

## Research Article

# Continued persistence of non-native ascidians in Southern California harbors and marinas

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## Abstract

Non-native ascidians have long dominated the artificial structures in southern California's (United States) marinas and harbors. To determine the change in ascidian abundance and community composition over the last several decades, in 2019–2020 we replicated surveys from 1994–2000. We then created nMDS plots using the abundance data collected in the 1994–2000 and 2019–2020 surveys to compare the two groups. Range and average abundance per species were analyzed to determine trends and changes in ascidian community composition. Of the species used for comparison, four are native, three are cryptogenic, and 12 are non-native. As predicted by Lambert and Lambert, non-native species have persisted in southern California; however, ranges and abundances have changed. The only native species found consistently in both sets of surveys, *Ascidia ceratodes*, remained rare in 2019–2020, with an unchanged average abundance. Several non-native species increased in abundance or remained common. The non-native colonial species *Polyandrocarpa zorritensis* had the greatest influence on the dissimilarity between the surveys, increasing from rare in 1994–2000 to more common in 2019–2020, and spreading north to Santa Barbara. Several non-native species confined to San Diego in the 1994–2000 surveys have also spread north, such as *Botrylloides giganteus* and *Styela canopus* which were found in Santa Barbara in 2019–2020. A formerly unidentified *Aplidium* sp. has now been identified as the non-native *Aplidium accarense*. There have also been additional introductions since 2000, including *Ascidia* cf. *virginea* and the first report of *Asciella aspersa* in the NE Pacific. The overwhelming trends of the surveys indicate that we will continue to see an increase and persistence of newly introduced non-natives in Southern California marinas, with possible continued northward expansion.

**Key words:** *Aplidium accarense*, *Ascidia* cf. *virginea*, *Asciella aspersa*, invasive species, introductions, nonindigenous, *Polyandrocarpa zorritensis*, rapid assessment survey

## Introduction

Ascidians (Phylum Chordata, Class Ascidiacea) are a group of invertebrates important in the ecology of a number of marine benthic communities (Lambert 2005a). Many species have become successfully introduced around the world via anthropogenic vectors, with a few causing extensive ecological and economic

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damage (Zhan et al. 2015). Non-native ascidians have effected declines in native species richness (Aldred and Clare 2014), altered benthic community structure (Lambert and Lambert 2003; Bullard et al. 2007; Lambert 2009; Aldred and Clare 2014), and disrupted the link between pelagic and benthic communities (Lengyel et al. 2009; Mercer et al. 2009). Economically, ascidians are a major problem for the aquaculture industry: *Styela clava* (Herdman, 1881) was estimated to cost the New Zealand green-lipped mussel industry NZ\$23.9 million over a 24-year period (Soliman and Inglis 2018).

The transport of these species is associated with boating traffic, both commercial and recreational, as well as aquaculture (Wasson et al. 2001; Lin et al. 2015; López-Legentil et al. 2015; Zhan et al. 2015). The reason for this is that ascidians are sessile as adults; their only motile phase is a short-lived, non-feeding tadpole larva with extremely limited dispersal ability (Lambert 2005a). Once ascidians arrive in a new location, anthropogenic activities such as overfishing, coastal development, and aquaculture can lead to changes that contribute to their success and rapid establishment (Zhan et al. 2015). Many ascidian species are known to tolerate a wide variety of environmental conditions, including pollution (Lambert 2005a; Pineda et al. 2012a, b).

The region from Santa Barbara to San Diego in California includes two of the world's largest and busiest ports – the Los Angeles and Long Beach Harbors – which have the highest boat traffic in the United States (Arhami et al. 2009). Repeated surveys in this region from 1994–2000 (Lambert and Lambert 1998, 2003; Cohen et al. 2005; Lambert 2007) determined that non-native ascidians have dominated the artificial structures in southern California harbors for several decades. Native species, which were once abundant on artificial structures (Ritter and Forsyth 1917; Van Name 1945), are now rare (Lambert and Lambert 1998, 2003). The amount of boat traffic, coupled with the historical data, makes southern California an important area to study the impact of non-native ascidians.

San Francisco Bay has a high invasion rate for non-native species and has been rated the most invaded estuary on the west coast (Cohen and Carlton 1998). As southern California marinas are among the highest traffic ports in the world, and have the same transfer methods as San Francisco Bay, it is both relevant and indeed imperative to monitor the impact of non-native fouling communities in the region. Replicating the surveys done by Lambert and Lambert from 1994–2000 gives vital insight into changes to the community composition of ascidians in Southern California, allowing us to follow trends and make predictions for the future of this region.

## Materials and methods

### Overview

The goal of this study was to compare the distribution and abundance of ascidian species in southern California over a 20+ year period, and to document the current distribution and abundance of both native and non-native ascidians in harbors and marinas. We replicated ascidian surveys conducted from 1994–2000 (Lambert and Lambert 1998, 2003; Lambert 2007; Lambert and Lambert unpublished data) in 2019–2020. In doing so, we can compare the current and historical distribution of species in the same locations. We also included a survey by G. Lambert from 2011 as a middle point in the comparison for San Diego (Mission Bay and San Diego Bay) locations (G. Lambert, unpublished data).

## Re-surveys

Surveys were conducted from Santa Barbara Harbor to San Diego Bay (Figure 1) in August–November 2019 and August–September 2020, with each site surveyed only once during this time frame (Table 1). Survey techniques follow Lambert and Lambert (1998, 2003). In all surveys, ropes and floating docks from every section of each marina were sampled. This examination process took between 30 minutes and 2 hours, depending on the size of the marina. At the end of each survey, abundance of each species was determined based on the number of individuals observed: (1) rare: one or a few specimens observed; (2) common: species frequently observed but not overly abundant; and (3) abundant: species occurring frequently and in large numbers. Both sets of surveys used the same abundance scale, although no species had the highest abundance category from the Lambert and Lambert publications (complete cover of large portions of substrate) in 2019–2020.



**Figure 1.** Harbors and bays in southern California where surveys were conducted. Alamitos Bay (not shown) is in between Long Beach/Los Angeles Harbor and Newport Harbor. The arrow represents 72 kilometers.

## Species Identifications

Most species were identified on site. Individuals or colonies not immediately recognizable were relaxed in menthol and preserved in 10% seawater formalin for morphological identification; subsamples were preserved in 95% ethanol for barcode sequencing. Field identifications were subsequently verified morphologically and/or by barcoding the mitochondrial cytochrome oxidase 1 locus (CO1). Morphological identifications were accomplished using published species descriptions (Ritter and Forsyth 1917; Van Name 1945; Berrill 1950; Saito et al. 1981; Saito et al. 2001; Abbott et al. 2007).

We also identified samples to species by sequencing the mitochondrial cytochrome oxidase 1 (mtCO1) gene. DNA was extracted using the Nucleospin Tissue Kit (Macherey Nagel). PCR amplification was performed using either OneTaq DNA Polymerase (New England Biolabs) or Phusion High-Fidelity DNA Polymerase (New England Biolabs). OneTaq reactions comprised the following ingredients: 25  $\mu$ l total reaction volume with 14.5  $\mu$ l of nuclease-free water (New England Biolabs), 5  $\mu$ l of 5X buffer (New England Biolabs), 0.5  $\mu$ l of 10 mM dNTPs, 0.5  $\mu$ l of 10  $\mu$ M primer of each primer, 2  $\mu$ l of OneTaq and 2  $\mu$ l of DNA template. Phusion reactions were as follows: 20  $\mu$ l total reaction volume with 10.8  $\mu$ l of nuclease-free water (New England Biolabs), 4  $\mu$ l HF buffer (New England Biolabs), 0.4  $\mu$ l of 10 mM dNTPs, 0.6  $\mu$ l of 100% DMSO, 1  $\mu$ l of each 10  $\mu$ M primer, 0.2  $\mu$ l

**Table 1.** Marinas visited during 2019–2020, as well as the date on which each site was surveyed.

Marina Name and Abbreviation	Harbor	City	Coordinates	Date Surveyed
Santa Barbara Harbor (SBH)	Santa Barbara	Santa Barbara	34.4036, -119.6936	8/29/2020
Ventura Harbor Entrance: Island Packers (VHE)	Ventura	Ventura	34.2444, -119.2652	8/30/2020
Ventura West Marina (Back Bay) (VHYC)	Ventura	Ventura	34.247, -119.26	8/30/2020
Channel Islands Sportfishing (Entrance) (PHE)	Channel Islands	Oxnard	34.1632, -119.2225	8/30/2020
Anacapa Isle Marina (Back Bay) (PHA)	Channel Islands	Oxnard	34.1736, -119.2257	8/30/2020
Fisherman's Village (MDR)	Marina Del Rey	Marina Del Rey	33.9726, -118.4462	9/1/2020
King Harbor Marina (KH)	King	Redondo Beach	33.8512, -118.3977	9/1/2020
Cabrillo Way Marina (LAW)	Long Beach/Los Angeles	San Pedro	33.7229, -118.278	9/1/2020
22 <sup>nd</sup> Street Landing Marina (LAW)	Long Beach/Los Angeles	San Pedro	33.72528, -118.2804	8/21/2019
Alta Sea	Long Beach/Los Angeles	San Pedro	33.72494, -118.2744	9/30/2019
Newmark's Yacht Center (LBI)	Long Beach/Los Angeles	Wilmington	33.76442, -118.2491	9/30/2019
Spinnaker Cove	Alamitos Bay	Long Beach	33.76703, -118.1242	10/12/2019
Alamitos Bay Marina (ABE)	Alamitos Bay	Long Beach	33.75239, -118.1113	8/25/2019
Balboa Island (NH1)	Newport	Newport Beach	33.60767, -117.8971	9/21/2019
Newport Fun Zone (NH1)	Newport	Newport Beach	33.60358, -117.9	9/21/2019
Newport Back Bay (NH3)	Newport	Newport Beach	33.61967, -117.8999	9/5/2020
Ocean Institute (DPH)	Dana Point	Dana Point	33.46092, -117.7063	9/27/2019
North Harbor (ON)	Oceanside	Oceanside	33.2116, -117.3961	9/25/2020
South Harbor	Oceanside	Oceanside	33.20569, -117.3896	10/1/2019
Santa Clara Boat Ramp (MB6)	Mission Bay	San Diego	32.78186, -117.2497	11/17/2019
Mission Bay Yacht Club (MB5)	Mission Bay	San Diego	32.77914, -117.2474	11/17/2019
Bahia Point Marina (MB4)	Mission Bay	San Diego	32.77303, -117.2483	10/17/2019
Dana Basin Boat Ramp (MB2)	Mission Bay	San Diego	32.76644, -117.2353	10/17/2019
South Shores Boat Ramp (MB3)	Mission Bay	San Diego	32.76447, -117.2179	10/17/2019
Seaforth Landing (MB1)	Mission Bay	San Diego	32.76272, -117.2359	11/17/2019
Sunroad Resort Marina (SD4)	San Diego Bay	San Diego	32.7251, -117.1914	9/26/2020
Shelter Island Police Docks (SD5)	San Diego Bay	San Diego	32.7091, -117.2345	9/26/2020
Pepper Park Boat Launch (SD1)	San Diego Bay	San Diego	32.6501, -117.1111	9/26/2020
Pier 32 Marina (SD1)	San Diego Bay	San Diego	32.6524, -117.109	9/26/2020
Chula Vista Boat Launch Ramp (SD2)	San Diego Bay	San Diego	32.6214, -117.1033	9/27/2020
Chula Vista Marina (SD2)	San Diego Bay	San Diego	32.6254, -117.1024	9/27/2020
Fiddler's Cove Marina (SD3)	San Diego Bay	San Diego	32.65212, -117.1495	9/27/2020

of Phusion and 2  $\mu$ l of DNA template. Each DNA sample was amplified with one of two PCR primer pairs: Tun\_forward/Tun\_reverse2 (Stefaniak et al. 2009) or LCO1490/HC02198 (Folmer et al. 1994). Tun primers were only used with One-Taq polymerase, using this protocol: 94 °C for 1 min, 60 $\times$  (94 °C for 10 sec, 50 °C for 30 sec, 72 °C for 50 sec), 72 °C for 10 min. Folmer primers were only used with Phusion polymerase, using this protocol: 98 °C for 30 sec, 35 $\times$  (98 °C for 10 sec, 48 °C for 30 sec, 72 °C for 30 sec), 72 °C for 5 min. PCR products were incubated with 1  $\mu$ l each of Exonuclease I (New England Biolabs) and Antarctic Phosphatase (New England Biolabs) at 37 °C for 1 hour, followed by 90 °C for 10 min. The PCR products were sequenced by Eurofins Genomics, using the Sanger dideoxy termination method. Forward and reverse sequences were edited and combined into a consensus sequence using Codon Codes Aligner (Codon Code Corporation).

Sequences were compared to those available in GenBank using the megablast algorithm. A query with 98–100% identity to a subject identified on GenBank was considered to be the same species as the sample on GenBank. Because GenBank sequences can be mis-identified, we only used GenBank identifications in which the submitting author is globally recognized as an ascidian taxonomy expert, and this expert verified the morphology independently. Sequences that had no matches on GenBank were identified by morphology only.

## Datasets

The abundance data 1994 to 2000 were taken from previously published surveys (Lambert and Lambert 2003; Lambert 2007), and from unpublished records of the same surveys. These surveys used the same abundance scale as noted above for the 2019–2020 surveys, although no species had the highest abundance category (complete cover of large portions of substrate) in 2019–2020. The abundances for a specific species at a given site were averaged across years, for all years that were surveyed, to give an average abundance for that species for the entire 1994–2000 period. If a site was not surveyed every year between 1994–2000, only the years surveyed were included in the average. Most of the 1994–2000 surveys were conducted twice a year, in the fall and spring (see Table 3 in Lambert and Lambert 2003); abundances recorded in the spring were often depressed from winter rains. For this reason, 2019–2020 surveys were only conducted in the fall, so only the surveys conducted in the fall during 1994–2000 were included in the comparison. To compare a single time point from the 1994–2000 surveys to the single time point from the 2019–2020 surveys for each site, we used fall 1997 abundance data (Lambert and Lambert 2003). The survey from 1997 was chosen for the comparison, as it is the earliest date in which all sites were surveyed.

We used the list of sites sampled in 1994–2000 as a guide for the 2019–2020 surveys; however, several sites were surveyed in 2019–2020 more thoroughly than in 1994–2000. For example, the site NH1 in the 2019–2020 survey refers to Newport Harbor's Inner Harbor in the 1994–2000 survey. This geographical area was sampled in one location in 1994–2000, and five locations in 2019–2020. In these cases, we averaged abundances across the corresponding sites in 2019–2020 to obtain a single value for each site. This value could then be compared to the corresponding single value for 1994–2000, averaged across fall surveys. Several locations surveyed in 2020, including Oceanside South Harbor and Alta Sea Dock, did not have counterparts in the 1994–2000 surveys, and thus were excluded from the comparative analysis.

We also used abundance data from 2011, recorded by one of the authors (G. Lambert) in San Diego (San Diego Bay and Mission Bay) (Sorensen et al. 2013

for San Diego Bay; data for Mission Bay 2011 unpublished). We compared these 2011 data to both 1994–2000 and 2019–2020 data in the same analysis. The survey site at J Street in San Diego Bay was not included in the comparisons, as there is no counterpart in the 2011 survey.

There were some differences in species identifications between the 1994–2000, 2011, and 2019–2020 surveys, and these differences were addressed to ensure a one-to-one species comparison. *Molgula ficus* (Macdonald, 1859) was recorded in 1994–2000 as *Molgula verrucifera* (Ritter & Forsyth, 1917), but these records were corrected in Lambert 2007. In the 1994–2000 surveys, *Botrylloides giganteus* (Peres, 1949) is referred to as *Botrylloides perspicuum*; this identification was corrected in Rocha et al. 2019. The species referred to as *Ciona intestinalis* (Linnaeus, 1767) in Lambert and Lambert (1998, 2003) has since been corrected; it is *Ciona robusta* (Hoshino & Tokioka, 1967) (Brunetti et al. 2015). *Botrylloides violaceus* (Oka, 1927) was not correctly identified in time for inclusion in the Lambert and Lambert 1998 publication but most of the distribution records were correctly included in Table 3 in Lambert and Lambert 2003.

## Analyses

To visualize changes in species abundances, we created several non-metric multidimensional scaling (nMDS) plots using PRIMER v.7 multivariate statistical software (Clarke and Gorley 2015). First, we created a plot of the pairwise comparison, between 1994–2000 and 2019–2020. Next, we created a plot using data from San Diego only, which included all three of the surveys (1994–2000, 2011, 2019–2020). The plots included the species recorded in the surveys: *Ascidia ceratodes* (Huntsman, 1912), *Ascidia* sp. 1 (described in Lambert and Lambert 1998), *Ascidia zara* (Oka, 1935), *Botrylloides giganteus*, *Botryllus schlosseri* (Pallas, 1766), *Ciona robusta*, *Ciona savignyi* (Herdman, 1882), *Diplosoma listerianum* (Milne Edwards, 1841), *Distaplia occidentalis* (Bancroft, 1899), *Microcosmus squamiger* (Michaelsen, 1927), *Molgula ficus*, *Molgula manhattensis* (De Kay, 1843), *Perophora annectens* (Ritter, 1893), *Polyandrocarpa zorritensis* (Van Name, 1931), *Styela canopus* (Savigny, 1816), *Styela clava*, *Styela gibbsii* Stimpson, 1864, *Styela plicata* (Lesueur, 1823), and *Symplegma reptans* (Oka, 1927). Not all species recorded in 1994–2000 or 2019–2020 were included in the comparisons. Because of uncertainties in identification at the time analyses were conducted, *Aplidium californicum* (Ritter & Forsyth, 1917), *Aplidium accareense* (Millar, 1953), *Ascidia* cf. *virginea*, *Ascidiella aspersa* (Müller, 1776), *Botrylloides violaceus*, *Didemnum* sp. white, and *Perophora japonica* (Oka, 1927) were excluded from the comparisons. *Botrylloides diegensis* (Ritter & Forsyth, 1917) and *Didemnum vexillum* (Kott, 2002) were found and correctly identified in 1994–2000 but not recorded, and were therefore excluded.

For all nMDS plots, the samples were not standardized, and a square root transformation was used to fit an S17 Bray-Curtis resemblance matrix. To compare the communities between time periods in a statistical framework, an ANOSIM analysis was performed with each nMDS plot using the same parameters. To determine individual species contributions to the sample dissimilarity between time periods, a SIMPER analysis was completed along with each nMDS plot. The SIMPER analyses were run using the S17 Bray-Curtis resemblance matrix to find the average dissimilarity between paired samples in each group, which was then separated into the percent contribution of each species.

We also plotted the change in abundance between time periods averaged across sites for each species using R 4.0.4 (R Core Team 2019). We found the average

abundance for each species across all sites in both the 1994–2000 and 2019–2020 surveys. The difference between these average abundances from 1994–2000, and the 2019–2020 average abundances was then recorded and implemented into an R script to plot the changes along the y-axis. As the 1994–1997 survey averages were subtracted from the 2019–2020 survey averages, a positive number indicates an increase in abundance, and a negative number indicates a decrease in abundance.

## Results

### Species identification

Table 2 lists the species from all surveys organized by order. mtCO1 sequences are available on GenBank (Accession Numbers [MW872258–MW872314](#), [MZ782781–MZ782802](#); Table 2). No sequence was obtained for entries labeled NA under Accession Number in Table 2.

**Table 2.** All the ascidian species found in the surveys, organized alphabetically by order, with origin, authority, year of first report, and GenBank accession numbers of each species. The species listed were found in at least one survey year, but are not all included in the one-to-one comparisons. Of these 25 species, 17 are classified as non-native, three as native, and five as cryptogenic. The introduction status of each species follows Chapman and Carlton (1991). In determining the status of each species included in this study (native, introduced, or cryptogenic), we utilized in part the Smithsonian Environmental Research Center’s National Estuarine and Marine Exotic Species Information System (NEMESIS), to which one of us (GL) contributed a major part over the years. (<https://invasions.si.edu/nemesis/>).

Order / Species	Origin	Solitary/ Colonial	Year of First Report in California	Source	Accession Number
<b>Aplousobranchia</b>					
<i>Aplidium accarensense</i> (Millar, 1953)	Non-native	Colonial	2019	Present Study	<a href="#">MW872264</a> , <a href="#">MW872289–MW872291</a> , <a href="#">MW872297–MW872298</a> , <a href="#">MW872301</a> , <a href="#">MW872303</a> , <a href="#">MW872304</a> , <a href="#">MW872308</a> , <a href="#">MW872309</a> , <a href="#">MW872311</a>
<i>Didemnum vexillum</i> Kott, 2002	Non-native	Colonial	1996	Lambert 2009	NA
<i>Didemnum</i> sp. white NA	Cryptogenic	Colonial	NA	NA	NA
<i>Diplosoma listerianum</i> (Milne Edwards, 1841)	Cryptogenic	Colonial	1917	Ritter and Forsyth 1917	<a href="#">MW872288</a> , <a href="#">MW872295</a> , <a href="#">MW872296</a>
<i>Distaplia occidentalis</i> Bancroft, 1899	Native	Colonial	1917	Ritter and Forsyth 1917	NA
<b>Phlebobranchia</b>					
<i>Ascidia ceratodes</i> (Huntsman, 1912)	Native	Solitary	1917	Ritter and Forsyth 1917	<a href="#">MW872261–MW872263</a> , <a href="#">MW872268–</a> <a href="#">MW872269</a> , <a href="#">MW872273–MW872275</a> , <a href="#">MW872280–MW872284</a> , <a href="#">MW872292–</a> <a href="#">MW872293</a> , <a href="#">MW872299–MW872300</a> , <a href="#">MW872305</a> , <a href="#">MZ782793–MZ782796</a>
<i>Ascidia zara</i> Oka, 1935	Non-native	Solitary	1984	Lambert and Lambert 1998	<a href="#">MZ782781–MZ782792</a>
<i>Ascidia</i> sp. 1 NA	Non-native	Solitary	1983	Lambert and Lambert 1998	NA
<i>Ascidia</i> cf. <i>virginea</i> NA	Non-native	Solitary	2020	Present study	<a href="#">MW872278</a>
<i>Ascidella aspersa</i> (Müller, 1776)	Non-native	Solitary	2019	Nydam et al. 2022	<a href="#">MW872258</a> , <a href="#">MW872260</a> , <a href="#">MW872267</a> , <a href="#">MW872271</a> , <a href="#">MW872272</a> , <a href="#">MW872276</a> , <a href="#">MW872277</a> , <a href="#">MW872307</a> , <a href="#">MW872313</a> , <a href="#">MW872314</a> , <a href="#">MZ782797</a> , <a href="#">MZ782798</a>

Order / Species	Origin	Solitary/ Colonial	Year of First Report in California	Source	Accession Number
<i>Ciona robusta</i> Hoshino & Tokioka, 1967	Cryptogenic	Solitary	1915	Ritter and Forsyth 1917 (as <i>C. intestinalis</i> )	MZ782799, MZ782800
<i>Ciona savignyi</i> Herdman, 1882	Non-native	Solitary	1985	Lambert and Lambert 1998	MZ782801
<i>Perophora annectens</i> Ritter & Forsyth, 1917	Native	Colonial	1917	Ritter and Forsyth 1917	NA
<i>Perophora japonica</i> Oka, 1927	Non-native	Colonial	2011	G Lambert, unpublished	MW872302
Stolidobranchia					
<i>Botryllus schlosseri</i> (Pallas, 1766)	Cryptogenic	Colonial	1965	Lambert and Lambert 1998	NA
<i>Botrylloides diegensis</i> Ritter & Forsyth, 1917	Cryptogenic	Colonial	1917	Ritter and Forsyth 1917	MW872270, MW872285
<i>Botrylloides giganteus</i> (Pérès, 1949)	Non-native	Colonial	1997	Lambert and Lambert 2003	MW872286–MW872287
<i>Botrylloides violaceus</i> Oka, 1927	Non-native	Colonial	1997	Lambert and Lambert 1998	MW872259
<i>Microcosmus squamiger</i> Michaelsen, 1927	Non-native	Solitary	1986	Lambert and Lambert 1998	MW872265–MW872266
<i>Molgula ficus</i> (Macdonald, 1859)	Non-native	Solitary	1994	Lambert 2007	NA
<i>Molgula manhattensis</i> (De Kay, 1843)	Non-native	Solitary	1984	Lambert and Lambert 1998	NA
<i>Polyandrocarpa zorritensis</i> Van Name, 1931	Non-native	Social	1994	Lambert and Lambert 1998	NA
<i>Symplegma reptans</i> (Oka, 1927)	Non-native	Colonial	1991	Lambert and Lambert 1998	MW872294, MW872306
<i>Styela canopus</i> (Savigny, 1816)	Non-native	Solitary	1972	Lambert and Lambert 1998	NA
<i>Styela clava</i> Herdman, 1881	Non-native	Solitary	1933	Abbott and Johnson 1972	MZ782802
<i>Styela gibbsii</i> Stimpson, 1864	Native	Solitary	1927	Johnson and Snook (1927)	NA
<i>Styela plicata</i> (Lesueur, 1823)	Non-native	Solitary	1915	Ritter and Forsyth 1917	NA

## Diversity and distribution in 2019–2020

Several species recorded in the 2019–2020 surveys which were not included in the comparison to historical data were found consistently in southern California marinas throughout the survey. *B. diegensis* was recorded at an average abundance of 1.95 across the 2019–2020 surveys and was consistently present or common across survey sites (with the exception of Kings Harbor) (Suppl. material 1). More recently introduced species *Ascidella aspersa*, *Ascidia* cf. *virginea*, and *Perophora japonica* were all recorded several times throughout the 2019–2020 surveys. The first California record for *P. japonica* was in 2003 in northern California (Lambert 2005b) but was not found in southern California during the 1994–2000 surveys.

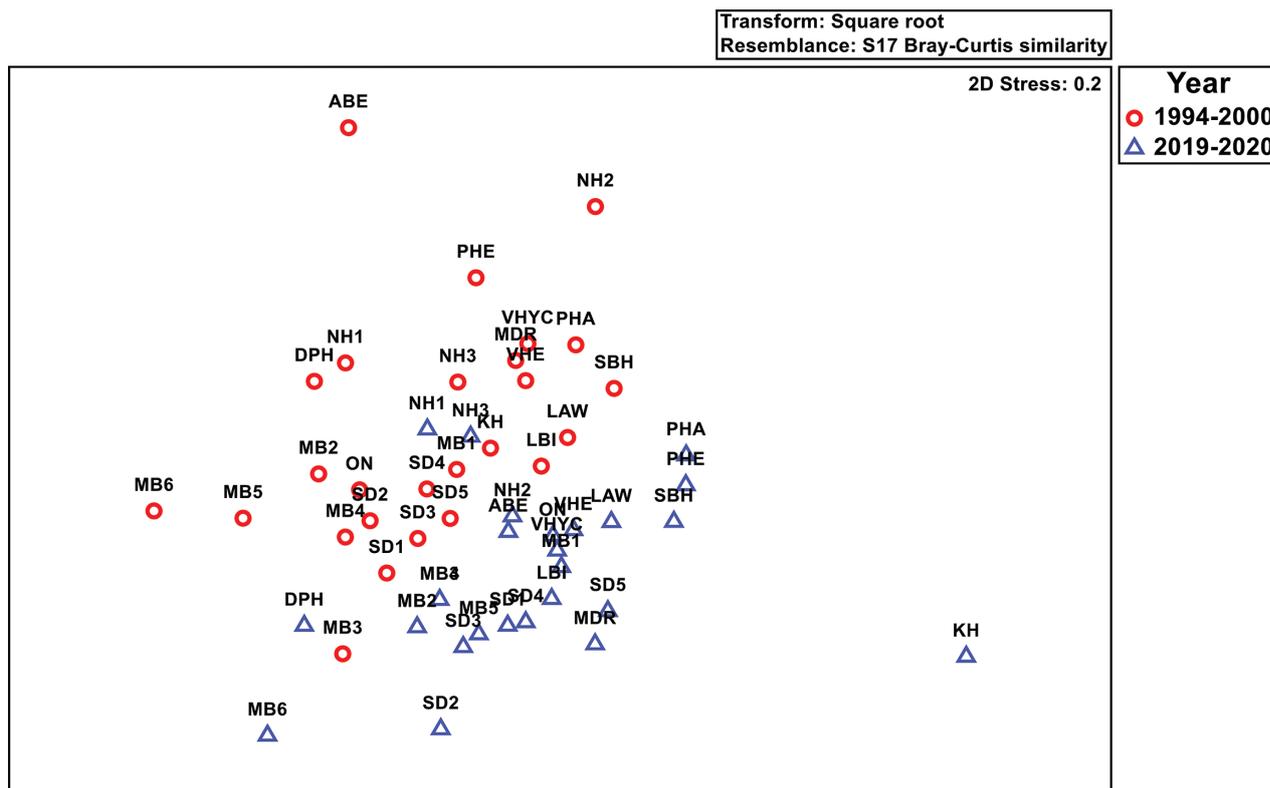
**Table 3.** The geographic ranges for each species in both the 1994–2000 and 2019–2020 surveys. For all species used in the comparison, three contracted in range, six expanded in range, eight did not change in range, and two are no longer present.

Species	Range 1994–2000	Range 2019–2020	Expansion/ Contraction
<i>Ascidia ceratodes</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Ascidia</i> sp. 1	San Diego Bay- Santa Barbara Harbor	Not Recorded	No Longer Present
<i>Ascidia zara</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Botryllus schlosseri</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Botrylloides giganteus</i>	San Diego Bay	San Diego Bay- Santa Barbara	Expansion
<i>Ciona robusta</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara	No Change
<i>Ciona savignyi</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Diplosoma listerianum</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Distaplia occidentalis</i>	San Diego Bay- Los Angeles Harbor	Alamitos Bay- Los Angeles Harbor	Contraction
<i>Microcosmus squamiger</i>	San Diego Bay- Ventura Harbor	San Diego Bay- Long Beach	Contraction
<i>Molgula ficus</i>	San Diego Bay- Port Hueneme	San Diego Bay- Santa Barbara Harbor	Expansion
<i>Molgula manhattensis</i>	Newport Harbor- Ventura Harbor	San Diego Bay- Newport Harbor	Expansion
<i>Perophora annectens</i>	San Diego Bay- Oceanside Harbor	Not Recorded	No Longer Present
<i>Polyandrocarpa zorritensis</i>	San Diego Bay- Redondo Beach	San Diego Bay- Santa Barbara Harbor	Expansion
<i>Styela canopus</i>	San Diego Bay- Oceanside Harbor	San Diego Bay- Santa Barbara Harbor	Expansion
<i>Styela clava</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Santa Barbara Harbor	No Change
<i>Styela gibbsii</i>	Long Beach Impound Marina	Alta Sea Dock	No Change
<i>Styela plicata</i>	San Diego Bay- Santa Barbara Harbor	San Diego Bay- Ventura Harbor	Contraction
<i>Symplegma reptans</i>	San Diego Bay- Long Beach	San Diego Bay- Los Angeles Harbor	Expansion

## Changes in species abundance

The nMDS plot visualizing differences in community composition of all species from 1994–2000 to that of the 2019–2020 surveys, displays two distinct clusters with slight overlap and a few outliers (Figure 2). The points representing 2019–2020 survey sites are more tightly clustered than the points representing the averages from sites in 1994–2000. The ANOSIM analysis indicates a difference between the two groups ( $R = 0.282$ ,  $p$ -value = 0.001, 999 permutations). The slight overlap in clusters is caused by several sites, like NH1 (Newport Inner Harbor) and NH3 (Newport Upper Harbor) in the 2019–2020 survey, that are more similar in abundances to sites from the average of 1994–2000 than to other sites in the 2019–2020 surveys (Figure 2). King Harbor Marina (KH) is the biggest outlier in the 2019–2020 survey, as it is separated from the main cluster comprising surveys. There are also several sites in the 1994–2000 surveys which are closer in proximity to the 2019–2020 surveys. The most obvious is MB3, Mission Bay South Shores Boat Launch.

The SIMPER analysis indicates that there are eleven species with similar contributions to the dissimilarity between groups, making up 75.13% of the cumulative contribution to this dissimilarity. Of these eleven species, 6 increased and 5 decreased in abundance (Figure 3). *P. zorritensis* is the species with the highest contribution to the dissimilarity (Suppl. material 3), with an increase from an average abundance of 0.87 in the earliest survey of 1994–2000 to 1.45 in 2019–2020. This increase indicates that *P. zorritensis* was classified as common on more occasions in 2019–2020 than in the earliest surveys recorded in 1994–2000 (Figure 3). *M. squamiger* has the second highest contribution to the dissimilarity between the

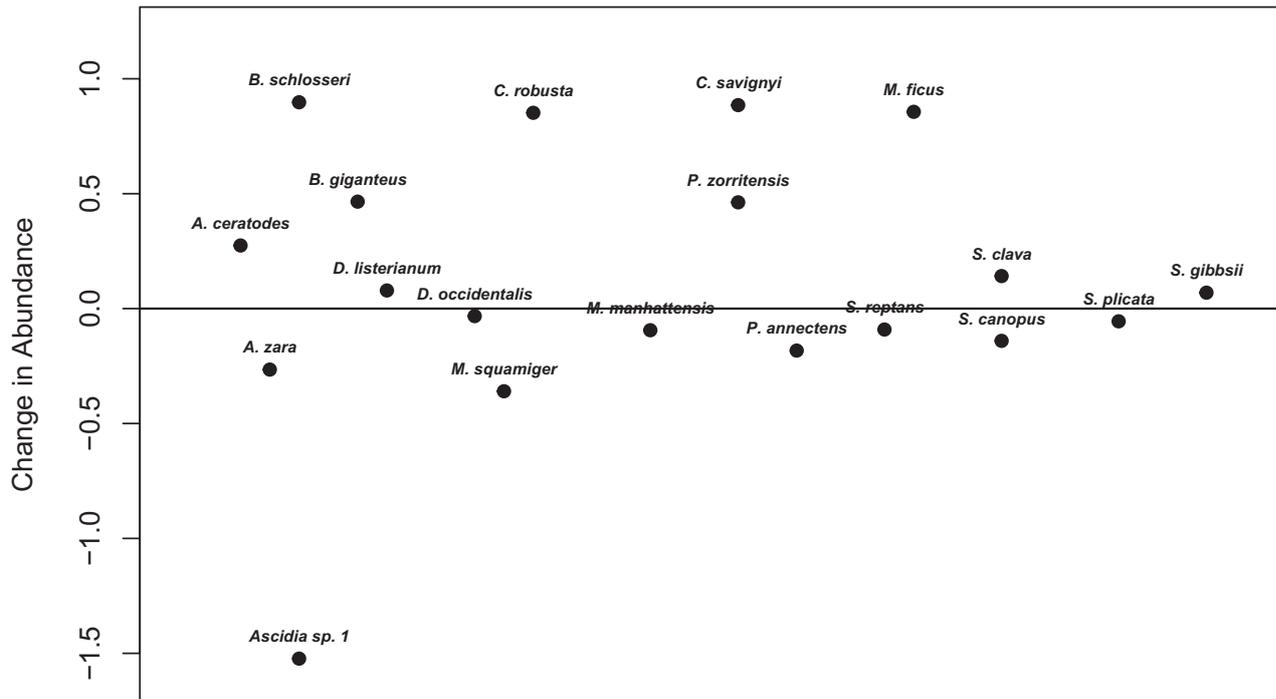


**Figure 2.** nMDS plot displaying differences in community composition between the 1994–2000 surveys and the 2019–2020 survey. The sites used for comparison, and their corresponding abbreviations, are consistent with the 1994–2000 survey. The following sites and abbreviations are used: Santa Barbara Harbor (SBH), Ventura Harbor Entrance (VHE), Ventura Harbor Pierpont Yacht Club (VHYC), Port Hueneme Entrance (PHE), Port Hueneme Anacapa Island Marina (PHA), King Harbor (KH), Marina Del Rey (MDR), LA Harbor Watchorn Marina (LAW), Long Beach Impound Marina (LBI), Alamitos Bay Entrance (ABE), Newport Harbor Inner Harbor (NH1), Newport Harbor Lido Peninsula (NH2), Newport Harbor Upper Bay (NH3), Dana Point Harbor (DPH), Oceanside North Harbor (ON), Mission Bay Seaforth Landing (MB1), Mission Bay Dana Landing (MB2), Mission Bay South Shores Boat Ramp (MB3), Mission Bay Bahia Point (MB4), Mission Bay Yacht Club (MB5), Mission Bay Santa Clara Boat Launch (MB6), San Diego Bay 24<sup>th</sup> Street National City (SD1), San Diego Bay J Street Chula Vista (SD2), San Diego Bay Fiddlers Cove (SD3), San Diego Bay Harbor Island (SD4), and San Diego Bay Shelter Island (SD5). This plot includes the species recorded in both surveys, across all sites. Group 1, the blue triangles, represents the abundances from the 2019–2020 survey, with each point representing a specific site. The red triangles represent the abundances recorded at the sites from the 1994–2000 survey. The plot shows clustering of each group with slight overlap, indicating that many of the sites within each group are closer in abundance to each other than they are to the other group.

groups, with a 0.38 abundance point decrease from 1.51 in the 1994–2000 surveys to 1.13 in 2019–2020 (Figure 3).

The year-to-year comparison between the Fall 1997 survey and 2019–2020 survey for all species shows an overlap between the groups, with less distinguishable clusters (Suppl. material 4). Visually, there is more overlap of the two groups than in the plot of the average abundances in 1994–2000 and 2019–2020 (Figure 1). The sites from the 2019–2020 survey form a tight cluster within the 1997 survey sites (Suppl. material 4). The difference between groups remains significant, as the ANOSIM gives an R statistic of 0.166 with a p-value of 0.001, 999 permutations. The species with the highest contributions to the dissimilarity between time periods, as evidenced by the SIMPER analysis, mirrors that of the analysis for 1994–2000 and 2019–2020. The three highest contributors (*P. zorriventris*, *C. robusta* and *M. squamiger*) have similar contribution percentages.

The 2011 survey conducted in San Diego provides an intermediate point between the surveys conducted in 1994–2000 and those in 2019–2020. The



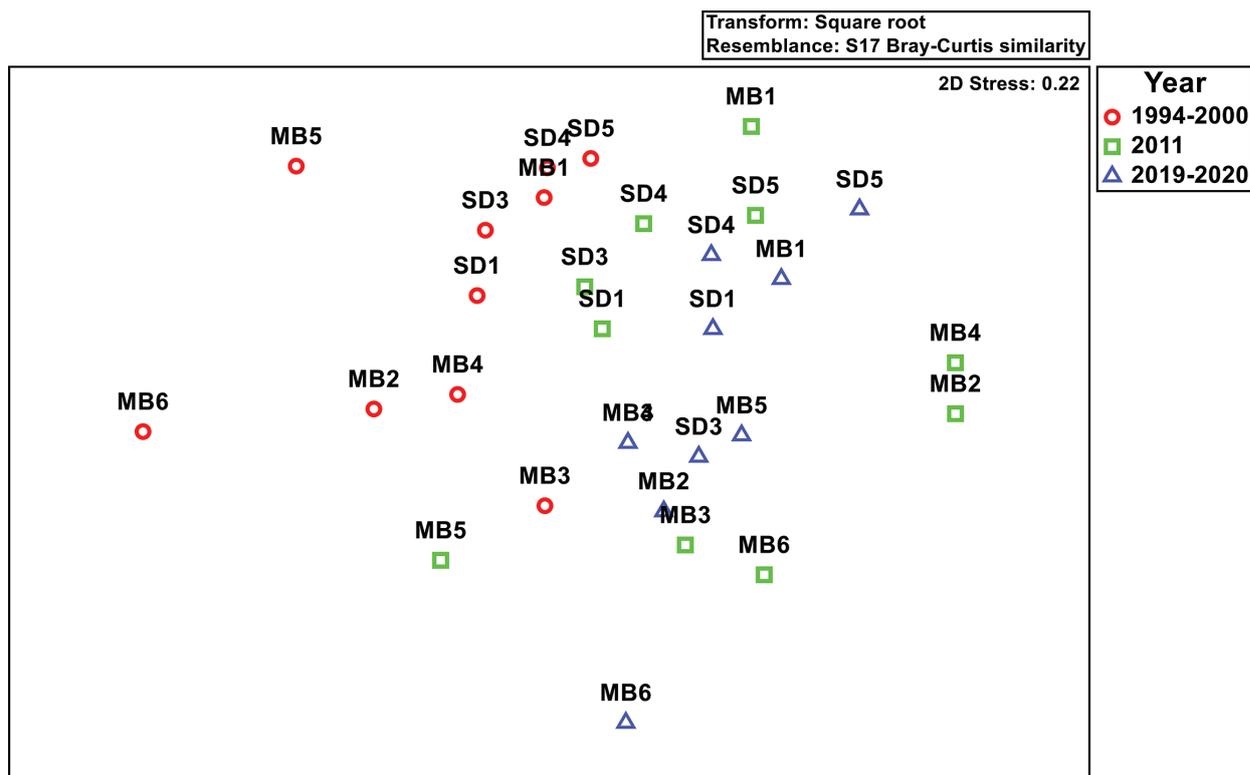
**Figure 3.** Graph of the change in abundance for each species from 1994–2000 to 2019–2020. Each point in the graph represents a species, and the abundance point increases or decreases from the 1994–2000 survey to the 2019–2020 survey. Species are displayed in alphabetical order along the x-axis.

nMDS for all sites and species recorded in San Diego, over three time periods (1994–2000, 2011, 2019–2020), shows obvious visual clusters with a few outliers (Figure 4). The nMDS shows two separate clusters: one includes both the 2019–2020 surveys and 2011 survey, which is separated from the other cluster, composed of sites from the 1994–2000 surveys (Figure 4). All outliers, with the exception of Mission Bay Yacht Club (MB5) from the 1994–2000 surveys, are closer to their respective clusters than to the other cluster. As these outlying sites are still closer in abundance to the other sites in the same cluster (1994–2000 or 2019–2020 and 2011), this further indicates a difference in abundance between groups.

The ANOSIM analysis for this nMDS plot provides a global test, as well as three pairwise tests for the difference in year groups. The global test value is statistically significant (R statistic = 0.262,  $p = 0.001$ , 999 permutations). The pairwise test between 1994–2000 and 2019–2020 and the pairwise test between 1994–2000 and 2011 shows similar results (R statistic = 0.419,  $p = 0.001$ , 999 permutations vs. R statistic = 0.252,  $p = 0.002$ , 999 permutations). The pairwise test between 2011 and 2019–2020 shows greater similarity between these two groups, but remains significant at  $p = 0.05$  (R statistic = 0.123,  $p = 0.043$ , 999 permutations).

The SIMPER analysis for this nMDS plot provides three pairwise tests between groups. The SIMPER analysis between the average of 1994–2000 and 2019–2020 indicates that *D. listerianum* has the largest contribution to dissimilarity in San Diego over this period. The average abundance of *D. listerianum* in San Diego decreased from 1.04 in 1994–2000, to 0.52 in the 2019–2020 surveys.

*Ciona robusta* is the species with the largest contribution to dissimilarity between the 1994–2000 surveys and 2011, increasing from an average abundance of 0.66 in 1994–2000 to 1.28 in 2011. Despite the greater similarity between 2011 and 2019–2020 surveys, the SIMPER analysis indicates that the level of



**Figure 4.** nMDS plot displaying differences between community composition in the 1994–2000 survey, the 2011 survey, and the 2019–2020 survey. The sites used for comparison, and their corresponding abbreviations, are consistent with the 1994–2000 survey. The sites and abbreviations are the same as in Fig. 2. Red represents the 1994–2000 sites, green the 2011 sites, and blue represents the 2019–2020 survey sites. The clusters of sites for 2019–2020 occur in between two clusters of 2011 survey sites. There are two visual clusters, one of the 1994–2000 data, and the other consisting of both the 2011 and 2019–2020 data.

dissimilarity between groups can be attributed to the same several species. The native species *A. ceratodes* has the highest contribution to dissimilarity between the groups, having decreased in abundance from an average abundance of 0.91 in 2011, to 0.38 in 2019–2020.

### Range changes

The geographic ranges for each species were documented in both the 1994–2000 and 2019–2020 surveys, and are shown in Table 3. For all species used in the comparison, 3 contracted in range, 6 expanded in range, 8 did not change in range, and 2 are no longer present.

## Discussion

### Diversity and distribution of native species

It is becoming increasingly rare to find native species on the docks of marinas, especially those with preferences for a natural habitat (Lambert and Lambert 2003). Historically, native species were found abundantly in southern California (Ritter and Forsyth 1917; Van Name 1945), and several of the most commonly found species on the docks are no longer present. For example, *S. gibbsii* and *P. annectens* were both considered abundant by Ritter and Forsyth in 1917 and Van Name in 1945, but were extremely rare or not present in the 1994–2000 and 2019–2020

surveys. As the marinas are overwhelmed by non-native species, we see a decrease and even disappearance of several native species on the docks, probably starting before the 1994–2000 survey.

### *Aplidium californicum*

All *Aplidium* colonies we sequenced in the 2019–2020 surveys were *Aplidium* cf. *accarensis*, and *Aplidium californicum* was never observed. It was common though patchy in the 1994–2000 surveys (Suppl. material 2).

### *Ascidia ceratodes*

*A. ceratodes* is the only native species to be consistently present in the marinas we surveyed. It was considered rare in both the 1994–2000 and 2019–2020 surveys, although it was common or even abundant at several locations in August 2000, and common in Port Hueneme (Fall 1995) and Santa Barbara (Fall 1994) (Suppl. material 2). This species did not change in abundance between time periods (Figure 3).

In 1917, Ritter and Forsyth recorded *A. ceratodes* as *A. californica*, classifying the species “fairly common” in the whole of southern California and everywhere in San Diego. The natural habitat of *A. ceratodes* is on the underside of rocks and is often only visible at low tide (Ritter and Forsyth 1917). In 1945, Van Name noted that *A. ceratodes* was found abundantly on floats and piles of marinas, as well as in water up to 30 meters deep in San Diego (Van Name 1945). One of the limitations of our survey was the limited access to deeper water. While we were able to survey submerged ropes, which reached beyond the surface buoys and docks, several marinas did not have hanging ropes, and ropes rarely reached beyond a few meters in depth. In 2019–2020, *A. ceratodes* was recorded mostly as rare, and was almost never recorded in groups. Lambert and Lambert (1998) described *A. ceratodes* as rarer in abundance than recorded in Ritter and Forsyth (1917), and much less common and also smaller in southern California than in northern California.

### *Distaplia occidentalis*

*D. occidentalis* is a colonial species found throughout the NW Pacific coast, from San Diego to Alaska (Ritter and Forsyth 1917; Van Name 1945; Lambert et al. 1996; Lamb and Hanby 2005; Abbott et al. 2007; Lambert 2019). *D. occidentalis* was present in both the 1994–2000 and 2019–2020 surveys, however it was consistently rare and the colonies were small. As it was recorded only a few times in both surveys, the average abundance was consistent over the time period (Figure 3). Despite being historically common on littoral rocks north of southern California (Van Name 1945), *D. occidentalis* was not found north of Los Angeles in either survey.

### *Perophora annectens*

*P. annectens* was recorded as rare or common at several sites in San Diego during the 1994–2000 and 2011 surveys, but was not recorded during in the 2019–2020 surveys (Suppl. materials 1, 2). *P. annectens* is a social species (Lambert and Lambert 1998), which naturally occurs on low intertidal and shallow subtidal rocks along the entire NE Pacific coast (Van Name 1945; Lambert et al. 1996; Lamb and Hanby 2005; Abbott et al. 2007; Lambert 2019). In 1917, Ritter and Forsyth

described *P. annectens* as “by no means rare on the southern coast”; however, since the 1994–2000 surveys by Lambert and Lambert, *P. annectens* has been consistently rare, or not present, in marinas.

### *Styela gibbsii*

The abundance of *S. gibbsii* is difficult to determine from a survey of marinas, as this native species has stricter habitat limitations (Ritter and Forsyth 1917). As recorded by both Ritter and Forsyth (1917) and Van Name (1945), *S. gibbsii* was found commonly along the entire Pacific coast, but primarily in deeper waters. *S. gibbsii* was recorded as rare in a single location during both the 1994–2000 and 2019–2020 surveys (Suppl. materials 1, 2). It was recorded at the Long Beach Impound Marina in 2000 and at the Alta Sea Dock in 2019. It is entirely possible that despite being nearly absent from the marinas in these surveys, *S. gibbsii* is present at depths.

### *Styela montereyensis*

*Styela montereyensis* (Dall, 1872) is common subtidally from southern California to British Columbia (Van Name 1945; Lamb and Hanby 2005; Abbott et al. 2007; Lambert 2019) but was recorded only once in Santa Barbara, spring 1997, on a floating dock with substantial water current (Suppl. material 2) and occasionally on floating docks in Washington State with considerable water movement, along the Strait of Juan de Fuca (GL unpublished). Very large specimens were recorded commonly in 2019 subtidally offshore during the LA/Long Beach biodiversity survey.

## Abundance and distribution of non-native ascidians

As described in the results of the 1994–2000 surveys (Lambert and Lambert 2003), the leading trend within these surveys is the long-term persistence of non-native species in southern California marinas. Despite this continued persistence, the species abundances have changed since the 1994–2000 surveys. As hypothesized by Lambert and Lambert (2003), several persistent non-natives in southern California have remained abundant, and in some cases have spread north. A significant example is *Botrylloides giganteus*, now recorded as far north as Santa Barbara but only in San Diego by 2000 (Lambert and Lambert 2003).

An *Aplidium* sp. was regularly collected on southern California docks by one of the authors (GL) in the 1990's, but a morphological match was never found in the literature. The same species was also found in the 2019–2020 survey. A recent paper by Montesanto et al. (2021) contributed *Aplidium accareense* sequences to GenBank, which were a 100% match to the sequences we generated from the southern California *Aplidium*. We compared the morphological characters of our *Aplidium* species to the descriptions of *Aplidium accareense*, and confirmed that our *Aplidium* species is *Aplidium accareense*. Montesanto et al. (2021) reviewed all the past records for this species, which included West Africa, Brazil and Venezuela, while adding their new records for the Mediterranean. This is the first California and indeed the first Pacific record for *A. accareense*. Its morphology agrees well with the very detailed description in Montesanto et al. (2021) except for a color difference; the Mediterranean specimens are yellowish while the southern California specimens have orange zooids clearly visible through the transparent tunic. Montesanto et al. (2021) acknowledged the color difference between their specimens and the various zooid colors of the West African and South America specimens and attributed it to an intra-specific variability; we feel that the orange color of our specimens is

another intra-specific difference, given that the CO1 sequences are 100% identical to the Mediterranean samples.

*Ascidia* sp. 1 (described in Lambert and Lambert 1998) is the only non-native species recorded in 1994–2000 which was not recorded in 2019–2020. We cannot rule out the possibility that it was still present in 2019–2020, despite not seeing it in the surveys. *Ascidia* sp. 1 remained common in San Diego from 1994–2000, when subsequent to the fall of 1997 this species almost completely disappeared from all other harbors (Lambert and Lambert 2003). With the results of the 2019–2020 surveys, it is reasonable to assume that the population did not recover from this initial disappearance, which may have occurred due to cooler water temperatures (Lambert and Lambert 2003).

While *Polyandrocarpa zorritensis* has remained persistent in southern California over the last 30 years, it is the species with the greatest amount of change since the initial 1994–2000 surveys. This species is highly adaptive, and tolerant to extreme temperature and salinity changes, making it a formidable invader to temperate coastal areas (Lambert and Lambert 1998). In 1998, Lambert and Lambert had only found *P. zorritensis* in San Diego, but hypothesized that the species would continue to persist in southern California, spreading further north, as has been shown for other non-native ascidians first recorded in southern California (Tracy et al. 2017). By 2003, *P. zorritensis* was found in several sites in Los Angeles. The 2019–2020 surveys further support this hypothesis in that *P. zorritensis* was found in several sites as far north as Santa Barbara Harbor where it was recorded as rare. The 2019–2020 survey also indicates that *P. zorritensis* has increased in abundance since 1994–2000.

*Microcosmus squamiger*, with the ability to endure wide ranges of temperature and salinity, was expected to increase in abundance in southern California, as well as spread further north (Lambert and Lambert 2003; see Tracy et al. 2017). *M. squamiger* was recorded as common or abundant on more occasions in 1994–2000 than in 2019–2020 (Suppl. materials 1, 2). Despite a decrease in abundance, *M. squamiger* has remained persistent in southern California, and was consistently recorded at marinas from Los Angeles to San Diego. In the 1994–2000 surveys, *M. squamiger* was recorded as far north as Ventura Harbor; however, it was not recorded north of LA Harbor in 2019–2020.

*Molgula ficus* was first recorded in southern California in 1994, and is thought to have been introduced to San Diego Bay around the same time (Lambert 2007). Throughout the 1994–2000 surveys, *M. ficus* increased in abundance and spread as far north as Port Hueneme by fall 1997 (Lambert 2007). Following these same patterns in 2019–2020, *M. ficus* has increased in average abundance and has spread further north to Ventura and Santa Barbara. It was first recorded in San Francisco Bay in October 2005 (Lambert 2007; Tracy et al. 2017).

In 1994–2000, *Styela plicata* was recorded as the most abundant non-native species and was present at every site surveyed (Lambert and Lambert 1998, 2003). *S. plicata* remained similarly abundant in 2019–2020, and has thus remained consistently common in southern California marinas. *Botrylloides giganteus* (previously referred to as *B. perspicuum* but corrected by Rocha et al. 2019), was the most recent non-native species recorded in the 1994–2000 surveys, and was first discovered in San Diego in 1997 (Lambert and Lambert 2003). It was locally abundant in San Diego Bay in 2011 (Sorensen et al. 2013), and was recorded as rare or common at several sites in San Diego Bay in 2019–2020. *B. giganteus* has also spread northward since the 2011 survey, and was recorded as far north as Santa Barbara Harbor in the 2019–2020 survey (Suppl. material 1). Similarly, non-native species *P. zorritensis*, *Styela clava*, and *Ciona robusta* have all increased in abundance from the earliest surveys in 1994–1997. Several persistent invaders, like *S. plicata* and *Molgula manhattensis*, have maintained a steady abundance within the harbors.

## New introductions

New species introductions have influenced the changing community composition in southern California. *Ascidella aspersa* (Müller, 1776) was recorded for the first time in southern California in the 2019–2020 surveys (Nydham et al. 2022). *A. aspersa* is a solitary ascidian native to Europe. Berrill (1928) noted that *A. aspersa* was confined to Europe including the Mediterranean. Molecular phylogenetic analyses of invasive populations in Japan and native populations in Europe discerned two *Ascidella* species genetically as well as by morphological differences (Nishikawa et al. 2014, 2019). Our samples of *A. aspersa* have >99% mtCO1 identity with those of *A. aspersa* on the east coast of the United States and Canada (LeBlanc et al. 2020), in Europe (Nishikawa et al. 2014; López-Legentil et al. 2015; Miralles et al. 2016; Couton et al. 2019) and also in Japan (Nishikawa et al. 2014; Shito et al. 2020), where it has been a persistent invasive causing serious damage to the scallop aquaculture industry in Hokkaido (Kanamori et al. 2017). Our samples also agree morphologically with those in Europe and Japan (Nishikawa et al. 2014; Nydam et al. 2022). In the United States, *A. aspersa* was first discovered in New England in the 1980s (Altman and Whitlatch 2007; JT Carlton pers. comm.), and has been a persistent invader on the NE coast of the U.S., often outcompeting native ascidians on artificial structures (Salem Sound Coastwatch 2011). See Nydam et al. (2022) for a more complete analysis of the introduction of *A. aspersa* worldwide as well as in southern California.

An unknown *Ascidia* species (distinct from *Ascidia* sp. 1 in the 1994–2000 surveys), was found in San Diego in 2019–2020. The mtCO1 sequence of this species had >99% sequence identity to *Ascidia virginea* (Müller, 1776) from Catalunya, Spain (Accession Number [KF309647](#), López-Legentil et al. 2015), from Florida, USA (Accession Numbers [ON055288](#)–[ON055294](#), Nydam unpublished), and from Norway (Accession Numbers [ON062301](#) and [ON062302](#)) using GenBank's BLAST tool. *A. virginea* is native to northern Europe (Thompson 1933; Berrill 1950; Millar 1966, 1970) and has a significantly different morphology from this recent southern California introduction. Therefore confirmation of the existence of this species in southern California as well as the recent specimens from Spain will require further morphological study.

An unidentified species in the *Diplosoma* genus was found once in Mission Bay (2019) and once in San Diego Bay (2020). The closest mtCO1 match on GenBank is *Diplosoma listerianum*, with only 91% sequence identity. This species has a much thicker tunic than *Diplosoma listerianum*, and has yellow zooids in a black tunic. Identification of this species will require collection of additional samples.

A *Symplegma* species was found once in San Diego Bay (2020). This species is unique morphologically and genetically from all other described *Symplegma*, but the mtCO1 barcode is nearly identical to a sample from Brazil (R Rocha pers. comm.). The tunic is clear, and zooids have gold pigmentation in an anterior/posterior line down the center, with gold pigmentation also outlining the transverse vessels.

## Changes in community composition

Warmer water sea surface temperatures can impact the composition of benthic communities that include ascidians (Dijkstra et al. 2010). In snapshot readings from the 1994–2000 surveys, the average temperature recorded for fall surveys was 20.51 °C, with a range of 13.5 °C to 27 °C (Lambert and Lambert 2003). For the 2019–2020 surveys, temperature data from SCCOOS Automated Shore Stations at Scripps Pier (San Diego), Newport Pier, Santa Monica Pier, and Stearns Wharf

(Santa Barbara) were used for comparison to the 1994–2000 surveys. The average temperature across stations, for both years was 19.03 °C, and temperatures ranged from 11.63 °C to 26.58 °C. The 1994–2000 surveys saw more extreme temperature shifts, as they spanned a longer time period. As the 2019–2020 temperature data was recorded every day of the survey season, it is more thorough than the snapshots recorded in 1994–2000. The slightly lower average temperatures and ranges recorded in 2019–2020 than in 1994–2000 could be a contributing factor to the community abundance changes. However, the average temperatures recorded in 2019–2020 fell within the range of temperatures recorded in 1994–2000. Although average temperature is not likely to explain the dissimilar communities surveyed in this study, climate change can impact fouling communities through the variation in minimum and maximum water temperatures throughout the year (Stachowicz et al. 2002). Similarly, water temperature fluctuations tend to have a positive effect on non-native ascidian recruitment, but do not have the same effect on native species recruitment (Sorte and Stachowicz 2011). The highest temperatures recorded in the fall during 1994–2000 were in 1997, when the first report of *B. giganteus* in southern California was recorded (Lambert and Lambert 2003). This was also the first year in which *P. zorriventis* was recorded north of Oceanside Harbor (Lambert and Lambert 2003). In 2019–2020, the highest temperatures were recorded at Scripps Pier (San Diego) at approximately 25–26.6 °C, and the lowest at Stearns Wharf (Santa Barbara) at approximately 11.6–12 °C. The highest temperatures were recorded in August–September (25–26.6 °C), and lowest in November (11.6–12 °C) across all piers for both years (SCCOOS graphs). These high temperatures in August–September correspond with the first record of *P. zorriventis* as far north as Santa Barbara Harbor.

Salinity levels have also been associated with patterns of invasion on the California coast (Ruiz et al. 2011). Salinity levels were measured as snapshots during the 1994–2000 surveys, and using SCCOOS data for 2019–2020. They averaged 33.47 ppt for fall surveys in 1994–2000 (Lambert and Lambert 2003) and 33.52 ppt in 2019–2020 (SCCOOS). As the salinity levels were consistent across surveys, salinity is not a likely contributor to the dissimilarity in community composition between surveys. Spring minimum salinities had a big effect on the 1994–2000 surveys (Lambert and Lambert 2003) though species presence and abundances had usually recovered completely by the fall surveys. In 2019–2020 only fall surveys were conducted.

Further exploration of the changes in ascidian community composition could be done through the analysis of other environmental factors. Water pollution and other human disturbances could influence the community dynamics (Sorte and Stachowicz 2011). Commercial ship hulls, sea chests and ballast water have largely contributed to the increasing number of non-native species in ports and marinas (Cohen and Carlton 1995, 1998; Cohen et al. 1998, 2001), and is most likely how *A. aspersa* was introduced to southern California. *A. aspersa* is known as the first invasive ascidian to cause serious damage to aquaculture in Japan, and can withstand both temperature and salinity fluctuations (Nishikawa et al. 2019). Based on the current trends in both ascidian composition and climate change, we may expect to see an increase of *A. aspersa* in southern California with possible northward movement to Santa Barbara, and future successful introductions of additional non-native ascidians. Thus, periodic monitoring will remain important.

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## Authors' contribution

G.L. conducted the original surveys. M.L.N. conceived of the idea to re-survey all the original sites and planned the surveys. M.L.N. and C.L.N. conducted surveys and performed analyses necessary for species identification. C.L.N. performed data analyses and wrote the manuscript with input from all authors.

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## Supplementary material 1

All the ascidian species recorded in the 2019–2020 surveys and the abundances recorded at each site

Authors: Claire L. Nichols, Gretchen Lambert, Marie L. Nydam

Data type: table (Excel file)

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Link: <https://doi.org/10.3391/ai.2023.18.1.101962.suppl1>

## Supplementary material 2

Native ascidian species recorded during 1994–2000 surveys

Authors: Claire L. Nichols, Gretchen Lambert, Marie L. Nydam

Data type: table (Excel file)

Explanation note: *Aplidium* sp. A is now considered to be the non-native *Aplidium accarense*. *Diplosoma listerianum* was formerly considered native; it is now recognized as a non-native introduction.

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## Supplementary material 3

The percent contribution of each species to the dissimilarity between the 1994–2000 and 2019–2020 surveys

Authors: Claire L. Nichols, Gretchen Lambert, Marie L. Nydam

Data type: figure (docx file)

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## Supplementary material 4

nMDS plot of the comparison between the abundances recorded in 1997 and the abundances recorded in the 2019–2020 survey

Authors: Claire L. Nichols, Gretchen Lambert, Marie L. Nydam

Data type: figure (PNG image)

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