Two new nonindigenous isopods in the Southwestern Atlantic: Simultaneous assessment of population status and shipping transport vector

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**ABSTRACT**

The Southwestern Atlantic is often perceived as remote region, yet it is not immune to biological invasions. Patchy information on historical community composition hinders our ability to identify introductions to coastal ecosystems in this region. Hull fouling is an under-managed shipping vector that likely continues to transport large numbers of marine species worldwide. The port of Mar del Plata is a comparatively well-studied shipping and commercial hub that may serve as an observatory to monitor new introductions to the Argentine coast. Following detection of nonindigenous isopods during preliminary port sampling in 2007–2008, we organized regular port surveys aimed at assessing the level of population establishment and evaluated hull fouling as a potential introduction vector in the Port of Mar del Plata. In 2011–2012, we conducted 12 monthly dive surveys of port isopod communities in combination with three opportunistic surveys (two in-water, one in dry-dock) of hull fouling communities attached to a domestic research vessel during consecutive port calls at its base in this port. Replicate biofouling samples from underwater dock structures and the vessel's hull were collected by scraping invertebrates in 20 × 20 cm quadrats (even surfaces) and 1000 cm\textsuperscript{3} of biofouling organisms (uneven surfaces). Both in port- and hull-fouling communities, we discovered the presence of the nonindigenous isopods *Dynamene edwardsi* and *Paracerceis sculpta*. This report constitutes the first detection of these two global marine invaders in American and Argentine waters, respectively. They likely represent relatively recent introductions to this corner of the world’s oceans, yet our data indicate that both species are currently well established in Mar del Plata. These results demonstrate (for the first time in the case of *D. edwardsi*) the potential for hull fouling to disperse both species, and raise a warning on their potential expansion to other Southwestern Atlantic ports and Antarctica in a near future. Research on the marine communities of the Southwestern Atlantic is pressingly needed to establish pre-invasion communities and detect new introductions. Simultaneous surveillance of ports (invasion hubs) and vessels (vectors) can effectively detect invaders and inform prevention efforts in this region.

1. Introduction

New aquatic and marine introductions are discovered every year, and ports around the world operate as hubs for the reception and dispersal of NIS globally (Carlton and Geller, 1993; Ruiz and Carlton, 2003; Schwindt et al., 2014). Notwithstanding these risks, prevention and management efforts are impeded by the lack of basic information on the biology of newly introduced populations and introduction vectors. Shipping vectors are widely recognized as prime pathways for the spread of marine nonindigenous species (NIS) (Ruiz and Carlton, 2003; Minchin et al., 2009). International management guidelines have largely mitigated the intensity of ballast water as an introduction vector,

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but other introduction pathways remain intense. This is the case of hull fouling, which, despite a recent international focus on this vector (e.g., International GloFouling project recently announced by the International Maritime Organization; visit www.imo.org) remains far from attaining similar management standards (Sylvester et al., 2011; Cuesta et al., 2016). Thus, port monitoring combined with vector assessment, particularly hull fouling, remains central to NIS early detection and prevention programs in marine ecosystems (Carlton and Geller, 1993; Bishop and Hutchings, 2011).

The Southwestern Atlantic remains as a largely understudied marine region. It has for some time been considered relatively safe from biological invasions due to its remoteness and comparative low shipping activity, but mounting information reveals a growing list of NIS for this area (Orensanz et al., 2002; Schwindt et al., 2014). This list is likely underestimated and biased towards visible organisms such as reef forming polychaetes (Ficopomatus enigmaticus (Fauvel, 1923)), shrimps (Palaeamon macrodactylus Rathbun, 1902), barnacles (Amphibalanus amphitrite (Darwin, 1854)), and macro-algae (Undaria pinnatifida (Harvey) Suringar, 1873) (Orensanz et al., 2002). Further research is needed to get a more realistic picture of the NIS complement in this corner of the world, particularly regarding inconspicuous organisms. In Argentina, Mar del Plata port is one of the most studied marine environments (Orensanz et al., 2002). Its important national and international marine traffic has favored the entrance of several invasive species, mainly amphipods and isopods, to this port (Roux and Bastida, 1990; Rumbold et al., 2015a, 2017). While these organisms likely introduce substantial changes to native ecosystems, detailed information on the biology, density, introduction vectors and even the identity of many of these invaders is missing.

Isopods are peracarid crustaceans that can be present in almost all types of terrestrial and aquatic habitats from the tropics to the poles (e.g. deep-sea, tidal flats, estuaries and fresh-water ecosystems) (Brandt, 2012; Broly et al., 2013). In marine ecosystems, these organisms are one of the most abundant groups and represent an important trophic link between primary producers and higher trophic levels (Wernberg et al., 2013). Many isopods possess typical traits of NIS, employing ships as vectors and colonizing ports and nearby marine environments worldwide (Carlton and Iverson, 1981; Hewitt and Campbell, 2001). However, some aspects of their population dynamics, reproductive traits and ecology, are still poorly known. Dynamene edwardsi (Lucas, 1849) and Paracerceis sculpta (Holmes, 1904) have been recognized as NIS in several marine environments (Vieira et al., 2016; Marchini et al., 2017). While several authors pointed as hull fouling as a potential transportation vector for both species, their factual presence in hull fouling communities has scarcely been documented (e.g., Marchini et al., 2017).

The objectives of the present study are: (1) to report the presence of two NIS, D. edwardsi and P. sculpta in Mar del Plata port; (2) to analyze their population biology in order to determine whether populations of these species are well established or transient; and (3) to study the presence of both species in vessel hull-fouling communities to assess their potential as a vector for introduction and secondary spread.

2. Material and methods

2.1. Study area

The Port of Mar del Plata (38° 20′S - 57° 32′W) is a semi-enclosed area (1.4 km²), limited by two artificial breakwaters (north and south) made of orthoquartzite and concrete blocks delimiting an approximately 300 m wide inlet, which serves a large commercial fleet (Fig. 1). Its mean water depth is about 5 m (ranging between 3 and 10 m), and the bottom is composed mainly of fine sand and silt. Water temperatures vary between 9.3 and 20.2 °C. Industrial, sewage effluents, and an intense fishing activity have led into the formation of a polluted area characterized by high levels of organic matter, hydrocarbons, copper and butyltin residues, turbidity, low pH, dissolved oxygen, and salinity (Laitano et al., 2015). Despite these conditions, artificial structures (e.g. ship hulls, breakwaters, wooden docks and marinas) host a rich fouling community composed by ascidians, algae, and polychaete tubes that provide refuge for fishes, flatworms, mollusks, nematodes and crustaceans (Rivero et al., 2005; Albano et al., 2013; Rumbold et al., 2017).

2.2. Field sampling and laboratory procedures

In order to assess the presence of exotic isopods, we conducted preliminary surveys of the benthic invertebrate communities of the Port of Mar del Plata in November 29th, December 29th 2007, and February 19th 2008. On each sampling date, divers scraped into containers organisms in 20 × 20 cm quadrats (3 replicates) placed over the concrete walls of the port’s north breakwater. Following detection of NIS isopods in those samples, we organized a schedule of periodic sampling of benthic communities in this port in order to evaluate the degree to which those organisms were established in Mar del Plata. Between March 2011 and March 2012, monthly port samples were taken from wooden docks of yacht club “Club Náutico Mar del Plata”. Uneven underwater surfaces precluded the use of quadrats at the yacht club, and hence samples were collected scraping into a container 1000 cm³ of biofouling from wooden dock structures. Each date, 5 replicate samples were obtained from a depth < 1 m. To assess potential vectors for the introduction and secondary spread of these organisms, we combined port surveys with surveys of vessel hull-fouling invertebrates in the same port. R/V Puerto Deseado is a medium-size (length 76 m) oceanographic vessel that regularly operates between Southern Brazil and Antarctica, using the Navy base of Mar del Plata as base port (http://www.conicet.gov.ar/wp-content/uploads/2014/05/Folleto-BOPD-CONICET.pdf). On June 26th and September 9th 2011, we conducted underwater surveys of R/V Puerto Deseado while docked in the Navy base of Mar del Plata; and on September 14th 2011 we conducted a third hull survey at the SPI dry-dock facility (Fig. 1). Hull fouling samples were collected by divers scraping into a bag all organisms in 20 × 20 cm quadrats. To preclude the dispersion of material in the water, soft organisms scraped from R/V Puerto Deseado were sucked...
into a large syringe (620 ml) by the action of a plunger. Dry-dock samples were obtained from nearby hull surfaces scraping 20 × 20 cm quadrats within a few hours after the vessel had been taken out of the water to undergo maintenance. Each sampling date, up to two hull fouling samples were taken from fouled underwater at the edge of the keel from the stern to the bulbous bow, leading, trailing edges, lateral sides of the rudder, rope guards and propellers to obtain isopods; other location inspected included the bilge keel, bow thrusters, sea-chests, bulbous bow, dry-docking support strips (DDSS, without antifouling paint), and main hull shell, although no isopods where retrieved from them and will not be discussed any further (see Results; Fig. 2). In dry-dock, random video transects were recorded from each location sampled to estimate percent cover and average densities (see below).

In the laboratory, samples were washed through a 65 μm (hull fouling) or 0.35 μm (port samples) mesh sieve and drained for 15 min. Yacht-club samples were weighed on a digital balance (± 0.01 g accuracy) to calculate densities as individuals 1000 g−1. Since hull fouling samples were not taken randomly but from spots densely covered by organisms, average isopod densities were estimated using video recorded data on overall fouling coverage at each location. The percent cover was estimated for each underwater location of the hull as the mean of 2–20 random still shots from the video recordings. Percent cover in each still shot was estimated superimposing 25–50 regularly distributed points on the photograph and counting the percentage of points where biofouling was present. Abundances in the 20 × 20 cm quadrats were multiplied by the average percent cover in each location and scaled to a 100 × 100 cm quadrat to obtain average densities (ind. m−2) for each location. These values were multiplied by the surface area of each location, obtained from vessel maps, and combined across locations to estimate total abundances for the whole ship.

Isopods in port and hull-fouling samples were sorted using a stereoscopic microscope. All Dynamene edwardsi and Paracerceis sculpta individuals were identified using taxonomic guides (Holdich, 1968; Loyola e Silva et al., 1999; Ariyama and Otani, 2004; Vieria et al., 2016). Voucher specimens were deposited at the Invertebrates General Collection of Centro Nacional Patagónico (CENPAT-CONICET), Argentina (D. edwardsi catalog number CNP-INV 1322; P. sculpta catalog number: CNP-INV 1327). Specimens of each species were dissected and photographed (dorsal and lateral view) using trinocular stereo and scanning electron microscopy (SEM; JSM-6460 at 15 Kv accelerating voltage). Organisms were classified into sex and age groups: juveniles, males, ovigerous females (i.e., with eggs in the marsupium) and non-ovigerous females (i.e. without marsupium). Individuals were measured from the frontal edge of the cephalon to the posterior end of the pleotelson (total length) under a stereoscopic microscope with a micrometric ocular (± 0.01 mm accuracy).

3. Results

3.1. Density of D. edwardsi and P. sculpta in wooden docks of Mar del Plata Yacht Club

A total of 607 individuals of Dynamene edwardsi (Fig. 3) were recorded from March 2011 to March 2012 (males: 181 males, females: 228 females and juveniles: 198). Mean density of D. edwardsi was 34.26 ± 47.83 ind. 1000 g−1. The highest density was recorded in April 2011 (155 ind. 1000 g−1) while no specimens were found in October (Fig. 4). Overall, all sexes and stages registered their highest

Fig. 2. Lateral view of R/V Puerto Deseado showing the sampled areas from underwater locations (dot line).

Fig. 3. Dynamene edwardsi (Lucas 1849). Adult male: dorsal view (A) and lateral view (B); adult female: dorsal view (C) and lateral view (D). Scale bars: 1 mm.
port of Mar del Plata during the study. Ovigerous females were recorded only from March to June 2011 at
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1 (460 ind. 1000 g
-

1; Fig. 4). Male, female and juvenile abundances ranged 0–5 ind. 1000 g
-

1 (Fig. 5B). Only 1 ovigerous female was recorded in May 2011 representing 18% of all females sampled. The mean size of males was 6.82 ± 1.21 mm (range 4.63–8.38 mm) and were larger than females (5.21 ± 1.20 mm, range 3.75–8.13 mm). Juveniles showed a mean size of 2.79 ± 0.50 mm (range 2.00–3.63 mm).

3.2. Density and location of isopods in hull fouling assemblages of the R/V Puerto Deseado

Nine (out of 40) samples collected from hull underwater locations of R/V Puerto Deseado contained a total of 37 isopods, which translates into densities of up to 134 ind. m
-

2 in some locations and ca. 4000 individuals potentially transported on the whole ship (Tables 1 and 2). Twenty-eight of these isopods were sampled while the ship was in the water; while 9 were retrieved in dry-dock. Isopods were most often found along the rudder (5 samples, 12 individuals), although the rope guard hosted the largest abundances (21 individuals in one sample); other locations where isopods were found were the propeller blades, and the rear and front (bulbous bow) edges of the keel (Table 1). The greatest concentration of individuals was recorded in the rope guard (up to 134 ind m
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2), trailing edge of the rudder (125 ind. m
-

2 and keel (24 ind. m
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2). The leading edge, sides of the rudder, and propeller hosted comparatively lower densities (< 2 ind. m
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2) (Table 2). Rudder and keel surfaces hosted the largest abundances of non-established NIS isopods (Tables 1 and 2). All isopods found in hull fouling communities of R/V Puerto Deseado belonged to NIS groups (Sphaeroma serratum (Fabricius 1787)), some of which had not been previously reported in Argentine waters (Paracerceis sculp
ta) or in America (Dynamene edwardsi) prior to the present work; or species for which clear evidence of being native or introduced to Southwestern Atlantic coast is wanting (i.e. Idotea balitica (Pallas 1772)). In contrast with port samples, hull samples contained noticeably more male than female P. sculp
ta, with the presence of one ovigerous specimen on the keel; while the only D. edwardsi individual retrieved was a male (Table 1).

4. Discussion

The present paper constitutes the first record of Paracerceis sculp
ta in the Argentine coast and Dynamene edwardsi throughout the Americas (North and South America). The information available suggests that both species are recent additions to the benthic fauna of the Southwestern Atlantic. The benthic ecosystems of Mar del Plata, where the two species have been found, have been extensively studied and their invertebrate community is well known compared to other areas (Orensanz et al., 2002; Rumbold et al., 2017). Particularly, isopod populations have been repeatedly surveyed since the detection of the nonindigenous isopod Sphaeroma serratum in this port in 1964 (Bastida, 1968; Roux and Bastida, 1990). Given that D. edwardsi and P. sculp
ta inhabit the same type of habitat as S. serratum, they should be detectable by the same sampling methods. The invariable absence of D. edwardsi and P. sculp
ta from samples that captured S. serratum in surveys prior to the present study (e.g., campaigns in 1986–1987 and 2001; Kittlein, 1991; Rivero et al., 2005) strongly suggests that the former were not present in Mar del Plata at least until 2001.

The presence of adult individuals and ovigerous females in the berth throughout the year and in most of our hull fouling samples clearly

had a mean length of 5.43 ± 0.82 mm (range 3.63–8.38 mm) and were larger than females, which reached in average 4.48 ± 0.77 mm (range 3.63–8.13 mm). The mean size of juveniles was 2.69 ± 0.58 mm (range 1.25–3.50 mm).

A total of 53 specimens of Paracerceis sculp
ta (Fig. 6) were recorded in March–July 2011 and December 2011 through March 2012 (males: 12, females: 25 and juveniles: 16); with no specimens found during the rest of the sampling period. Mean density was 3.01 ± 4.52 ind. 1000 g
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1 (ranging 0–8 ind. 1000 g
-

1; Fig. 4). Male, female and juvenile abundances ranged 0–5 ind. 1000 g
-

1 (Fig. 5B).

Fig. 4. Monthly variation of harbour water temperature and total density (mean ± standard deviation) of Dynamene edwardsi and Paracerceis sculp
ta in the Port of Mar del Plata during the study.

Fig. 5. Monthly variation of male, female, and juvenile density (mean ± standard deviation) of (A) Dynamene edwardsi and (B) Paracerceis sculp
ta in the Port of Mar del Plata during the study.

densities in April 2011 (c. 40–60 ind. 1000 g
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1), showing values lower than 40 ind. 1000 g
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1 during the rest of the study period (Fig. 5A). Ovigerous females were recorded only from March to June 2011 at abundances < 3 ind. 1000 g
-

1 (4–11% respect to total females). Males
suggests that the two species are well established in the Port of Mar del Plata. Abundances of both species in the port follow a seasonal pattern, characterized by peaks in the summer and early autumn, and low values throughout the cold season. This pattern most probably reflects the effect of the temperature, which is the primary factor modulating the populations of other well established NIS and cryptogenic species in Mar del Plata port, such as the amphipods *Monocorophium acherusium* (Costa, 1853) and *Erichtonius brasiliensis* (Dana, 1853), the isopod *S. serratum* and the tanaidacean *Tanais dulongii* (Audouin, 1826) (Kittlein, 1991; Rumbold et al., 2015b, 2016, 2017). Given that hull surveys were conducted during the winter, higher abundances of *D. edwardsi* and *P. sculpta* than observed here should be expected during warm months.

The possibility that *D. edwardsi* and *P. sculpta* can be dispersed within hull fouling communities has been suggested many times (e.g. Hewitt and Campbell, 2001; Picker and Griffiths, 2011; Marchini et al., 2017), and the association of *P. sculpta* with hull fouling communities has been informed in government reports (Montelli and Lewis, 2008). Yet, this is the first time these associations have been reported in the widely-available scientific literature, and in the case of *D. edwardsi*, the first time the species has been effectively observed attached to a vessel’s hull. The presence of a *P. sculpta* ovigerous female on the keel underscores the high introduction potential of this vector, since at least in theory, only one individual would suffice to seed a NIS population. It is unclear, however, whether R/V Puerto Deseado has been the actual introduction vector in Mar del Plata. In fact, the preceding southern-most record for *P. sculpta* in South America (Bombinhas, Santa Catarina, Brazil; Loyola e Silva et al., 1999) is outside of R/V Puerto Deseado’s habitual routes; while *D. edwardsi* has altogether never been reported in the Western Atlantic before, which technically puts the species out of the reach of a domestic vessel (Vieira et al., 2016). Consequently, we postulate that port populations detected here likely seeded the populations fouled to the hull of R/V Puerto Deseado - and not the other way round; although this and other similar medium-size vessels could clearly contribute to the future dispersal of the two species regionally, including sensitive areas such as Antarctica.

Immediately before our hull surveys, R/V Puerto Deseado conducted navigations across open waters in southern Brazil and over the continental shelf nearby Mar del Plata. Reappearance of *P. sculpta* in the hull across sampling events suggests that this isopod can cope the physicochemical stress (hydrodynamic sheer forces, changes in temperature and salinity) associated with regional navigation. We found *P. sculpta* in association with the rudder (leading, trailing edges, and lateral sides) and the trailing edge of the rear keel, well-known niche locations where biofouling organisms commonly accumulate (Sylvester and MacIsaac, 2010). Dense aggregations of encrusted organisms in niche locations can alleviate environmental stress (e.g., through creation of a tridimensional habitat, and protection against direct exposure to antifouling paint) and facilitate non-sessile organisms including isopods (Floerl et al., 2014; Mendez et al., 2015). While repeated re-colonization from the port is also a possible alternative explanation for the persistence of these organisms across sampling periods, a conservative standpoint from the prevention perspective has to consider the possibility that these organisms are able to endure navigation-borne stress in niche locations.

*P. sculpta* has three types of male morphs, which are genetically determined (Shuster, 1989; Shuster and Wade, 1991): α-males, the largest and most ornamented; β-males, smaller than α-males, not ornamented and with short and flattened uropods; and γ-males, smaller than α- and β-males and females, also without ornamentation, and with short uropods. Type α males are the most long-lived of all morphs, surviving on average 34% longer than β- and almost 80% longer than γ-males in wild populations in California (calculated from data on Shuster and Wade, 1991). Interestingly, only the type α was found in our samples, in accordance with observations from invasive populations in

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**Table 1**

Isopod abundances (ind. sample⁻¹) sampled from hull fouling assemblages on wetted surfaces of R/V Puerto Deseado while the ship was moored in the water and in dry-dock in the Port of Mar del Plata between June and September 2011. Hull location and specimen codes: Keel.Bulb = edge of the keel at the bulbous bow, Keel.Trail = trailing edge of the keel at the stern, Prop = propeller blades, Rope = rope guard; Rud = lateral sides of the rudder; Rud.Lead = leading edge of the rudder; Rud.Trail = trailing edge of the rudder; M = male; F = female (* = ovigerous); J = juvenile; unspecified (*S. serratum*) = unknown.

<table>
<thead>
<tr>
<th>Species</th>
<th>June</th>
<th>September</th>
<th>September (dry-dock)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. edwardsi</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. sculpta</em></td>
<td>3(2 M, 1 J)</td>
<td>2(2 M)</td>
<td>0</td>
</tr>
<tr>
<td><em>S. serratum</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>I. balbica</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

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Fig. 6. *Paracerceis sculpta* (Holmes 1904). Adult male: dorsal view (A) and lateral view (B); adult ovigerous female: dorsal view (C) and lateral view (D). Scale bars: 1 mm.
Europe, Australia, Asia, North, and South America (Brazil) (Hewitt and Campbell, 2001; Ariyama and Otani, 2004; Munguía and Shuster, 2013; Marchini et al., 2017). Individuals of D. edwardsi found in Mar del Plata also belonged to a single, long-setae morph. This too suggests the underlying functioning of transport bottlenecks, although we cannot find a probable mechanism for selection in this case.

More and more new species are found in coastal ecosystems across the globe, whose expansion is assisted by a network of ports, different types of vessels, and shipping patterns (Floerl et al., 2009). While domestic vessels are not involved in introductions between continents, they likely have a very effective role in secondary regional dispersion of NIS. Sailing patterns characterized by extended port periods, low sailing speeds, absence of long oceanic transits, and longer periods between hull cleaning operations as compared with commercial vessels, increases their chances to gather hull fouling (Sylvester and MacIsaac, 2010; Sylvester et al., 2011). Despite this high risk, newly introduced NIS may remain undetected due to a dearth of studies promoted by the challenges associated to hull-fouling sampling. Our results confirm the role of the port of Mar del Plata as a gateway for marine invasions into South America. The combination of port and vessel sampling has proven effective for the detection of new introductions, and is therefore recommended in this and other ports. Sampling of other Southwestern Atlantic ports is needed to corroborate whether the two sphaeromatid isopods detected here are restricted to Mar del Plata or have spread elsewhere.

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