843, one *Thylaeodus* Mörch, 1860 and three *Thylacodes* Guettard, 1770 (Rios 2009; Spotorno et al. 2012; Breves-Ramos 2012). Brazilian oceanic islands have been comparatively well-studied compared to coastal regions, but the group remains insufficiently known on Brazil (Spotorno and Simone 2013).

The genus *Eualetes* has only two described species: *Eualetes tulipa* (Rousseau in Chenu, 1843) and *Eualetes centiquadrus* (Valenciennes, 1846) (MolluscaBase 2018). *Eualetes centiquadrus* has been encountered on the Pacific coast of Colombia (OBIS 2018), while *E. tulipa* has been identified in Hawaii (as *Vermetus alii* Hadfield and Kay in Hadfield et al. 1972; Coles et al. 2009), the Atlantic and Pacific coasts of Panama (Miloslavich 2018), Venezuela (Miloslavich and Penchaszadeh 1992) and recently in India (Jebakumar et al. 2015) but only as an introduced and invasive species.

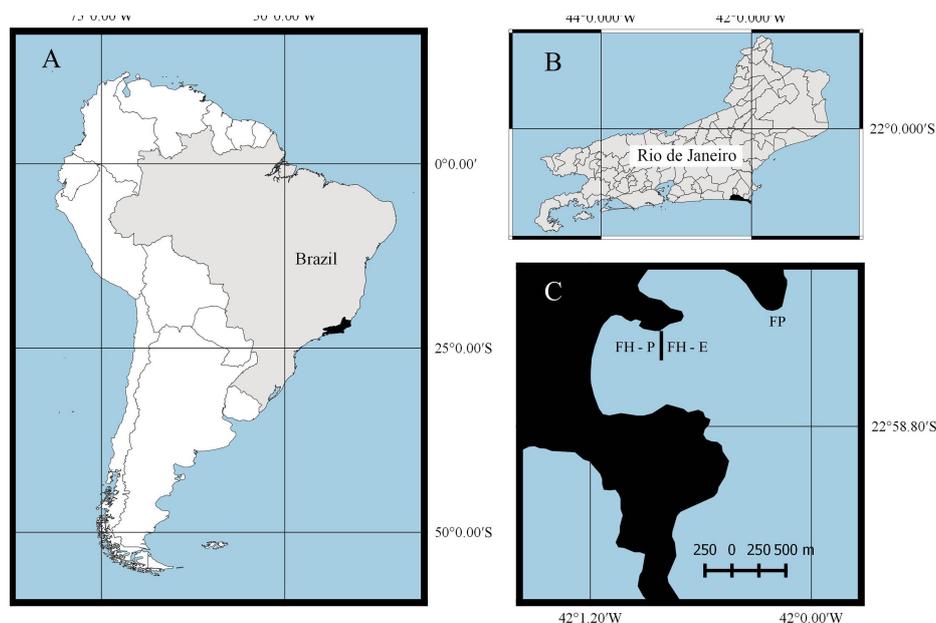


Figure 1. A. Location of study region in relation to South America and south-eastern Brazilian coast. B. Rio de Janeiro state and Cabo Frio region. C. Anjos Bay with the three surveyed sites: Forno Harbour protected (FH - Prot), Forno Harbour exposed (FH - Exp) and Forno Point (FP).

We encountered one unusual shallow population of large vermetids in 2009 inside the Forno Harbour, at Arraial do Cabo, Brazil growing on the breakwater, pillars, and other artificial and natural substrates. It was firstly identified as *Thylacodes decussatus* (Gmelin, 1791) but, in 2013, Dr. Spotorno-Oliveira (*pers. comm.*) identified it as *Eualetes tulipa*. The present report details its distribution when it was originally detected at Arraial do Cabo and basic information to understand its current (Spotorno-Oliveira et al. 2017) and potential future distribution, which is similar to that reported for *Tubastraea* spp. (Batista et al. 2017). We therefore describe here the density, bathymetry, and local distribution of *E. tulipa*, and discuss factors that could be specifically related to its introduction and the large numbers of individuals found near the Forno Harbour.

Materials and methods

Study site

The present study was conducted on the Cabo Frio region, at Arraial do Cabo/Anjos Cove Rio de Janeiro State, Brazil, at three sites (Figure 1): Forno Harbour (FH) breakwater (22°58'22.39"S; 42°00'49.44"W) on its protected and exposed walls and Forno Point (FP) (22°58'06.64"S; 42°00'21.05"W). Forno Harbour is dominated by artificial substrates such as the breakwater, the pillars of the harbour and from the fisherman docks and many wooden boats. Few corals are attached to the breakwater. Forno Point, like almost all other areas is dominated by natural hard substrates such as rocky shore, large boulders and coral species (Ferreira et al. 2001). In this site we also record artificial substrates like one floating restaurant and longlines of a

shell farm. Although this region is influenced by upwelling, the study site is in a tropical region with sea surface temperatures ranging from 19–26 °C (mean of ~ 22.5 °C) (Skinner et al. 2007, 2011; Guimaraens et al. 2008). Authorization to conduct the research at Reserva Extrativista Marinha de Arraial do Cabo (RESEX AC) was given by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO authorization #25024).

Sampling

We encountered specimens of *E. tulipa* growing on different natural and artificial substrates in Forno Harbour, with many reproductive individuals with egg capsules, as well as recently settled individuals (Figure 2); the present survey considered only individuals attached to breakwater boulders or natural substrates. We investigated the bathymetric distribution, abundance, and size structure of *E. tulipa* population using scuba. Protected and exposed sides were investigated on the FH breakwater. We used two horizontal transect lines (30 m) at four depths adjusted to high tide: 2, 4, 6, and 8 m, counting all *E. tulipa* individuals inside 1.2 m² quadrats (1.2 wide and 1.0 long). The number of transects was determined by the length of the breakwater. To determine the population size structure, we divided each transect into two sides (left and right) and then randomly selected fifteen 0.5 × 1.0 m quadrats to measure shell tube apertures (using callipers) for population structure analysis. Because of their low abundance at 2 and 4 m, only individuals observed at the 6 and 8 m depths were measured. The surveys were performed during May 2010, one year approximately after the first observation of the animals on the field. The *E. tulipa* specimens were deposited in the malacology collection at the National Museum at the Federal University of Rio de Janeiro (MNUFRJ voucher #19373, collector LFSkinner, 27/ VII/ 2011).

We also took measurements of sea water temperatures using IButton[®] sensors at a depth of 2 m, programmed to record temperatures at 30 minute intervals. Temperatures were recorded from June to November (except September), totalling 4,105 records.

Data analysis

The data of *E. tulipa* distributions along the transects at different depths were analysed using three-way ANOVA, considering the factors: transect (1 or 2), location (protected or exposed), and depth (2, 4, 6 and 8 m); followed by the Student-Neuman-Keuls (SNK) test (Underwood 1997). The ANOVA and SNK tests were performed using Statistica V. 7.0 software. Only density and aperture size data from the harbour site were considered, as only two individuals were recorded at Forno Point. We also performed chi-square tests to analyse size class distributions (as evaluated by shell aperture measurements).

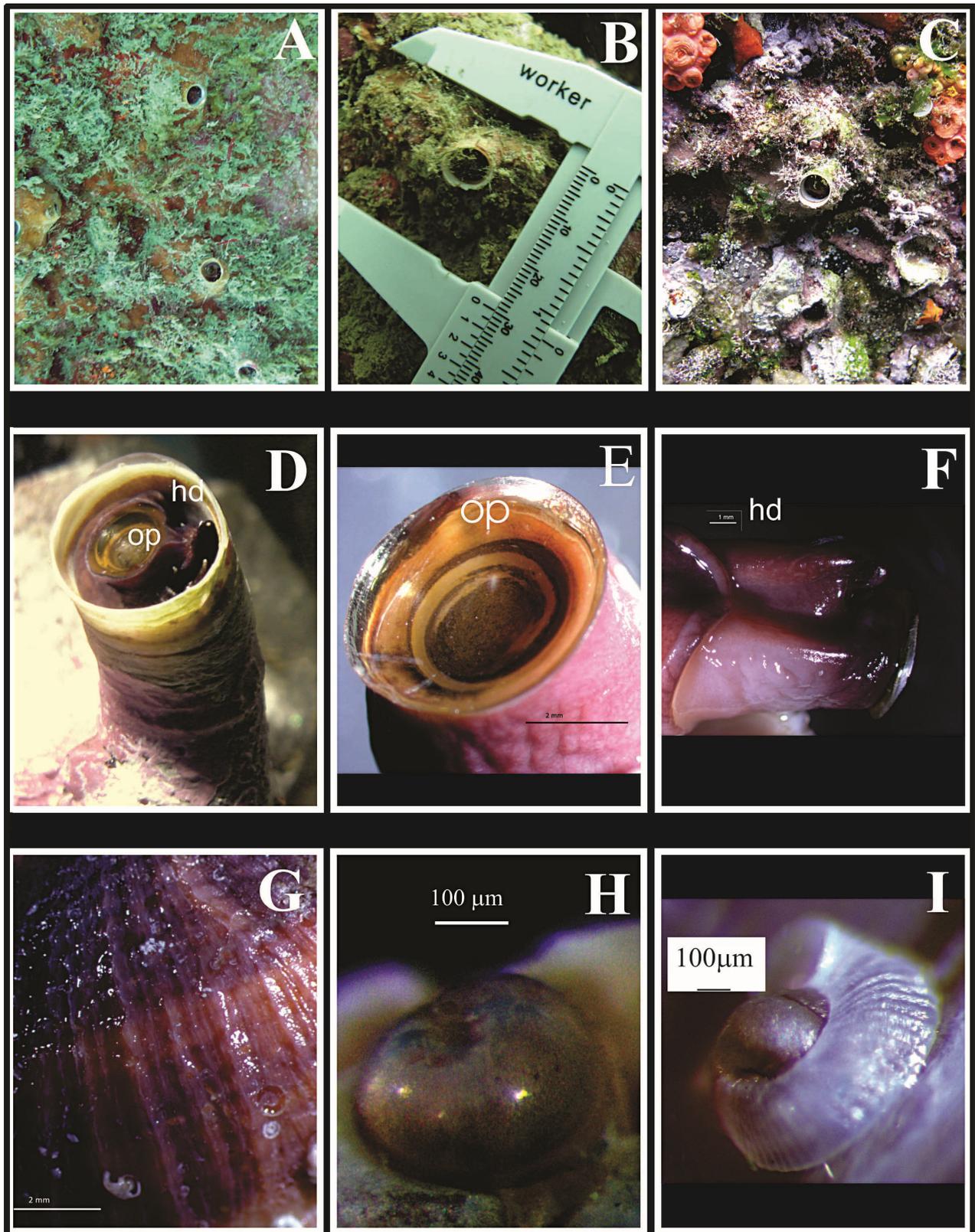


Figure 2. A, B and C: *Eualetes tulipa* individuals attached to breakwater rocky substrata surrounded by many fouling species; D: Detailed view of the opening of the shell, with operculum (op) and head (hd); E: Operculum (op); F: Head (hd) in lateral view; G: Recruits of *E. tulipa* attached to adult shell; H: Protoconch of 1day old recruit of *E. tulipa* on adult shell; I: Detail of the sculptures of the shell on 1day old recruit. Photographs by L.F. Skinner.

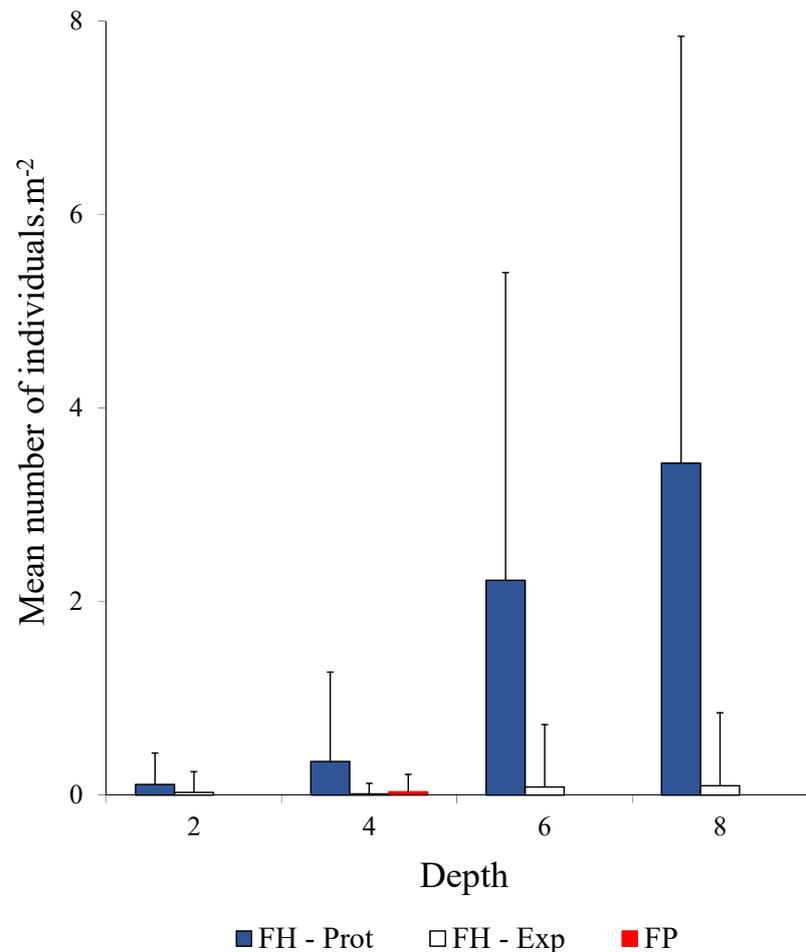


Figure 3. Mean density (ind m⁻²) of *Eualetes tulipa* counted in different depths on Forno Harbour protected (FH - Prot) and exposed (FH - Exp) sides and at Forno Point (FP).

Results

Water temperature

Sea surface temperatures ranged from 19.5 to 25 °C from June through November 2010. The highest water temperatures were recorded in October and November, and the lowest in October and August. The mean water temperature ranged from 21.4 °C (June, July and October) to 21.8 °C (November). 99% of recorded temperatures were in the 20–23 °C range, with only 0.15% lower than 20 °C and 0.85% higher than 23 °C.

Abundance and depth distribution

We recorded a total of 494 *E. tulipa* individuals in the three dive survey sites. The highest number of individuals was recorded inside the harbour area on the protected side (440 ind), with decreasing numbers towards the opposite side of the breakwater (52 ind); only two individuals were recorded at 4 m depth on Forno Point, on natural boulders. The highest densities were recorded along the deeper transects, with a density of 4.0 ind m⁻² in the protected side at 8 m. The highest density at the exposed side was at the 6 m depth (Figure 3). The ANOVA (Table 1) revealed significant

Table 1. Univariate Tests of Significance for the number of individuals at each quadrat related to depth (2, 4, 6 or 8 m), transect (1 or 2), and wave exposure (protected or exposed) for *Eualetes tulipa* at Forno Harbour, Cabo Frio region.

	SS	df	MS	F	p
Intercept	433.200	1	433.2000	76.03	0.000*
Depth	340.533	3	113.5111	19.92	0.000*
Transect	6.533	1	6.5333	1.14	0.285
Wave exposure (Protected or Exposed)	374.533	1	374.5333	65.73	0.000*
Depth × Transect	16.867	3	5.6222	0.98	0.399
Depth × Wave exposure	308.200	3	102.7333	18.03	0.000*
Transect × Wave exposure	1.633	1	1.6333	0.28	0.593
Depth X Transect × Wave exposure	14.833	3	4.9444	0.86	0.458
Error	2643.667	464	5.6976		

Table 2. Minimum, maximum, mean size (in mm) standard deviation (s.d.) of shell aperture and number (N) of *Eualetes tulipa* on protected and exposed sites at Forno Harbour, Cabo Frio.

	FH Inside		FH Outside	
	6 m	8 m	6 m	8 m
Minimum	5	4	3	4
Maximum	17	11	17	12
mean	7.75	7.85	7.75	7.88
s.d.	3.16	1.74	3.16	1.65
N	20	83	20	73

Table 3. Univariate Tests of Significance for size of shell aperture (mm) Sigma-restricted parameterization Effective hypothesis decomposition. Depth: 6 or 8 m; Wave exposure: exposed or protected. * denotes significant difference

	SS	df	MS	F	p
Intercept	8010.234	1	8010.234	1879.76	0.000*
Depth	0.022	1	0.022	0.01	0.942
Wave exposure	0.001	1	0.001	0.00	0.986
Error	839.476	197	4.261		

differences related to depth, wave exposure (protected × exposed), and depth × wave exposure interactions. The SNK post hoc test showed that densities at 2–4 m were significantly different from those at 6–8 m.

The maximum and mean aperture sizes of the shells were similar between breakwater sides and depths (Table 2), with no significant differences between them (Table 3). The smallest (3 mm) and the largest aperture sizes (17 mm) were recorded at 6 m in both sides.

The analyses of the size structures of the shell openings at each side at 6 and 8 m (Figure 4) indicated different patterns. The highest proportions of smaller sized individuals (< 5 mm) were recorded at 6 m for both sides. Intermediate aperture sizes (6 to 8 mm) were observed in higher proportions at 8 m, while the numbers of large individuals (> 9 mm) were similar at both depths. The analysis of the cumulative proportions of the size classes (Table 4) showed that more than 50% of the individuals were included in size classes I and II (up to a shell aperture of 8 mm). There were, however, differences between wave exposure and depths. At 6 m, the cumulative proportion increased up to the 9–11 mm class, while at 8 m, the highest proportion was found in the 6–8 mm class, followed by 9–11 mm. The chi-square test results showed that differences in size class distributions

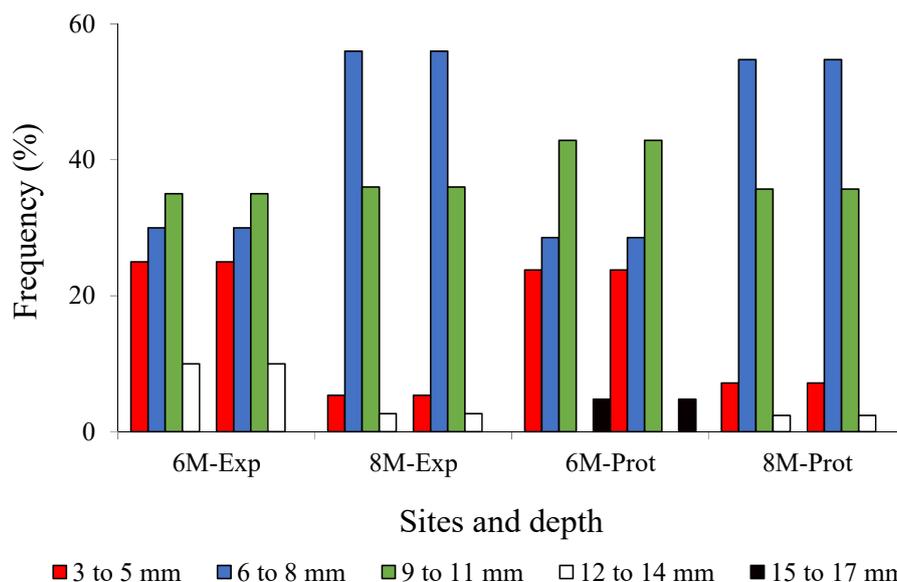


Figure 4. Shell aperture size class distributions for *Eualetes tulipa* from Forno Harbour, at 6 and 8 m depth, Protected (P) or Exposed (E).

Table 4. Cumulated proportion (%) of individuals at different classes of shell aperture (mm) from 6 and 8 m deep. E: exposed side; P: protected side.

Size class (mm)	6m-E	8m-E	6m-P	8m-P
3 to 5	25.0	5.3	23.8	7.1
6 to 8	55.0	61.3	52.4	61.9
9 to 11	90.0	97.3	95.2	97.6
12 to 14	100.0	100.0	95.2	100.0
15 to 17	100.0	100.0	100.0	100.0

could not be attributed to chance ($X^2 = 28.093$; $df = 12$; $P = 0.0054$), as the proportions were non-equally distributed, with higher proportions of low aperture-size individuals.

Discussion

This is the first record of depth and population structure of the recently-introduced vermetid *Eualetes tulipa* on the Brazilian coast. This species was first collected on the Brazilian shoreline at Amarante Harbour in 2005, but its first mention in the literature was made by Spotorno-Oliveira and Skinner (2013) for Arraial do Cabo. It is important to note that the distance between those two locations is approximately 3,000 km, and they are located in different biogeographical realms (Spalding et al. 2007), which would apparently present many barriers to their natural transposition in just four years. Those factors reinforce an introduction hypothesis.

Our surveys indicated that *E. tulipa* individuals were mostly aggregated on the breakwater at Forno Harbour on both its protected (inner) and exposed (outer) sides. Individuals that had settled on the granite rocks of the breakwater close to “encrusting algae”, didemnid ascidians and other fouling species were usually covered by those organisms as recorded by other authors on other regions (Hadfield et al. 1972; Osman 1987; Calvo and Templado 2005).

The surveyed population could be considered to be well-established in the harbour, as we encountered large numbers of both young and adult individuals (as indicated by shell aperture sizes). Additionally, our surveys and lab dissections showed reproductive animals and many settlers on adult shells, that reinforces their fitness to the area.

At Arraial do Cabo, we only recorded *E. tulipa* up to 8 m depth since this is the mean depth around investigated sites, specially Forno Harbour. But in all other records, *E. tulipa* has been record from the intertidal on Hawaii (Hadfield et al. 1972) to subtidal zones, up to 5 m in the Caribbean and India (Miloslavich and Penchaszadeh 1992; Jebakumar et al. 2015) and up to 16 m on a pillar wall at Amarante Harbour (Spotorno-Oliveira et al. 2017).

The highest density (4.0 ind.m⁻²) recorded here (as a first record) is much lower than the density recorded in Hawaii (also as a first record of the species there – 170 ind.m⁻²) (Hadfield et al. 1972), in India (45 to 55 ind.m⁻² up to 1 m in depth) (Jebakumar et al. 2015), or Venezuela (ca. 30 ind.m⁻² in shallow water, to 9.0 ind.m⁻² in deeper [5 m]) (Miloslavich and Penchaszadeh 1992). Those densities indicate that *E. tulipa* can quickly expand its population and form large aggregates. Spotorno-Oliveira et al. (2017) reported densities of up to 24 ind.cm⁻² at Forno Harbour in 2017, which corresponds to 2,400 ind.m⁻² – an approximately 600 fold population density increase inside the harbour breakwater, only five to seven years after our initial survey. If these numbers are correct, it could be enough to consider *E. tulipa* as very invasive in the region. The largest known vermetid population in Rio de Janeiro State is that of *P. varians* at Angra dos Reis, ranging from 100,000 to 300,000 ind.m⁻² (Breves et al. 2016, 2017; Breves and Junqueira 2017) at a nuclear power plant site. Those extremely high densities are apparently related to the high water temperatures at the discharge point, indicating the importance of that environmental factor for some vermetid species. High densities of *E. tulipa* in literature are also associated to regions with high temperatures, usually above 23 °C and up to 30 °C (Miloslavich and Penchaszadeh 1992; Jebakumar et al. 2015; NOAA 2018).

In contrast to the density measurements, individuals growing at Arraial do Cabo (17 mm aperture) were larger than those from India (15 mm) (Jebakumar et al. 2015), Venezuela (12 mm) (Miloslavich and Penchaszadeh 1992), or Hawaii (8.0 mm) (Hadfield et al. 1972), which could reflect their low density. Other differences between the populations may be related to certain aspects of their reproduction and reflect differences between the biogeographical regions where *E. tulipa* has been recorded. Water temperatures at Arraial do Cabo ranged from 19.5 to 25.0 °C in agreement with other citations in the literature for that region (Guimaraens and Coutinho 2000; Skinner et al. 2007), from 23.0 to 29.0 °C in Hawaii (Coles et al. 2009), from 26.4 to 30 °C in Venezuela (Miloslavich and Penchaszadeh 1992), and from 26 to 30 °C in India (Jebakumar et al. 2015), so the

population of *E. tulipa* at Arraial do Cabo is subject to a “colder” environment than other populations. The reproductive differences among populations of *E. tulipa* in those regions, as summarized by Spotorno-Oliveira et al. (2017), indicated that the Arraial do Cabo population produces fewer egg capsules, but with higher numbers of veliger larvae per capsule than seen in Hawaii and the Caribbean. Arraial do Cabo, on the eastern tropical South America coast, experiences upwelling and therefore increased food availability (Valentin 2001; Fernandes et al. 2017). The intensities and indirect influences of upwelling are known to affect the growth and reproduction of other zoological groups in the region, such as Echinodermata, Cirripedia, and Porifera (Carvalho and Ventura 2002; Skinner et al. 2011; Cavalcanti et al. 2013), and most likely also affect *E. tulipa*. Hadfield (1989) likewise reported similar influences of lower water temperature on the number of eggs per capsule in the vermetid *Petalochonchus montereyensis* Dall, 1919 when comparing California and Washington populations.

According to Miloslavich and Penchaszadeh (1992) *E. tulipa* can reproduce all year round, and settlement can occur up to 24 h after hatching. Other vermetid species show similar reproductive patterns, mainly in shaded communities in relatively calm waters (Osman 1987; Calvo and Templado 2005), similar those found inside the harbour. All of the descriptions of *E. tulipa* in the literature indicate higher settlement rates in protected environments (Hadfield et al. 1972; Calvo and Templado 2005).

We noted that young individuals (with the smallest aperture sizes) tend to settle close to large individuals, promoting an aggregated distribution pattern. Some recruits were recorded growing on adult shells, indicating some kind of preference for settlement, and perhaps a signalling effect. That situation was also noted during observations in the laboratory after larval release, with larvae preferentially settling on adult shells rather than on other substrates such as glass or granite blocks (*pers. obs.*).

The distribution of *E. tulipa* in Arraial do Cabo, with high densities both inside and outside the harbour breakwater, but few (or no) individuals at other sites, suggests that its introduction occurred some years before 2009. The harbour itself was probably the introduction site, and the vectors were probably monobuoys or ship hulls. The presence of only two individuals at Forno Point, more distant from the harbour, is consistent with the circulation in Anjos Bay (Savi 2007; Paixão and Sousa Junior 2011), and indicates that dispersal and colonization to other sites in the region is occurring. This suggestion is confirmed by the data of Spotorno-Oliveira et al. (2017) that indicates an increase in density inside harbour and the spread to Anjos Beach rocky reef. This pattern of spread is similar to that recorded for *Tubastraea coccinea* since its detection in 1999 by Ferreira (2003). Only one year after its detection the number and size of colonies

had tripled. Fifteen years later, *T. coccinea* is widely distributed at Anjos Cove following the circulation pattern (Batista et al. 2017).

Many non-indigenous *species* have been detected in that region since the 2000s, such as the cnidarians *Tubastrea* spp. (Ferreira 2003; Ferreira et al. 2006) and *Chromonephthea braziliensis* van Ofwegen, 2005 (Ofwegen 2005), the bivalve *Isognomon bicolor* (C. B. Adams, 1845) (López et al. 2014), ascidians (Rocha and Costa 2005; Skinner et al. 2013), and polychaetes (Costa-Paiva et al. 2007; Skinner et al. 2012). Many of those introductions have been associated with increased shipping traffic to Forno Harbour. The harbour was previously limited mostly to operations related to salt transfer from Rio Grande do Norte State on the north-eastern coast of Brazil to Rio de Janeiro State. Since the late 1990s, however, it has experienced activities related to the oil industry and shipping traffic, and the numbers of oil platforms have increased significantly in the region.

Vermetids have been recorded as introduced and as invasive species in many places (Strathmann and Strathmann 2006; Carlton and Eldredge 2009; Weinberger et al. 2010; Pati et al. 2011; Miloslavich 2018). At Angra dos Reis (Rio de Janeiro State, Brazil), within the natural range of distribution of *P. varians*, the increased density of this species in the early 2000's led it to be considered an invasive species since it promotes changes on the community structure (Moysés et al. 2007; Breves-Ramos et al. 2010; Teixeira et al. 2012). Bieler et al. (2017) described on one shipwreck at Florida Keys, a new species of Vermetidae with high invasive potential that was only detected during dives in 2012 and 2014, indicating a very recent introduction in sites that had been surveyed regularly for more than 20 years. Introduced vermetids can pose additional problems on hard substrates in tropical areas, as they could compete for space and impact important organisms such as corals (Phillips and Shima 2010).

The complex taxonomy of the Vermetidae makes the identification of introduced and invasive species difficult. *Eualetes tulipa*, for example, was previously identified as a new species named *Vermetus alii* Hadfield and Kay, 1972 (Hadfield et al. 1972). It was recognized as non-native and identified as *E. tulipa* by Bieler in Carlton (1999). It was identified as *Vermetus* sp. by Miloslavich and Penchaszadeh (1992), and earlier in this survey as *Thylacodes decussatus* (Gmelin, 1791) and, only after detailed observations were those records recognized as being *E. tulipa*.

Organic matter originating from domestic sewage discharged into the sea off Anjos Beach (Paixão and Sousa Junior 2011) increases the nutrient contents of those waters, which could promote the development of vermetid adults and larvae (Littler and Murray 1975, 1978). The high concentrations of heterotrophic bacteria found at Anjos Bay is correlated with the high levels of dissolved organic matter in its waters (Pereira et al. 2010; Cury et al. 2011). In other cases, sewage discharge can have negative

impacts, such as those recorded with *Dendropoma cristatum* (Biondi, 1859) (Terlizzi et al. 2005) – although that does not seem to be the case in the present study.

The population age structure measured by shell aperture sizes suggests recent recruitment events, due to the presence of large animals (> 12 mm shell aperture) and at least two recruitment pulses (shell openings from 8 to 11 mm) appear to have occurred – a situation that also supports the recent introduction hypothesis.

Based on *E. tulipa* density, distribution, and the substrata colonized, we can conclude that this population was introduced some years before 2009. Data from Spotorno-Oliveira et al. (2017) indicates that this species is spreading in the region and that monitoring and/or the removal of those animals from the region will be crucial to preventing long-term ecological damage.

Acknowledgements

We would like to acknowledge to Dr. Spotorno-Oliveira for *Eualetes tulipa* identification. Thanks to Porto do Forno for access of our team to its installation to perform the survey and dives. This study was funded by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E.26/110.250/2010, E.26/111. 454/2011). Thanks also for all valuable comments of the referees.

The publication of this article is supported by the Open Access Publishing Fund of the International Association for Open Knowledge on Invasive Alien Species (INVASIVESNET).

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