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Rapid Communication

The simultaneous introduction of the tunicate *Styela clava* (Herdman, 1881) and the macroalga *Undaria pinnatifida* (Harvey) Suringar, 1873, in northern Patagonia

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Abstract

Determining which factors contribute to the establishment and spread of an introduced species is a challenge with substantial management implications, especially when that species is found associated with another introduced species. Here, we report for the first time the presence of two introduced species, the macroalga *Undaria pinnatifida* (Harvey) Suringar, 1873 and the clubbed tunicate *Styela clava* (Herdman, 1881), in San Antonio Bay (northern Patagonia), where apparent facilitation by the tunicate may be contributing to the establishment of the algae. Because *S. clava* is an ecosystem engineer and acts as a facilitator for the settlement (and further growth) of other species, it may create habitats that are more suitable for *U. pinnatifida* invasion at locations not yet colonized by the algae.

Key words: *Styela clava*, *Undaria pinnatifida*, facilitation, San Matías Gulf, Patagonia introduced species

Introduction

There are many mechanisms by which introduced species could be integrated into native assemblages, and facilitation has been increasingly recognized as one of these mechanisms (Altieri et al. 2010; White and Orr 2011). This is especially true in harsh environments such as coastal Patagonia (Bertness et al. 2006), where some species may facilitate each other by providing shelter or refuge, reducing predator populations, or by ameliorating stressful conditions (Locke et al. 2007; Thompson and Schiel 2012). When these facilitative interactions occur between introduced species it can lead to increased introduction rates or increased population and community effects, which is referred to as an "invasional meltdown" (*sensu* Simberloff 2006).

The macroalga *Undaria pinnatifida* (Harvey) Suringar (hereafter referred to as *Undaria*), originally from northeastern Asia, has become a successful introduced species in several countries worldwide. It is considered one of the worst 100 invasive species (Lowe et al. 2000), with an opportunistic life cycle (i.e. a short life span, high numbers of propagules, and high growth rate) (Valentine et al. 2007). *Undaria* was accidentally introduced to Argentina in 1992 (Casas and Piriz 1996), and since then it has expanded towards the north and south of the country (Pereyra et al. 2014a). The clubbed tunicate *Styela clava* (Herdman, 1881) (hereafter referred to as *Styela*), originally from the northwestern Pacific Ocean, has been accidentally introduced into many countries worldwide, including Denmark, France, Ireland, Spain, United Kingdom, New Zealand, USA, and Canada (Davis and Davis 2007; Goldstien et al. 2011). *Styela* can dominate the fouling communities in the locations where it has been introduced and causes great concern due to the ecological and economic damages it can produce at high densities (Wong et al. 2011, and references therein).

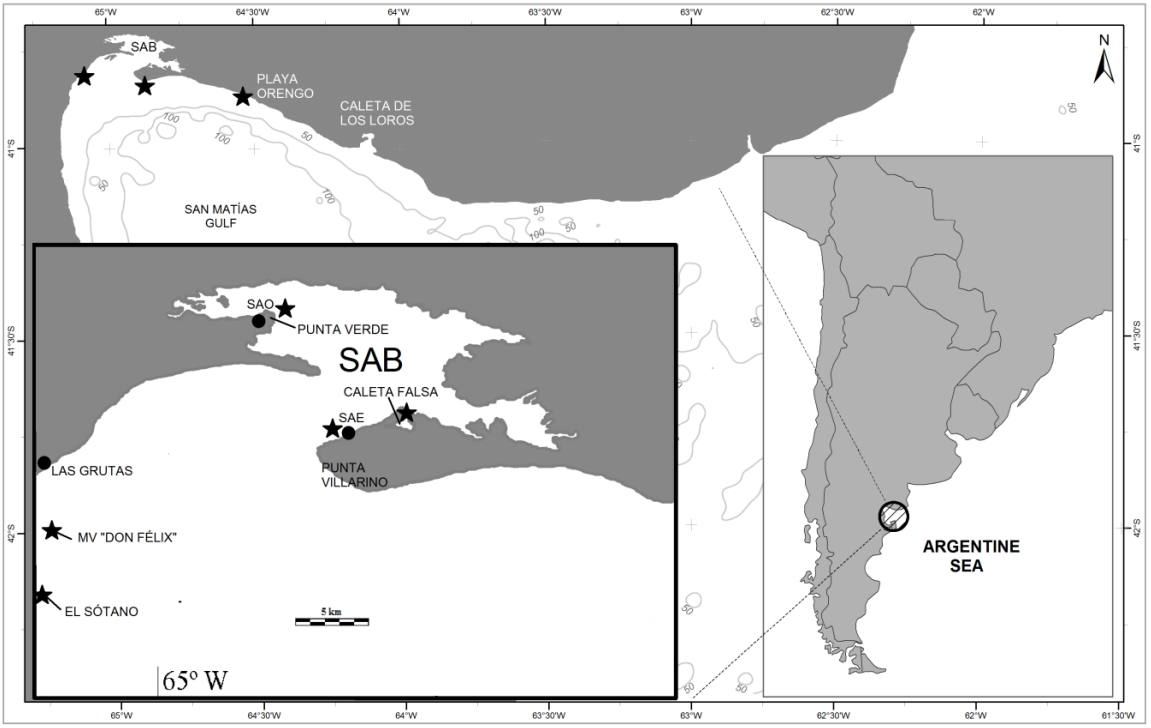

Figure 1. Map showing the sampling location in Northern Patagonia, Argentina: San Antonio Bay (SAB), San Antonio Este Port (SAE), and San Antonio Oeste (SAO). Stars indicate locations where *Styela clava* was found. For details see supplementary material Table 1S.

In this note, we document the expansion (secondary spread) of *Undaria* into San Antonio Bay, northern Patagonia (Figure 1), and the first finding of *Styela* in the southwestern Atlantic Ocean.

Methods

This study was conducted at San Antonio Bay (SAB), Argentina (40°45´S, 65°55´W), a macrotidal system with up to 9 m tidal amplitude, several inner channels, and extensive flats mainly composed of sand and pebbles (Figure 1, supplementary material Table 1S). SAB is a harsh, hypersaline, environment (Isacch et al. 2006; Martinetto et al. 2010). Average seawater temperatures show a regular cyclic annual pattern, with the lowest in the austral winter (August: ~5ºC) and the highest in midsummer (February: ~25ºC). The bay and the surrounding areas have been recognized as an important biodiversity reserve and are part of the Western Hemisphere Shorebird Reserve Network International (WHSRN, Morrison and Ross 1989).

As part of a project aimed at monitoring the expansion of *Undaria* into the San Matías Gulf, where the species was estimated to have arrived in 2010 (Pereyra et al. 2014a), three surveys were carried out from June to October 2014 on an aggregation recently found in Punta Verde (Pereyra et al. 2014b). Individuals of *Undaria* were mechanically removed from the shore of the main inner channel and by SCUBA diving along the central part of the channel. Samples of sporophytes were collected haphazardly and taken to the laboratory.

Once in the laboratory, the maximum length and width of sporophytes and sporophylls were measured. The maximum length of sporophytes was measured from the base of the midrib to the proximal end of the stipe, whereas the maximum width was measured as the maximum width of the midrib. The developmental stage of each sporophyte was determined according to Casas et al. (2008).

The presence of *Styela* was recorded for the first time in October 2013 while performing field surveys for other projects, and its association with *Undaria* became apparent in June 2014. Once the individuals collected were confirmed as belonging to *Styela* (based on detailed macro- and microscopic analyses of 10 individuals), exploratory surveys were performed from September

to November 2014 in the inner channel of SAB and at other points in the northwestern San Matías Gulf (Figure 1). In two of these sites (Punta Verde and Caleta Falsa), individuals of *Styela* were collected for additional examination and measurement in the laboratory. Opportunistic surveys were performed from the coast and with SCUBA divers in San Antonio Este, Las Grutas, El Sótano and the shipwreck *Don Felix*, to confirm the presence or absence of *Styela* and *Undaria*. In Punta Verde most *Styela* were observed hanging from the top of coastal caves on the rocky shore, thus a specific sampling method was designed to estimate density. A malleable wire was used to draw an area of 0.5 × 0.5m^2 (n = 7) onto the surface of the caves and all the individuals within such area were detached and immediately sampled. Each *Styela* was measured (distance in mm from the peduncle base to the tip of siphons) with the individuals submerged in sea water.

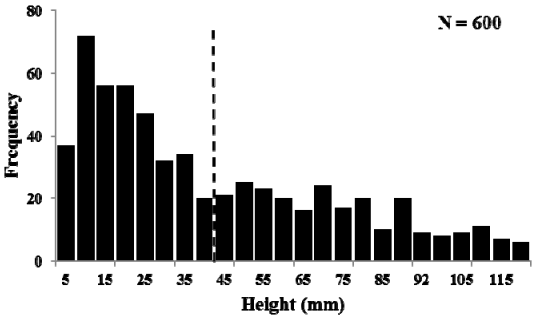

Figure 2. Size-frequency distribution of *Styela clava* in samples from Punta Verde and Caleta Falsa. The dashed line indicates the size at sexual maturation (cf. Parker et al. 1999).

Results and discussion

The specimens of *Styela* collected in this study were consistent with previous morphological descriptions of size and shape (Millar 1960; Davis and Davis 2008): a club-shaped body with a short stem-like stolon; appearance is leathery and rough; the siphons are terminal and marked with four dark brown stripes; about 30 oral tentacles of similar size, showing keels; the dorsal tubercle has an anterior opening, slightly oriented into the left; the dorsal lamina is continuous and plain edged; the branchial folds sum to 20–25 on the folds and 7–10 between them; an average of six stigmata per mesh; the anal border is very lobulated; and the gonads are three on the left and six on the right side of the body, respectively.

Undaria was identified following the description by Okamura (1915). The midrib, pinnate division of the blades, and the undulated sporophyll in the basal region of the sporophyte are characteristic of this species, and no other macroalga in the region has a comparable tallus (Borasso de Zaixso 2013).

Most *Undaria* in the aggregations located in Punta Verde (SAB) were found attached to a rocky area (approximately 50 m) along the main channel. The remaining individuals were found in the central portion of the channel and among the scattered rocks in the surrounding area. The presence of *Undaria* was only confirmed in this channel. We collected 1,883 individuals, and all the development stages were identified. From the individuals collected, 68% corresponded to the early stages 0 (43%) and I (25%), less than 2% corresponded to stage IV, and the remaining corresponded to stages II (10.5%) and III (19.5%).

The presence of *Styela* was confirmed for the intertidal areas in the main inner channel of SAB and associated with a variety of different types of hard substrate. Its presence on hard substrates was also confirmed in Punta Villarino, Caleta Falsa, and the docks of San Antonio Este Port (Figure 1). *Styela* was also found in subtidal areas (14–20 m deep) at the *Don Felix*, and on mussel beds of El Sótano and Playa Orengo. *Styela* was not found in Caleta de los Loros.

The mean density of *Styela* in Punta Verde was 84 ind.m^{-2} (SD = 83.0 ind.m^{-2}). Although preliminary, these densities are some of the highest reported for this species in natural and semi-natural environments (Lützen 1999 reports densities of up to 100 ind.m^{-2} for northern hemi-sphere). Simkanin et al. (2012) stated that there are no differences in density for *Styela* between natural and semi-natural habitats, but our results show densities an order of magnitude larger than the greatest density reported by Simkanin et al. (2012). Densities in artificial structures are usually much higher; they can exceed 400 ind. m^{-2} (Lützen 1999; Wong et al. 2011). Although not measured in this study, we observed both high densities (at dock pillars and buoys) and low densities (in a shipwreck) on artificial structures. This can be due to both that it is a recent introduction or to the level of human activity (i.e. vessels movements) in each of these sites.

Figure 3. Images of (a) *Styela clava* with inhalant siphons open and epibionts present in the SAB; (b) *S. clava* with juveniles (arrow) and an individual of *Undaria pinnatifida* (asterisk); and (c) *S. clava* smothered and partially decomposed by the holdfast of *U. pinnatifida* (arrows point to remaining tunic adhered to the holdfast; asterisk shows the orange mantle of *S. clava*).

The size frequency distribution of *Styela* collected in Punta Verde and Caleta Falsa showed a dominance of recent recruits (Figure 2), which were mainly attached to the adults (Figure 3a, b). In fact, *Styela* served as substrate for a variety of organisms including *Undaria* (Figure 3b-c). Interestingly, 86% of the *Undaria* found in Punta Verde, mainly composed of recruits (Stages 0 and I), were observed attached to *Styela*. Other attachment substrates for *Undaria* were pebbles, bivalves, gastropods, and other (native) tunicates. Such a noticeable interaction has not been previously documented, even considering that these two species share some areas where both were introduced (see http://www.europe-aliens.org for European distributions; Russell et al. 2008; Goldstien et al. 2011 for New Zealand examples). In this study, we found up to 66 *Undaria* juveniles (stage 0) on a single *Styela* specimen, and adult sporophytes of *Undaria* (stages III and IV) were found overgrowing and smothering the *Styela* to which they were attached (Figure 3). Interestingly, most of the largest *Undaria* collected were attached to *Styela*, which suggest that competition for space may also be involved. However, *Styela* can colonize substrates unavailable for *Undaria* (e.g. rocky edges and caves). Which mechanism (i.e. facilitation versus competition) is more important is the objective of current studies in the area.

Several features of our observations should be highlighted. First, this is the first report of *Styela* for the southwestern Atlantic Ocean; in South America it was reported only for Venezuela (Montes and Prietos Arca 2005; Pérez et al. 2007). Two recent studies (Simkanin et al. 2012; Schwindt et al. 2014) do not report the presence of *Styela* for the southwestern Atlantic Ocean. Since Schwindt et al. (2014) finished their sampling in the port of San Antonio Este in 2007, it is safe to conclude that the species arrived at the area afterwards. Moreover, surveys conducted in the area until 2012 also did not detect *Styela* (MT pers. observation). Second, Simkanin et al. (2012) reported that *Styela* rarely escapes from the human-made structures where it is commonly found adjacent to natural or semi-natural areas. In the present study, *Styela* was found not only in the inner channel of SAB, but also on mollusk beds of El Sótano and Playa Orengo (Figure 1), and in other localities with human-made structures (e.g. docks, buoys, moorings). Our results suggest that *Styela* is widely distributed in the northwest of San Matías Gulf and particularly in the inner zone of SAB. Third, since the presence of *Styela* was not reported in other areas, we hypothesize that it arrived on the Argentine coasts through the port of San Antonio Este because this port concentrates most of the international trade in the area (Schwindt et al. 2014 and references therein). Our surveys confirmed the presence of *Styela* on the port structures, on the hulls of vessels docked there and in the neighboring area of Caleta Falsa. Finally, since surveys have been regularly conducted in the area during the last four years and *Undaria* has not been previously observed, we are confident that this is the first report of *Undaria* for SAB. The recent arrival of *Undaria* at SAB could be due to a higher propagule pressure from Playas Doradas (90 km southwards from SAB), an area in San Matías Gulf recently colonized by this species (Pereyra

et al. 2014a). However, as *Undaria* has not been reported from other areas between these sites, other sources (e.g. recreational and small commercial vessel activity) should not be discarded. In short, we hypothesize that international commercial shipping is the primary vector for the introduction of *Styela* to the port of SAE. The spread of *Styela* within SAB may be the result of the movement of smaller vessels.

In New Zealand, where *Undaria* is also introduced, recruitment of *Undaria* is greatly enhanced by facilitative organisms such as turf algae (Thompson and Schiel 2012). Since *Styela* is an ecosystem engineer detected before *Undaria* in SAB, and it acts as a substrate for *Undaria* settlement, this tunicate may have created habitats that are more suitable for the establishment of *Undaria*. Interestingly, *Styela* itself also has been facilitated by another invasive species (Locke et al. 2007); in this case by the reduction of a predatory snail by a non-native crab. These observations are consistent with the invasional meltdown hypothesis (Simberloff 2006) that predicts additive and synergistic effects between introduced species. This hypothesis will be further studied in the SAB, with additional surveys and experimental manipulation.

Undaria and *Styela* are both recognized as global invaders with negative ecological and/or economic effects documented in several regions (Lowe et al. 2000; Lambert 2007). The presence of juvenile and reproductively active individuals in SAB indicates that these species are able to complete their life cycles (Goldstien et al. 2011; Dellatorre et al. 2014). Given the relatively short time period since these species arrived in the area (about 2013 for Styela and 2014 for *Undaria*), we consider both as 'introduced' in the invasion continuum (Blackburn et al. 2011). The localized distribution of *Undaria* in the area surveyed suggests that population control is still possible, but these efforts could be compromised if *Styela* is having a facilitative effect. *Styela*, on the other hand, already has a widespread distribution, which makes mechanical control unfeasible. Nevertheless, monitoring and localized control efforts should not be rejected, mainly in ports, since the most prevalent vector in the area is domestic shipping. Finally, it should be noted that if *Styela* has a facilitative effect on *Undaria,* it may be only a matter of time before *Undaria* reaches the areas already colonized by *Styela*.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Survey locations and observations of non-native species.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2015/Supplements/BIR_2015_Pereyra_etal_Supplement.xls