Biological Journal of the Linnean Society, 2023, **XX**, 1–17
https://doi.org/10.1093/biolinnean/blad156
Advance access publication 25 November 2023
Original Article

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Parallel phenotypic plasticity and divergent ecological strategies in morphologically and molecularly similar sympatric sponge species

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ABSTRACT

How can phenotypic plasticity promote or impede adaptive change and diversification? Answering this key question can be experimentally intractable, but closely related clonal species allow a direct approach: experimentally provoking phenotypic plasticity to assess the adaptive significance of both plastic traits and species differences. Two common Caribbean sponge species, *Aplysina fulva* and *A. cauliformis*, are nearly indistinguishable molecularly, and share growth form, habitat, and geographical range. This raises questions about species boundaries, within-species variability, and mechanisms of speciation. To distinguish phenotypic plasticity from genetic variation, and learn how plasticity might influence adaptive—and divergent—evolution, I: (i) quantified morphological and ecological characters, (ii) revealed phenotypic plasticity by growing clonemates in different environments, and (iii) related plastic morphological features to ecological function. Characters included skeletal fibre density, biomechanical properties, vulnerability to parasites and predators, wound healing, transport pathways, propagation by fragments, population dynamics, and growth and survival in settings differing in food, sunlight, predators, and water motion. Transplanting both species to a different environment elicited parallel plasticity in the same traits. Combined comparative and experimental data reveal integrated suites of ecologically relevant characters that clearly distinguish these species and allow interpretation of adaptive significance of plastic characters that may underlie divergence.

Keywords: adaptive design; *Aplysina fulva*; *Aplysina cauliformis*; Caribbean coral reefs; ecological function; ecological speciation; skeletal meshes; sympatric congeners

INTRODUCTION

Phenotypic plasticity is a crucial aspect of the ecology and evolution of organisms that is increasingly a focus for both experimental and theoretical work (e.g. West-Eberhard 2003, Pigliucci 2005, Whitman and Agrawal 2009, Forsman 2015). Assessing how phenotypic plasticity might promote or impede adaptive change in populations and influence speciation is often experimentally intractable. However, this remains a key question for understanding evolution. Evolution might be slowed if phenotypic plasticity shields adaptive characters from selection; or diversification might be facilitated if phenotypic plasticity allows a population to colonize new habitat, with subsequent selection for characters that enhance success in that habitat (e.g. West-Eberhard 1989, 2003, Price *et al.* 2003, Whitman and Agrawal 2009). In ecological contexts, phenotypic plasticity can cushion effects of environmental challenges by allowing adaptive responses at sites that are undergoing changes and by allowing populations to live in more than one habitat type (e.g. Miner *et al.* 2005, Gratani 2014). In addition to its importance for evolution and ecology, phenotypic plasticity can make it difficult to delimit species or assign names to particular specimens, resulting in mistakenly lumping distinct species in ecological studies, in pharmaceutical development of natural products, and in conservation planning (Todd 2008 for coral examples).

One must demonstrate whether a trait's plastic responses to different environments are adaptive to understand its ecological role and its evolution but, as Richards *et al.* (2006) remarked for plants, 'Merely showing that there are phenotypic differences on average among a group of plants grown in different environments is not adequate to demonstrate or quantify phenotypic

Received 2 June 2023; revised 17 September 2023; accepted 18 October 2023

plasticity'. A solely observational approach can confound plasticity, i.e. the capability to alter in response to changed circumstances, with variation, i.e. a pattern that can result from a variety of mechanisms such as genetic variation among populations or differential mortality of variants. Moreover, increasing our understanding of how plasticity may influence adaptive evolution depends on taking an integrated, whole organism approach, rather than focusing on single traits (e.g. Wund 2012, Forsman 2015).

Sponges are frequently referred to as 'phenotypically plastic' in appreciation of, and frustration about, their malleability of form in response to various environmental circumstances. However, this often refers to observed variation between individuals (i.e. possibly based in genetic differences), rather than demonstrated plasticity of individuals in response to changed circumstances (i.e. demonstrated to not be based in genetic differences). Two complementary approaches to variation in sponges have been taken: (i) combining molecular, morphological, and ecological characters to reliably distinguish closely related sympatric species, and (ii) quantifying variation in populations of a single species in different circumstances and correlating this variation to environmental factors, and in some cases testing plasticity by experimental transplants. This study combines these approaches.

Controlling for genotype in transplant experiments can allow confident detection of relatively subtle plastic changes. Thus, pairs of closely related clonal species offer an unusual opportunity to experimentally probe otherwise intractable questions such as: How is the capacity for plasticity in a particular trait shared within a clade, i.e. when do closely related species show parallel responses to the same stimulus, and when do divergent responses reflect differentiation between species? Phenotypic plasticity comparisons among related species can also be a powerful tool for better understanding adaptive design (e.g. Piersma and Drent 2003).

Sponges of the genus *Aplysina* are typical, ecologically important members of hard-bottom marine communities throughout the Mediterranean and tropical and subtropical Atlantic and eastern Pacific. *Aplysina* species diversity is greatest in the tropical western Atlantic. Species of *Aplysina* can be among the most abundant (by volume) sponges in Caribbean coral reef communities (e.g. Alcolado 1990, Wulff 2006a, b). *Aplysina* is known for compounds of pharmaceutical interest, as well as antimicrobial bacterial symbionts (e.g. Hentschel *et al.* 2001). *Aplysina fulva* (Pallas, 1766) and *A. cauliformis* (Carter, 1882) share an erect, narrow-branched growth form and are common on tropical western Atlantic coral reefs, where they co-occur at some sites, while at other sites only one is present (Fig. 1).

Persistent ambiguity in delimitating these species, both morphologically and molecularly, combined with the question of how two so similar species coexist, motivated a strategy that can be readily applied to other biodiversity quandaries. Simultaneously probing the plasticity of morphological and ecological characters in both species can help to illuminate adaptive significance of distinguishing characters.

My goals were to: (i) show how experimentally provoked phenotypic plasticity can help to distinguish taxonomically challenging sponge species, (ii) explore how plasticity per se is shared or differs between closely related species, and (iii) understand the coexistence and divergence of closely related and very similar species, while (iv) offering insight into the adaptive significance of previously unstudied traits and how suites of morphological and ecological traits may be integrated. These goals were accomplished by: (i) evaluating within- and between-species variation in morphology and ecology, including skeletal and tissue characters, growth rate, survival, fragment survival, population dynamics, wound healing, life histories, and vulnerability to vigorous wave action, predators, and parasites; (ii) transplanting individuals of both species from their normal coral reef habitat to mangroves to quantify phenotypic plasticity in morphological and ecological characters by comparing individuals of the same genotype grown in contrasting environments; and (iii) revealing the adaptive significance of morphological characters by relating them to ecological characters.

MATERIAL AND METHODS

Taxonomic history of Caribbean branching *Aplysina* species (F. Aplysinidae, O. Verongiida)

De Laubenfels (1936) was the first to report in detail on these species as populations of living animals, rather than solely as museum specimens. He clearly distinguished two species with narrow branches: *Verongia fulva*, soft, bright yellow, fleshy, slightly spongy, and turning dark blue on contact with air, vs. *V. longissima*, dull yellow, relatively hard, with branches that tend to anastomose, and little colour change on exposure to air. These species were later confirmed as members of the genus *Aplysina* (Wiedenmayer 1977, Bergquist 1980).

Kaye and Ortiz (1981), and Kaye and Reiswig (1985) distinguished narrow, multibranched, and anastomosing (i.e. de Laubenfels' *V. longissima*) from thicker single rod or minimally branching erect forms of the relatively stiff, non-colour-changing *Aplysina*, calling them, respectively, *V. longissima* and *V. cauliformis* in reference to Carter (1882). In Curaçao, Biggs (2013) worked with two distinct forms, and Zea *et al.* (2014) proposed two forms in their online guide. On the basis of metabolite analysis, Puyana *et al.* (2015) distinguished lilac creeping vs. brown erect forms of *A. cauliformis*. Hechtel (1965) suggested retaining 'the name *V. longissima* for the sponges reported by de Laubenfels from several localities' until types are re-examined; and Wiedenmayer (1977), referring to *A. cauliformis*, suggested that '*A. longissima* sensu de Laubenfels requires a different name'. These forms have been combined as *A. cauliformis*, and the species name *longissima* dismissed because Carter's specimen represents *Callyspongia tenerrima* Duchassaing & Michelotti, 1864. Carter (1882) complicated things by describing *Luffaria cauliformis* sp. nov. as having the 'clear golden amber-colour' fibres that place it in Aplysinidae, and applying the name *Aplysina cauliformis* sp. nov. to a sponge that 'appears to be the same as *Callyspongia tenerrima,* de F. et M'. Carter included in *L. cauliformis* sponges that are 'simple or branched irregularly; erect, straggling, or repent … uniting with each other where in contact, and with all other kinds of objects in their course', but these are *not* characteristics of *C. tenerrima*. There is increasing agreement (e.g. Puyana *et al.* 2015) about two distinct forms of relatively stiff narrow-branched Caribbean *Aplysina*, one lavender in colour with multiple anastomosing branches, and the other dull yellow/brown and erect with minimal branching. A third ramose form, with thick (up to 5 cm) branches, has been called *Aplysina rigida* and

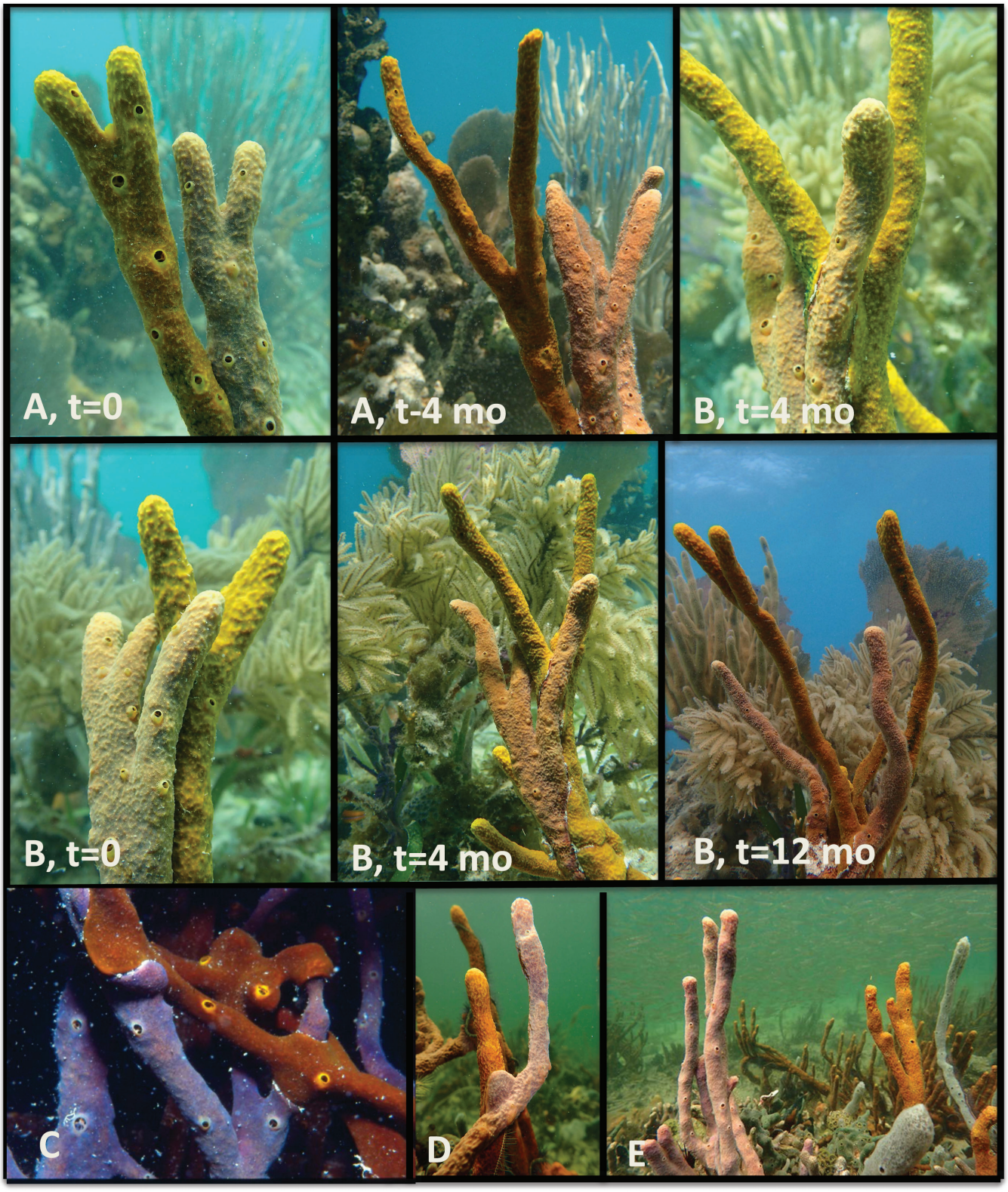

Figure 1. A, *A. fulva* (ochre yellow) and *A. cauliformis* (tan-lavender) individuals growing intertwined and adherent to each other, Blue Ground Range, Belize, at $t = 0$ and $t = 4$ months; *A. fulva* grew 1.85× faster during the 4 months. B, another pair of *A. fulva* and *A. cauliformis* individuals growing adherent to each other at $t = 0$, $t = 4$ months, $t = 4$ months (close-up), and $t = 12$ months; *A. fulva* grew 1.74× faster during the 4 months. C, *A. fulva* and *A. cauliformis* individuals adhering to each other 2 years after being transplanted to mangrove prop roots, Twin Cayes, Belize, D, E, *A. fulva* and *A. cauliformis* growing together on two different reefs in Bocas del Toro, Panama. Note the green tint to the water in Bocas del Toro.

studied in Barbados by Leys and Reiswig (1998) and in Curaçao by Biggs (2014), but is not included by Pinheiro *et al.* (2007) or Cruz-Barazza *et al.* (2012) among western Atlantic *Aplysina*. This study focuses on the narrow-branched anastomosing *A. cauliformis*, corresponding to *V. longissima* of de Laubenfels (1936), as the form most morphologically similar to, and often co-occurring with, *A. fulva*.

Multiple names have not caused recent confusion for *A. fulva*, but it has a complex history, reported in detail by Pinheiro *et al.* (2007); and Pallas' types were destroyed in a fire in 1758. A neotype was designated from Búzios, RJ, Brazil (Pinheiro *et al.* 2007), from a population with morphologies and colours that are somewhat outside the range of variation seen in Caribbean populations (e.g. Wulff 1986: fig. 5). In Caribbean populations consistency is found in circular or elliptical cross-sections of branches and relative invariance of branch width within a branch segment (e.g. Wulff 1990: fig. 1), as well as ochre yellow colour, and dark bruising. Although Pinheiro *et al.* (2007) observed black bruising where *Oreaster reticulatis* fed on a Brazilian *A. fulva*, matching Caribbean observations, the Brazilian sponges have flattened stubby branches with wide bases tapering to narrow tips and various colours (Pinheiro *et al.* 2007: fig. 7; J. Wulff pers. obs. at Búzios).

Schmidt *et al.* (2005) suggested recent divergence of Caribbean and eastern Pacific *Aplysina* species because they could not distinguish among seven species (including *A. fulva* and *A. cauliformis*) using 18S rRNA and ITS2 secondary structure prediction. The coexistence of morphologically distinct Caribbean species led them to consider interbreeding unlikely, in spite of extreme genetic similarity. Heim *et al.* (2007) concurred that the inability to distinguish western Atlantic *Apysina* species reflects recent radiation. Erwin and Thacker (2007) added ITS2 and the 5′ end of the 28S subunit, and scored 13 morphological characters, finding only slight differences in guanine/cytosine, oscular arrangement, and fibre diameters. Lamarão *et al.* (2010) distinguished *A. fulva* and *A. cauliformis* by migration patterns of the ITS1 region on 12% but not 8% single-strand conformational polymorphism (SSCP) gels; and sequencing revealed only a single nucleotide difference. Using mitochondrial DNA (mtDNA), COI, and rDNA ITS1-5.8-ITS2, Cruz-Barraza *et al.* (2012) concluded that eastern Pacific *Aplysina* species are monophyletic, and placed *A. fulva* plus Caribbean *A. fistularis* as sister to *A. cauliformis* plus Pacific species, suggesting that *A. cauliformis* represents the lineage from which eastern Pacific *Aplysina* species arose. For the first mitochondrial genome comparison of congeners in the Demospongiae, Sperling *et al.* (2012) provided a complete mtDNA sequence for *A. cauliformis* to match a published sequence for *A. fulva* (Lavrov *et al.* 2008). Mitochondrial genome length and structure did not differ, and there were only six confirmed nucleotide differences. Although they suggested that these may be the same species, they acknowledged differences reported by Erwin and Thacker (2007) and Lamarão *et al.* (2010), and concluded that these are distinct species. Approaches beyond current molecular techniques appear to be required to further understand relationships of these ecologically important sponges.

Ecological and morphological comparisons of *A. fulva* and *A. cauliformis*, and experimental probes of plasticity

Study sites

Sites were chosen on the Belize Barrier Reef and in Bocas del Toro, Panama, where coral reefs with both *Aplysina* species are near mangroves, which provide a contrasting habitat for probing phenotypic plasticity. *Aplysina* spp. are not members of the typical mangrove root sponge fauna, although they are rarely found at unusual sites where mangroves are very closely associated with reefs (e.g. Rützler *et al.* 2000, Wulff 2005, Rogers 2017). Mangroves differ from reefs in having more dissolved organic carbon (DOC) and picoplankton (i.e. sponge food), less sunlight, reduced water movement, and different and many fewer spongivores. Transplanting clonemates of reef-dwelling sponges into mangroves allowed direct comparison of ecological and morphological plasticity of both *A. fulva* and *A. cauliformis*. Strimaitis (2012) compared summer picoplankton and abiotic factors between coral reefs and mangroves at these sites. In Belize, picoplankton density, total nitrogen (N), and DOC were higher in the mangroves of Twin Cayes than on a nearby (4 km) coral reef in the Blue Ground Range by factors of 2.23×, 2.98×, and 2.15×, respectively, and sunlight intensity was 8.12× higher on the reef than in the mangroves (Strimaitis 2012). In Bocas del Toro, Panama, near the field station of the Smithsonian Tropical Research Institute on Isla Colón, the mangrove and reef water column characteristics were very similar due to close proximity (10 m). Picoplankton density, total N, and DOC in the mangroves were similar to those on the reef (respectively 0.98×, 1.06×, and 1.01×); and light intensity on the reef was only 2.09× higher (Strimaitis 2012). The water column in Bocas del Toro is more nutrient-laden than in Belize, and comparisons of picoplankton density, total N, and DOC reflect this (respectively 2.23×, 1.5×, and 1.3× higher in Panama than in Belize). Light intensity on the reef in Belize is 1.35× higher than in Panama for the same reason (Fig. 1D, E). Coral reef sites in Belize represented a range of exposures to rough water. The most important piscine spongivores in the Caribbean, angelfishes, were only present on reefs, but much less common spongivorous trunkfishes were also sometimes present among mangroves. Spongivores are rare in Bocas del Toro.

Growth and survival

Growth and survival were compared in five experiments lasting 8–24 months, with a total of 216 sponges (Table 1). Experiments were deployed on reefs ranging from exposed to relatively calm in Belize and Panama, and at mangrove sites in Belize (Twin Cayes) and Panama (Isla Colón). All fragments were cut to the same initial size, and each included an intact branch tip. On reefs, fragments were attached, using narrow (1 mm) beaded cable ties, to coral rubble anchored securely near their parent sponges. In three experiments, fragments of the same size and genotypes as those grown on their home reef were transplanted (using narrow beaded cable ties) to CPVC pipes suspended among mangrove prop roots. Sponges were never removed from water. At each time interval, lengths and widths of every branch segment (i.e. whenever diameter changed by > 1 mm) were measured, in the field, to the nearest millimetre. Specific growth rates (i.e. size increase standardized by initial size) were analysed for each experiment by the nonparametric Wilcoxon rank-sum test. Mortality of one member of some genotype pairs led me to abandon paired statistical testing in favour of using all of the growth data in each experiment. Hoping to make photographic documentation of relative growth rates of these two species in the identical microhabitat, a search was made for pairs of

Table 1. Number of participants in growth rate experiments.

	Aplysina fulva		*Aplysina cauliformis*		
Habitat	Reef	Mangrove	Reef	Mangrove	Months
(A, B) Mangroves vs. semi-exposed reef, Belize	17 (9, 9)	17 (10, 6)	15 (8, 6)	15 (8, 5)	12, 20
(C) Mangroves vs. adjacent reef, Panama	20 (11)	20 (11)	19 (9)	19 (10)	12
(D) Mangroves vs. exposed reef, Belize	10 (8)	10 (10)	10 (5)	10 (6)	8
(E) Mangroves, Belize		9 (7)		9 (7)	13
(F) Semi-exposed reef, Belize	8 (7)		8 (5)		24

In each experiment all sponges were the same initial length and had intact growing tips. In experiments comparing growth and survival on the coral reef vs. mangroves, individuals transplanted to the mangroves were the same genotypes as those remaining on the reef. Numbers in parentheses are the number of surviving individuals at the end of the measurement period. Letters correspond to data graphs in Figure 2. Sponge samples for measurement of plasticity of tissue, skeletal, biomechanical properties, and of predator deterrence were taken from sponges in these experiments 4 years after transplantation to the mangroves.

A. fulva and *A. cauliformis* individuals adhering to each other. Two examples on a reef in Belize were each marked with a narrow cable tie for reference, and measured and photographed at $t = 0$, 4, and 12 months (Fig. 1A, B).

To compare repair of wounds, such as are made by spongivorous fishes, wounds (0.9 × 0.5 cm) mimicking trunkfish bites were made in each of five individuals per species. Repair was monitored with measurements and photographs were made for 7 days.

Reattachment and survival of loose fragments were compared at a vigorous water movement reef site at the seaward edge and a moderate–calm site behind the seaward edge of the Belize Barrier Reef. For each experiment, fragments, 10 cm long, were cut from 16 individuals of each species and scattered in a flat area of coral rubble and medium massive corals. Each fragment was examined daily for 2 weeks for adherence to solid substratum and signs of mortality (partial or complete).

Population dynamics

Populations of both species were monitored for 10 years on a shallow reef in the Blue Ground Range of the Belize Barrier Reef. At yearly censuses all individuals on seven patch reefs were mapped and their biomass was estimated by taking sets of external measurements, allowing calculation of volume by conglomerations of appropriate geometric solids, primarily cylinders. Data from the first 6 years have been presented in the context of recovery after mass mortality of the entire sponge fauna of 54 species (Wulff 2013: fig. 7).

Tissue and skeleton characteristics

Four years after the transplants to the mangroves were made, samples were taken from branches that had grown during the years in the mangroves, as well as from branches of sponges of those same genotypes that had remained on the reef. The samples were preserved in 4% formalin in seawater, and transferred to 70% ethanol after 24 h. Paraffin-embedded portions were sectioned at 10–15 μm and stained with haematoxylin/eosin.

Distinctive transport pathways typical of *Aplysina* spp. (Leys and Reiswig 1998), identified by texture and colour, run parallel to the long axes of branches. Cross-sectional areas of single transport pathways were measured in series of sections to confirm that diameters were consistent along their length. The clearest slice from each individual ($N = 4$ from the reef, $N = 4$ from mangroves) was chosen for measurement of short and long diameters of every pathway, to calculate cross-sectional area as an ellipse. Data are reported, for each slice, as a ratio of the combined area of all transport pathways to total branch cross-sectional area.

Skeletal mesh size (i.e. skeletal fibre densities) and widths of individual fibres and their piths were measured on samples from the reef ($N = 4$), and from their clonemates ($N = 4$), that had grown for 4 years in the mangroves. Skeletons were prepared by allowing starfish, *Oreaster reticulatus* (Linnaeus, 1758), to digest the tissue, exposing the fibres. Five cross-sections, each 3 mm thick, were cut from different portions of each branch and affixed to microscope slides to facilitate counting and measurement using light microscopy. All of the fibres that were continuous through the plane of the slice were counted. For skeletal fibre densities, the number of fibres in each cross-section were standardized by cross-sectional area (circle or ellipse) of the branch at that point. Cross-sections of individual fibres and their pith were not always perfectly circular, so long and short diameters were measured (10 fibres per specimen, i.e. 40 per species) to calculate cross-sectional areas as ellipses.

Resistance to damage by vigorous water movement

Two biomechanical properties, extensibility (ratio of length extended to original resting length at the moment of breaking) and breaking strength (force exerted in kg per mm^2 cross-sectional area at the moment of breaking), were measured using a device that gripped the ends of 8-cm-long branch sections with cushioned clips, one stationary and the other movable, aligned with a ruler and attached to a spring scale. Steady stretching until breakage yielded measures of maximum extension of the branch and force exerted at the moment of breaking for sponges collected from a reef ($N = 16$ for *A. fulva*, $N = 17$ for *A. cauliformis*). Biomechanical plasticity was explored by measuring extensibility and breaking strength for branches that had grown in the mangroves for 4 years. Because a differently calibrated spring scale was used at that time, an additional set of measurements on sponges collected directly from the reef was required to make comparisons. Accidental loss overboard of mangrove-grown *A. cauliformis* confined the plasticity comparison to *A. fulva* ($N = 10$ for reef-grown *A. fulva*, $N = 8$ for mangrove-grown *A. fulva*, and $N = 9$ for reef-grown *A. cauliformis*).

Internal polychaete parasites

Branch segments, 0.5 cm long (from at least 4 cm below the tip), were collected from sponges of both species on a coral reef in Belize (*A. fulva* $N = 24$, *A. cauliformis* $N = 13$), and after 4 years in the mangroves (*A. fulva* $N = 10$, *A. cauliformis* $N = 7$), and preserved in 75% ethanol. All polychaete worms, *Haplosyllis spongicola* (Grube 1855), that are typical parasites of *Aplysina* spp. (Tsurumi and Reiswig 1997), were removed from the tissue, while finely dissecting the entire sample on a microscope stage. Whole worms were counted, as well as fragments with heads (each representing a parasite individual). Worm densities were standardized by total volume of the sponge sample.

Predators

Palatability to trunkfishes, angelfishes, parrotfishes, and starfish was previously evaluated (Wulff 2021) by making both species available on shallow coral reefs, seagrass meadows, and mangroves in Belize and Panama. To test the hypothesis that production of defences against reef-dwelling angelfishes and parrotfishes is a plastic response to their presence, 12 fragments from each species, cut from branches grown in the mangroves for 4 years, were made available to reef-dwelling spongivores on anchored coral rubble.

RESULTS

Growth and survival

Differences in growth rates between the species were striking and consistent. On coral reefs, *A. fulva* always grew significantly faster than *A. cauliformis*: (i) in Belize on a windward-facing reef, where storm waves are somewhat damped ($P < .01$, Fig. 2A, B); (ii) on a shallow reef in Bocas del Toro, Panama, that is well protected from storm waves but exposed to water that is increasingly polluted by sewage and sediment ($P < .05$, Fig. 2C); (iii) on an exposed reef on the seaward edge of the Belize Barrier Reef ($P < .025$, Fig. 2D); and (iv) on a small, somewhat protected reef in the lee of Carrie Bow Cay ($P < .025$, Fig. 2F). Growth of *A. fulva* was also significantly faster than *A. cauliformis* for individuals transplanted from coral reefs to mangrove prop roots at Twin Cayes, Belize ($P < .025$, Fig. 2A; $P < .005$, Fig. 2B; $P < .01$, Fig. 2D; $P < .01$, Fig. 2E), and also for individuals transplanted from a reef in Bocas del Toro to nearby mangroves ($P < .05$, Fig. 2C).

Transplantation of the same sets of genotypes monitored on reefs to a habitat richer in picoplankton, N, and DOC among mangroves in Belize resulted in significantly faster growth for both species. In the first year, *A. fulva* grew 3.6× more and *A. cauliformis* grew 3.7× more in the mangroves than on the reef ($P < .01$ for each species, Fig. 2A). By 20 months, the size disparity was extreme: *A. fulva* had grown 7.6× more and *A. cauliformis* 7.5× more in the mangroves than on the reef (Fig. 2B; data presented in the context of growth data on 12 sponge species in Wulff 2017: figs 1, 2). In another set of experiments, *A. fulva* grew 2.7× faster and *A. cauliformis* grew 5.7× faster during 8 months in the mangroves than on a relatively exposed coral reef ($P < .01$ for each species, Fig. 2D). In Bocas del Toro, Panama, both species grew more slowly in the mangroves ($P = .025$ *A. fulva*, $P < .05$ *A. cauliformis*, Fig. 2C).

Photos of the two species in the pairs that were naturally adherent to each other (i.e. in exactly the same microhabitat) illustrate the growth rate differences in the experiments (Fig. 1A, B). Specific growth over 4 months of *A. fulva* in Fig. 1A photos was 1.85× faster than that of the *A. cauliformis* to which it adhered (0.76 vs. 0.41), and growth of *A. fulva* in Fig. 1B photos was 1.74× that of the *A. cauliformis* to which it adhered (0.87 vs. 0.5).

Growth rate differences were not reflected in wound healing, which did not differ between species. In both species surface pinacoderms were replaced, with pieces of skeletal fibres poking through, by 24 h (Fig. 3A, C); and on day 5 only some colour and a thin layer of tissue volume remained to be restored (Fig. 3B, D).

Fragments of *A. fulva* suffered more mortality and damage than those of *A. cauliformis* at both the calmer and rougher sites (Fig. 4). At the calmer site, 3/16 *A. fulva* lost portions of their tissue 1.5–6 cm long but no *A. cauliformis* were damaged by the end of 2 weeks (Figs 3E, 4). At the rougher site four *A. fulva* fragments had lost all of their tissue after 2 weeks, but no *A. cauliformis* were dead. In both experiments, damage to fragments was associated with their having not yet reattached themselves.

Population dynamics

Population dynamics in terms of total live volume over 10 years were very similar for these species (Fig. 5). Both declined abruptly during a community-wide mortality of unknown cause in 2008, and again in 2011 during a dense phytoplankton bloom. After the 2008 decline they recovered only partially before the 2011 bloom, which killed 71% of the sponge biomass in this coral reef community of 54 sponge species (Wulff 2013). Neither *Aplysina* species had fully recovered 5 years after the second event, when their total volumes were only 33.8% (*A. fulva*) and 26% (*A. cauliformis*) of their volumes in 2006 (Fig. 5).

Tissue and skeleton characteristics

On the reef, both species devoted the same proportion of total cross-sectional branch area to transport pathways (Fig. 6). These pathways responded plastically to increased food availability in both species, occupying significantly greater proportions of their branch cross-sectional area 4 years after they were transplanted to the mangroves from the reef ($P < .05$, Mann–Whitney test for both species). The plastic response was parallel in the two species, so they remained the same in the proportion of total cross-sectional area devoted to transport pathways after transplantation (Fig. 6).

Densities of skeletal fibres per unit cross-sectional area (which varies inversely with mesh size) were compared using the nonparametric Wilcoxon rank-sum test, with all measurements within each of the four species-by-habitat categories combined for the rankings. Fibre density was significantly greater for *A. cauliformis* than for *A. fulva* (Figs 3F, G, 7A; $P < .001$) for individuals that remained on the coral reef, as well as for clonemates of those same individuals after they had grown for 4 years in the mangroves ($P < .001$). The density of skeletal fibres increased significantly in individuals of both species that were transplanted to the mangroves ($P < .01$, for both species), relative to their clonemates that remained on the reef during that 4-year period. Variation within individuals was substantial, as shown in Fig. 7B, illustrating the importance of collecting data from several places within each of several specimens when comparing between

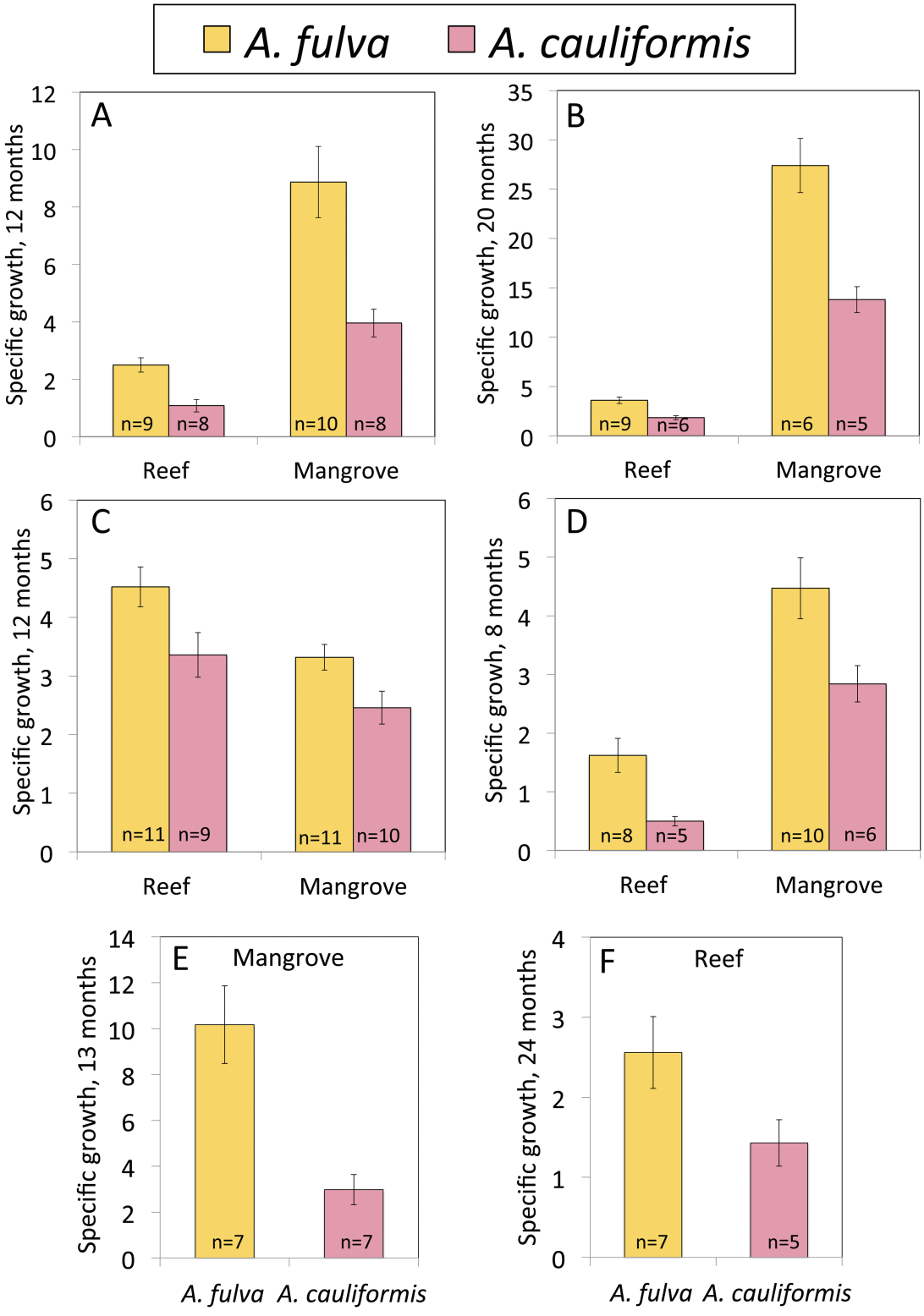

Figure 2. Specific growth rates at various sites and over various time periods; all experiments comparing between habitats are controlled for genotype and initial size. A, *A. fulva* and *A. cauliformis* grown on their home reef and among mangrove prop roots for 12 months; B, the same experiments as in A, after 20 months (presented also in the context of 10 other species in Wulff 2017); C, *A. fulva* and *A. cauliformis* grown on their home reef in Panama, and among mangrove prop roots about 10 m away for 12 months; D, *A. fulva* and *A. cauliformis* grown on their shallow home reef and among mangroves 4 km distant for 8 months; E, *A. fulva* and *A. cauliformis* collected in the Blue Ground Range, Belize, grown among mangrove roots at Twin Cayes, Belize for 13 months; F, *A. fulva* and *A. cauliformis* grown on a shallow reef near Carrie Bow Caye, Belize, for 24 months. Note the different *y*-axis scales. In every experiment, *A. fulva* grew significantly more rapidly than *A. cauliformis*. In Panama, both species grew significantly more slowly in the mangroves; however, in all experiments in Belize, both species grew significantly more rapidly in the mangroves.

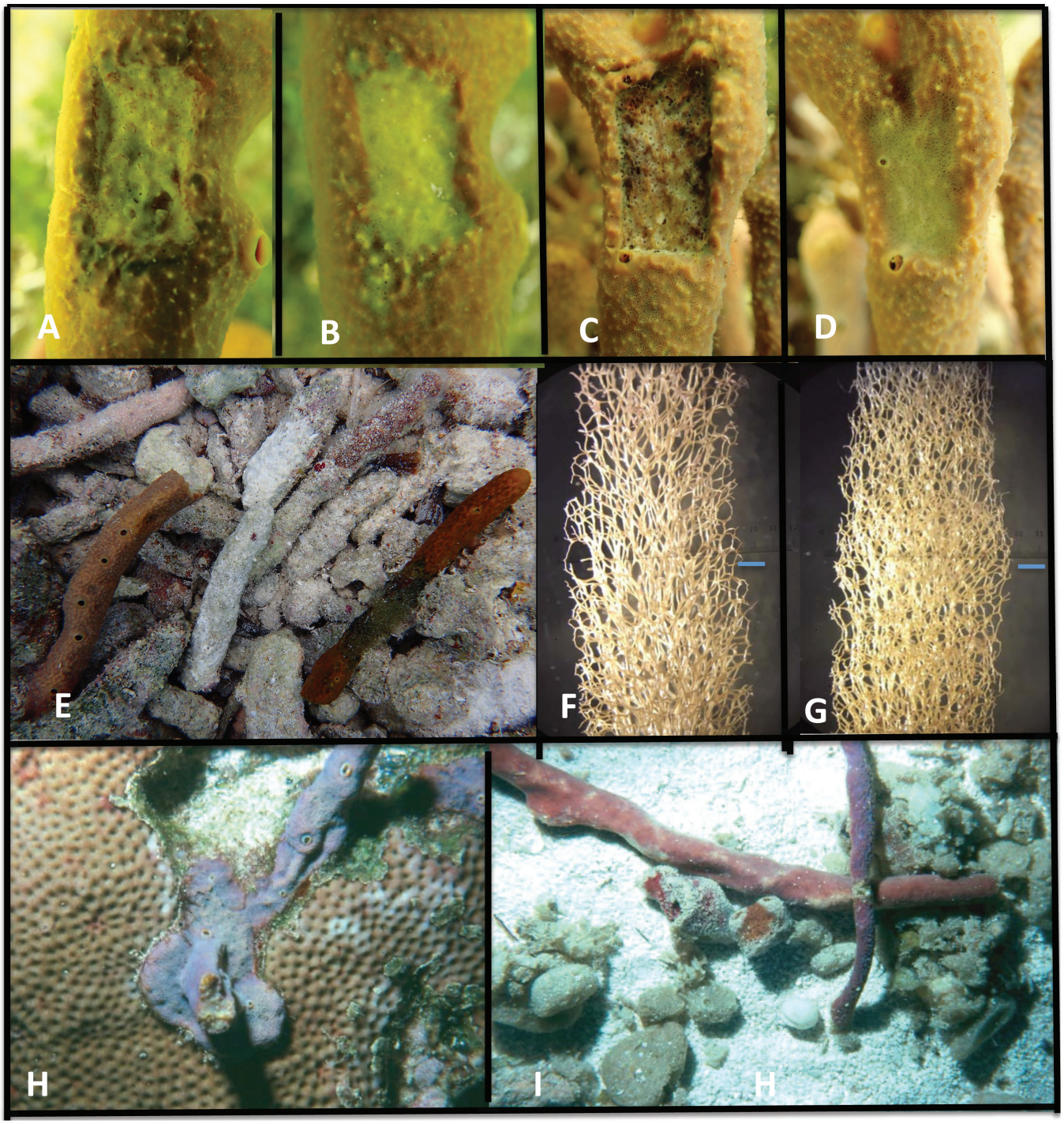

Figure 3. A, *A. fulva* 1 day and B, 5 days after experimental wounding. C, *A. cauliformis* 1 day and D, 5 days after experimental wounding, Bocas del Toro, Panama. Note that differences in density of skeletal fibres can also be seen by comparing the surfaces of the living sponges. E, fragment reattachment and survival experiment, Little Clearwater Reef, Belize, *A. cauliformis* on the left and *A. fulva* on the right, with the skeleton visible where the tissue has died. F, *A. fulva* and G. *A. cauliformis* skeletons with tissue digested off by *Oreaster*; scale bars are 1 mm. H, *A. cauliformis* base on a fore-reef coral at Discovery Bay, Jamaica, after Hurricane Allen, a few weeks after the storm; the stub from which a branch broke off has healed. I, *A. cauliformis* fragments on the fore-reef after Hurricane Allen, alive and relatively undamaged amidst dead skeletons of at least a dozen other sponge species 1 week after the storm.

species, and urging caution (at least five cross-sections should be evaluated) in using this character for assigning an individual specimen to a particular *Aplysina* species.

Comparisons of individual fibres (tested using the nonparametric Mann–Whitney test) were inconsistent. Cross-sectional areas (μm^2) did not differ significantly between reef-dwelling *A. fulva* (mean 64.2, SE 4.3) and *A. cauliformis* (mean 74.4, SE 4.4); and after 4 years in the mangroves they did not change in *A. fulva* (mean 64.1, SE 3.2) but decreased significantly ($P < .05$) in *A. cauliformis* (mean 51.5, SE 1.8). Pith comprised a

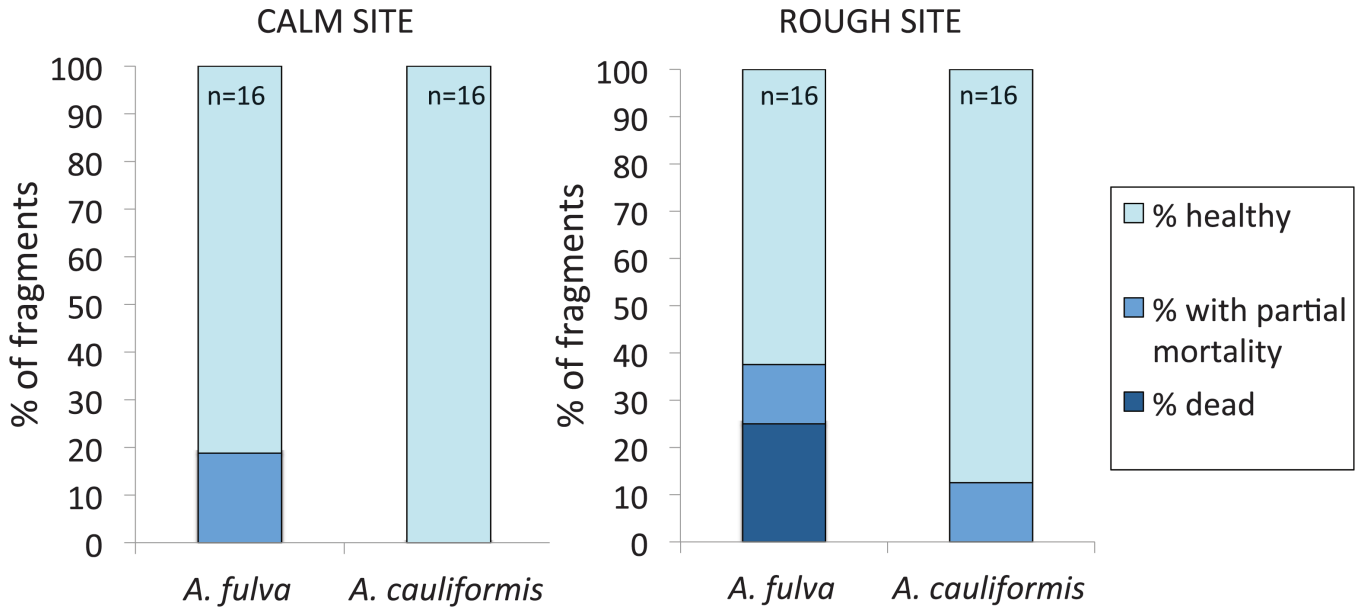

Figure 4. Survival, partial survival, and reattachment of loose fragments of *A. fulva* and *A. cauliformis* were recorded for 2 weeks after scattering fragments (10 cm long) of each species at two sites (rougher vs. calmer water movement).

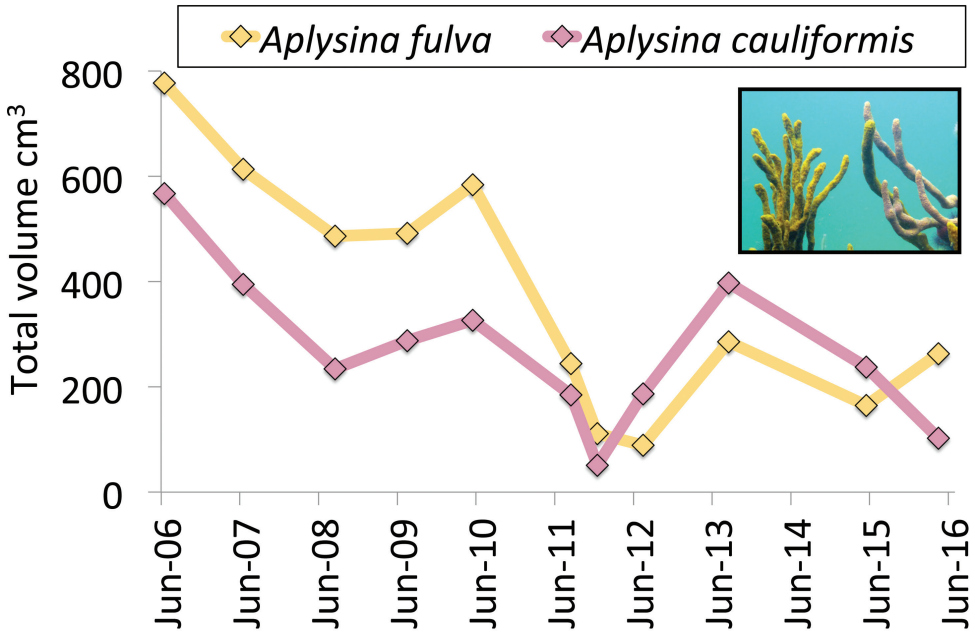

Figure 5. Population dynamics of *A. fulva* and *A. cauliformis* over 10 years on a shallow reef in the Blue Ground Range, Belize, in terms of total volume of live tissue. Every sponge individual was mapped and measured for volume on seven small patch reefs at time intervals that were in most cases about 12 months long.

significantly greater ($P < .001$) proportion of the cross-sectional areas of individual fibres in *A. fulva* (mean 40.9%, SE 1.8) than in *A. cauliformis* (mean 17%, SE 19.7) for individuals on the coral reef. However, after 4 years in the mangroves, pith constituted a significantly greater proportion of fibres for reef-grown than mangrove-grown *A. fulva* (mean 26.6%, SE 6.6) but not *A. cauliformis* (mean 19.7%, SE 2.8), erasing the difference between the species.

Resistance to damage by vigorous water movement

Extensibility but not breaking strength differed significantly between the species (Mann–Whitney $P < .01$; Fig. 8). Extensibility of reef-grown *A. fulva* (mean 0.131, SE 0.011) and *A. cauliformis* (mean 0.09, SE 0.032) in the second set of biomechanical experiments also differed significantly (Mann–Whitney $P < .05$), confirming this difference between species (spring scale calibration differences resulted in actual values differing from those in

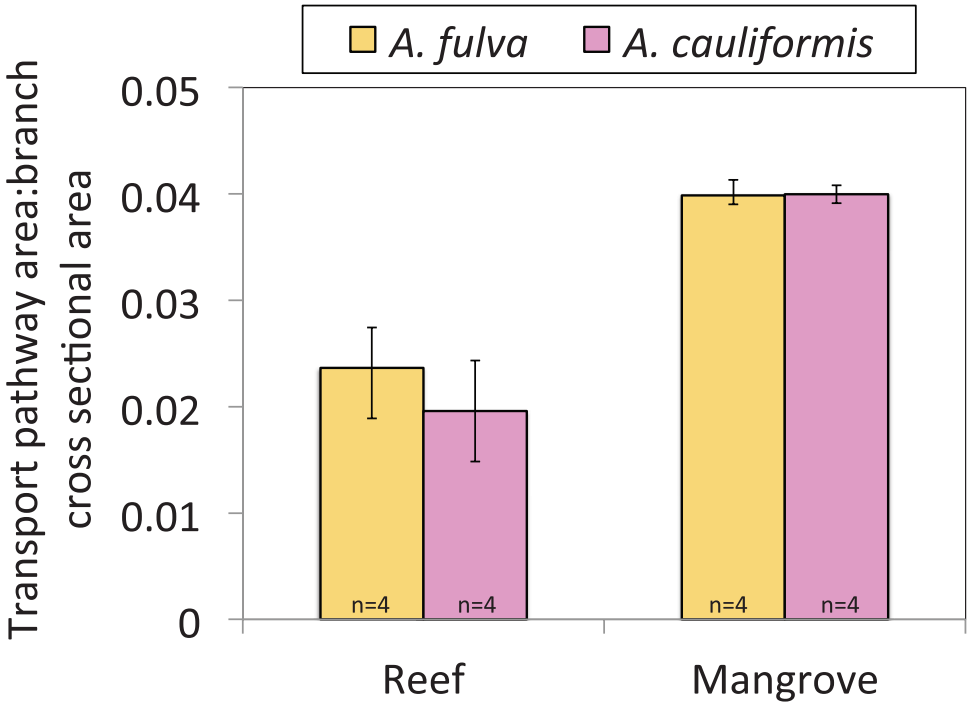

Figure 6. Ratio of the cross-sectional area of transport pathways to total branch cross-sectional area of branches of *A. fulva* and *A. cauliformis*. Mangrove individuals were the same set of genotypes as those collected from their home habitat of the coral reef, and the portions collected had entirely grown in the mangroves during the 4 years since the transplant. Differences between species were not significant in either habitat, but both species had significantly higher ratios of transport pathway area to total cross-sectional area after 4 years in the mangroves ($P < .05$ for each species).

the initial between-species comparison; Fig. 8). *Aplysina fulva* that had grown in the mangroves for 4 years were less extensible (mean 0.112, SE 0.01) than those remaining on the reef (mean 0.131, SE 0.011), but this difference was not significant.

Internal polychaete parasites

Parasitic polychaete density did not differ significantly between species on the reef or in the mangroves, and polychaete density did not change in either species in response to transplantation from reef to mangroves and living in those different habitats for 4 years (Fig. 9).

Predation

Neither experiments nor observations detected differences in predation on these two *Aplysina* species by angelfishes, parrotfishes, trunkfishes, and a starfish (Wulff 2021). Individuals grown in the angelfish-free mangroves for 4 years did not lose resistance to fish predation, and were not consumed when transplanted back to the reef. Plasticity in palatability to the large seagrass starfish, *Oreaster reticulatis*, could not be evaluated because *Aplysina* spp. exposed to this predator did not get a second chance.

DISCUSSION AND CONCLUSIONS

Aplysina fulva and *A. cauliformis*: morphological and ecological similarities

The molecular and morphological similarities of *A. fulva* and *A. cauliformis* suggest that they have very recently diverged (Schmidt *et al.* 2005, Heim *et al.* 2007, Cruz-Barazza *et al.* 2012, Sperling *et al.* 2012). Ecological similarities are also striking. The species co-occur at many sites (Fig. 1; Erwin and Thacker 2007, Gochfeld *et al.* 2007, Wulff 2013, Freeman *et al.* 2015, Edmunds *et al.* 2020), where subtle colour differences can be the sole visual distinction. At the two sites where long-term population dynamics of both have been followed, they fluctuated in tandem (Fig. 5; Wulff 2013, Edmunds *et al.* 2020). Life histories of both species are dominated by asexual propagation by fragments created by physical disturbance and pathogens (Wulff 1990, 1991, 2006b, 2006c, Olson *et al.* 2006), and their gamete production is very low. Tsurimi and Reiswig (1997) sampled every week for a year and found oocytes in only nine and spermatocytes in only one out of 207 *A. cauliformis* specimens, and maximum oocyte density was only 7.6/mm^3. Similarly, Leong and Pawlik (2011) sampled monthly and found oocytes in only 1/60 *A. fulva* and 2/60 *A. cauliformis* and no spermatocytes. These *Aplysina* species did not differ in breaking strength (Fig. 8); and both are susceptible to the same pathogen (Gochfeld *et al.* 2012), internal polychaete worm parasites (Fig. 9), and large seagrass-dwelling starfish (Wulff 2021). They are equally favoured by trunkfish, and particular individuals within populations of both species are consistently favoured (Wulff 1994, 2021); in addition, they healed wounds mimicking bites of the trunkfish that feed on them equally well (Fig. 3A, B, C, D). Finally, these species devoted the same proportion of tissue to transport pathways (Fig. 6), a focal tissue characteristic because of their functional role in moving nutrients to growing tips (Leys and Reiswig 1998).

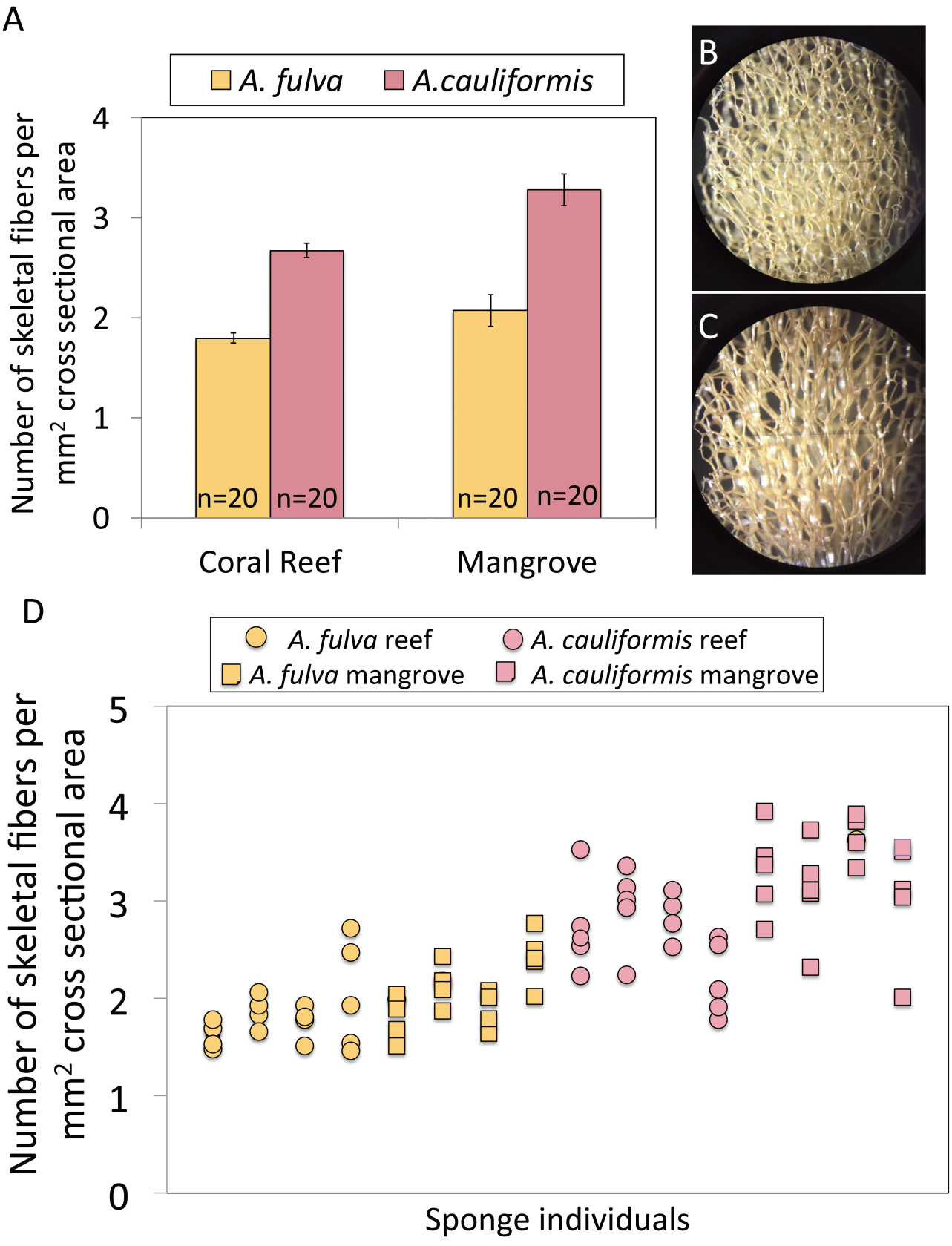

Figure 7. Density of skeletal fibres per cross-sectional area of branches for *A. fulva* and *A. cauliformis* that were collected from their home habitat of the coral reef as well as after the same genotypes had grown for 4 years among mangrove prop roots. All fibres that were severed in cutting the section were counted for five sections in each of four sponges representing each species-by-habitat combination. A, density is significantly different, by the Wilcoxon rank-sum test (all measurements within each species-by-habitat category were combined for the rankings), within species between habitats ($P < .01$ for both species), and between species within habitats ($P < .01$ for the reef, $P < .001$ for the mangroves); B, *A. cauliformis* skeletal meshes; C, *A. fulva* skeletal meshes; D, data points plotted individually to show the variation within and between sponge individuals. In spite of the significant differences between species in each habitat, the overlap between species suggests caution in use of this as a diagnostic character.

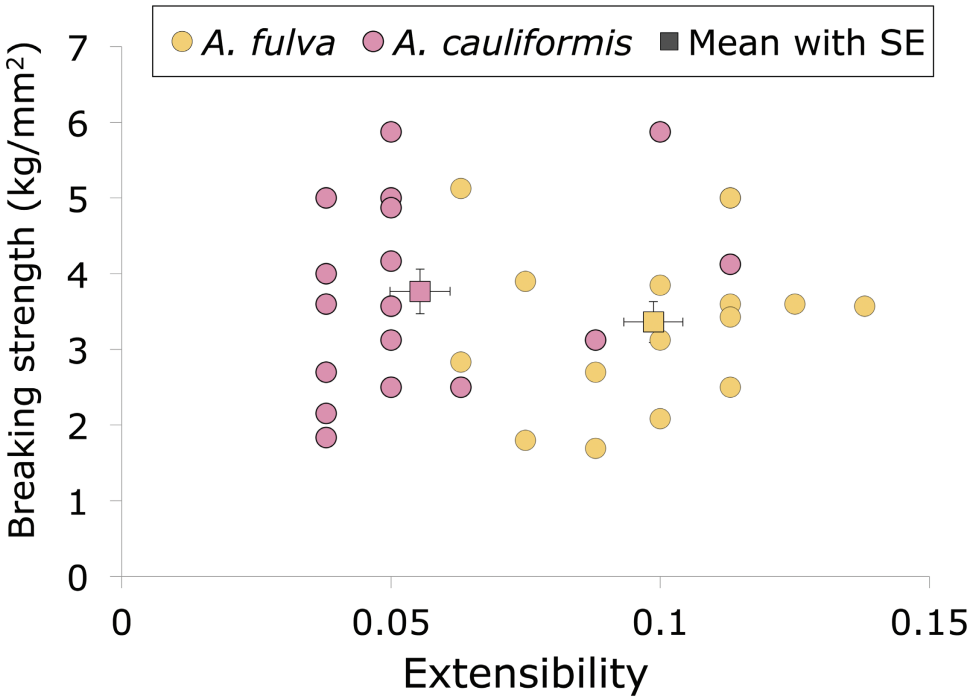

Figure 8. Breaking strength (force/cross-sectional area at breaking) and extensibility (length extended/initial length at breaking) for *A. fulva* ($N = 16$) and *A. cauliformis* ($N = 17$) branches. Each circle indicates a sponge individual, and means and SEs of the means are indicated with squares. These species differ significantly in extensibility ($P < .01$), but not in breaking strength.

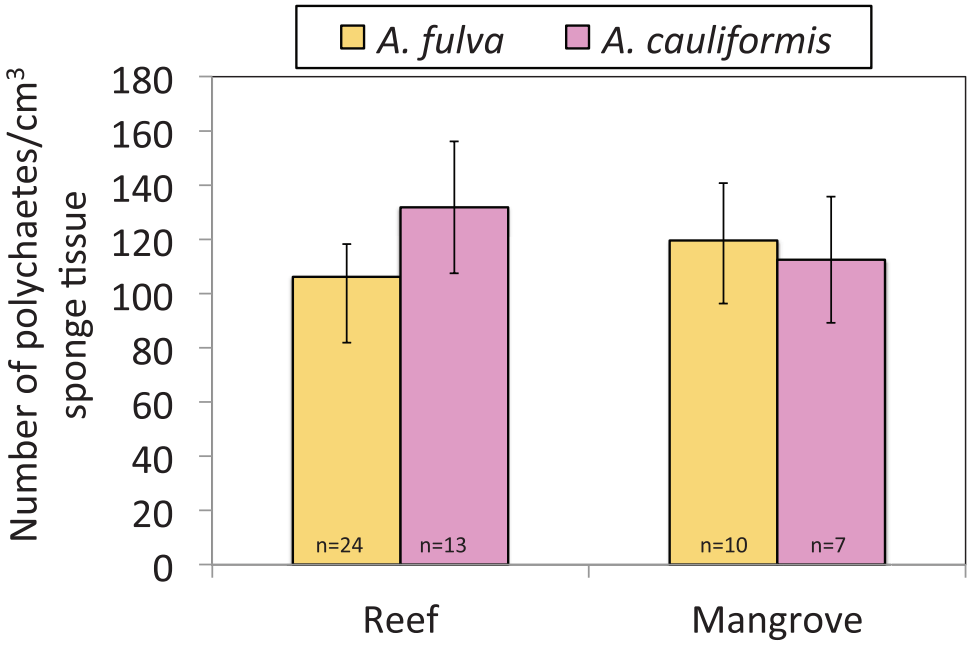

Figure 9. Density of polychaete parasites, *Haplosyllis spongicola*, in *A. fulva* and *A. cauliformis*, showing no significant differences between species or between habitats in sponge samples that were collected from both the reef and mangroves, 4 years after transplants were made to the mangroves.

Aplysina fulva and *A. cauliformis* differences: divergent strategies

Boury-Esnault *et al.* (2013) sparked appreciation of the value of combining ecological, morphological, and molecular characters (i.e. integrative taxonomy) to clarify boundaries in character-sparse sponges. These *Aplysina* species add to the accumulating examples: clear ecological differences led to a previously unconsidered morphological character (i.e. mesh size), and ultimately revealed divergent suites of characters that suit these species for different ends of a habitat continuum.

Extensibility differed consistently, with the more extensible *A. fulva* less likely to be fragmented by physical disturbance (Fig.

8), increasing its success in shallow water. *Aplysina cauliformis* is more vulnerable to breakage, but its loose fragments survive better than those of *A. fulva* (Fig. 4). Differences in extensibility that suit *A. fulva* for shallower water and *A. cauliformis* for deeper water prompted attention to details in distribution reports, which confirmed that at sites with only one of these species, *A. fulva* was the sole species at relatively shallow sites (e.g. Guna Yala near San Blas Point, Panama, Wulff 2006a; Dry Tortugas, de Laubenfels 1936; the lagoon at Bimini, Bahamas, Wiedenmayer 1977), and *A. cauliformis* was the sole species at relatively deep sites (e.g. forereef at Discovery Bay, Jamaica, Wulff 2006b; Marsagantupo, Guna Yala, Panama, Wulff and Buss 1979; coral reefs north of Puerto Rico, de Laubenfels 1936; Navassa, Wulff and Swain 2006; and some sites near Lee Stocking Island, Bahamas, Olson *et al.* 2006, Easson *et al.* 2013).

Propagation by fragmentation dominates the life histories of both species, but mechanistic details differ in important ways. Previous studies at sites with only one branching *Aplysina* species offer informative comparisons with other sponge species. In two fragment dispersal experiments, in Guna Yala, Panama, survival in the first few weeks, when fragments were loose and readily moved by water, was much lower for *A. fulva* than for two other branching species, *Iotrochota birotulata* and *Amphimedon compressa* (Wulff 1985). However, survival after week 4 (for 7 weeks in one experiment and 1 year in another) was proportional for all three species, showing that *A. fulva* fragments are more vulnerable only before they reattach. The strategy of *A. fulva* for avoiding this unattached stage is for branches to bend down to the substratum and attach before becoming severed. The percentage of successful fragment production by severing basal or repent portions, rather than upright branches, was higher for *A. fulva* than the other two species, and successful initiation of new repent branches by erect branches bending down vs. breaking off was much higher for *A. fulva* (Wulff 1990: figs 1, 5, 6). Rapid repopulation of cleared quadrats (37% by numbers and 56% by volume in one year) by *A. fulva* was facilitated by repent branches (Wulff 1991). In a plot where clonemates were identified and mapped, *A. fulva* genotypes characterized by repent branches were represented by more physiologically independent clonemates than were genotypes with all upright branches (Wulff 1986: fig. 5). These data further confirm that repent branches increase success of asexual propagation by *A. fulva*. By contrast, after a major hurricane in Jamaica, many *A. cauliformis* fragments were alive in the midst of piles of dead skeletons of dozens of other species (Fig. 3H, I). At a site where those live fragments were trapped among branching corals, rather than swept off the reef, *A. cauliformis* was propagated by the hurricane (Wulff 2006b). Less extensible (i.e. more readily broken) branches do not suit this species for living in shallow, chronically wave-washed sites, but when infrequent major storms wreak havoc in deeper water, *A. cauliformis* fragments are excellent survivors.

Differences in both extensibility and fragment survival may arise from the sole clear morphological difference between these species: mesh size/density of skeletal fibres, a feature not used to distinguish between *Aplysina* species until this study. Consistently more fibres per cross-sectional area (i.e. smaller meshes) in *A. cauliformis* (Figs 3F, G, 7) may decrease extensibility, resulting in increased vulnerability to fragmentation by physical disturbance. However, greater fibre density may also help loose fragments avoid fatal battering and bestow increased stiffness that allows attached *A. cauliformis* to maintain an erect stance, helping ensure adequate light for photosymbionts in deeper water.

The advantages of more fibres per cross-sectional area may be expensive. Costs of sponge skeletal elements have been surmised before, when increased spicules and other skeletal elements were associated with decreased growth or reproduction (e.g. Uriz *et al.* 1995, Meroz-Fine *et al.* 2005). As is characteristic of the order Verongiida, *Aplysina* species do not have spicules, and so spongin fibres are the only skeletal elements. In all 10 comparisons, *A. cauliformis* grew significantly more slowly (Fig. 2). This cannot be explained by differences in predators, parasites, or nutrient-ferrying transport pathways, none of which differed between species. If skeletal fibres are expensive, differences in mesh size/fibre density are a plausible explanation, as increased allocation to fibres could reduce allocation to growth. A difference in nutrient acquisition was identified by Freeman *et al.* (2015) using stable isotopes. Both species assimilated C and N from their microbial symbionts, but host and symbiont metabolisms were more tightly coupled in *A. cauliformis*, which derived a higher percentage of its C from symbionts. Growth rates (73 days) only differed under <5% of ambient light. While isotopic niches of these congeners are intriguingly different, in the context of 12 other common species, they are relatively similar (Freeman *et al.*, 2020).

Two distinctive strategies emerge from the combined differences between these narrow branched *Aplysina* species (Table 2). Suited for shallow water, *A. fulva* branches resist breakage from physical disturbance. Its less stiff branches bend to the substratum, where they are sometimes severed by sediment, disease, or predation, propagating the genotype more reliably than by loose fragments that survive poorly until they reattach. Better suited for deeper water, *A. cauliformis* branches are more readily broken, but their erect stance may help ensure

Table 2. Ecological and morphological similarities and differences between *A. fulva* and *A. cauliformis*.

***Aplysina fulva*, *A. cauliformis* similarities:**
vulnerability to spongivores (angelfishes, parrotfishes, trunkfishes, starfish)
internal polychaete parasites
wound repair
population dynamics during 10 years (on a reef where they co-occur)
*transport pathways
***Aplysina fulva*, *A. cauliformis* differences:**
habitat depth (much overlap, but *A. cauliformis* deeper)
fragment survival (*A. fulva* worse)
fragmentation style (reattachment pre- vs. post-severing)
*growth rate (*A. fulva* faster)
*skeletal fibre density (*A. cauliformis* denser)
*extensibility (*A. fulva* higher)

Traits that were demonstrated to be plastic by genotype-controlled experiments are marked with an asterisk (*). All plastic traits were plastic in the same direction and to the same extent in both species.

access of photosymbionts to adequate sunlight. In addition, if a major storm breaks branches, loose fragments survive well.

Context: ecological distinction between pairs of sympatric congeneric sponge species and phenotypic plasticity in sponges

Studies of ecological differences between sponge congeners and studies of phenotypic plasticity in sponges have been fruitful but separate research directions for sponges, providing context for how these two lines of inquiry can be meshed. Subtle ecological and morphological distinctions between other pairs of sympatric congeneric sponge species in life histories, skeletal elements, growth, and chemical defences have been related to seasonal changes, substratum type, depth, water column materials, water flow, and responses to predators (e.g. Hartman 1957, Barbieri *et al.* 1995, Wulff 2006d, Blanquer *et al.* 2008, Bavestrello *et al.* 2009, Muricy *et al.* 2019, Vicente *et al.* 2020).

Phenotypic plasticity is often invoked to explain variation in sponges. Actual demonstrations of plasticity, involving environmental alteration, are less common, but span a variety of environmental situations. Wilkinson and Vacelet's (1979) transplantation of five Mediterranean species to different light, current, and sediment conditions spurred a variety of species-specific shape and growth rate changes. Transplantation to more vigorous water motion spurred production of thicker spicules in *Halichondria panicea* (Palumbi 1986) and *Cinachyrella australiensis* (McDonald *et al.* 2001), and greater spicule density in *Tetilla* sp. (Meroz-Fine *et al.* 2005). Spicule content in *Cliona varians* was increased by predator exposure and decreased by protection from predators (Hill and Hill 2002). Transplantation of the sponge/red alga association *Haliclona caerulea*/*Jania* to different depths resulted in larger attachments, smaller more dense oscules, and narrower spicules at the shallowest depth; and inside cages branching differed and algal contribution diminished (Carballo *et al.* 2006). Transplantation to water columns that are richer in nutrients has resulted in faster growth in deeper water (Trussell *et al.* 2006: *Callyspongia vaginalis*) and in mangroves (Wulff 2017: 12 coral reef species); and a temporal switch to more food in the Antarctic water column speeded sponge growth (Dayton *et al.* 2013: *Anoxycalyx joubini*). Temporal changes in spicule, cell, and surface characters occurred with seasons in *Chondrilla* aff. *nucula* in Brazil (Cavalcanti *et al.* 2007). Transplantation to deeper sites caused morphological changes in Mediterranean *Chondrosia reniformis* (Gökalp *et al.* 2020) and Caribbean *Ircinia felix* and *Aplysina fistularis* (Maldonado and Young 1998). Exposure to predators increased chemical deterrence in *Plakortis angulospiculatus* (Slattery *et al.* 2016), and severe wounding caused bioconversion of a possibly antipathogen metabolite in a Mediterranean *Aplysina* species (Thoms *et al.* 2006).

Aplysina fulva and *A. cauliformis* did not differ in predator and parasite responses, nor were those responses plastic. Otherwise, however, these *Aplysina* species fit well into context from other sponge species in ecological distinctions between closely related sympatric sponge species (depth distribution, water flow regime, and life histories) as well as in plastic responses of individual species to altered conditions (growth rates, skeletal elements, and overall morphology).

Phenotypic plasticity, integrated suites of adaptive characters, coexistence, and diversification

Two themes that have been explored separately for sponges are combined in this study: (i) ecological differences between sympatric congeners, and (ii) plasticity of individuals within a species in response to environmental changes. One previous study combined these approaches: Wilkinson and Vacelet (1979) confirmed morphologically similar Mediterranean *Aplysina aerophoba* and *A. cavernicola* as distinct species when phenotypic plasticity induced by transplantation matched their habitat preferences for illuminated vs. shaded microhabitats. Plastic responses to an alternative environment of *A. fulva* and *A. cauliformis* likewise confirm species distinctions, but their parallel plasticity in every character contrasts intriguingly with divergent plasticity in the Mediterranean *Aplysina* spp.

Unlike the case with the many phylogenies for vertebrates, arthropods, molluscs, and other groups on which speciation events can be precisely located, our understanding of sponge speciation is relatively meagre. Many cryptic species have been successfully distinguished, and genetic structure has been revealed, sometimes on quite small scales, within some species (Uriz and Turon 2012). A few within-genus or family phylogenies have been based on the confluence of a variety of molecular techniques (e.g. Melis *et al.* 2016, Kelly *et al.* 2021, Kenny and Itskovitch 2021), but sponges often stymie attempts to refine phylogenies due to few clear characters and inapplicability of molecular techniques useful in other groups (Uriz and Turon 2012, Boury-Esnault *et al.* 2013). Further refinement of branch points for the phylogeny of Caribbean *Aplysina* by current molecular techniques appears to be out of reach due to very recent divergence (e.g. Schmidt *et al.* 2005, Heim *et al.* 2007, Cruz-Barazza *et al.* 2012).

Thirty years ago Bavestrello and Sarà (1992) suggested that phenotypic plasticity in sponges might precede speciation. They distinguished two sympatric species of Mediterranean cave-dwelling *Petrosia* using a comprehensive set of morphological characteristics, including aquiferous system branching patterns, proportions of different spicule types, spicule morphometrics, and overall morphology. By relating morphological to ecological differences in cave microhabitats, they distinguished intraspecific variation from phenotypic plasticity by comparing three populations. Bavastrello and Sarà (1992) interpreted their data as support for parasympatric speciation in *Petrosia*, with divergence preceded by phenotypic plasticity for coping with water movement.

Do the ecological characters and experimental approaches to phenotypic plasticity presented here suggest a similar route to speciation in branching Caribbean *Aplysina* species? Parallel plasticity (i.e. in the same direction and to the same degree) in response to transplantation suggests strongly that plasticity for skeletal fibre density, extensibility, growth rate, and proportions of tissue devoted to transport pathways was present ancestrally. Plasticity in the transport pathways has not resulted in divergence of narrow-branched Caribbean *Aplysina* in this character, but it will be interesting to see if the tropical Eastern Pacific species, inhabiting seasonally much richer water that is patchily distributed, have diverged in response to food availability. Plasticity in skeletal fibre density, and consequently extensibility and

growth rate, however, may have paved the way for divergence in the western Atlantic.

In advocating a mechanistic approach, viewing organisms as integrated wholes, Wund (2012) pointed out advantages of working with examples of diverged species in the same environment so reactions to different environments could be observed in both the ancestral and derived species. The designation of either of these *Aplysina* species as ancestral has not been possible with current techniques, but we can consider the process of diversification simply as a divergence. Initial divergence within an adaptively plastic species can occur in a population that does not experience alternative environments (e.g. Pfennig *et al.* 2010). For branching *Aplysina* species, this could be either a deep reef at a site lacking shallow hard substrata, or a shallow reef that bottoms out in seagrass or sediment. Both are common circumstances in the Caribbean. A preponderance of propagation via fragments that are unable to traverse sedimented areas can lead to separation of populations after a chance (very low probability) colonization; and the combination of some sexual reproduction combined with disproportionate asexual propagation of those genotypes particularly well suited to the site could have led to divergence. Confirming some sexual reproduction, possibly only successful at long time intervals, in population dynamics dominated by asexual propagation, the 60 largest *A. fulva* individuals within a 10 × 20-m area represented 13 clones, and 20 of 30 smaller individuals were also members of those same clones (Wulff 1986).

One scenario casts the more extensible, shallow-water *A. fulva* as ancestral. Plasticity for density of skeletal fibres could result in increased fibre density, and thus enhanced fragment survival and also a more erect stance, beneficial for photosymbiont-bearing species in deeper water where gorgonians, corals, and other sponges might otherwise overshadow them. However, the advantages of more fibres per cross-sectional area are probably not cost-free: increased vulnerability to breakage concomitant with increased fibre density that decreases extensibility might force restriction to deeper water in areas with high water movement, with the cost of more skeletal fibres decreasing growth rates.

An alternative 'plasticity first' scenario casts the less extensible, deeper-water *A. cauliformis* as ancestral. Plasticity for skeletal fibre density/extensibility could provide breakage-resistance in shallow water, but possibly force restriction to shallow water because branches are too limp to reliably collect adequate sunlight in deeper water. A bonus could be faster growth due to decreased expenditure on skeletal fibres. Compensation for lower fragment survival might be gained as less stiff branches droop to the substratum, securing additional attachment points, so asexual propagation can proceed without risking mortality of unattached fragments.

Integrated suites of traits that promote thriving on either shallow or deeper reefs place this example well among other examples in which plasticity influences habitat use and consequently diversification (e.g. Pfennig *et al.* 2010). Plastic reactions to transplantation suggest the ecological traits 'extensibility' and 'fragment survival' may be integrated via the morphological trait 'fibre density/mesh size', which had not been used to distinguish these species before. Growth rates were influenced by food abundance, but in the context of possible trade-offs between competing functions, growth rate reflects phenotypically plastic choices between allocation to size, reproduction, skeletal reinforcement, and defences against predators (Whitman and Agrawal 2009). In this case growth rates may be integrated into overall strategies in terms of allocation to fewer vs. more skeletal fibres (i.e. larger vs. smaller skeletal meshes), a clear example of how phenotypic plasticity comparisons among related species can be a powerful tool for better understanding adaptive design (e.g. Piersma and Drent 2003). This multipronged approach to ecology, morphology, and phenotypic plasticity of *A. fulva* and *A. cauliformis* has divulged shared directions and degrees of plasticity and revealed integrated suites of adaptive characters that improve survival in subtly different habitats and suggest possible paths of speciation.

ACKNOWLEDGEMENTS

I am deeply grateful to the field stations that have facilitated years of intense underwater study: Carrie Bow Cay in Belize, and Smithsonian Tropical Research Institute stations in Guna Yala and Bocas del Toro, Panama. Erin Crawford, Katie Kramer, and Kelly Vasbinder helped to measure skeletal fibres, transport pathways, and parasites. Brendan Biggs and Anna Strimaitis contributed to some of the reef censuses, and Anna also embedded the specimens, and she and Colin Wulff assisted with biomechanical evaluations. Many thoughtful comments on the manuscript were provided by Egbert Leigh and three reviewers. I am grateful for funding by the National Science Foundation [grant number 0550599]; the Marine Science Network of the Smithsonian Institution, supported in part by the Hunterdon Oceanographic Research Endowment; and the Caribbean Coral Reef Ecosystems Program (CCRE) of the National Museum of Natural History, Smithsonian Institution.

DATA AVAILABILITY

All of the data are presented in the paper in Figures 4, 5, 7, and 8. The raw data on which means and standard errors in Figures 2, 6, and 9 are based are available on request from the author.

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