



Seasonal changes in facilitation between an ascidian and a kelp in Patagonia

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ABSTRACT: Positive interactions between species may change in outcome over time and may differ for the two interacting species. We explored the seasonal patterns of facilitation between an ascidian (*Styela clava*) and a macroalga (*Undaria pinnatifida*) by following their association for a complete sporophytic phase. In addition, we investigated how the relationship affected the morphometric parameters of *U. pinnatifida*, and whether the association had a negative outcome for *S. clava*. We performed monthly surveys in San Antonio Bay (Argentina) for nearly 1 yr to evaluate *U. pinnatifida* density (both the total and mature individuals exclusively) on different substrates to determine whether *U. pinnatifida* was more closely associated with *S. clava* than with inert substrates (i.e. consolidated and nonconsolidated). Moreover, we compared the morphometry of *U. pinnatifida* between substrates (*S. clava* vs. rock) and collected *S. clava* individuals overgrown by *U. pinnatifida* and classified them by their holdfast coverage to assess their condition (dead or alive). A higher total density of *U. pinnatifida* was associated with *S. clava* than with other substrates from autumn to spring. *U. pinnatifida* individuals growing on *S. clava* were smaller than those growing on rock. Finally, the probability of *S. clava* dying increased for individuals with higher holdfast coverage, although the scarcity of dead individuals found suggested a negligible influence. Our findings indicate that whereas *U. pinnatifida* clearly benefitted from this interaction by appearing earlier in the field and presenting higher densities that could benefit the population when associated with *S. clava*, for *S. clava* the relationship appeared more neutral.

KEY WORDS: *Styela clava* · *Undaria pinnatifida* · Facilitation · Population-level effect · Individual-level effect · Patagonia · Argentina

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1. INTRODUCTION

Negative interspecific interactions are involved in the structuring of communities (Irving & Bertness 2009, Silliman & He 2018). Nevertheless, in stressful environments, positive interactions may play a significant role as well (Bertness & Callaway 1994, Bruno et al. 2003). Facilitative interactions are often

defined as interspecific, non-trophic interactions that benefit one species and do not harm another (Bruno et al. 2003). Furthermore, it is widely recognized that the outcome of interspecific relationships can change with changing environmental or biological conditions (Silliman & He 2018, Gastaldi et al. 2020). Such changes in the interactions are well documented in marine environments, where habitat-forming species

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have a positive effect (on the biomass or the diversity of species) in high intertidal areas, and neutral effects in the low intertidal zone, both for algae (Molina-Montenegro et al. 2005) and for other benthic organisms (Uyà et al. 2020). In addition, the effects of interspecific interactions can vary throughout the life cycle of the interacting species (Schiffers & Tielbörger 2006, Allegranza et al. 2016, Klanderud et al. 2021), but this phenomenon has seldom been considered in marine environments (Bulleri 2009).

In studies concerning facilitation processes, it has also been interesting to document how the facilitator species may affect the development or growth of the facilitated organism (cf. Peterson et al. 1984, Bulleri 2009, Purcell et al. 2016). For example, in species with density-dependent growth, if the association between those species results in a higher density of individuals (Thomsen et al. 2018), that effect could translate into a limited growth of the facilitated species, i.e. individuals that grow more densely exhibit smaller morphometric parameters (Gao et al. 2014). In addition, studies of facilitative interactions have been dominated by unidirectional approaches (Schöb et al. 2014). Reciprocal effects between interacting species are well documented, though mainly for plant–plant positive interactions, and have demonstrated a wide range of feedbacks (positive, negative, or neutral) by facilitated species on facilitators (Pugnaire et al. 1996, Armas & Pugnaire 2005, Cranston et al. 2012, Schöb et al. 2014). Understanding not only how a facilitation relationship affects population parameters of the facilitated species (e.g. recruitment, growth), but also how the relationship affects the facilitator, is therefore essential.

Most facilitation studies performed in marine environments were carried out with organisms capable of generating dense aggregates or entire new habitats, e.g. mussels, corals, and seagrasses (Gribben et al. 2019). Ascidiates, however, have received less attention as facilitators, despite being capable of providing refuge and resources to other species (Rimondino et al. 2015). On Argentine rocky shores, an interaction occurs between the solitary ascidian *Styela clava* Herdman, 1881 (hereafter *Styela*) and the macroalga *Undaria pinnatifida* (Harvey) Suringar, 1873 (hereafter *Undaria*) in which the latter uses the former as a basibiont (Pereyra et al. 2017). These species are native to northeast Asia (Abbott & Johnson 1972, Akiyama & Kurogi 1982). Both were accidentally introduced, presumably by shipping activity (Piriz & Casas 1994, Pereyra et al. 2015). Through a short-term experiment, Pereyra et al. (2017) provided evidence for facilitation at the start of the sporophytic

phase, but in the present work, we investigated whether shifts in this interaction take place over time. In addition, since *Undaria* grows on a wide variety of substrates, including rock, bivalve shells, and artificial and live substrates (Wotton et al. 2004, Forrest & Blakemore 2006, Thomsen et al. 2018), we were interested in how growth on *Styela* can affect the morphometric parameters of *Undaria*. If *Styela* increases the density of *Undaria* as was observed with other invertebrates (Thomsen et al. 2018), the ascidian could affect the growth of *Undaria* because of the density-dependent growth of the kelp (Gao et al. 2014). Finally, studying the effect of that interaction on both participating species is essential for gaining a better understanding of the possible outcomes in the relationship between the 2 species in this type of facilitation.

We conducted field sampling for 11 mo from the time that the first sporophytes appeared in the area until most became senescent—hereafter called the sporophytic phase—with the objective of assessing changes in the sign of the facilitation between *Styela* and *Undaria*, and how that interaction affects both species. We hypothesized that seasonal changes occur in the facilitative interaction between *Styela* and *Undaria* throughout the sporophytic phase of the macroalga. In this context, we predicted that (1) the density of *Undaria* on *Styela* would be higher than on other available substrates at the beginning of the sporophytic phase of the kelp, but that the pattern would be the opposite when *Undaria* reaches maturity; (2) *Undaria* growing on *Styela* would be smaller than those growing on rock; and (3) the probability of *Styela* dying would be greater as the level of coverage by *Undaria* increases.

2. MATERIALS AND METHODS

2.1. Study area and species investigated

The study was performed in San Antonio Bay, a protected marine area of 80 km², located in the San Matias Gulf, northern Argentine Patagonia (40° 46' S, 64° 54' W). San Antonio Bay is dominated by tidal currents, with a semidiurnal macrotidal regime (up to 9 m amplitude; Alliot et al. 2000) and is characterized by its high diversity of benthic species, mainly mollusks (Güller & Zelaya 2017). Among the Mollusca, mytilids, such as *Brachidontes rodriguezii* and *Perumytilus purpuratus*, are the dominant species on the rocky shores, and although ophiuroids, crabs, snails, sea stars, and sea urchins in addition to algae

such as *Corallina officinalis*, *Ulva* sp., and *Dictyota* sp. are also common, their distributions are patchier (Narvarte 2006).

We carried out the study near the mouth of the bay, a sector that is characterized by a substrate of a wide range of grain sizes, i.e. silt, sand, and gravel, including granules, pebbles, and cobbles (Salas et al. 2016). In this location, the interaction between the studied species was documented for the first time, with both species being found in relatively high abundances (Pereyra et al. 2015). *Undaria* (Phaeophyceae, Laminariales) has a biphasic life cycle with a heteromorphic alternation of generations, i.e. a microscopic gametophyte and a macroscopic sporophyte (Epstein & Smale 2017). *Styela* is a solitary ascidian with an elongated oval body and a long tapering peduncle terminating in a discoid holdfast (Lützen 1998).

2.2. Facilitation throughout the sporophytic phase of *Undaria*

To evaluate the facilitation between *Undaria* and *Styela*, we performed monthly surveys during low tide in the intertidal and shallow subtidal (up to 0.5 m deep), from March 2018 through January 2019. Sampling was carried out using quadrats (30 × 30 cm), arranged in a random, stratified manner. We divided the study area into 2 sectors according to the dominant substrate, namely (1) a consolidated substrate sector (dominated by rocky outcrops) and (2) a non-consolidated substrate sector (dominated by sand and cobble in different proportions), and randomly deployed 50 quadrats in each one every month. For each quadrat, we counted the number of individuals of *Undaria*, and registered the occurrence of *Styela* in order to obtain the number of *Undaria* growing in quadrats with and without *Styela* in each sector. We collected all individuals of *Undaria* in the quadrats for further processing in the laboratory, where they were preserved in aquaria with aerated seawater until further processing (from 5 to 10 h). Each *Undaria* was classified by its developmental stage after Casas et al. (2008): stage 0, no stipe or defined holdfast; stage 1, poorly developed holdfast; stage 2, sporophyll not yet developed; stage 3, fully developed sporophyll.

We evaluated the total density of *Undaria* and the density of the mature macroalga (stage 3) relative to the substrate throughout the sporophytic phase. The variable substrate was defined as having 3 levels: (1) *Styela*: quadrats from the consolidated sector containing *Styela* individuals; (2) consolidated substrate: quadrats from the consolidated sector with no *Styela*;

and (3) nonconsolidated substrate: quadrats from the nonconsolidated sector with no *Styela* present (Fig. 1). Because we found only 4 quadrats from the nonconsolidated sector containing *Styela*, we excluded this circumstance as a fourth level from the analysis. We used a Scheirer-Ray-Hare test, an extension of the Kruskal-Wallis test (Sokal & Rohlf 1995) because the data were zero-inflated and presented highly heterogeneous variances (Levene test, $p < 0.001$) and thus did not conform the parametric test assumptions that could be corrected by data transformation. The Scheirer-Ray-Hare test was performed with the function 'scheirerRayHare' in the R package 'rcompanion' (Mangiafico 2021) in a crossed design involving 'sampling month' and 'substrate' as independent variables, and the number of *Undaria* individuals as the response variable.

We also compared the number of *Undaria* individuals between substrates and months. We compared *Styela* vs. consolidated substrate to evaluate the use of *Styela* as substrate. In addition, we compared the use of consolidated vs. nonconsolidated substrate to determine if *Undaria* was more closely associated with hard substrates, as is usually described for the species (Epstein & Smale 2017). These analyses were performed with Kruskal-Wallis tests through the use of the 'Kruskal-test' function in the R package 'stats' (R Core Team 2020).

2.3. Morphometric differences in *Undaria*

To evaluate the potential effect of *Styela* as a basibiont on the morphometry of *Undaria*, we randomly collected mature individuals (stage 3) of *Undaria* growing atop *Styela* and on rock ($n = 14$ on each substrate) in July 2018. For each *Undaria* individual, we measured (in mm) lamina length, lamina width, stipe length, stipe width, and sporophyll width with a measuring tape. In addition, the total wet and dry weights and the sporophyll wet weight (in g) were registered. For the dry weight, individuals were weighed after drying in an oven at 60°C for 48 h. The different measurements used in this study are those that are usually considered in ecological and commercial studies to characterize algal growth (Casas et al. 2008, Gao et al. 2014, de Leij et al. 2017).

Morphometric variables were compared between *Undaria* growing on *Styela* and on a rocky substrate with a multivariate permutational analysis of variance (PERMANOVA) by means of the 'adonis' function in the R package 'vegan' (Oksanen et al. 2019), through the use of Euclidean distances and 999

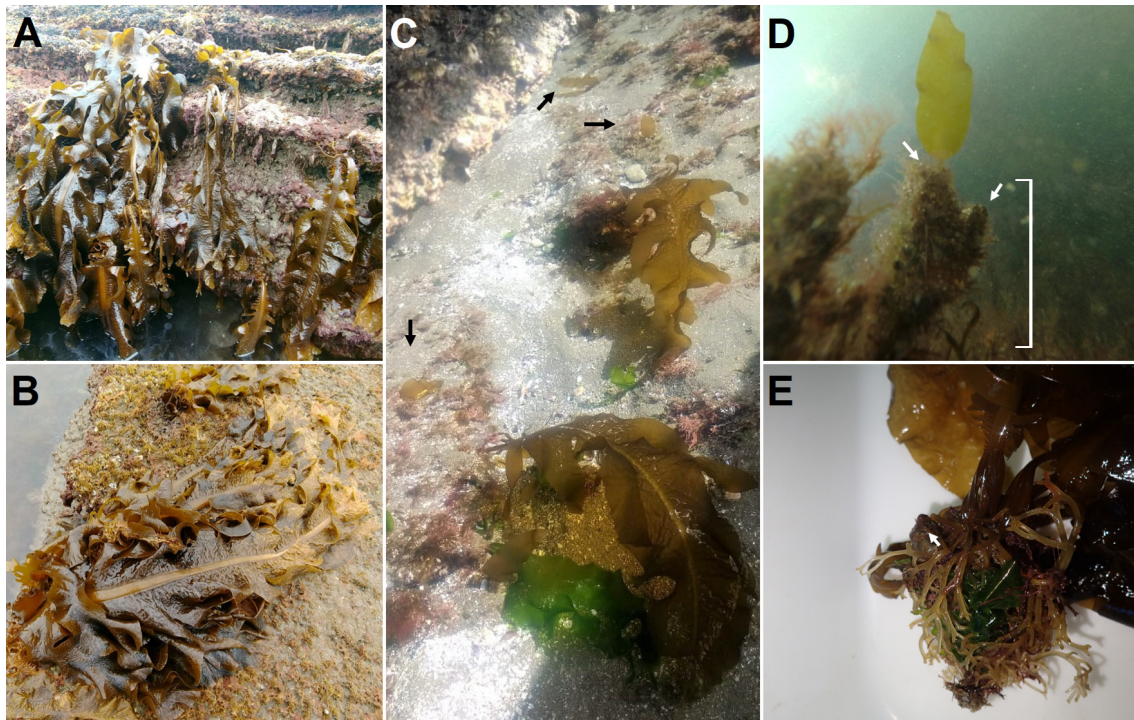


Fig. 1. *Undaria pinnatifida* growing on each of the substrates evaluated: (A,B) consolidated substrate, (C) nonconsolidated substrate (black arrows point to recruits), (D,E) *Styela clava* (white arrows point to *S. clava* siphons and the white bracket demarcates the *S. clava* body)

unrestricted permutations of the raw data. The variables were standardized after Becker et al. (1998), with the scale function from the R base package (R Core Team 2020). The assumption of multivariate homogeneity of group dispersion (variances) was tested with the 'permutest' function (Anderson 2017) in the package 'vegan'. As a post hoc test, a SIMPER analysis was used to identify the main morphometric response variables contributing to the dissimilarity by means of the simper function of the R package 'vegan'.

2.4. Holdfast coverage of *Undaria* on *Styela*

To assess the effect of *Undaria* overgrowth on *Styela*, individuals of *Styela* that were overgrown by *Undaria* were randomly collected every month during low tide, from April 2018 through January 2019 (throughout the sporophytic phase of *Undaria*). Colonized individuals of *Styela* were collected in a different shallow subtidal zone (up to 0.5 m deep) from the one described in Section 2.2, to avoid a potential disturbance in the sampling area. Individuals of *Styela* were classified in the following manner in accordance with their overgrowth (i.e. the percent cover-

age) by *Undaria* holdfasts: (I) 0–25%, *Styela* with a small *Undaria* individual without a holdfast; (II) 25–50%, *Undaria* holdfast covering up to half of the *Styela* surface; (III) 50–75%, holdfast covering more than half of the *Styela* surface, but the siphons remaining uncovered; (IV) 75–99%, holdfast covering everything except the *Styela* peduncle; (V) 100%, *Styela* totally covered; for more details, see Table S1 in the Supplement, www.int-res.com/articles/suppl/m693p095_supp.pdf). The condition of *Styela* upon collection (i.e. dead vs. alive) was also assessed. Individuals that responded to being pricked with a probe, either by opening and closing the siphons or the expulsion of water through the siphons or by remaining turgid when squeezed, were considered alive; otherwise, they were regarded as dead.

We used a generalized linear mixed model (GLMM; Bolker et al. 2009) to identify the effect of the *Undaria* holdfast coverage on *Styela*. This test is an extension of a generalized linear model that allows the violation of independence assumption between observations. The levels of coverage were used as the fixed factor and the condition of *Styela* as the response variable with binomial error distribution. The *Undaria* holdfast coverage occurred at 5 levels and was considered an ordered factor because the state of the

holdfast overgrowth was sequential, i.e. the holdfast in level 5 had to have gone through levels 4 and 3 previously. To deal with temporal autocorrelation, the month of collection was included as a random factor. The effects of holdfast overgrowth were assessed through the use of Akaike's information criterion (Burnham & Anderson 2002) by means of the function 'aictab' in the R package 'AICcmodavg' (Mazerolle 2020). GLMMs were calculated through the 'glmer' function in the R package 'lmer4' (Bates et al. 2015). All analyses were performed in R version 3.6.3 (R Core Team 2020).

3. RESULTS

3.1. Facilitation throughout the sporophytic phase of *Undaria*

Upon evaluation of the total density of *Undaria*, the data revealed an interaction between month and substrate use (Schierer-Ray-Hare test, $H_{20,1058} = 63.230$, $p < 0.001$) suggesting that the density of *Undaria* on

each substrate was dependent on the month of the year (Fig. 2).

For simplicity, we express the differences in *Undaria* density by citing 1 month per season. Those months, accordingly, were representative of the differences evidenced for the remaining months of each season (cf. Tables S2–S5 for a detailed comparison of every month). In autumn (April), the density of *Undaria* was 97 % higher in the *Styela* quadrats than in the consolidated-substrate quadrats (Kruskal-Wallis, April: $H_1 = 37.4$, $p < 0.001$; Fig. 2). Similar results were found in winter (July), with a 24 % higher density in the *Styela* quadrats than in the consolidated-substrate quadrats (Kruskal-Wallis, July: $H_1 = 4.9$, $p = 0.026$) and 72 % higher in the consolidated-substrate quadrats than in the nonconsolidated-substrate quadrats (Kruskal-Wallis, July: $H_1 = 5.7$, $p = 0.016$). In spring (October), the density of *Undaria* was 28 % higher in the *Styela* quadrats than in the consolidated-substrate quadrats (Kruskal-Wallis, October: $H_1 = 4.7$, $p = 0.029$). Finally, in summer (January), we found no evidence that *Undaria* density in the consolidated-substrate quadrats differed from that in the *Styela* quadrats (Kruskal-Wallis, January: $H_1 = 3.5$, $p = 0.061$) or the nonconsolidated-substrate quadrats (Kruskal-Wallis, January: $H_1 = 1.6$, $p = 0.212$).

Upon observing the density of the different developmental stages of *Undaria* associated with each evaluated substrate, we found that density was higher in *Styela* quadrats for every developmental stage (Fig. 3). Mature *Undaria* occurred from only June onwards. These results also revealed an interactive effect of substrate and month on the density of mature *Undaria* (Schierer-Ray-Hare test, $H_{20,1058} = 42.3$, $p = 0.002$), suggesting that the density of mature *Undaria* associated with the different evaluated substrates varies throughout the evaluated months. Mature *Undaria* were affected by substrate only during June and August, exhibiting a 35 and 39 % higher density, respectively, in the consolidated-substrate quadrats than in the nonconsolidated-substrate quadrats (Kruskal-Wallis, June: $H_1 = 4.8$, $p = 0.027$; August: $H_1 = 4.3$, $p = 0.02$; see Table S4 for details). Throughout the study period, the densities of mature

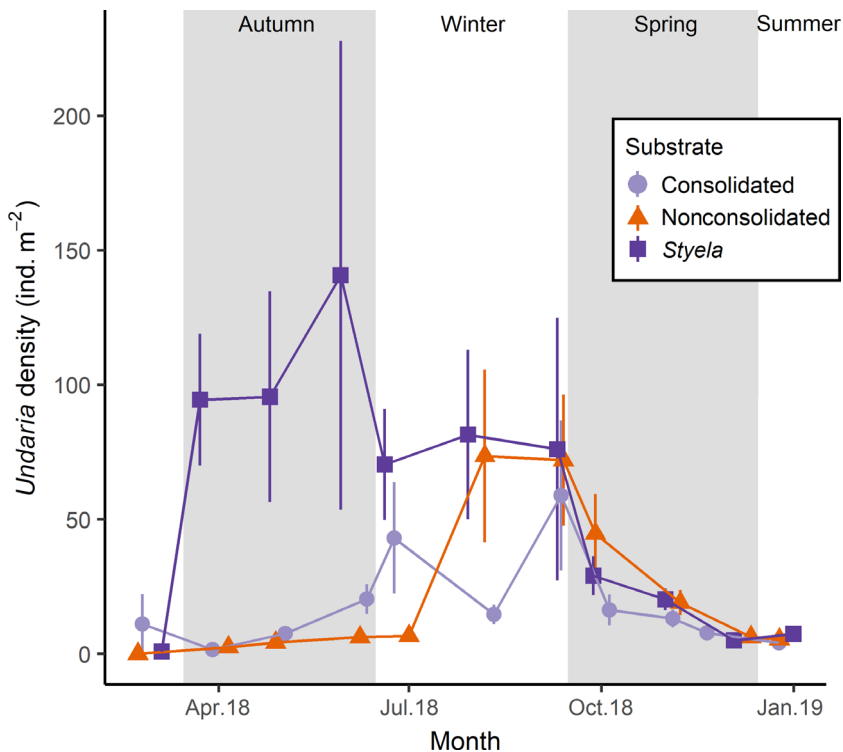


Fig. 2. *Undaria pinnatifida* density on different substrates through the sporophytic phase (mean \pm SE). Shaded and unshaded areas mark the seasons. Symbols are jittered to reduce the overlap of points and error bars. The months on the x-axis are those for which results are provided in the main text. $n = 1100$ quadrats

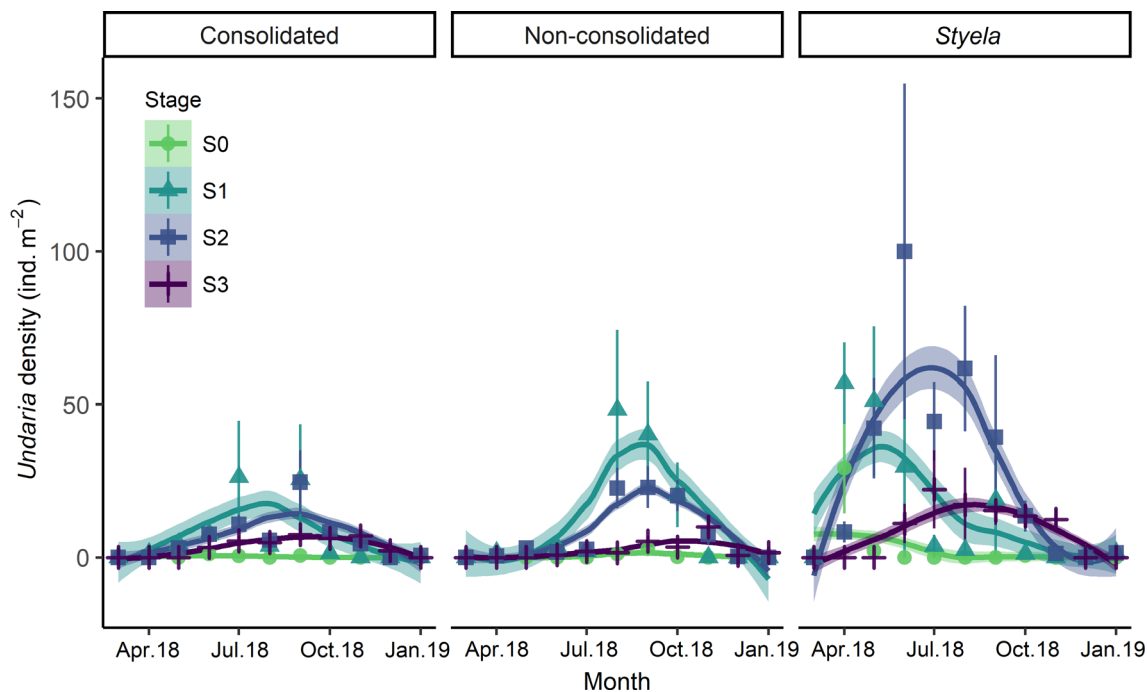


Fig. 3. *Undaria pinnatifida* density at each developmental stage on different substrates throughout the sporophytic phase. Stages are: 0, no stipe or defined holdfast; 1, poorly developed holdfast; 2, sporophyll not yet developed; 3, fully developed sporophyll. n = 1100 quadrats

Undaria were not significantly different between the *Styela* and the consolidated substrates (see Table S5 for details).

3.2. Morphometric differences in *Undaria*

Our data revealed that *Undaria* individuals growing on *Styela* were morphometrically different from those growing on rock (PERMANOVA, $F_{1,28} = 9.9$, $p = 0.001$). *Undaria* growing on *Styela* were smaller than those growing on rock with respect to all metrics used (Fig. 4), and all the morphometric variables evaluated contributed to the dissimilarity (SIMPER, $p \leq 0.01$), except the stipe width (SIMPER, $p = 0.05$).

3.3. *Undaria* holdfast coverage on *Styela*

We examined 270 individuals of *Styela* with different levels of *Undaria* holdfast coverage. Only 12 of the *Styela* individuals collected (4.4%) were dead (Fig. 5). However, the data revealed an association between *Undaria* holdfast coverage on the condition of *Styela* (Table 1), although the size of the effect was very small since only 13% of the individuals from categories IV or V were dead (Fig. 5).

4. DISCUSSION

Our results suggest that, although the facilitation between *Styela* and *Undaria* is maintained throughout the sporophytic phase, changes occur in that interaction. *Undaria* sporophytes are found in the substrates in a differing fashion during the year-long phase, with the total density of *Undaria* in *Styela* quadrats being higher for almost the entire time—except in summer, when this pattern changes and the total densities become similar on all of the substrates evaluated. In addition, from the first half of the sporophytic phase until the end, the density of mature individuals of *Undaria* is higher in quadrats with *Styela* than in any other substrate quadrats. Our observations indicate that *Styela* facilitates *Undaria* during the entire sporophytic phase—first with a higher density of juveniles of *Undaria* and then with a greater proportion of mature individuals—by serving as a beneficial substrate. Nevertheless, *Undaria* growing on *Styela* ends up being smaller than when growing on a rocky substrate, a difference which might be interpreted as a negative effect of this relationship. In addition, our results did not suggest any negative effect from the interaction on *Styela* because, although the higher proportion of dead *Styela* individuals presented a high degree of coverage by

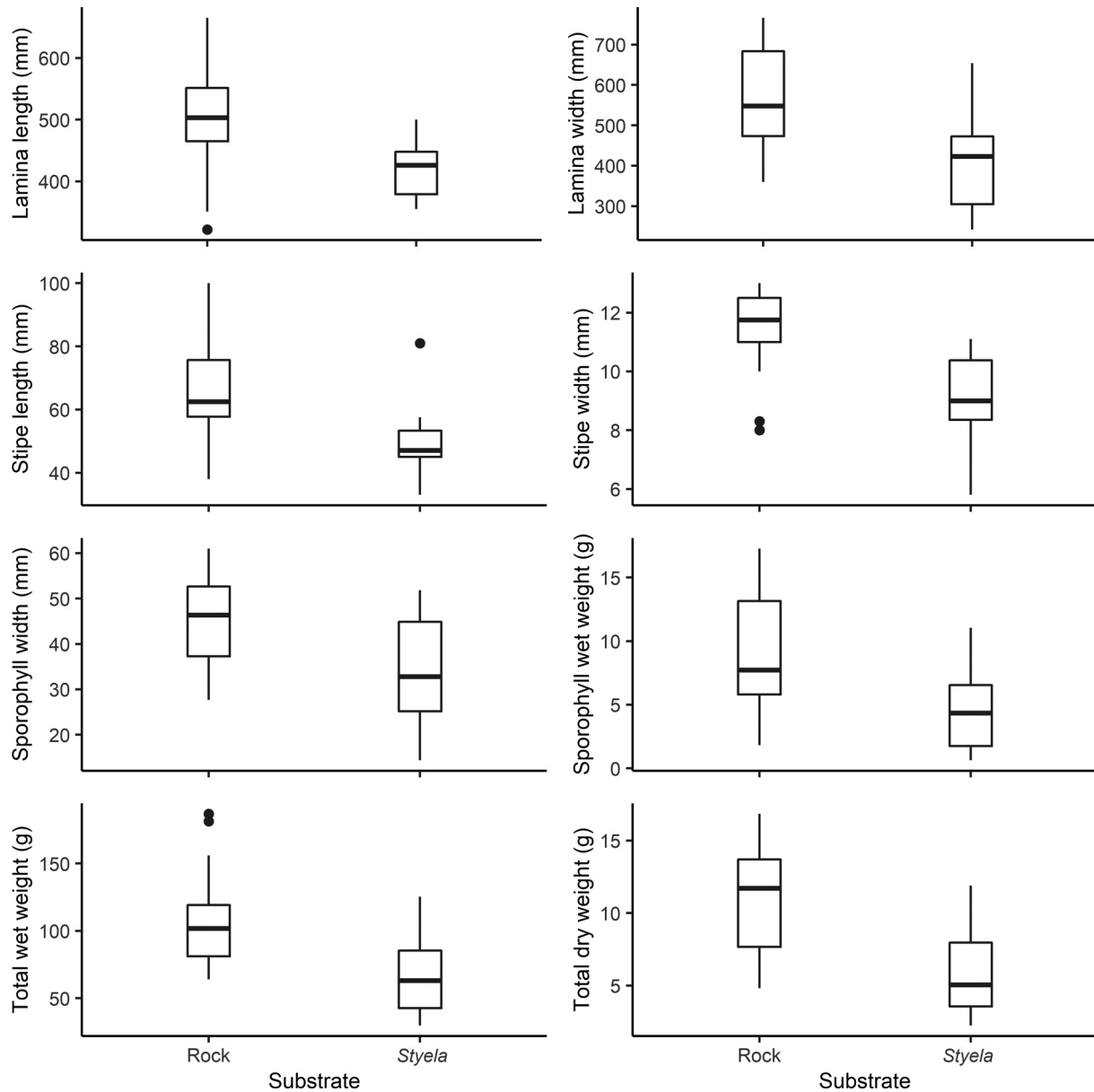


Fig. 4. Morphometric differences between *Undaria pinnatifida* growing on rock and on *Styela clava*. Each box represents 50% of the central data and the thick horizontal line denotes the median. The whiskers represent maximum and minimum values without the outliers, and the black points mark the outliers. $n = 14$ individuals of *U. pinnatifida* per substrate

Undaria, the number of dead individuals of *Styela* found in the current study was still quite low.

4.1. Facilitation throughout the sporophytic phase of *Undaria*

Undaria was more associated with *Styela* during the first half of the sporophytic phase (autumn to early spring), but this decreased at the end of the sporophytic phase to the point of being indistinguish-

able from other substrates by summer. Nonetheless, an evaluation of the number of individuals of *Undaria* reaching maturity indicated that *Styela* quadrats still had the highest densities of mature *Undaria*.

The presence of *Undaria* almost exclusively in *Styela* quadrats at the beginning of the sporophytic phase could favor the kelp competitively (Thompson & Schiel 2012). *Undaria* is considered a poor competitor for space, and in the presence of other canopy-forming macroalgae, it cannot readily become established in new environments (Thompson & Schiel 2012).

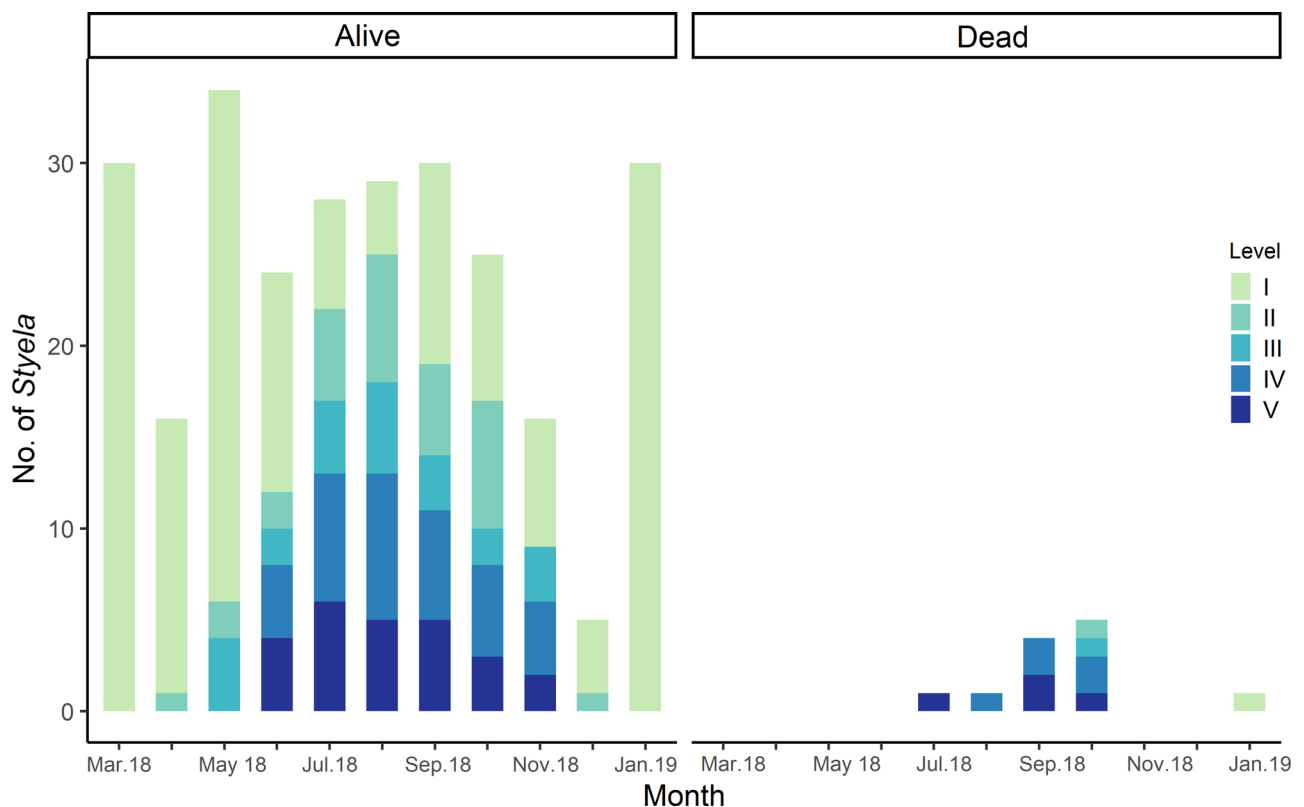


Fig. 5. *Styela clava* condition (alive vs. dead) in relation to the level of *Undaria pinnatifida* overgrowth represented by the *U. pinnatifida* holdfast coverage throughout the study period (coverage Category I: 0–25% of *Styela* surface covered by *U. pinnatifida*; other coverage categories as in Table 1)

If that establishment does occur, it is only transient (South et al. 2015). Thus, growing associated with *Styela* could give *Undaria* a competitive advantage by providing an opportunity to emerge before other algae. In a similar study, Thomsen et. al. (2018) reported that mussels could facilitate *Undaria*, thus resulting in the maintenance of kelp cover through the sporophytic phase. Something similar may occur in the present study site, with *Styela* benefitting *Undaria* by facilitating it in periods when the cover or abundance of the kelp is negligible. Since solitary tunicates have the ability to increase organic matter deposits via biodeposition (Qi et al. 2015), the high density of *Undaria* recruits found growing in *Styela* quadrats at the beginning of the sporophytic phase could be due to the biological properties of *Styela* compared to bare substrate (Qi et al. 2015, Pereyra et al. 2021).

The density of *Undaria* in *Styela* quadrats decreases from the middle to the end of the sporophytic phase. This pattern may be related to the high mortality of recruits in kelps, which may be even higher when initial densities are high (Schiel & Foster 2006). In addition, kelp fecundity and recruitment are consid-

ered high, but the survival of recruits is extremely low (Primo et al. 2010). Nevertheless, the decrease in the population density of *Undaria* could be due to the natural death of individuals, as mortality is common in summer as a result of rising temperatures (Thornber et al. 2004, Casas et al. 2008).

Table 1. Parameter estimates \pm SE and 95% confidence interval limits (CL) for explanatory variables describing variation in the probability of *Styela clava* individuals dying relative to the level of coverage by *Undaria pinnatifida* (Category II: 25–50% of the *Styela* surface covered by *Undaria* holdfasts; III: 50–75% coverage; IV: 75–99% coverage; V: 100%, *Styela* completely covered). Significant ($p < 0.05$) explanatory variables are represented in **bold**

Explanatory variable (coverage)	Parameter estimate	CL	
		Lower	Upper
Intercept	5.405 \pm 1.149	3.69	8.65
Category II	–1.534 \pm 1.489	–4.88	1.79
Category III	–2.042 \pm 1.495	–5.44	1.28
Category IV	–3.189 \pm 1.196	–6.31	–1.15
Category V	–3.312 \pm 1.227	–6.48	–1.19

Based on our results, this interaction could be understood as a zero-sum game, where the benefits of the first half of the sporophytic cycle are canceled by the end of the cycle. Notwithstanding, even if the total density of *Undaria* individuals growing on *Styela* quadrats decreased to become equal to densities of the other substrates, the proportion of mature individuals was still higher on *Styela* quadrats than on any other substrate quadrats, a difference that implies *Styela* presence leads to more individuals with the ability to reproduce. Considering all of these different aspects, we conclude that *Undaria* benefits from *Styela* from the standpoint of the overall population level of the kelp during the entire sporophytic phase.

4.2. Morphometric differences in *Undaria*

Undaria individuals that grew on *Styela* were smaller than those growing on rock. This difference was expected because kelp species typically exhibit high morphological plasticity (Wernberg & Thomsen 2005, Fowler-Walker et al. 2006), and higher densities of *Undaria* growing on *Styela* might translate into smaller morphometric structures as a result of limited space (Schiel & Foster 2006, Gao et al. 2014). Competition for space could have consequences not only for growth of individual *Undaria*, but also for its reproductive success (de Leij et al. 2017). The sporophyll is the reproductive structure of *Undaria*, and, as spore production is correlated with sporophyll area (Primo et al. 2010), the smaller sporophylls of *Undaria* growing on *Styela* may have lower fitness (or spore production) than those growing on rock. Nevertheless, to speculate how this difference would affect the reproductive potential of *Undaria* would be premature, and should be done carefully until the effect on the fitness of the kelp is evaluated. Another likely explanation for the reduced size of *Undaria* on *Styela* (vs. on rock) may be a selective dislodgment process by the strong tidal currents in the main channel, which affects mainly large individuals (cf. Thomsen 2004). However, we did not see dead individuals of *Undaria* attached to *Styela* when they were dislodged following the greatest spring tide. For this reason, it seems unlikely that *Undaria* is dislodged more when attached to *Styela* than to other substrates.

While growing on *Styela* could be detrimental for individual growth of *Undaria*, the higher densities on that substrate could be beneficial for the *Undaria* population as a whole (Schiel & Foster 2006). For kelps, high population density may increase dispersal distances as well as spore and gametophyte den-

sities, enhance fertilization, reduce physical stress in intertidal habitats, and suppress the recruitment and growth of competitors (McConnico & Foster 2005, Schiel & Foster 2006, Tatsumi et al. 2022). Our results suggest that, even when the *Undaria* individuals growing on *Styela* were smaller, the density of the mature stages was higher on *Styela* than on other substrates, implying that *Styela* could boost the population growth of *Undaria*. We note that the sample size for comparing the morphometry of *Undaria* growing on *Styela* and rock was small ($n = 14$ per substrate), and thus the conclusions should be accepted with caution. The estimation of the effect size, however, has a high certainty (Baguley 2004) because the differences between the groups were verified statistically.

4.3. *Undaria* holdfast coverage on *Styela*

Our study revealed a higher proportion of dead individuals when *Undaria* holdfasts covered *Styela* completely. Despite this, in view of the low proportion of dead *Styela* found, we conclude that the ascidian tolerates being covered by the *Undaria* holdfast, contrary to what was originally hypothesized by Pereyra et al. (2017). As result, we propose that, with respect to lethality, the outcome of the interaction for *Styela* was essentially neutral.

Although ascidians could have defenses against epibiosis, including the presence of antifouling compounds, such as vanadium, on their tunics (Stoecker 1978, Hirose et al. 2001), they are capable of surviving overgrowth by epibionts if their siphons are not covered (Claar et al. 2011). Despite this, we found live *Styela* individuals with their siphons fully covered by *Undaria* holdfasts. The high degree of epibiosis that *Styela* presents (Rodríguez 2020) could be due to the very low concentration of vanadium and consequently, its poor antifouling defenses (Curtin et al. 1985). In addition, *Styela* has irregular longitudinal wrinkles and grooves in the tunic, features that would favor the accumulation of organic matter and thus facilitate the settlement of epibionts even more (Filip 2020).

Despite these observations, the possible outcomes of the interaction for *Styela* need to be experimentally tested. While we only examined lethal outcomes of the coverage by *Undaria*, *Styela* individuals could experience a reduction in fitness if, for example, their reproductive output is compromised by affecting gonad development, spawning, and recruitment (Bourque et al. 2007). In addition, other aspects as the dislodgment of *Styela* should be tested, because

this could be modified (as the strength of attachment) by the epibiosis of *Undaria*. It is important to note that our survey did not allow us to determine the cause of death of *Styela*. For example, the age of the *Styela* individuals could have influenced our results, but we think this is unlikely, since *Styela* has a relatively short life cycle (up to 2 yr; Morris et al. 1980, Lambert & Lambert 1998) and a relatively low growth rate (1–15 mm mo⁻¹; Morris et al. 1980). Thus, since the collected *Styela* individuals were about the same size, it was unlikely that they belonged to different cohorts.

Lastly, positive effects on *Styela* should also be tested. Positive outcomes for the basibiont have been described before in marine environments (Thomsen et al. 2022 and references therein). *Styela* could benefit from the interaction by a reduction in predation or physical stress that *Undaria* may offer. Ascidians are commonly preyed on (Giachetti et al. 2022), and we have regularly observed in the field dead individuals of *Styela* showing signs of predation, but this was not the case with the individuals tested here. A possibility that remains to be explored is that *Undaria* offers protection against predation to *Styela* by growing over it, as has been documented for similar cases, e.g. *Microcosmus sabatieri* overgrown by an encrusting sponge (Voultsiadou et al. 2010). Thus, to determine the net effect of the interaction for *Styela*, we need to better understand the effects on the ascidian, both positive and negative, beyond lethal effects.

4.4. Varied outcomes of the interaction

The current study adds evidence documenting changes in outcome and magnitude of the effects occurring in the facilitation relationship between *Styela* and *Undaria* over time, as related to biotic or abiotic contexts. However, a gap still remains in our understanding of how these interactions manifest at other marine sites because context dependency could well be related not only to time but also to the site where the interaction occurs (Catford et al. 2022). *Undaria* using *Styela* as a basibiont has been documented in other locations, but the interaction was not the focus of the study (Thornber et al. 2004) or was studied as competition (Farrell & Fletcher 2006). In general, our results provide evidence for how facilitation changes over time in a marine system, at least for *Undaria*, and contribute new observations with respect to how the interaction affects both species. *Undaria* seems to benefit from its association with *Styela*, although the effects on reproductive outcome

will need further investigation. *Styela* does not seem to be harmed by *Undaria*, although possible negative effects not tested in this study should be further evaluated. Since the 2 species have only recently been introduced into San Antonio Bay, *Undaria* and *Styela* are still adapting to their interactions with resident species, as well as to the new abiotic environment. Perhaps the plasticity of *Undaria* and the facilitative interaction with *Styela* are only 2 adaptive responses to this new set of selective pressures among others that can aid the 2 species in the successful invasion of this new environment in Patagonia.

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LITERATURE CITED

- Abbott PD, Johnson JV (1972) The ascidians *Styela barnharti*, *S. plicata*, *S. clava* and *S. montereyensis* in Californian waters. *Bull S Calif Acad Sci* 71:95–105
- Akiyama K, Kurogi M (1982) Cultivation of *Undaria pinnatifida* (Harvey) Suringar, the decrease in crops from natural plants following crop increase from cultivation. *Bull Tohoku Reg Fish Res Lab* 44:91–100
- Allegrezza M, Corti G, Cocco S, Pesaresi S, Chirico GB, Saracino A, Bonanomi G (2016) Microclimate buffering and fertility island formation during *Juniperus communis* ontogenesis modulate competition–facilitation balance. *J Veg Sci* 27:616–627
- Alliata S, Schnack EJ, Isla FI, Lizasoain GO (2000) Desarrollo secuencial de formas de fondo en un régimen macromareal. *Lat Am J Sedimentol Basin Anal* 7:95–107
- Anderson MJ (2017) Permutational multivariate analysis of variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online* 2017:1–15
- Armas C, Pugnaire FI (2005) Plant interactions govern population dynamics in a semi-arid plant community. *J Ecol* 93:978–989
- Baguley T (2004) Understanding statistical power in the context of applied research. *Appl Ergon* 35:73–80
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Becker RA, Chambers JM, Wilks AR (1998) The new S language: a programming environment for data analysis and graphics. *Wadsworth & Brooks/Cole*, Pacific Grove, CA
- Bertness MD, Callaway R (1994) Positive interactions in communities. *Trends Ecol Evol* 9:191–193
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear

- mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135
- ✦ Bourque D, Davidson J, MacNair NG, Arsenault G, LeBlanc AR, Landry T, Miron G (2007) Reproduction and early life history of an invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. *J Exp Mar Biol Ecol* 342:78–84
- ✦ Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends Ecol Evol* 18: 119–125
- ✦ Bulleri F (2009) Facilitation research in marine systems: state of the art, emerging patterns and insights for future developments. *J Ecol* 97:1121–1130
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference—a practical information-theoretic approach, 2nd edn. Springer, New York, NY
- ✦ Casas G, Piriz ML, Parodi ER (2008) Population features of the invasive kelp *Undaria pinnatifida* (Phaeophyceae: Laminariales) in Nuevo Gulf (Patagonia, Argentina). *J Mar Biol Assoc UK* 88:21–28
- ✦ Catford JA, Wilson JR, Pyšek P, Hulme PE, Duncan RP (2022) Addressing context dependence in ecology. *Trends Ecol Evol* 37:158–170
- ✦ Claar DC, Edwards KF, Stachowicz JJ (2011) Positive and negative effects of a dominant competitor on the settlement, growth, and survival of competing species in an epibenthic community. *J Exp Mar Biol Ecol* 399:130–134
- ✦ Cranston BH, Callaway RM, Monks A, Dickinson KJM (2012) Gender and abiotic stress affect community-scale intensity of facilitation and its costs. *J Ecol* 100:915–922
- ✦ Curtin MA, Kustin K, Robinson WE (1985) Iron accumulation in tunicate blood cells. II. Whole body and blood cell iron uptake by *Styela clava*. *Biol Bull (Woods Hole)* 169: 152–163
- ✦ de Leij R, Epstein G, Brown MP, Smale DA (2017) The influence of native macroalgal canopies on the distribution and abundance of the non-native kelp *Undaria pinnatifida* in natural reef habitats. *Mar Biol* 164:156
- ✦ Epstein G, Smale DA (2017) *Undaria pinnatifida*: a case study to highlight challenges in marine invasion ecology and management. *Ecol Evol* 7:8624–8642
- ✦ Farrell P, Fletcher RL (2006) An investigation of dispersal of the introduced brown alga *Undaria pinnatifida* (Harvey) Suringar and its competition with some species on the man-made structures of Torquay Marina (Devon, UK). *J Exp Mar Biol Ecol* 334:236–243
- Filip MM (2020) Epibiota comparison between two biogenic substrates: the non-native tunicate *Styela clava* and the native blue mussel *Mytilus edulis*. MSc dissertation, University of Bremen
- ✦ Forrest BM, Blakemore KA (2006) Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257:333–345
- ✦ Fowler-Walker MJ, Werneberg T, Connell SD (2006) Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? *Mar Biol* 148:755–767
- ✦ Gao X, Endo H, Taniguchi K, Agatsuma Y (2014) Effects of experimental thinning on the growth and maturation of brown alga *Undaria pinnatifida* (Laminariales; Phaeophyta) cultivated in Matsushima Bay, northern Japan. *J Appl Phycol* 26:529–535
- ✦ Gastaldi M, Firstater FN, Romero MA, Pereyra PJ, Narvarte MA (2020) Seasonality dictates changes in the ecological interactions among spatial dominants. *Mar Biol* 167:176
- ✦ Giachetti CB, Battini N, Castro KL, Schwindt E (2022) The smaller, the most delicious: differences on vulnerability to predation between juvenile and adult of invasive ascidians. *Estuar Coast Shelf Sci* 268:107810
- Gribben PE, Angelini C, Altieri AH, Bishop MJ, Thomsen MS, Bulleri F (2019) Facilitation cascades in marine ecosystems: a synthesis and future directions. *Oceanogr Mar Biol Annu Rev* 57:127–168
- ✦ Güller M, Zelaya DG (2017) A hot-spot of biodiversity in Northern Patagonia, Argentina. *Biodivers Conserv* 26: 3329–3342
- Hirose E, Yamashiro H, Mori Y (2001) Properties of tunic acid in the ascidian *Phallusia nigra* (Asciidiidae, Phlebobranchia). *Zool Sci* 18:309–314
- ✦ Irving AD, Bertness MD (2009) Trait-dependent modification of facilitation on cobble beaches. *Ecology* 90:3042–3050
- ✦ Klanderud K, Meiner E, Goldberg DE, Michel P, Berge A, Guittar JL, Vandvik V (2021) Vital rates in early life history underlie shifts in biotic interactions along bioclimatic gradients: an experimental test of the Stress Gradient Hypothesis. *J Veg Sci* 32:e13006
- ✦ Lambert CC, Lambert G (1998) Non-indigenous ascidians in southern California harbors and marinas. *Mar Biol* 130: 675–688
- ✦ Lützen J (1998) *Styela clava* Herdman (Urochordata, Asciacea), a successful immigrant to North West Europe: ecology, propagation and chronology of spread. *Helgol Meeresunters* 52:383–391
- ✦ Mangiafico S (2021) rcompanion: Functions to support extension education program evaluation. <http://rcompanion.org>
- ✦ Mazerolle MJ (2020) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). <https://cran.r-project.org/package=AICcmodavg>
- ✦ McConnico LA, Foster MS (2005) Population biology of the intertidal kelp, *Alaria marginata* Postels and Ruprecht: a non-fugitive annual. *J Exp Mar Biol Ecol* 324:61–75
- ✦ Molina-Montenegro MA, Muñoz AA, Badano EI, Morales BW, Fuentes KM, Cavieres LA (2005) Positive associations between macroalgal species in a rocky intertidal zone and their effects on the physiological performance of *Ulva lactuca*. *Mar Ecol Prog Ser* 292:173–180
- Morris RH, Abbott DA, Haderlie EC (1980) Intertidal invertebrates of California. Stanford University Press, Stanford, CA, p 207–208
- Narvarte MA (2006) Biology and fishery of the whelk *Buccinanops globulosum* (Kiener, 1834) in northern coastal waters of the San Matías Gulf (Patagonia, Argentina). *Fish Res* 77:131–137
- ✦ Oksanen J, Blanchet GF, Friendly M, Kindt R and others (2019) vegan: community ecology package. <https://github.com/vegandevs/vegan>
- ✦ Pereyra PJ, Narvarte MA, González R (2015) The simultaneous introduction of the tunicate *Styela clava* (Herdman, 1881) and the macroalga *Undaria pinnatifida* (Harvey) Suringar, 1873, in northern Patagonia. *Biol Invasions Rec* 4:179–184
- ✦ Pereyra PJ, de la Barra P, Gastaldi M, Saad JF, Firstater FN, Narvarte MA (2017) When the tiny help the mighty: facilitation between two introduced species, a solitary ascidian and a macroalga in northern Patagonia, Argentina. *Mar Biol* 164:185
- ✦ Pereyra PJ, de la Barra P, Saad JF, Gastaldi M and others (2021) Unravelling facilitation among introduced species, a mechanistic approach. *Biol Invasions* 23:3483–3496

- ✦ Peterson CH, Summerson HC, Duncan PB (1984) The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, *Mercenaria mercenaria*. J Mar Res 42:123–138
- Piriz ML, Casas G (1994) Occurrence of *Undaria pinnatifida* in Golfo Nuevo, Argentina. Applied Phycology Forum 10:4
- ✦ Primo C, Hewitt CL, Campbell ML (2010) Reproductive phenology of the introduced kelp *Undaria pinnatifida* (Phaeophyceae, Laminariales) in Port Phillip Bay (Victoria, Australia). Biol Invasions 12:3081–3092
- ✦ Pugnaire FI, Haase P, Puigdefabregas J (1996) Facilitation between higher plant species in a semiarid environment. Ecology 77:1420–1426
- ✦ Purcell S, Conand C, Uthicke S, Byrne M (2016) Ecological roles of exploited sea cucumbers. Oceanogr Mar Biol Annu Rev 54:367–386
- ✦ Qi Z, Han T, Zhang J, Huang H, Mao Y, Jiang Z, Fang J (2015) First report on *in situ* biodeposition rates of ascidians (*Ciona intestinalis* and *Styela clava*) during summer in Sanggou Bay, northern China. Aquacult Environ Interact 6:233–239
- R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- ✦ Rimondino CM, Torre L, Sahade R, Tatián M (2015) Sessile macro-epibiotic community of solitary ascidians, ecosystem engineers in soft substrates of Potter Cove, Antarctica. Polar Res 34:24338
- Rodríguez EA (2020) Macrofauna epibionte de la ascidia solitaria *Styela clava* (Herdman, 1881) en el Golfo San Matías. Thesis, Escuela Superior de Ciencias Marinas, Universidad Nacional del Comahue, San Antonio Oeste
- ✦ Salas MC, Defeo O, Narvarte MA (2016) Attachment features of mytilids in ecosystems with mobile substrate: *Brachidontes rodriguezii* in San Antonio Bay (Patagonia, Argentina). J Mar Biol Assoc UK 96:1449–1456
- ✦ Schiel DR, Foster MS (2006) The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. Annu Rev Ecol Syst 37:343–372
- ✦ Schippers K, Tielbörger K (2006) Ontogenetic shifts in interactions among annual plants. J Ecol 94:336–341
- ✦ Schöb C, Michalet R, Cavieres LA, Pugnaire FI and others (2014) A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs. New Phytol 202:95–105
- ✦ Silliman BR, He Q (2018) Physical stress, consumer control, and new theory in ecology. Trends Ecol Evol 33:492–503
- Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. W.H. Freeman, New York, NY
- ✦ South PM, Lilley SA, Tait LW, Alestra T, Hickford MJH, Thomsen MS, Schiel DR (2015) Transient effects of an invasive kelp on the community structure and primary productivity of an intertidal assemblage. Mar Freshw Res 67:103–112
- ✦ Stoecker D (1978) Resistance of a tunicate to fouling. Biol Bull (Woods Hole) 155:615–626
- ✦ Tatsumi M, Mabin CJT, Layton C, Shelamoff V, Cameron MJ, Johnson CR, Wright JT (2022) Density-dependence and seasonal variation in reproductive output and sporophyte production in the kelp, *Ecklonia radiata*. J Phycol 58:92–104
- ✦ Thompson GA, Schiel DR (2012) Resistance and facilitation by native algal communities in the invasion success of *Undaria pinnatifida*. Mar Ecol Prog Ser 468:95–105
- ✦ Thomsen MS (2004) Species, thallus size and substrate determine macroalgal break force and break location in a low-energy soft-bottom lagoon. Aquat Bot 80:153–161
- ✦ Thomsen MS, Alestra T, Brouckhoff D, Lilley SA, South PM, Schiel DR (2018) Modified kelp seasonality and invertebrate diversity where an invasive kelp co-occurs with native mussels. Mar Biol 165:173
- ✦ Thomsen MS, Altieri AH, Angelini C, Bishop MJ, and others (2022) Heterogeneity within and among co-occurring foundation species increases biodiversity. Nat Commun 13:581
- ✦ Thornber CS, Kinlan BP, Graham MH, Stachowicz JJ (2004) Population ecology of the invasive kelp *Undaria pinnatifida* in California: environmental and biological controls on demography. Mar Ecol Prog Ser 268:69–80
- ✦ Uya M, Bulleri F, Wright JT, Gribben PE (2020) Facilitation of an invader by a native habitat-former increases along interacting gradients of environmental stress. Ecology 101:e02961
- ✦ Voultsiadou E, Kyrodinou M, Antoniadou C, Vafidis D (2010) Sponge epibionts on ecosystem-engineering ascidians: the case of *Microcosmus sabatieri*. Estuar Coast Shelf Sci 86:598–606
- ✦ Wernberg T, Thomsen MS (2005) The effect of wave exposure on the morphology of *Ecklonia radiata*. Aquat Bot 83:61–70
- ✦ Wotton DM, O'Brien C, Stuart MD, Fergus DJ (2004) Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. Mar Pollut Bull 49:844–849

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