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Life history attributes of a global invader: factors contributing to the invasion potential of *Didemnum vexillum*

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ABSTRACT: The colonial ascidian *Didemnum vexillum* has dispersed from its native Japan to many temperate coastal regions of the world, yet many features of its life history remain poorly studied. Using *D. vexillum* populations in the northeastern USA, several features of growth and sexual reproduction, as well as asexual reproduction via the unusual feature of detachment of larva-laden tendrils were examined from 2008 to 2011. Recruits of *D. vexillum* grown on PVC panels in Groton, CT, between July and September 2011 reached sexual maturity in 50 to 62 d. A natural, overwintering *D. vexillum* population in Noank, CT, sampled weekly from 12 May to 14 July 2008 first had reproductive structures on 19 May; 7 wk later the first recruits were found on recruitment panels at the same dock. Examination of paired samples of encrusting and tendril growth forms collected on 22 July 2010 in Newport, RI, revealed no significant differences in densities of reproductive structures between growth forms. Fifty-nine percent of tagged tendrils in Westport, MA, detached in 2 wk. One hundred percent of tendrils reattached within 48 h to PVC panels in the laboratory, while only 1 in 80 tendrils tethered to bare substrates (rock or concrete) at nearby field sites reattached to the substrate. Tendrils tethered to natural rock were rapidly consumed by predatory snails. Further laboratory experiments found a minimum of 8 h of undisturbed contact with the substrate was necessary for *D. vexillum* tendrils to reattach. Overall, we found that the tendril growth form is an important factor in the population biology of *D. vexillum* because it increases surface area for feeding and reproducing zooids in a space-limited environment.

KEY WORDS: *Didemnum vexillum* · Life history cycle · Fragmentation · Sexual reproduction · Asexual reproduction · Dispersal

INTRODUCTION

Native to the coastal waters of northern Japan (Stefaniak et al. 2012), the colonial ascidian *Didemnum vexillum* Kott, 2002 has successfully invaded temperate regions of coastal North America, northwestern Europe, and New Zealand (Lambert 2009, Stefaniak et al. 2009) over the past 4 decades. *D. vexillum* readily grows on natural and artificial substrates, including rock (Gittenberger 2007, Osman & Whitlatch 2007), cobble–gravel (Valentine et al. 2007b), eelgrass (Carman & Grunden 2010), pilings (Carman & Grunden 2010), and aquaculture gear (Lambert 2009). The presence of *D. vexillum* can cause serious problems for aquaculture operations (e.g. Auker 2010, Carman et al. 2010, Fletcher et al. 2013b). Its apparently unique ability to colonize subtidal cobble–gravel substrates (e.g. Valentine et al. 2007b, Mercer et al. 2009) over large portions of the seafloor has also raised concerns about its ability to interfere with the foraging activities and escape swimming of economically important finfish and shellfish (e.g. Bullard et al. 2007a, Valentine et al. 2007b).

Given its rapid spread to temperate coastal areas throughout the world, a number of studies have examined factors contributing to *D. vexillum*'s invasion

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success. These have included the effects of predators and competitors in controlling its abundance and distribution (e.g. Osman & Whitlatch 2007, Carman et al. 2009, Dijkstra & Harris 2009, Epelbaum et al. 2009, Forrest et al. 2013) as well as various life history features of the species (e.g. Valentine et al. 2007a, 2009, Auker & Oviatt 2008, Edwards & Stachowicz 2010, Fletcher & Forrest 2011, Fletcher et al. 2013a,b).

To be considered an invasive species, exotic species must continue to spread beyond the original invasion point (Kolar & Lodge 2001, Sakai et al. 2001). Dispersal distance via larvae is generally short in ascidians (Jackson 1986); therefore, other methods of dispersal are likely necessary to produce widespread populations. Non-native ascidians are undoubtedly moved by humans on boat hulls and via aquaculture transfers (e.g. Acosta & Forrest 2009), but some species may also have evolved life history traits to augment dispersal. Reproduction of daughter colonies in *D. vexillum* is both sexual (via larvae) and asexual (via colony fragmentation) (Millar 1971). In some locations, *D. vexillum* colonies produce long tendrils from the encrusting portion of the colony (Fig. 1). These tendrils can fragment and presumably disperse, settle, and reattach elsewhere (e.g. Bullard et al. 2007a, Lambert 2009). Tendrils extending from *D. vexillum* colonies often have constrictions along their length, separating thicker portions of the tendril (Kott 2002, Coutts & Forrest 2007, Reinhardt et al. 2012), which may increase the probability of fragmentation.

Fig. 1. *Didemnum vexillum* tendrils at Westport Point, Massachusetts, July 2011. Tag in the central tendril. Scale bar = 1 cm

Laboratory and field experiments have demonstrated that mechanically produced fragments readily reattach (Bullard et al. 2007b). Also, large colonies of *D. vexillum* have been observed on substrates below and adjacent to sources of tendrils (e.g. Coutts & Forrest 2007). These results have led to the hypothesis that natural fragmentation of tendrils greatly increases the dispersal and invasibility of *D. vexillum* through tendril detachment, transport, and reattachment (Minchin & Sides 2006, Bullard et al. 2007a, Lambert 2009, Martin et al. 2011). Reattachment rates and times have been tested several times in the laboratory and in the field for mechanically produced fragments of encrusting colonies (Bullard et al. 2007b, Valentine et al. 2007b, Hopkins et al. 2011, Carman et al. 2014). However, rates of natural detachment and reattachment of tendrils have never been quantified. Transport of adults that are full of brooded larvae may be more effective than planktonic larval dispersal for establishing new populations (e.g. brooding versus broadcast spawning periwinkle snails; Johannesson 1988). When only a few larvae of an exotic species are introduced to a new location, they necessarily must reproduce from a few isolated individuals (Sakai et al. 2001). However, the release of brooded larvae after a gravid adult dispersal event can lead to an immediate, dense second generation population, increasing the probability of population survival (Johannesson 1988).

Transport of larvae-packed tendrils may also increase the distance that *D. vexillum* can naturally disperse. Colonial ascidians commonly have larvae that remain in the water column for less than 3 h (Jackson 1986) and are competent to settle immediately following release (Jackson 1986, Svane & Young 1989, Fletcher & Forrest 2011). Short free-swimming larval stages are generally thought to translate into short dispersal distances (Jackson 1986). Natural long-distance dispersal likely relies on movement of adult colonies (Jackson 1986, Worcester 1994) such as fragmentation and transport of tendrils in *D. vexillum*. Whether larvae are being packed into tendrils for long-distance transport is unknown, as there is no direct evidence regarding the relative reproductive output of the tendril and encrusting growth forms of the species.

Recruitment studies indicate that *D. vexillum* likely has an annual reproductive cycle (e.g. Osman & Whitlatch 2007, Valentine et al. 2009, Fletcher et al.

2013b). However, the time period required for young-of-the-year colonies to become reproductively active has not been quantified; neither has the length of the reproductive cycle (sexual maturity to first recruitment). Since short generation time is considered to be a character of a good invader (Sakai et al. 2001), multiple generations per year could increase the invasion potential of *D. vexillum*. The objective of this study was to quantify these and other aspects of the life history cycle of *D. vexillum* in order to examine the relative importance of sexual and asexual pathways to its reproduction and distribution, and the implications for the invasion success of this species.

MATERIALS AND METHODS

Sexual reproductive and dispersal pathway

Time to sexual maturity

To determine the length of time from recruitment to sexual maturity (presence of testes and ovaries and/or eggs), in July 2011, *Didemnum vexillum* larvae were settled onto 100 cm^2 PVC panels, and gardened to a density of 5 recruits $panel^{-1}$ to reduce intra-specific spatial competition. These *D. vexillum* colonies were grown in the field off Bushy Point, Groton, Connecticut (depth ~5 m; 41° 18.919′ N, 72° 03.069′ W) suspended in the water column (~2 m off the bottom), isolated from benthic predators, approximately 100 m from the nearest hard substrate that could be a source of competitors′ larvae. Three *D. vexillum* colonies were collected at intervals of 1 to 2 wk, relaxed with menthol, and preserved in 10% formalin in sea water. Colonies were transferred to 95% ethanol for dissection. For colonies younger than 23 d, the entire colony was examined. For colonies between 23 and 68 d old, a subsample of the colony was examined. Colonies were first photographed through a dissecting microscope for later calculation of the area dissected using the software ImageJ (Abramoff et al. 2004, Rasband 2012). Reproductive structures (testes [immature and mature], ovaries and/or small eggs, large eggs, embryos, and larvae; Fig. 2) were dissected from the tunic and counted. Counts of reproductive structures were standardized by the area of the dissected colony subsample. Temperature was measured every 2 h with a HOBO Pendant temperature data logger (Onset).

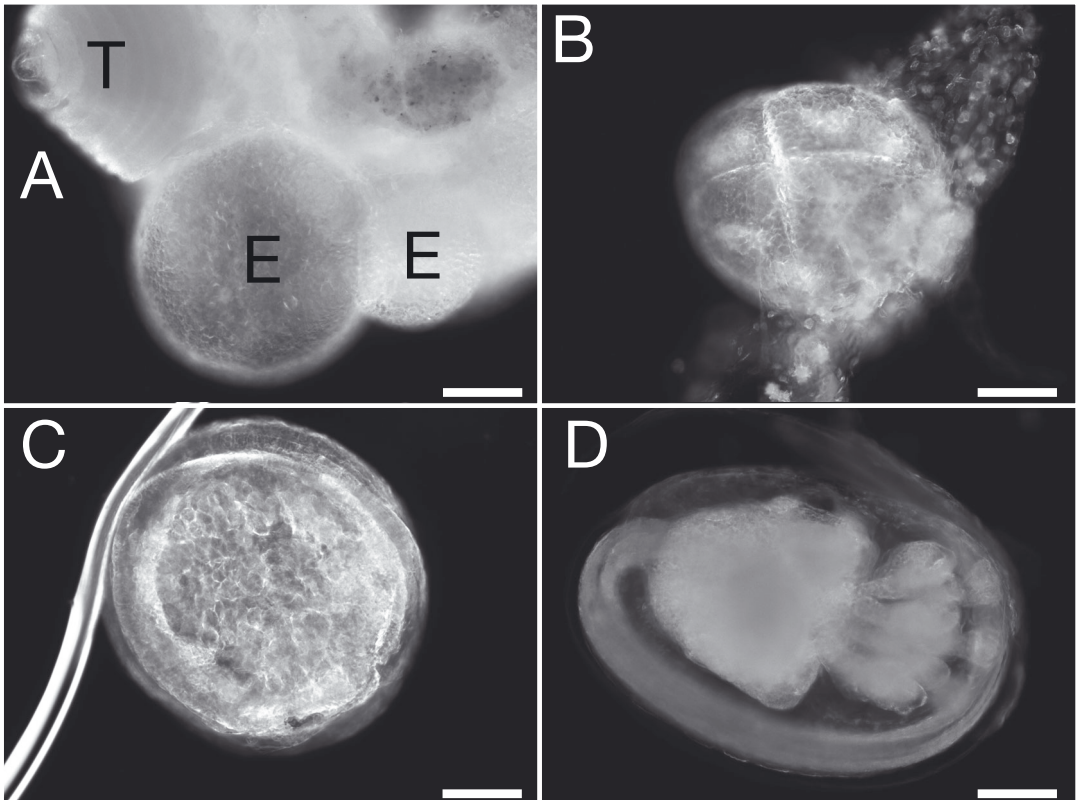

Fig. 2. *Didemnum vexillum* reproductive structures showing (A) mature testes (T) with spermiduct and large eggs (E); (B) embryo; (C) tailbud embryo (line along left side of image is the edge of an air bubble); and (D) larva. All scale bars = 100 µm

Length of reproductive cycle

The length of the reproductive cycle was operationally defined as the time between finding the first reproductive structures in *D. vexillum* colonies and finding the first *D. vexillum* recruits on recruitment panels at the same location. To determine the length of the reproductive cycle, three 1 to 2 cm^2 pieces of naturally occurring encrusting adult colonies were collected weekly using SCUBA, starting 12 May 2008, from the pilings of a fixed dock in Noank, Connecticut (depth up to 3 m; 41° 19.47′ N, 71° 59.09′ W). Samples were preserved as above and subsamples of each colony processed the same way as 'time to sexual maturity' samples.

Four PVC settlement plates (100 cm^2) were deployed from the same dock to determine the time of initial recruitment of *D. vexillum*. Recruitment plates were examined under 2× magnification in the field, with the number of *D. vexillum* recruits recorded, and the plates replaced with clean plates weekly. Temperature was recorded at a nearby dock every 2 h using a HOBO Pendant temperature data logger. Salinity was measured weekly with a hand refractometer.

Potential encrusting colony and tendril reproductive output

To compare potential reproductive output of the tendril and encrusting growth forms, 3 paired samples consisting of a piece of encrusting colony and an attached tendril were collected subtidally (depth ≈ 5 m) using SCUBA, from pilings in Newport, Rhode Island (41° 29.47′ N, 71° 19.4′ W) on 22 July 2010. Samples were preserved as above. A small (<1 cm^2 surface area) subsample of each encrusting and tendril growth form pair was processed the same way as the 'time to sexual maturity' samples. The number of each reproductive structure was standardized per zooid. Each standardized reproductive structure was compared between encrusting and tendril growth forms using a paired *t*-test (SigmaPlot v.11; Systat Software).

Asexual reproductive and dispersal pathway

Tendril detachment

Tendril detachment experiments were conducted in July and August 2011 at a fixed wooden dock in Westport Point, Massachusetts near the mouth of the Westport River (41° 30.82′ N, 71° 04.63′ W). A total of 21 individual tendrils were haphazardly selected and tagged at the base and tip with numbered fish T-bar tags (Aquatic Ecosystems). Length (total and to first constriction), width (greatest and first constriction), thickness (greatest), and number of constrictions were measured for each tagged tendril. Base tags were relocated after 2 wk, and remaining tendrils remeasured. Initial measurements of tendrils that had detached (termed 'Lost') and tendrils that were still attached (termed 'Present') after 2 wk and initial vs. final measurements of Present tendrils were compared using *t*-tests (SigmaPlot v.11). Temperature and salinity were measured at tendril tagging and after 2 wk.

Tendril reattachment

Naturally produced tendrils were collected by gently pulling them by hand from encrusting colonies. Tethers made of 10 cm of fishing line (Trial 1) or heavy-duty thread (Trial 2) were attached to one end of each tendril. Tendrils were then deployed at Bushy Rock (a relatively high flow [10 to 15 cm s^{-1}; Hamilton 2005] environment south of Bushy Point, Groton, Connecticut; 41° 18.918′ N, 72° 03.191′ W; depth ≈ 2 m) and a floating concrete dock at UConn Avery Point (a low flow [1 to 2 cm s^{-1}; Hamilton 2005] environment, 41° 18.983′ N, 72° 03.660′ W; depth ≈ 30 cm). At Bushy Rock, the fouling community on the natural rock is dominated by macroalgae; the concrete at the Avery Point dock is dominated by invertebrates. To standardize the contact surface between sites, the substrates were scraped clean of encrusting organisms at each site. Tendrils were deployed so the entire length of the tendril was in contact with the substrate. Reattachment was tested at 24 and 50 h (Trial 1; 10 to 12 Aug 2011) and at 24 and 43 h (Trial 2; 14 to 16 Sep 2011) after deployment by squirting water at the submerged tendrils with a pipette (after Bullard et al. 2007b). Colonies that did not move when water was squirted at them were defined as 'reattached.' For each location–time–trial combination, 10 tendrils were tested (total N = 80 tendrils).

Reattachment ability of tendrils was also measured in a flow-through laboratory sea water tank at UConn Avery Point (deemed a minimal flow environment). For laboratory trials (14 to 16 Sep 2011), 20 tendrils were laid flat on PVC panels lining the bottom of the tank. Reattachment was tested at approximately 24 and 48 h, as with the field-deployed ten-

drils (n = 10 for each time period). To test for the minimum contact time needed for tendril reattachment, 19 tendrils from Trial 2 at the Avery Point dock were retrieved and laid on PVC panels in the seawater tank and tested for reattachment after 2, 4, 8, and 16 h on the panels ($n_{2,4,8\ h}$ = 5, n_{16h} = 4; 17 to 18 Sep 2011). Tendrils unattached after the initial time intervals were rechecked for reattachment after an additional 20 to 24 h of undisturbed contact time.

RESULTS

Sexual reproductive and dispersal pathway

Time to sexual maturity

Under control treatment conditions, newly recruited *Didemnum vexillum* colonies first had testes at 50 d, and had both testes and ovaries (sexual maturity) at 62 d. The first immature testes (lacking visible spermiduct) were seen in colonies collected 50 d after recruitment (Table 1). Mature testes and ovaries and/or small eggs, as well as a small number of large eggs were present 62 d after recruitment. A large increase in female reproductive structures occurred between 62 and 68 d after recruitment. Temperatures ranged from 19 to 21°C.

Length of reproductive cycle

Dissections of naturally occurring *D. vexillum* colonies from 2008 revealed that 7 wk elapsed between the first presence of reproductive structures (19 May) and the first presence of *D. vexillum* recruits on the recruitment panels (7 July; Table 2). Among-colony variability was high for all of the reproductive structures across the time series, likely because sampled colonies came from a naturally occurring population, where colony ages were unlikely to be synchronized. However, clear patterns did emerge. No reproductive structures were found on the first sampling date (12 May). Both male (immature testes) and female (ovaries and/or small eggs) structures were first present on 19 May, though male reproductive structures appeared to mature 2 wk prior to the first presence of large eggs (Table 2). Temperature ranged from ~13 to 24°C over the course of the experiment (~19.5°C on 7 July); salinity ranged from 27 to 32.

Table 1. Time from recruitment to sexual maturity in *Didemnum vexillum*: mean (±1 SE) number of each reproductive structure cm^{-2} of colony surface area over time. No reproductive structures were found in colonies younger than 50 d (n = 3 colonies age^{-1}). Temperature ranged from 19 to 21°C

Structures	Colony age (d)		
	50	62	68
Immature testes	21.4 (10.7)	71.9 (1.4)	13.6 (4.7)
Mature testes	0 (0)	109.5 (8.0)	140.5 (42.9)
Ovaries/eggs: small	0 (0)	23.4 (3.2)	83.4 (24.1)
Eggs: large	0 (0)	1.5 (0.9)	76.3 (26.8)
Embryos	0 (0)	0 (0)	1.4 (0.8)

Potential encrusting colony and tendril reproductive output

Zooid densities were greater in encrusting growth forms (470.7 ± 14.8 cm^{-2}) than in tendril growth forms (316.5 ± 75.8 cm^{-2}), and it was difficult to precisely measure the surface area of the tendril subsamples. Therefore, we standardized each reproductive structure by number per zooid. The densities of reproductive structures in encrusting and tendril growth forms were not significantly different (Fig. 3, Table 3).

Asexual reproductive and dispersal pathway

Tendril detachment

Of base tags that were relocated after 2 wk (17 of 21 deployed), 59% of the tagged tendrils had detached from the encrusting portion of the colony. While there were no significant differences in the initial measurements between Lost and Present tendrils (Table 4), Lost tendrils tended to be thinner at constrictions points than tendrils that were still attached after 2 wk (Present: 4.8 ± 0.4 mm, Lost: 3.7 ± 0.4 mm). There was also a reduction in total length (Initial: 76.3 ± 32.4 mm, Week 2: 51.4 ± 27.9 mm; t = 2.316, df = 6, p = 0.06) of the Present tendrils after 2 wk, suggesting a partial loss of the tendrils. Partial tendril loss is supported by the loss of 5 out of 7 tip tags for tendrils still present after 2 wk. Including partial tendril loss, tendril detachment after 2 wk increased from 59 to 88%. Water temperature was 23.9°C at initial tagging and 24.7°C 2 wk later. Salinity was 30 on each date.

Tendril reattachment

Of the 80 tendrils tethered at Bushy Rock (n = 40) and the Avery Point dock (n = 40), only a single ten-

Table 2. Length of *Didemnum vexillum* reproductive cycle: mean (±1 SE) number of reproductive structures cm^{-2} of colony surface area over time. First recruits were found on 7 July 2008 (n = 3 colonies $date^{-1}$) The length of the reproductive cycle was operationally defined as the time between finding the first reproductive structures in *D. vexillum* colonies and finding the first *D. vexillum* recruits on recruitment panels at the same location

Date	Immature testes	Mature testes	Ovaries/eggs: small	Eggs: large	Embryos	Embryos: tailbud	Larvae
12 May	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
19 May	112.1 (58.2)	0 (0)	100.5 (62.3)	0 (0)	0 (0)	0 (0)	0 (0)
26 May	25.9 (23)	3 (3)	14.9 (14.9)	0 (0)	0 (0)	0 (0)	0 (0)
2 June	0 (0)	51.7 (11.7)	18.6 (11.9)	0 (0)	0 (0)	0 (0)	0 (0)
9 June	0 (0)	40.9 (20.9)	13.5 (8.1)	8.3 (8.3)	0 (0)	0 (0)	0 (0)
16 June	0 (0)	18.9 (9.6)	7.5 (6.2)	2.8 (2.8)	0 (0)	0 (0)	0 (0)
23 June	0 (0)	83.7 (20.1)	27.8 (16.4)	3.2 (3.2)	0 (0)	0 (0)	0 (0)
30 June	0 (0)	104.9 (53.7)	77.4 (39.4)	81.1 (51.9)	57.7 (51.8)	0 (0)	0 (0)
7 July	23.5 (15.5)	122 (75.6)	78.6 (39.6)	72.7 (64.4)	21.7 (21.7)	0 (0)	0 (0)
14 July	0 (0)	179.2 (38.5)	136.8 (37.5)	164.9 (77.5)	44.5 (26.6)	0 (0)	9.9 (9.9)
21 July	0 (0)	184.3 (43.2)	56.7 (10.7)	119.9 (64.2)	55 (39.5)	2.1 (2.1)	22.3 (14.5)

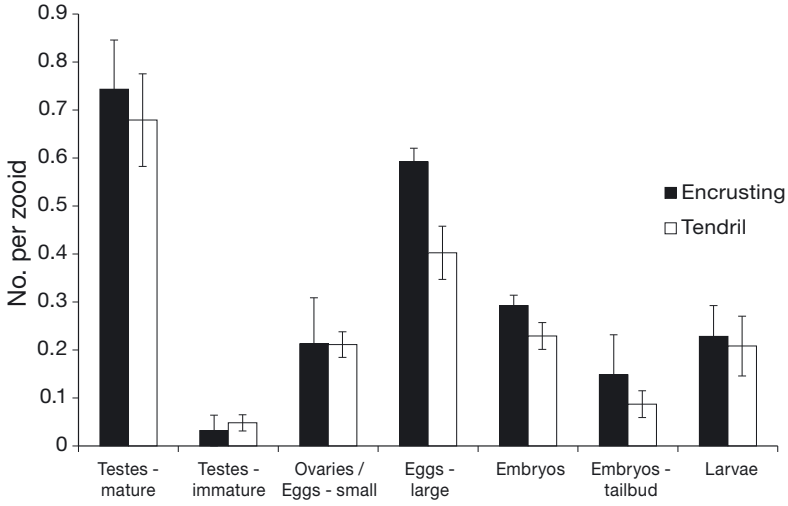

Fig. 3. Potential reproductive output of different *Didemnum vexillum* growth forms: comparison of reproductive structures in paired tendril/encrusting *D. vexillum* samples. No significant differences were found. Mean (±1 SE) number of reproductive structures per zooid (n = 3 pairs of tendril and encrusting growth forms)

Table 3. Potential reproductive output of different *Didemnum vexillum* growth forms. Comparison of density ($zooid^{-1}$) of reproductive structures in paired tendril/encrusting *D. vexillum* samples (paired *t*-tests, n = 3 pairs)

Structure	*t*	df	p
Immature testes	–0.927	2	0.452
Mature testes	3.985	2	0.058
Ovaries/eggs: small	0.0168	2	0.988
Eggs: large	2.748	2	0.111
Embryos	1.300	2	0.323
Embryos: tailbud	0.749	2	0.532
Larvae	0.202	2	0.859

dril (located at Bushy Rock) reattached to the substrate. The reattached tendril, which was already degraded 50 h after deployment, had completely disappeared 6 d after deployment. Tendrils deployed at Bushy Rock degraded quickly (within 24 h), likely due in part to predation by dove snails *Costoanachis* spp. that were observed foraging on the tendrils within 24 h of deployment. Tendrils at the Avery Point dock (where dove snails were not observed) degraded more slowly (by ~50 h; n = 4) or not at all (n = 36).

In the laboratory, 100% of tendrils tested at 24 and 43 h were reattached. When tendril reattachment was tested at shorter time intervals, 0% were reattached at 2 or 4 h, 20% reattached at 8 h, and 75% reattached at 16 h. After 20 to 24 h of additional undisturbed contact time to control for reattachment ability, only 16% of tendrils had not attached to the substrate. None of the tendrils in the laboratory degraded.

DISCUSSION

We found that the tendril growth form plays an important role in the invasion potential of the colonial ascidian *Didemnum vexillum*, though it may not be important in the way previously hypothesized. While *D. vexillum* tendrils can readily detach from

Table 4. *Didemnum vexillum* tendril detachment: differences in initial measurements (mean ± SE) between tagged *D. vexillum* tendrils that were lost (n = 10) or still present (n = 7) after 2 wk. *t*-tests or Mann-Whitney *U*-tests revealed no significant differences between tendrils lost or still present after 2 wk for any initial measurements

	Lost tendrils	Present tendrils	Test statistic	df	p
Length: total	74.2 (12.1)	76.3 (12.2)	$t = -0.117$	15	0.908
Length to 1st constriction	39.7 (7.5)	49.0 (11.9)	$t = -0.696$	10	0.502
Width: greatest	9.4 (0.7)	10.9 (1.0)	$t = -1.186$	15	0.254
Width at 1st constriction	3.7 (0.4)	4.8 (0.4)	$t = -2.044$	10	0.068
Thickness: greatest	3.6 (0.3)	3.6 (0.3)	$U = 35$		0.956
No. of constrictions	0.7 (0.2)	0.7 (0.2)	$U = 34.5$		1.000

parent colonies, our data do not support the hypothesis that tendril reattachment is a major contributor to the invasion success of *D. vexillum*. However, given the length of time needed for a full generation from larval-based sexual reproduction, the brooded larvae traveling within detached tendrils can play an important role in the dispersal, reproduction, and invasion success of *D. vexillum*.

Laboratory trials showed that at least 8 h of constant, direct contact with a substrate is necessary before tendrils reattach. Tethered tendrils in the field did not reattach to the substrate and were quickly consumed by predatory snails at the natural substrate site. *D. vexillum* colonies do not produce tendrils at all locations. If tendrils are not common and do not readily reattach in the field, their reattachment may not contribute substantially to *D. vexillum* dispersal. However, this study only examined tendril reattachment on flat, bare substrates. Tendrils could potentially reattach in the field if they became wedged in a crevice, entangled in protrusions from the substrate (e.g. algae, eelgrass, upright sessile fauna), or settled in an area of low current velocity. Carman et al. (2014) found that *D. vexillum* fragment reattachment was enhanced by the presence of eelgrass at water temperatures between 3 and 6°C.

The main role of tendrils to *D. vexillum*'s success as an invader may be in the way tendrils can increase the density of larvae dispersing to a particular area. *D. vexillum* larvae can travel up to (and possibly exceed) 250 m, but the density of recruits drops off rapidly as distance from the larval source increases (Fletcher et al. 2013a). By combining the results of the time to sexual maturity and the length of the reproductive cycle, we found that a minimum of 4 mo is needed for a complete *D. vexillum* sexual reproduction cycle. However, in colonial organisms, size and age are often disassociated from each other (e.g. Millar 1971). If the time to sexual maturity is linked to colony size instead of colony age, decreased growth due to competition and predation (L. M. Stefaniak & R. B. Whitlatch unpubl. data) could increase the length of the sexual reproduction cycle, possibly up to the 1 yr suggested by recruitment studies (e.g. Osman & Whitlatch 2007). Competition and predation also significantly reduce survival of *D. vexillum* juveniles (L. M. Stefaniak & R. B. Whitlatch unpubl. data), which lowers the chances that a single larva that dispersed to a new location would survive to sexual maturity. By transporting large numbers of brooded larvae to new locations, the number of individuals in a new population may be increased (e.g. Worcester 1994). An increased initial population size increases the probability that some of the population will survive long enough to reproduce (Johannesson 1988).

Tendril detachment and transport may also increase the distance that larvae are transported in the field. While colonial ascidian larvae only swim for a few minutes or hours (Millar 1971), Morris & Carman (2012) demonstrated that small, mechanically produced *D. vexillum* colony fragments remain viable for reattachment and reproduction after being suspended in laboratory aquaria for 4 wk. Even though fragment sinking rates suggest *D. vexillum* fragments would quickly settle to the bottom (Fletcher et al. 2013a), re-suspension of unattached fragments is possible. However, since reproductive effort is not being concentrated in the tendrils, the tendril growth form may be a secondary reproductive strategy for dispersal rather than a primary one.

The tendril growth form is also important in the population biology of *D. vexillum*, even without detachment and transport. The primary limiting resource in hard substrate communities where *D. vexillum* is often found is space. In a 2-dimensional, space-limited environment, tendril morphology can greatly increase the surface area of a colony. Increasing colony surface area increases the number of feeding and reproducing zooids, which will subsequently increase the overall reproductive output of the colony. Also, growth rates in 2-dimensional, encrusting colonial ascidians are limited by the circumference:surface area ratio because the colonies are only growing at the periphery of the colony (Stoner 1989). The 3-dimensional tendril growth form in

D. vexillum has the potential for growth over the entire surface area of the colony.

Additionally, this study demonstrates the importance of measuring life history rates in the field. Larval duration times measured in the laboratory have long been acknowledged to be overestimates of those in the field (Svane & Young 1989), and growth rates for ascidians may be underestimated in the laboratory. Fletcher & Forrest (2011) found 4 to 6 zooids per juvenile *D. vexillum* colony after 4 wk, reportedly similar to other lab-raised colonial ascidians. Our study found that colonies grown in the field, isolated from competitive overgrowth and predation pressure, had an average of 35.6 ± 4.2 and 229.4 ± 40.8 zooids at 23 and 31 days of age, respectively. If processes such as sexual maturation time and length of reproductive cycle are more closely linked to colony growth and size than age, estimates made in the laboratory could be very different from what is actually happening in the field.

In conclusion, because reattachment of tendrils requires an extended period of contact, and our data suggest reattachment may be uncommon in the field, the primary function of tendrils in the dispersal and establishment of new populations may not be reattachment in distant locations. Instead, their primary function may be to increase the number of recruits settling in a single location, potentially resulting in an increased probability of population survival. The tendril growth form also is important in the population biology of *D. vexillum* in that it increases space for feeding and reproducing zooids in a space-limited environment. However, our study only examined tendril reattachment on bare, flat surfaces, conditions not commonly found on natural substrates or even on artificial substrates that have been submerged for a few months. Therefore, further research is needed to determine field tendril reattachment when the tendrils become entangled in algae or eelgrass. Entanglement may increase reattachment success of tendrils in the field, making this process more important in the invasion success of *D. vexillum*.

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