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Turbulence exposure recapitulates desperate behavior in late-stage sand dollar larvae

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Abstract

Background: A common life cycle pattern in benthic-dwelling taxa in the ocean is an obligate *pre-competent* dispersal period of microscopic propagules followed by their entry into a *competent* period, during which they are capable of transitioning back to the sea floor at settlement. The behavior of larvae during their competent periods varies: some taxa are characterized by discriminating larvae that will only accept high quality habitat in which to settle, whereas larvae from other taxa may initially be discriminating, but will become increasingly 'desperate' to settle as they age. Larvae in this latter class, if they fail to encounter optimal settlement habitat, are observed to accept increasingly sub-optimal habitat as their competent period progresses.

Results: Here we show that a brief exposure of competent sand dollar larvae (*Dendraster excentricus*) to a high intensity of turbulent shear shifts larvae into less discriminating settlement behavior. Specifically, turbulence-exposed larvae will settle in response to a wider variety of settlement cues, and will do so more rapidly than will their unexposed siblings. We also report that turbulence causes larvae to sink to the bottom in greater numbers, presumably increasing their exposure to cues on the substrate.

Conclusions: We conclude that fundamental aspects of larval life history and habitat selection are sensitive to short term environmental exposure. We discuss these findings in the context of how larvae might more generally utilize transient features of their environment to make key life history decisions. Such a perspective has implications for issues as diverse as aquaculture, population recovery following disturbance, and the sensitivity and response of marine organisms to climate change.

Keywords: Metamorphosis, Settlement, Competence, Fluid dynamics, Larval behavior, Desperate larva hypothesis, Echinodermata, Pluteus, Recruitment, Sensory biology

Background

The typical marine life cycle –from kelp, to clownfish, to clams– involves a sedentary or sessile adult that releases offspring that disperse. In animals, the dispersing form is often called a larva, which subsequently undergoes a metamorphic process as it settles back into benthic habitat and begins its juvenile stage. During their dispersal period, larvae face numerous challenges: acquiring food, avoiding predation, navigating oceanic flows that can carry them away from suitable adult habitat, recognizing such habitat when they have reached a stage capable of transitioning to

the benthos, and attaching and settling successfully therein (reviewed in [1]).

The developmental stage at which larvae are capable of transitioning and settling into a suitable benthic location is called *competence*. What constitutes an appropriate settlement location for a given larva can vary both among species and through that larva's *competent period* itself (see Methods for our definitions of these and related terms). Broadly speaking, competent larvae can be considered to fall on a spectrum of choosiness with respect to their settlement location. At one end of the spectrum are larvae that exercise marked habitat selectivity: such larvae will accept "Death Before Dishonor" [2], and continue dispersing as competent larvae either until either a high quality benthic habitat is detected or

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they perish. At the other extreme of the spectrum are non-discriminating larvae that exhibit no habitat selectivity at all; they will simply settle on the first available solid surface they encounter. Less extreme cases involve larvae for whom choosiness is modulated as they proceed through their competent period [2–6]. Early competent larvae of this type tend to be discriminating, but as time passes and no high quality habitats are found, such larvae become less selective and may eventually settle onto any number of solid surfaces (the so-called Desperate Larva Hypothesis [2–6]). In other words, as larvae of this type age, they behave more 'desperately' with respect to site selection, and thus act increasingly akin to the extreme non-discriminators. Indeed, the observation of so-called 'spontaneous settlement' (i.e., settlement in the absence of any specific trigger) in aging cohorts of larvae has been used as a means of characterizing larvae as behaving 'desperately' (reviewed in [2]). We note that this concept of 'desperation' is about a *decline in choosiness*, and should not be taken to suggest that such larvae are operating out of "fear" or "panic", as one might otherwise infer from the vernacular use of the term.

Age is not the only variable that can modulate a larva's decision to either settle or not settle. Food availability [7], exposure to toxins [8], low pH [9] and low salinity [10], for example, can heighten or suppress a given larva's response to a local settlement cue. In recent work, we and colleagues have identified a new modulator of settlement behavior, one that operates through an unanticipated pathway and is perhaps best described by means of its effects on larvae. If *pre-competent* sand dollar or sea urchin larvae (defined by their lack of response to a local settlement inducer) are exposed to a brief pulse of turbulent shear characteristic of the conditions beneath breaking waves, they immediately transition to competence, and thus become responsive for the first time to local settlement cues [11, 12].

Although there is still much to learn about the physiological underpinnings of this novel modulator of settlement, there are a number of its attributes that are readily explored. In addition to turbulence exposure invoking an early transition to competence [11, 12], here we examine if it also modulates the selectivity of larvae through the competent period itself. We examine this possibility in a species of sand dollar from the NE Pacific, *Dendraster excentricus*, in which late-stage competent larvae have been observed to settle in the absence of any specific inducer (i.e., "spontaneously"), and thus express some 'desperate' behavior [2, 13]. Our results indicate that turbulence exposure increases the occurrence of such non-selective settlement behavior in advanced *D. excentricus* larvae. We discuss how larval experience modulates larval decision-making to enhance their prospects of successful recruitment into adult populations.

Results

Early competent sand dollar larvae do not exhibit 'desperate' larval behaviors

We first used observations of spontaneous settlement (i.e., settlement in the absence of any specific inducer) to assess whether *D. excentricus* larvae early in the competent period changed their settlement selectivity (i.e., behaved 'desperately') following turbulence exposure. In previous work, we found that *D. excentricus* larvae reared at room temperature attained competence between 10 and 12 days post fertilization (dpf), and that the time to competence is shortened by approximately 20% if they are given a 3 minute exposure to 6 W/kg of turbulent shear [12]. Therefore, using a Monterey CA (USA)-derived larval cohort from 2015 (see Methods), we first exposed larvae at 10 dpf either to 6 W/kg turbulent shear or no turbulence (control), and then transferred the larvae either directly into excess KCl –to assess their state of competence– or into 0.45 μm-Millipore filtered sea water (MFSW, no cue) –to examine spontaneous settlement, and thus assess their degree of 'desperation'. 59% (95 pct CI: 44–74%) of these control larvae and 78% (95 pct CI: 69–86%) of turbulence-exposed larvae settled in response to excess KCl (Fig. 1). Nevertheless, we detected virtually no spontaneous settlement in their sibling larvae left only in MFSW, with [2% settled ± 2% standard error of the mean (s.e.m.)] or without (0% ± 0% s.e.m.) previous turbulence exposure (Fig. 1). Because only 10% (95 pct CI: 0–20%) of this same cohort of larvae settled in response to excess KCl at 9 dpf (data not shown), we conclude that these larvae at 10 dpf had just reached what we call the early competent phase (see Methods for our definitions of these terms).

Fully competent larvae exposed to turbulence exhibit an enhancement of apparently 'desperate' larval behaviors

Using a second cohort (February 2017) of larvae reared at room temperature from the same population as in Fig. 1 (Monterey), we first established that they were at the early competent phase on day 10 pf, as judged by the proportion of larvae settling (76% [95 pct CI 63–90%]) following excess KCl exposure. We continued to feed them for 1 day (until 11 dpf), at which point nearly all of the larvae were competent: 96% ± 4% (s.e.m.) settled following excess KCl exposure (Fig. 2). Then, we exposed these 11 dpf larvae to either 6 W/kg turbulent shear or no turbulence (control), and transferred them (4 replicates each, $N = 16$–24 larvae per replicate) either into MFSW (to assay spontaneous settlement, and hence 'desperate' behavior [2, 6]) or into a natural settlement inducer: a 40% extract of sand dollar sand (see Methods). We monitored settlement at 1 and 16 h after transfer into their settlement medium.

At 1 h, we saw a 2.4-fold increase in the proportion of larvae that had settled in sand dollar sand extract if they
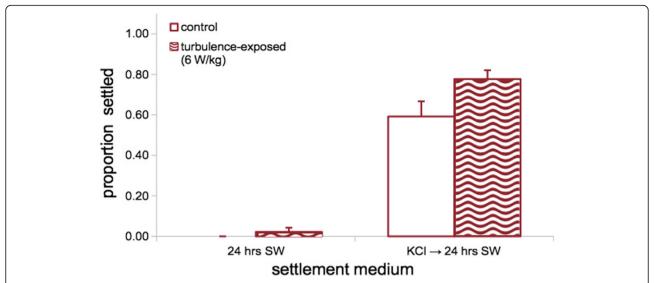
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Fig. 1 Early competent sand dollar larvae do not display 'desperate' behaviors at settlement, with or without turbulence exposure. We either exposed *D. excentricus* larvae 10 dpf (reared at ~ 20 ℃) to 3 min of 6 W/kg turbulent shear (*wavy bars*) or did not (*open bars*). Then, we transferred exposed and control larvae into one of two settlement conditions: 24 h in MFSW alone (*left side of graph*) or a 1 h exposure to 40 mM excess KCl in MFSW –to assess competence– followed by a 24 h recovery in MFSW (*right side of graph*). We detected an effect both of turbulence exposure ($F_{1,12} = 5.36$, $p < 0.02$) and settlement medium ($F_{1,12} = 228.93$, $p < 0.001$) on proportion of larvae settled, but no clear interaction ($F_{1,12} = 3.41$, $p = 0.09$). Error bars are ± the standard error of the mean (s.e.m.)

had been previously been exposed to 6 W/kg turbulent shear (Fig. 2). By 16 h, 100% of larvae settled in the sand extract, with or without previous turbulence exposure. This latter outcome was expected for competent larvae and a strong cue.

Using a three-way ANOVA, we detected a three-way interaction between turbulence exposure, settlement medium and time in cue ($F_{1,24} = 6.192$, $p < 0.05$). Subsetting these data, we found that turbulence exposure increased settlement in the 0% sand cue treatment at 1 and 16 h ($F_{1,12} = 14.152$, $p = 0.011$). In the 40% sand cue treatments, we detected a significant interaction between turbulence and time ($F_{1,12} = 11.188$, $p < 0.05$), indicating
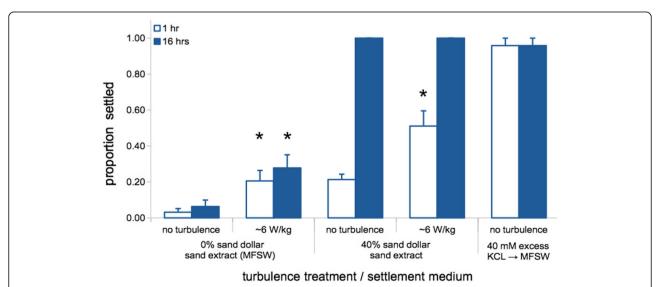

Fig. 2 Fully competent sand dollar larvae exposed to turbulence are less choosy about settlement substrate, and thus behave like 'desperate' larvae. We either exposed *D. excentricus* larvae 11 dpf (reared at ~ 20 ℃) to 3 min of 6 W/kg turbulent shear or did not ("no turbulence"). Then, we transferred exposed and control larvae into one of two settlement conditions: 0% extract of sand from sand dollar aquaria (MFSW; no inducer) or 40% extract of sand from sand dollar aquaria (strong natural inducer), and counted the numbers settled at 1 and 16 h. We also exposed a separate set of control (no turbulence) larvae for 1 h to 40 mM excess KCl in MFSW, followed by recovery in MFSW (right side of graph). More than 95% of these latter larvae settled, confirming that the larvae in this experiment were indeed fully competent. Error bars are + s.e.m. *Asterisks* indicate comparisons within settlement medium at the indicated time point between turbulence exposed (6 W/kg) larvae and the corresponding controls (no turbulence): *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

that turbulence has an effect on larvae at 1 h, but all larvae settled regardless of turbulence by 16 h. The unexpected result here is the MFSW treatment, in which 28% (± 7% s.e.m) of the larvae settled spontaneously by 16 h when previously exposed to just 3 mins of intense (6 W/kg) turbulent shear, whereas only 6% (± 4% s.e.m.) of the corresponding control larvae (no turbulence) had settled spontaneously by 16 h (Fig. 2).

In this experiment, we monitored an additional behavior associated with but distinct from settlement per se: the proportion of larvae on the bottom of the dish (and hence in contact with a potential substrate inducer of settlement) following turbulence exposure. For this analysis, we analyzed all runs by turbulence exposure (6 W/kg versus control) before transfer into their settlement treatment conditions. Turbulence exposure resulted in a 2-fold increase in the proportion of larvae on bottom when compared to controls (56% ± 8% s.e.m. versus 26% ± 9% s.e.m.; $Z = -5.343$, $p < 0.001$, $n = 8$ replicates each of turbulence-exposed and unexposed larvae, approximately 21 larvae per replicate). Note that the distinction between this *knock-down* behavior and *settlement* is that, by our definition (see also Methods) a sand dollar larva has only *settled* when it has begun the irreversible process of transformation into a juvenile. By contrast, the vast majority of larvae *knocked down* in our experiment -but deprived of a settlement inducer- are up and swimming again as intact larvae in fewer than 30 min (Ferner et al. in review). We did not observe any lethality of larvae following turbulence exposure (data not shown).

D. excentricus larvae from a different population, reared at lower temperature showed a similar effect

To assess if the preceding results would manifest more widely in *D. excentricus*, and to ensure that the results were not in part due to the relatively high rearing temperature used in the previous experiment (Fig. 2), we reared larvae from an Orcas Island WA (USA) population at a more typical summer seawater temperature (13–15 °C) until they had reached competence (approximately 20 dpf, data not shown). We continued to feed and rear these larvae until 40 dpf, and confirmed that the larvae were still competent (13/13 larvae settled in a 40 mM excess KCl test). We exposed these 40 dpf larvae to either to 6 W/kg turbulent shear or no turbulence (control), and then transferred them into one of two settlement media (3 replicates each, $N = 12$–17 larvae per replicate): MFSW (no sand extract), or a 2-fold (i.e., 200%) extract of non-sand dollar bed sand (see Methods). In order to compare these settlement media with a full strength positive cue, we similarly exposed control (non-turbulence exposed) larvae to a 30% sand dollar sand extract. We monitored settlement at 1.5, 3, 7.5 and 19 h after transfer into their respective settlement medium.

By 7.5 h, 100% of the larvae in sand dollar sand cue had settled (Fig. 3), confirming that these larvae were fully competent and behaving as expected. Using these larvae, we first compared the three settlement media (MFSW, non-sand dollar sand extract, and 30% sand dollar sand extract) for their relative effectiveness as inducers of settlement, looking at maximal settlement induction (i.e., the 19 h time point; Fig. 3). Our results indicate that, in the absence of turbulence, sand dollar sand extract is a more potent settlement inducer than the other two settlement media (versus non-sand dollar sand extract: $t_{10} = -11.62$, $p < 0.001$, versus MFSW: $t_{10} = -20.8$, $p < 0.001$), and non-sand dollar sand is a more potent settlement inducer than MFSW alone ($t_{10} = -20.8$, $p < 0.001$).

Next, we examined the effect of turbulence on maximal settlement (the 19 h time points; Fig. 3) in MFSW and non-sand dollar sand. When larvae were deprived of a specific cue (i.e., exposed only to MFSW), no larvae settled spontaneously unless they had first been exposed to 6 W/kg of turbulent shear for 3 mins, in which case nearly 20% of the larvae had settled spontaneously by 19 h ($t_{10} = 6.163$, $p = 0.002$). This same analysis at 19 h showed no effect of turbulence in the non-specific sand cue treatment ($t_{10} = 0.233$, $p > 0.8$). However, these latter larvae settled more rapidly if they had previously been exposed to turbulent shear ($t_{10} = 6.16$, $p < 0.001$; Fig. 3). Specifically, we detected a change in slope of the regression in the non-sand dollar sand treatment. We had intended to conduct a similar analysis on the rate of settlement in MFSW, comparing turbulence-exposed and unexposed larvae. However, as we detected no settlement at all in any unexposed larvae in MFSW (Fig. 3), this planned contrast was rendered irrelevant.

We also monitored the proportion of larvae that remained temporarily on the bottom of the dish following turbulence exposure in these trials. Again, we analyzed all runs by turbulence exposure (6 W/kg versus control) regardless of settlement substrate. In these more advanced larvae, turbulence exposure resulted in a 10-fold increase in the proportion of larvae on bottom when compared to controls (84% ± 5% s.e.m. versus 8% ± 3% s.e.m.; $Z = -7.189$, $p < 0.001$, $n = 6$ replicates of turbulence-exposed larvae and $n = 9$ replicates of unexposed larvae, approximately 14 larvae per replicate).

Discussion

From afar, benthic marine organisms across phyla and even kingdoms exhibit remarkable similarities in the overall trajectory of their life histories: macroscopic benthic adults release numerous microscopic propagules that disperse and, if fortunate, recruit back to benthic adult populations to themselves reproduce. Yet upon closer examination, marine organisms can exhibit surprising diversity in these same life history trajectories.
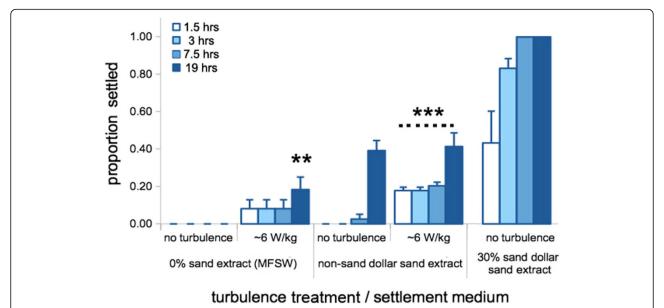
Fig. 3 Fully competent sand dollar larvae exposed to turbulence are less choosy about settlement substrate, and thus behave like 'desperate' larvae. We either exposed *D. excentricus* larvae 40 dpf (reared at ~ 14 °C) to 3 min of 6 W/kg turbulent shear or did not ("no turbulence"). Then, we transferred exposed and control larvae into one of two settlement conditions: 0% extract of sand from sand dollar aquaria (MFSW; no inducer) or 200% extract of sand from a beach without sand dollars (poor quality natural inducer), and counted the numbers settled at 1.5, 3, 7.5 and 19 h. We also exposed a separate set of control (no turbulence) larvae to a strong natural inducer (30% extract of sand from sand dollar aquaria; *right side of graph*). This treatment not only confirms that the larvae were fully competent (100% of larvae settled by 8 h), but indicates the expected rate of settlement response in a strong cue as a basis of comparison to the sub-optimal cues described above. Error bars are ± s.e.m. *Asterisks* as in Fig. 2. *Dotted line and Asterisks* above non-sand dollar sand data indicates an accelerated response to this cue in turbulence-exposed larvae (see the text)

Even closely related animal species with almost identical adults can have reproductive life histories that differ in profound ways [14]: releasing many small eggs versus few large ones, requiring planktonic food to complete development or developing entirely on yolk reserves, and producing larvae that have strict habitat requirements at the crucial settlement transition as compared with nondiscriminatory larvae. Understanding the evolutionary forces that shape these shared features as well as the varied ones is fundamental to our understanding of larval biology and marine ecosystems more broadly.

Here, we investigated a larval life history trait –the competent period– common to the vast majority of marine invertebrate taxa [15], and explored how critical larval behaviors during that period can be modulated by short-term environmental experience. In most studied marine invertebrate taxa with a distinct larval stage, the larval period can be divided operationally into a phase when larvae are not responsive to local inducers of the settlement transition (the *immature* and *precompetent* phases, sensu [12]) and a *competent* phase when the larvae are responsive and will settle in response to such inducers. In prior work, we showed that the larvae of two common NE Pacific echinoids -the purple urchin *Strongylocentrotus purpuratus* and the Pacific sand dollar *Dendraster excentricus*– can be induced to suddenly transition from precompetence to competence with a mere 3 min

exposure to turbulent shear characteristic of the nearshore conditions where they live as adults [11, 12]. In the current study, we examined how such turbulence exposure might impact larval decision-making in the competent period itself, during which a larva irreversibly commits to life on the sea floor.

Our findings reported here indicate that a brief exposure of competent *D. excentricus* larvae to intense turbulent shear causes them to settle more rapidly in response to high quality settlement cues (i.e., those indicative of good adult habitat), and also makes them more likely to respond to sub-optimal cues. Indeed, our experiments reported here for 'spontaneous settlement' in Millipore-filtered sea water controls (MFSW in Figs. 2 and 3) indicates that turbulence exposure itself can be considered akin to a settlement cue in older competent *D. excentricus* larvae (c.f [11]). In sum, turbulence exposure makes such advanced larvae less choosy about and more responsive to local settlement inducers. In other words, turbulence exposure in late stage *D. excentricus* larvae recapitulates 'desperate' larval behaviors.

'Desperate' versus 'stoic' larval behavior

The commonality of competent periods among marine invertebrates overlays substantial diversity in larval behaviors within the competent phase. One strategy involves what we here call 'stoic' larvae that would rather

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meet "Death Before Dishonor" [2] and will remain as larvae until a high quality settlement location is found, or eventually perish if such a site is never encountered. A distinct strategy involves larvae that behave 'desperately' as they age [2–6]: while they tend to be discriminating early in their competent period, they will become increasingly less choosy if deprived of settlement cues, often settling 'spontaneously' on the first solid surface they encounter if it is late during their competent period.

The canonical 'desperate' larva has a non-feeding life history [6, 16], and thus has a prescribed set of energetic reserves –derived from its mother– to fuel its embryonic and larval development, as well as its energetically-costly metamorphic transition to a feeding juvenile. The selective argument for the desperate behavior is thus that as such a larva continues to swim and disperse and use up energy, it would reach a point where settling into even marginal habitat would be its only viable chance of survival and reproduction, however slim [5, 6, 16]. Yet such a strategy is not limited to non-feeding larvae. Any costs associated with remaining in the dispersal phase (e.g., the presence of predators or the lack of food) could select for a reduction in choosiness as the competent period proceeds (e.g. [2, 5, 17, 18],). On the flip side, the 'stoic' larvae that follow a Death Before Dishonor strategy are characterized by high settlement site selectivity, where settlement in inappropriate habitats would mean poor to no chance of survival [2].

Bishop and colleagues [2] took a comparative phylogenetics approach to the issue of 'desperate' versus 'stoic' ("Death Before Dishonor") larval behavior and their ecological correlates. They found a highly significant association between the occurrence in species of observed spontaneous settlement and how specific a settlement cue their larvae require: taxa with spontaneous settlement generally had low cue specificity, whereas those without observed spontaneous settlement had high cue specificity. In other words, larvae that are known to behave as habitat generalists at the time of site selection displayed 'desperate' behaviors late during the competent period, while those known to behave as habitat specialists at the time of site selection maintained 'stoic' behaviors as their competent period proceeded, matching the expectations outlined above.

Our findings reported herein add a further layer of complexity to this general trend. When a larva of a shoreline species like the Pacific sand dollar senses that is near to shore, it undergoes a shift in behavior along the stoic -- > non-discriminatory axis towards more 'desperate' behavior. The cue that we used that indicates shoreline approach is a 3 minute pulse of high intensity turbulence, an intensity that a larva could only expect to encounter under breaking waves. Thus, one possibility is that high-intensity turbulence operates as a habitat-scale

signature (see also [19]) to larvae that they have reached the vicinity of a wave-exposed coast. The unexpected twist suggested by our data reported here is that such a short-duration, habitat-scale signature appears to immediately induce behaviors that, according to the Desperate Larva Hypothesis, typically emerge gradually as larvae age.

We note that the turbulence intensity that we used in this study (6 W/kg) is quite high, and unlikely to be encountered in locations other than within breaking waves on the shoreline (e.g. [11]). Still, while *D. excentricus* sand dollar beds are not found within the high-intensity surf zone itself, many *D. excentricus* populations –including our main study population in Monterey– are found just adjacent to areas characterized by intense crashing waves. Therefore, it is conceivable that a sand dollar larva could use an exposure to such intense fluid turbulence as a proxy for approach to a neighborhood of potentially suitable shoreline habitat, and to respond accordingly.

In addition to fluid turbulence, we can also imagine other contributing indicators of a larva's approach to potentially suitable habitat in various oceanic ecosystems and conditions. For example: warm water in shallow embayments, lower salinity in coastal zones with heavy run-off, the characteristic sound of water flow over oyster beds [20], pH fluctuations associated with diurnal patterns of photosynthesis in tide pools [21], and even break-down products of kelp in high energy surf zones [22] could each function as a habitat scale cue for larvae. As such, we feel that we have here only scratched the surface of the ways in which patterns in larval behavior at settlement could be modulated by short term experience of competent larvae at a variety of scales [1].

Lower cue specificity or increased cue sensitivity?

A careful consideration of the results we report can lead to one of two interpretations for how the brief turbulence exposure is interacting with larval behavior in response to settlement cues. We have mainly been interpreting the results we report herein as suggesting that turbulence exposure causes sand dollar larvae to accept a wider range of settlement cues. Our findings in particular that turbulence-exposed larvae demonstrate increased settlement in the absence of specific cues (Figs. 2 and 3), and more robust settlement on a nonspecific sand cue (Fig. 3), appear to support this interpretation. Nevertheless, other aspects of these data might support a slightly different reading of our results. Although a greater proportion of our turbulence-exposed larvae settled on a non-specific sand cue during the first several hours of exposure, prolonged exposure led to equivalent proportions settled in turbulence-exposed and control larvae (Fig. 3). This finding suggests a possible alternative explanation for our overall results:

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turbulence exposure merely decreases the threshold sensitivity of larvae to a cue to which they were previously sensitive (c.f., [23–26]). Indeed, in this conception, settlement by turbulence-exposed larvae in sea water alone (Figs. 2 and 3) can be interpreted as merely a lowering of the threshold for spontaneous settlement, and the more rapid settlement in the high quality sand cue after turbulence exposure (Fig. 2) is also consistent with this alternative interpretation (c.f., [26, 27]).

But are these two alternative explanations truly different? Without knowing the physiological mechanisms through which larvae evaluate and then decide whether or not to settle, one could imagine that an observation of cue fidelity in a given larva is nothing more than a high threshold for inducing the settlement behavior in this larva on alternate, lower quality cues.

There is evidence in the literature for a cellular mechanism through which marine invertebrate larvae across phyla respond to habitat cues: nitric oxide/cyclic-GMP (NO-cGMP) signaling. The overall observation is that nitric oxide generally functions as a repressor of settlement, and when nitric oxide levels drop or its signaling activity is blocked, the settlement response is unmasked ([28] for review). It is possible that a brief turbulence exposure causes a rapid deactivation of nitric oxide signaling, and thus an initiation of settlement behaviors. Still, responses in the NO-cGMP signaling system in the context of settlement have not yet been shown to be sensitive to short term environmental conditions in the way we hypothesize. As such, a full appreciation of the implications of our findings awaits improved understanding of how -in a cellular-physiological sense- larvae make decisions at this crucial life history transition that is so common to the broadest array of organisms in the sea.

Is precocious competence akin to precocious desperation?

As mentioned above, our previous work on turbulence and settlement [11, 12] focused on the precocious activation of competence by turbulence exposure in echinoid larvae. One way of describing our prior observations is that a brief exposure to intense turbulence hastens the onset of behaviors that would have occurred in those larvae -in the absence of turbulence- at a later date [12]. This description could just as well apply to the observations reported herein: that turbulence hastens the onset of 'desperate' behaviors, such as spontaneous settlement, that eventually occur in many competent *D. excentricus* larvae that have been in culture for long periods [2, 13]. In that respect, the mechanism by which turbulence induces precocious competence may be the same as that by which turbulence induces a decrease in settlement selectivity in already competent larvae.

Sink first settle later?

In addition to its effect on settlement itself, we here report on an associated behavior that resulted from turbulence exposure: the larvae temporarily sank to the substrate. In some mollusk larvae, turbulence causes retraction of the swimming apparatus (the velar lobes), which then leads the shelled larvae to sink [29]. Other mollusk larvae may actively swim downwards when exposed to turbulence [30, 31], but the results are variable. In our experiments, we did not have the opportunity to observe the sinking per se; we simply counted the larvae on the bottom of the dish within a few minutes after recovery from the Taylor-Couette device. Still, these observations as a whole are consistent with a general scenario in which nearshore-destined larvae first encounter a high intensity of fluid turbulence and sink to the substrate, where they are then exposed to local (e.g., chemical or tactile) cues associated with the benthos [29, 32]. Our results reported previously [11, 12] and herein add an additional twist: the aforementioned encounter of turbulence by larvae in the water column not only increases a larva's likelihood of being exposed to a local settlement inducer on the substrate ([29, 32–36] and results reported herein), it also increases the receptivity of a larva to that settlement inducer once it arrives there.

Implications for aquaculture

Lower cue fidelity in competent larvae following turbulence exposure could have important implications for the shellfish aquaculture industry. A common challenge in aquaculture efforts is synchronous culture of larvae to competence, and ideally, settlement of juveniles during a short window of exposure to a settlement cue (e.g., [37]). In many aquaculture settings a cohort of larvae spawned on the same day might reach competence over the course of several weeks. As such, a single larval culture might on a given day have a mixture of *pre-competent* larvae refractory to any cue, *early competent* larvae with high cue specificity, and *later competent* larvae that settle more readily.

Our findings here suggest that one might be able to synchronize the settlement of such cultures merely by providing a brief turbulence exposure. Our unpublished data suggest that shaking of sand dollar larvae in flasks for 3 minutes can have similar effects as specific turbulence exposure in our Taylor-Couette device. Such a technique could be worth exploring in an aquaculture context to see if one could increase the settlement success of a cohort of various types of shellfish larvae, many of which are known to exhibit settlement-associated behaviors in response to fluid turbulence (e.g., [30, 35, 36, 38–40]).

Conclusions

An obligate larval dispersal period followed by the attainment of competence and then settlement to the

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sea-floor is a common life cycle pattern among the majority of marine invertebrate phyla [15]. Because larvae have likely evolved independently at least several times among invertebrates [41], their common life cycle patterns have evolved in parallel. At that key settlement transition, however, different larvae show profound differences in behavior with respect to settlement site selection, even among closely related species, from less discriminating larvae to those that exhibit high selectivity. Here, we show that those broader behavioral types are themselves subject to modulation based on short term environmental experience by their late stage larvae.

Greater attention is lately being given to the ways in which settlement behavior and dynamics can impact marine populations and community structure. In particular, recent studies have singled out larval settlement as a key process sensitive to warming and ocean acidification (reviewed in [42]). This focus is understandable: settlement and metamorphosis in the marine realm are both ontogenetically and behaviorally complex, with larvae integrating external signals with their internal state to make appropriate, context-responsive settlement decisions.

We here present one example of how short term environmental exposures (specifically, intense turbulent shear) can modulate this decision-making process. It seems a small stretch to hypothesize that environmental changes such as increased temperature and decreased pH could alter these environmentally sensitive settlement decisions in ways that could be difficult to predict, and which could have cascading consequences for population dynamics. As such, detailed explorations into this critical and common yet diverse and modulatable behavior will yield increasingly important insights into the ecology and evolution of life in the ocean.

Methods

Experiment overview, definitions of terms and study species

Adults of the Pacific sand dollar *Dendraster excentricus* (Eschscholtz) live gregariously in intertidal or shallow subtidal beds throughout much of the Northeastern Pacific Ocean, in both wave-exposed and protected locations [43]. Their planktotrophic larvae have a very wide temperature tolerance range –from 8 °C through at least 24 °C ([44], Hodin J, unpublished)– and when fed ad libitum at approximately 19–22 °C, they develop synchronously and reach competence in quiescent laboratory conditions at approximately 11–12 days after fertilization [12].

Because of our focus on the transition of larvae from a planktonic to benthic phase, we here provide definitions of several relevant terms regarding life cycle transitions in the ocean [1]. We define metamorphosis as a more-or-less radical morphological transformation between two multicellular life stages [45]. In echinoids (sea urchins and sand dollars) with feeding larvae, the morphological transformation (larva

to juvenile) begins several days or even weeks before the habitat shift (planktonic to benthic); we refer to echinoid metamorphosis as the *entire period from the first formation of juvenile structures ("rudiment") in the larva until the juvenile begins to feed on the sea floor*. The most dramatic event of metamorphosis is settlement: the (in most cases *irreversible*) transition that the larva makes from the planktonic to the benthic zone, at which point it is called a juvenile. This process of settlement is what we measured throughout.

Based on previous work, we define the competent period as *beginning when a larva first becomes responsive to localized settlement cues* [12]. When rearing batches of larvae, there is always within and among batch variation in the time at which individual larvae attain competence. Therefore, it is useful for us to establish criteria for defining the degree to which batches or cohorts of larvae are competent. We here define a batch or cohort of sand dollar larvae as early precompetent when fewer than 1/3 of the larvae have attained competence, as judged by their response to a 1 h induction with 40 mM excess KCl in 0.45 μm Millipore-filtered sea water (MFSW): *competent larvae*, under such exposure, settle and then complete their metamorphic transformation into functional juveniles in MFSW. By this same criteria, in a batch of late precompetent larvae, 1/3 of the larvae have attained competence. Similarly, in a batch of early competent larvae, 2/3 of the larvae have attained competence, and fully competent batches are those in which 100% of the larvae have attained competence.

Note that for the purposes of this manuscript, if the 95% confidence intervals (CI) surrounding the mean proportion settled of a given batch of larvae overlaps 0.33, then we consider that batch to be late precompetent. Likewise, if the 95% CI surrounding the mean proportion settled of a different batch of larvae overlaps 0.67, then we consider that batch early competent and so on. We would reject a batch of larvae in this context as too variable for our purposes in which the 95% CI surrounding the mean proportion settled was ambiguous with respect to category. This situation did not arise in the present study.

Our overall approach was to rear larvae through their feeding larval stage using standard methods [44, 46], and on various days during the competent stage, subject a subset of them to a brief turbulence exposure of a specified intensity. Immediately after exposure, we transferred the larvae into different settlement inducing media: MFSW, extracts of sand from inside or outside sand dollar beds, or excess KCl in MFSW. We then quantified settlement as it relates to turbulence exposure and settlement medium.

Source populations, collection and permits

We collected adult *D. excentricus* adults from two geographically distinct populations: 1) a subtidal population

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(~ 100 m offshore and 1.5 m below the surface at mean lower low water) approximately 30 m east of Municipal Pier 2 in downtown Monterey CA, USA; 2) an intertidal population on Crescent Beach in East Sound, Orcas Island, WA, USA. We collected adults in Monterey by snorkeling on 23 July 2015 and 10 February 2017, and transported them to Hopkins Marine Station ("HMS"; Pacific Grove CA, USA). Adults in East Sound were collected at low tide in July 2016, and transported to Friday Harbor Labs ("FHL"; Friday Harbor, WA, USA). In both locations, we kept sand dollars partially buried within fine sediments (collected from the adult beds) in flowing sea water aquaria until spawning. The Monterey population in February 2017 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.

Our collections in California were made in accordance with Stanford and HMS policy (http://web.stanford.edu/group/dbr/HMLR.html) and under a collecting permit issued by the California Department of Fish and Wildlife to Dr. Christopher Lowe at HMS. Our collections in Washington were made under the auspices of state statute (House Bill 68, R.C.W.28.77.230, 1969 Revision R.C.W.28B.20.320), which includes the collection location on Orcas Island as part of the larger Marine Biological Preserve (MBP), with FHL as the managing agency (https://fhl.uw.edu/research/research-policies/). As such, the director of FHL approved the collection of sand dollars in 2016.

Larval culture

We fertilized our experimental sand dollar individuals as follows. We obtained gametes by intracoelomic injection of (depending on the size of the adult) between 0.2 and 0.5 ml 0.55 M KCl in distilled water. For the 2015 spawning (Monterey sand dollars collected in July), sperm was collected dry and placed on ice; unfertilized eggs were washed 3 times and were transferred into individual 250 ml culture flasks containing 0.1 g/L ampicillin in 0.45 μm Millipore-filtered natural sea water (MFSW) with UV treatment. Storing eggs in this manner at ambient temperature (here ~ 14 °C) renders them fertilizable for a week or longer [12, 47, 48]. These stored gametes (spawned 26 August 2015) were then shipped overnight that same day from HMS to FHL. After arrival, we washed out the antibiotic with three rinses in MFSW before proceeding with fertilizations.

We set up fertilizations on 27 August 2015 (shipped Monterey gametes), 10 February 2017 (freshly collected Monterey sand dollars) and 4 May 2017 (Orcas Island sand dollars collected July 2016) at 13–14 °C using standard methods [44, 46]. For each larval culture, we set up single male-female crosses, each resulting in > 90% fertilization success. In August 2015, we allowed the newly fertilized eggs to warm up to room temperature (19–22 °C), and maintained at room temperature at FHL throughout larval development. In February 2017, we kept the embryos overnight at 14 °C, and the following day warmed them up to room temperature (19–22 °C), at which temperature they remained until experiments. In May 2017, we maintained larvae at sea table temperatures (13–15 °C) at FHL throughout.

We began feeding 4-arm pluteus larvae 1 day post fertilization (1 dpf). Initial larval densities were ~ 0.5–1 larva/ml, which we lowered to 0.2–0.3 larva/ml after rudiment invagination (6 arm larvae; 3 dpf at room temperature, 6 dpf at 13 °C). We fed larvae a mixture of 2500 cells/ml *Rhodomonas spp.* and 3000 cells/ml *Dunaliella tertiolecta* throughout, changing > 90% of the culture water by reverse filtration every 2 days and then re-feeding. For the February 2017 experiments, we transported 3 dpf larvae in a 1-gal water jug at ~ 1 larva/ml to Bodega Marine Laboratories (BML; Bodega Bay CA, USA), where they were replaced in gallon jars and maintained for the remainder of larval development.

At both FHL and BML, we cultured embryos and larvae in 0.45 μm MFSW in gallon jars, either with mechanical stirring [44, 46] or on a gyratory shaker platform.

Generating turbulence in the laboratory

To generate fluid turbulence in the laboratory (quantified in terms of the energy dissipation rate, in units of W/kg), including intensities characteristic of wave-battered coasts, we employed a Taylor-Couette cell [49]. This apparatus consists of two vertically oriented, co-axial cylinders separated by a 3.5 mm gap that contains seawater (described in greater detail in [11]). We held the stationary inner cylinder, and thus the water in the gap, at either 19–21 °C (September 2015 and Feb 2017) or 13–15 °C (June 2017) by means of a circulating water stream from a temperature-controlled water bath passing through the cylinder's interior. During operation, the outer cylinder rotated at a prescribed speed causing relative motion between the cylinders and thereby shearing the seawater between them. At the rotation speeds employed for testing sand dollar larvae, the sheared flow was turbulent [11].

Larval exposure to turbulent shear

In all experiments described herein, we exposed larvae to a single turbulence intensity of approximately 6 W/kg, which corresponds to a rotation rate of 500 r.p.m. at 20 °C and 550 r.p.m. at 14 °C (the difference is due to the increased viscosity of sea water at 14 °C). This intensity is substantially in excess of those used in most laboratory examinations of larval responses to turbulence (e.g., [40, 50]), approaching the largest values measured under actively breaking waves on steep rocky

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coasts during non-storm conditions [51]. See the Discussion for our justification for using these high turbulence intensities.

We first concentrated larvae by reverse filtration and selected 15–35 larvae into individual 125-ml glass beakers at a density of 1 larva per 3–4 ml MFSW. Larvae in these experiments were developing synchronously; we only eliminated larvae from consideration if they were obviously delayed in development (< 5% of the larvae; data not shown). Otherwise, we chose larvae haphazardly. Then, we randomly assigned beakers to treatment and replicates, and began the exposures.

For each treatment run, 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 cell. We then subjected the entire water volume within the apparatus to the specified level of turbulent shear for 3 min. Immediately following each treatment run, we gently poured the larvae within the Taylor-Couette cell into a 1-L glass beaker already containing approximately 100 ml of MSFW (to minimize additional stimulus to larvae during the pour), rinsed the Taylor-Couette cell 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 settlement assay conditions. We generally recovered > 95% of the larvae, all of which we used in ensuing settlement assays. We then rinsed the Taylor-Couette cell 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 settlement assays. In previous studies, we compared *unmanipulated controls* to *handling controls* (i.e., 0 W/kg [= 0 r.p.m.] treatments), where we treated the larvae the same as those assigned to the turbulence exposure treatments, except we did not activate the Taylor-Couette cell during the 3 min that larvae were within it, thereby controlling for manipulations associated with transfer of larvae into and out of the apparatus. Since we have never observed any differences between larvae exposed to *unmanipulated* and *handling controls* in our studies ([11, 12] and additional unpublished data), we here eliminated the *handling controls*.

Quantification of settlement

Following turbulence treatments, we assessed settlement by transferring all larvae from a run to a single well of a pre-washed, non-tissue culture-treated 6-well plate (see [52]) containing 8 ml of one of three potential inducers of settlement: MFSW, extracts of sand in MFSW (see below), or 40 mM excess KCl in MFSW, all maintained at the rearing temperature. All exposures to the settlement inducers were continuous for up to 24 h, except for the KCl exposures, which were only for 1 h, followed by 24 h in 8 ml of MFSW (e.g. [12],). We scored a larva as settled if skin had begun to withdraw from the tips of the larval skeletal rod, as per standard assays (e.g., [11, 12, 18, 53, 54]). We verified continued withdrawal of skin 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.

As we transferred the larvae from a glass finger bowl into the settlement wells, we recorded the numbers of larvae swimming versus those contacting the bottom of the bowl. We used these counts to calculate the "proportion of larvae on bottom" for each trial.

Preparation of sand extracts

For *sand dollar sand* we used sand from the sand dollar holding aquaria at FHL (originally collected from the East Sound beds), either frozen and stored at – 20 °C in 50 ml conical tubes (BML experiments), or collected fresh from those aquaria (FHL experiments). We thawed the frozen sand at room temperature for > 1 h before use. For the *non-sand dollar sand* (a non-specific cue) tested in June 2017, we collected sand from an intertidal beach (+ 1 m) in front of the main FHL laboratory where no sand dollars are found. To prepare the sand cues, we added 25 ml of sand to 25 ml of MFSW in a 50 ml conical tube and shook it vigorously for 30 s, allowed the large sand particulates to settle out for 10 s, and then decanted off the opaque supernatant into a new tube. When we diluted this supernatant 1:1 with MFSW, it resulted in what we term a 100% sand extract. We then used this extract for settlement tests at various dilutions in MFSW within a few hours of preparation. We conducted sand extract exposure trials in 6-well plates in which the remaining sediment suspended in the sand extract had been allowed to settle out for at least 30 min, rendering the water above reasonably transparent. In the June 2017 experiments, we prepared the *non-sand dollar sand* cue at approximately the same opacity as an effective *sand dollar sand* cue; we asked a colleague not involved in the study to evaluate these preparations of different sand cues for their approximately equivalent opacity. As it happened, a "200%" *non-sand dollar sand* cue (i.e., the poured-off supernatant *not diluted* 1:1 with MFSW) was deemed by this individual to be as opaque as a 30% *sand dollar sand* cue.

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Statistical analyses

We assessed the effect of turbulence exposure (6 W/kg versus unexposed controls) on proportion of larvae on the bottom by a logistic regression with turbulence as a fixed factor. For our early competent larval dataset (2015; Fig. 1), we also used a logistic regression to assess the effect of turbulence exposure and settlement medium (MFSW, excess KCl) on proportion of larvae settled, with turbulence and settlement medium as fixed factors. For the other settlement data (Feb 2017 and June 2017: Figs. 2 and 3, respectively), the logistic regression model would not converge (due to lack of linearly separable data; not shown).

Therefore, in those cases, we assessed the effect of turbulence exposure (6 W/kg versus unexposed controls), settlement medium (MFSW, sand extracts), and time after turbulence exposure on proportion of larvae settled by ANOVA. For the Feb 2017 dataset (Fig. 2), we treated time after exposure (1 or 16 h) as categorical, and ran a 3-way ANOVA (settlement medium, turbulence exposure, time). Because we detected a significant three-way interaction (see Results), we subsetted the data by settlement medium in order to determine how the effect of turbulence changes over time between the different settlement media, using Bonferroni corrections for multiple comparisons.

For the June 2017 dataset (Fig. 3), we compared the final proportion of larvae settled (19 h) in the different treatments using a two-way ANOVA, with settlement medium and turbulence treatment as the two factors. Because we detected a significant interaction between settlement medium and turbulence treatment ($F_{1,10} = 17.58$, $p < 0.002$), we separated the data both by settlement medium and turbulence treatment for further pairwise comparisons using a Tukey HSD test and the appropriate Bonferroni corrections. Note that we identified the specific pairwise comparisons that we made here in advance of gathering these data, based on questions of interest that motivated the June 2017 experimental design, deriving from results obtained in Feb 2017 (Fig. 2).

To determine if turbulence exposure and settlement medium could impact the rate at which larvae subsequently settled, we conducted a two-way ANCOVA with time after exposure as a covariate and settlement medium (MFSW, non-sand dollar sand extract) and turbulence treatment as the two factors. We uncovered a significant three-way interaction between time, settlement medium, and turbulence exposure, suggesting that the rate at which larvae settled was different among treatments ($F_{1,40} = 15.21$, $p < 0.001$). We then used planned contrasts to test whether the rate at which larvae settled over time was different between turbulence treatment and UMC for each of the two settlement media. As indicated above, these planned contrasts were in place before we gathered the data in June 2017.

We treated each spin (exposure) in the Taylor-Couette cell as as a random effect to account for the multiple larvae within each spin. 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. We performed all statistical analyses using R (version 3.4.2) and the lme4 package.

Availability of data and materials
Data are available in the National Science Foundation Biological and Chemical Oceanography Data Management Office (BCO-DMO) data repository at the following link: https://www.bco-dmo.org/dataset/740414.

Authors' contributions
J.H. planned the experiments, collected and analyzed the data, prepared the figures and wrote and revised the manuscript. M.C.F. planned the experiments, collected the data and discussed/commented on the manuscript. G.N. led our statistical analyses of the data and discussed/commented on the manuscript. B.G. planned the experiments, supervised the experiments at BML and discussed/commented on the manuscript.

Ethics approval and consent to participate
The research presented here does not require any approval and therefore complies with all necessary regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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References

1. Hodin J, Ferner MC, Heyland A, Gaylord B. I feel that! Fluid dynamics and sensory aspects of larval settlement across scales. In: Carrier TJ, Reitzel AM, Heyland A, editors. Evolutionary ecology of marine invertebrate larvae. New York: Oxford University Press; 2018. 13, pp 190–207.
2. Bishop C, Huggett M, Heyland A, Hodin J, Brandhorst BP. Interspecific variation in metamorphic competence in marine invertebrates: the significance for comparative investigations of regulatory systems. Integr Comp Biol. 2006;46:662–82.
3. Wilson DP. The settlement of *Ophelia bicornis* Savigny larvae. J Mar Biol Assoc UK. 1953;32:209–33.
4. Knight-Jones EW. Decreased discrimination during settling after a prolonged planktonic life in larvae of *Spirorbis borealis* (Serpulidae). J Mar Biol Assoc UK. 1953;32:337–45.
5. Toonen RJ, Pawlik JR. Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae) II: testing the desperate larvae hypothesis. Mar Ecol Prog Ser. 2001;224:115–31.
6. Marshall DJ, Keough MJ. Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Mar Ecol Prog Ser. 2003;255:145–53.
7. Pechenik JA, Estrella MS, Hammer K. Food limitation stimulates metamorphosis of competent larvae and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*. Mar Biol. 1996;127:267–75.
8. Lam C, Neumann R, Shin PK, Au DW, Qian PY, Wu RS. Polybrominated diphenylethers (PBDEs) alter larval settlement of marine benthic polychaetes. Environ Sci Technol. 2010;44:7130–7.
9. Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ. Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol Lett. 2012;15:338–46.
10. Dineen JF Jr, Hines AH. Interactive effects of salinity and adult extract upon settlement of the estuarine barnacle *Balanus improvisus* (Darwin, 1854). J Exp Mar Biol Ecol. 1992;156:239–52.
11. Gaylord B, Hodin J, Ferner MC. Turbulent shear spurs settlement in larval sea urchins. Proc Natl Acad Sci U S A. 2013;110:6901–6.
12. Hodin J, Ferner MC, Ng G, Lowe CJ, Gaylord B. Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence. R Soc Open Sci. 2015;2:150114.
13. Highsmith RC. Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. Ecology. 1982;63:329–37.
14. Strathmann RR. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annu Rev Ecol Syst. 1985;16:339–61.
15. Hadfield MG, Carpizo-Ituarte EJ, del Carmen K, Nedved BT. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. Am Zool. 2001;41:1123–31.
16. Elkin C, Marshall DJ. Desperate larvae: influence of deferred costs and habitat requirements on habitat selection. Mar Ecol Prog Ser. 2007;335:143–53.
17. Botello G, Krug PJ. Desperate larvae revisited: age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod Alderia sp. Mar Ecol Prog Ser. 2006;312:149–59.
18. Mos B, Dworjanyn SA. Early metamorphosis is costly and avoided by young, but physiologically competent, marine larvae. Mar Ecol Prog Ser. 2016;559:117–29.
19. Fuchs HL, Gerbi GP. Seascape-level variation in turbulence- and wave-generated hydrodynamic signals experienced by plankton. Prog Oceanogr. 2016;141:109–29.
20. Lillis A, Eggleston DB, Bohnenstiehl DR. Oyster larvae settle in response to habitat-associated underwater sounds. PLoS One. 2013;8:e79337.
21. Bensoussan N, Gattuso JP. Community primary production and calcification in a NW Mediterranean ecosystem dominated by calcareous macroalgae. Mar Ecol Prog Ser. 2007;334:37–45.
22. Trapido-Rosenthal HG, Morse DE. L-α, ω-Diamino acids facilitate GABA induction of larval metamorphosis in a gastropod mollusc (*Haliotis rufescens*). J Comp Physiol B. 1985;55:403–14.
23. Rittschof D, Branscomb ES, Costlow JD. Settlement and behavior in relation to flow and surface in larval barnacles, *Balanus amphitrite* Darwin. J Exp Mar Biol Ecol. 1984;82:131–46.
24. Coon SL, Fitt WK, Bonar DB. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. Mar Biol. 1990;106:379–87.
25. Gibson G. Why be choosy? Temporal changes in larval sensitivity to several naturally-occurring metamorphic inducers in the opistobranch *Haminaea callidegenita*. J Exp Mar Biol Ecol. 1995;194:9–24.
26. Swanson RL, Marshall DJ, Steinberg PD. Larval desperation and histamine: how simple responses can lead to complex changes in larval behaviour. J Exp Biol. 2007;210:3228–35.
27. Jackson DJ, Ellemor N, Degnan BM. Correlating gene expression with larval competence, and the effect of age and parentage on metamorphosis in the tropical abalone *Haliotis asinina*. Mar Biol. 2005;147:681–97.
28. Bishop CD, Biggers WJ. In Haliotis, NO means YES. Front Mar Sci. 2014;1:51. https://doi.org/10.3389/fmars.2014.00051.
29. Fuchs HL, Mullineaux LS, Solow AR. Sinking behavior of gastropod larvae (*Ilyanassa obsoleta*) in turbulence. Limnol Oceanogr. 2004;49:1937–48.
30. Fuchs HL, Hunter EJ, Schmitt EL, Guazzo RA. Active downward propulsion by oyster larvae in turbulence. J Exp Biol. 2013;216:1458–69.
31. Wheeler JD, Helfrich KR, Anderson EJ, Mullineaux LS. Isolating the hydrodynamic triggers of the dive response in eastern oyster larvae. Limnol Oceanogr. 2015;60:1332–43.
32. Abelson A, Denny M. Settlement of marine organisms in flow. Annu Rev Ecol Syst. 1997;28:317–39.
33. Chia FS, Koss R, Bickell LR. Fine structural study of the statocysts in the veliger larva of the nudibranch, *Rostanga pulchra*. Cell Tissue Res. 1981;214:67–80.
34. Denny MW, Shibata MF. Consequences of surf-zone turbulence for settlement and external fertilization. Amer Nat. 1989;134:859–89.
35. Crimaldi JP, Thompson JK, Rosman JH, Lowe RJ, Koseff JR. Hydrodynamics of larval settlement: the influence of turbulent stress events at potential recruitment sites. Limnol Oceanogr. 2002;47:1137–51.
36. Fuchs HL, Gerbi GP, Hunter EJ, Christman AJ. Waves cue distinct behaviors and differentiate transport of congeneric snail larvae from sheltered versus wavy habitats. Proc Natl Acad Sci U S A. 2018;115:E7532–40.
37. Carl C, Poole AJ, Williams MR, de Nys R. Where to settle–settlement preferences of *Mytilus galloprovincialis* and choice of habitat at a micro spatial scale. PLoS One. 2012;7:e52358.
38. Welch JM, Forward RB. Flood tide transport of blue crab, *Callinectes sapidus*, postlarvae: behavioral responses to salinity and turbulence. Mar Biol. 2001;139:911–8.
39. Pernet F, Tremblay R, Bourget E. Settlement success, spatial pattern and behavior of mussel larvae *Mytilus spp.* in experimental "downwelling" systems of varying velocity and turbulence. Mar Ecol Prog Ser. 2003;260:125–40.
40. Wheeler JD, Chan KYK, Anderson EJ, Mullineaux LS. Ontogenetic changes in larval swimming and orientation of pre-competent sea urchin *Arbacia punctulata* in turbulence. J Exp Biol. 2016;219:1303–10.
41. Strathmann RR. Hypotheses on the origins of marine larvae. Annu Rev Ecol Syst. 1993;24:89–117.
42. Nagelkerken I, Munday PL. Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. Glob Chang Biol. 2016;22:974–89.
43. Mooi R. Sand dollars of the genus *Dendraster* (Echinoidea: Clypeasteroida): phylogenetic systematics, heterochrony, and distribution of extant species. Bull Mar Sci. 1977;61:343–75.
44. Strathmann MF. Reproduction and development of marine invertebrates of the northern Pacific coast. Seattle, WA: University of Washington Press; 1987.
45. Hodin J. Expanding networks: signaling components in and a hypothesis for the evolution of metamorphosis. Integr Comp Biol. 2006;46:719–42.
46. Strathmann RR. Culturing larvae of marine invertebrates. In: Carroll JD, Stricker AS, editors. Developmental biology of the sea urchin and other marine invertebrates: methods and protocols. Totowa NJ: Humana Press; 2014. p. 1–25.
47. Epel D, Vacquier VD, Peeler M, Miller P, Patton C. Sea urchin gametes in the teaching laboratory: good experiments and good experiences. Methods Cell Biol. 2004;74:797–823.
48. Kiyomoto M, Hamanaka G, Hirose M, Yamaguchi M. Preserved echinoderm gametes as a useful and ready-to-use bioassay material. Mar Environ Res. 2014;93:102–5.
49. Taylor GI. Stability of a viscous liquid contained between two rotating cylinders. Phil Trans R Soc Lond A. 1923;223:289–343.
50. Fuchs HL, Solow AR, Mullineaux LS. Larval responses to turbulence and temperature in a tidal inlet: habitat selection by dispersing gastropods? J Mar Res. 2010;68:153–88.

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51. Gaylord B. Hydrodynamic context for considering turbulence impacts on external fertilization. Biol Bull. 2008;214:315–8.

52. Herrmann K, Siefker B, Berking S. Sterile polystyrene culture dishes induce transformation of polyps into medusae in *Aurelia aurita* (Scyphozoa, Cnidaria). Methods Cell Sci. 2003;25:135–6.

53. Sato Y, Kaneko H, Negishi S, Yazaki I. Larval arm resorption proceeds concomitantly with programmed cell death during metamorphosis of the sea urchin *Hemicentrotus pulcherrimus*. Cell Tissue Res. 2006;326:851–60.

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