Zoological Journal of the Linnean Society, 2020, **190**, 737–756. With 6 figures.

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

JASON HODIN[1*], MATTHEW C. FERNER[2] and BRIAN GAYLORD[3,4]

[1]*Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA*
[2]*Estuary & Ocean Science Center, San Francisco State University, Tiburon, CA 94920, USA*
[3]*Bodega Marine Laboratory, University of California at Davis, Bodega Bay, CA 94923, USA*
[4]*Department of Evolution and Ecology, University of California at Davis, Davis, CA 95616, USA*

Received 13 March 2019; revised 17 September 2019; accepted for publication 24 October 2019

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

*Corresponding author. E-mail: larvador@uw.edu

'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

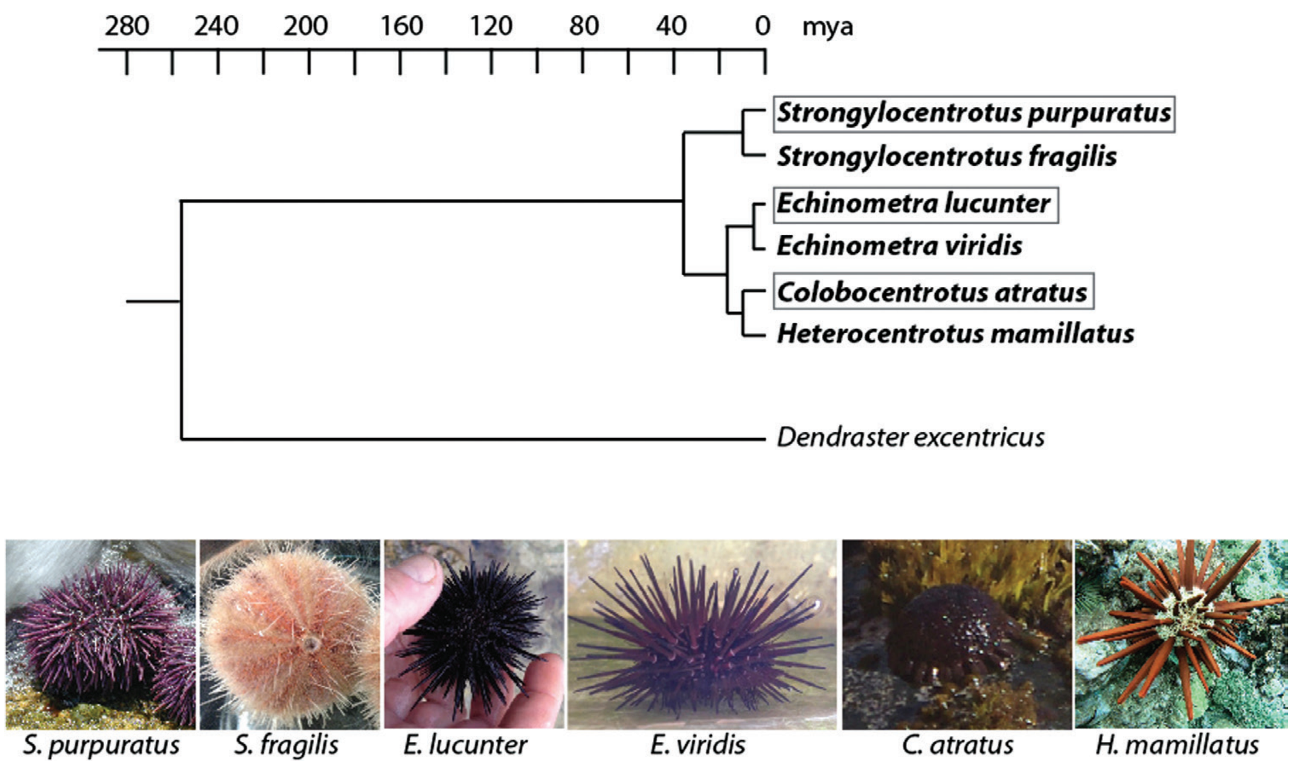

Figure 1. Composite phylogenetic hypothesis for the six study species (bold lettering and pictured), plus a more distantly related echinoid (*Dendraster excentricus*; plain lettering) mentioned throughout the text. Our six study species consist of three pairs of closely related species that differ in 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

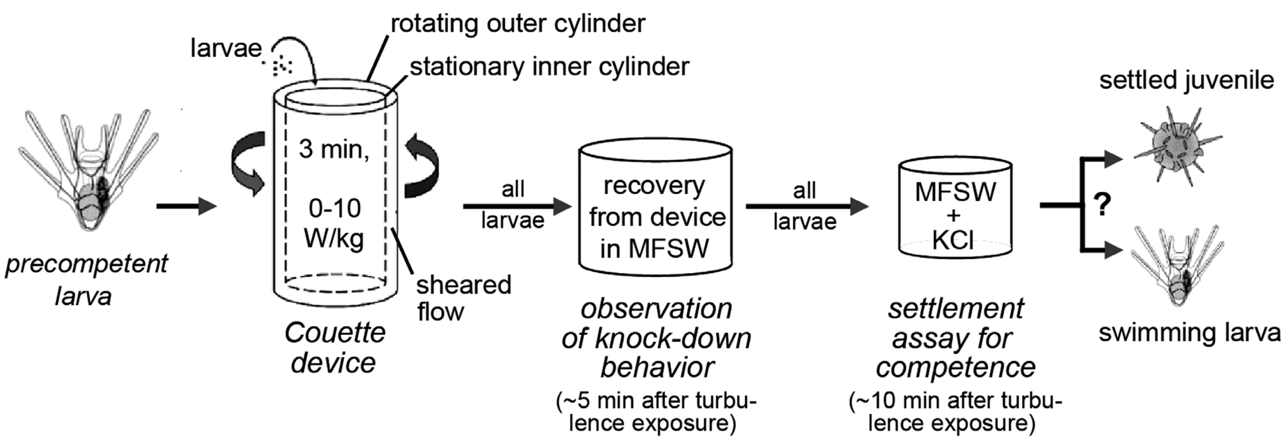

Figure 2. Schematic diagram of our turbulence treatment methodology and knockdown and competence assays. Abbreviation: MFSW, Millipore-filtered sea water.

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.

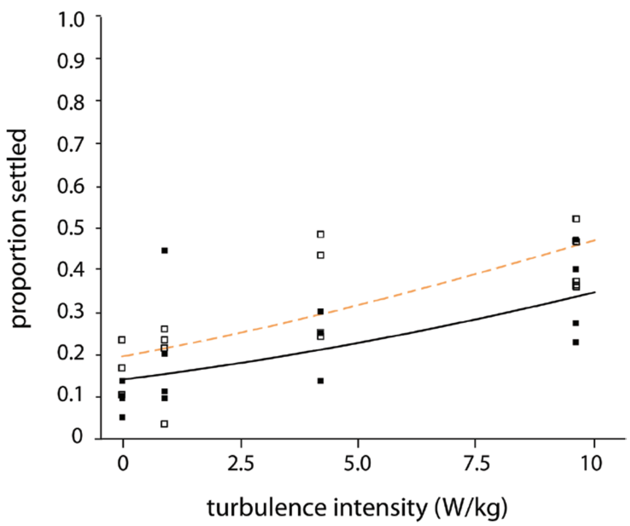

Figure 3. Induction of competence as judged by settlement response over a range of turbulence intensities in early precompetent larvae of two *Echinometra* species from Panamá. Each square is a replicate turbulence exposure of 15–30 larvae. Open squares and dashed brown line indicate *Echinometra viridis*. 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 (± SEM) was 0.16 (± 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%

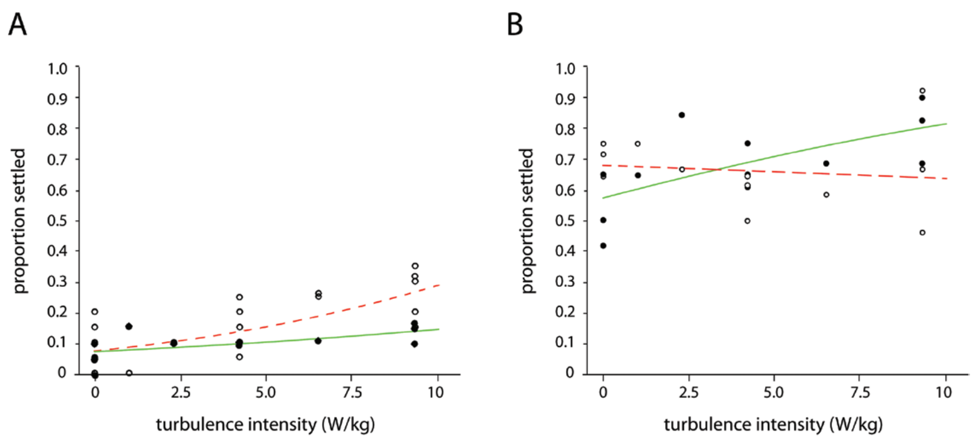

Figure 4. Induction of competence over a range of turbulence intensities in larvae of two closely related urchins from Hawai'i: *Heterocentrotus mamillatus* (open circles and dashed red line) and *Colobocentrotus atratus* (filled circles and continuous green line). Each circle is a replicate turbulence 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

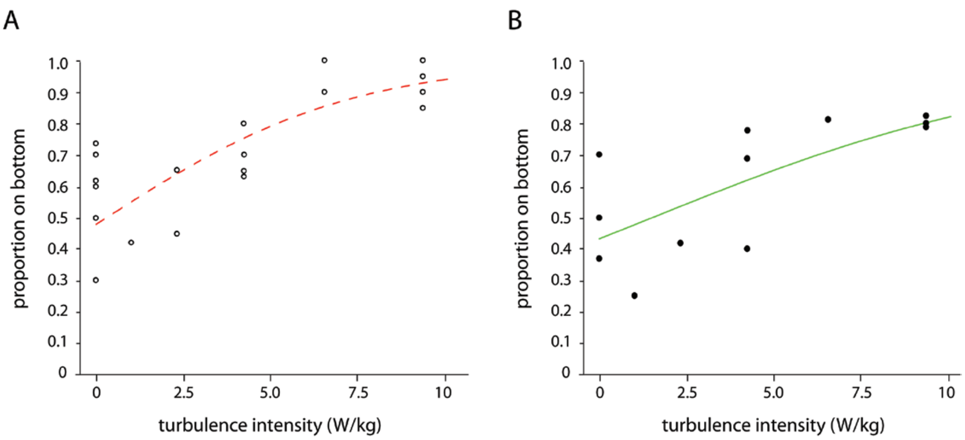

Figure 5. Knockdown response of early precompetent *Heterocentrotus mamillatus* larvae (A) and late precompetent *Colobocentrotus atratus* larvae (B). Lines and symbols are as in Figure 3. Both species showed a positive knockdown response to increasing turbulence ($P < 0.001$; see main text), but, as 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).

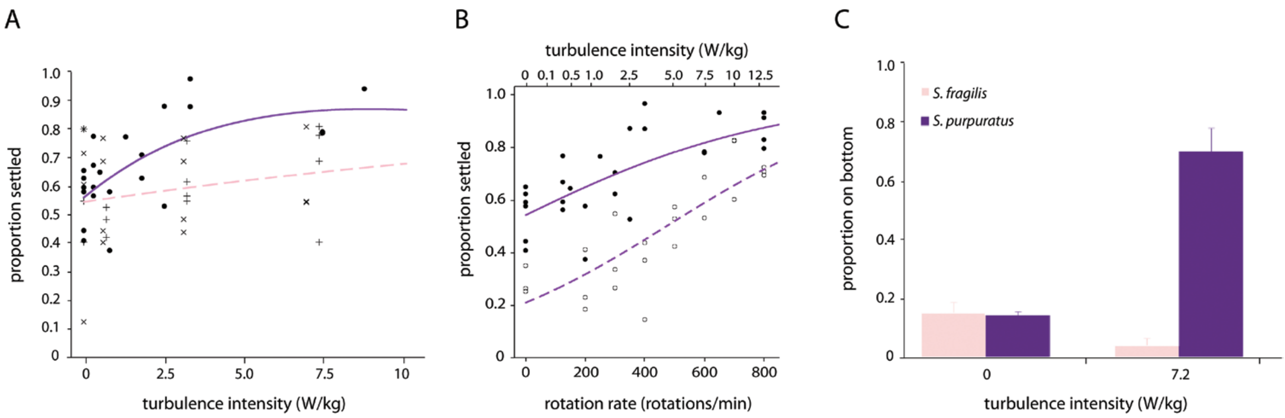

Figure 6. Induction of competence (A, B) and knockdown response (C) over a range of turbulence intensities in larvae of two *Strongylocentrotus* species from the north-east Pacific. A, B, each symbol is a replicate turbulence exposure of 15–40 larvae. A, induction of competence by turbulence in late 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 northeast 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 ~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

We are grateful to Athula Wikramanayake, Chris Lowe, Jim Truman, Lynn Riddiford, Merrill Hille, the Maui Ocean Center, Smithsonian Tropical Research Institute-Bocas and the University of Miami and their marine laboratory (Rosenstiel School of Marine and Atmospheric Science) for access to facilities. We thank Acadio Castillo, Brian Cheng, Bruno Pernet, Denis Mahaffy, Dennis Dunn, Jim Luecke, John Gorman, Lisa Komoroske, Louise Hodin, Mark Loos and Trevor Fay (Monterey Abalone Company) for assistance with collecting urchins. Kevin Uhlinger, Doug Pace and Annie Jean Rendleman conducted fertilizations and kindly provided us with purple and pink urchin embryos. Richard Pryor at the Transportation Safety Administration (TSA) in Fort Lauderdale and Davelyn Gordon and Robert Vickers at TSA in Maui facilitated our transport of larvae in carry on luggage. Miscellaneous thanks to Alex Lowe, Beatriz Velazquez, Bicicletaria Ixa, Carol Vines, Cory Bishop, Dan Swezey, David Epel, Eric Sanford, Gary Cherr, Haris Lessios, Joe Newman, Justin McAllister, Karen Chan, Kirk Sato, Kitty Brown, Matthew Hirano, Melissa Pespeni, Noam Gundle, Phil Gillette, Rachel Collin, Richard Strathmann and Wai Pang Chan. Gabriel Ng assisted with experiments and advised on statistics. The 2019 Friday Harbor Laboratories ZooBot students, the associate editor Rachel Przeslawski and two anonymous reviewers provided helpful feedback on an earlier version of the manuscript. We are grateful to Rahul Biswas and the University of Washington Biostatistics Consulting team for assistance with the bootstrap analysis and related statistical issues. This work was supported by National Science Foundation (NSF) grants (OCE-1356966, OCE-1357077 and OCE-1357033 to B.G., M.C.F., J.H. and Chris Lowe), an award under the Federal Coastal Zone Management Act, administered by the National Oceanic and Atmospheric Administration Office for Coastal Management (to San Francisco State University) and a University of Washington Royalty Research Fund award (to J.H.). The authors declare that they have no competing interests, financial or otherwise.

REFERENCES

Akaike H. 1978. A Bayesian analysis of the minimum AIC procedure. *Annals of the Institute of Statistical Mathematics* **20:** 9–14.

Amador-Cano G, **Carpizo-Ituarte E**, **Cristino-Jorge D. 2006.** Role of protein kinase C, G-protein coupled receptors, and calcium flux during metamorphosis of the sea urchin *Strongylocentrotus purpuratus*. *Biological Bulletin* **210:** 121–131.

Appelbaum L, **Achituv Y**, **Mokady O. 2002.** Speciation and the establishment of zonation in an intertidal barnacle: specific settlement vs. selection. *Molecular Ecology* **11:** 1731–1738.

Bates D, **Maechler M**, **Bolker B**, **Walker S. 2015.** Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67:** 1–48.

Biermann CH, **Kessing BD**, **Palumbi SR. 2003.** Phylogeny and development of marine model species: strongylocentrotid sea urchins. *Evolution & Development* **5:** 360–371.

Bierne N, **Bonhomme F**, **David P. 2003.** Habitat preference and the marine-speciation paradox. *Proceedings of the Royal Society B: Biological Sciences* **270:** 1399–1406.

Bishop CD, **Erezyilmaz DF**, **Flatt T**, **Georgiou CD**, **Hadfield MG**, **Heyland A**, **Hodin J**, **Jacobs MW**, **Maslakova SA**, **Pires A**, **Reitzel AM**, **Santagata S**, **Tanaka K**, **Youson JH. 2006.** What is metamorphosis? *Integrative and Comparative Biology* **46:** 655–661.

Cameron RA, **Tosteson TR**, **Hensley V. 1989.** The control of sea urchin metamorphosis: ionic effects. *Development, Growth & Differentiation* **31:** 589–594.

Carpizo-Ituarte E, **Salas-Garza A**, **Pares-Sierra G. 2002.** Induction of metamorphosis with KCl in three species of sea sea urchins and its implications in the production of juveniles. *Ciencias Marinas* **28:** 157–166.

Carr MH. 1991. Habitat selection and recruitment of an assemblage of temperate zone reef fishes. *Journal of Experimental Marine Biology and Ecology* **146:** 113–137.

Chia F-S. 1978. Perspectives: settlement and metamorphosis of marine invertebrate larvae. In: Chia F-S, Rice ME, eds. *Settlement and metamorphosis of marine invertebrate larvae*. New York: Elsevier, 283–285.

Chia F-S, **Koss R**, **Bickell LR. 1981.** Fine structural study of the statocysts in the veliger larva of the nudibranch, *Rostanga pulchra*. *Cell and Tissue Research* **214:** 67–80.

Cowen RK, **Sponaugle S. 2009.** Larval dispersal and marine population connectivity. *Annual Review of Marine Science* **1:** 443–466.

Crisp DJ. 1974. Factors influencing the settlement of marine invertebrate larvae. In: Grant PT, Mackie AM, eds. *Chemoreception in marine organisms*. New York: Academic Press, 177–265.

Dade WB. 1993. Near-bed turbulence and hydrodynamic control of diffusional mass transfer at the sea floor. *Limnology & Oceanography* **38:** 52–69.

Darwin CR. 1869. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*, *5th edn*. London: John Murray.

Delany J, **Myers AA**, **McGrath D**, **O'Riordan RM**, **Power AM. 2003.** Role of post-settlement mortality and 'supply-side' ecology in setting patterns of intertidal distribution in the chthamalid barnacles *Chthamalus montagui* and *C. stellatus*. *Marine Ecology Progress Series* **249:** 207–214.

Denny MW. 1988. *Biology and the mechanics of the wave-swept environment*. Princeton: Princeton University Press.

Denny MW, **Gaylord B. 1996.** Why the urchin lost its spines: hydrodynamic forces and survivorship in three echinoids. *The Journal of Experimental Biology* **199:** 717–729.

Denny MW, **Nelson EK**, **Mead KS. 2002.** Revised estimates of the effects of turbulence on fertilization in the purple sea urchin, *Strongylocentrotus purpuratus*. *Biological Bulletin* **203:** 275–277.

Denny MW, **Shibata MF. 1989.** Consequences of surf-zone turbulence for settlement and external fertilization. *The American Naturalist* **134:** 859–889.

Ebert TA. 1982. Longevity, life history, and relative body wall size in sea urchins. *Ecological Monographs* **52:** 353–394.

Feddersen F. 2012. Observations of the surf-zone turbulent dissipation rate. *Journal of Physical Oceanography* **42:** 386–399.

Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* **125:** 1–15.

Ferner MC, **Hodin J**, **Ng G**, **Gaylord B. 2019.** Brief exposure to intense turbulence induces a sustained life-history shift in echinoids. *The Journal of Experimental Biology* **222:** jeb187351.

Foote AD. 2018. Sympatric speciation in the genomic era. *Trends in Ecology & Evolution* **33:** 85–95.

Fuchs HL, **Christman AJ**, **Gerbi GP**, **Hunter EJ**, **Diez FJ. 2015.** Directional flow sensing by passively stable larvae. *The Journal of Experimental Biology* **218:** 2782–2792.

Fuchs HL, **Gerbi GP**, **Hunter EJ**, **Christman AJ. 2018.** Waves cue distinct behaviors and differentiate transport of congeneric snail larvae from sheltered versus wavy habitats. *Proceedings of the National Academy of Sciences of the United States of America* **115:** E7532–E7540.

Fuchs HL, **Hunter EJ**, **Schmitt EL**, **Guazzo RA. 2013.** Active downward propulsion by oyster larvae in turbulence. *The Journal of Experimental Biology* **216:** 1458–1469.

Fuchs HL, **Mullineaux LS**, **Solow AR. 2004.** Sinking behavior of gastropod larvae (*Ilyanassa obsoleta*) in turbulence. *Limnology & Oceanography* **49:** 1937–1948.

Fuchs HL, **Solow AR**, **Mullineaux LS. 2010.** Larval responses to turbulence and temperature in a tidal inlet: habitat selection by dispersing gastropods? *Journal of Marine Research* **68:** 153–188.

Gage JD, **Tyler PA. 1991.** *Deep-sea biology: a natural history of organisms at the deep-sea floor*. New York: Cambridge University Press.

Gaylord B. 2008. Hydrodynamic context for considering turbulence impacts on external fertilization. *Biological Bulletin* **214:** 315–318.

Gaylord B, **Denny MW. 1997.** Flow and flexibility – I. Effects of size, shape and stiffness in determining wave forces on the stipitate kelps *Eisenia arborea* and *Pterygophora californica*. *The Journal of Experimental Biology* **200:** 3141–3164.

Gaylord B, **Hodin J**, **Ferner MC. 2013.** Turbulent shear spurs settlement in larval sea urchins. *Proceedings of the National Academy of Sciences of the United States of America* **110:** 6901–6906.

Gaylord B, **Reed D**, **Raimondi PT**, **Washburn L. 2006.** Macroalgal spore dispersal in coastal environments: mechanistic insights revealed by theory and experiment. *Ecolological Monographs* **76:** 481–502.

Gaylord B, **Reed D**, **Raimondi PT**, **Washburn L**, **McLean S. 2002.** A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. *Ecology* **83:** 1239–1251.

George R, **Flick RE**, **Guza RT. 1994.** Observations of turbulence in the surf zone. *Journal of Geophysical Research: Oceans* **99:** 801–810.

Gorospe KD, **Karl SA. 2015.** Depth as an organizing force in *Pocillopora damicornis*: intra-reef genetic architecture. *PLoS ONE* **10:** e0122127.

Grant PR. 1999. *Ecology and evolution of Darwin's finches*, *2nd edn*. Princeton: Princeton University Press.

Grosberg RK. 1981. Competitive ability influences habitat choice in marine invertebrates. *Nature* **290:** 700–702.

Grosberg RK. 1982. Intertidal zonation of barnacles: the influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology* **63:** 894–899.

Gross TF, **Williams AJ**, **Terray EA. 1994.** Bottom boundary layer spectral dissipation estimates in the presence of wave motions. *Continental Shelf Research* **14:** 1239–1256.

Hadfield MG. 2011. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science* **3:** 453–470.

Hadfield MG, **Paul VJ. 2001.** Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BJ, eds. *Marine chemical ecology*. Boca Raton: CRC Press, 431–461.

Hendler G, **Miller JE**, **Pawson DL**, **Kier PM. 1995.** *Sea stars, sea urchins, and allies: echinoids of Florida and the Caribbean*. Washington: Smithsonian Institution Press.

Herrmann K, **Siefker B**, **Berking S. 2003.** Sterile polystyrene culture dishes induce transformation of polyps into medusae in *Aurelia aurita* (Scyphozoa, Cnidaria). *Methods in Cell Science* **25:** 135–136.

Heyland A, **Hodin J. 2014.** A detailed staging scheme for late larval development in *Strongylocentrotus purpuratus* focused on readily-visible juvenile structures within the rudiment. *BMC Developmental Biology* **14:** 22.

Hodin J, **Ferner MC**, **Heyland A**, **Gaylord B. 2018a.** I feel that! Fluid dynamics and sensory aspects of larval settlement across scales. In: Carrier TJ, Reitzel AM, Heyland A, eds. *Evolutionary ecology of marine invertebrate larvae*. New York: Oxford University Press, 190–207.

Hodin J, **Ferner MC**, **Ng G**, **Gaylord B. 2018b.** Turbulence exposure recapitulates desperate behavior in late-stage sand dollar larvae. *BMC Zoology* **3:** 9.

Hodin J, **Ferner MC**, **Ng G**, **Gaylord B. 2018c.** Sand dollar larvae show within-population variation in their settlement induction by turbulence. *Biological Bulletin* **235:** 152–166.

Hodin J, **Ferner MC**, **Ng G**, **Lowe CJ**, **Gaylord B. 2015.** Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence. *Royal Society Open Science* **2:** 150114.

Hodin J, **Heyland A**, **Mercier A**, **Pernet B**, **Cohen DL**, **Hamel J-F**, **Allen JD**, **McAlister JS**, **Byrne M**, **Cisternas P**, **George SB. 2019.** Culturing echinoderm larvae through metamorphosis. In: Foltz KR, Hamdoun A, eds. *Echinoderms, part A. Methods in cell biology*, **Vol. 150**. New York: Elsevier, 125–169.

Hoover JP. 2010. *Hawaii's sea creatures: a guide to Hawaii's marine invertebrates*. Honolulu: Mutual Publishing.

Hopkins MJ, **Smith AM. 2015.** Dynamic evolutionary change in post-Paleozoic echinoids and the importance of scale when interpreting changes in rates of evolution. *Proceedings of the National Academy of Sciences of the United States of America* **112:** 3758–3763.

Hunt HL, **Scheibling RE. 1996.** Physical and biological factors influencing mussel (*Mytilus trossulus*, *M. edulis*) settlement on a wave-exposed rocky shore. *Marine Ecology Progress Series* **142:** 135–145.

Hunt HL, **Scheibling RE. 1997.** Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series* **155:** 269–301.

Jenkins SR. 2005. Larval habitat selection, not larval supply, determines settlement patterns and adult distribution in two chthamalid barnacles. *Journal of Animal Ecology* **74:** 893–904.

Karp-Boss L, **Boss E**, **Jumars PA. 1996.** Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanography and Marine Biology: an Annual Review* **34:** 71–107.

Kier PM, **Grant RE. 1965.** Echinoid distribution and habits, Key Largo Coral Reef Preserve, Florida. *Smithsonian Miscellaneous Collections* **149:** 1–68.

Kingsford MJ, **Leis JM**, **Shanks A**, **Lindeman KC**, **Morgan SG**, **Pineda J. 2002.** Sensory environments, larval abilities and local self-recruitment. *Bulletin of Marine Science* **70**(Suppl. 1): 309–340.

Kinjo S, **Shirayama Y**, **Wada H. 2004.** Phylogenetic relationships and morphological diversity in the family Echinometridae (Echinoida, Echinodermata). In: Heinzeller T, Nebelsick JH, eds. *Echinoderms: Munchen*. Leiden: A.A. Balkema, 527–530.

Kiyomoto M, **Hamanaka G**, **Hirose M**, **Yamaguchi M. 2014.** Preserved echinoderm gametes as a useful and ready-to-use bioassay material. *Marine Environmental Research* **93:** 102–105.

Koehl MAR. 2007. Mini review: hydrodynamics of larval settlement into fouling communities. *Biofouling* **23:** 357–368.

Koehl MAR, **Strother JA**, **Reidenbach MA**, **Koseff JR**, **Hadfield MG. 2007.** Individual-based model of larval transport to coral reefs in turbulent, wave-driven flow: behavioral responses to dissolved settlement inducer. *Marine Ecology Progress Series* **335:** 1–18.

Lenth R. 2018. *Emmeans: estimated marginal means, aka least-squares means*. *R package version 1.1.2*. Available at: https://CRAN.R-project.org/package=emmeans

Levins R. 1968. *Evolution in changing environments. Some theoretical explorations*. Princeton: Princeton University Press.

Lillis A, **Eggleston DB**, **Bohnenstiehl DR. 2013.** Oyster larvae settle in response to habitat-associated underwater sounds. *PLoS ONE* **8:** e79337.

Losos JB, **Leal M**, **Glor RE**, **de Queiroz K**, **Hertz PE**, **Schettino LR**, **Lara AC**, **Jackman TR**, **Larson A. 2003.** Niche lability in the evolution of a Caribbean lizard community. *Nature* **424:** 542–545.

Lueck RG, **Osborn TR. 1985.** Turbulence measurements in a submarine canyon. *Continental Shelf Research* **4:** 681–698.

McCartney MA, **Keller G**, **Lessios HA. 2000.** Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. *Molecular Ecology* **9:** 1391–1400.

McDonald KA, **Vaughn D. 2010.** Abrupt change in food environment induces cloning in plutei of *Dendraster excentricus*. *Biological Bulletin* **219:** 38–49.

McPherson BF. 1969. Studies on the biology of the tropical sea urchins, *Echinometra lucunter* and *Echinometra viridis*. *Bulletin of Marine Science* **19:** 194–213.

Montgomery EM, **Hamel J-F**, **Mercier A. 2018.** Larval nutritional mode and swimming behaviour in ciliated marine larvae. *Journal of the Marine Biological Association of the United Kingdom* **99:** 1027–1032.

Morgan SG, **Fisher JL**, **Miller SH**, **McAfee ST**, **Largier JL. 2009.** Nearshore larval retention in a region of strong upwelling and recruitment limitation. *Ecology* **90:** 3489–3502.

Mortensen T. 1943. *A monograph of the Echinoidea. III, 3. Camarodonta. II. Echinidae, Strongylocentrotidae, Parasaleniidae, Echinometridae*. Copenhagen: C.A. Reitzel.

Mos B, **Dworjanyn SA. 2016.** Early metamorphosis is costly and avoided by young, but physiologically competent, marine larvae. *Marine Ecology Progress Series* **559:** 117–129.

Nickols KJ, **White JW**, **Largier JL**, **Gaylord B. 2015.** Marine population connectivity: reconciling large-scale dispersal and high self-retention. *The American Naturalist* **185:** 196–211.

Ogden NB, **Ogden JC**, **Abbott IA. 1989.** Distribution, abundance and food of sea urchins on a Leeward Hawaiian reef. *Bulletin of Marine Science* **45:** 539–549.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology: an Annual Review* **30:** 273–335.

Pechenik JA. 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177:** 269–297.

Pineda J, **Porri F**, **Starczak V**, **Blythe J. 2010.** Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity. *Journal of Experimental Marine Biology and Ecology* **392:** 9–21.

Pineda J, **Reyns N. 2018.** Larval transport in the coastal zone: biological and physical processes. In: Carrier TJ, Reitzel AM, Heyland A, eds. *Evolutionary ecology of marine invertebrate larvae*. New York: Oxford University Press, 141–159.

R Core Team. **2017.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Raubenheimer B, **Elgar S**, **Guza RT. 2004.** Observations of swash zone velocities: a note on friction coefficients. *Journal of Geophysical Research: Oceans* **109:** C01027.

Rogers-Bennett L. 2007. The ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. In: Lawrence JM, ed. *Edible sea urchins: biology and ecology*. Amsterdam: Elsevier, 393–425.

Rüber L, **Verheyen E**, **Meyer A. 1999.** Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences of the United States of America* **96:** 10230–10235.

Sanford E, **Kelly MW. 2011.** Local adaptation in marine invertebrates. *Annual Review of Marine Science* **3:** 509–535.

Santos R, **Flammang P. 2006.** Morphology and tenacity of the tube foot disc of three common European sea urchin species: a comparative study. *Biofouling* **22:** 187–200.

Sato KN, **Levin LA**, **Schiff K. 2017.** Habitat compression and expansion of sea urchins in response to changing climate conditions on the California continental shelf and slope (1994–2013). *Deep-Sea Research Part II: Topical Studies in Oceanography* **137:** 377–389.

Sato Y, **Kaneko H**, **Negishi S**, **Yazaki I. 2006.** Larval arm resorption proceeds concomitantly with programmed cell death during metamorphosis of the sea urchin *Hemicentrotus pulcherrimus*. *Cell and Tissue Research* **326:** 851–860.

Schmidt PS, **Rand DM. 1999.** Intertidal microhabitat and selection at Mpi: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. *Evolution* **53:** 135–146.

Simpson SD, **Meekan MG**, **McCauley RD**, **Jeffs A. 2004.** Attraction of settlement-stage coral reef fishes to reef noise. *Marine Ecology Progress Series* **276:** 263–268.

Stocking JB, **Rippe JP**, **Reidenbach MA. 2016.** Structure and dynamics of turbulent boundary layer flow over healthy and algae-covered corals. *Coral Reefs* **35**:1047–1059.

Strathmann MF. 1987. *Reproduction and development of marine invertebrates of the northern Pacific coast*. Seattle: University of Washington Press.

Sun X, **Hedgecock D. 2017.** Temporal genetic change in North American Pacific oyster populations suggests caution in seascape genetics analyses of high gene-flow species. *Marine Ecology Progress Series* **565:** 79–93.

Sutherby J, **Giardini JL**, **Nguyen J**, **Wessel G**, **Leguia M**, **Heyland A. 2012.** Histamine is a modulator of metamorphic competence in *Strongylocentrotus purpuratus* (Echinodermata: Echinoidea). *BMC Developmental Biology* **12:** 14.

Sutherland P, **Melville WK. 2015.** Measuring turbulent kinetic energy dissipation at a wavy sea surface. *Journal of Atmospheric and Oceanic Technology* **32:** 1498–1514.

Taylor GI. 1923. Stability of a viscous liquid contained between two rotating cylinders. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **223:** 289–343.

Truelove NK, **Kough AS**, **Behringer DC**, **Paris-Limouzy CB**, **Box SJ**, **Preziosi RF**, **Butler MJ. 2017.** Biophysical connectivity explains population genetic structure in a highly dispersive marine species. *Coral Reefs* **36:** 233–244.

Turelli M, **Barton NH**, **Coyne JA. 2001.** Theory and speciation. *Trends in Ecology & Evolution* **16:** 330–343.

Underwood AJ. 1997. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge: Cambridge University Press.

Welch JJ. 2010. The "Island Rule" and deep-sea gastropods: re-examining the evidence. *PLoS ONE* **5:** e8776.

Wellenreuther M, **Clements KD. 2008.** Determinants of habitat association in a sympatric clade of marine fishes. *Marine Biology* **154:** 393–402.

Wheeler JD, **Chan KYK**, **Anderson EJ**, **Mullineaux LS. 2016.** Ontogenetic changes in larval swimming and orientation of pre-competent sea urchin *Arbacia punctulata* in turbulence. *The Journal of Experimental Biology* **219:** 1303–1310.

Wheeler JD, **Helfrich KR**, **Anderson EJ**, **Mullineaux LS. 2015.** Isolating the hydrodynamic triggers of the dive response in eastern oyster larvae. *Limnology & Oceanography* **60:** 1332–1343.

Woodin SA. 1991. Recruitment of infauna: positive or negative cues? *American Zoologist* **31:** 797–807.

Xuereb A, **Benestan L**, **Normandeau É**, **Daigle RM**, **Curtis JMR**, **Bernatchez L**, **Fortin MJ. 2018.** Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RADseq, in a highly dispersive marine invertebrate (*Parastichopus californicus*). *Molecular Ecology* **27:** 2347–2364.

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.

Figure S2. Representative images of our six study species. A, competent *Echinometra viridis* (*Ev*) larva. B, *Echinometra lucunter* (*El*) larva at soft tissue stage v [fivefold ectoderm; see the Supporting Information (Table S1) and Heyland & Hodin (2014) for staging information]. C, *Heterocentrotus mamillatus* (*Hm*) larva at approximately skeletogenic stage 1 (image pieced together from two photographs). D, typical barrel-shaped morphology of a competent *Colobocentrotus atratus* (*Ca*) larva, with its ciliated band adopting a more circumferential arrangement (as indicated by the arrangement of the adjacent red pigment cells). E, *Strongylocentrotus purpuratus* (*Sp*) and *Strongylocentrotus fragilis* (*Sf*) larvae, both at approximately skeletogenic stage 4, showing the larger relative body size in *Sf*. F, *Ev* larva settling in response to excess potassium. Black arrowhead indicates skin withdrawal from an arm tip, a clear sign that this larva has begun to settle irreversibly. G, H, close-up view of the rudiment in *Hm* larvae. G, soft tissue stage viii (primary podia touching). White arrowhead indicates where adjacent podia are in contact. H, skeletogenic stage 8, with incomplete second tube foot ring (TF ring; white arrowhead) and adult prespine (white arrow). I, close-up view of skeletogenic stage 9 *Sf* larva flattened under a coverglass to reveal all rudiment skeleton, including incomplete second TF ring (black arrowhead) and adult spine with three cross-hatches (black arrow). J, close-up view of skeletogenic stage 8 *El* rudiment, with complete second TF ring (white arrowhead) and adult pre-spine (white arrow). K, L, *Ca* close-up views. K, single TF end plate with four complete rings in a recently settled juvenile. L, skeletogenic stage 8 rudiment with a third TF ring forming (white arrowhead) and a spine primordium + base (white arrow). In comparison to *Hm* in panel H, panel L shows the more rapid TF skeletal development relative to spine growth in *Ca* (see Supporting Information, Fig. S3). M, recently settled *Sf* juvenile, with its remarkably extended tube feet; the TF with its tip indicated by the white arrow is ~700 μm long. Panels C, D, F, H and J–L are cross-polarized light micrographs. Scale bar in A corresponds to each panel as follows: 100 μm (A); 135 μm (B); 85 μm (C, D); 115 μm (E); 70 μm (F); 20 μm (G); 16.5 μm (H); 50 μm (I); 30 μm (J); 12 μm (K); 14 μm (L); and 160 μm (M).

Figure S3. Comparison of ontogenetic stage progression in the six study species. Here, we characterized the relative timing of appearance of two types of skeletal elements in the echinus (juvenile) rudiments within late-stage larvae: adult-type spines (*x*-axis) and tube foot (TF) skeletal end plates (*y*-axis). The TF end plates are organized into concentric rings that are added sequentially as ontogeny proceeds (for details, see Heyland & Hodin, 2014). Owing to the way we gathered these staging data, they are not amenable to statistical analysis, but the trends indicate intriguing interspecies differences that are correlated with habitat. Two of the three species with higher relative energy exposure in their adult habitats (*Colobocentrotus atratus* and *Strongylocentrotus purpuratus*), when compared with their lower-energy counterparts (*Heterocentrotus mamillatus* and *Strongylocentrotus fragilis*, respectively), exhibited precocious development of TF end plates relative to adult spine development (higher intercepts in the former). In our third species pair there was no obvious difference in intercept, but the higher-energy *Echinometra lucunter* exhibited an increased apparent slope of the linear trend line when compared with the lower-energy *Echinometra* viridis, indicating that TF skeletal development proceeded more rapidly in the former. Interestingly, the three species pairs each appear clustered, indicating a phylogenetic signal in their response, overlaid by the apparent differences within the species pairs.