



## Early detection of marine invasive species following the deployment of an artificial reef: Integrating tools to assist the decision-making process

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### ABSTRACT

Early detection and rapid response plans are a set of principles to reduce the establishment, spread and impact of invasive species and it is a critical step in management in marine ecosystems. Two potentially invasive ascidians attached to the hull of a recently sunk fishing vessel were early detected in Patagonia. With the aim of assisting in the management decision-making process during the early steps of a rapid response, we conducted several analyses through different approaches. First, we identified the species through classic taxonomical and genetic analyses. Then, we evaluated the regional and international shipping connectivity to study potential donor regions and finally, we used species distribution models (SDMs) to predict the potential distribution of these species. The potentially invasive ascidians were identified as *Styela clava* and *Styela plicata*, and this is the first record for both species in the Nuevo gulf, Patagonia Argentina. Both species have a widespread distribution around the world with strong ecological and economic impacts documented. Shipping traffic analysis suggested that *S. plicata* could have arrived by secondary spread from regional ports, while the arrival of *S. clava* was likely to be associated with international shipping traffic. Furthermore, the SDM predicted that *S. clava* has suitable coastal areas along the entire Southwestern Atlantic shoreline, where it is currently absent. On the contrary, the SDM predicted that further southward spread of *S. plicata* is unlikely, being limited by the minimum annual temperature. We discussed the different approaches, tools, and expertise integrated in this work in the light of the decision-making process for the early detection of marine invasive species in the Southwestern Atlantic. Moreover, we call attention to the increased creation of artificial habitats through the intentional sinking of ships and the potential consequences of these actions in the conservation of marine ecosystems.

### 1. Introduction

The historical accumulation of marine introductions mediated by shipping traffic in coastal areas and estuaries, together with anthropogenic stressors such as coastal urbanization and marine and land pollution has turned these ecosystems into some of the most altered in the world (Carlton, 2010; Carlton and Ruiz, 2015). Despite the international, regional and national regulations, the number of reported introduced species is constantly increasing (Mead et al., 2011; Chainho et al., 2015; Schwindt et al., 2020; Teixeira and Creed, 2020), partially

due to the intensification of international shipping traffic which is expected to increase between 240 and 1209% by 2050 (Sardain et al., 2019). A conceptual framework used on vector risk analysis has highlighted that, because a high propagule pressure (i.e. the number of individuals introduced and/or the number of introduction events of any species (Lockwood et al., 2005)), routes with a higher shipping traffic are more likely to result in new introductions events than those with lower connectivity (Kaluza et al., 2010; Seebens et al., 2013). Some prevention strategies focused on reducing ship-mediated propagule pressure of invasive species have been implemented. The Ballast Water

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Management (BWM) Convention (IMO, 2004; IMO, 2017) is the newest regulation at the international level directed to establish general standard management mechanisms to minimize the introduction of invasive species by ships. However, this regulation is insufficient to intercept all marine invasive organisms, partly because national and regional governments are still far behind their capacities to cope with international management standards and because hull biofouling (i.e. organism attached to the exterior of hull, anchors or sea chests) remains largely unregulated (Zabin et al., 2018; Georgiades et al., 2020). It is widely documented in the literature that once an introduced species becomes invasive, the implementation of different management strategies is highly costly, especially in marine ecosystems, given the nature of this environment (Culver and Kuris, 2000; Wotton et al., 2004). Thus, when a potential invasive species is early detected (i.e. observing and documenting an invasive species before its establishment), a rapid response is more cost effective and likely to succeed than other actions that can be taken after the species becomes established and widely dispersed (Simberloff et al., 2013; Reaser et al., 2020).

Early detection and rapid response (EDRR) plans are defined as a set of principles to reduce the establishment, spread and impact of invasive species. Among the first steps, EDRR plans require the correct taxonomic identification of the species, allowing the access to key information about the biology and ecology of the organism for further management decisions (Bortolus, 2008; Campbell et al., 2018; Lyal and Miller, 2020). Taxonomy provides the basic understanding of the components of biodiversity, and it is a key tool for effective management of invasive species which can only be implemented when these are correctly identified (Carlton, 2009; Schwindt and Bortolus, 2017; Lyal and Miller, 2020). Hence, the loss of taxonomic expertise in the highly diverse and poorly known marine taxa implies a reduction in the capacity to evaluate the response of decision-makers to the introduction of invasive species (Campbell et al., 2018). Moreover, the risk of misidentifying species, may lead to a cascade of errors in the management decision process and a waste of resources (Bortolus, 2008; Bush et al., 2021). A subsequent informative tool is to integrate the available global data through spatially explicit models to predict potential scenarios of spreading and colonization (Morissette et al., 2020; Reaser et al., 2020). Species distribution models (SDMs) are useful and cost-effective tools that provide an integrated analysis to predict potential areas where a given species can spread once it is introduced. Thus, these analyses help to prioritize locations for surveillance and monitoring (Peterson, 2003; McGeoch et al., 2016; Goldsmit et al., 2018).

As part of the agreement with the Convention on Biological Diversity, Argentina is committed to achieve the Aichi Target 9 related to invasive exotic species. The project “Strengthening of Governance for the Protection of Biodiversity through the Formulation and Implementation of the National Strategy on Invasive Alien Species (GCP/ARG/023/GFF)” is coordinated by the Ministry of Environment and Sustainable Development of Argentina since 2016 and aims to promote public policies to minimize the impact of biological invasions on biodiversity, culture, economy and health (<https://www.argentina.gob.ar/ambiente/biodiversidad/especiesinvasoras>). The National Strategy on Invasive Alien Species (NSIAS) has been formulated from a multiple and participatory approach, building capacities of individuals and institutions, education and communication programs for its implementation. Included in the National Strategy, an EDRR plan to be carried out in marine ports and surrounding areas was discussed and agreed among stakeholders in 2018. Although this plan is still very recent, the first field experience (Schwindt et al., 2019), highlighted the importance of monitoring specific targets and, improving communication among working groups. In line with the adaptive management and precautionary principles, the main focus of this plan is to prevent and minimize the spread and impact of marine invasive species. The newest review of marine and estuarine introduced species showed that their number have increased 4.5 times since 2002 (Schwindt et al., 2020) and while an important number of records within this publication were gathered from

the literature, many of the new reports came from the permanent research efforts in port areas, marinas and hull biofouling vessels (Bigatti et al., 2014; Schwindt et al., 2014; Cianis et al., 2018; Rumbold et al., 2018, 2020; Albano and Obenat, 2019; Giachetti et al., 2019; Castro et al., 2020; Meloni et al., 2020).

In January 2019 during the monitoring for the field validation of the EDRR within the NSIAS framework, individuals of two different solitary ascidians were found attached on the hull of a fishing vessel recently sunk for recreational activities in northern Patagonia, Argentina (42°S). Preliminary field identifications coincided with two species of the genus *Styela* already introduced in two ports of Argentina; *S. clava* recorded for the first time at San Antonio Bay, Patagonia (40°S) in 2013 (Pereyra et al., 2015) and *S. plicata* recorded in Mar del Plata port (38°S) in 2007 (Albano and Obenat, 2019). As other solitary ascidians, because of their high reproduction and growth rates, both species are successful colonizing artificial structures being a threat to aquaculture operation worldwide. They form heavy aggregates on aquaculture gear, increasing the cost of this activity, and compete with bivalves for food and oxygen, reducing their tissue weight (Bannister et al., 2019). They might also dominate benthic communities reducing their richness and diversity (Aldred and Clare, 2014; Zhan et al., 2015). The aim of this work was to piece together several analyses to assist the decision-making process about the better management actions to be taken during the early steps of a rapid response. In particular we focused on: 1) the identification of the species through classic taxonomy and genetic analysis, 2) the evaluation of the regional and international shipping connectivity to study potential donor regions and 3) the prediction of the potential distribution of these ascidian species using SDMs. We discussed the implications of the results in the light of the importance of early detection and rapid response plans for the management of marine invasive species and for the conservation of marine ecosystems.

## 2. Materials and methods

### 2.1. Study site

All the specimens were collected from a ship that was recently and deliberately sunk near Puerto Madryn port in the Nuevo gulf (Patagonia, Argentina, Fig. 1). The area has an annual mean sea surface temperature of 13.3/14.3 °C, varying between 9 °C in winter (July–August) and 21 °C in summer (January–March), and a salinity ranging between 33.5 and 33.9. The shipwreck *Hu Shun Yu 809* was a squid fishing ship captured by the Prefectura Naval Argentina (National Maritime Authority) in April 2015 while found illegally fishing in the Argentinian Exclusive Economic Zone. Soon after the ship was captured and towed to the port, the hull was inspected for the presence of potentially invasive species, but only pelagic gooseneck barnacles were found (ES pers. obs.). Finally, the vessel was intentionally sunk to create an artificial underwater park for scuba diving in December 2017.

### 2.2. Record details

In January 2019, one individual of a potential species of *Styela* was found by scientific divers at a depth of 24 m (at low tide) attached to the bow bulbous of the shipwreck *Hu Shun Yu 809* (Fig. 2a). In May 2019, during another exploratory sampling of the entire ship, four additional individuals and a single specimen of another potential *Styela* species were collected from the stern deck. All the individuals were transported in insulated containers with sea water within an hour to the CCT CONICET-CENPAT Aquarium to be photographed and preserved for further analyses.

### 2.3. Identification through morphological analysis

Specimens were relaxed with menthol during several hours and fixed in formalin solution 10%. Siphon fragments were fixed and stored in

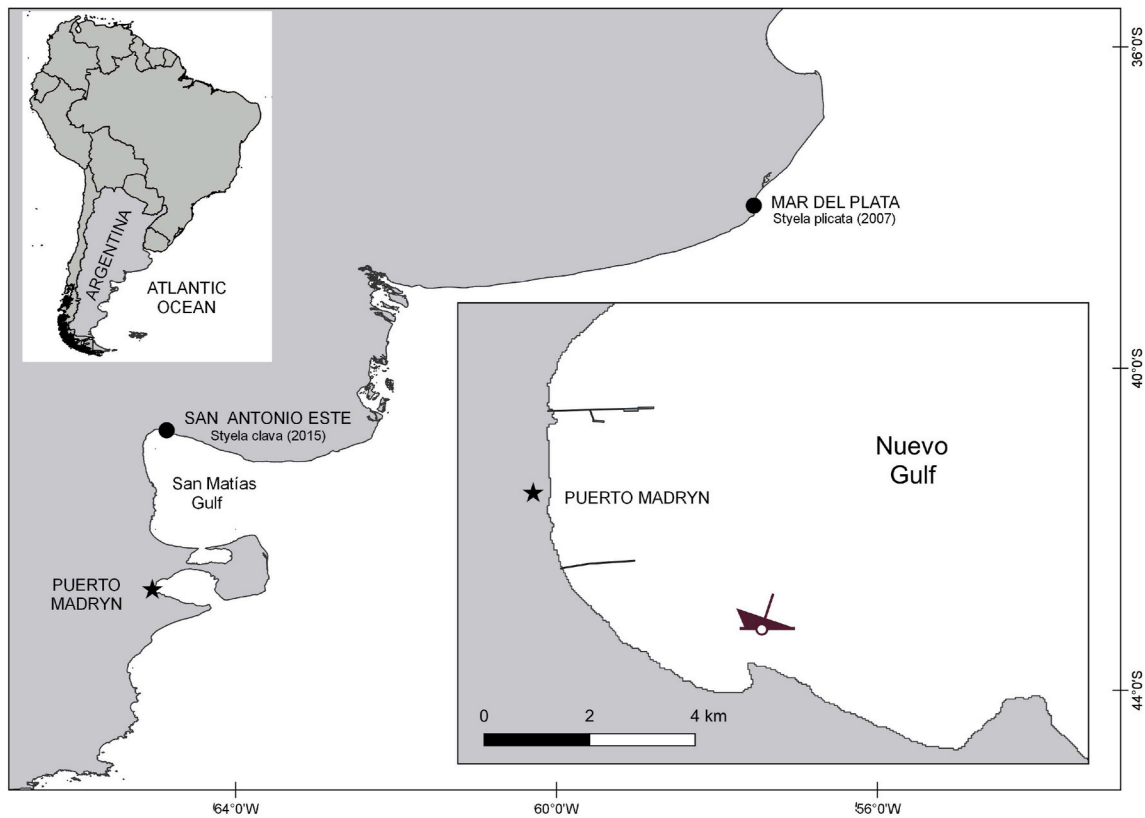


Fig. 1. Map showing the location of the shipwreck where individuals of two potential species of *Styela* were found, and the ports of Argentina where *S. plicata* and *S. clava* were first detected, Mar del Plata and San Antonio Este respectively. Dates refers to the year of first detection for each species in Argentina.

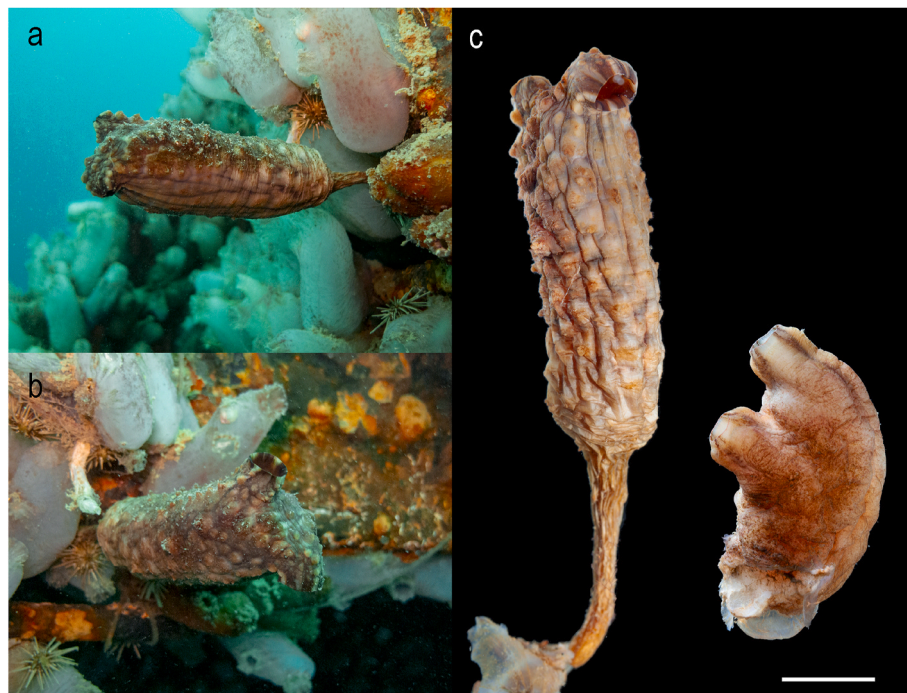


Fig. 2. Specimens of two *Styela* species from Puerto Madryn, Argentina. a) and b) *Styela clava* attached to the hull of the shipwreck Hu Shun Yu 809 c) *Styela clava* (left) and *Styela plicata* (right), collected for taxonomical identification. Scale bar: 2 cm. Photos by N. Battini.

96% ethanol for further genetic analysis (see below). Specific taxonomic literature was used to identify two individuals of *Styela* sp. 1 and one individual of *Styela* sp. 2 (Monniot et al., 1991; da Rocha et al., 2012).

The description of species was conducted following Herdman (1881), Carlisle (1954), Millar (1960), Van Name (1945) and Kott (1985). All specimens were deposited in the Invertebrate Collection of the

IBIOMAR-CONICET (INV-CNP) (see Results section for collection numbers).

## 2.4. Identification through genetic analysis

One specimen of *Styela* sp. 1 and one from *Styela* sp. 2 were used for genetic analysis. All steps to obtain DNA sequences were performed in the Laboratory of Molecular Biology (IDEAUS-CONICET, Argentina) and are detailed in the Supplementary material. DNA sequence data was edited and aligned using CodonCode (CodonCode Corp., MA, USA) and Clustal W (Thompson et al., 1994), respectively. DNA sequences of the putative species and related taxa were downloaded from GenBank and are listed on Table S1.

Estimates of evolutionary divergence over sequence pairs of the COI mitochondrial gene between *Styela* sp. 1, *Styela* sp. 2 and several species of the genus, were calculated using “p-distance” (Kimura, 1980) in MEGA v5 (Tamura et al., 2011). Bayesian phylogenetic reconstruction based on the COI mitochondrial gene was carried out using Mr Bayes 3 (Ronquist and Huelsenbeck, 2003). Details of the analyses are listed in Supplementary material. The software Figtree v 1.4 (Morariu et al., 2009) was used to edit the tree.

## 2.5. Shipping connectivity

The intensity of regional shipping connectivity associated with Puerto Madryn port was studied in detail, given the previous reports of both invasive *Styela* species in two of these ports (Mar del Plata and San Antonio Este, Fig. 1). In addition, because these species could have been introduced from other worldwide donor regions, the international shipping connectivity of Puerto Madryn port was also analysed. The number of arrivals during a 5-year period (from January 2013 to December 2018) was studied for regional and international vessels, classifying each visit by category of vessel and bioregion of origin. Vessels categorised as commercial vessels included merchant vessels (bulk carriers, tankers, container ships, cruise ships). Non-trading vessels included a wide variety of types, such as tugs, dredges, research vessels and mainly recreational vessels. Commercial fishing vessels included all vessels engaged in the capture of wild stocks of living marine resources, such as fishing (general), trawler (all types) and fish factory vessels. All maritime traffic data were extracted from the maritime authority database, Prefectura Naval Argentina. The database was revised for potential errors, such as duplications, spelling mistakes on port names or inconsistencies between a port name and the country where it is located. Arrivals of fishing and recreational vessels for which the origin and destination ports were the same, were not considered, as well as arrivals of vessels coming from freshwater ports. Finally, for every international marine-coastal port a unique province was assigned following the bioregionalization proposed by Spalding et al. (2007). Probable donor regions were identified through the quantification of the total number of vessels (as a proxy for propagule pressure) incoming from each province during the whole analysed period.

## 2.6. Potential distribution

### 2.6.1. Occurrences

After the species were positively identified, the potential distribution of both species was studied by using Species Distribution Models (SDM). Global occurrence records were extracted from the Global Biodiversity Information Facility (<http://www.gbif.org>), the Ocean Biogeographic Information System (<http://www.iobis.org>) and specific scientific literature accessed through Google Scholar, the Aquatic Sciences and Fisheries Abstracts (ASFA) and Scopus. Records were rigorously scrutinized and removed whenever they were dubious or incorrect due to species misidentifications or errors in the original data. Given that spatial autocorrelation leads to biases in model evaluation (Dormann, 2007), autocorrelated records were removed prior to the analyses. After

this procedure, 261 and 272 non-autocorrelated occurrences were extracted for *Styela clava* and *Styela plicata*, respectively, which were used for further distribution modelling (Table S2).

### 2.6.2. Environmental predictors

Environmental variables directly affecting the two *Styela* species were selected for the SDMs, including depth, distance to the shore, temperature, salinity and chlorophyll concentration as a proxy for food availability. For climatic variables, the annual mean, minimum, maximum and range at the bottom mean depth were considered. All the variables except for distance to the shore were derived from BioOracle v.2.0 database (<http://www.bio-oracle.org>) (Thiyagarajan and Qian, 2003; Assis et al., 2018) at a spatial resolution of 5 arcmin (~10 km), while distance to shore was derived from Marspec (<http://www.marspec.org>) (Sbrocco and Barber, 2013) at a 2.5 arcmin resolution (~5 km). Given that correlation between environmental predictors can severely affect modelling outputs (Dormann et al., 2012), the collinearity among predictors was calculated and whenever the variables were highly correlated ( $|r| > 0.8$ ), only one was selected. Based on these analyses, 10 variables were selected for *Styela clava* and 9 for *Styela plicata* (Table S3). Further details on environmental information are provided in the Supplementary material.

### 2.6.3. Modelling procedure

Given that there are differences between the algorithms used in SDMs, an ensemble modelling approach was used to model the distribution of both *Styela* species. These ensembles were fitted using ‘biomod2’ package (Thuiller et al., 2009) in R (R Development Core Team, 2020), combining six different presence-only or presence-background modelling algorithms: generalised linear models (GLM), general boosted regression models (GBM), multiple adaptive regression splines (MARS), maximum entropy (MaxEnt), random forests (RF) and surface range envelope (SRE) with default parameters. The performance of each model was evaluated using cross-validation, with 80% of the occurrences points randomly selected to train the model and the remaining 20% to validate it. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the individual models, which were then combined into an ensemble by scaling their predictions with a binomial GLM, using their training AUC as an average weighing parameter following Chapman et al. (2019). In that way, better performing models had a larger influence on the final ensemble. Moreover, poorly performing models were excluded from the final ensemble, based on modified z-scores, with models with  $z < -1$  rejected (Chapman et al., 2019). Even though the AUC can be a misleading parameter to evaluate model performance (Lobo et al., 2008), it provides a valuable estimator to compare different algorithms evaluated on the same data, and thus a good weighting parameter (Chapman et al., 2019).

To select pseudoabsences, two different perspectives were combined, following Chapman et al. (2019): the accessible area and habitat unsuitability selection criteria (Thuiller, 2004; Anderson and Raza, 2010; Barve et al., 2011). For each algorithm, ten different sets of pseudoabsence points were selected based on the combination of the previously mentioned criteria (see Supplementary material for more detailed explanation about pseudoabsence selection) and for each set, ten different model runs were performed for each algorithm, each one with a different train/test data split, resulting in 600 individual models for each species. The importance of each variable was calculated following ‘biomod2’ default procedure.

## 3. Results

### 3.1. Identification through morphological analysis

Taxonomic analyses based on morphology confirmed the preliminary identification made during the field assessment, and both species corresponded to the genus *Styela*. Individuals named as *Styela* sp.



1 were identified as *Styela clava* and the individual named as *Styela* sp. 2 was identified as *Styela plicata*. These records are the first for *S. clava* in the Nuevo gulf, and the first for *S. plicata* in Patagonia.

*Styela clava* Herdman (1881). Material examined: Puerto Madryn, Argentina INV- CNP 3854, 2 specimens.

The specimens of Puerto Madryn resembles the external shape of *S. clava* as described by Herdman (1881) and Carlisle (1954). They are club shaped and pedunculate organisms, with a length of 13.5 cm and two anterior siphons with four lobes each. Siphons presented longitudinal stripes, four white and four rich chocolate brown. The body presented a brown color, with yellowish and reddish tones, covered by paler mamillations in the anterior half (Fig. 2b). Specimens examined presented a row of 32–40 of simple oral tentacles with keels. The pharynx presented 4 folds of each side, with 20–30 branchial vessels on them, and 9–11 vessels between them, and six stigmata per mesh. The dorsal lamina was continuous with a plain edge, and the dorsal tubercle presented an anterior opening turned slightly to the left, similar to those described by Millar (1960). Both individuals were hermaphrodite with the gonads located at both sides of the body. On the right, we found 5–6 ovaries, while on the left we found 3–5 (Van Name, 1945; Abbott and Johnson, 1972; Millar, 1960). The testes were grouped in small compact white lobes, several around each ovary. The individuals of this species that occur in other locations frequently have several epibiont organisms, including small native bivalves (*Musculus viator*) living embedded within the tunic (Lazari et al., 2019), nevertheless the individuals collected in Puerto Madryn had no visible organisms associated.

*Styela plicata* (Lesueur, 1823). Material examined: Puerto Madryn,

Argentina INV-CNP 3855, 1 specimen.

The external appearance of this ascidian followed the descriptions of Van Name (1945) and Kott (1985). The individual, with a length of 6.8 cm, presented a clean tunic, free of epibionts, white and yellowish with large prominences. Both siphons presented four lobes with a dark brown stripe each (Fig. 2c). The specimen presented a row of approximately 27 simple tentacles, of two orders. The dorsal tubercle was C-shaped with an anterior opening, and the dorsal lamina was continuous and plain-edged (Van Name 1945; Kott 1985). The pharynx presented four folds in each side, with 12–14 longitudinal vessels on the folds, 7–8 between folds, and 8–10 stigmata per mesh. The specimen of Puerto Madryn presented four gonads on the right side of the body and two on the left side. The testes were long and branched located along the tubular ovary. The stomach was large and plicated, with more than 10 longitudinal folds; the gut loop was narrow and deep (Kott, 1985).

### 3.2. Identification through genetic analysis

The genetic distance between *Styela* sp. 1 (Genbank: MT423971) and two sequences of *Styela clava* was  $0.30\% \pm 0.002$  (Table S4), while with other more distantly related species ranged between 19.6% and 27.3% (Table S4). The genetic distance between *Styela* sp. 2 (Genbank: MW326670) and two sequences of *Styela plicata* was  $0.60\% \pm 0.007$ , while with other related species ranged between 19.8% and 24.2% (Table S5).

In the COI phylogeny, *Styela* sp. 1 forms a well-supported monophyletic clade together with sequences of *S. clava* (Figure S1). In the

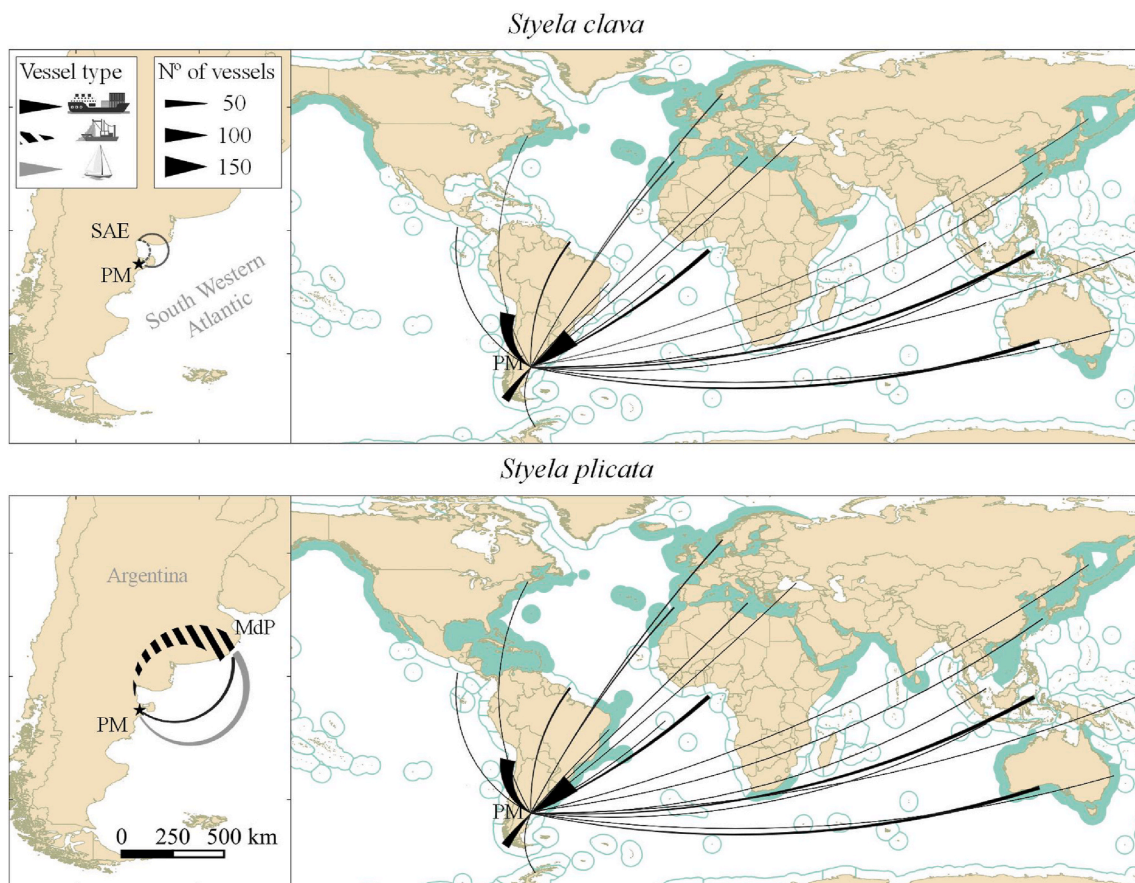


Fig. 3. Shipping connectivity with Puerto Madryn port (PM, marked with a star) during the 2013–2018 period. Thickness of the arrow corresponds to the number of vessels arrived to Puerto Madryn. On the left, vessels that arrived from the other two ports of Argentina where *Styela clava* (San Antonio Este, SAE) and *Styela plicata* (Mar del Plata, Mdp) were reported, according to type of vessel (commercial, fishing and recreational). On the right, international commercial vessels that arrived by province of origin (arrows start at the centroid of each province as defined by Spalding et al. (2007)). Provinces with at least one record of each species are painted in green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

same way, *Styela* sp. 2 forms a well-supported monophyletic clade together with sequences of *S. plicata* (Figure S1).

### 3.3. Shipping connectivity

Analysis of intraregional connectivity between Puerto Madryn port and the two ports where either *Styela* species were reported, showed that during the period 2013–2018, Puerto Madryn and Mar del Plata ports were connected mainly by fishing (221 arrivals) and recreational vessels (45 arrivals), while the arrivals of cargo vessels and commercial vessels were much lower (13 arrivals) (Fig. 3). The number of vessels coming from San Antonio Este port was negligible regardless of the category of the vessel (Fig. 3), with 1 commercial vessel and 3 fishing vessels arrivals recorded.

The international traffic analysis showed that Puerto Madryn port received 464 international commercial vessels during the five-year period studied (2013–2018). More than 35% of the arrivals corresponded to bulk carrier/general cargo vessels, followed by container vessels (34%) and cruise ships (29%). In contrast, tanker vessels were uncommon (0.86%). Regarding the port of origin, Puerto Madryn port received vessels from a large variety of provinces (Fig. 3). The most frequent origins were the two neighboring provinces, the Warm Temperate Southwestern Atlantic (Uruguay and southern Brazil) with 153 arrivals and the Warm Temperate Southeastern Pacific (Central and Northern Chile to Peru), with 115 arrivals. In addition, Puerto Madryn port received 63 vessels from the southernmost region of Chile, corresponding to the Magellanic province (Fig. 3). Arrivals from the remaining 19 provinces ranged from 1 to 50 vessels during the five years

period (Fig. 3). The number of vessels arrived from provinces which had at least one record of *S. clava* was 26 (~6%) while 200 vessels (43%) arrived from provinces with records of *S. plicata*.

### 3.4. Potential distribution

Based on the species distribution models for *S. clava* and *S. plicata*, both species have a very widespread potential distribution (Fig. 4). For *S. plicata*, potential distribution is throughout tropical and subtropical regions while in the case of *S. clava* throughout more temperate regions (Fig. 4). Accordingly, the environmental predictor that most contributed to the models in the case of *S. plicata* was the minimum annual temperature, while in the case of *S. clava* was the maximum annual temperature, both at the mean bottom depth (Figure S2). Predictors related to salinity, chlorophyll concentration and geography had little effect on the potential distribution of both species (Figure S2). Individual models ( $n = 600$ ) showed variable performance with AUC values ranging from 0.438 to 0.995 for *S. clava* (mean  $\pm$  sd =  $0.789 \pm 0.118$ ) and from 0.448 to 0.998 for *S. plicata* ( $0.882 \pm 0.138$ ). However, these AUC values were proportional to the contribution of each model to the final ensembles, and models with lower AUC values ( $<0.670$  for *S. clava* and  $<0.744$  for *S. plicata*) were excluded from these ensembles.

The tropical and subtropical distribution of *S. plicata* explains the differences in the potential distribution predicted across the Southwestern Atlantic (SWA), with *S. plicata* extending further north than *S. clava* (Fig. 4). Particularly in the studied region, when the final ensemble was thresholded using the tenth percentile training presence

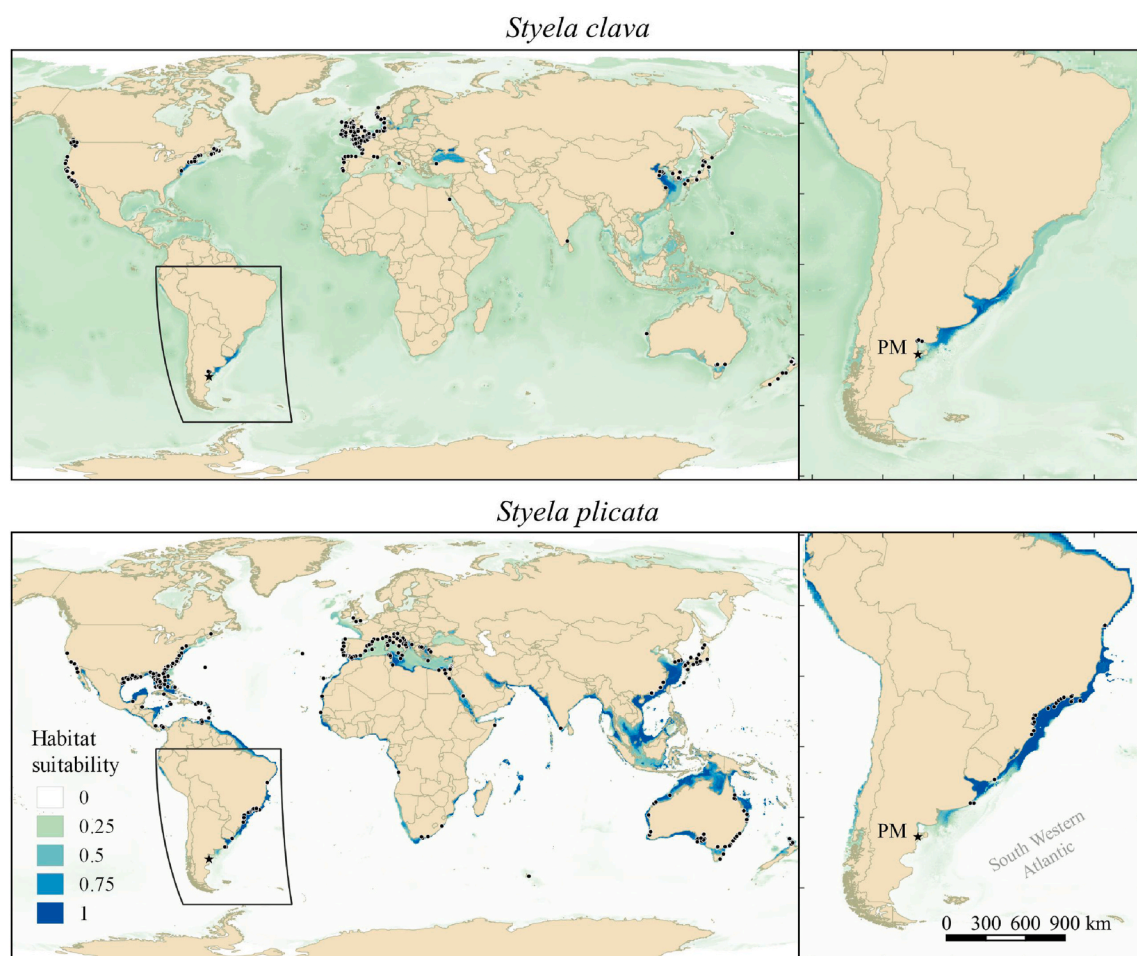


Fig. 4. Global forecasted potential distribution for *Styela clava* and *S. plicata*. Close up shows the Southwestern Atlantic. Black dots indicate occurrence points for each species and the star indicates the location of the new record for both species found in this study (Puerto Madryn (PM), Argentina). Habitat suitability values represent a weighted mean for rescaled individual models based on their AUC.



logistic criteria, models predicted the occurrence of *S. clava* in the Nuevo gulf but not that of *S. plicata* (Fig. 5). According to this ensemble, the southernmost suitable locality for *S. plicata* along the SWA is the San Matías gulf, which is located approximately 100 km north to the point where the individual was collected.

#### 4. Discussion

The preliminary identification of the individuals early found on the shipwreck was confirmed through concordant molecular and morphological analysis, and this is the first record for both *Styela clava* and *Styela plicata* in Puerto Madryn. The intensity and origin of the shipping traffic suggests that *S. plicata* could have arrived by secondary spread from regional ports, while the arrival of *S. clava* is likely to be associated with international shipping traffic. Furthermore, our distribution models predict that both ascidian species might reach a notably widespread potential distribution across the SWA. Particularly, the model predicts that *S. clava* has coastal suitable areas along the entire SWA shoreline, where it is currently absent, indicating a high south and northward potential expansion. On the contrary, the model predicts that further southward spread of *S. plicata* is unlikely, being the minimum annual temperature the limitation for its distribution.

*Styela plicata* is a conspicuous species growing on artificial structures throughout the world (Shenkar and Swalla, 2011). Its presence attached to docks and pilings in marinas and small ports (Pineda et al., 2016; Spagnolo et al., 2019) and also on hull boats (Wyatt et al., 2005; Lambert, 2007), suggests that it could have secondary spread as a biofouling organism through recreational vessels (Pineda et al., 2011; Brine et al., 2013). This species was first detected in Mar del Plata port in 2007, where today is a well-established species in fouling communities on breakwaters and wooden pilings from the yacht club (Albano and Obenat, 2019). This port is not only one of the main economic hubs for national fisheries, but also holds the most important marina of the country (Albano, 2012; Rumbold et al., 2020), considered as a hotspot for marine invasive species (Schwindt et al., 2020). The high intensity of regional connectivity between our study site and the Mar del Plata port strongly suggests that *S. plicata* was likely transported by fishing or recreational vessels. However, because species introductions are inherently difficult to predict, we cannot discard an independent newly introduction through international ships. Our results showed that the

most frequent origins of the international vessels were two neighboring countries, Brazil and Uruguay, where *S. plicata* is a well-established invasive species (da Rocha and Kremer, 2005; Corrêa de Barros et al., 2009; Scarabino et al., 2018). In Uruguay, this species is present in La Paloma port, and in Brazil it was recorded living on the rocky substrata, piers and marinas of Paranaguá Bay and on artificial floating structures in Rio de Janeiro port (da Rocha and Kremer, 2005; Orichio et al., 2019). Knowing which is the most likely scenario, is important for managing new invasions as they lead to different kind of actions; while one focus on restricting or inspecting international ships, the other focuses on reducing the spread from invaded areas via regional maritime traffic. Further studies are needed to disentangle the donor area for *S. plicata*, since genetic sequences are incomplete for regional localities. Our hypothesis is that the arrival of *S. plicata* in the study area is a result of secondary spread from regional ports. Although this hypothesis needs to be tested in the future, it is supported by the high intensity of regional connectivity, the fact that fishing and recreational vessels usually spend several days moored in the ports, increasing the risk of colonization by fouling organisms, and the fact that these ships are usually poorly maintained regarding cleaning of the hull (Godwin, 2003; Davidson et al., 2012; Moser et al., 2017).

The distribution model analyses successfully identified the current locations of *S. plicata* around the world, showing that the species has a widespread distribution with areas susceptible to invasion largely occupied. Nonetheless, predictions for *S. plicata* show that this species may expand even northern of its current distribution in the SWA. In addition, the Southeast Pacific coast is a suitable environment for its establishment, where is presently not reported among the non-indigenous tunicates (Pinochet et al., 2017). The potential distribution model did not predict the presence of *S. plicata* at the Nuevo gulf. Indeed, it indicated that the collected individual was 100 km outside its predicted southern distribution boundary. Thus, a further southward expansion seems very unlikely. According to the model, minimum annual temperature plays the most important role in determining the potential distribution of *S. plicata* which could be limited by the range of temperature needed for reproduction from 11 °C to 28 °C (West and Lambert, 1976). Although the minimum mean temperature in the study site (9 °C in winter), is lower to the reported as optimal for *S. plicata*, the establishment of this species in other invaded areas has been attributed in part to its tolerance to wide changes in temperature (Thiyagarajan and Qian, 2003; Pineda, 2012). Indeed, invasive species can surprise with a habitat changes or by tolerating suboptimal conditions during their establishment and spread in the invaded ranges (Schwindt et al., 2009). In this study, and despite the apparent suboptimal conditions for the survival of *S. plicata*, we found a mature adult individual growing on the shipwreck. Other individuals might have already colonized nearby structures, and considering the predicted distribution models at these latitudes, we suggest that an extensive monitoring is needed to determine whether the species is established and, if necessary, a rapid localized removal before individuals become abundant. Because invasion events are unique, the timeframe to achieve eradication depends on multiples environmental and socio-economic variables that are context-specific (Simberloff, 2003). It is important to remark that early detection and rapid response schedules do not end with control measures, and different surveys need to be carried out through time to assess the efficiency of the actions and to prevent the recolonization of the species.

The arrival of *Styela clava* to Puerto Madryn through secondary spread seems unlikely due to a limited shipping connectivity with San Antonio Este port, accumulating only four vessels in five years. In addition, larvae of *S. clava* rarely swim more than few centimetres before settlement, which occurs ~12 h after hatch, and because of this, larvae are not able to spread long distances by natural vectors (Davis and Davis, 2007; Darbyson et al., 2009). The introduction of this species in Argentina is relatively recent, as it was first recorded in 2013 in San Antonio Bay, and now has successfully and abundantly colonized piers

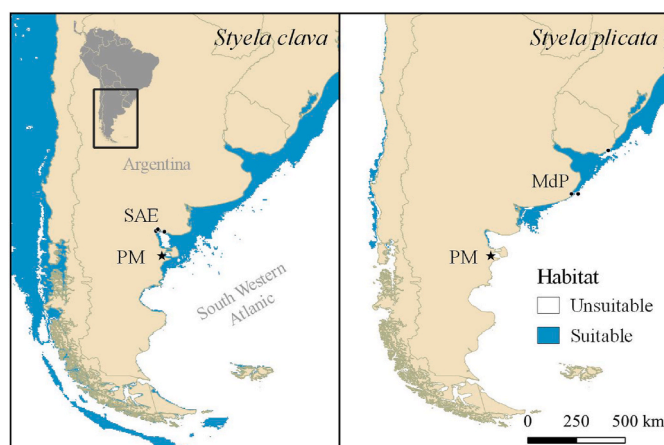


Fig. 5. Predicted habitat suitability for *Styela clava* and *S. plicata* at a regional scale within South America. Predictions were thresholded using the tenth percentile training presence logistic criteria to produce binary presence/absence maps. Black dots indicate occurrence records for both species, and the star indicates the locality where the new specimens were collected in this study (Puerto Madryn, PM, Argentina). For each species, the ports where they were recorded for the first time are shown (SAE: San Antonio Este; MdP: Mar del Plata).

and subtidal habitats (Pereyra et al., 2015). The hypothesis of a new introduction event is consistent with the analysis of shipping traffic of international commercial vessels. Puerto Madryn port received vessels from a large variety of regions where *S. clava* is currently considered an invasive species, such as the coast from Denmark to Portugal (Davis and Davis, 2007), the Mediterranean Sea (Davis and Davis, 2005), Australia (Hewitt et al., 2004), the Atlantic coast of North America (Sephton et al., 2011) and also from the northwest Pacific Ocean where *S. clava* is native (Davis and Davis, 2005). The identification of donor regions has always some degree of uncertainty in marine bioinvasions (Ruiz et al., 2000), and further studies with a complete dataset of molecular sequences are needed to disentangle the donor area for *S. clava*. The population genetic reconstructions have been utilized to infer the colonization scenarios more accurately (e.g. Geller et al., 2008; Dupont et al., 2009; Darling et al., 2012). Although the connectivity with each potentially donor region was not particularly intense, analysing the shipping traffic considering all the regions where *S. clava* is already present, as well as the poor connectivity at a regional scale, leads us to hypothesize that a new introduction event, instead of secondary spread, may have occurred from outside the country.

The potential distribution model analyses revealed that *S. clava* is globally less expanded than its congener *S. plicata*. According to the predictions, the Southeast Pacific coast is also a highly suitable environment for the establishment of *S. clava*, where as far as we know it is absent and it is thus a vulnerable area for future invasions. Although future environmental changes could modify the potential predicted distributions (Lyons et al., 2020; Zhang et al., 2020), *S. clava* is expected to achieve a continuous distribution throughout the Southern Cone unless effective management strategies are implemented. Indeed, the model predicted the occurrence of *S. clava* in the Nuevo gulf, suggesting that this species has not fully realized its potential distribution along Patagonia, which may extend further south through the San Jorge gulf (44°S), one of the most productive marine ecosystems in Argentina.

Interestingly, the finding of *S. clava* in this study occurred during the monitoring of the shipwreck in search for the invasive oyster *Crassostrea gigas*, which had been recently detected by recreational scuba divers (Schwindt et al., 2019). Indeed, as a set of nesting dolls we found the individual of *S. plicata* during a second and more exhaustive search for *S. clava*. This highlights the importance of surveillance programs with established schedules and not occasional or fortuitous samplings, and the training and field expertise of the people in charge of them to early detect exotic species (Figure S3) (Lyal and Miller, 2020). Building capacities for the early detection of invasive species is an important component of management programs and requires specific investment (Figure S3) (Frey et al., 2015; McGeoch et al., 2016; Morissette et al., 2020). In this sense, citizen science has also proven to yield good results in conservation plans, providing an opportunity for casual observers to aid in EDRR programs (Waugh, 2009; Crall et al., 2010; Pagès et al., 2019), as it happens with the emerging of online communities through the identification of photographic observations of biodiversity (Martinez et al., 2020). For example, a local outreach campaign with recreational divers yielded fruitful results to detect an introduced oyster (Schwindt et al., 2019). Divers were able to learn about the identification of the species and more than ten reports of oysters were received during the next six months since the early detection. This experience shows that collectively, citizen science is a powerful tool not only for early detection, but also for maximizing rapid response efforts (Crall et al., 2010).

For conspicuous and easily recognized species as those we studied here, citizen science initiatives can be conducted for many potential invasive species on artificial structures. In addition, localized and quick removals aimed to eradicate these species can be also coordinated with recreational divers, taking advantage of their valuable skills and deep knowledge of the local marine environment (Figure S3). Therefore, the efficiency of prevention, early detection and rapid response strategies are improved by the correct training personnel in monitoring and by the application of coordinated citizen science programs. Considering our

findings and discussion, we strongly recommend stakeholders to work in preparedness measures (Figure S3). This means to be prepared with the laws and policies, human resources, and a coordinated structure that is ready to take action, before the potential arrival of an invasive species, including a clear establishment of the roles, funding commitments, and responsibilities (Burgiel et al., 2020; Burgos-Rodríguez and Burgiel, 2020). A first step toward an integrated management within the National Strategy on Invasive Alien Species in Argentina is the publication of the best practices guide for small vessels and nautical equipment (<https://bit.ly/3xlttdtw>). This guide provides the cleaning methods for hull and equipment and the steps to follow during an early detection directed to prevent new species introductions as well as to reduce spread of invasive species already introduced.

The inspection of the submerged surfaces of the fishing vessel two years before it was sunk, together with the monitoring of the port area, allowed us to assume that *S. clava* and *S. plicata* arrived after 2015. Moreover, the location of the ascidians on the hull and deck surfaces over the floating level indicates that the colonization occurred after the vessel was sunk, suggesting a very recent arrival. Artificial structures, such as this recently sunk ship, can provide colonisable space for the settlement of sessile invasive species, acting as stepping stones for their spread into natural habitats (Apte et al., 2000; Bulleri and Airoldi, 2005; Dafforn, 2017), as it was recently documented for the sun coral *Tubastraea tagunensis* in Brazilian coral reefs (Soares et al., 2020). Tourism and recreational activities are considered an important pathway for the spread of invasive species in marine environments (Bax et al., 2001; Clarke Murray et al., 2011; Anderson et al., 2015). Particularly, recreational diving is among the fastest growing touristic activities in the world (Davenport and Davenport, 2006) for which obsolete ships have been intentionally sunk to become artificial reefs (Davidson et al., 2008; Miranda et al., 2020). In addition, diving boats that travel between shipwrecks usually also visit natural sites and even marine protected areas, acting as a secondary spread vector for invasive species (Parretti et al., 2020; Soares et al., 2020). In the light of our findings, together with the evidence of the impact of artificial habitats on marine ecosystems (Aguilera, 2018), we call for the attention of the governments and other stakeholders for a deeper discussion on integrated coastal management (Barragán Muñoz, 2019). The creation of artificial reefs for recreational diving need to be developed in agreement with the Convention of Biological Diversity conservation goals (Miranda et al., 2020), which promulgates that measures should take place to manage pathways to prevent the introduction and establishment of invasive species (UNEP, 2011).

In sum, here we integrated information obtained through different approaches, tools, and expertise for the early detection of marine invasive species. This highlights the importance of implementing coordinated EDRR programs, and the necessity for governments and other stakeholders to consider all preparedness measures to implement them. In particular, shipping connectivity analysis is a powerful tool that can improve preventive management strategies, through risk assessments with potential donor regions of invasive species (Faulkner et al., 2017). We also draw the attention to the increasing intentional sinking of ships for the creation of artificial habitats and the potential consequences of these actions in marine ecosystems. The United Nation's Sustainable Development Goal 14 focus on assessing and reducing cumulative pressure to the ocean, thus proactive measures, as those discussed in this work, are urgently needed to attain the conservation of marine coastal areas.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2021.113333>.

## Authors contribution

Karen L. Castro, Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration. Nicolás Battini, Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Clara B. Giachetti, Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Berenice Trovant, Investigation, Writing – review & editing. Mariana Abelando, Investigation, Writing – review & editing. Néstor G. Basso, Investigation, Writing – review & editing. Evangelina Schwindt, Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

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