Choosing the right home: settlement responses by larvae of six sea urchin species align with hydrodynamic traits of their contrasting adult habitats

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Ocean organisms as diverse as seaweeds and sea cucumbers exhibit life cycles in which dispersal occurs primarily via microscopic larvae or spores, with adults exhibiting limited or even no dispersal. In benthic animals, the larval stage concludes with irreversible settlement into the benthos. The decision of where and when to settle is thus one of substantial import. Prior work has shown that settlement in two shoreline echinoids (a sea urchin and a sand dollar) is unexpectedly sensitive to an environmental feature (intense fluid turbulence) that can be considered as a signal to larvae of their arrival in the neighbourhood of the hydrodynamically energetic habitats in which these taxa live as adults. Here, we used a comparative approach to explore the evolution of turbulence responsiveness in late-stage echinoid larvae. We examined three pairs of closely related sea urchins that differ in the energetic exposure of their adult habitats and found that larval responsiveness to turbulence was more pronounced in urchins that settle in more hydrodynamically exposed locations. These results raise the possibility that evolutionary differences in larval responsiveness to environmental indicators of appropriate adult habitat might reinforce or even provide a mechanism for vicariance in the ocean.

ADDITIONAL KEYWORDS: Caribbean geography – deep-sea evolution – distribution – echinoid physiology – Hawaiian Islands – larval behaviour – metamorphosis – rocky shores – sensory perception – sympatric speciation.

INTRODUCTION

In the *Origin of Species*, Darwin noted that closely related species tend to be similar in form and habitat, and thus may compete intensely. This situation often leads to one of two outcomes: one species outcompetes the other, or one or both species evolve modifications to reduce competition (Darwin, 1869). One route to the latter outcome is 'niche partitioning', which is well documented in taxa such as rift-lake cichlids and Caribbean anole lizards (e.g. Rüber *et al.*, 1999; Losos *et al.*, 2003). Alternatively, competition can be avoided by geographical separation (i.e. allopatry), as famously exemplified by Darwin's finches and their species-specific use of particular Galápagos Islands (Grant, 1999).

In the marine realm there are numerous instances of both scenarios for reducing ecological overlap and competition. For example, niche partitioning operates in young-of-the-year rockfish (family Sebastidae) that recruit into different subtidal habitats within kelp forests (Carr, 1991), whereas allopatry characterizes related species of snails occupying shallow- vs. deep-water habitats (e.g. Welch, 2010).

The biphasic life histories that typify many marine taxa add a layer of complexity to this issue of related species avoiding ecological overlap, because adults and their planktonic larvae often occupy distinct habitats. This feature means that although adults of congeneric oceanic species may be allopatric—with different species specializing, for example, in deep sea, protected bay or rocky-shore habitats—their larvae might nevertheless co-occur during their planktonic period and, in that specific sense, be considered

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'sympatric' for a portion of their ontogeny. If those larvae then survive and successfully recruit back into their respective benthic habitats, they re-establish allopatry or niche partitioning in each generation (e.g. Wellenreuther & Clements, 2008), and thus avoid interspecific competition as adults.

The habitat specificity that maintains geographical separation among biphasic marine taxa would be reinforced if larvae are discriminating as to features of sites they select at the conclusion of their pelagic period. Indeed, there is predicted to be strong selection on the processes by which larvae choose a definitive settlement location (Pechenik, 1999); poor decisions by larvae about where and when to settle would be likely either to be fatal or to result in reduced fitness. It is therefore no surprise that much work has focused on environmental features used by larvae to decide where to settle, from the presence of olfactory cues indicative of a conspecific adult or a required food source, to local flow dynamics suitable for filter feeding, to the texture of a substrate favourable for burrowing or attachment (Crisp, 1974). Furthermore, it is clear that larvae from different species prioritize different cues, as one would predict for larvae searching for habitats tailored to their own needs (Appelbaum *et al.*, 2002; Bierne *et al.*, 2003). Likewise, larvae respond negatively to cues indicative of a poor settlement location (Woodin, 1991), although negative cues have been less explored.

Localized settlement cues, both positive and negative, share the common feature that most are detectable only after a larva arrives close to the benthos. For example, larvae of species whose adults inhabit wave-exposed shores settle in dynamic intertidal locations where breaking waves induce strong water mixing. Beyond a few centimetres from such potential settlement sites, olfactory cues originating at the seabed would be quickly dispersed and diluted (e.g. Denny & Shibata, 1989; Koehl *et al.*, 2007). This situation raises the question as to whether larvae might also exploit information available at larger scales, before reaching the immediate vicinity of benthic habitat, to increase their chances of arriving and settling there (reviewed by Kingsford *et al.*, 2002; Hodin *et al.*, 2018a). Such an ability would not only be selectively advantageous, but also could contribute to the maintenance of geographical separation among species.

In fact, a growing body of literature suggests that larvae do respond to cues at broader spatial scales that represent the 'neighbourhood' of suitable settlement sites. For example, some larvae respond positively to characteristic sounds, such as waves impacting tropical reefs or water flowing over oyster beds (Simpson *et al.*, 2004; Lillis *et al.*, 2013). Wave motions and fluid turbulence also provide neighbourhood-scale information that could be useful to larvae during settlement (Chia *et al.*, 1981; Ebert, 1982; Fuchs *et al.*, 2004, 2010; Gaylord *et al.*, 2013). For example, high-intensity turbulence produced by large breaking waves occurs most prominently in the surf zones of rocky shores, is found reliably in few other locations and affects settlement of larvae that prefer such habitats as adults (Gaylord *et al.*, 2013; Hodin *et al.*, 2015, 2018b, c; Ferner *et al.*, 2019). The strong vertical mixing characteristic of such sites may also interact with larval responses by enhancing the transport of larvae to the substratum in these habitats (Denny & Shibata, 1989), increasing the likelihood that larvae will encounter local, seafloor-associated cues.

The potential importance of neighbourhood-scale information for larval settlement is reinforced by recent findings regarding the reactions of larvae to high-intensity turbulence. A mere 30–180 s of exposure can cause echinoid (sea urchin and sand dollar) larvae to transition immediately from the precompetent state, in which larvae are not yet responsive to local settlement cues, to the competent state, in which they are responsive to such cues and can settle out of the plankton (Gaylord *et al.*, 2013; Hodin *et al.*, 2015, 2018c; Ferner *et al.*, 2019). In this regard, exposure of larvae to intense turbulence, before their arrival at the seafloor, might prime them to be able to respond quickly and efficiently to appropriate seafloor-associated chemical cues once they reach the seabed.

For echinoids, the intensities of turbulence that prompt this shift to competence are comparable to those found under breaking waves (George *et al.*, 1994; Raubenheimer *et al.*, 2004; Gaylord, 2008; Feddersen, 2012; Gaylord *et al.*, 2013; Sutherland & Melville, 2015). Furthermore, Ferner *et al.* (2019) recently reported that this turbulence-induced life-history shift from precompetence to competence is functionally permanent and is accompanied by a behavioural 'knockdown' response, in which larvae remain temporarily on the substratum after exposure to turbulence (see also Hodin *et al.*, 2018c). Together, the competence shift and the knockdown response might increase the likelihood that larvae will both contact settlement cues on the seafloor and be able to react to them appropriately once they have arrived there.

In addition, recently reported genetic variation for turbulence responsiveness in the north-east Pacific sand dollar, *Dendraster excentricus* (Eschscholtz, 1831), suggests that the manner in which echinoid larvae respond to turbulence might be subject to selection (Hodin *et al.*, 2018c). When summed, these findings raise the general hypothesis that larvae from related species whose adults occupy distinct habitats would differ in their responses to neighbourhood-scale indicators of those same habitats, in ways that would

encourage geographical separation of the adults of different species (Hodin *et al.*, 2018a).

Here, we test this hypothesis in three contrasting pairs of closely related echinoid species that live in adult habitats of differing hydrodynamic exposure (Fig. 1).

1. The rock-boring urchin *Echinometra lucunter* (Linnaeus, 1758) (family Echimometridae) is found in the intertidal and shallow subtidal zones of exposed shores in Caribbean and surrounding waters, whereas its apparent sister species (McCartney *et al.*, 2000; see Supporting Information, Supplemental Methods), the reef urchin *Echinometra viridis* (A. Agassiz, 1863), inhabits somewhat deeper and more protected regions (Hendler *et al.*, 1995). This species pair represents a modest contrast of hydrodynamic intensity across habitat.

2. The shingle or helmet urchin, *Colobocentrotus atratus* (Linnaeus, 1758) (family Echimometridae), is an intertidal specialist on highly wave-impacted shores throughout the Indo-Pacific, whereas the red slate pencil urchin *Heterocentrotus mamillatus* (Linnaeus, 1758) is from a sister genus (Kinjo *et al.*, 2004; see Supporting Information, Supplemental Methods) but obligatorily subtidal in a wide range of habitats (Mortensen, 1943; Ogden *et al.*, 1989; J. Hodin, personal observation). This species pair represents an intermediate contrast of hydrodynamic intensity across habitat.

3. The purple urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857) (family Strongylocentrotidae), is an intertidal specialist on exposed shores in the north-eastern Pacific Ocean. In contrast, one of its sister species (Biermann *et al.*, 2003; see Supporting Information, Supplemental Methods), the fragile or pink urchin *Strongylocentrotus fragilis* (Jackson, 1912), is found only in deeper waters, usually well below 50 m (Sato *et al.*, 2017), where wave-driven flows are negligible (Denny, 1988; Gaylord & Denny, 1997). This species pair represents a strong contrast of hydrodynamic intensity across habitat.

Using these three contrasted pairs of urchins, we examine the induction of competence of their late-stage
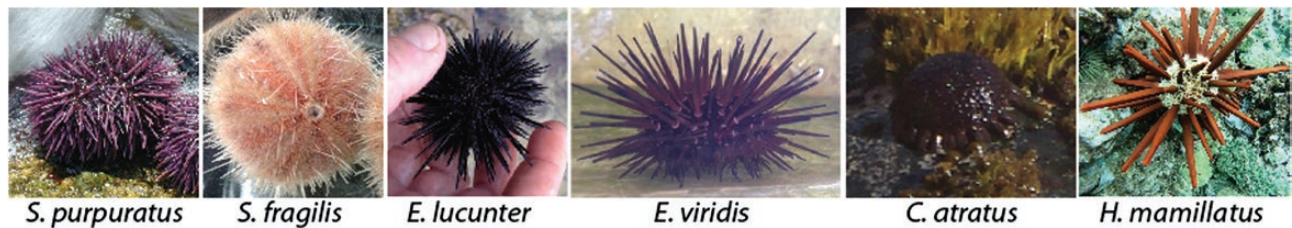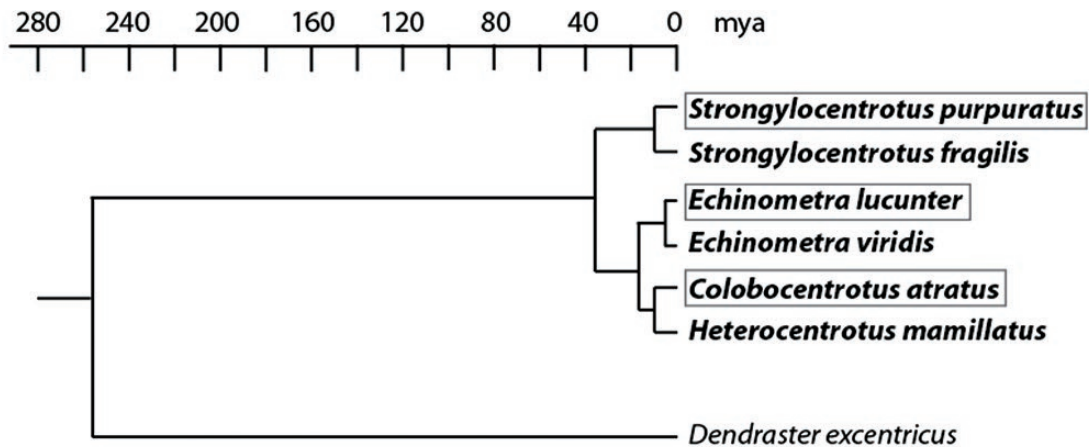
Figure 1. Composite phylogenetic hypothesis for the six study species (bold lettering and pictured), plus a more distantly related echinoid (*Dendraster excentricus*; plain lettering) mentioned throughout the text. Our six study species consist of three pairs of closely related species that differ in energetic exposure of their typical adult habitats: the three species with boxes around their names are from higher-energy adult environments than their unboxed counterparts. Topology and divergence time estimates are adapted from McCartney *et al.* (2000), Biermann *et al.* (2003), Kinjo *et al.* (2004) and Hopkins & Smith (2015). Abbreviation: mya, millions of years ago. All photographs are by J. Hodin, except *Heterocentrotus mamillatus* photograph by Xiwang Clements.

larvae in response to transient exposures to strong turbulence, which can be expected to operate as one of a number of environmentally relevant aspects of hydrodynamic intensity (see, e.g. Fuchs *et al.*, 2015). In particular, we explore whether larvae whose adults live in wave-exposed habitats show increased sensitivity and greater responsiveness to turbulence than related species with adults in more protected habitats. We hypothesize that species pairs that dwell in more highly contrasted habitats will exhibit greater differences in their turbulence responses than species pairs representing less extreme habitat contrasts. We also test the propensity of the larvae of two of the three species pairs to be knocked down (i.e. to remain temporarily on the substratum) after exposure to turbulence, and again predict that exposed-coast species will show an enhancement of this effect relative to their counterparts from less-exposed shores. And finally, we conduct a morphological staging comparison of our six target species to evaluate the hypothesis that taxa that settle in exposed locales exhibit heterochronic advancement in the ontogeny of their attachment structures relative to their sheltered counterparts. Together, these efforts represent a first exploration of whether turbulence might not only serve as an important environmental indicator to larvae of their proximity to suitable habitat, but also whether variation in responsiveness to turbulence might represent a plausible contributor to vicariance and allopatry in the ocean.

MATERIAL AND METHODS

EXPERIMENTAL OVERVIEW, TERMINOLOGY AND GENERATION OF TURBULENCE IN THE LABORATORY

We conducted a series of laboratory experiments to evaluate late-stage echinoid larval responses to turbulence by initially exposing larvae to turbulent water motion and then assessing resultant changes in larval competence (see Introduction for definition) and associated behaviours (Fig. 2). We exposed larvae to turbulence using a Taylor–Couette cell (Taylor, 1923; Karp-Boss *et al.*, 1996; Denny *et al.*, 2002), a standard device used to produce and study both laminar and turbulent flows in the laboratory. This instrument consisted of two concentrically nested cylinders separated by a 3.5 mm gap filled with seawater, into which we introduced larvae by handheld pipette. Relative rotation of the two cylinders sheared the water in the gap between the cylinders, and did so strongly enough to generate turbulent flow. Turbulence generated by the Taylor–Couette cell recreates many of the features of natural turbulence produced beneath breaking waves, where kinetic energy is translated down through ever-smaller eddies to the smallest scales of fluid motion until that turbulent energy is dissipated by viscosity. More intense turbulence results in higher levels of energy dissipation, indexed in watts per kilogram (= $m^2\ s^{-3}$), and a broader energy cascade that sustains eddies of tinier size, smaller than (hence detectable by) marine larvae.

Given that we are interested in larval responses to conditions ranging from calmer waters to those observed on wave-swept rocky coasts, we tested larval responses to a range of turbulence intensities (energy dissipation rates from 0 to > 10 W kg^{-1}). This range extends from quiescent conditions, to dissipation rates measured in surf zones of gently sloping beaches or in the crests of white caps, to exceptional values comparable to some of the largest rates recorded under breaking waves on steep, rocky shores (George *et al.*, 1994; Raubenheimer *et al.*, 2004; Gaylord, 2008; Gaylord *et al.*, 2013; see also Sutherland & Melville, 2015). We recorded larval behaviours after exposure to turbulence and transferred the larvae into seawater containing chemical inducers of settlement to evaluate
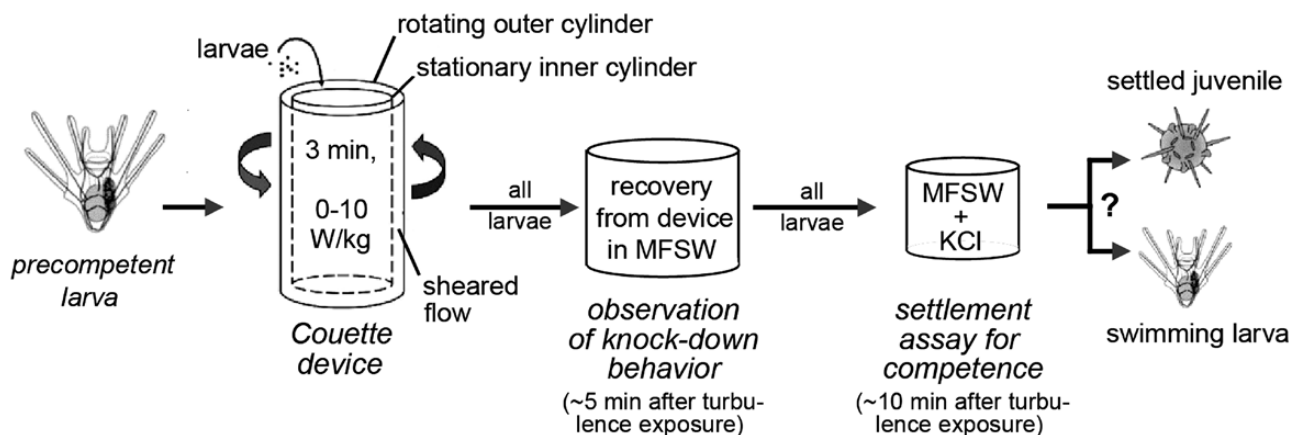
Figure 2. Schematic diagram of our turbulence treatment methodology and knockdown and competence assays. Abbreviation: MFSW, Millipore-filtered sea water.

the extent to which turbulence exposure prompted an early transition of larvae into competence and a subsequent progression to the juvenile stage.

We define settlement as the most dramatic stage of metamorphosis, where the larva makes its irreversible habitat transformation to the benthos (Chia, 1978; Hodin *et al.*, 2018a; for other definitions, see Bishop *et al.*, 2006). This process of settlement, and the associated knockdown behaviour of echinoid larvae contacting the substratum, is what we measured throughout the present study.

SOURCE OF ADULTS FROM OUR CONTRASTING SPECIES PAIRS

Detailed collection locales and other population information for our three contrasted species pairs (and details regarding the larval cohort codes referenced in Table 1) can be found in the Supporting Information (Supplemental Methods). We present a precis of this species information and their collection locales below.

Species contrast 1

We collected *E. viridis* from a protected site and *E. lucunter* from exposed sites in Bocas del Toro Island, Panamá in 2015, and *E. lucunter* from an exposed site in Conch Key, FL, USA in 2016.

Species contrast 2

We collected *C. atratus* adults by hand from an exposed intertidal site on the south shore of Maui, HI, USA, and *H. mamillatus* adults in a nearby subtidal locale (5–10 m depth) by SCUBA, both in 2018.

Species contrast 3

Our *S. purpuratus* adults were collected from two shallow-water locales: a wave-exposed intertidal site in Clallam Bay, WA, USA, with the urchins maintained year-round at Friday Harbor Laboratories (Friday Harbor, WA, USA), and a shallow kelp forest locale by SCUBA near to and maintained year-round at Hopkins Marine Station (HMS; Pacific Grove, CA, USA). Given that the urchins in each of these laboratory colonies are mixtures of urchins collected over many years, we do not know the collection date for the particular *S. purpuratus* urchins used in the present study. Our *S. fragilis* adults came from two deep-water populations: at 120–150 m in Monterey Canyon (CA, USA) in 2016 and at 160 m off Long Beach, CA, USA in 2017.

FERTILIZATION, LARVAL REARING AND STAGING

We undertook spawning, initiation of cultures and larval rearing for each of the species according to standard protocols (Strathmann, 1987): intracoelomic injection of a 0.5 M KCl solution (~0.1 mL per 10 mL of urchin volume); collection of eggs in 0.45 µm Millipore-filtered sea water (MFSW) held at ambient temperature; collection of sperm dry (i.e. with minimal sea water) and stored at 4 °C; and single male × female fertilizations subsequently mixed in equal proportions after making post-hatching density counts, to establish cultures at an approximate initial density of one larva per 1 mL MFSW in glass containers. Once larvae reached the four-arm pluteus stage, we initiated feeding with a mixture of 3000 cells/mL of *Rhodomonas* spp. and 2500 cells/mL of *Dunaliella tertiolecta* Teodor. We exchanged > 95% of the culture water every 2 days by gentle reverse filtration and then fed the larvae as before. We reared larvae in 3 or 1.5 L glass jars with either gentle mechanical stirring or on a gyratory shaker platform. For detailed larval rearing methodologies, see Hodin *et al.* (2019).

During water changes, we examined a few larvae at ×100 magnification to note the initiation of the development of juvenile structures (the invagination of an organ known as the echinus rudiment), at which point we reduced the larval density over the course of two water changes to one larva in 4–5 mL (see Table 1). From that point on, we tracked the progression of rudiment structures regularly, according to the published staging scheme for *S. purpuratus* (Heyland & Hodin, 2014), based upon juvenile structures that appear in a consistent manner within the echinus rudiment. Given that some structures in the rudiment (such as the developing tube feet) might have functional importance for larvae settling in exposed vs. protected habitats, we developed modified staging schemes for the other five study taxa to look for heterochronies or other alterations in the formation of juvenile structures within the rudiments of late-stage larvae. Details on our larval staging methodology can be found in the Supporting Information (Supplemental Methods).

Once larvae reached the stage with adult spines (skeletogenic stage 8 in *S. purpuratus*; Heyland & Hodin, 2014), we began to test a subset to see whether ≥ 10% of the larvae had become competent to settle. Once this threshold was passed, we initiated turbulence exposure experiments as described below. See Table 1 for fertilization and rearing details for each of the larval cohorts used for the present study.

Transport of larvae among experimental locations involved one or two trips, depending on context. Our transportation methods, which used tissue culture flasks, resulted in minimal disturbance. Dates for the transport steps for each larval cohort are listed in Table 1, and details of our transport protocol can be found in the Supporting Information (Supplemental Methods).

Table 1. Experimental design for the 14 larval cohorts used in this study

Larval cohort	Species	Collection date	Fertilization date	Cross design	Rearing temperature (°C)	Larval age (dpf) at:		
						Transport step	Density reduction	Experiments
Sp1	*S. purpuratus*	Unknown*	5 September 2011	3M × 1F	16	1, 26	14	32–33
Sp2	*S. purpuratus*	Unknown*	11 January 2012	2M × 2F	16	8, 21	14	27–28
Sp3	*S. purpuratus*	Unknown*	2 January 20153	1M × 3F	14–16	22	18	30
Sp4	*S. purpuratus*	Unknown*	22 May 2018	3M × 1F	12–14	None	13	33
Sf1	*S. fragilis*	23 February 2016	9 March 2016	2M × 1F	14	1–2, 41	17	49
Sf2	*S. fragilis*	23 February 2016	9 March 2016	2M × 1F	11	1–2, 41	17	55
Sf3	*S. fragilis*	2 March 2017	6 March 2017	2M × 1F	14	0–1	17	49
El1	*E. lucunter*	13 January 2015	13 January 2015	2M × 2F	23–28	None	7	13
El2	*E. lucunter*	25 January 2015	26 January 2015	2M × 2F	25–27	1–2, 9	8	18
El3	*E. lucunter*	4 February 2016	10 February 2016†	1M × 1F	22–25	12	8	18–21
Ev1	*E. viridis*	10 January 2015	10 January 2015	1M × 2F	23–28	None	9	13
Ev2	*E. viridis*	25 January 2015	26 January 2015	2M × 4F	25–27	1–2, 9	8	14–16
Ca1	*C. atratus*	5 March 2018	7 March 2018	1M × 1F‡	24	1	12	49–64
Hm1	*H. mamillatus*	6 March 2018	7 March 2018	1M × 1F‡	24	1	12	38–64

We refer throughout the text to the larval cohort code, as listed in the first column.
Abbreviations: dpf, days postfertilization; F, female; M, male.
*The collection dates for *S. purpuratus* are 'unknown' because these urchins maintained at Friday Harbor Laboratories are a mixture of adults collected over the course of several years, and we do not know the collection dates of particular urchins used in the present study.
†Spawning and fertilization were always on the same day except for El3, in which case on 8 February 2016 we stored sperm at 4 °C and a dilute suspension of eggs in 200 µM sulfamethoxazole + 10 µM trimethoprim in sterile Millipore-filtered sea water (MFSW); eggs stored in this way remain fertilizable for 1 week or longer (Kiyomoto *et al.*, 2014). We rinsed stored eggs three times with MFSW before fertilization.
‡For both *C. atratus* and *H. mamillatus*, we set up and reared three separate M × F fertilizations, using different females and males in each cross.

TURBULENCE EXPOSURES

For each study species, we focused on larvae where most individuals in a cohort were precompetent, because our interest was in the transition from this stage to competence and, in particular, the effects of turbulence in modulating this transition. Note that we distinguish between 'precompetent' individuals vs. even younger 'immature' ones (see Hodin *et al.*, 2015). We exposed batches of precompetent larvae to either 0 W kg^{-1} control conditions (see below) or one of a range of turbulence intensities, corresponding to energy dissipation rates from 0.3 to 10 W kg^{-1} in the Taylor–Couette device. For the within-species comparison in *S. purpuratus* (see below), we tested turbulence intensities ≤ 13 W kg^{-1}. We selected the upper values (5 W kg^{-1} and above) based on peak intensities of turbulence that have been measured in the field on exposed rocky shores, and which also elicited maximal responses in our previous studies (Gaylord, 2008; Gaylord *et al.*, 2013; Hodin *et al.*, 2015). These intensities are substantially in excess of those used in most laboratory examinations of larval responses (e.g. Fuchs *et al.*, 2013; Wheeler *et al.*, 2016), but are relevant to our study species (especially *S. purpuratus* and *C. atratus*) with respect to the conditions they might experience in their approach to their wave-impacted shoreline habitats.

On the day of experimental trials, we first concentrated larvae by gentle reverse filtration and selected 15–45 larvae into individual 125 mL glass beakers at a density of one larva per 3–4 mL MFSW. Although most of the larvae that we reared developed synchronously to competence, some batches of larvae developed asynchronously. For more asynchronous batches, we imposed a more stringent selection for larvae that appeared to be nearing competence but were not adhered to the substratum (for our selection criteria to enrich for precompetent larvae in *S. purpuratus*, see Gaylord *et al.*, 2013). Otherwise, we selected larvae haphazardly for assignment to beakers. Then, we randomly assigned beakers to treatments and replicates and began the exposure trials.

For each exposure trial (= replicate), we gently poured the entire contents of a 125 mL beaker into a finger bowl and used a glass Pasteur pipette to introduce all of the larvae into 150 mL of MFSW within the Taylor–Couette device (see Fig. 2). We then subjected the entire water volume within the apparatus to the specified intensity of turbulence for a duration of 3 min. Immediately after each trial, we gently poured the larvae within the Couette device into a 1 L glass beaker already containing ~100 mL of MSFW (to minimize additional stimuli to larvae during the pour), rinsed the Taylor–Couette device one or two times with MFSW of the appropriate temperature to capture any remaining larvae, concentrated the entire recovered volume back down to ~100 mL by gentle reverse filtration, and poured this remaining volume into a small glass bowl for further observations and subsequent transfer directly into settlement assay conditions, as described below. We generally recovered > 95% of the larvae from the Taylor–Couette device, all of which we used in ensuing settlement assays. We then rinsed the Taylor–Couette device thoroughly with distilled water to ensure that no living larvae were transferred to subsequent trials, and we initiated the next trial.

We used two types of controls: handling controls and unmanipulated controls. For handling controls (0 W kg^{-1}), we treated the larvae in the same way as those assigned to the turbulence exposure treatments, except that we did not activate the Taylor–Couette device during the 3 min that larvae were within it, thereby controlling for manipulation effects. For each unmanipulated control trial, we transferred larvae directly from their 125 mL holding beaker into a small glass bowl for behavioural observations, and then into their settlement containers, thus skipping all manipulations involving the Taylor–Couette device. For the species tested here, we used only unmanipulated controls once we had verified, as in our previous studies (Gaylord *et al.*, 2013; Hodin *et al.*, 2015), that the unmanipulated control-treated larvae did not differ in their settlement responses from handling controls (data not shown).

KNOCKDOWN ASSAY

Some echinoid larvae (e.g. *D. excentricus* and *S. purpuratus*) will sink to the bottom and remain there after exposure to intense turbulence (Hodin *et al.*, 2018b, c; Ferner *et al.*, 2019). This 'knockdown effect' reverses in ~30 min if no settlement inducer is provided, and the larvae then resume normal swimming (Ferner *et al.*, 2019). Here, we explored this effect in several of the focal taxa of the present study.

After retrieval of *S. purpuratus*, *S. fragilis*, *C. atratus* and *H. mamillatus* larvae from the Taylor–Couette device, and before transferring them into settlement conditions, we recorded the numbers of larvae swimming vs. those in contact with the bottom of the recovery bowl (see Fig. 2). We used these counts to calculate the proportion of larvae knocked down by each trial to look for any interspecific differences. Unfortunately, we do not have this information for the two *Echinometra* species, because we were not yet documenting the knockdown effect at the time of those experiments (2015–2016).

COMPETENCE/SETTLEMENT ASSAY

We assessed competence of the larvae using a standard approach of exposing larvae to elevated

potassium in seawater, which causes competent larvae to settle (Cameron *et al.*, 1989; Carpizo-Ituarte *et al.*, 2002; Amador-Cano *et al.*, 2006; Sutherby *et al.*, 2012; Gaylord *et al.*, 2013; Hodin *et al.*, 2015, 2018b, 2019). To do so, we transferred all larvae from a trial into a single well of a prewashed, non-tissue culture-treated six-well plate (see Herrmann *et al.*, 2003), maintained at the rearing temperature and containing 8 mL of MFSW with excess potassium (i.e. excess KCl in MFSW; see Fig. 2).

After a 1 h exposure to the species-specific excess potassium concentration (reported by Hodin *et al.*, 2019; see also Supporting Information, Supplemental Methods), we transferred all larvae to 8 mL of MFSW for recovery. At the time of transfer, we scored a larva as settled if tissue had begun to withdraw from the tips of the larval skeletal rods, according to standard assays (e.g. Sato *et al.*, 2006; Sutherby *et al.*, 2012; Gaylord *et al.*, 2013; Hodin *et al.*, 2015, 2019; Mos & Dworjanyn, 2016). In some of the tested species (*S. fragilis* was the most extreme example), it took a few hours in recovery for larvae to begin to show skin withdrawal from larval arms and other signs of settlement. Therefore, in all species, we verified continued withdrawal of tissue over several hours and eventual adoption of the definitive juvenile morphology, including emergent and active tube feet and spines. Thus, our recorded settlement data is from 15–24 h after exposure to excess potassium. Larvae from all treatments that we had scored as not settled (i.e. that had not transitioned from precompetence to competence as a result of the turbulence exposure) had resumed swimming, and we detected no postsettlement mortality in the 15–24 h after exposure.

We verified for four of the species (*S. purpuratus*, *C. atratus*, *E. viridis* and *E. lucunter*) that the concentrations of KCl used were approximately as effective as a natural cue (see Supporting Information, Fig. S1). The natural cues we used were as follows: live fronds of the coralline algae *Calliarthron tuberculosum* (Postels & Ruprecht) E.Y.Dawson for *S. purpuratus* (see Gaylord *et al.*, 2013); live fronds of what we believe to be the red intertidal turf alga *Melanamansia glomerata* (C.Agardh) R.E.Norris for *C. atratus*; and, for the two *Echinometra* species, pulverized small intertidal rocks with live biofilm collected from nearshore regions in Panamá where the two species co-occurred.

IDENTIFYING COMPARABLE BATCHES OF LARVAE FOR INTERSPECIFIC COMPARISONS

A challenge in the experiments reported herein was to ensure that we were comparing larvae from different species at a comparable developmental stage relative to competence, which in *S. purpuratus* is not correlated strongly with any of the rudiment stages described above (J. Hodin, unpublished data). Based on prior experiments (Hodin *et al.*, 2015) indicating that the response of an echinoid larva to turbulence can change substantially in the days approaching competence, we used the proportion of larvae settled in 0 W kg^{-1} controls as our baseline for comparison (see also Hodin *et al.*, 2018b). In other words, if ~25% of the control (0 W kg^{-1}) larvae in 'species A' settled on a certain day after fertilization, we sought to compare those larvae with 'species B' on whatever day these latter larvae were also at ~25% settlement in their controls. Specifically, we considered batches of tested larvae (both within and between species) to be comparable only if they exhibited no clear evidence for any difference in their proportion settled in the controls ($P > 0.25$; see Underwood, 1997). We used this approach for structuring both the competence and the knockdown analyses.

STATISTICS

We conducted all statistical analyses with R v.3.5.2 (R Core Team, 2017) using the lme4 and emmeans packages (Bates *et al.*, 2015; Lenth, 2018). We used either a quadratic or a logistic mixed-effects model to analyse data owing to the binomial nature of our response variable (larvae settled or knocked down, or not). In all cases, we treated each exposure of a group of 15–40 larvae as a random intercept. We began by using a quadratic model for each set of data, given the robust quadratic response that we previously modelled for the turbulence–settlement responses in the sand dollar, *D. excentricus* (Hodin *et al.*, 2015). However, in every comparison that we present here, the Akaike information criterion (AIC) score (Akaike, 1978) was lower for the logistic model (data not shown); therefore, we report our analyses here only using the logistic model. For all of the settlement data, and for the knockdown data in the Hawai'ian urchins, we treated turbulence intensity (in watts per kilogram) as a continuous variable. For each interspecific comparison, we determined whether the response for each species (i.e. the slope) over a range of turbulence intensities was significantly positive or negative, and whether there was any interaction between the slopes in the two species.

For the one intraspecific ontogenetic comparison (the responses of early precompetent vs. late precompetent larvae in *S. purpuratus*), we took a slightly different approach. Early precompetent larvae have a lower background settlement rate than late precompetent larvae (see Hodin *et al.*, 2018b); therefore, the approach we used for the interspecies comparisons of only comparing batches of larvae with comparable settlement proportions in controls would not apply.

Furthermore, we were not asking here whether the slopes of the responses differ, but instead whether larvae change in their sensitivity to turbulence as they age. Therefore, for this analysis we compared the inflection points of the dose responses at the two stages to determine whether they were statistically distinguishable.

To do this, we compared the AIC scores of a quadratic and a logistic mixed-effects model, with developmental stage as a variable and again treating each exposure of a group of 15–40 larvae as a random intercept; the AIC score for the logistic model was lower (262.5 vs. 263.6). We estimated the approximate inflection points of the logarithmic responses for each of the two stages (early and late) by identifying the values of the independent variable for each of the two curves corresponding to the greatest slope. To determine whether the inflection point estimate for the early precompetent stage was statistically greater than that for the late precompetent stage, we ran a non-parametric bootstrap algorithm (using the boot.ci function in R) to generate 10 000 estimates for the inflection points for each of the two stages in addition to the difference between the inflection points of the two stages. This bootstrap procedure sampled with replacement from the dataset of each developmental stage (early or late precompetent) separately, and from each sample for the two stages in order to obtain the inflection point estimates. The reported P-value of the comparison is based on the proportion of the 10 000 difference estimates (i.e. inflection point for early precompetent minus the inflection point for late precompetent) that were zero or less. We also used the 10 000 estimates for the inflection points to give 95% confidence intervals (CIs) of the individual inflection point estimates that we report for the two stages. Note that in order for the models to converge in this analysis (unlike the interspecific comparisons), we ran the mixed-effects models using rotation rate (rotations per minute) of the Taylor–Couette device as the independent variable. Therefore, our initial inflection point estimates were in units of rotation rate, which we then reported as watts per kilogram using the conversion equations provided by Gaylord *et al.* (2013).

For the two *Stongylocentrotus* species, we tested the knockdown responses at only two turbulence intensities: 0 and 7.2 W kg^{-1}. Here, we used a categorical logistic regression, scoring knockdown behaviour as a binomial response, with the turbulence intensity as a categorical variable. We also included replicates as a random intercept to account for the multiple larvae within each exposure trial (replicate).

For all data, we assessed normality using Shapiro tests and q–q plots, and visually inspected residuals plotted against predicted values to check for heteroscedasticity.

RESULTS

SPECIES CONTRAST 1: *E. LUCUNTER* AND *E. VIRIDIS*

Larvae of protected-shore *E. viridis* (larval batch Ev2; Table 1) and of exposed-shore *E. lucunter* (El2) from Panamá at the early precompetent stage (~10% settlement in controls; Fig. 3) showed a modest increase in competence induction with increasing turbulence. For *E. viridis* at 14 days postfertilization (dpf), the slope was 0.12 (± 0.04 SEM; $Z = 3.082$; $P = 0.002$) log odds of settling per unit increase of watts per kilogram; for *E. lucunter* at 18 dpf, the slope was 0.13 (± 0.03; $Z = 3.160$; $P < 0.002$). Comparing these slopes revealed no clear differences in the response ($Z = 1.379$; $P = 0.17$).

More advanced precompetent *E. viridis* larvae (40% settlement in controls; 16 dpf; batch Ev2) continued to show increased settlement (competence induction) in response to turbulence (0.06 ± 0.03 log odds of settling; $Z = 2.367$; $P < 0.02$). A comparison between the slopes of the responses in *E. viridis* at 14 and 16 dpf revealed no clear differences ($Z = -1.725$; $P = 0.085$). We were unable to compare the more advanced *E. viridis* with comparably staged *E. lucunter* owing to insufficient numbers of larvae.
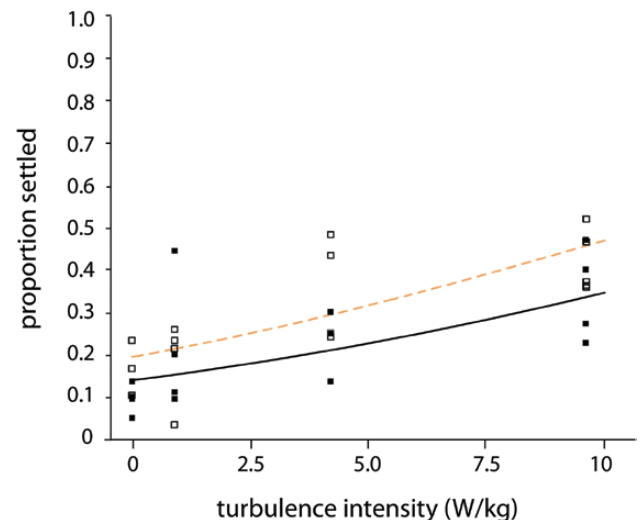
Figure 3. Induction of competence as judged by settlement response over a range of turbulence intensities in early precompetent larvae of two *Echinometra* species from Panamá. Each square is a replicate turbulence exposure of 15–30 larvae. Open squares and dashed brown line indicate *Echinometra viridis*. Filled squares and continuous black line indicate *Echinometra lucunter*. Both species showed a positive response to turbulence ($P < 0.005$), but their responses did not differ from one another statistically ($P = 0.17$; see main text). The 0 W kg^{-1} treatments shown here are unmanipulated controls (see Material and Methods section for details).

We detected differences in the turbulence responses of *E. lucunter* larvae from different populations (Panamá and Florida; larval batches El2 and El3, respectively, in Table 1), with the Panamá larvae (El2 at 18 dpf; see above) displaying a significantly more robust settlement response to turbulence ($Z = -2.057$; $P < 0.04$) than did the Florida larvae (El3 at 18–21 dpf; 0.03 ± 0.02 log odds of settling; $Z = 1.485$; $P = 0.14$). This apparent difference between populations is reminiscent of similar findings with larvae of the Pacific sand dollar, *D. excentricus* (Hodin *et al.*, 2018c).

Our staging analyses for *E. lucunter* and *E. viridis* revealed modest differences in the relative timing of appearance of juvenile structures in both species relative to *S. purpuratus* (see Supporting Information, Table S1), and the two species also differed from one another. Specifically, relative to formation and elongation of the adult spines, ontogeny of the tube foot skeletal end plates (see Supporting Information, Fig. S2) appeared to proceed more quickly in *E. lucunter* when compared with *E. viridis* (see Supporting Information, Fig. S3).

### SPECIES CONTRAST 2: *C. ATRATUS* AND *H. MAMILLATUS*

We detected stage-specific differences in the induction of competence by turbulence when comparing the intertidal *C. atratus* with the subtidal *H. mamillatus*. At the early precompetent stage (~10% settlement in controls), *H. mamillatus* larvae (39 and 41 dpf in different crosses; see Table 1) showed a modest increase in competence induction with increasing turbulence: the slope ($\pm$ SEM) was 0.16 ($\pm$ 0.04; $Z = 4.332$; $P < 0.001$) log odds of settling per unit increase of watts per kilogram (Fig. 4A). At this developmental stage, the response to turbulence in *C. atratus* larvae (49 dpf) was not significantly positive by standard criteria (0.08 ± 0.06 log odds of settling; $Z = 1.255$; $P = 0.2$; Fig. 4A). Nevertheless, we detected no clear difference in slope between the two species at the early precompetent stage ($Z = 1.218$; $P = 0.22$).

In contrast, at the late precompetent stage (64 dpf), the two species showed differing responses in their competence induction by turbulence ($Z = -2.117$; $P = 0.034$; Fig. 4B). Late precompetent *H. mamillatus* larvae showed no consistent induction of competence with increasing turbulence, with a slope of -0.02 ± 0.05 log odds of settling ($Z = -0.373$; $P = 0.7$), whereas late precompetent *C. atratus* larvae showed a robustly positive response, with a slope of 0.12 ± 0.04 log odds of settling ($Z = 2.764$; $P = 0.006$).

Both species took longer than expected to reach these precompetent stages, a trend that probsbly arose for two main reasons: (1) we reared these larvae at a relatively low temperature to limit bacterial infections; and (2) we noticed substantial bouts of larval cloning (see McDonald & Vaughn, 2010) in both species, which rendered the cultures less synchronous and delayed.

At the early precompetent stage, ~80% of the control (0 W kg⁻¹) *C. atratus* larvae were on the bottom of the recovery bowl, which allowed insufficient scope to detect any additional 'knockdown' behaviour attributable to turbulence. In comparison, only ~50%
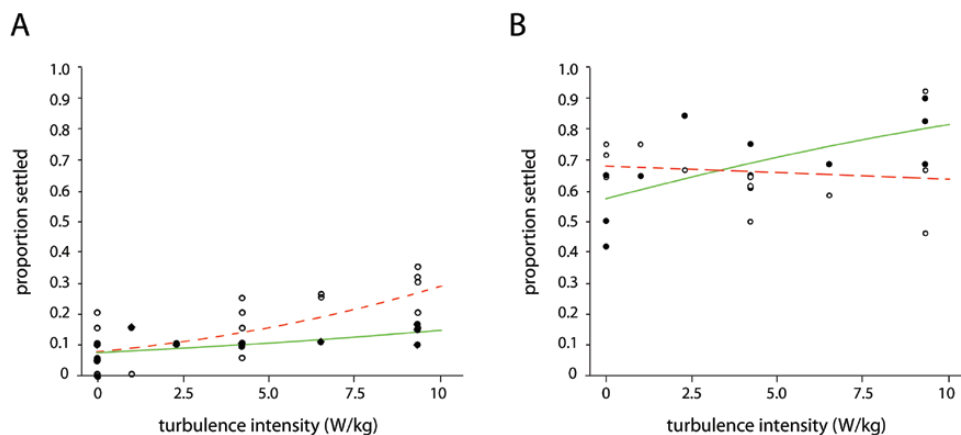
Figure 4. Induction of competence over a range of turbulence intensities in larvae of two closely related urchins from Hawai'i: *Heterocentrotus mamillatus* (open circles and dashed red line) and *Colobocentrotus atratus* (filled circles and continuous green line). Each circle is a replicate turbulence exposure of 13–21 larvae. A, early precompetent larvae. Although only *H. mamillatus* showed a significant positive response to increasing turbulence ($P < 0.001$), the responses of the two species did not differ statistically ($P = 0.22$; see main text). B, late precompetent larvae. Here the situation was reversed, with only *C. atratus* exhibiting a positive settlement response to turbulence ($P = 0.006$), but in this case the responses (slopes) in the two species differed statistically ($P = 0.034$; see main text). The 0 W kg⁻¹ treatments shown here are handling controls (see Material and Methods section for details).

of control *H. mamillatus* larvae were on the bottom at the early precompetent stage (Fig. 5A). At the late precompetent stage, the situation was reversed, with > 90% of *H. mamillatus* control larvae on the bottom vs. ~45% for *C. atratus* (Fig. 5B). This situation prevented explicit comparison of the responses of the two species. Nevertheless, both species showed a positive knockdown response to turbulence. In *H. mamillatus* early precompetent larvae, the slope (± SEM) of the response was 0.28 (± 0.04; $Z = 7.591$; $P < 0.001$) log odds of being on the bottom per unit increase in watts per kilogram (Fig. 5A). For late precompetent larvae of *C. atratus*, the slope of the response was 0.18 ± 0.04 log odds of being on the bottom ($Z = 4.210$; $P < 0.001$; Fig. 5B).

As in the two *Echinometra* species, we again noted differences in the relative timing of appearance of juvenile structures in both Hawai'ian species relative to *S. purpuratus* (see Supporting Information, Table S1), and the two Hawai'ian species also differed from one another. In this case, *C. atratus* larvae, whose larvae settle in higher-energy locales, exhibited an earlier relative appearance of tube foot end plate skeletal structures ('rings') when compared with *H. mamillatus* larvae, a difference that persisted throughout the remainder of larval development (see Supporting Information, Figs 2H, K, L, 3).

### SPECIES CONTRAST 3: *S. PURPURATUS* AND *S. FRAGILIS*

We observed substantial differences in the manner in which late precompetent larvae of the intertidal *S. purpuratus* responded to turbulence when compared with late precompetent larvae of the deep-sea *S. fragilis*. This late precompetent stage (~55% settlement in the controls; see Fig. 6A) was reached at 27–28 dpf in larval batch Sp1, 32–33 dpf in Sp2, 49 dpf in the 14 °C-reared batch Sf1 and 55 dpf in the 11 °C-reared batch Sf2 (see Table 1). As observed in our previous studies, *S. purpuratus* showed a robust settlement response (competence induction) to increasing turbulence: the slope (± SEM) of the response was 0.22 (± 0.06; $Z = 3.887$; $P < 0.001$) log odds of settling per unit increase of watts per kilogram (Fig. 6A). In contrast, *S. fragilis* exhibited no obvious settlement response to increasing turbulence, with a slope of 0.06 ± 0.04 log odds of settling ($Z = 1.458$; $P = 0.15$). There was a clear difference between the slopes of the responses for the two species ($Z = 2.419$; $P = 0.016$).

For *S. purpuratus*, we detected differential sensitivity to turbulence in its induction of competence in early (~10% settlement in the controls) vs. late precompetent larvae (~55% settlement in the controls; see above), observed across a range of turbulence intensities (Fig. 6B). From the logarithmic responses shown in Fig. 6B, we estimated the mean respective inflection points (and 95% CIs based on 10 000 bootstrap replicates) as a proxy for the threshold of the turbulence response at each stage. For early precompetent larvae, this threshold energy dissipation rate was 4.2 W kg^{-1} (95% CI, 3.3 to 6.2 W kg^{-1}). For the late precompetent larvae, the inflection point was a negative value: –0.05 W kg^{-1} (95% CI, –0.5 to 0 W kg^{-1}). Although such negative energy dissipation rates were not in themselves meaningful, what this analysis revealed was that the logarithmic function
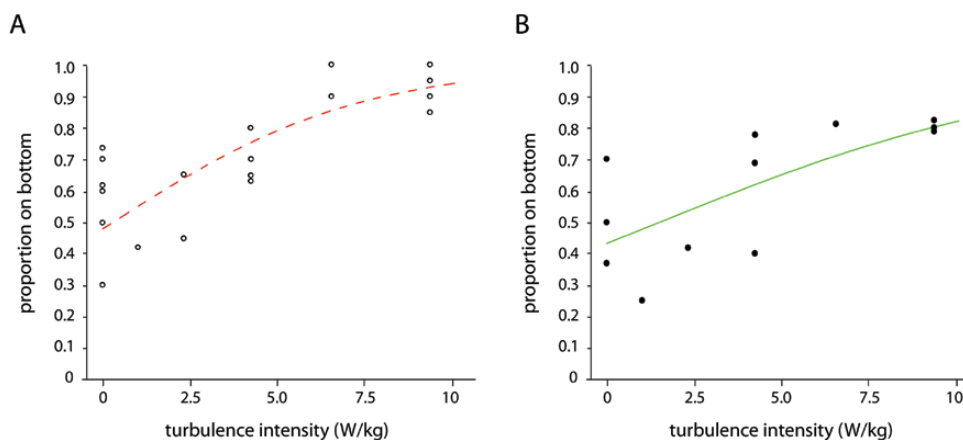
Figure 5. Knockdown response of early precompetent *Heterocentrotus mamillatus* larvae (A) and late precompetent *Colobocentrotus atratus* larvae (B). Lines and symbols are as in Figure 3. Both species showed a positive knockdown response to increasing turbulence ($P < 0.001$; see main text), but, as explained in the main text, we were unable to compare responses between the two species explicitly, because their larvae were at different stages. Each circle represents a replicate exposure of 18–20 larvae. The 0 W kg^{-1} treatments shown here are handling controls (see Material and Methods section for details).
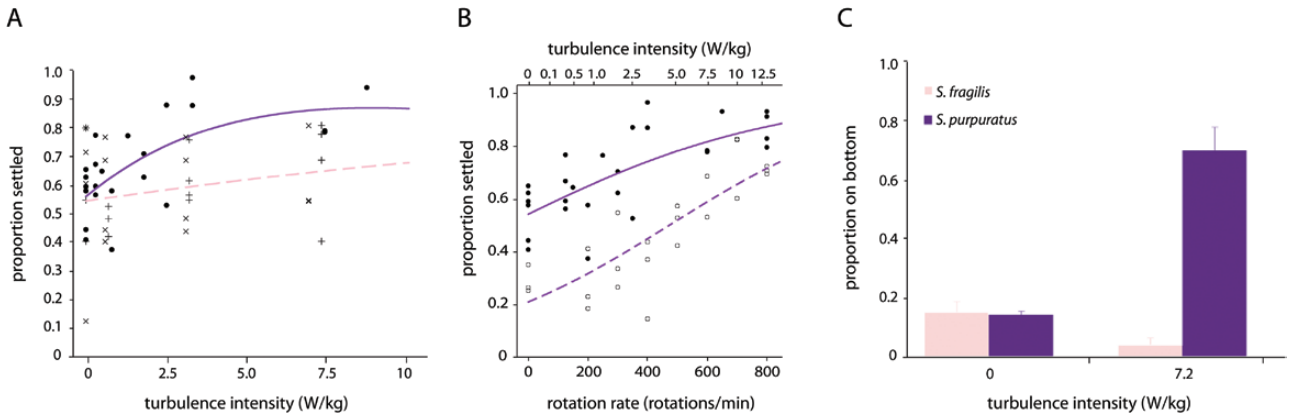
Figure 6. Induction of competence (A, B) and knockdown response (C) over a range of turbulence intensities in larvae of two *Strongylocentrotus* species from the north-east Pacific. A, B, each symbol is a replicate turbulence exposure of 15–40 larvae. A, induction of competence by turbulence in late precompetent larvae of *Strongylocentrotus purpuratus* (filled circles and continuous purple line) and *Strongylocentrotus fragilis* (two styles of crosses and pink dashed line). *Strongylocentrotus fragilis* larvae are from two separate batches (see Table 1) whose responses did not differ: **+** symbols are from batch Sf1; × symbols are from batch Sf2. Only *S. purpuratus* larvae showed a positive settlement response to increasing turbulence ($P < 0.001$), which was statistically different from the response in *S. fragilis* ($P = 0.016$; see main text). The 0 W kg^{-1} treatments shown here are unmanipulated controls in *S. fragilis* and handling controls in *S. purpuratus* (see Material and Methods section for details). B, comparison of the positive turbulence–settlement response of *S. purpuratus* larvae at two different ontogenetic stages: early precompetent (open circles and dashed line) and late precompetent (filled circles and continuous line). As explained in the Material and Methods section, we modelled these responses using the rotation rate of the Taylor–Couette cell (lower *x*-axis); corresponding watts per kilogram values are shown on the upper *x*-axis (note that the upper axis is non-linear, and that the 0 W kg^{-1} treatments shown here are handling controls). The inflection points for the responses at the two stages are statistically different from one another ($P < 0.001$), suggesting that the more advanced larvae were more sensitive to turbulence (see main text). C, comparison of the knockdown response in *S. purpuratus* (purple bars) and *S. fragilis* (pink bars) in unmanipulated control (0 W kg^{-1}) and turbulence-exposed (7.2 W kg^{-1}) larvae. The fivefold increase in larvae on the bottom for *S. purpuratus* after turbulence exposure was significant ($P < 0.001$), as was the corresponding threefold decrease in *S. fragilis* larvae ($P = 0.03$). The responses in the two species also differed from one another ($P < 0.001$; see main text). Each bar represents three replicate exposures of 15–25 larvae.

for the late precompetent larvae was already falling at the lowest turbulence intensities that we tested (0.3 W kg^{-1}), whereas the corresponding logarithmic response started falling in early competent larvae only above ~3.3 W kg^{-1}. In sum, the more advanced *S. purpuratus* larvae were propelled to competence at a lower threshold energy dissipation rate when compared with the less advanced larvae ($P < 0.001$). These findings of an ontogenetic shift in turbulence sensitivity in *S. purpuratus* represent an additional example of a pattern we previously reported for the Pacific sand dollar, *D. excentricus* (Hodin *et al.*, 2015).

Late precompetent *S. purpuratus* larvae (larval batch Sp4) showed a strong knockdown response to turbulence: five times as many larvae were on the bottom after recovery from the turbulence exposure than in controls (Fig. 6C; $Z = 5.353$; $P < 0.001$). Late precompetent *S. fragilis* larvae (Sf3) showed the opposite effect: there were one-third as many larvae on the bottom in the turbulence-exposed treatments when compared with the controls (Fig. 6C; $Z = -2.171$; $P = 0.03$). In other words, the turbulence treatment

caused *S. fragilis* larvae to swim rather than sink. There was a clear difference between these responses in the two species ($Z = 4.948$; $P < 0.001$). Note that for each species in this analysis, we used only a single high-intensity treatment of 7.2 W kg^{-1} in comparison with untreated larvae (0 W kg^{-1}).

We documented a number of differences in the relative timing of the appearance of juvenile structures in the two species (see Supporting Information, Table S1). Once again, the contrasted species whose larvae settle in higher energy locales (in this case, *S. purpuratus* relative to *S. fragilis*) exhibited more rapid development of tube foot skeletal end plates (rings; Supporting Information, Figs S2I, S3).

DISCUSSION

Our results indicate that intense fluid turbulence (a neighbourhood-scale environmental feature of nearshore oceanic habitat) has species-specific effects on traits tied to a key life-history transition in sea

urchin larvae. Pairs of related sea urchin species (Fig. 1) that differ in their adult habitat characteristics exhibit different larval responses to turbulence. Specifically, species whose adults dwell in wave-swept, shallow locales respond to turbulence by sinking to the substratum and transitioning to competence, the stage in which they are ready to settle into the benthos, whereas their sister taxa from deeper or more protected habitats show less dramatic or no such responses.

Furthermore, the turbulence responses parallel the magnitude of the individual habitat contrasts. The pair of *Echinometra* species, which is the pair with the least extreme contrast in adult habitat, show no consistent differences in their larval response to turbulence. The two Hawai'ian species (from the sister genera *Colobocentrotus* and *Heterocentrotus*) have moderately contrasting adult habitats, and we see differing larval responses between the species in their induction of competence (in the predicted direction), but no obvious differences in sinking behaviour. Finally, the two north-east Pacific *Strongylocentrotus* species represent the greatest habitat contrast, and here only the species whose adults dwell in high-energy intertidal locales, *S. purpuratus*, exhibit either an induction of competence or a sinking response. Indeed, our evidence suggests that exposure to intense turbulence induces larvae of the deep-sea species, *S. fragilis*, to swim in greater numbers rather than sink, indicating that exposure to turbulence might even be a deterrent to settlement in this species. In each species pair, our staging analysis also uncovers a morphological correlate of the habitat contrast: the higher-energy-adapted species in each pair shows enhanced relative development of the tube foot skeletal end plates, structures that may be important in maintaining adhesion under flow (Santos & Flammang, 2006).

In sum, our results suggest that larvae seeking a settlement habitat on the shore could cue in on turbulence as an indicator of their shoreline approach, whereas deeper-dwelling species show less or no response in this way, in line with their less turbulent settlement locales. These findings suggest that differences in habitat between incipient or closely related species could be reinforced or even induced by the differing responses of their late-stage larvae to neighbourhood-scale environmental features, such as turbulence. This scenario raises the possibility of a previously unrecognized potential mechanism for evolution by vicariance in the ocean, which we discuss below.

BACK IN THE OLD NEIGHBOURHOOD: LARVAL RESPONSES ACROSS SCALES

The typical marine life cycle in taxa as diverse as animals and kelp involves benthic adults with limited or no movement that release propagules that disperse for a time in the plankton and then re-enter the benthos in a process called settlement. In some cases, such propagules have limited swimming ability and thus are at the mercy of prevailing flows (see e.g. Gaylord *et al.*, 2002, 2006; Montgomery *et al.*, 2018), whereas in other cases plankton exhibit clear behavioural mechanisms that enhance the probability of successful settlement into appropriate habitats (reviewed by Pineda & Reyns, 2018). For example, in the upwelling systems that characterize some continental margins, if a larva rises to the surface it will tend to be carried offshore, whereas if it sinks it will be preferentially carried back onto the continental shelf. Therefore, by controlling its vertical position in the water column, that larva could remain close to shore throughout its larval period, or return there near its completion, thus increasing chances for successful settlement (see Morgan *et al.*, 2009; for review, see Pineda & Reyns, 2018). In such cases, larval swimming behaviours can have consequences for larval dispersal and, ultimately, for successful settlement across 'macro scales' of many kilometres from their ultimate settlement site (Hodin *et al.*, 2018a).

Once a larva is near to shore and approaching competence to complete metamorphosis and settle back into the intertidal zone, that larva can respond to intermediate scale features characteristic of the neighbourhood of the nearshore. Fluid turbulence is one such feature that has been particularly well studied. Turbulence increases as waves overturn in their approach to the shoreline, and our results here and in previous work (Gaylord *et al.*, 2013; Hodin *et al.*, 2015, 2018b, c) indicate that late-stage echinoid larvae from shoreline-dwelling adults exhibit two complementary responses to brief pulses of intense turbulence. The first response carries the larvae to the substratum: they remain there, even in the absence of a settlement inducer, for ~30 min on average, before they resume swimming (Ferner *et al.*, 2019). This behavioural response is consistent with observations in molluscs of downward larval swimming in response to turbulence (Fuchs *et al.*, 2004, 2015; Wheeler *et al.*, 2015) and with modelling studies indicating that strong mixing associated with turbulence can carry larvae rapidly to the bottom from some distance above (Denny & Shibata, 1989). The second larval response to turbulence is a different one both phenomenologically and temporally: echinoid larvae that are not yet competent to settle (i.e. are 'precompetent') suddenly and permanently (Ferner *et al.*, 2019) transition to competence in response to intense fluid turbulence.

The precocious induction of competence at the 'neighbourhood scale' of metres to a kilometre from a suitable settlement site is synergistic with the behavioural and other mechanisms by which turbulence

directs larvae to the substratum (Hodin *et al.*, 2018a). Furthermore, larval contact with the substratum means potential contact with local 'larval-scale' benthic cues, be they chemical or physical, that are known to trigger settlement in competent larvae (for reviews, see Crisp, 1974; Pawlik, 1992; Hadfield & Paul, 2001; Koehl, 2007; Hadfield, 2011; Hodin *et al.*, 2018a).

Taken together, an emerging view of larval life histories is that larvae can be masters of their fate not only by swimming vertically between water masses associated with kilometre-scale transport, and by testing seafloor-associated chemical cues over dimensions of millimetres, but also by responding to a variety of neighbourhood-scale processes. Indeed, the last of these might represent a particularly crucial juncture, where the response of a larva could mean the difference between finding a suitable settlement site on the shore or being carried back out to sea. The further possibility that neighbourhood-scale cues might be evaluated differently by larvae seeking different settlement habitats remains largely unexplored (but see Fuchs *et al.*, 2018), and never previously through the use of an explicitly comparative methodology (*sensu* Felsenstein, 1985).

DIFFERENCES IN TURBULENCE EFFECTS MIRROR THE MAGNITUDE OF HABITAT CONTRASTS

Of the three species pairs we examined, the pair with the least extreme contrast in their adult habitats is the *Echinometra* species pair, and our results likewise suggest that their respective responses to turbulence do not differ notably. Although *E. viridis* is generally described in the literature as a species that tends to inhabit deeper and more protected waters than *E. lucunter* (e.g. Hendler *et al.*, 1995), *E. viridis* is reported to co-occur with *E. lucunter* on outer patch reefs in the Florida Keys (McPherson, 1969), and there is a single report of them co-occurring in the nearshore off Molasses Key (Kier & Grant, 1965). Although we did not visit Molasses Key, our observations elsewhere in the Florida Keys were similar to those of McPherson (1969); we did not encounter *E. viridis* in the nearshore in any of several sites where we found *E. lucunter*. In contrast, in and around Bocas del Toro island, Panamá, we unexpectedly found numerous *E. viridis* alongside *E. lucunter* in every nearshore location in which we found the latter. We also found *E. viridis*, but not *E. lucunter*, on the protected side of Bocas del Toro island in shallow reefs among mangroves, as expected.

In sum, *E. viridis* seems to be somewhat generalist in its distribution, from exposed shores, to patch reefs, to deeper and calmer waters. *Echinometra lucunter* is more specialized to the exposed nearshore and is also found in patch reefs off the Florida Keys. Based on measurements of surf-zone turbulence in a variety

of habitats, we would expect both of these species commonly to encounter energy dissipation rates up to $\sim 10^{-2}$ W kg^{-1} within their more exposed adult habitats (George *et al.*, 1994; Feddersen, 2012; Gaylord *et al.*, 2013; Sutherland & Melville, 2015). In contrast, the subtidal, protected lagoon location in Panamá from which we collected *E. viridis* for the present study would be expected typically to experience three or more orders of magnitude lower mean energy dissipation rates (see, e.g. Stocking *et al.*, 2016).

The Hawai'ian species represent a greater adult habitat contrast than our *Echinometra* pair, and they also exhibit differences in their respective turbulence responses: *C. atratus* larvae show a more robust induction of competence by turbulence than do *H. mamillatus* larvae. *Colobocentrotus atratus* inhabits some of the most extreme high-energy shores on the planet (see, e.g. Denny & Gaylord, 1996), but it would not be accurate to describe *H. mamillatus* as inhabiting only calm or protected waters. Although *H. mamillatus* is obligatorily subtidal (Mortensen, 1943; Ogden *et al.*, 1989; Hoover, 2010), we have encountered individuals commonly on reef crests, in addition to isolated individuals in relatively shallow nearshore environments impacted regularly by breakers and seasonally heavy wave action. Therefore, the positive sinking response to turbulence and weakly positive competence induction by turbulence in *H. mamillatus* is consistent with some of their adult habitats in Hawai'i. With regard to *C. atratus*, early precompetent larvae did not show a strong settlement response to turbulence, but late precompetent larvae did. It might be that the extremely violent habitat that *C. atratus* settles into makes them more reticent to settle precociously, and that it is only immediately before they would reach competence in the absence of turbulence that these larvae are sensitive to activation of competence by turbulence.

We can anticipate that *C. atratus* will often experience energy dissipation rates of 1–10 W kg^{-1} or greater, consistent with measurements on other rocky intertidal shores (see Gaylord *et al.* 2013). In contrast, the subtidal *H. mamillatus* might be expected typically to encounter energy dissipation rates of 10^{-2} W kg^{-1} or lower, associated with frictional interactions of currents and orbital wave velocities with the substratum (e.g. Dade, 1993; Gross *et al.* 1994). Large turbulence intensities associated with wave breaking on shore would not be expected routinely for *H. mamillatus* in their adult habitats.

The habitat distinction associated with our *Strongylocentrotus* species pair was the most highly contrasted of our study taxa. *Strongylocentrotus purpuratus* individuals are commonly encountered in highly energetic, wave-impacted intertidal and shallow subtidal rocky shores in the north-east Pacific Ocean

(Rogers-Bennett, 2007), where energy dissipation rates approaching 10 W kg⁻¹ have been measured (Gaylord *et al.*, 2013; see also Sutherland & Melville, 2015). However, *S. fragilis* is restricted to much more quiescent conditions and reportedly never occurs above 50 m depth (Sato *et al.*, 2017). It can be expected that typical energy dissipation rates encountered in such deep habitats will be 10^{-6} W kg⁻¹ or lower (e.g. Lueck & Osborn, 1985). Of the three 'lower-energy' species that we studied, only *S. fragilis* exhibited neither a sinking behaviour nor an induction of competence across the full range of turbulence intensities tested.

In sum, our data on larval responses to turbulence indicate that larvae whose adults live in high-energy habitats are more responsive to intense turbulence than larvae whose adults dwell in calmer waters and that the magnitude of our observed differences in these responses mirrors the degree of habitat contrast among the three species pairs. Additional species contrasts in echinoids and non-echinoids will need to be examined to determine whether this intriguing pattern holds across a broader array of invertebrate taxa.

TURBULENCE AND SETTLEMENT: A PATHWAY TO VICARIANCE?

Whether new species originate more often allopatrically or not remains a robustly debated topic, in both terrestrial and aquatic ecosystems (Foote, 2018). In marine animals with planktonic dispersal stages, especially in clades with feeding larvae, it has long been assumed that allopatric speciation is unlikely, because the larvae can maintain high gene flow over large geographical distances, thus preventing local adaptation (Levins, 1968). As indicated above, this concept is increasingly being questioned, with accumulating evidence for local retention of larvae (e.g. Morgan *et al.*, 2009; Nickols *et al.*, 2015; reviewed by Pineda & Reyns, 2018) in addition to numerous examples of genetic structure even in species with long-lived larvae (e.g. Sun & Hedgecock, 2017; Truelove *et al.*, 2017, Xuereb *et al.*, 2018; reviewed by Cowen & Sponagule, 2009; Sanford & Kelly, 2011).

One potential stumbling block in this arena is that the terms 'sympatric' and 'allopatric' are often defined only loosely in the ocean. Allopatry is easiest to define in the context of island biogeography, as obvious physical separation between populations and species (Turelli *et al.*, 2001). In the ocean, an analogous situation is seen in deep-water hydrothermal vents: unique habitats that can be separated from one another by vast stretches of open ocean (Gage & Tyler, 1991). Nonetheless, the great majority of benthic species live on the continental shelves, and their separation from related species nearby is either by depth along the continental slope or by habitat type within a depth range, or both. Given that many marine animals (particularly invertebrates) are either sessile or sedentary as adults, a relatively short physical distance between habitats might still ensure lack of contact between adults dwelling in those respective habitats, or between their gametes in the case of broadcast spawners. The result would be a lack of opportunity for mating, hence reproductive isolation.

We suggest that a consideration of larvae and larval behaviour could be helpful in untangling the broader issues with respect to animal speciation in the ocean. As a first conceptualization, it seems plausible to consider the plankton as large assemblages of interacting organisms, both closely and distantly related, some transiently planktonic and others holoplanktonic. In this conception, two closely related species whose adults spawn around the same time of year might be considered 'sympatric' during their larval period, although their respective benthic stages might be separated by subhabitat or even by a considerable distance. Therefore, planktonic stages face the challenge of re-establishing their distinct adult distributions at every generation and, in particular, at the key settlement stage.

Although larvae of some species may not be discriminating at settlement, thereby requiring that physical separation of heterospecific adults be sustained purely by 'postsettlement' processes (e.g. Hunt & Scheibling, 1997; Delany *et al.*, 2003; see also Schmidt & Rand, 1999; Gorospe & Karl, 2015), diverse field and laboratory observations indicate that presettlement processes are often key determinants of adult distribution (e.g. Grosberg, 1981, 1982; Hunt & Scheibling, 1996; Bierne *et al.*, 2003; Jenkins, 2005; reviewed by Pineda *et al.*, 2010). Our results suggest that turbulence could operate before settlement as a neighbourhood-scale process that could also influence or drive evolutionary changes in adult distributions in the ocean.

For example, our most extreme contrasted species pair, *S. purpuratus* and *S. fragilis*, do not co-occur as adults. Nevertheless, their respective larvae are long lived (1–2 months or more in the plankton), their reproductive seasons coincide in the late winter and early spring, and larvae of both species develop normally in surface water conditions, such as those used in our larval cultures. Therefore, it is likely that their larvae co-occur in the plankton and might be carried together by prevailing flows into the nearshore where they experience increases in fluid turbulence. Our data suggest that advanced *S. purpuratus* larvae will respond positively to this turbulence exposure by transitioning to competence and sinking to the substratum, where they will settle if they encounter a suitable local cue, such as the coralline alga, *Calliarthron tuberculosum* (Gaylord *et al.*, 2013). In contrast, *S. fragilis* larvae neither sink nor transition to competence after encountering

intense fluid turbulence. They remain swimming and, if fortunate, could therefore be carried back out to waters of greater depth, hence towards their own preferred settlement habitat.

Whether additional neighbourhood-scale cues might facilitate their movement into deeper habitats remains unknown and warrants attention. For instance, increased pressure, reduced oxygen or lower pH might all be useful environmental indicators for *S. fragilis* larvae. Other marine species from different habitats might focus on still different classes of neighbourhood-scale cues, such as sound or salinity. If so, neighbourhood-scale larval responses might be a key feature for both habitat differentiation and successful settlement in a wide range of marine taxa. If this is the case, it raises the possibility that larval neighbourhood-scale responses might reinforce or even drive the evolution of habitat specificity, and possibly vicariance, in an appreciable array of marine taxa.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Staging schemes for the six echinoid species studied here.
Figure S1. Comparison of effectiveness of natural cues (12–24 h exposure) relative to excess potassium in seawater (1 h exposure, 12–24 h recovery) in four of our study species. Note that, as explained in the main text (and by Hodin *et al.*, 2019), the minimal effective excess potassium concentrations differ for different echinoid species. Arabic numerals inside the bars are numbers of exposed larvae in these side-by-side settlement tests. In

most cases, we did not replicate these particular exposures; we were mainly interested in verifying that exposure to excess potassium was a conservative minimum estimate of the timing [in days postfertilization (dpf)] at which these larvae became competent to settle. A, settlement responses in *Echinometra viridis* (larval cohort Ev1; see Table 1) at 13 dpf to either: 8 mL of 70 mM excess KCl in Millipore-filtered sea water (MFSW) or 8 mL of MFSW with 0.5 g crushed live rock collected in an intertidal location in Panamá where *E. viridis* and *Echinometra lucunter* co-occurred. B, settlement responses in *E. lucunter* (larval cohort El1) at 13 dpf after 3 min of shaking in an Erlenmeyer flask (~240 repetitions min^{-1}) to simulate turbulence; settlement conditions as in A. C, settlement responses in *Colobocentrotus atratus* larvae reared in 2014 (24–28 °C, 28 dpf) after a 3 min treatment with 16 W kg^{-1} turbulence, and then exposed to either 8 mL of 100 mM excess KCl in MFSW or 8 mL of MFSW containing 27 mg of the intertidal turf alga *Melanamansia glomerata* collected alongside *C. atratus* at our study site in Maui. D, settlement responses in *Strongylocentrotus purpuratus* larvae reared in 2012 (14 °C, 29 dpf, as reported by Gaylord *et al.*, 2013), exposed in three replicates each either to 8 mL of 70 mM excess KCl or to 8 mL of MFSW in chambers with their surfaces coated with a 7-day-old biofilm and containing 100 mg of the coralline alga, *Calliarthron tuberculosum*. Error bars in D are SEM.

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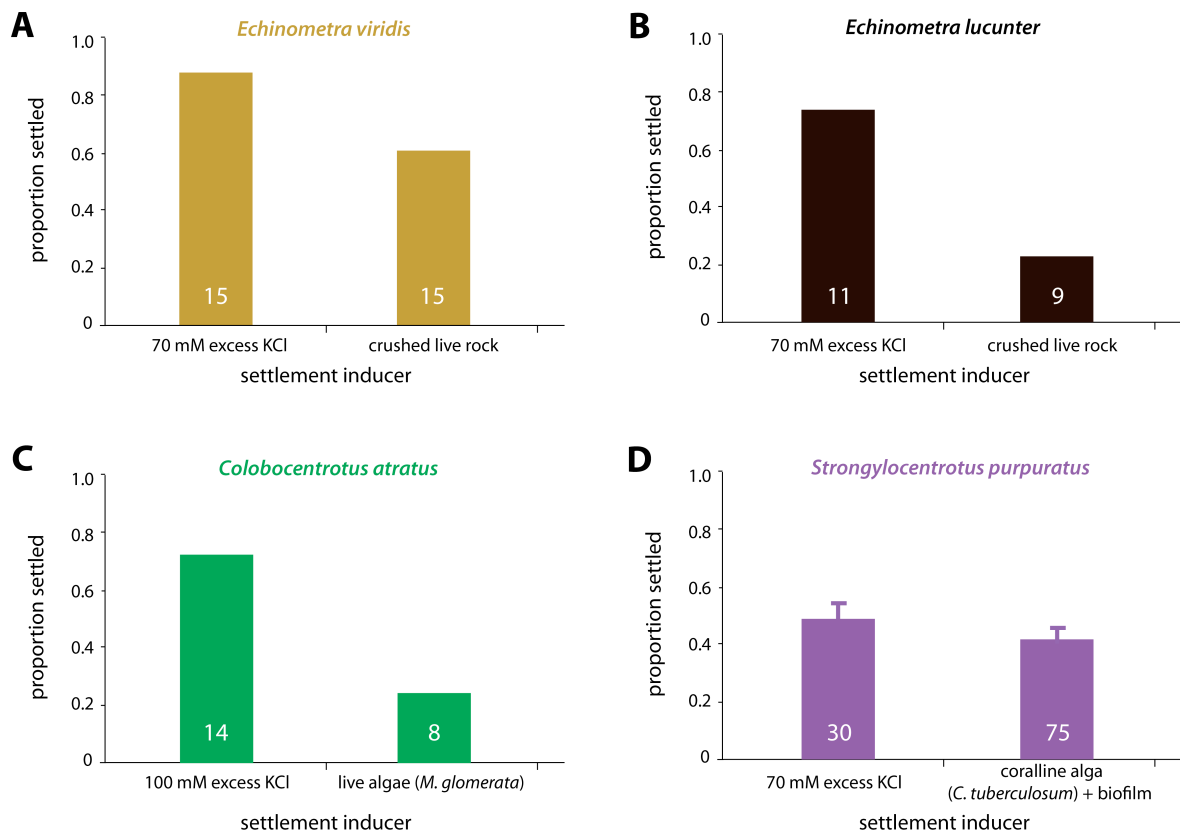
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Table S1. Staging schemes for the six echinoid species studied here.

stage	S. purpuratus – 14°C dpf	S. purpuratus – 14°C description	S. fragilis – 14°C dpf	S. fragilis – 14°C description	E. lucunter – 26°C dpf	E. lucunter – 26°C description	E. viridis – 26°C dpf	E. viridis – 26°C description	C. atratus – 24°C dpf	C. atratus – 24°C description	H. mamillatus – 24°C dpf	H. mamillatus – 24°C description
i	14	invagination	17	invagination	7	invagination	7	invagination	14	invagination	15	invagination
ii	15	contact	19	contact	9	contact	8	contact	15	contact	16	contact
iii	16	flattened contact	21	flattened contact	10	flattened contact	9	flattened contact	15.5	flattened contact	16.5	flattened contact
iv	17	5-fold mesoderm	23	5-fold mesoderm	11	5-fold mesoderm	10	5-fold mesoderm	16	5-fold mesoderm	17	5-fold mesoderm
v	18.5	5-fold ectoderm	25	5-fold ectoderm	11.5	5-fold ectoderm	10.5	5-fold ectoderm	16.5	5-fold ectoderm	17.5	5-fold ectoderm
vi	20	pp	27	pp	12	pp	11	pp	17	pp	18	pp
vii	22	pp folded	30	pp folded	13	pp folded	12	pp folded	17.5	pp folded	18.5	pp folded
viii	23	pp touching	32	pp touching	14	pp touching	13	pp touching	18	pp touching	19	pp touching
1	22	spic dot	27	spic dot	13	spic dot	11	spicules	18	spic / TF spic dot	19	spicule dot
2	22.5	spic	28	spic	13.5	spic	11.5	MB / TF spic dot	19	MB / TF spicules	20	spicule
3	23	MB / TF spic dot	29	MB	14	MB / TF spic dot	12	TF spicules	20	TF incompl 1st	22	MB / TF spic
4	24	TF spicules	31	TF spic dot / ad spp spic dot	14.5	TF spicules	13	TF incompl 1st / ad spp spic dot	22	TF compl 1st / ad spp spic dot	23	adult spp spic dot
5	25	TF incompl 1st / adult spp	33	TF spicules / adult spp	15	TF incompl 1st / adult spp spic dot	13.5	TF compl 1st / adult spp	23	TF incompl 2nd / adult spp	24	TF incompl 1st
6	26	TF compl 1st / adult spp+base	37	TF incompl 1st / adult spp+base	15.5	TF compl 1st / adult spp	14	adult spp+base	25	TF compl 2nd	26	TF compl 1st / adult spp
7	27	TF 2nd<½ compl / adult pre-spine	39	TF compl 1st / adult pre-spine	16	TF incompl 2nd / adult spp+base	15	TF 2nd<½ compl / adult pre-spine	26	adult spp+base	28	TF incompl 2nd / adult spp+base
8	28	adult spine	41	adult spine	16.5	TF compl 2nd / adult pre-spine	15.5	adult spine	28	TF incompl 3rd / adult pre-spine	29	TF compl 2nd / adult pre-spine
9	29	TF 2nd >½compl	43	TF incompl 2nd	17	adult spine	16	TF compl 2nd	30	TF compl 3rd	31	TF incompl 3rd / adults spine
10	30	TF compl 2nd	45	TF compl 2nd	17.5	TF incompl 3rd	17	TF incompl 3rd	32	TF incompl 4th / adult spine	33	TF compl 3rd / TF incompl 4th

Notes: Modified staging schemes based upon the published staging scheme for *S. purpuratus* (Heyland & Hodin, 2014; henceforth "H&H14"), as reproduced here in the first three columns. *Roman numerals* in the first column (*stages i–viii*) are the "soft tissue stages" as defined in H&H14, Table 1. *Arabic numerals* in the first column (*stages 1–10*) are the "skeletogenic stages" as defined in H&H14, Table 2. *dpf* should be considered approximations. We chose an example temperature (*first* row) here for the *dpf* data as this varies based on temperature. For example, we reared *S. fragilis* larval batch Sf2 (see Table 1, main text) at 11°C. These larvae took approximately 30% longer to reach comparable stages when compared to the 14°C-reared batches Sf1 and Sf3 (data not shown). Text in the *description* columns are shorthand for the soft tissue or skeletal structures upon which we base our staging scheme; this shorthand corresponds to the shorthand designations used in H&H14, Tables 1 and 2. For full descriptions, line drawings and definitions of these structures, see H&H14. Following H&H14, we did not utilize all skeletal features for the staging scheme, such as the juvenile spines, lantern skeleton and the extra rudiment structures such as the right side spines, pedicellaria and aboral juvenile skeleton. Abbreviations: ad-adult; compl-complete; incompl-incomplete; MB-multibranched spicules; pp-primary podia; spic-spicule; spp-spine primordium; spp+base-spine primordium + base;TF-primary tube foot; 1st, 2nd, 3rd, etc.-refer to the number of concentric TF skeletal rings (see H&H14). Staging order for the five new species are based upon all of the larval batches indicated in our Table 1, main text. Numbers of larvae examined for these analyses: *S. fragilis*, N=369; *E. lucunter*, N=313; *E. viridis,* N=160; *C. atratus*, N=90; *H. mamillatus*, N=107.
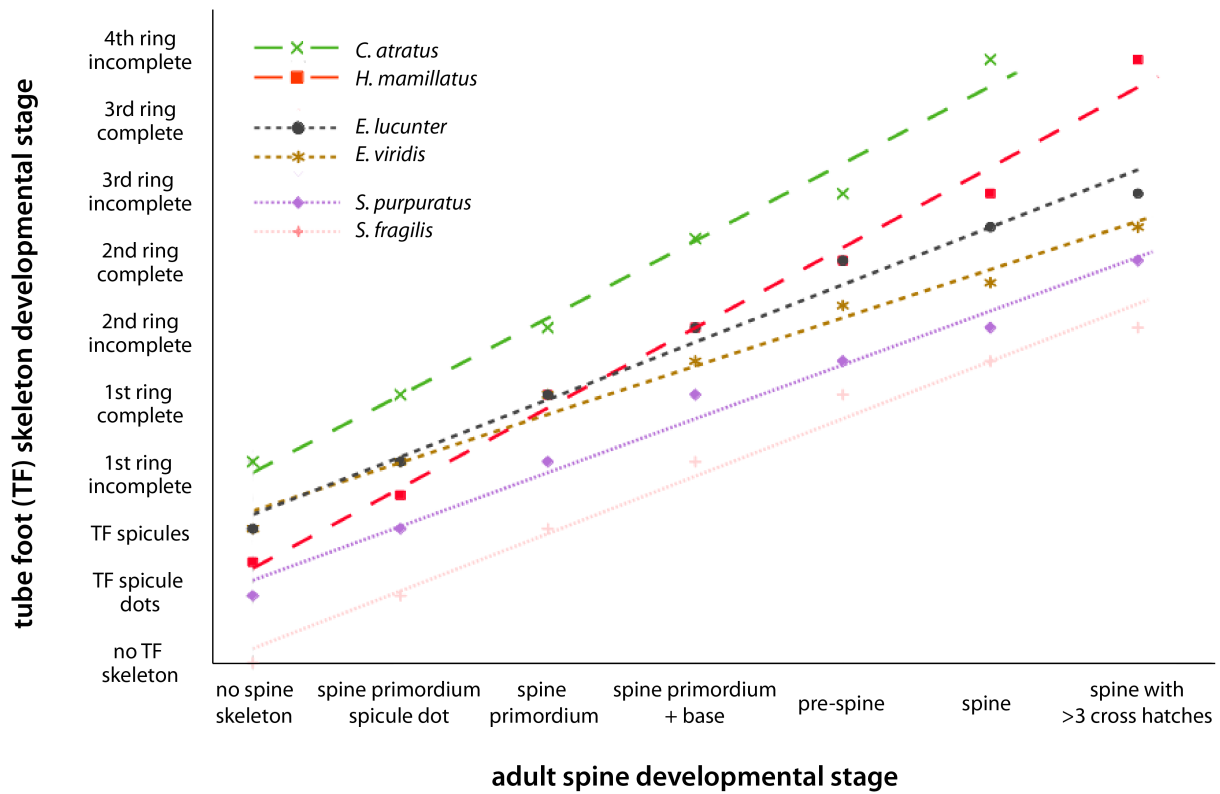
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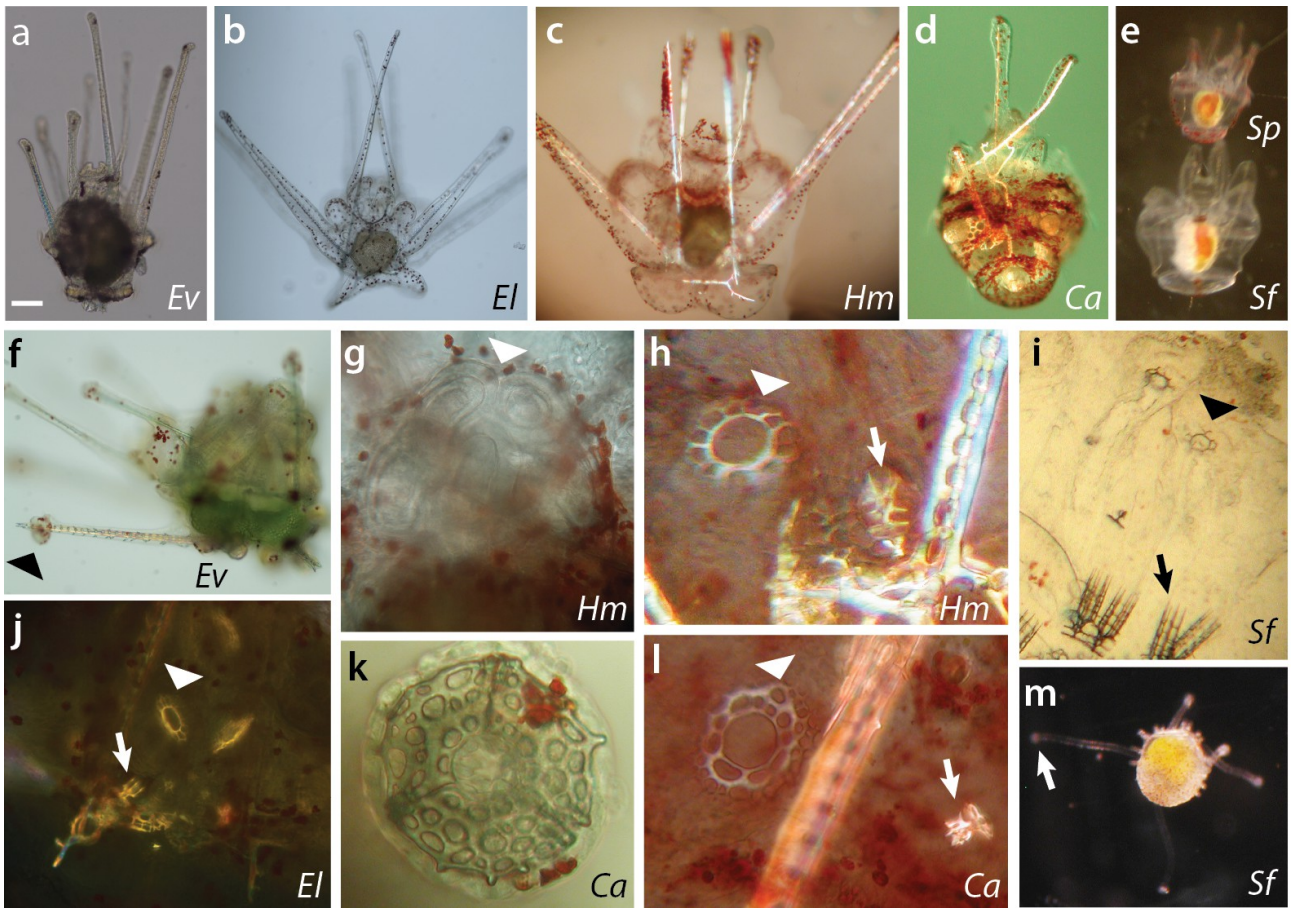
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Detailed Information Concerning the Sources of Adults for our Contrasting Species Pairs, and Apparent Relationships of these Pairs of Species

Species contrast 1.–We collected *E. viridis* in January 2015 by snorkeling to a shallow (<3m) subtidal population adjacent to mangroves on the protected side of Bocas del Toro island (Panamá), near to the Smithsonian Tropical Research Institute field station (STRI-Bocas; approximately 9.3517 N, 82.2589 W). We maintained adults in flowing seawater aquaria at STRI-Bocas until spawning. We collected *E. lucunter* from two locations on exposed locations on and near Bocas del Toro island (Panamá) in January 2015, and one location in southern Florida in February 2016 ("El_" refers to larva cohort codes in Table 1): El1- on 13 January 2015 by snorkeling on the Northeast side of Carenero Island (Panamá) at ~1m depth (approximately 9.3418 N, 82.2301 W); El2- on 25 January 2015 by hand at low tide at Flat Rock Beach, Bocas del Toro island, Panamá, north of Paki Point Beach (approximately 9.3822 N, 82.2353 W); and El3- by snorkel on 4 February 2016 from an East-facing rock wall just West of the Long Key Bridge in Conch Key FL, USA, at ~1m depth (approximately 24.7886 N, 80.8874 W). Until spawning, we held the adults in flowing seawater either at STRI-Bocas (El1 and El2) or the University of Miami's Rosenstiel School of Marine and Atmospheric Science (Miami FL, USA; El3).

E. lucunter is quite common both on Bocas del Toro Island (Panamá) along exposed rocky shores, and also among concrete rubble and other shallow subtidal rocks on the exposed (East- and Southeast-facing) shores of Virginia Key, Miami FL (USA). We found several additional populations of *E. lucunter* at each of these locales, but we did not use any in experiments since none of the urchins that we collected at these other locations were gravid. Interestingly, in Bocas del Toro, *E. viridis* co-occurs with *E. lucunter* at every nearshore location where we found the latter, which was unexpected (McPherson, 1969; Hendler *et al.*, 1995; H. Lessios, pers. comm.; but see Kier & Grant, 1965). By contrast, we did not find a single *E. lucunter* individual in the protected Bocas

del Toro location from which we collected *E. viridis* for this study. In Florida the situation was different; we did not encounter *E. viridis* at any of our *E. lucunter* collection sites. As a result, our *Echinometra* species pair when considered over its entire range represented a less extreme contrast than we were anticipating.

Relationships. *E. lucunter* and *E. viridis* are sister species according to a recent phylogenetic hypothesis (McCartney et al. 2000).

Species contrast 2.–We collected *C. atratus* Linnaeus adults by hand at low tide on 5 March 2018 from the outer jetty in Ma'alaea Harbor (Maui HI, USA; approximately 20.7902 N, 156.5113 W). We collected *H. mamillatus* Linnaeus adults by SCUBA on 6 March 2018 along the Ma'alaea water intake pipeline at ~5–6 m (Maui HI, USA; approximately 20.7885 N, 156.5124 W). We maintained adults of both species until spawning flow-through aquaria with heavy oxygenation (necessary for their short term survival in captivity) at the Maui Ocean Center (MOC; Ma'alaea, Maui HI, USA). Per the MOC collection permit, we returned *H. mamillatus* adults to the field 24 hrs after collection, as they do not survive well in captivity.

Relationships. Although *C. atratus* and *H. mamillatus* are in different genera, they are strongly supported as sister genera in a molecular analysis that included most extant genera in the Echinometridae (see Figure 1 in Kinjo et al., 2004). The two described species of *Colobocentrotus* are extremely similar in morphology and habitat, being restricted to high energy intertidal locales (Aggasiz 1908; Ebert 1982; Thet 2004). Likewise, the two described *Heterocentrotus* species are subtidal, though *H. trigonarius* is described as inhabiting relatively more exposed locales than *H. mamillatus* (Ebert 1982). *H. trigonarius* is not found in Hawai'i - they would be an interesting subject for future work, as would the species from the only other described genus in the clade, *Zenocentrotus*, known only from Niuafoou Island in the Tonga Archipelago (Clark 1931; Bronstein et al. 2018), and whose relative affinity to *Colobocentrotus* and *Heterocentrotus* has not been studied, nor has their habitat distribution been described. In sum (and in the absence of relevant phylogenetic and habitat information on *Zenocentrotus*), it seems fair to conclude that the habitat shift between subtidal and high energy intertidal occurred in either the

stem *Colobocentrotus* or stem *Heterocentrotus* lineage.

Species contrast 3.–The *S. pupuratus* adults we used for larval cohorts Sp1, Sp2 and Sp4 (see Table 1) were collected from a large intertidal population at Slip Point, Clallam Bay WA, USA, maintained in subtidal cages suspended off a floating dock at Friday Harbor Labs (FHL; Friday Harbor WA, USA) and fed with live bull kelp (*Nereocystis luetkeana*) throughout the year. The adults we used for larval cohort Sp3 were collected by SCUBA near to Hopkins Marine Station (HMS; Pacific Grove CA, USA), maintained in flow-through sea tables at HMS, and fed with live giant kelp (*Macrocystis pyrifera*) throughout the year. Because the urchins in each of these lab colonies are mixtures of urchins collected over many years, we do not know the collection date for the particular *S. purpuratus* urchins used in this study.

We reared *S. fragilis* larvae from two adult populations: one collected on 23 February 2016 at 120–150 m in spot prawn traps in Monterey Canyon (CA USA) and used for larval cohorts Sf1 and Sf2 (see Table 1); the second collected by trawl on 2 March 2017 at 160m off Long Beach CA, USA (within 1km from 33.70 N, 119.34 W), and used for larval cohort Sf3 (see Table 1). Adults were held in flow-through sea water aquaria at HMS and California State University -Long Beach (CA USA), respectively, until spawning. At HMS, there was a 2-week delay between collection and spawning, during which time the urchins were fed live giant kelp (*M. pyrifera*).

Relationships. The most recent phylogenetic hypothesis of the *Strongylocentrotus* genus is that of Biermann et al. (2003). Their Figure 2 shows the best supported topology by maximum likelihood methods, in which *S. droebachiensis* + *S. pallidus* + *S. fragilis* is the sister clade to *S. purpuratus*.

Embryo and Larval Transport Protocol

In most of our larval rearing episodes, we transported larvae at least once from the location where we fertilized

the gametes to the location where we conducted the turbulence-settlement trials. In some cases, we did two such transports: one from the fertilization location to the larval rearing location, and a second later in larval development in advance of the turbulence-settlement trials. We have had good success transporting embryos and larvae (at ~1 larva/ml) –by both ground and air– in 1L or smaller tissue culture flasks, filled to the brim with MFSW, and the tops secured with Parafilm® and then with plastic wrap and a rubber band. See Table 1 in the main text for a breakdown of the transport steps for each of the larval cohorts used for this study.

Species-Specific Excess KCl Concentrations

The effective concentration for settlement induction in *S. purpuratus* is 70 mM excess KCl in MFSW (see Gaylord *et al.*, 2013). For the other five species, we first needed to determine the minimum effective excess KCl concentration (ranging from 50 to 120 mM excess KCl) using competent larvae. For these species, the minimum effective excess KCl concentrations were as follows: *E. lucunter* and *E. viridis*– 70 μM; *S. fragilis*– 100 μM; *C. atratus* and *H. mamillatus*– 120 μM. We used the species-specific excess KCl concentration for all turbulence-settlement experiments.

Staging Schemes for Late Larval Development in our Six Study Species (Detailed Methods)

Starting at the dates when we reduced the density in the larval cultures (see Table 1), and at subsequent water change dates, we chose a haphazard subset of 5–10 larvae from each larval cohort, mounted them live on slides with raised cover glass, and examined them in a binocular microscope at 100x magnification and higher. Soft tissue stages (such as the first appearance of 5-fold symmetry) are easily observable by regular or differential interference contrast (DIC) optics. Skeletal structures in the rudiment are visible by employing simple cross polarization. For each larva, we scored the soft tissue stage and categorized the visible skeletal structures. We

continued staging larvae until they reached the stage at which we conducted turbulence trials. See Heyland and Hodin (2014) for detailed methodologies and descriptions of rudiment stages and structures.

All six species proceeded through the soft tissue stages in a manner comparable to *S. purpuratus*, but the same was not true for the skeletogenic stages. To establish the skeletogenic staging schemes for each species relative to the published *S. purpuratus* scheme, we rank ordered the appearance of each of the skeletal structures. If the first appearance of two structures for a given species coincided in this data set, then the appearance of those two structures were combined to define a single stage, and assigned an approximate (or interpolated) date of first appearance on the day when half the larvae had reached the stage in question. We also used the raw, rank-ordering data to analyze the relative appearance of spine versus tube foot skeletal structures in all six species.

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