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Colonial ascidians strongly preyed upon, yet dominate the substrate in a subtropical fouling community

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Abstract

Higher diversity and dominance at lower latitudes has been suggested for colonial species. We verified this pattern in species richness of ascidians, finding that higher colonial-to-solitary species ratios occur in the tropics and subtropics. At the latitudinal region with the highest ratio, in south-eastern Brazil, we confirmed that colonial species dominate space on artificial plates in two independent studies of five fouling communities. We manipulated settlement plates to measure effects of predation and competition on growth and survivorship of colonial vs. solitary ascidians. Eight species were subjected to a predation treatment, i.e., caged vs. exposed to predators, and a competition treatment, i.e., leaving vs. removing competitors, to assess main and interactive effects. Predation had a greater effect on growth and survivorship of colonial compared to solitary species, whereas competition did not show consistent patterns. We hypothesize that colonial ascidians dominate at this subtropical site despite being highly preyed upon because they regrow when partially consumed and can adjust in shape and space to grow into refuges. We contend that these means of avoiding mortality from predation can have large influences on diversification patterns of colonial species at low latitudes, where predation intensity is greater.

Keywords: Ascidians, Coloniality, Competition, Latitudinal Gradient, Marine Diversity, Predation, Tunicates

Introduction

Colonial animals reproduce asexually to construct colonies out of repeated, functionally autonomous modules, called zooids or polyps, that remain physiologically attached to one another [1]. Coloniality occurs in a wide range of animal taxa, such as corals, hydroids, bryozoans, entoprocts, pterobranch hemichordates, and ascidians. While sponges are often not considered true colonies, they are modular organisms that share many of these characteristics. Modular construction and clonal growth enable escape from senescence, extreme regenerative capacity, potential for colony fusion, and for decoupling between aggregate size and constraints on module size, permitting indeterminate growth [2–4]. These consequences of coloniality lead to colonial species often differing in their abundance and diversity compared to solitary species, depending on the environment [5]. For example, colonial species are generally excluded from the intertidal zone and soft substrata, but they dominate the hard-substratum communities of many shallow seas and frequently exhibit higher diversity than solitary forms [6]. Colonial species are reported to be more abundant and diverse at lower latitudes compared to solitary species [5–8]. For example, stony corals and ascidians, which comprise both solitary and colonial forms, show a ratio of colonial:solitary species of 0.75 in temperate waters near Great Britain and 2.97 in the Caribbean [5,9]. These biogeographical differences in colonial and solitary species suggest adaptations of these two life forms to selective pressures that vary with habitat and latitude.

It is widely accepted that biotic interactions, such as predation and competition, decrease in intensity as latitude increases [10]. Colonial species use different strategies to survive these interactions than solitary species. Colonial species exhibit exponential growth and can propagate indeterminately when unconstrained, giving them a competitive advantage over solitary species [6,11,12]. Colonial species are also generally less susceptible to fouling and overgrowth than solitary species. For instance, colonial ascidians in the Bermuda islands were found to be less susceptible to recruitment of epibionts than solitary species [13]. Colonial propagation allows for increased viability after significant damage from predation, whereas the ability of solitary species to survive damage is orders of magnitude less [5]. For example, cheilostome bryozoans in Scotland have a higher survival rate after tissue damage compared to nearby urchins and polychaetes [14]. Since colonial species differ from solitary species in their abilities to survive competition and predation, which both vary with latitude, this suggests that these ecological factors may underlie the trends in colonial and solitary species diversity and abundance.

Ascidians (phylum Chordata, subphylum Tunicata, class Ascidiacea) are one of the few animal groups that possess closely-related solitary and colonial species, providing an opportunity to employ a comparative approach to study the ecological significance of two different life forms. Ascidians are sessile and inhabit a range of natural (i.e. rocky shores, coral reefs) and artificial (i.e. floating docks, ship hulls) substrates worldwide [15]. They include numerous invasive species, with non-indigenous ascidians often comprising a dominant component of fouling communities in marinas [16,17]. Among the colonial taxa, ascidian species exhibit a range of forms, from stalked, upright, mound-building and multi-lobed forms to flat sheet-like species [18]. Colonial species also show differences in colony integration; some are “social”, with individual zooids only connected at the base, while other species are highly-integrated, with zooids embedded in a common tunic. Colony forms and levels of integration are widespread. Of the three ascidian suborders, two (Stolidobranchs and Phlebobranchs) possess both colonial and solitary species. The third suborder, the Aplousobranchs, lack solitary species, with one exception in which budding was secondarily lost [19]. It has been documented that colonial ascidians, among other colonial species, dominate the substrate in the tropics [6]. Colonial ascidian species also outnumber solitaries in tropical environments, characterizing 80% of species diversity [15,20].

To further document the pattern colonial vs. solitary ascidian species richness across latitude, we analysed worldwide occurrence data of ascidian species. In order to understand how biotic interactions influence the dominance and richness of colonial and solitary species, we examined ascidians of a fouling community in south-eastern Brazil, coincidentally corresponding to the latitude with the largest proportion of colonial ascidian species. To confirm and broaden our understanding of the ecology of this benthic community, we examined its composition over time and found a general dominance of colonials. Lastly, we conducted a manipulative experiment to measure the effects competition and predation on the fitness of individual solitary ascidians and colonies measured by survivorship and growth. We selected species of different growth forms and levels of integration in order to examine the ecological effects of these different morphological strategies While this study was conducted in artificial habitats, the diversity and access to animals at the chosen sites provides a unique opportunity to examine the ecology of solitary and colonial ascidians, which could be further tested in natural systems.

Methods

Latitudinal distribution:

Ascidian data was retrieved from the Ocean Biogeographic Information System (http://www.iobis.org). Entries were checked for valid genus names against the Ascidiacea World Database (http://www.marinespecies.org/ascidiacea/). Ratios of colonial to solitary species counts were calculated for each 10° latitudinal bin (Figure 1).

Dominance and species richness in SE Brazil:

We examined the coverage and species richness of sessile colonial and solitary species of all animal phyla in SE Brazil, corresponding to the latitude where the proportion of colonial-to-solitary ascidian species was the highest. To determine patterns of dominance and richness of colonial vs. solitary forms in the São Sebastião Channel, we included in this study data obtained from plates exposed to predators at five distinct sites from the years 2007 (Segredo) and 2010 (Feiticeira, Figueira, Yacht Club Ilhabela-YCI and Curral) (Supplementary Figure 1). At all sites, panels were suspended (at least 1.5m deep and 2m from the bottom) from artificial structures (moorings at Segredo; jetties at Curral, Feiticeira and Figueira; and floating platforms at YCI), which are covered by a diverse fouling community that can supply settlers to the experimental plates. From 30x30cm plates in Segredo, and 15x15cm plates in Curral, Feiticeira, Figueira and YCI, we used the 10x10cm central area to register the number of species and to take photos to calculate the percent cover of taxa present. For Segredo, data were obtained from the same plates in different sampling events, and therefore coverage and richness were compared between life forms (colonial x solitary) and across time (30, 60, 90, 120, 150 and 180 days; n=6) using a Repeated Measures ANOVA, conducted in R [21] with the ezANOVA package [22]. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, (coverage: W=0.002, $p < 0.05$; richness: W=0.182, $p < 0.05$) thus Greenhouse–Geisser corrections were applied (coverage: $\varepsilon=0.369$, richness: $\varepsilon=0.621$) [23,24]. For significant interactions we highlighted the differences in a descriptive way [25]. 2010 data were obtained from independent sets of panels after 30 (n=12) and 100 days (n=18), with coverage and richness being compared between life forms and across time for Feiticeira, Figueira, and YCI, and only between life forms for Curral (all panels were lost after 30 days), using a two- or one-way

ANOVA in the software Systat. Although data normality and homoscedasticity was not achieved even after square-root transformation, the data was balanced and sufficiently replicated so we used ANOVA tests because they are robust for such deviations [26] and more powerful compared to non-parametric tests [25].

Effects of predation and competition on growth and survivorship:

We selected representative species of the ascidian fouling community along the São Sebastião Channel to capture as much diversity of life histories and forms as possible to limit results that may be due to morphological or phylogenetic constraints (Figure 2). Solitary species included representatives of two families: *Styela plicata* (Styelidae) and *Herdmania pallida* (Pyuridae), whereas colonial species included representatives of four families: *Botrylloides niger* (Styelidae), *Polyclinum constellatum,* and *Aplidium accarense* (Polyclinidae), and *Didemnum galacteum* (Didemnidae). Colonial species selected represent different degrees of zooid integration/separation; four species bear zooids arranged into systems with a shared cloacal aperture and embedded completely in a common tunic (*P. constellatum, A. accarense, D. galacteum,* and *B. niger*). Of those, *B. niger* has a vascular system connecting all zooids of the colony, and thus represents a higher level of colonial integration. Based on colony morphology, species can also be categorized by “morphological strategy” or “functional group” [5,27]. Four species (*D. galacteum, B. niger*, *P. constellatum,* and *A. accarense*) grow either as “sheets”, encrusting the substrate in two dimensions, or as “mounds”, remarkably growing in a vertical dimension as well; whereas the other two species (*C. oblonga* and *P. zorritensis*) grow as “trees” or “runners”. The latter have a basal tunic or stolons that allow them to grow either branching encrusted or erect, and occasionally the branches become so dense that they form more massive "mounds". Whereas the biota present at this highly diverse subtropical site did not provide replicate pairs of evolutionary divergent species to avoid lineage specific effects that may be unrelated to form, it did provide species that are distributed among major monophyletic groups of ascidians: the two solitary species used are in the families Styelidae and Pyuridae, the two mound-like colonial forms are both aplousobranchs in the family Polyclinidae, whereas the two social and two sheet-like compound forms are divided between aplousobranchs and stolidobranchs (Figure 2) [18,28]. We used only adults of the different life forms, excluding any possible effects on larval stages and initial recruitment into the fouling community.

Solitary and colonial species were collected and cultured for three months in preparation for the manipulative experiment at the YCI (February–May, 2016; Supplementary Figure 1). For solitary species, gametes were dissected from a single pair of reproductive adults and in vitro fertilization was conducted [29]. Larvae were allowed to settle on petri dishes, which had previously been scraped with sandpaper to provide a rough settlement surface. The petri dishes containing the metamorphosed juveniles were attached onto temporary PVC panels and allowed to grow at Praia do Segredo for three months in cages submerged at 2m. For colonial species, small pieces of colonies were initially "planted" to the centre of the underside of a temporary PVC panel with plastic cable ties and allowed to grow into a larger colony for three weeks.

The experiment was conducted along a stretch of floating concrete platforms at the YCI (May–August, 2016). Each species was attached to a 30x30cm PVC panel, randomly distributed, and suspended horizontally 3m deep and 1m apart (see Supplementary Figure 2). By using flat panels, we excluded effects of pre-existing spatial complexity. For the encrusting colonial species, a single large colony from a temporary plate was sub-cloned (i.e., cut into smaller genetically-identical pieces) into 1.5x1.5cm squares and "planted" to the centre of the panels

(Supplementary Figure 2C). For the two social species (*C. oblonga* and *P. zorritensis*) five zooids connected by their tunic were attached to the centre of the panels using two small cable ties (Supplementary Figure 2D). For solitary species, four or six three-month-old juveniles of *S. plicata* or *H. pallida* respectively were attached 5 cm apart with superglue to the downward face of the same panel (Supplementary Figure 2E). Predation was manipulated using screen cages (30x30x8cm, and 75x75mm mesh) (Supplementary Figure 2B). Half of the panels were enclosed by full-cages, excluding large predators (mainly fish), while the other half were enclosed by open-cages, which were built in the same way as full-cages but lacking the roof, allowing access of predators while controlling for any eventual cage artefact [30]. Competition was manipulated by removing all surrounding settlers (including barnacles, mussels, bryozoans, and other ascidians) using a paint scraper every two weeks, since fouling species settled but did not have time to grow significantly into the focal specimen during this time window. For each species, predation and competition treatments were varied orthogonally to assess main effects and interactions (treatments were “full-cage/cleaned,” “full-cage/uncleaned,” “open-cage/cleaned,” and “open-cage/uncleaned” with n=4 replicates per treatment).

Survivorship of each focal species was recorded, and photographs of the panels (with ruler) were obtained every other week. At the same time, the panels in the “cleaned” treatments were scraped and cages were cleaned to prevent build-up of fouling species. The area of the focal species (i.e., the region of interest) was measured in the photos using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). In order to assess whether the area measurements were reliably capturing the growth of the ascidians, we determined if area was correlated to dry mass of each species, measured after baking specimens at 60°C for 24hr. At the beginning of the experiment, for each species three randomly chosen additional specimens that had been prepared for the study were used for dry mass. At six weeks, for each solitary species, one individual from each panel was removed for dry mass. At the end of the experiment, all remaining specimens, including both solitary and colonial species, were collected and their dry mass was determined. Using these data, we generated area-by-dry-mass growth curves, which show that area is a correlate of mass for all species except *P. zorritensis*, in which there were too few data points to generate a correlation (Supplementary Figure 3).

We used a loglinear model to understand the association between life-form (colonial or solitary), predation, competition, and the survival of the ascidians after 6 weeks. We used this period because we lost several replicates after 8 weeks. To fit a loglinear model, we converted our four-way contingency table in a table with a column for cell frequencies where each row represents a unique combination of the four variables and used it as response variable in a generalized linear model (GLM) with a Poisson distribution. We tested two models, one with two-way and other with three-way interactions, and compared them using analysis of deviance. We present here only the three-way model, once we observed significant difference between the two models and the two-way model expected frequencies did not satisfy the loglinear model. Results are interpreted by analysis of deviance.

To compare the effects of competition and predation on growth of each treatment among life-form, the area of each species at each time point was standardized by converting the raw region of interest area into a z-score. Pseudoreplicates of the solitary forms were removed from the data and only one individual per panel was randomly selected for the analyses. Here, only the first eight weeks of data were used in our analyses because we lost several replicates after this time. Repeated measures ANOVA was conducted as before. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, W=0.3398, $p < 0.05$, thus Greenhouse–Geisser corrections were applied (ε=0.6585).

Results

Latitudinal trends in colonial and solitary species counts

We found decreasing colonial-to-solitary species ratios toward the poles, but two peaks on either side of the equator (Figure 1). The highest proportion of colonial to solitary ascidian species was 1.96 in the 15–25°S latitude bin. Solitary species outnumbered colonial species (ratio < 1) in the 45–55°N bin and further north and in the 35–45°S bin and further south. In contrast, the tropical and subtropical regions generally showed a higher proportion of colonial species (ratio >1).

Dominance and richness of colonial and solitary species in Brazil

Two of us (EAV & GMD) have been studying community structure on settlement plates in the São Sebastião Channel in SE Brazil for over a decade [30–33]. Here, we documented that colonial animals dominate over solitary forms and show higher species richness at five sites in this fouling community (Figure 3, Supplementary Tables 1 and 2). These separate investigations took place in two separate years and on panels in which animals were exposed to predators. Although showing the same number of colonial and solitary species in the first 30 days, colonial species outnumbered solitary species at Segredo after 60 days, being almost 6 times more numerous after 180 days (Figure 3A, Supplementary Table 1). In 2010 at four additional sites, Feiticeira, Curral,Figueira, and YCI, colonial species displayed higher percent cover and richness compared to solitary species at both 30 and 100 days of submergence (Figure 3B, Supplementary

Table 2). The trends in the effects of submergence time on solitary vs. colonial coverage varied among sites. At Segredo colonial coverage and richness increased between 30 to 90 days, while solitary species decreased with submergence time; Feiticeira and YCI showed an increase in coverage and number of both life forms between 30 and 100 days; Figueira showed an increase in both colonial and solitary species number but no changes in cover between 20 and 100 days. Despite some variation, these results demonstrate a general dominance and diversity of colonial species during the establishment of fouling communities that is maintained over the years at a subtropical site.

Effects of predation and competition on colonial and solitary ascidians

Competition did not affect survival regardless of the other factors. The proportion of ascidians surviving after 6 weeks depended on its life-form and predation regime. Survival of colonial but not of solitary ascidians was reduced when exposed to predators (Supplementary Table 3, Figure 4). More than 60% of colonies survived after 12 weeks when protected against predation, but only 40% when exposed to predators (n=16 per treatment, Figure 4A). Around 40% or less of solitary ascidians survived the 12-week study, without a major difference in survivorship across any of the treatments (Figure 4B).

Colonial ascidians protected against predators achieved larger sizes than those exposed, suggesting an effect of predation on growth (Figure 5, RM-ANOVA: Form*Predation*Time, $p<0.05$, Supplementary Table 4). Representatives of all the colonial growth forms, including mound-like (*A. accarense*, *P. constellatum),* social (*P. zorritensis*), and sheet-like (*D. galacteum*), grew substantially more in the predator-protected treatments (Figure 5A-F). However, *C. oblonga* (social) and *B. niger* (sheet-like) did not show differences between predator-protected and exposed as great as other species. Both solitary species in predator-protected treatments showed similar growth to those in the exposed treatments (Figure 5G-H). This suggests that different forms (colonial vs. solitary) respond differently to predation, with many colonials being more susceptible than solitary species (RM-ANOVA: Form*Predation*Time, $p<0.05$, Supplementary Table 4). Competition did not have a significant effect on growth of any life form (RM-ANOVA, Supplementary Table 4).

Discussion

Latitudinal gradient in the ratio of colonial to solitary ascidian species

It has been suggested that diversity and dominance of colonial animals are higher in the tropics (i.e. lower latitudes) compared to solitary species, which are more abundant in temperate waters (i.e. higher latitudes), however this trend has not been clearly documented [5–8]. Colonial ascidians, in particular, have been noted to be more species-rich in the tropics, comprising 80% of total species diversity [15]. Here, using OBIS data, we confirmed that colonial ascidians are more species-rich than solitary species in tropical and subtropical latitudes. Less than half of all ascidian species are colonial near the poles and nearly two times more species are colonial at low latitudes. However, the ratio shows two peaks – one on either side of the equator. The 5°N–5°S latitudinal bin that spans the equator, on the other hand, shows lower ratios of colonial-to-solitary ascidian species. It is unclear why the tropics show a depression in the ratio, but it may be due to some bias such as undersampling, since the number of total species recorded in this equatorial zone is slightly lower compared to nearby latitudes. Alternatively, the bimodal distribution may represent a real phenomenon, as other studies have found multiple peaks in marine species diversity that are offset from the equator [34]. Nonetheless, the ratio is still larger in tropical and subtropical regions than most temperate and polar latitudes. Biotic interactions, including predation, are also known to be greater in lower latitudes [10,35]. Thus, we hypothesize that colonial vs. solitary species diversification may be linked to one or more ecological differences that vary with latitude.

Colonial forms dominate and show higher species richness compared to solitary forms, yet are more susceptible to predation in a subtropical fouling community

At the latitude with the largest ratio of colonial-to-solitary ascidian species (subtropical SE Brazil), we found that colonial species (of a variety of phyla) were several times more numerous and taking up to 70–90% of the substrate space. This trend, with colonial species dominating the substrate and showing higher diversity, was consistent over multiple years and sites 2–15km apart in SE Brazil. Although our study was restricted to artificial substrate and the resemblance with natural systems is still a matter for further investigation, the results obtained here are clear and may not only provide insights for coloniality patterns but also for invasiveness strategies, as most of the organisms growing in these artificial substrates are currently considered cryptogenic species [36].

By manipulating predation and competition at one of the same sites in SE Brazil, we were able to determine if these ecological factors affected the growth and survivorship of colonial and solitary ascidians differently on the experimental panels. Since competition has been reported to favour colonial over solitary forms [5,6], we predicted that competition would affect growth and survivorship of solitary more than colonial species. Surprisingly, our data did not show any effect of competition on either solitary or colonial life forms. Although we have a procedural limitation of not using true settlers, and thus not allowing competition effects to take place on early and more susceptible stages, our experimental panels were fully occupied by 6-8 weeks which clearly imposes some competition pressure even for larger organisms. However, if only heavy crowding generates a significant effect on growth, our study may not have included enough time to capture competition effects. The only colonial ascidian species in this community for which competition effects have been documented is *Didemnum perlucidum*, but such an effect is only evident after 18 weeks [37].

Our data suggests that predation had a greater effect on growth and survivorship of colonial compared to solitary ascidian species. Thus, colonial ascidians may employ fewer defensive strategies against predation (morphological or chemical defenses) than solitary species. This is surprising, given that ascidians are studied for the presence of defensive metabolites [13,38–41]. Few studies have directly compared the relative strength of defenses between solitary and colonial species. While colonial ascidians from Bermuda show less palatability than solitary species [13], colonial ascidians from the Gulf of Mexico have been shown to have higher palatability to fishes than solitary ascidians [42]. At our site the most observed predators are fish, including *Abudefduf saxatilis* (Linnaeus, 1758), *Stephanolepis hispidus* (Linnaeus, 1766), and *Diplodus argenteus* (Valenciennes, 1830) [30,43,44]. Here, solitary species were less predated by fish likely due to anatomical defenses, such as tougher tunics or camouflage [13,42]. In addition to morphological defences, solitary species have been known to protect themselves from predation via an "escape in size" strategy, in which animals prevent predators from being able to bite them by growing rapidly past a size-sensitive threshold [6,45]. One of the solitary species used in this study, *Herdmania pallida*, has numerous tunic spicules that probably deters predators similar to other *Herdmania* species [46,47].

Solitary species may be particularly vulnerable to predation at early life stages [48–50]. For example, while 1–3-day-old juveniles (0.5–1mm in diameter) were killed by predators in less than an hour, 4 week young adults of the solitary ascidian *Molgula manhattensis* (approx. 5mm) survived after exposure to predators [50]. Mortality of early life stages of solitary ascidians was not explored in our study because the specimens were already three months old when the experimental regimes started.

We found that colonial species are less resistant to predators than solitary species in a region where colonial species are both dominant and more species-rich compared to solitary species. We hypothesize that predation acts as a selective force on solitary forms, with only those species that can withstand predation being maintained, while predation seems to be less important in restricting the occurrence of colonial species. This may help explain why even with high predation the diversity of colonials is much higher than that of solitary species. Further, this suggests that colonial species have alternative strategies to survive predation to maintain higher dominance and diversity in this system. Colonial species, due to their unique growth mode, have extreme re-growth potential; they have the ability to survive partial or even major damage to the colony by regenerating lost parts. Some colonial species even have the potential to fuse to become chimeras, which may be considered a survival strategy since mortality often decreases with colony size [12]. Colonial species can also grow laterally to take advantages of crevices and other cryptic environments that may act as spatial refuges from predators [51,52]. Such a protective mechanism of “running away” into refuges can been termed “escape in space” [53,54]. In addition, colonial species can scale back (e.g., some undergo hibernation) when conditions are poor, then regrow [55,56]. This is similar to the idea of “temporal escape” or “escape in time” proposed by Lubchenco and Gaines (1981), in which colonies may withstand occasional disturbance, such as a dearth of resources [51] or the presence of predators, by temporally modulating growth [53,54]. As these alternative protective strategies are linked to the colonial life style, we hypothesize that coloniality provides non-defensive mechanisms to tolerate disturbances, allowing colonial species to avoid mortality and eventually dominate the substrate. In regions where predation is intense, coloniality may provide alternative protective strategies, which could explain the large diversity and dominance of these organisms even in the study area, where predation appears to be strong [30,43,57].

Defenses are known to be more common in tropical benthic ecosystems (although there are exceptions, see [58]), where it has been suggested that high rates of predation favour more strongly defended prey than in temperate and polar regions [59]. For example, physical and behavioral defenses due to increased fish predation are known at low latitudes; toxicity of sponges and sea cucumbers is inversely related to latitude [60], littoral gastropods show reduced foraging time at lower latitudes as a means of avoidance of shell-crushing fish, which are more abundant in the tropics [61,62]. We suggest that coloniality-linked survival strategies promoted by higher predation at lower latitudes (i.e., propagative regeneration or clonal expansion to escape and avoid predators) may act as drivers of colonial diversity observed in the tropics. Since predation is more intense in the tropics where diversity and dominance of colonial species is greatest, predation may be driving the origin and diversification of coloniality as a survival strategy. Studies at additional latitudes would help test this hypothesis.

Data accessibility

The datasets supporting this article have been uploaded as part of electronic supplementary material.

Authors' contributions

L.S.H. designed and performed the field and laboratory work for the 2016 manipulative study, and performed statistical analyses for the 2016 study. E.A.V. carried out fieldwork and analysis for the 2007-2010 field studies. G.M.D. helped with experimental design and statistical analysis. F.B. was involved in the experimental design and helped with logistics. L.S.H. wrote the manuscript with contributions from all the other authors.

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Figure 1: Ratio of colonial to solitary ascidian species counts within 10° latitudinal bins.

Based on data from the Ocean Biogeographic Information System database. Number of total ascidian species (n) in each bin is indicated on bars.

Figure 2: Ascidian species used in study

(A) Ascidian phylogeny [63,64]. Species used in this study are underlined. Boxes demarcate ascidian orders. Columns to the right indicate whether each species is colonial (“C”) or solitary (“S”),degree of colony integration, and morphological strategy/functional group. (B-I) Photos of species used in this study. (B) *Styela plicata.* (C) *Herdmania pallida.* (D) *Didemnum galacteum*. (E) *Botrylloides niger.* (F) *Polyclinum constellatum.* (G) *Aplidium accarense*. (H) *Clavelina oblonga*. (I) *Polyandrocarpa zorritensis*. Scale bars: (B-C) approx. 10mm, (E) 14mm, (G) 30mm, (I) approx. 5mm.

Figure 3: Colonial forms dominate the substrate in SE Brazilian fouling community.

(A) Mean percent cover (±SE) and (B) richness (±SE) of colonial (black) and solitary (grey) species on panels over 180 days of submergence at Segredo (2007) and after 30 and 100 days of submergence at Feiticiera, Figueira, YCI, and Curral (2010).

Figure 4: Survivorship of colonial and solitary ascidians in predation and competition treatments.

Proportion of colonial (A) and solitary (B) ascidians alive in each treatment over 12 weeks (n=16 plates per treatment).

Figure 5: Growth of ascidians in predation and competition treatments.

Mean area (± SE) of social colonial (A-B), mound-forming colonial (C-D), sheet-like colonial (E-F), and solitary (G-H) ascidians over 56 days: (A) *Clavelina oblonga*. (B) *Polyandrocarpa zorritensis*. (C) *Polyclinum constellatum*. (D) *Aplidium accarense*. (E) *Didemnum galacteum*. (F) *Botrylloides niger*. (G) *Styela plicata*. (H) *Herdmania pallida*. n=4 replicate plates per treatment.

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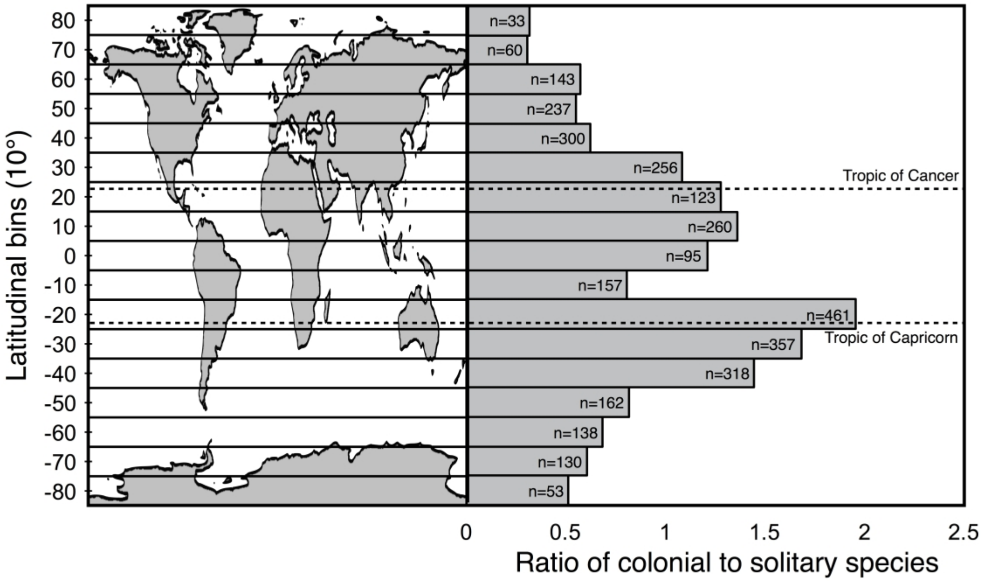

Figure 1: Ratio of colonial to solitary ascidian species counts within 10° latitudinal bins. Based on data from the Ocean Biogeographic Information System database. Number of total ascidian species (n) in each bin is indicated on bars.

446x259mm (300 x 300 DPI)

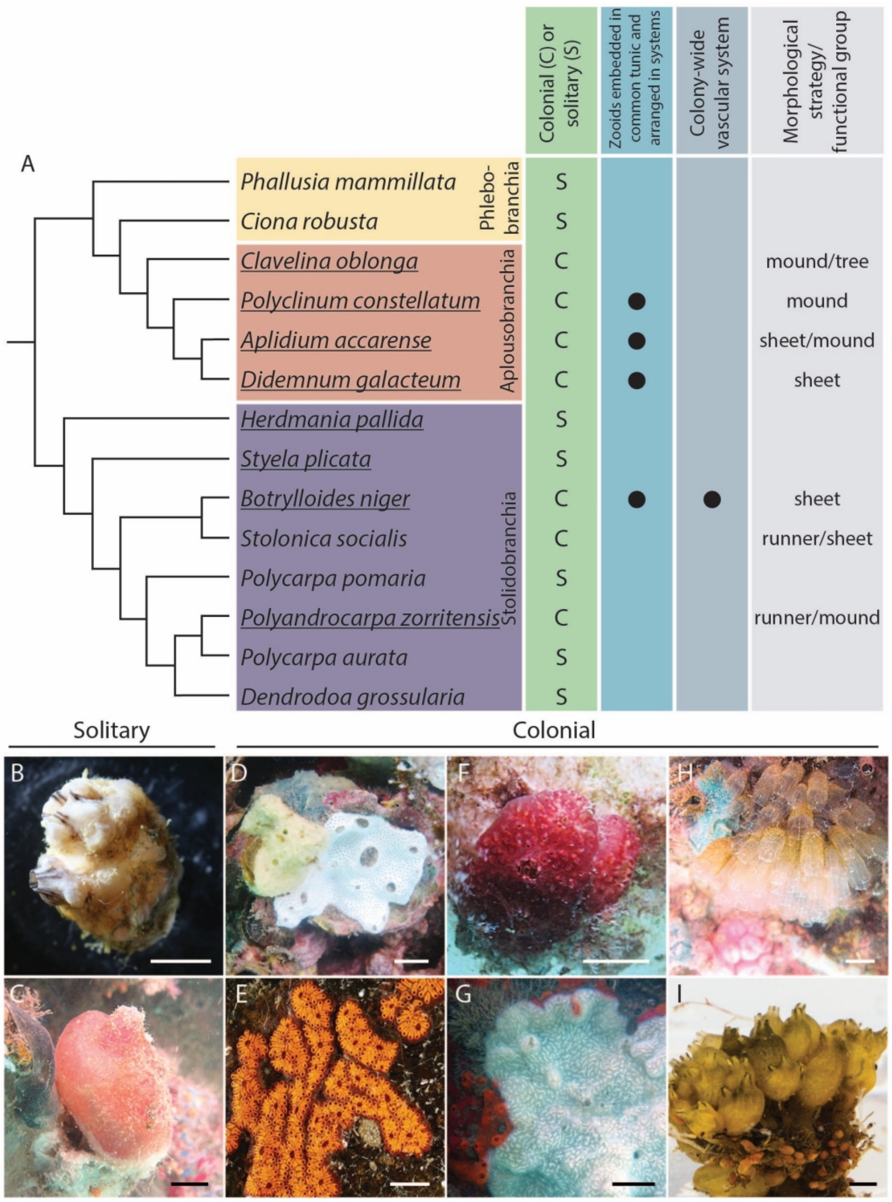

Figure 2: Ascidian species used in study

(A) Ascidian phylogeny [63,64]. Species used in this study are underlined. Boxes demarcate ascidian orders. Columns to the right indicate whether each species is colonial ("C") or solitary ("S"),degree of colony integration, and morphological strategy/functional group. (B-I) Photos of species used in this study. (B) Styela plicata. (C) Herdmania pallida. (D) Didemnum galacteum. (E) Botrylloides niger. (F) Polyclinum constellatum. (G) Aplidium accarense. (H) Clavelina oblonga. (I) Polyandrocarpa zorritensis. Scale bars: (B-C) approx. 10mm, (E) 14mm, (G) 30mm, (I) approx. 5mm.

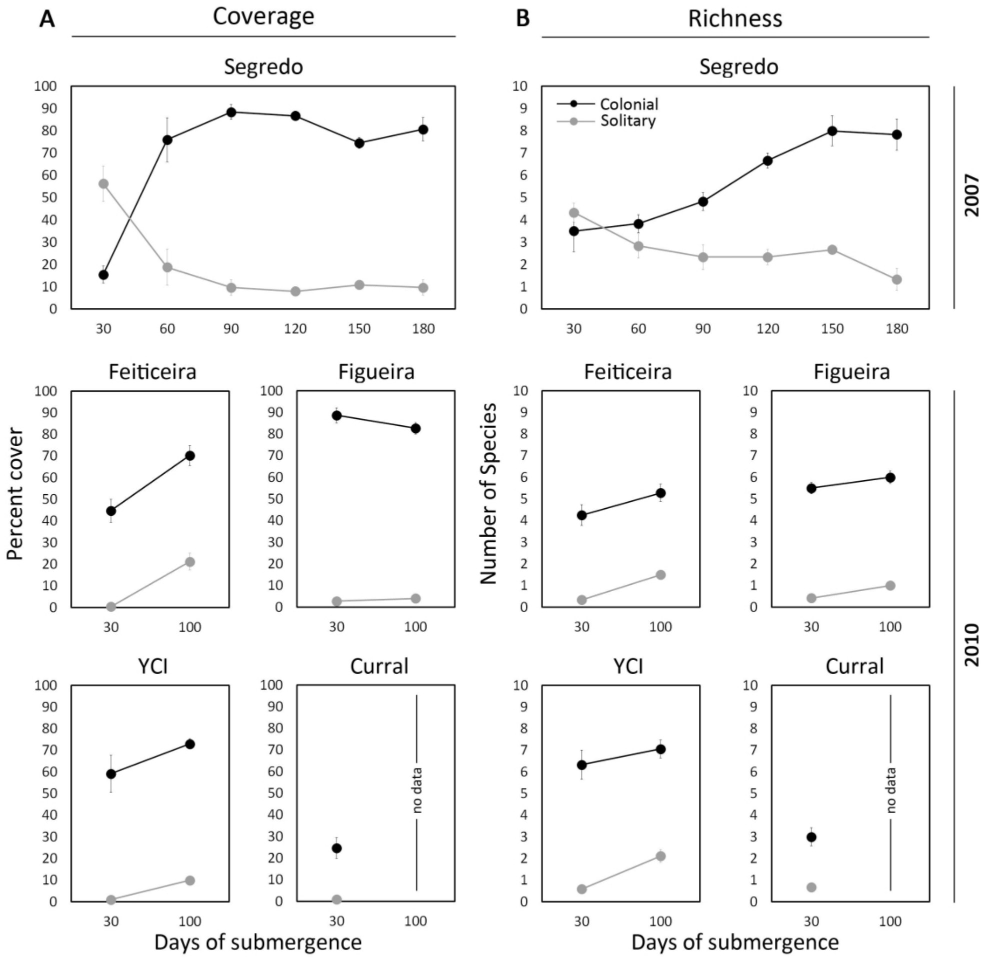

Figure 3: Colonial forms dominate the substrate in SE Brazilian fouling community.
(A) Mean percent cover (±SE) and (B) richness (±SE) of colonial (black) and solitary (grey) species on panels over 180 days of submergence at Segredo (2007) and after 30 and 100 days of submergence at Feiticiera, Figueira, YCI, and Curral (2010).

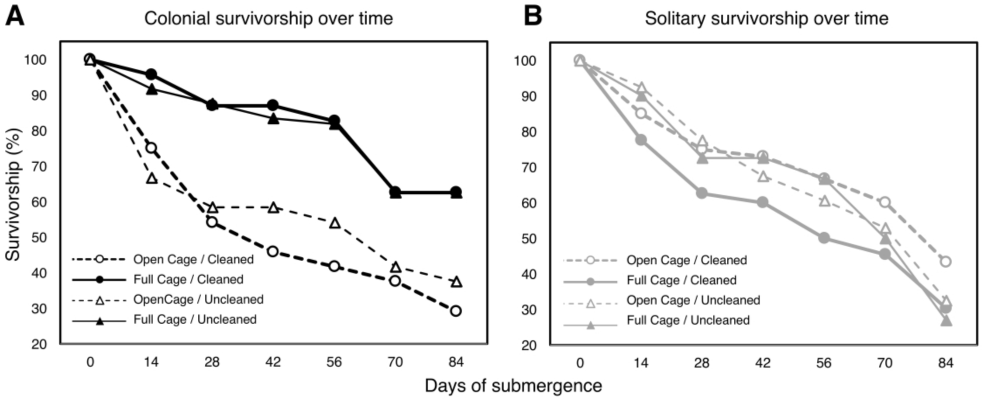

Figure 4: Survivorship of colonial and solitary ascidians in predation and competition treatments. Proportion of colonial (A) and solitary (B) ascidians alive in each treatment over 12 weeks (n=16 plates per treatment).

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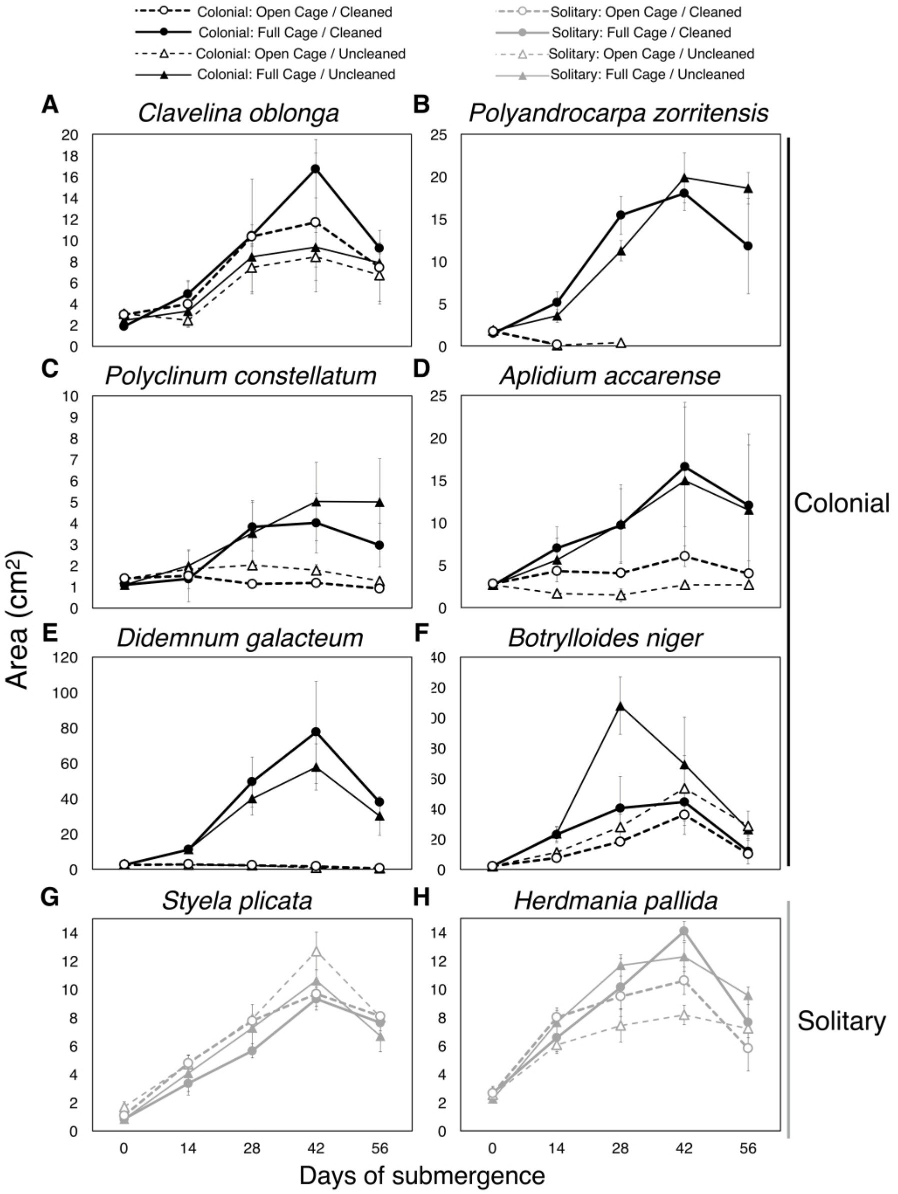

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458x599mm (300 x 300 DPI)