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INVASIVE SPECIES - ORIGINAL PAPER

Spatial and temporal dynamics of ascidian invasions in the continental United States and Alaska

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Abstract Species introductions have increased dramatically in number, rate, and magnitude of impact in recent decades. In marine systems, invertebrates are the largest and most diverse component of coastal invasions throughout the world. Ascidians are conspicuous and well-studied members of this group, however, much of what is known about their invasion history is limited to particular species or locations. Here, we provide a large-scale assessment of invasions, using an extensive literature review and standardized field surveys, to characterize the invasion dynamics of non-native ascidians in the continental United States and Alaska. Twenty-six non-native ascidian species have established documented populations on the Pacific, Atlantic and Gulf coasts (spanning 25–57°N). Invader species richness is greatest for the Pacific coast (19 spp.), followed by the Atlantic (14 spp.) and Gulf (6 spp.) coasts, and decreases towards higher latitudes. Most species (97 %) expanded their range after initial introduction, although the direction and latitudinal extent of secondary spread varied. Temporal analyses, based on literature reported first records and repeated field surveys, show an increase in recorded non-native ascidians at continental, regional, and local scales. Our results underscore that non-native species continue to establish and spread, and the transfer of biofouling organisms on underwater surfaces of vessels is an active and potent vector that remains largely unmanaged. More broadly, we suggest that ascidians provide a tractable and important indicator group for evaluating invasion dynamics and management strategies.

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Introduction

In coastal environments, the observed rate of invasions has increased steadily in the past century, largely due to a range of human-mediated vectors including commercial shipping, aquaculture transfers, recreational boating and intentional release (Cohen and Carlton 1998; Ruiz et al. 2000; Wasson et al. 2001; Ruiz et al. 2015). Although few aquatic ecosystems are free from invaders, not all regions and habitats are invaded to the same extent (Ruiz et al. 1997). Patterns of invasion vary over latitudinal and regional scales. For instance, polar habitats are less invaded than temperate ones (Ruiz and Hewitt 2009), and bays and estuaries are invaded more often than exposed open coasts (Wasson et al. 2005; Preisler et al. 2009; Ruiz et al. 2009). While there is some discussion of invasion patterns across regions and habitats, contemporary analyses of the spatial extent and temporal spread of marine invaders at large spatial scales are rare, especially when combining extensive field surveys and literature synthesis.

Invertebrates represent the largest and most diverse component of marine invasions throughout the world (Molnar

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et al. 2008). They can be transported by multiple vectors, increasing the likelihood of successful introduction and establishment. For instance, many invertebrates can be carried as planktonic larvae in ballast water aboard commercial ships or as sessile adult stages attached to ships' hulls and sea chests, recreational boats, or shellfish aquaculture stock. Ascidians comprise one of the most conspicuous and well-documented groups of invertebrate invaders, making them a model for studying broad scale invasion patterns and dynamics (see Zhan et al. 2015 for review).

Ascidians (Phylum Chordata, Sub-Phylum Tunicata, Class Ascidiacea) are diverse and abundant members of marine communities, with approximately 3000 described species worldwide (Shenkar and Swalla 2011). They are hermaphroditic, sessile, filter feeders and are found in a variety of habitats from shallow water to the deep sea (Millar 1971; Monniot et al. 1991; Lambert 2005a). They can be solitary or colonial in body form and their life history includes a short lecithotrophic larval phase and a sessile adult form (Svane and Young 1989). They settle on a wide variety of hard substrates including rocky benthos, coral reefs, mangroves, algal fronds, bivalve shells, and man-made structures such as pilings, docks, seawalls, and boat hulls (Millar 1971; Lambert 2005a; Davidson et al. 2010). Given the short dispersal phase of ascidians (minutes to hours) and the numerous ascidian records from beyond their native range, analyses of this group can provide unique insight into the consequence of anthropogenic transport on marine species distributions.

Around the globe, there are 80 ascidian species that are known to be non-native in parts of their documented range (Shenkar and Swalla 2011; Zhan et al. 2015). Some of these species are invasive with increased concern about their potential economic and ecological impacts (Lambert 2007a; McKindsey et al. 2007). For instance, a number of non-native ascidians have been found to displace native species (Stachowicz et al. 2002; Castilla et al. 2004; Blum et al. 2007), overgrow cultured bivalve molluscs (Ramsay et al. 2008; Rius et al. 2011), and alter benthic community structure (Castilla et al. 2004; Valentine et al. 2007). Many of these impacts are reported from anthropogenic habitats, such as marinas, docks, pilings, and aquaculture gear, where these species often flourish (Lambert and Lambert 1998; Lutzen 1999; Lambert 2002; Simkanin et al. 2012). However, some species have invaded natural benthic habitats, where they can compete with native species for space and resources (Castilla et al. 2004; Pereyra et al. 2015).

In this study, we provide an overview and contemporary analysis of non-native ascidian biogeography in the United States, and North America more broadly. Our goal is to contribute insight into the invasion dynamics of a globally widespread group of invaders, which have wide-ranging economic and ecological impacts. Specifically, we characterize spatial and temporal patterns of ascidian introductions by assessing region of origin, introduction dates, arrival locations, transport vectors, and subsequent spread. We focus particular attention on large-scale patterns across coasts, species, and bays.

Materials and methods

To generate a full record of ascidian invaders, we compiled species lists using two separate and complementary methods: an extensive literature review and standardized field surveys. We focused our search on established species that are known to be non-native in the continental United States and Alaska (hereafter referred to as the US). We excluded cryptogenic species (i.e. native/non-native status unknown; see Supplementary Table 1) from analyses and utilized the most recent biogeographical data available to collate species lists. A species was classified as established when: (1) there were multiple records over multiple years for a location, (2) local populations were reportedly numerous and successfully reproducing, or (3) the species was reported as established in the literature or through personal communication (see Ruiz et al. 2000 for greater detail).

Literature review

Non-native ascidian records were compiled through an extensive literature review and synthesis of marine invaders in North America (Table 1). The resulting information is contained within the National Marine and Estuarine Species Information System (NEMESIS), a Smithsonian Institution database created over the past 15 years. NEMESIS is an ongoing effort that includes biogeographical data for more than 400 introduced marine and estuarine species. Data collated and reviewed within the database come from a wide range of sources, including: published papers, unpublished reports and theses, records from long-term monitoring efforts, museum specimens, and communications with marine taxonomists to verify collected information. For each non-native ascidian species we assembled information on: native region, dates of first record per coast and per bay, subsequent occurrence records with dates and locations, and potential vectors of introduction. This synthesis includes data and information from over 7000 Ascidiacea references, worldwide. Information gathered during this extensive review is publicly available at http://invasions.si.edu/nemesis/databases.html. Detailed occurrence records for California are also publicly available as part of the California Non-native Estuarine and Marine Organisms (Cal-NEMO) database at http://invasions.si.edu/nemesis/calnemo/intro.html.

Table 1 Taxonomic and biogeographic information for the 26 ascidian species introduced and established in the US

Species	Order	Body form	Native range	Introduced coast	Date of first record	Location of first record	Citation for first record
Ascidia sydneiensis	P	S	Indo-Pacific	A	1898	Santa Marta, Columbia	Van Name (1921)
Ascidia zara	P	S	NW Pacific	P	1984	LA/Long Beach, CA	Lambert and Lambert (1998)
Ascidiella aspersa	P	S	NE Atlantic	A	1983	Cape Cod Canal, MA	James T. Carlton pers comm.
Botrylloides giganteum	S	C	SW Pacific	P	1997	San Diego, CA	Lambert and Lambert (1998)
Botrylloides violaceus	S	C	NW Pacific	P,A	P: 1966 A: 1980	P: Santa Barbara, CA A: Groton, CT	P: Lambert and Lambert (1998) A: Whitlatch and Osman (2000)
Botryllus schlosseri	S	C	NE Atlantic	P	1947	San Francisco, CA	Carlton (1979)
Ciona robusta	P	S	NW Pacific	P	1897	San Diego, CA	Carlton (1979)
Ciona savignyi	P	S	NW Pacific	P	1985	LA/Long Beach, CA	Lambert and Lambert (1998)
Clavelina lepadiformis	A	C	NE Atlantic	A	2009	New London, CT	Reinhardt et al. (2010)
Corella inflata	P	S	NE Pacific	P	2003	Coos Bay, OR	Ruiz et al. unpublished
Didemnum perlucidum	A	C	Indo-Pacific	A,G	A: 2004 G: 1999	A: Miami, FL; G: Stetson Bank, TX	A: Ruiz et al. unpublished G: Culbertson and Harper (2000)
Didemnum psammatodes	A	C	Indo-Pacific	A,G	A: 1988 G: 2004	A: Indian River, FL G: South Padre Is, TX	A: Bingham (1992) G: Lambert et al. (2005)
Didemnum vexillum	A	C	NW Pacific	P,A	P: 1993 A: 1982	P: San Francisco, CA A: Damariscotta, ME	P: Cohen (2005) A: Dijkstra and Nolan (2011)
Diplosoma listerianum	A	C	Unknown	P,A	P: 1899 A: 1975	P: San Diego, CA A: Groton, CT	P: Eldredge (1966) A:James T. Carlton pers comm.
Diplosoma sp. aff. spongiforme	A	C	NE Atlantic	A,G	A: 2002 G: 2002	A: Indian River, FL G: Tampa Bay, FL	A: Ruiz et al. unpublished G: Ruiz et al. unpublished
Ecteinascidia turbinata	P	C	Unknown	A	1961	Wachapreague, VA	US National Museum of Natural History
Microcosmus squamiger	S	S	Indo-Pacific	P	1986	LA/Long Beach, CA	Lambert and Lambert (1998)
Molgula citrina	S	S	Arctic Boreal	P	2008	Kachemak Bay, AK	Lambert et al. (2010)
Molgula ficus	S	S	Indo-Pacific	P	1994	San Diego, CA	Lambert (2007b)
Molgula manhattensis	S	S	Western Atlantic	P	1949	Tomales Bay, CA	Carlton (1979)
Perophora japonica	P	C	NW Pacific	P	2003	Humboldt Bay, CA	Lambert (2005b)
Polyandrocarpa zorritensis	S	C	Unknown	P,A,G	P: 1994 A: 1986 G: 2002	P: Oceanside, CA A: Indian River, FL G: Clearwater, FL	P: Lambert and Lambert (1998) A: Dalby and Young (1992) G: Lambert et al. (2005)
Styela canopus	S	S	Indo-Pacific	P,A,G	P: 1972 A: 1852 G: 1879	P: San Diego, CA A: Boston, MA G: 'off Southern FL'	P: Lambert and Lambert (1998) A: Stimpson (1852) G: US National Museum of Natural History
Styela clava	S	S	NW Pacific	P,A	P: 1933 A: 1970	P: Newport Beach, CA A: Beverly, MA	P: MacGinitie and MacGinitie (1968), A: Berman et al. (1992)

Table 1 continued

Species	Order	Body form	Native range	Introduced coast	Date of first record	Location of first record	Citation for first record
Styela plicata	S	S	Unknown	P,A,G	P: 1915 A: 1880 G: 1877	P: San Diego, CA A: Charleston, SC G: Cedar Key, FL	P: Ritter and Forsyth (1917) A/G: US National Museum of Natural History (East and Gulf)
Symplegma reptans	S	C	NW Pacific	P	1991	Los Angeles, CA	Lambert and Lambert (2003)

Order is represented as 'A' for Aplousobranchia, 'P' for Phlebobranchia and 'S' for Stolidobranchia. Body form is represented as 'S' for solitary and 'C' for colonial. Introduced coast is represented by 'P' for Pacific, 'A' for Atlantic, and 'G' for Gulf. Dates and locations of first record are valid for the full North American range (Mexico, the US and Canada) of non-native ascidians

Field surveys

Standardized surveys were conducted in 22 bays in the US, spanning 24–57°N on the Pacific, Atlantic and Gulf coasts. Sites were surveyed for subtidal fouling species over a 14 year time period (2000–2014), with most bays (17) sampled once during this time, and five bays sampled repeatedly over a number of years (see Table 2). In each bay, at least 100 PVC settlement plates, 14 × 14 cm in size, were deployed and examined to determine the presence of fouling organisms, including ascidian species (except in Portsmouth, New Hampshire where 16, 10 × 10 cm plates were deployed, see Dijkstra and Harris 2009; Dijkstra et al. 2011). Each plate was sanded on one side. Plates were suspended from man-made structures (e.g. docks, marinas, buoys, bridges, piers) in bays and harbors in a horizontal, downward position (using a brick weight), sanded side facing the benthos. All plates were deployed in late spring or early summer, during the usual peak of larval recruitment (colonization), and remained in the field for three months to allow sufficient community development. Once retrieved, plates were processed to identify the full suite of fouling organisms, including both sessile and mobile invertebrates. Processing involved recording easily identifiable species in the field, while unidentifiable or questionable species were collected and preserved for subsequent identification in the laboratory. If a species was especially unusual or difficult to identify, voucher specimens were sent to a taxonomic expert for identification.

Data analyses

Data from the extensive literature review and field surveys were collated and analyzed to examine invasion patterns across coasts and bays. Dates of first record were assigned based on the first date of collection or documented introduction of an established population. Dates and locations of first record are valid for the full North American range (Mexico, the US and Canada) of non-native ascidians. If these were not reported, dates of written documents or publications were used. These dates are the best known information that is currently available, but we recognize that dates may be affected by the timing of sampling, taxonomic expertise of the sampler, and lags in publication times.

We examined the latitudinal extent of species' current continuous non-native ranges on the Pacific and Atlantic/Gulf coasts of North America—including distributions spanning Mexico, the US, and Canada. These data were acquired using occurrence records reported throughout the literature review and synthesis. Atlantic and Gulf coasts were combined in this analysis because the coastlines are continuous and species ranges generally extended across both coasts. If a section of a species range was considered

Table 2 List of the 22 bays in the US where fouling plate surveys were carried out, including years sampled and the number and identity of the non-native ascidian species recorded

Coast	Site name	Latitude (°N)	Year(s) sampled	# of species recorded	Species recorded on fouling plates	Additional established species	Total species
West	San Diego Bay, CA (SD, CA)	32.73	2000, 2013	15	*A. zara, B. violaceus, B. schlosseri, C. robusta, C. savignyi, D. vexillum, D. listerianum, M. squamiger, M. ficus, M. manhattensis, P. zorritensis, S. canopus, S. clava, S. plicata, S. reptans*	*B. giganteum, P. japonica*	17
	Mission Bay, CA (MI, CA)	32.78	2013	13	*A. zara, B. violaceus, B. schlosseri, C. robusta, C. savignyi, D. vexillum, D. listerianum, M. squamiger, M. ficus, P. zorritensis, S. clava, S. plicata, S. reptans.*	*B. giganteum, S. canopus*	15
	Long Beach, CA (LB, CA)	33.77	2003	13	*A. zara, *B. giganteum, B. violaceus, B. schlosseri, C. robusta, C. savignyi, D. listerianum, M. squamiger, M. ficus, M. manhattensis, P. zorritensis, S. clava, S. plicata*	*S. canopus*	14
	Morro Bay, CA (MB, CA)	35.37	2013	4	*B. violaceus, B. schlosseri, D. vexillum, D. listerianum*	*C. robusta, M. manhattensis*	6
	San Francisco Bay, CA (SF, CA)	37.62	2000, 2001, 2011, 2012, 2013	10	**A. zara, B. violaceus, B. schlosseri, C. robusta, C. savignyi, C. inflata, D. vexillum, D. listerianum, M. manhattensis, S. clava*	*P. zorritensis*	11
	Bodega Bay, CA (BB, CA)	38.33	2012	8	*A. zara, B. violaceus, B. schlosseri, C. robusta, D. vexillum, D. listerianum, M. manhattensis, *P. japonica*	*C. savignyi, S. clava*	10
	Humboldt Bay, CA (HB, CA)	40.72	2003	6	*B. violaceus, B. schlosseri, C. savignyi, D. listerianum, M. manhattensis, P. japonica*	*C. robusta, C. inflata, D. vexillum, M. citrina, S. canopus*	11
	Coos Bay, OR (CB, OR)	43.37	2000	4	*B. violaceus, B. schlosseri, D. listerianum, M. manhattensis*	*C. inflata, D. vexillum, M. citrina, S. clava*	8
	Puget Sound, WA (PS, WA)	47.72	2000	6	*B. violaceus, B. schlosseri, C. savignyi, *D. listerianum, M. manhattensis, S. clava*	*D. vexillum*	7
	Ketchikan, AK (KT, AK)	55.34	2003	1	*B. violaceus*	*B. schlosseri*	2
	Sitka, AK (ST, AK)	57.05	2001	2	*B. violaceus, *B. schlosseri*	*D. vexillum*	3

Table 2 continued

Coast	Site name	Latitude (°N)	Year(s) sampled	# of species recorded	Species recorded on fouling plates	Additional established species	Total species
	Biscayne Bay, FL (BB, FL)	25.57	2004	5	**A. sydneiensis, D. perlucidum,* *D. sp. aff. spongiforme, *S. canopus, S. plicata*	*D. psammatodes*	6
East	Indian River, FL (IR, FL)	28.06	2005	5	**D. perlucidum, D. psammatodes, *P. zorritensis, S. canopus, S. plicata*	*D. sp. aff. spongiforme*	6
	Jacksonville, FL (JX, FL)	30.33	2001	2	*S. canopus, S. plicata*		2
	Pensacola Bay, FL (PB, FL)	30.42	2002	3	*D. perlucidum, S. canopus, S. plicata*		3
	Charleston Harbor, SC (CH, SC)	32.74	2002	2	**S. canopus, S. plicata*		2
	Chesapeake Bay, VA (CB, VA)	37.58	2000, 2001, 2014	1	*S. plicata*	*B. violaceus*	2
	Narragansett Bay, RI (NB, RI)	41.47	2001	5	*A. aspersa, B. violaceus, D. listerianum, S. canopus, S. clava*	*D. vexillum*	6
	Portsmouth, NH (PT, NH)	43.07	2001, 2013	3	*B. violaceus, D. vexillum, D. listerianum*	*A. aspersa, S. clava*	5
Gulf	Tampa Bay, FL (TB, FL)	27.75	2002, 2012, 2014	3	*D. perlucidum, *S. canopus, S. plicata*	*D. sp. aff. spongiforme*	4
	Corpus Christi, TX (CC, TX)	27.80	2002	2	**S. canopus, S. plicata*		2
	Galveston Bay, TX (GB, TX)	28.47	2002	1	**S. canopus*		1

Species marked with a * are first records for that bay. Additional established species, known from the extensive literature review and unpublished records, are also noted. In many cases, these species were introduced after plate sampling was conducted cryptogenic or there was a large gap in known occurrences (i.e. greater than a marine ecoregion, as in Spalding et al. 2007), we considered the last confirmed and continuous introduction record to be the range limit (see reported north and south range edges in Supplementary Table 2). This is a conservative estimate and further research in under sampled regions (e.g. sections of Central America and Mexico) may expand the latitudinal extent for some species.

For each non-native ascidian species, we characterized the vector(s) associated with the initial invasion record per bay sampled. Vectors were assigned per species based on life history characteristics (i.e. larval duration and adult settlement patterns), historical vector activity within bays, and date of first record relative to human activities. For some non-native ascidian species, multiple vectors were considered possible. Vectors in our analysis included (1) Ballast water—the ballast tanks (water, sediments and surfaces) of ships; (2) Vessel biofouling—the hulls and underwater surfaces, including sea chests, of vessels; (3) Oyster accidental—accidental transfers with Oyster transplants or equipment; and (4) Fisheries accidental—accidental transfers with aquaculture species or equipment that are not oyster related. For the vessel biofouling vector, we could not easily distinguish the roles of commercial or recreational vessels as sources of introduction in some bays; thus, our analysis treats them as one group. All statistical analyses were conducted in Sigma Plot version 12.3 (Systat Software Inc., San Jose, CA, USA) and PRIMER version 7 (PRIMER-E Ltd, Plymouth, UK).

Results

Literature review: invasion patterns across coasts

We recorded 26 non-native ascidian species established in the US (Table 1). In total, half of these species (13 spp.) were colonial and half were solitary species. A majority (12 spp.) were in the order Stolidobranchia, while eight were Phlebobranchia, and six were Aplousobranchia. Geographically, non-native ascidian richness was highest on the Pacific Coast (19 spp.), followed by the Atlantic (14 spp.) and Gulf (6 spp.) coasts. Most species were reported from only one coast (16 spp.), but ten species were found on multiple coasts. Species native to the Western Pacific and Indo-Pacific dominated non-native ascidian assemblages on all three coasts, comprising 68 % of non-native ascidians on the Pacific coast, and 50 % on both the Atlantic and Gulf coasts (Fig. 1).

Few non-native ascidians were reported from North American waters earlier than 1900 (7 spp.) and most of these were discovered in historical shipping centers at lower-latitudes (25–35°N) on the Atlantic and Gulf coasts

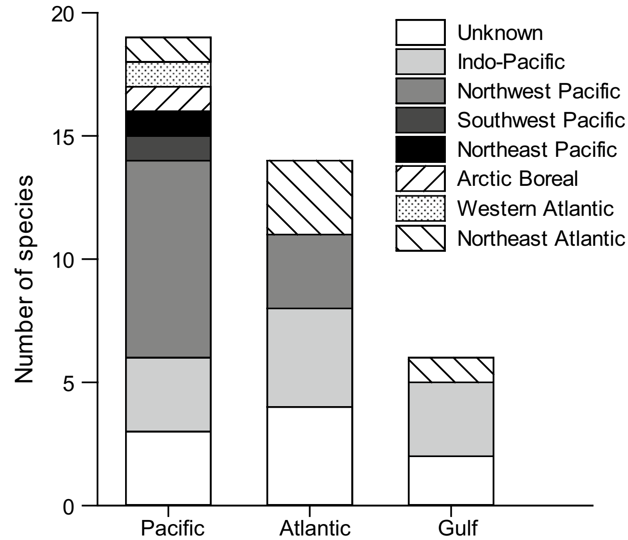

Fig. 1 The native range of ascidians introduced to the Pacific, Atlantic and Gulf coasts of the US

(Fig. 2a,b). The rate of discovery was relatively low until around 1950, when a steady increase began that continues to the present. A large part of this increase coincides with several targeted sampling efforts which have been initiated in recent decades (Fig. 2b).

On the Pacific coast, southern California (San Diego to Santa Barbara) was the region of first occurrence for 13 of 19 non-native ascidians (Fig. 3a), whereas on the Atlantic coast, half of the documented non-native ascidians (7 of 14 spp.) were first reported from New England (Connecticut to Maine; Fig. 3b). Overall, 100 % of the ascidian species introduced on the Pacific coast and 93 % on the Atlantic/Gulf coast spread beyond initial introduction locations. The ranges of most species expanded in both a north and south direction (11 spp. Pacific and 9 spp. Atlantic/Gulf coasts), with fewer species expanding in one direction only (8 spp. Pacific and 4 spp. Atlantic/Gulf) and only one species not being reported beyond its initial introduction site (on the Atlantic/Gulf) (Fig. 3a,b). The most widespread non-native ascidians on the Pacific coast of North America are *Botrylloides violaceus* (spanning 41° of latitude), *Botryllus schlosseri* (spanning 31°), and *D. vexillum* (spanning 26°) (Fig. 3a). On the Atlantic and Gulf coasts, the most widespread species are *S. canopus* (spanning 33°), *S. plicata* (spanning 26°), and *Didemnum psammatodes* (spanning 24°) (Fig. 3b).

Field survey: invasion patterns across bays

A total of 118 occurrence records for 24 non-native ascidian species were reported during fouling plate surveys conducted in 22 bays across the continental US and Alaska (Table 2). At least 14 of these occurrences represent 'first

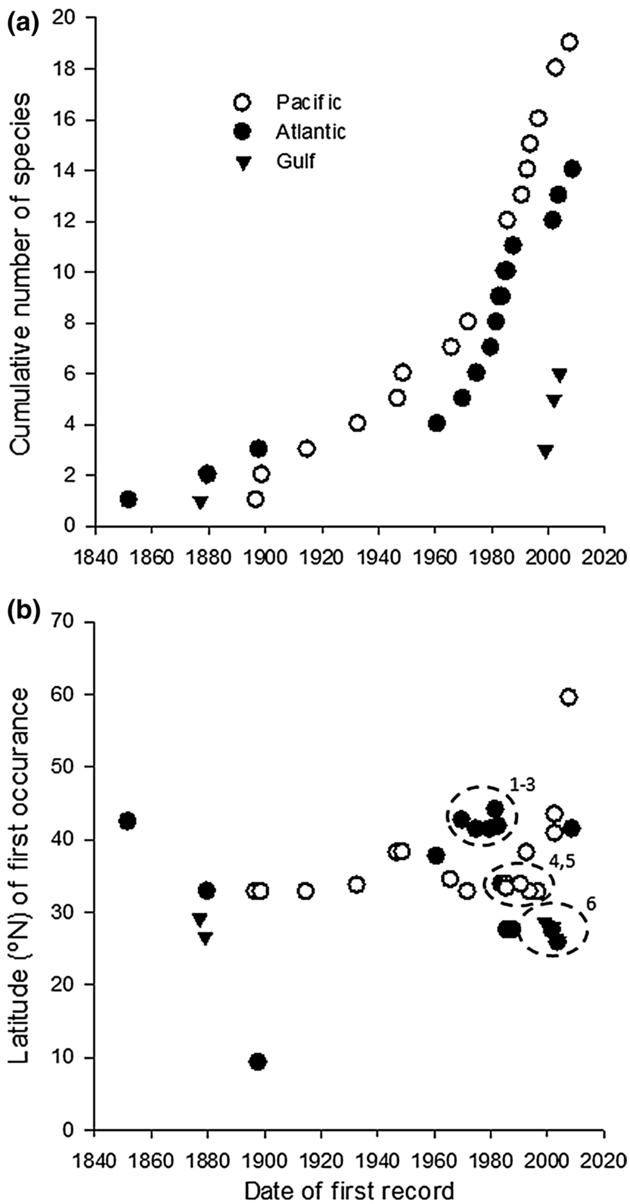

Fig. 2 Ascidian invasions through time shown as: **a** the cumulative number of species reported on each coast since 1840 and **b** the latitude of first occurrence for each species record per coast. *Dotted circles* represent research which lead to increases in non-native species discovery. References for first records include: [1]Berman et al. 1992, [2]Whitlatch and Osman 2000, [3]James T. Carlton personal communication, [4]Lambert and Lambert 1998, [5]2003, [6]Lambert et al. 2005

records' for the bay or region being sampled. Two additional non-native species are known from US waters, but were not recorded during plate surveys, *Molgula citrina* (Pacific coast) and *Clavelina lepadiformis* (Atlantic coast). Both species are recent invaders, with dates of introduction in the US being 2008 and 2009, respectively, and were detected after field surveys were conducted.

Multivariate analyses of non-native ascidian richness per bay show a clear distinction between established community assemblages across coasts (ANOSIM, Global $R = 0.469$, $P < 0.001$; Fig. 4). Specifically, species assemblages on the Pacific coast were significantly different from those on the Atlantic (ANOSIM, $R = 0.483$; $P < 0.002$) and Gulf coasts (ANOSIM, $R = 0.8$; $P < 0.005$); however, there was little distinction between non-native ascidian communities present on the Gulf and Atlantic coasts (specifically sites from South Carolina south; ANOSIM, $R = -0.177$, $P = 0.848$; Fig. 4). SIMPER analysis indicates that *Styela plicata*, *Styela canopus* and *Didemnum vexillum* contributed most to differences between Pacific and Atlantic coasts; while *B. violaceus*, *S. canopus* and *D. vexillum* contributed most to differences between Pacific and Gulf coasts.

In bays on the Pacific Coast, richness patterns indicated a latitudinal trend of decreasing ascidian invasions with increasing latitude ($f = 29.88 + -0.496*x$; $r^2 = 0.751$), which was not the case on the Atlantic coast, where there was no trend ($f = 3.817 + 0.005*x$; $r^2 = 0.003$; Fig. 5). On the Pacific coast, San Diego Bay (California) had the greatest non-native ascidian richness with 17 species, followed by nearby Mission Bay with 15 species, and San Francisco Bay with 14 species (Table 2; Fig. 5). On the Atlantic coast, three sites: Biscayne Bay (Florida), Indian River (Florida) and Narragansett Bay (Rhode Island) had the highest richness of non-native ascidians, with 6 species each.

In the 22 sampled bays, ascidian species were introduced through a number of human-mediated vectors including ballast water, vessel biofouling (ships and boats) and as hitchhikers with aquaculture species (Fig. 6). By far, the most frequent mechanism for introduction was through transport as biofouling on the hulls and sea chests of transiting vessels and boats. On the Pacific coast, accidental introductions with imported commercial Japanese oysters and movement of aquaculture equipment (i.e. Oyster accidental) also appeared to be important potential vectors for non-native ascidians (Fig. 6). Some species have the potential to arrive through multiple vectors, such as with both imported oysters and on vessel hulls (see Ruiz et al. 2011 for further discussion).

Temporal comparisons from two repeated, standardized plate surveys at five bays—two on the Pacific Coast, two on the Atlantic Coast, and one on the Gulf Coast—showed an increase in the number of detected non-native ascidians within four of the five bays over 12–13 years (Fig. 7). The exception was Chesapeake Bay, where detected ascidian richness declined from two species to one over 13 years from 2000 to 2013.

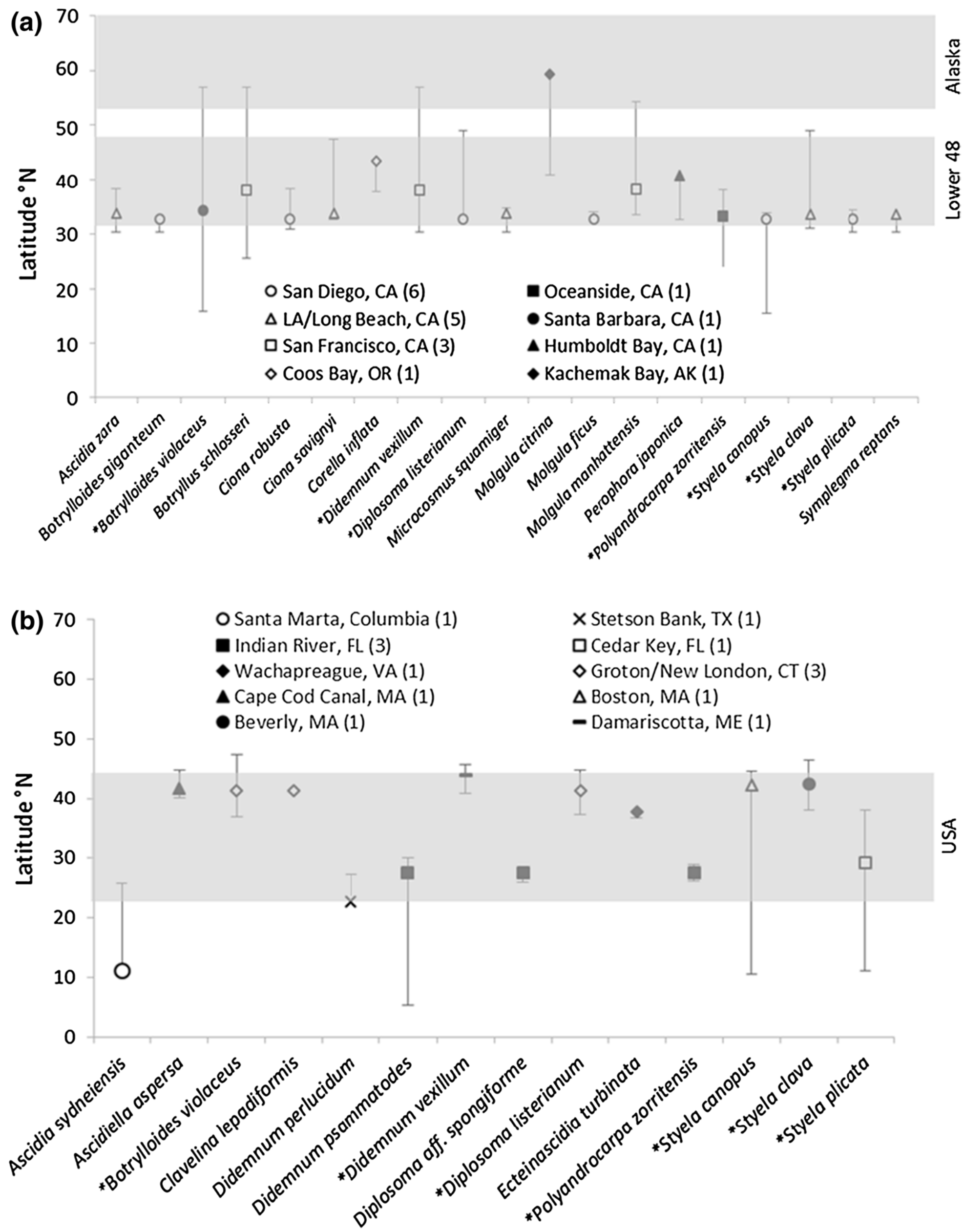

Fig. 3 The current North American range of ascidians introduced and established in the US. The latitude and location of first record for each coast: **a** Pacific and **b** Atlantic/Gulf is shown as *symbols*. *Error bars* represent the full latitudinal extent (°N) of species' continuous known non-native ranges. Atlantic and Gulf coasts are combined because the coastlines are continuous and species generally extended across both coasts. The total number of species with first records in each bay is shown in parentheses after the location name. *Shaded areas* represent continuous US territory on each coastline. Species marked with *asterisk* are introduced on the Pacific and Atlantic/Gulf coasts

Discussion

Combining an extensive literature review and broad scale field surveys we provide insight into the invasion dynamics of 26 non-native ascidian species established in the US, and North America more broadly. Although our study provides an accurate and comprehensive assessment based on current knowledge, this also represents a conservative minimum estimate of total non-native ascidian richness and distribution, for a number of reasons. First, the taxonomic resolution and biogeographic information for many ascidian species is still advancing and new records are likely to be added simply as a result of new taxonomic and genetic information being acquired (e.g. Brunetti et al. 2015; Vandepas et al. 2015; Yund et al. 2015). Second, although marine non-native species are relatively well studied in the US (Ruiz et al. 2000, 2015), there are some areas where systematic surveys for coastal invaders have not been conducted on a large scale. For instance, there is limited information on invaders in Delaware Bay and New York Harbor, which are areas with high commercial shipping activity. This limited knowledge is particularly true of tropical regions, such as sections of the Gulf of Mexico and Central America, which have not been as extensively studied as further north. As a result, the southern (low latitude) range extents for some non-native ascidians are likely

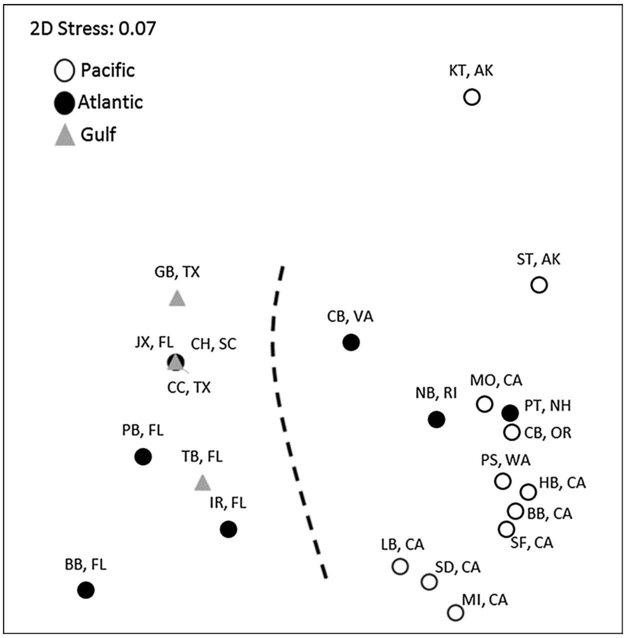

Fig. 4 A non-metric multidimensional scaling (nMDS) plot of non-native ascidian communities across bays in the US (including species occurrences reported from fouling plate surveys and the extensive literature review). We included the full presence/absence dataset for species which are native or cryptogenic to one portion of the US, but introduced to another (e.g. *Botryllus schlosseri*, *Corella inflata*, *Molgula citrina*, and *Molgula manhattensis*). Location abbreviations match those listed in Table 2

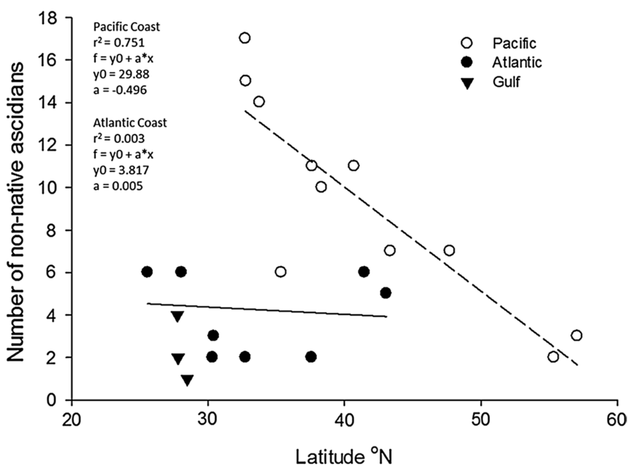

Fig. 5 The number of non-native ascidians across latitudes where field surveys were conducted within bays on the Pacific, Atlantic, and Gulf coasts of the US

underestimated. Thus, increased surveys in these under-represented locations will likely lead to new records and greater understanding of invasion patterns.

Non-native ascidian richness is greatest on the US Pacific coast, mirroring previous large scale analyses across

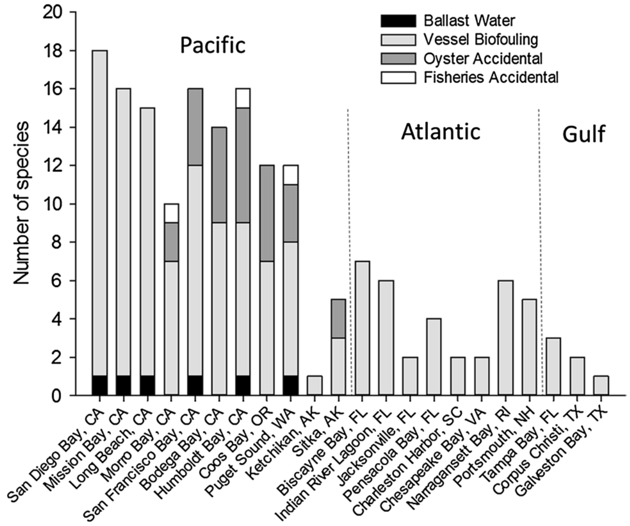

Fig. 6 The number of non-native ascidian species attributed to each invasion vector within the 22 bays where field surveys were conducted on the Pacific, Atlantic and Gulf coasts of the US. Some species may have been transported by multiple vectors and were therefore counted multiple times

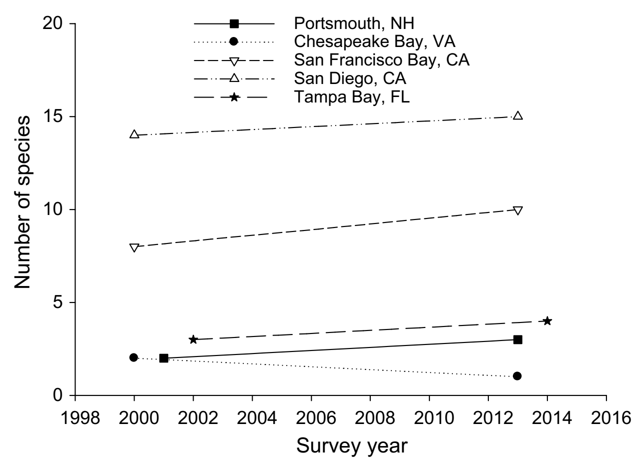

Fig. 7 The number of non-native ascidians recorded during repeated surveys at five sites in the US. Survey sites included two bays on the Pacific: San Diego and San Francisco, California (CA); two on the Atlantic: Chesapeake Bay, Virginia (VA) and Portsmouth, New Hampshire (NH); and one on the Gulf: Tampa Bay, Florida (FL)

all groups of introduced marine taxa (Ruiz et al. 2000, 2015). More specifically, southern California is a hotspot for ascidian invasions (e.g. Tracy and Reyns 2014). Interestingly, non-native ascidian richness is inversely correlated to latitude on the Pacific coast, but not on the Atlantic. However, sites further north than Portsmouth, New Hampshire at 43°N were not sampled during this study. Sites in New England (Rhode Island—Maine) and Atlantic Canada have been extensively surveyed for non-native ascidians (LeGresley et al. 2008; Sephton et al. 2011; Massachusetts Office of Coastal Zone Management 2013; Moore et al. 2014). Newfoundland at 46–51°N is reported to have three non-native ascidians—reflecting invasion levels similar to sites from high latitudes on the Pacific coast (Ketchikan, Alaska (AK) has two non-native ascidians and Sitka, AK has three). The three non-native ascidian species established in Newfoundland are *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis* (Callahan et al. 2010; Ma et al. 2011; Sargent et al. 2013). However, the invasion history of two of these species, *B. schlosseri* and *C. intestinalis*, is unresolved and in some cases they have been considered cryptogenic in this region (Zhan et al. 2010; Yund et al. 2015; Bouchemousse et al. 2016; this study—see Supplementary Table 1). Cooler water temperatures and shorter reproductive seasons at northern latitudes may limit the spread of invasions in these environments, but current northern range limits may also reflect relatively low historical propagule supply by vessels and aquaculture activities (de Rivera et al. 2011; Ruiz et al. 2011). The relative contribution of these factors to the northern distribution of non-native invertebrates is not presently clear.

The rate of discovery for non-native ascidians in the US has accelerated since 1950. This trend matches the discovery rate of ascidians on a global scale, regardless of native or non-native population status, suggesting that a rise in taxonomic expertise may contribute strongly to the observed temporal patterns (Shenkar and Swalla 2011). The survey and identification work of Charles and Gretchen Lambert—termed here as the Lambert effect—has been instrumental in increasing ascidian knowledge throughout the US. Their research greatly increased the number of non-native ascidian records for southern California (Lambert and Lambert 1998, 2003) and the Gulf Coast (Lambert et al. 2005); undoubtedly influencing large scale temporal patterns (see Fig. 2). However, new introductions and sustained coastwise spread also contribute to the increasing discovery rate. New records of ascidian invaders continue to accumulate (e.g. Lambert 2007b, 2009; Lambert et al. 2010; Cohen et al. 2011) and repeated replicate plate surveys show increased non-native ascidian occurrences in the last decade, all evidence that non-native species are continuing to establish and spread in North America. Further, a large scale assessment across taxonomic groups documented a 16–25 % increase in total marine invasions from 1999 to 2010 (Ruiz et al. 2015). Some of this is likely increased detection and expertise, but some is clearly the continued arrival of newly introduced species. Disentangling these two drivers is difficult, but repeated systematic surveys at the same locations (such as the plate surveys conducted here) can begin to tease these mechanisms apart.

Most non-native ascidians in the US (on all three coasts) are native to the Western Pacific, which is also a global biodiversity hotspot for ascidian species (Shenkar and Swalla 2011). High non-native ascidian richness in California may stem from direct and extensive trade links with Asia, especially Japan, including shipping and historical oyster imports, which transfer marine biota. A large number of commercial ships traverse the Pacific Ocean exchanging goods between the two continents (Carlton and Geller 1993; Verling et al. 2005), increasing the opportunity for hitchhiking species to be introduced. Further, historical oyster imports from Asia introduced a number of species to the Pacific Coast (specifically in and around San Francisco Bay) prior to 50 years ago, when the vector mostly ceased (Carlton 1979; Ruiz et al. 2013; Grosholz et al. 2015). Once established, the likelihood that these species spread to other locations substantially increases. Non-native ascidian species have been reported on varied introduction vectors including commercial ships' hulls in niche areas such as sea-chests (Fofonoff et al. 2003; Coutts and Dodgshun 2007; Frey et al. 2014), recreational boat hulls (Davidson et al. 2010; Clarke Murray et al. 2011), and aquaculture stock and infrastructure (Carver et al. 2003; McKindsey et al. 2007).

Most non-native ascidian species (97 % overall) spread coastwise following their initial introduction and establishment. However, species non-native ranges may illustrate multiple primary introductions from the native range, rather than secondary spread from the first/initial introduction site. Advances from population genetics show that this is the case for a number of species (Roman and Darling 2007), including the ascidian *S. clava* on the Pacific Coast (Goldstien et al. 2011; Darling et al. 2012). This interplay between multiple primary introduction sites versus secondary spread from initial introduction points, complicates coast-wide spread estimates. Analyses combining population genetics and detailed occurrence records will lead to more accuracy when assessing the invasion history of some species (e.g. Stefaniak et al. 2009), providing greater insight into vector dynamics and species post-establishment spread. For instance, a recent genetic analysis comparing populations of *B. violaceus* in North America illustrates differing colonization processes between Pacific and Atlantic coasts (Bock et al. 2011). Non-native populations in Washington and British Columbia were established by multiple primary introduction events from the species native range in Asia; whereas populations in Eastern Canada appear to have spread by contiguous stepping-stone movements through secondary introduction vectors (Bock et al. 2011).

In order for an invading species to spread beyond its initial introduction location, it needs to overcome barriers to dispersal (Blackburn et al. 2011). Because ascidians have short larval durations, their ability to naturally disperse quickly and over large distances is limited (Peterson and Svane 1995; Lambert 2005a; Fletcher et al. 2012). However, many non-native ascidian species have quickly expanded their ranges along the coast of North America illustrating their capacity for transfer by human-mediated vectors. Initial introduction locations for the 26 ascidian species are centered around historical shipping centers—San Diego and LA/Long Beach on the Pacific Coast, and Boston and New England on the Atlantic coast. Prior to the 1900s, many ocean going vessels were wooden hulled and carried solid ballast which was dumped before loading cargo, providing ample hard substrate for the settlement of marine invertebrates, such as ascidians (Carlton 1989; Carlton and Hodder 1995). In more recent times, recreational boats have become an important vector for the spread of non-native ascidians. Several recent assessments of the fouling communities attached to recreational boat hulls show that non-native ascidians are present (Davidson et al. 2010; Clarke Murray et al. 2011; Zabin et al. 2014). Many of the bays sampled during our surveys lack commercial shipping ports, but have large marinas and transient boating communities. This vector links larger bays and harbors to smaller, perhaps less invaded, ones, thereby increasing the non-native range of invaders (Davidson et al. 2010; Zabin et al. 2014) and possibly aiding the spread of ecologically impactful ascidians, such as *D. vexillum* (Bullard et al. 2007; McCann et al. 2013).

The expansion of aquaculture provides an additional vector for the local transport of invaders, as well as, a major new habitat for colonization by ascidian species (Carlton 1989; McKindsey et al. 2007; Rocha et al. 2009). In floating aquaculture habitats, widespread ascidian invaders, such as *B. violaceus*, *D. vexillum* and *S. clava*, are known to proliferate, reaching high densities and inflicting economic damage by fouling farmed shellfish stock (LeBlanc et al. 2003; McKindsey et al. 2007; Carman et al. 2010). Many states and provinces in North America have initiated restrictions and permitting requirements for shellfish aquaculture transfers, partly due to the large economic impacts caused by non-native species (e.g. Grosholz et al. 2015). Although, there is an indication that the strength of this vector has decreased through time (Ruiz et al. 2013), ascidians continue to have large impacts as nuisance species in aquaculture facilities (Ramsay et al. 2008; Carman et al. 2010; Adams et al. 2011) and control efforts are being developed to limit economic costs (Switzer et al. 2011).

As non-native ascidians continue to establish and spread throughout North America, the likelihood of having negative economic or ecological impacts on a new area increases. As species spread, they encounter novel environmental and biotic conditions which may spur impacts to occur (Simberloff et al. 2012). Moreover, the cumulative impact of a species is affected by the total area occupied, which often expands beyond the initial introduction point, as seen with ascidians. Thus both areal extent and per-capita effects are important dimensions in estimating impacts and targeting management efforts (Parker et al. 1999).

On a global scale, ships' ballast water is the only vector with explicit large-scale management practices in place to reduce the rate of new coastal invasions (Davidson and Simkanin 2012; Ruiz et al. 2015). As mentioned previously, the life history characteristics of ascidians (e.g. short non-feeding larval stage, sessile adult phase) suggest that they are unlikely to survive long-distance voyages within a ship's ballast tank. Although, ascidian larvae may survive shorter coastal transits (<24 h in duration, e.g. Simkanin et al. 2009) and *Ciona* larvae have been found in ballast water samples (Ruiz et al. unpublished). Vessel biofouling (on both commercial and recreational boats) and fouling on imported or transferred aquaculture stock and equipment are more potent and successful vectors for ascidian introductions (Williams et al. 2013). These vectors are not broadly regulated or managed to reduce species introduction and spread, though there is some management at regional scales (e.g. California State Lands Commission 2015; Grosholz et al. 2015) and emerging vessel biofouling regulations at national scales (Davidson et al. 2016). Ascidians, as conspicuous and well-studied invaders, are increasingly important indicator organisms in vector assessments (Aldred and Clare 2014) and can act as model organisms for studying the efficacy of management regulations for reducing invasive species spread.

Man-made habitats, such as docks, marinas and aquaculture sites, are focal areas for invasions and therefore provide important monitoring sites for detecting new species arrivals (Glasby et al. 2007; Ruiz et al. 2009). Little is known about the extent to which non-native ascidians are spreading from man-made structures into natural ecosystems, and what impacts they may be having on native marine communities. Many non-native marine invertebrates, including ascidians, appear to have limited ability to spread into nearby natural benthic habitats (Simkanin et al. 2012; Airoldi et al. 2015). Native predators or reduced propagule supply may limit non-native ascidian abundance in benthic habitats (Dumont et al. 2011; Forrest et al. 2013; Simkanin et al. 2013; Rogers et al. 2016). Importantly, the ascidian *D. vexillum* is an exception in such habitat restriction, and serves as an example of expansive colonization of natural habitat in many global regions (Bullard et al. 2007; Valentine et al. 2007; Carman and Grunden 2010). Further research is needed to understand what factors limit non-native species establishment in natural habitats and whether colonization patterns may change in response to human activities or other forcing factors.

Conclusion

Our analysis of ascidian invasions provides important insight into the large-scale invasion dynamics of an economically and ecologically impactful group of species. First, the Pacific coast, particularly southern California, is a hotspot for ascidian invasions and new invaders continue to be reported from this area. Second, across the continent, the number of ascidian invasions continues to increase despite widespread implementation of management protocols (i.e. shellfish transfer restrictions and ballast water exchange/treatment). Biofouling on commercial ships and recreational boats, considered the primary vector for coastwise spread of non-native ascidians, is currently unregulated. As species continue to establish and spread, the potential for negative ecological or economic impacts increases. Third, additional field surveys and taxonomic expertise are needed to fill in distribution gaps for under sampled regions, including portions of Mexico and Central America. Finally, fouling plate surveys provide an inexpensive, useful tool to detect new arrivals and evaluate range expansions of non-native marine invertebrates. Within this context, ascidians are particularly useful indicators for evaluating the spatial extent and temporal spread of invaders and testing the efficacy of management strategies used to minimize initial invasions, subsequent secondary spread, and potential impacts.

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