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RESEARCH ARTICLE

Brief exposure to intense turbulence induces a sustained life-history shift in echinoids

Matthew C. Ferner[1,*,‡], Jason Hodin[2,*], Gabriel Ng[3] and Brian Gaylord[3]

ABSTRACT

In coastal ecosystems, attributes of fluid motion can prompt animal larvae to rise or sink in the water column and to select microhabitats within which they attach and commit to a benthic existence. In echinoid (sea urchin and sand dollar) larvae living along wave-exposed shorelines, intense turbulence characteristic of surf zones can cause individuals to undergo an abrupt life-history shift characterized by precocious entry into competence – the stage at which larvae will settle and complete metamorphosis in response to local cues. However, the mechanistic details of this turbulence-triggered onset of competence remain poorly defined. Here, we evaluate in a series of laboratory experiments the time course of this turbulence effect, both the rapidity with which it initiates and whether it perdures. We found that larvae become competent with turbulence exposures as brief as 30 s, with longer exposures inducing a greater proportion of larvae to become competent. Intriguingly, larvae can remember such exposures for a protracted period (at least 24 h), a pattern reminiscent of long-term potentiation. Turbulence also induces short-term behavioral responses that last less than 30 min, including cessation of swimming, that facilitate sinking and thus contact of echinoid larvae with the substratum. Together, these results yield a novel perspective on how larvae find their way to suitable adult habitat at the critical settlement transition, and also open new experimental opportunities to elucidate the mechanisms by which planktonic animals respond to fluid motion.

KEY WORDS: *Dendraster excentricus*, Hydrodynamics, Larval settlement, Sensory ecology, *Strongylocentrotus purpuratus*, Surf zone

INTRODUCTION

Fluid mixing and the turbulent processes that underlie it are known to influence organism performance, survival and reproduction, and the nature of these influences can differ profoundly across an organism's life history. A spectrum of biomechanical studies has examined how turbulence affects external fertilization, chemical sensing, feeding, swimming, predator–prey interactions and larval settlement (e.g. see reviews by Kiørboe and Saiz, 1995; Karp-Boss et al., 1996; Koehl, 2007; Liao, 2007; Webster and Weissburg, 2009; Crimaldi, 2012; Guasto et al., 2012; Pécseli and Trulsen,

[1]San Francisco Bay National Estuarine Research Reserve and Estuary & Ocean Science Center, San Francisco State University, Tiburon, CA 94920, USA. [2]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA. [3]Bodega Marine Laboratory and Department of Evolution and Ecology, University of California at Davis, Bodega Bay, CA 94923, USA.
*These authors contributed equally to this work

‡Author for correspondence (mferner@sfsu.edu)

M.C.F., 0000-0002-4862-9663

2016). This research has done much to enhance our understanding of key themes in organismal design as they relate to interactions between aquatic fauna and their hydrodynamic environs.

An important subset of such work has focused on consequences for organisms of the intense fluid turbulence characteristic of wave-swept rocky shores. In these energetic habitats, flows can be both extremely rapid and extraordinarily dynamic over time and space, as arriving swells overturn and break, eventually degenerating into the frothing whitewater typical of exposed coastlines (Denny, 1995, 2016; Gaylord, 1999). We can quantify these levels of turbulence in terms of the energy dissipation rate, a measure of how fast kinetic water fluctuations are transferred from macroscopic to microscopic scales of motion. Extremes in energy dissipation rate under breakers crashing on shoreline rocks may reach nearly 10 W kg^{-1} (Gaylord, 2008; Gaylord et al., 2013; see also Stokes et al., 2004). These values greatly exceed those commonly encountered in offshore waters, which frequently span 10^{-9} to 10^{-3} W kg^{-1} (e.g. Oakey and Elliott, 1982; Terray et al., 1996; Fuchs and Gerbi, 2016). Such contrasts in turbulence intensities might therefore provide diagnostic information to planktonic organisms that experience these flows, indicating their position relative to the shoreline.

Early planktonic life stages of echinoids (sea urchins and sand dollars) have often served as useful experimental subjects for investigations into biological effects of marine turbulence, including turbulence characteristic of wavy habitats. Indeed, a suite of studies has examined effects of turbulence on the physics and ecology of broadcast spawning by sea urchins (e.g. Levitan et al., 1992; Crimaldi and Browning, 2004; see also Denny et al., 1992), and on their fertilization dynamics (Mead and Denny, 1995; Denny et al., 2002; Riffell and Zimmer, 2007; Gaylord, 2008). Research also has shown that turbulence influences fundamental behaviors such as swimming and feeding by echinoid larvae (e.g. Maldonado and Latz, 2011; Chan, 2012; McDonald, 2012; Wheeler et al., 2016).

Turbulence can additionally affect the transition of echinoid and other larvae from the plankton to the benthos as they prepare for settlement (e.g. Denny and Shibata, 1989; Mullineaux and Garland, 1993; Pawlik and Butman, 1993; Welch and Forward, 2001; Crimaldi et al., 2002; Pernet et al., 2003; Fuchs et al., 2010, 2013; Koehl et al., 2013; Hodin et al., 2018b,c). For echinoid larvae in particular, intense turbulence (>2 W kg^{-1}) found in the surf zones of wave-exposed coasts can speed the advancement of those larvae into a state called competence, in which they are capable of settling to the substratum and thus leaving the water column (Gaylord et al., 2013; Hodin et al., 2015, 2018a,c). In other words, exposure to intense turbulence causes echinoid larvae to transition abruptly and precociously from precompetence to competence (see Materials and Methods for detailed definitions). This previously unrecognized temporal plasticity in a critical and irreversible life-history event, one common to a broad array of animal and non-animal marine taxa, could have important implications for our understanding of the many oceanic species that have both a microscopic pelagic phase and a macroscopic benthic phase.

There are also functional issues to consider with respect to turbulence-initiated entry into competence and settlement. A common presumption regarding the selective advantage of a precompetent period is that larvae will face negative consequences if they transition to the benthos too early in ontogeny. For example, if larvae settle precociously, the resulting juveniles may be at a competitive disadvantage or experience increased predation risk (Pechenik, 1999). In contrast, if a precompetent larva were to bypass suitable juvenile habitat, it might never encounter it again. In the latter situation, it would benefit a larva to rapidly transition to competence, and thus acquire the ability to settle quickly if and when it reaches the vicinity of suitable habitat.

In this conception, precompetent sea urchin and sand dollar larvae would take advantage of strong turbulence as an environmental indicator that they are approaching suitable shoreline habitat (Chia et al., 1981), and despite their lack of full maturation, settlement therein might nevertheless be favored (Hodin et al., 2015). If this is the case, we expect that only the most intense values of turbulence would provide diagnostic utility for echinoid larvae seeking wave-exposed locales, in contrast with other types of larvae settling into calmer habitats (Fuchs et al., 2004, 2007). This point follows from the considerable temporal variability ('intermittency') of turbulence (Tennekes and Lumley, 1972), which causes distributions of energy dissipation rate from different environments to overlap except in their statistical tails (Fuchs and Gerbi, 2016).

Although we are beginning to understand connections of turbulence to settlement in echinoid larvae, there remains an important nuance that warrants careful exploration. Turbulence does not typically function as a habitat cue per se, in that it does not directly induce settlement as do standard benthic chemical cues. Instead, it primes late-stage sea urchin and sand dollar larvae to respond to chemical settlement inducers (Gaylord et al., 2013; Hodin et al., 2015). This priming function suggests that echinoid larvae might first respond to the signal of increased turbulence that would indicate their arrival into the surf zone, and then subsequently hone in on the more localized, seafloor-associated chemical cues that a wide range of larvae (including echinoids) use to make the final decision of where to settle (see reviews in Pawlik, 1992; Hadfield and Paul, 2001; Koehl, 2007; Hadfield, 2011).

The fact that competence can be triggered by intense turbulence raises additional, ecologically relevant issues. For example, it suggests that if a turbulence-primed larva does not subsequently detect a chemical cue diagnostic of appropriate habitat, it could be advantageous for that larva to decay in its initial priming, from competent back to precompetent, such that the larva is no longer responsive to chemical cues. Doing so could reduce the chance that the larva would complete its pelagic phase in a place devoid of appropriate habitat features. Similarly, one could imagine benefits to larvae in only responding to a minimum threshold of turbulence duration, so as to avoid an inappropriately early transition into the primed state.

Here, with an eye to these unknowns, we examine the temporal dynamics of the response of late-stage echinoid larvae to surf-zone levels of turbulence using controlled laboratory flows followed by assays of larval competence. In particular, we address three biomechanically motivated issues that bear on the critical step of settlement in these taxa. First, we ask whether the turbulence response requires an appreciable duration of exposure, or whether it can be sparked by a very brief one (less than a minute). Second, we ask whether the turbulence response perdures (is sustained) following its initiation, or whether larvae forgo their initial priming and revert to a precompetent state. Third, we ask whether and for how long larvae exhibit a distinct and rapid behavioral response following exposure to turbulence, in which larvae are carried to the substratum and remain in contact with it. These temporal aspects of larval responses to turbulence could have important and lasting implications for natural patterns of settlement and survival, and thus for the ecology of nearshore marine populations.

MATERIALS AND METHODS
Overview, study species and larval developmental state

We conducted a series of laboratory experiments to evaluate temporal aspects of late-stage echinoid larval responses to turbulence by first exposing larvae to turbulent water motion and then assessing resultant changes in larval competence. In all experiments, we used larvae from one of two echinoid species from the Northeast Pacific coast: *Dendraster excentricus* Eschscholtz 1831, the Pacific sand dollar, and *Strongylocentrotus purpuratus* (Stimpson 1857), the purple sea urchin. We exposed larvae to turbulence with a Taylor–Couette cell (Taylor, 1923; Karp-Boss et al., 1996; Denny et al., 2002), a device consisting of two nested cylinders separated by a 3.5 mm gap filled with seawater into which larvae were introduced 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. Given that we are interested in larval responses to conditions observed on wave-swept rocky coasts, we tested larval responses to intense turbulence (energy dissipation rates of $6-7$ W kg^{-1}, which extend just beyond those measured in surf zones of gently sloping beaches, or in the crests of white caps, to include some of the largest values recorded under breaking waves on steep rocky shores; see George et al., 1994; Raubenheimer et al., 2004; Gaylord, 2008; Gaylord et al., 2013; Sutherland and Melville, 2015). We then exposed larvae to a strong chemical inducer to evaluate the effects of turbulence exposure on larval competence and the shift to the juvenile stage.

Because our focus is on behaviors and developmental changes associated with the transition of larvae from a planktonic to benthic phase, we here provide definitions of several important terms (Hodin et al., 2018a). We define metamorphosis as a more-or-less radical morphological transformation between two multicellular life stages (Bishop et al., 2006). We refer to echinoid metamorphosis as the entire period of days or even weeks from the first formation of juvenile structures (known as the rudiment) in the larva until the juvenile begins to feed on the seafloor. The most dramatic event of metamorphosis is settlement: the irreversible habitat shift that an echinoid larva makes from the planktonic to the benthic zone, at which point that larva is now called a juvenile (see also Chia, 1978). This process of settlement, and the associated behavior of larvae contacting the substratum, is what we measured throughout the present study.

Although the developmental context for our study is the transition to competence, defined as the developmental state when larvae are capable of responding to settlement inducers by settling and completing metamorphosis, we note a relevant distinction as to the potential state of larvae prior to that shift. Before a larva is competent, it is either immature or precompetent. Immature larvae will not respond to a localized settlement cue under any circumstances, whereas developmentally more advanced precompetent larvae will immediately transition to competence when exposed to intense turbulence (>2 W kg^{-1} for 3 min), at which point they can respond to local cues (Hodin et al., 2015).

When rearing batches (cohorts) of larvae, there is always within- and among-batch variation in the time at which individual larvae attain competence. Therefore, for the experiments reported herein,

we examined batches of larvae where a certain proportion of the larvae were competent but the majority were not, indicating that there were precompetent larvae in the batch that might respond to turbulence by transitioning to competence. We note that competence, as it is defined for most species, is an operational definition based in large part on laboratory-reared larvae. It is formally possible that the timing of the onset of competence under laboratory conditions might differ from that in the field.

Source populations and collection
We collected adult *D. excentricus* from a subtidal population (∼1.5 m below the surface at mean lower low water) by snorkeling approximately 100 m offshore and 30 m east of Municipal Pier 2 in downtown Monterey, CA, USA, and transported them to Hopkins Marine Station (HMS; Pacific Grove, CA, USA), where they were maintained in flowing sea tables with sand until their use in fertilizations. In 2015 we collected 40 adults. In February 2017, the population had been seemingly severely impacted by recent storms; on that date we only located two adults from this formerly (over the preceding 10 years at least) very large population. Fortuitously, those two adults were one male and one female.

We acquired adult *S. purpuratus* at low tide from Slip Point (Clallam Bay, WA, USA) and maintained them in subtidal cages suspended off the floating docks at Friday Harbor Laboratories (FHL; Friday Harbor, WA, USA), fed throughout the year *ad libitum* with drift kelp (mainly blades of *Nereocystis luetkeana*).

Larval rearing
We followed standard methods to spawn and fertilize both species (Strathmann, 1987, 2014): intracoelomic injection with <1 ml of 0.5 mol l^{-1} KCl, collection of eggs in 0.45 µm Millipore-filtered seawater (MFSW; note that MFSW at HMS is also UV-treated), collection of sperm dry, individual male×female fertilizations, and then, if setting up mixed parentage cultures, mixture of embryos from independent crosses in approximately equal proportions. We cultured resulting embryos and early stage larvae at approximately 1 larva ml^{-1} in MFSW in glass jars, and fed them [starting 1 day post-fertilization (dpf) in *D. excentricus* and 3 dpf in *S. purpuratus*] with a mixture of 3000 cells ml^{-1} *Dunaliella tertiolecta* and 2500 cells ml^{-1} *Rhodomonas* spp. We cultured *S. purpuratus* at sea table temperatures (11–14°C) and *D. excentricus* at room temperature (19–22°C) throughout, unless otherwise indicated.

We removed 95% of the water by reverse filtration every 2 days, visually inspected and hand-cleaned the cultures as required, and then refilled the jars with MFSW and food as above. We stirred cultures either with a motor-driven stirring rack with paddles (Strathmann, 1987) or on a gyratory shaker platform. Following an established staging scheme (Heyland and Hodin, 2014), we reduced the density of cultures to ∼1 larva per 5 ml when they reached stage ii (contact of invaginating echinus rudiment with hydrocoel; approximately 3 dpf in *D. excentricus* and 15 dpf in *S. purpuratus*). We continued to culture the larvae at this density until late stages (∼30 dpf in *S. purpuratus*, ∼9 dpf in *D. excentricus*), at which point we conducted daily competence tests on a subset of the larvae from each batch using excess KCl in MFSW as described below. Once a small percentage of larvae in a batch had reached competence, we used larvae from that batch in turbulence exposure experiments as described below.

Overall experimental approach
We conducted five rounds of experiments from 2015 to 2017 (Table 1): three rounds of *D. excentricus* experiments at Bodega Marine Laboratory (BML; Bodega Bay, CA, USA), and two rounds of *S. purpuratus* experiments at FHL. Together, these experimental rounds encompassed trials where we examined the minimum time required to initiate an early transition to competence ('exposure time experiment'), whether or not the shift to competence perdured ('perdurance experiments'), and the proportion of larvae that were carried to the substratum immediately after turbulence exposure and the duration over which they remained there ('knockdown experiment'). The dates and cross designs for each round of larval rearing (i.e. larval cohort) are summarized in Table 1, and corresponding larval cohorts are designated as De1–3 for *D. excentricus* and Sp1–2 for *S. purpuratus*. Idiosyncratic information about two of our larval cohorts are as follows: for De3, after fertilization we held embryos at 14°C for the first 18 h of development and then warmed the hatched embryos up to their culturing conditions at room temperature (19–22°C); for Sp1, a colleague at FHL set up and maintained cultures for us until transporting them at ∼4 larvae ml^{-1} at 5 dpf to the University of Washington (Seattle, WA, USA). In Seattle, we thinned them out to ∼1 larva ml^{-1} and began feeding and caring for them as above. At 27 dpf, we transported them back to FHL for subsequent experiments.

Turbulence exposure protocol
In all five rounds of our study, we exposed larvae to a single intensity of turbulence of approximately 6–7 W kg^{-1} in a Couette device. We selected this value based on peak levels of turbulence that have been measured in the field on exposed rocky shores, and which also elicited maximal responses in our previous studies with larval settlement (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 more relevant to our study species and the conditions they likely experience in their approach to shoreline habitat.

Table 1. Experimental design for the five larval cohorts of *Dendraster excentricus* and *Strongylocentrotus purpuratus* used in this study

Larval cohort	Species	Collection date (mm-dd-yy)	Fertilization date (mm-dd-yy)	Cross design	Rearing temperature (°C)	Larval age (dpf) at:		
						Transport step	Density reduction	Experiments
De1	*D. excentricus*	07-23-15	07-24-15	2M×2F	19–22	3	3	11
De2	*D. excentricus*	07-23-15	02-21-16	3M×1F	19–22	1	3	9, 10
De3	*D. excentricus*	02-10-17	02-10-17	1M×1F	19–22	3	3	11
Sp1	*S. purpuratus*	Unknown	11-13-15	2M×1F	11–14	5, 27	15	35, 37
Sp2	*S. purpuratus*	Unknown	05-03-17	2M×2F	11–14	None	15	35

Note that we refer throughout the text to the larval cohort code, as listed in the first column. 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, so we do not know the collection dates of the particular urchins used to establish the larval cultures for this study.

We first concentrated larvae by reverse filtration and then selected and placed 15–45 larvae into individual 125-ml glass beakers at a density of 1 larva per 3–4 ml MFSW. *Dendraster excentricus* larvae in these experiments were developing very synchronously; we eliminated <5% of the larvae from consideration as being obviously delayed in development. *Strongylocentrotus purpuratus* larvae can develop somewhat asynchronously, so on the day of experimental trials with that species, we imposed a more stringent selection for larvae that appeared to be nearing competence, but were not adhered to the bottom [see also Gaylord et al. (2013) for our selection criteria to enrich for precompetent larvae]. Otherwise, we assigned larvae to beakers haphazardly. Then, we randomly assigned beakers to treatment 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 Couette device. We then subjected the entire water volume within the apparatus to the specified intensity of turbulence for a duration ranging from 30 s to 3 min, depending on the treatment. Immediately following each trial, we gently poured the larvae within the Couette device into a 1-liter glass beaker already containing approximately 100 ml of MSFW (to minimize additional stimulus to larvae during the pour), rinsed the Couette device 1–2 times with MFSW of the appropriate temperature to capture any remaining larvae, gently reverse-filtered the entire recovered volume back down to ~100 ml, and poured this remaining volume into a finger bowl for further observations and subsequent transfer into holding conditions (perdurance experiments) or directly into settlement assay conditions as described below. We generally recovered >95% of the larvae from the Couette device, all of which we used in ensuing settlement assays. We then rinsed the device thoroughly with distilled water to ensure that no living larvae were transferred to subsequent trials, and we initiated the next trial.

In concert with the treatment exposures, we exposed randomly selected batches of larvae to unmanipulated control conditions, where we poured the contents of the 125-ml beakers into finger bowls, as described above, and then immediately subjected them to holding conditions or settlement assays, as specified below. In previous studies (Gaylord et al., 2013; Hodin et al., 2015; J.H., unpublished data), we compared unmanipulated controls with handling controls (i.e. 0 W kg^{-1} treatments), where we treated the larvae the same as those assigned to the turbulence exposure treatments, except we did not activate the Couette device during the 3 min that larvae were within it, thereby controlling for manipulation effects. Because we never observed any differences between larvae exposed to unmanipulated and handling controls in those studies, we here eliminated the handling controls except in the knockdown experiment.

Competence and settlement assays

We assessed competence of the larvae in all exposure time and perdurance experiments by conducting settlement tests. We transferred all larvae from a trial to a single well of a pre-washed, non-tissue-culture-treated six-well plate (see Herrmann et al., 2003), maintained at the rearing temperature, and containing 8 ml of excess potassium in MFSW, which is a strong inducer of settlement in competent larvae (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, 2018c). The effective concentration for settlement induction in *D. excentricus* is 40 mmol l^{-1} excess KCl in MFSW (see Hodin et al., 2015); for *S. purpuratus* it is 70 mmol l^{-1} excess KCl in MFSW (see Gaylord et al., 2013).

After a 1-h exposure, we transferred all larvae to 8 ml of MFSW. 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, as per standard assays (e.g. Sato et al., 2006; Sutherby et al., 2012; Gaylord et al., 2013; Hodin et al., 2015; Mos and Dworjanyn, 2016). We verified continued withdrawal of tissue over the next several hours and eventual adoption of the definitive juvenile morphology, including emergent and active tube feet and spines. Larvae from all treatments that we had scored as not settled were living larvae, and we detected no post-settlement mortality.

Specific experiments
Exposure time experiment
To examine the minimum duration of turbulence exposure required to spark entry to competence, we exposed *D. excentricus* larvae to 0 s, 30 s, 1 min or 3 min of turbulence in the Couette device. We used larval cohort De1 (see Table 1). Following removal of the larvae from the device and their subsequent recovery in a finger bowl, we transferred them immediately into 40 mmol l^{-1} excess KCl in MFSW, and monitored settlement as described above.

Perdurance experiments
To determine whether the turbulence induction of competence is a temporary (and therefore reversible) or a sustained life-history shift, we held larvae for specified times following their turbulence exposure, and then assayed their state of competence following each hold period. If larvae remained competent despite their hold period, it would indicate that the turbulence-induced shift to competence was a sustained one. We used two separate protocols during these perdurance experiments (approaches 1 and 2 in Fig. 1), employing the second one to examine a potential complicating factor.

In our initial experiments (*D. excentricus*: larval cohorts De1 and De2; *S. purpuratus*: larval cohort Sp1; see Table 1), we undertook the post-turbulence exposure holds in the same six-well plates that we used for our settlement trials; however, instead of transferring the larvae directly into excess KCl, we first transferred them into wells with 8 ml of MSFW for a specified hold time of 1–24 h, after which we transferred them into excess KCl and proceeded as described above (shown as approach 1 in Fig. 1A). For the *S. purpuratus* experiment in which we held larvae for 0, 4, 11 or 24 h before settlement challenge, we employed a slight variant of approach 1 simply to increase the number of trials we could run in a single experiment. Here, we exposed 40 larvae (cohort Sp1 in Table 1) at a time to turbulence in the Couette device, and after recovery, haphazardly assigned the recovered larvae to four separate wells, with ~10 larvae in each well; three wells contained 8 ml of MFSW, and corresponded to the 4, 11 and 24 h holds, respectively, and the fourth well contained 40 mmol l^{-1} excess KCl in MFSW, i.e. the 0 h hold treatment. We exposed eight replicates of 40 larvae each to this treatment protocol, and eight replicates of 40 larvae each to unmanipulated control trials, hapahazardly split into the four hold times in the same way.

We employed approach 2 (Fig. 1B) to determine whether the larvae in the hold conditions described above might behave differently in wells of still water than in their standard culturing conditions. In these later experiments, we transferred four replicates of *S. purpuratus* larvae (cohort Sp2 in Table 1) following turbulence exposure and eight replicates of unexposed larvae into 125-ml beakers of MFSW at the same larval and food density as they had experienced throughout their late larval period. During the hold period, we mixed the beakers gently on a gyratory shaker platform. After the specified hold period, we gently poured the entire contents of the beaker into a glass finger bowl, at which point the larvae
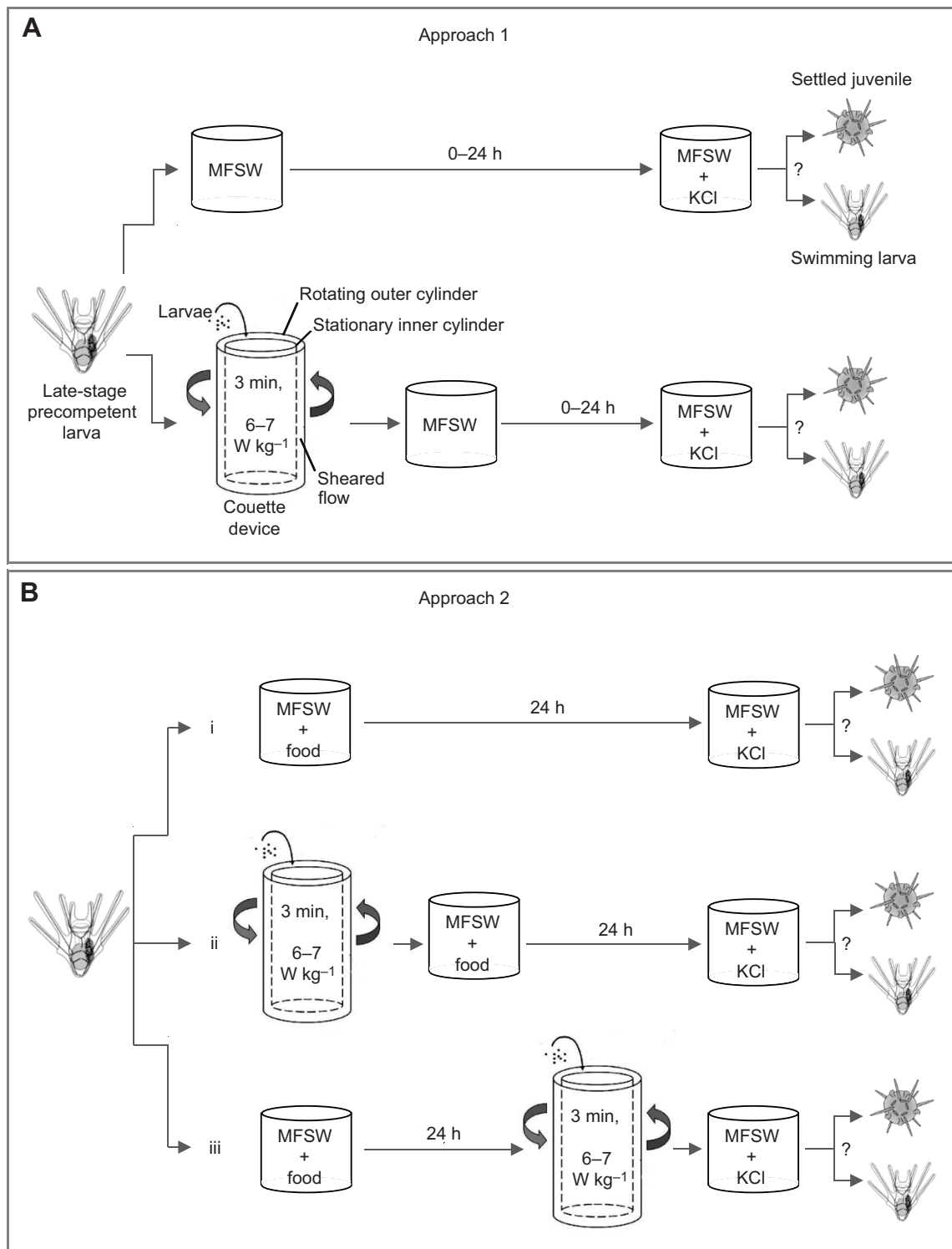
Fig. 1. Schematic of experimental methods for perdurance experiments using precompetent larvae. (A) We used approach 1 for our initial set of perdurance experiments to compare controls (upper arrow) with turbulence-treated larvae (lower arrow). After exposure of larvae to turbulence, we held the larvae in still Millipore-filtered seawater (MFSW) at 3–5 larvae ml⁻¹ for a specific hold period (0–24 h), followed by exposure of larvae at that same density to a strong settlement inducer and indicator of competence: excess potassium chloride (KCl) in MFSW. We treated controls the same as experimental larvae but did not expose them to turbulence. (B) We refined that protocol with approach 2. Here, all larvae were at the same age when tested for competence, and the hold conditions mimicked the rearing conditions the larvae had experienced throughout: with food and at low larval density (~1 larva per 5 ml). Using approach 2, we either (i) held them for 24 h in rearing conditions before the settlement assay, with no exposure to turbulence (control); (ii) exposed larvae to turbulence and then held them for 24 h in rearing conditions before the settlement assay; or (iii) held them for 24 h in rearing conditions before exposing them to turbulence followed directly by the settlement assay. See Table 1 and Materials and Methods for details on rearing conditions.

received one of three treatments. In our control treatment (i), we transferred four of the replicates of unexposed larvae after their 24 h hold directly into 70 mmol l^{-1} excess KCl in MFSW to assess what proportion of our larvae were already competent following the hold period; in treatment ii, we transferred the four replicates of larvae exposed the previous day to turbulence directly into 70 mmol l^{-1} excess KCl in MFSW to assess their state of competence after the hold period; and in treatment iii, we exposed the other four replicates of unexposed larvae after their 24 h hold to 3 min of 7 W kg^{-1} turbulence, and then after recovery from the device, transferred them immediately into 70 mmol l^{-1} excess KCl in MFSW.

Knockdown experiment

In prior work (Hodin et al., 2018b,c), we reported that immediately following turbulence exposure and recovery of larvae from the Couette device, *D. excentricus* larvae were knocked down and tended to remain on the substratum; these larvae appeared stunned, in that they moved very little or not at all for a time before recovering and beginning to swim again. Here, we repeated these tests with *S. purpuratus* larvae, and then examined further details of the phenomenon with *D. excentricus* larvae.

Following retrieval of *S. purpuratus* larvae (larval cohort Sp2; see Table 1) from the Couette device, and before transferring them into settlement or hold conditions, we recorded the numbers of larvae swimming or otherwise off the bottom versus those in contact with the bottom of the recovery bowl. We used these counts to calculate the proportion of larvae on the bottom for each trial.

In order to determine how long this knockdown effect of turbulence might last, we exposed 22–25 larvae per replicate of *D. excentricus* from larval cohort De3 (see Table 1) to 0 or 6 W kg^{-1} turbulence for 3 min (three replicate trials at each turbulence intensity), and then after gently concentrating the recovered larvae to approximately 100 ml MFSW by reverse filtration, we carefully transferred that entire volume into a finger bowl for further observations in a binocular microscope. At regular intervals over the subsequent 30 min, we scored all of the larvae as either on the bottom (i.e. contacting the bottom of the bowl) or not, thus monitoring recovery of the larvae from any knockdown effect that they may have experienced from the prior turbulence exposure.

Statistical analysis

We conducted all statistical analyses with R (https://www.r-project.org/; v3.4.2) using the lme4 (Bates et al., 2015) and emmeans (https://CRAN.R-project.org/package=emmeans) packages. We used a logistic mixed-effects model to analyze data owing to the binomial nature of our response variable (larvae settled or knocked down). In all cases, each exposure of a group of 15–40 larvae was treated as a random intercept.

For the exposure time experiment, we treated duration of turbulence exposure as both a categorical and continuous predictor, allowing us to determine whether any period of exposure resulted in an increase in settlement and whether there was a positive trend in settlement as exposure duration increased. We used a Bonferroni correction to account for this dual comparison.

We conducted three perdurance experiments following approach 1 (Fig. 1A). For the first two experiments with *D. excentricus* (larval cohorts De1 and De2 in Table 1), we treated turbulence exposure and hold times, along with the interaction, as categorical variables. If a significant interaction was detected, we used planned contrasts to determine whether there was a difference between turbulence exposure at each hold time and whether there was a difference between hold times at each turbulence exposure. For the experiment

with *S. purpuratus* using approach 1 (larval cohort Sp1 in Table 1), we also treated turbulence exposure as a categorical variable; however, we assumed that hold time was a continuous variable in determining whether the slopes between the two exposure treatments differed significantly. Here, in addition to exposure replicates, we also included days post-fertilization as a random intercept because we conducted the experiment on two separate days: 35 and 37 dpf. In the experiment with *S. purpuratus* (larval cohort Sp2 in Table 1) following approach 2 (see Fig. 1B), we treated the three different turbulence treatments as categorical variables and used a subsequent Tukey test to calculate which treatments were significantly different from each other.

To examine how turbulence treatment affected the knockdown behavior of larvae, we used larval cohort Sp2 and employed a categorical logistic regression. We scored knockdown behavior as a binomial response, with turbulence exposure (versus control) as a categorical variable. We also included replicates as a random intercept to account for the multiple larvae within each exposure trial (replicate).

When testing short-term behavioral effects of turbulence exposure on *D. excentricus* (recovery from knockdown; larval cohort De3 in Table 1), we treated minutes after exposure as a continuous variable, whereas turbulence exposure (versus control) was a categorical variable. We first shifted the exposure by 3 min, so that the intercepts of the model explicitly compared whether there was a difference in number of larvae knocked down at the first time point (which was 3 min after the completion of the exposure in the Couette device). We also included an interaction term between minutes after exposure and turbulence exposure to determine whether the slopes between the two turbulence exposures significantly differ.

RESULTS

Competence is induced by 30 s of exposure and the effect increases with exposure duration

We previously showed that, in contrast with the lack of responses by immature larvae, precompetent *S. purpuratus* (Gaylord et al., 2013) and *D. excentricus* larvae (Hodin et al., 2015) will immediately transition to competence following a 3 min exposure to strong turbulence (6–7 W kg^{-1}). Here (Fig. 2), we treated a batch of mostly precompetent *D. excentricus* larvae to turbulence exposures lasting from 30 s to 3 min, and compared their settlement proportions with those of unmanipulated controls (UMC). We analyzed these data in two ways (note that the *P*-values listed here are after Bonferroni correction). First, considering exposure time as a categorical variable, we found that settlement rates associated with each of the three exposure durations (30 s, 1 min and 3 min) were significantly different from the unspun controls (UMC versus 30 s: $Z=2.326$, $P=0.040$; UMC versus 1 min: $Z=2.703$, $P=0.014$; UMC versus 3 min: $Z=4.111$, $P<0.001$; $n=4$ replicate trials of each exposure duration and UMC, 19–25 larvae per replicate trial). Second, with duration as a continuous variable, we showed an increased response with increased duration, as judged by a significantly positive slope of the regression (0.609 log odds increase in settlement per minute of exposure; $Z=3.563$, $P<0.001$).

Induction of competence by turbulence is long-lasting

In prior experiments, we always tested larvae for competence immediately following turbulence exposure and recovery of the larvae from the Couette device. Here, we asked whether the observed induction of competence by turbulence exposure is a transient or a long-term life-history shift.

Our initial perdurance experiments (approach 1; Fig. 1A) demonstrated that the transition to competence by turbulence exposure is long-lasting. Competence, once induced, persisted for at
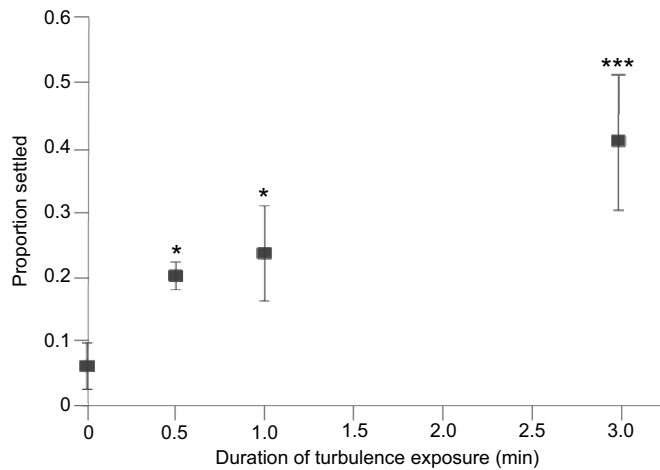
Fig. 2. Increased exposure duration leads to increased settlement responses in larval sand dollars (*Dendraster excentricus*). All three exposure durations (0.5, 1 and 3 min) to intense fluid turbulence (6 W kg^{-1}) resulted in enhanced settlement responses relative to unmanipulated controls (UMC; 0 min exposure). Furthermore, the positive slope of the regression ($P<0.001$) indicates that the response is enhanced with increasing exposure time. See Results for details on statistical comparisons. Error bars are ±s.e.m. Asterisks indicate significance of pairwise comparisons between turbulence exposures and unexposed controls: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

least 6 h in *D. excentricus* (turbulence: $Z=-4.265$, $P<0.001$; time: $Z=-0.877$, $P=0.381$; interaction: $Z=1.583$, $P=0.113$; Fig. 3A) and at least 24 h in *S. purpuratus* (turbulence: $Z=-3.055$, $P<0.01$; time: $Z=1.722$, $P=0.085$; interaction: $Z=-1.480$, $P=0.139$; Fig. 3C).

Importantly, we saw no evidence of a reversion to precompetence with lengthy hold times following exposure. Specifically, in the longer hold times for the turbulence-exposed larvae shown in Fig. 3B (*D. excentricus*) and 3C (*S. purpuratus*), we did not observe an attenuation of the settlement responses relative to the corresponding no-hold treatments (i.e. we did not see decreased settlement with longer holds after exposure).

Nevertheless, we did see evidence in controls for increasing settlement responses with longer hold times in *D. excentricus* (Fig. 3B), where we observed a strong effect of turbulence and a turbulence-by-time interaction (turbulence: $Z=-6.496$, $P<0.001$; time: $Z=1.728$, $P=0.084$; interaction: $Z=3.723$, $P<0.001$). Further planned contrasts within those data revealed that the difference between controls and turbulence-exposed larvae immediately after exposure (0 h: $Z=6.496$; $P<0.001$) was still present after 18 h ($Z=2.801$, $P<0.01$), but that the proportion settled showed a clear increase between 0 and 18 h in the control treatment only (control: $Z=-6.636$, $P<0.001$; turbulence-exposed: $Z=-1.728$, $P=0.084$). In *S. purpuratus* (Fig. 3C), there was a hint of a similar effect (i.e. an apparent increase in settlement in the controls only) after a 24 h hold, but not significantly so (turbulence-by-time interaction: $Z=-1.480$, $P=0.139$). These observations in both species suggest that unexposed larvae in our hold conditions may be transitioning to competence either through aging or owing to the relatively crowded starvation conditions under which we were maintaining these larvae during their hold periods, or both (see Materials and Methods and Fig. 1 legend).

Our modified perdurance experimental protocol (approach 2; Fig. 1B) addressed the potential crowding and starvation issues explicitly, and ensured that all larvae being compared were at the same age (in dpf). Under the modified conditions of approach 2, larvae from both turbulence treatments (exposed either before or after
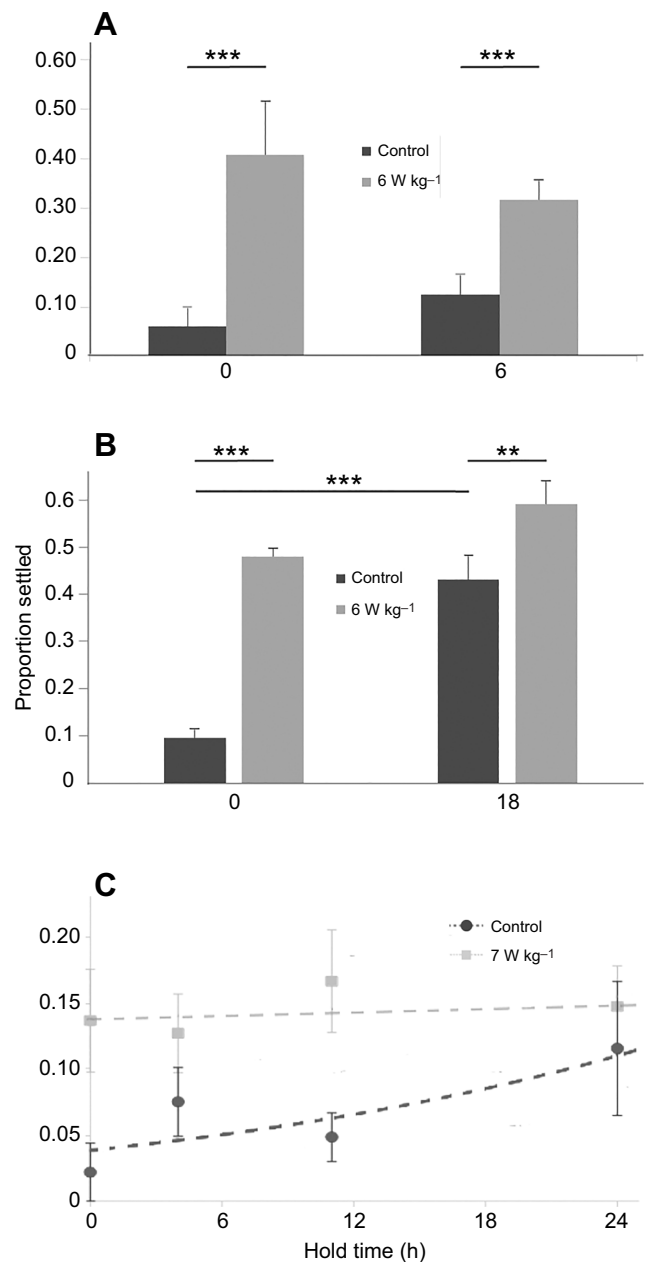
Fig. 3. Brief turbulence exposure causes long-term induction of competence in a sand dollar and a sea urchin. (A,B) *Dendraster excentricus*. We exposed larvae to unmanipulated control conditions or 6 W kg^{-1} turbulence for 3 min and tested for their settlement responses either 0 and 6 h after exposure (A: 4 replicate trials per treatment; 21–25 larvae per replicate trial) or 0 and 18 h after exposure (B: 4–7 replicate trials per treatment; 21–30 larvae per replicate trial). The induction of competence lasted for at least 6 h (A); by 18 h, the controls had become competent in large numbers, though still significantly fewer than in exposed larvae at 18 h. (C) *Strongylocentrotus purpuratus*. Here, we exposed eight replicates of 40 larvae each to 7 W kg^{-1} turbulence for 3 min or unmanipulated control conditions, and then haphazardly divided the recovered larvae approximately equally into four different hold times (0, 4, 11 and 24 h) followed by a competence test as above. We again see evidence for perdurance of turbulence-induced competence in exposed larvae. Asterisks in A and B indicate significant pairwise difference between the treatments connected by the solid lines above the graph: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Error bars are ±s.e.m. For statistical comparisons in C, see Results. All experiments here followed approach 1 (see Fig. 1A).
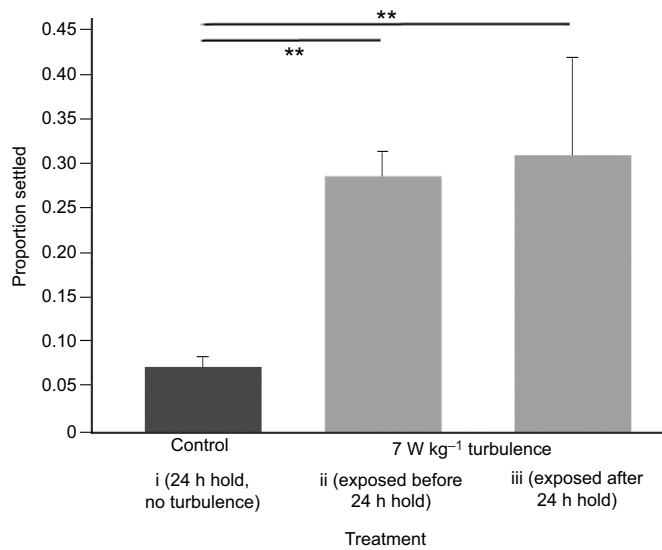
Fig. 4. Sea urchin larvae exhibit a sustained induction of competence following turbulence exposure. *Strongylocentrotus purpuratus*. See Fig. 1B (approach 2) for description of the control (i) and the two turbulence exposure treatments (ii, iii). With this improved protocol, we now see clear evidence that a 3 min exposure to 7 W kg^{-1} turbulence causes a lasting induction of competence. N=23–25 larvae per replicate trial, 4 replicate trials per treatment. Asterisks indicate pairwise comparisons between treatments connected by the bars: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Error bars are ±s.e.m.

a 24-h holding period; treatments ii and iii, respectively, in Fig. 1B) settled in significantly higher numbers when compared with control larvae [treatment i (control) versus ii: $Z=-3.247$, $P=0.003$; treatment i versus iii: $Z=-3.082$, $P=0.006$; Fig. 4]. However, we detected no difference between the two turbulence-exposed treatments (treatment ii versus iii: $Z=0.168$, $P=0.985$). In sum, these results again indicated that the induction of competence by turbulence is a sustained life-history shift, showing no evidence for attenuation even after 24 h.

A distinct behavioral response to turbulence reverses in fewer than 30 min

In our knockdown experiment, *S. purpuratus* larvae displayed a similar behavior to that seen by us in prior work on *D. excentricus* (Hodin et al., 2018b,c). Here, we found that 64±4% (mean±s.e.m.) of *S. purpuratus*

larvae remained on the bottom following a 3 min exposure to 7 W kg^{-1} turbulence, compared with 37±5% of unexposed control larvae ($Z=4.250$, $P<0.001$). Unlike the sustained life-history shift to competence, this knockdown effect of turbulence was reversible and quite short-lived. To determine the time course of this reversal, we observed several batches of exposed and unexposed *D. excentricus* larvae in the 30 min following the cessation of the exposure to turbulence (Fig. 5). In this experiment, five times as many exposed larvae were on the bottom at our first time check (~4 min after exposure) when compared with unexposed larvae (35±8% versus 7±4%, respectively) ($Z=4.173$, $P<0.001$). Over 30 min of subsequent observations, we detected a clear interaction of time and turbulence exposure ($Z=-2.839$, $P=0.0045$), with the proportion of exposed and unexposed larvae on the bottom converging by approximately 22 min after exposure (8±2% versus 6±4%, respectively).

DISCUSSION

Dispersing larvae returning to wave-swept shores must not only contend with highly dynamic flow environments, but may also use such conditions as an indication that they could be approaching suitable settlement habitat (Chia et al., 1981; Fuchs et al., 2004; reviewed in Hodin et al., 2018a). Our results reveal important temporal dynamics of larval responses to the intense turbulence characteristic of the surf zone of such high-energy shores. Turbulence exposures as brief as 30 s spurred early transitions to competence, with longer exposures inducing a greater proportion of larvae to become competent (Fig. 2). However, that heightened state of readiness to respond to chemical inducers of settlement does not fade after a larva reenters calmer waters; even 24 h later, turbulence-exposed larvae remain fully competent to complete metamorphosis (Fig. 4). In contrast, a distinct behavioral response following turbulence exposure that brings larvae into contact with the substratum persists for <30 min (Fig. 5).

We emphasize that our experiments do not reveal which fluid-dynamic aspects of turbulence might be responsible for the larval responses. Turbulence is characterized by strong velocity gradients (shear), rotational motions (vorticity), linear and angular accelerations, pressure gradients associated with those accelerations, and appreciable spatio-temporal variation in underlying coherent flow structures (Jumars et al., 2009). Ongoing and future experiments to isolate which of these or other features of turbulence operate as the
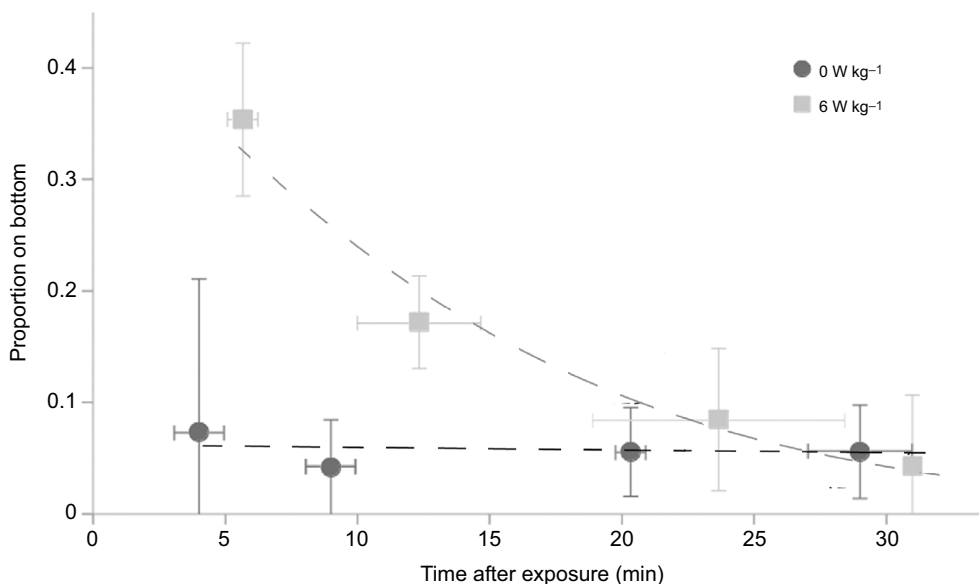

Fig. 5. The knockdown effect of turbulence exposure is reversible and short lived. *Dendraster excentricus*. We here exposed three replicates each of 23–25 larvae in the Couette device to 3 min of either 0 or 6 W kg^{-1} turbulence and, following recovery, we repeatedly recorded their position (either down contacting the bottom or up swimming in the recovery bowl) for 30 min. By approximately 20 min after turbulence exposure, the knockdown effect is no longer apparent. See Results for statistical comparisons. Error bars are ±s.e.m.

proximal trigger for precocious competence or for observed knockdown behaviors will be an important extension of this work (e.g. Fuchs et al., 2018).

Regardless of the precise drivers of the larval responses we have examined, their temporal characteristics have substantial implications for the ecology and biology of these animals. In the days leading up to nominal competence – the stage at which they will settle in response to local cues in the absence of turbulence – sand dollar larvae are increasingly responsive and sensitive to turbulence; following turbulence exposure, larvae immediately become competent and thus exhibit a significant shortening of the precompetent period (Hodin et al., 2015). Here, we further demonstrate that responsiveness increases with exposure time, suggesting that larvae in nature might respond differently to different durations of intense turbulence. For example, anomalous biogenic turbulence or other brief mixing events may not be sufficient to prompt a majority of late-stage larvae to suddenly attain competence, whereas more chronic exposure to strong turbulence within a series of breaking waves should trigger a greater proportion of larvae to become responsive to subsequent encounters with chemical settlement inducers. This kind of fail-safe mechanism preventing inappropriate responses could be advantageous to larvae given the generally irreversible nature of the settlement transition, and hence the need to make appropriate decisions at that transition (Keough and Downes, 1982).

What advantage might there be for larvae to remain competent for a protracted period following turbulence exposure, even in the absence of settlement inducers? One possibility may relate to species with nearshore adults whose typical habitat is not within the surf zone itself, yet whose larvae may still benefit from being primed to settle by turbulence exposure. One such example is our study population of *D. excentricus* in Monterey, which is located in a partially protected sand bed within 100 m of the surf zone. Such larvae, once exposed to turbulence, would retain a heightened state of sensitivity to chemical settlement inducers, thereby allowing them to remain primed to settle until they are moved out of the surf zone into more appropriate adult habitat.

It may be that relationships between the intensity or duration of surf-zone turbulence and the proximity of appropriate settlement habitat facilitate species-specific responses to turbulence that are adaptive for larvae in the context of other, perhaps less predictable environmental features that could help guide larvae from a pelagic to benthic existence. We might expect that different taxa should exhibit different sensitivities to turbulence exposure time and also to the temporal offset between exposure to turbulence and the subsequent induction of settlement.

Is the induction of competence by turbulence permanent?

Formally, we have not demonstrated that the induction of competence is permanent; to do so, we would have had to follow larvae for a number of days until all of the control larvae became competent in the absence of turbulence. If during that period, the exposed larvae showed no signs of an attenuated response (i.e. no reversion from competence to precompetence), then we could conclude with more certainty that the response was truly permanent. Nevertheless, we see no evidence in any of our experiments that an 18–24 h period following turbulence exposure is accompanied by any demonstrable regression to precompetence (see Figs 3 and 4). And because 18 h is nearly half of the time it takes for precompetent *D. excentricus* larvae to become competent in the absence of turbulence (Hodin et al., 2015), the evidence that we do have points to the response being effectively, if not formally, permanent.

Experimental advantages of decoupling exposure to turbulence and settlement inducers

One important outcome of our study was the refinement of a protocol for examining the perdurance of larval responses to turbulence exposure, which sharpened our understanding of the nature of the turbulence trigger. Initially, we examined the temporal persistence of larval responses by exposing larvae to turbulence and then holding those larvae in completely still, filtered seawater for various periods of time before offering chemical settlement inducers (approach 1; Fig. 1A). Despite resulting indications that turbulence exposure could yield lasting changes in larval readiness to settle, confounding effects of larval aging as well as possibly stressful holding conditions led us to develop a more robust protocol in which treatment and control larvae were held in conditions mimicking their larval rearing conditions – gentle shaking, with food, at low larval density – and tested at identical larval ages (approach 2; Fig. 1B). This modified protocol not only allowed us to demonstrate an effectively permanent life-history shift in larvae exposed to turbulence (Fig. 4), but it also provides a potential methodological tool for investigating the neurophysiological and other mechanistic underpinnings of the response to turbulence as well as of subsequent settlement decisions.

For example, to test whether the activation of stretch-activated ion channels (Christensen and Corey, 2007) is involved in initiating larval responses to turbulence, one could introduce chemical inhibitors of those channels in concert with turbulence exposure, wash out the inhibitor following exposure, and then hold the larvae in rearing conditions for an extended period before inducing settlement (e.g. up to 24 h); this should allow full recovery of the larvae from any non-specific effects of the inhibitor. If such larvae then fail to settle, one could have more confidence that the inhibitor effect was a specific one, indicating functional importance of that mechanism for the turbulence response. Another example would involve a search for the mechanism of the perdurance itself. Our observation of larvae 'remembering' the exposure to turbulence for a protracted period is reminiscent of long-term potentiation (LTP) in learning and memory. In both vertebrates (Sweatt, 1999) and invertebrates (Hawkins et al., 2006), LTP is accompanied by lasting changes in the phosphorylation state of protein kinases. Treatment of turbulence-primed larvae with phosphatase agonists (e.g. sodium selenate or regorafenib; Corcoran et al., 2010; Fan et al., 2014) may therefore cause these larvae to 'forget' their turbulence exposure and revert to precompetence.

The ability to subject larvae to such manipulations while delaying settlement and hence the completion of metamorphosis itself also provides a unique and relatively unambiguous method for elucidating the molecular and physiological changes within larvae affected by turbulence. Gene products extracted from larvae exposed to turbulence in the laboratory – and then held without inducing settlement – could be compared with extracts of control larvae of the same age to examine the occurrence and magnitude of gene transcription, translation or post-translational modifications (see Williams and Carrier, 2018) induced by turbulence exposure. The advantage of this protocol is that it avoids the confounding effect of the tremendous changes in cellular activity that occur as part of the settlement transition itself, during which larval tissues undergo apoptosis, juvenile tissues rapidly rearrange and grow, and the juvenile begins to function in its new benthic habitat (Chia and Burke, 1978; Hodin, 2006; Sato et al., 2006). Such experimental approaches could yield profound new insights into how larvae are affected by exposure to turbulence and, more generally, how they

interact with their environment to coordinate their irreversible transition to the benthos.

Perdurance of larval responses to turbulence also will allow for careful manipulation of other environmental features that demonstrate gradients among different marine environments (e.g. from deep sea to estuaries) such as temperature, food concentration, larval density and acoustic characteristics (see Hodin et al., 2018a for review). In this way, and as we discuss further below, we can begin to build a hierarchical understanding of the manner in which larvae assess the broad array of potential environmental cues to make key life-history decisions, and how those strategies vary among closely and distantly related taxa.

Furthermore, combining manipulations of the duration of turbulence exposure with manipulations of hold time following exposure (but before settlement induction) should allow for more targeted alignment of experimental treatments with species-specific time scales of larval transport and delivery to adult habitat (see Pineda and Reyns, 2018). Such approaches also may be useful in potential aquaculture applications in which short periods of turbulence exposure could help synchronize settlement in situations with variable rates of larval development (Hodin et al., 2018b).

Sensing the shoreline: cue hierarchies and reversible versus irreversible responses

We expect that many larvae of shoreline invertebrates experience a time lag between exposure to intense turbulence and chemical inducers in nature, in that they may pass through regions of high turbulence before final transport and delivery to suitable adult habitat. This situation could be especially true for species such as one of our target taxa, the Pacific sand dollar (*D. excentricus*), which often inhabits protected sandy sites directly adjacent to exposed shoals. The propensity to remain primed to settle after experiencing intense turbulent mixing therefore could benefit such species by allowing them to respond quickly to any subsequent exposure to appropriate settlement inducers, and also to be open to influence by positive as well as negative modulators (e.g. Woodin, 1991) of the irreversible settlement transition.

In contrast, permanent or even long-term induction of competence by turbulence could be disadvantageous if larvae of coastal species experience such conditions far away from shore (e.g. in surface waters during strong storm events; Gargett, 1989), particularly in larvae that are less discriminating about chemical inducers and might mistakenly respond to transient encounters with a piece of drift kelp or a floating log bearing an attached conspecific, for example (Crisp, 1974). Whether larvae primed by turbulence maintain their subsequent responsiveness to settlement inducers could be described as a trade-off associated with the low likelihood of encountering a high-quality settlement cue in appropriate adult habitat. LTP or memory of turbulence exposure therefore may itself be subject to selection, and could be an important detail to consider when examining the later stages of metamorphosis and their ecological implications.

Because larvae are likely exposed to many more classes of potential stimuli during their approach to adult habitat than just fluid turbulence, some of those stimuli could also serve as possible habitat-scale cues (Fuchs et al., 2010; Hodin et al., 2018a). Thus, we expect larvae to integrate multiple types of cues when assessing whether a given location seems like a promising place to commit to settlement. Previous studies on a range of behaviors have demonstrated how terrestrial and aquatic animals utilize and integrate multiple cue modalities (e.g. Dusenbery, 1992; Pluta and Kawasaki, 2008; Harley et al., 2011; Igulu et al., 2013; Ravi et al.,

2016; Lattenkamp et al., 2018), and specifically how cue hierarchies can lead to optimal decisions in dynamic marine environments (e.g. Kingsford et al., 2002; Woodson et al., 2007; Fuchs et al., 2010; Seuront, 2013).

Numerical modeling of such processes in marine invertebrate larvae, such as by varying the encounter rates of larvae with different environmental cues and then examining outcomes with larvae that differ in the degree of their selectivity and choosiness for various cues and settlement inducers, could be fruitful. Laboratory experiments using natural inducers and/or variable rearing conditions could help to elucidate the relative importance of different stimuli and potential habitat-scale cues, and could elicit a more natural level of variability in larval responses, as compared with more traditional experiments that rely on more uniform and benign rearing conditions and only highly inductive chemical settlement inducers.

Regardless of prior life experience, no single set or sequence of cues will be entirely reliable for every larva (Crisp, 1974), yet given the irreversible nature of settlement into the benthos for echinoids and for the vast majority of marine invertebrates, each individual larva should strive to make optimal decisions with the information available. Although some adult echinoids can move or relocate somewhat after settlement, in others that movement is extremely limited (e.g. Dumont et al., 2004) and it is likely that early juvenile survival will suffer if larvae make a poor decision about where and when to settle.

Short-term versus long-term behavioral responses to turbulence

In some cases, the behavioral effects of turbulence exposure are quite obvious, such as when larvae settle preferentially in locations characterized by strong or weak turbulence (e.g. Mullineaux and Garland, 1993), when they are knocked down to the substratum immediately after exposure (Fig. 5), or when downward swimming or sinking responses are displayed in response to hydrodynamic cues (Clay and Grünbaum, 2010; Fuchs et al., 2004; 2015). In any of these cases, it seems likely that larvae could capitalize on their newfound proximity to the bottom, where chemical inducers are available, facilitating attachment and ultimately completing metamorphosis.

Such short-term behavioral responses to turbulence exposure are worthy of more examination, yet they would seem to differ qualitatively from the long-lasting advancement in competence that we document in this study. The long-term nature of the latter response to turbulence suggests something distinct from more transient behavioral responses, instead indicating a life-history shift in response to environmental conditions.

We have previously shown that larvae that exhibit knockdown behavior following turbulence exposure are more likely to settle in response to a chemical inducer than larvae that remain swimming after exposure (Hodin et al., 2018c). Those results lead to the intriguing possibility that, despite the distinct temporal dynamics of the knockdown effect (short-term) and competence induction (long-term), the two behaviors might be physiologically connected. Such a finding would be reminiscent of how the early phase of LTP – driven by non-transcriptional changes in phosphorylation state of proteins – is distinct from but still inductive of the late phase, which is dependent on gene transcription (Lynch, 2004).

Conclusions

The ability of planktonic larval invertebrates to recruit into suitable adult habitat depends in part on their sensory responses to

environmental cues, including small-scale hydrodynamic fluctuations associated with waves and turbulence. Past research by ourselves and others has shown that larvae respond behaviorally to various aspects of fluid motion, which can result in passive or active movement of larvae toward the substratum where benthic settlement may occur. Our past work has also shown that echinoid (sea urchin and sand dollar) larvae respond to intense turbulence characteristic of energetic breaking waves in an distinct and unexpected manner: they suddenly become precociously responsive (i.e. 'competent' to respond) to olfactory settlement cues associated with the benthos. Together, these observations suggest a general scenario where larvae whose adults dwell in nearshore regions use exposure to extreme turbulence as a proxy for their arrival into the neighborhood of suitable adult habitat (Chia et al., 1981). Such exposure not only leads larvae to be carried to the substratum, where inducers of settlement can be encountered, but also heightens larval responsiveness to settlement inducers once they arrive there.

Here, we refine this general scenario by elucidating temporal dynamics of larval responses to turbulence. Although we observe both classes of responses to turbulence in late-stage echinoid larvae – remaining on the substratum and shifting to competence – the duration of these effects are quite distinct. The behavioral response that leads larvae to be carried to the bottom is quickly reversed: in the absence of chemical settlement inducers, turbulence-exposed larvae resumed swimming in fewer than 30 min. In contrast, such larvae remained precociously responsive to settlement inducers for at least 24 h relative to unexposed (control) larvae. From a mechanistic perspective, this observation of a sustained priming effect of turbulence is reminiscent of LTP in animal learning and memory. From an ecological perspective, the perdurance of the turbulence effect suggests that even for species whose adults live in calmer habitats near to but outside the surf zone, sustained readiness of larvae to respond precociously to settlement inducers could be an adaptive response to habitat-scale cues indicative of proximity to shoreline habitat. Further study is needed to understand how other informative habitat-scale cues might likewise influence larval decision-making during the critical and generally irreversible life-history shift from planktonic to benthic existence.

Data availability
Data are available from the National Science Foundation BCO-DMO: https://www.bco-dmo.org/project/472793

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