

Sand Dollar Larvae Show Within-Population Variation in Their Settlement Induction by Turbulence

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Abstract. Settlement—the generally irreversible transition from a planktonic phase to a benthic phase—is a critical stage in the life history of many shoreline organisms. It is reasonable to expect that larvae are under intense selection pressure to identify appropriate settlement habitat. Several decades of studies have focused mainly on local indicators that larvae use to identify suitable habitat, such as olfactory cues that indicate the presence of conspecifics or a favored food source. Our recent work has shown that the larvae of seashore-dwelling echinoids (sea urchins, sand dollars, and kin) can be primed to settle following a brief exposure to a broader-scale indicator of their approach to shore: an increase in fluid turbulence. Here we demonstrate that this priming shows within-population variation: the offspring of certain Pacific sand dollar (*Dendraster excentricus*) parents—both specific fathers and specific mothers, regardless of the other parent—are more responsive to turbulence than others. In particular, the observation of the effect correlating, in some cases, with specific fathers leads us to conclude that these behavioral differences are likely genetic and thus heritable. We also report that turbulence exposure causes larvae to temporarily sink to the bottom of a container of seawater and that larvae that respond in this way are also more likely to subsequently settle. We hypothesize a two-step scenario for the evolution of turbulence responsiveness at settlement and suggest that the evolutionary origin of these behaviors could be a driving force for population differentiation and speciation.

Introduction

Complex life histories are typical of the vast majority of shoreline-dwelling marine organisms, characterized by an adult phase in the benthos and a pelagic dispersal phase. In animals, the pelagic form is often termed a larva, which can disperse for days to months, span many kilometers, and, if fortunate, return to and settle into suitable shoreline habitat. From an evolutionary standpoint, it is clear that selection occurs throughout both phases and all associated steps (Pechenik, 1999). However, the process of larval settlement seems an especially portentous period. Because the dispersal potential of the adults themselves is limited or nil, population replenishment depends on appropriate decisions of larvae as to where and when to settle, making these literally life-or-death concerns.

Consistent with the observation that larvae may often be under intense selection pressure to make appropriate settlement decisions, diverse taxa employ a range of behaviors to maximize the likelihood of settling at a beneficial time and place (Crisp, 1974). The majority of published work on such behaviors has focused on those that occur at small scales—from millimeters to centimeters. At these scales, larvae may evaluate ambient light, substrate topography, hydrodynamic shear stress, and a range of chemical cues that might offer information regarding suitable juvenile and adult habitat (reviewed in Hodin *et al.*, 2018a). But what of broader scales? Do successful larvae only encounter suitable micro-scale habitats if they are lucky enough to be carried there by ambient flows? Or can larvae also employ behaviors at broader spatial scales that can increase their likelihood of successful recruitment into adult populations? If so, larvae might employ a stepwise approach to finding their way to preferred habitat, first responding behaviorally to signatures of “good neighborhoods” (perhaps spanning hundreds of meters; *e.g.*, estuaries *vs.* open coasts,

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Abbreviations: BML, Bodega Marine Laboratory; dpf, days post-fertilization; FHL, Friday Harbor Laboratories; HMS, Hopkins Marine Station; MFSW, 0.45- μ m Millipore-filtered seawater; RT, room temperature.

shallow vs. deep benthos), then narrowing their search strategies until they can use the aforementioned small-scale cues before they finally commit to a given settlement location (Hodin *et al.*, 2018a).

Behavioral observations and modeling work suggest that larval responses to environmental factors, which vary over relatively broad scales, may enhance the return of larvae to suitable juvenile and adult habitats (Denny and Shibata, 1989; Fuchs *et al.*, 2010, 2013), as predicted by Chia *et al.* (1981). Moreover, larvae may react to environmental features in a manner that alters the duration of their dispersal phase. In particular, intense turbulence characteristic of that measured under breaking waves at the shore causes two common Northeast Pacific shoreline echinoids—the Pacific sand dollar *Dendraster excentricus* and the purple urchin, *Strongylocentrotus purpuratus*—to undergo an unexpected and rapid life-history shift: so-called precompetent larvae that had been non-responsive to settlement inducers suddenly become competent to respond to such inducers and settle (Gaylord *et al.*, 2013; Hodin *et al.*, 2015). This novel finding indicates a pathway whereby larvae might increase their likelihood of successful settlement in locations appropriate for juvenile survival.

In accordance with the notion that settlement traits are likely to be important ones upon which selection might act, we uncovered evidence in *D. excentricus* for substantial variation in the turbulence response of larvae among batches (larval cohorts) deriving from distinct populations of adults. Specifically, we observed differences among larval rearing episodes in their propensity for their precompetent larvae to transition to competence and settle following turbulence exposure (Hodin *et al.*, 2015; JH, unpubl. obs.). Furthermore, certain batches of *D. excentricus* larvae from a given population were essentially unresponsive to turbulence in terms of settlement behavior, whereas other batches from that same population seemed to demonstrate a robust settlement response following turbulence exposure.

In the present study, we address the hypothesis that this variation derives at least in part from within- and among-population genetic variation in how turbulence activates competence. We discuss the possibility that such variation could provide the raw material for habitat selection leading to allopatry.

Materials and Methods

Experiment overview and study species

Adults of the Pacific sand dollar *Dendraster excentricus* (Eschscholtz, 1831) live gregariously in intertidal or shallow subtidal beds throughout much of the northeastern Pacific Ocean, in both wave-exposed and protected locations (Mooi, 1997). Their planktotrophic larvae have a wide temperature tolerance range—from 8 °C to at least 24 °C (M. F. Strathmann, 1987; JH, unpubl. obs.)—and when fed *ad libitum* at approximately 19–22 °C, they develop synchronously and

reach competence in quiescent laboratory conditions at about 12 days post-fertilization (dpf) (Hodin *et al.*, 2015).

Our previous work has demonstrated that precompetent sand dollar larvae (for definitions, see Hodin *et al.*, 2015, 2018a) become competent to settle when exposed to 3 minutes of intense fluid turbulence. Here we examine whether different genotypes show a weaker or stronger response to turbulence in this way. Our overall approach was to rear larvae through their feeding larval stage by using standard methods (M. F. Strathmann, 1987; R. R. Strathmann, 2014); and as larvae approached the competent stage, subject a subset of them to a brief turbulence exposure of a specified intensity. Immediately after turbulence exposure, we transferred the larvae into a chemical inducer of settlement (and an indicator of competence; Hodin *et al.*, 2015): 40 mmol L⁻¹ excess KCl in 0.45- μ m Millipore-filtered natural seawater (MFSW). We then quantified settlement as it related to turbulence exposure and genotype.

Source populations and collection

We collected adult *D. excentricus* adults from five geographically distinct populations in the Northeast Pacific (Fig. 1). Populations 1 and 2 were at sheltered locations within the inland waters of the Salish Sea, with calm hydrodynamic exposure; populations 3–5 were in outer coast locations, routinely exposed to high-energy waves and swell.

1. “Orcas”: a mid-intertidal population on Cannon Beach in East Sound, Orcas Island, Washington; latitude, longitude: 48.6933, -122.8985.

2. “Seattle”: a high intertidal sand dollar bed (~0.5 vertical meters above the mean lower low water mark) near Me-Kwa-Mooks Park in west Seattle, Washington; latitude, longitude: 47.5655, -122.4097.

3. “Moclips”: a shallow subtidal to low intertidal population near the mouth of the Moclips River, Moclips, Washington; latitude, longitude: 47.2462, -124.2224.

4. “Bodega Head”: a subtidal population (~5 m deep) off of Doran Beach in Bodega Bay, California; latitude, longitude: 38.3113, -123.0427.

5. “Monterey”: a subtidal population (~100 m offshore and 1.5 m below the surface at mean lower low water) about 30 m east of Municipal Pier 2 in downtown Monterey, California; latitude, longitude: 36.6032, -121.8889.

We collected Monterey adults by snorkeling on May 27, 2014, and July 23, 2015, and transported them to Hopkins Marine Station (HMS; Pacific Grove, CA). Bodega Head adults were collected by SCUBA on January 14, 2012, and transported to Bodega Marine Laboratory (BML; Bodega Bay, CA). We collected Moclips adults near the low tide mark (-0.85 m below mean lower low water) on June 6, 2016, and transported them to Friday Harbor Labs (FHL; Friday Harbor, WA). We collected Seattle adults on July 6, 2015, and transported them to the University of Washington (Seattle, WA). Orcas adults



Figure 1. Location of the five Northeast Pacific sand dollar (*Dendraster excentricus*) populations used in this study. Monterey, Bodega Bay, and Moclips are outer coast populations exposed to higher energy; Orcas and Seattle are protected locales within the Salish Sea.

were collected on June 6, 2015, July 17, 2015, June 3–9, 2016, and July 16, 2016, and in each case transported to FHL. Everywhere except Seattle, we kept adult sand dollars partially buried within fine sediments (collected from the adult beds) in flowing seawater aquaria until spawning; in Seattle, we fertilized the sand dollars on the day of collection and returned the adults to their sand dollar bed the following day.

Larval culture

We set up fertilizations of gametes deriving from our experimental sand dollar individuals as follows. We obtained gametes by intracoelomic injection of between 0.2 and 0.5 mL of 0.55 mol L⁻¹ KCl in distilled water, depending on the size of the adult. For the experiments in which we were explicitly examining within-population variation (the Monterey 2015 and Orcas-Moclips June 2016 experiments), as well as the experiment in which we took a first look at among-population variation (the Orcas-Moclips June 2016 experiment), we conducted single male-female crosses. We also conducted a single male-female cross in the Bodega Head experiment, because those were the only gravid individuals that day. In all other cases

(Monterey 2014, Seattle 2015, Orcas July 2016, and Orcas May 2017), we combined hatched embryos in equal proportions from three or four individual male-female crosses, thus yielding mixed-parentage cultures. We conducted all fertilizations at 13–14 °C using standard methods (M. F. Strathmann, 1987; R. R. Strathmann, 2014). We then allowed the newly fertilized eggs to warm to room temperature (RT, 19–22 °C) and maintained the embryos and larvae at RT throughout larval development. The one exception was the experiment comparing settlement in knocked-down *versus* swimming larvae following turbulence exposure (Orcas July 2016), for which we cultured the larvae at 13–15 °C throughout.

We note that the typical ocean temperatures experienced by *D. excentricus* larvae from the populations we studied are in the 12–16 °C range, and thus substantially cooler than the lab temperatures (19–22 °C) that we used for the genetic crosses that formed the bulk of this study. Because the vast majority of our prior experiments with turbulence and settlement in this species have been at 19–22 °C (Hodin *et al.*, 2015, 2018b; MCF, JH, GN, and BG, unpubl. data), and because we have shown that 14 °C-reared sand dollars show a similar response (Hodin *et al.*, 2018b), we are confident that rearing temperature is not a factor in the results that we report herein. We do note that water temperatures where the sand dollar bed is located on Orcas can regularly exceed 20 °C at around low tide in summertime (Henry, 2017), so 19–22 °C is not an “unnatural” temperature *per se*.

We began feeding four-arm pluteus larvae 1 dpf. Initial larval densities were ~0.5–1 larva mL⁻¹, which we lowered to 0.2–0.3 larva mL⁻¹ after rudiment invagination (see Heyland and Hodin, 2014), which occurred at the 6-arm stage in all of our larvae (3 dpf at RT, 6 dpf at 13 °C). We fed larvae a mixture of 2500 cells mL⁻¹ *Rhodomonas* spp. and 3000 cells mL⁻¹ *Dunaliella tertiolecta* throughout, changing >90% of the culture water by reverse filtration every 2 d and then re-feeding. We cultured embryos and larvae in 0.45- μ m MFSW (with UV treatment at HMS), in gallon jars either with mechanical stirring (M. F. Strathmann, 1987; R. R. Strathmann, 2014) or on a gyratory shaker platform. As larvae approached competence (starting at about 8 dpf at RT and 18 dpf at 14 °C), we subjected a haphazardly chosen subset to daily competence tests using 40 mmol L⁻¹ excess KCl in MFSW. When at least 10% of larvae in a batch were competent by this criterion, we planned turbulence-exposure trials as described below.

Genetic cross designs

Monterey 2015 experiment. We set up 4 reciprocal, single male-female crosses at HMS using 2 males (M1, M2) and 2 females (F1, F2), reared them as above, and then transported the larvae 3 dpf in 0.5-gallon jars with lids at ~1 larva mL⁻¹ to BML. Upon arrival, we changed the water in the cultures, and, for each cross, we counted out exactly 300 larvae into each of

3 replicate jars with 1 L MFSW (thus, 1 larva per 3.3 mL), for a total of 12 jars (3 replicate jars for each of the 4 crosses). We increased the volume at the next water change (5 dpf) to 1500 mL (thus, 1 larva per 5 mL), shifted the location of the 12 jars on the stirring rack (as we also did at each subsequent water change) in an attempt to limit jar effects, and maintained them at this density until 9 dpf, when we conducted turbulence-exposure trials with 2 of the 3 replicates from each cross.

Orcas-Moclips June 2016 experiment. Here we set up single within- and between-population crosses using 2 males and 2 females from each locale, for a total of 16 crosses. After hatching, we haphazardly chose 4 of the 8 hybrid (Orcas \times Moclips) crosses and discarded the other 4: we have room on our larval stirring rack for only 12 jars and wanted them all to be stirred together to eliminate differences in stirring as a possible environmental source of variation. Thus, when we began feeding the cultures 1 dpf, we had 4 Moclips \times Moclips crosses, 4 Orcas \times Orcas crosses, and 4 hybrid crosses, with 1 jar per cross. We cultured them (larval density, shifting jar positions, *etc.*) as described above through days 11 and 12, when we conducted turbulence-exposure trials. Note that we did not replicate crosses in this design for the reasons indicated above, and we thus did not analyze any of our results by cross. Instead, we grouped the crosses by mother, father, or population as our source of replication and thus to test for jar effects.

Generating turbulence in the laboratory

To generate fluid turbulence in the laboratory (quantified in terms of the energy dissipation rate, in units of $W\ kg^{-1}$), including intensities characteristic of wave-battered coasts, we employed a Taylor-Couette cell (Taylor, 1923). This apparatus consists of two vertically oriented, coaxial cylinders separated by a 3.5-mm gap that contains seawater (described in greater detail in Gaylord *et al.*, 2013). During operation, the cylinders rotate at a prescribed speed relative to one another, thereby shearing the seawater between them. At the rotation speeds employed for testing sand dollar larvae, the sheared flow was turbulent (Gaylord *et al.*, 2013).

Larval exposure to turbulent shear

In all experiments described herein, we exposed larvae to one of a range of turbulence intensities (from 0 to 12 $W\ kg^{-1}$) for 3 minutes. This range of intensities spans those characteristic of calm waters to those measured under actively breaking waves on steep, rocky coasts (Gaylord, 2008). The latter are substantially in excess of those used in most laboratory examinations of larval responses to turbulence (*e.g.*, Fuchs *et al.*, 2010; Wheeler *et al.*, 2016) and are modestly more intense than analogous measurements from gently sloping, sandy beaches (George *et al.*, 1994; Terray *et al.*, 1996; Raubenheimer *et al.*, 2004; Fedderson, 2012; Sutherland and

Melville, 2015). We first concentrated larvae by reverse filtration and transferred 15–35 larvae each 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 eliminated larvae from consideration only if they either were obviously delayed in development or showed the morphological hallmarks (see Hodin *et al.*, 2015) of competent larvae (<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 exposure (trial), 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 minutes. Immediately following each exposure trial, we gently poured the larvae within the Taylor-Couette cell into a 1-L glass beaker already containing approximately 100 mL of MFSW (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 \sim 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 some trials, we compared “unmanipulated controls” to “handling controls” (0 $W\ kg^{-1}$ [=0 rpm] 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 minutes that larvae were within it, thereby controlling for manipulations associated with transfer of larvae into and out of the apparatus. We have never observed any differences between larvae exposed to unmanipulated and handling controls in our studies (Gaylord *et al.*, 2013; Hodin *et al.*, 2015; JH, unpubl. obs.).

Quantification of settlement

Following turbulence treatments, we assessed settlement by transferring all larvae from an exposure trial to a single well of a pre-washed, non-tissue culture-treated 6-well plate (see Herrmann *et al.*, 2003) containing 8 mL of 40 mmol L^{-1} excess KCl in MFSW, maintained at the rearing temperature.

KCl exposures were for 1 hour, followed by recovery in 8 mL of MFSW. After the 1-hour KCl exposure, 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., Sato *et al.*, 2006; Sutherby *et al.*, 2012; Gaylord *et al.*, 2013; Hodin *et al.*, 2015; Mos and Dworjanyan, 2016). We verified continued withdrawal of skin over the next several hours and eventual adoption (with a final scoring at 12+ hours in recovery) 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 alive and appeared healthy, and we detected no post-settlement mortality.

Studies on mollusc larvae have identified a sinking or active downward propulsion behavior associated with turbulence exposure (Fuchs *et al.*, 2004, 2010, 2013, 2015a, b; Wheeler *et al.*, 2015). In our study conditions, we could not directly observe such behaviors during turbulence exposure. Instead, 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 (i.e., the proportion of larvae knocked down by each trial).

Statistical analyses

We assessed the effect of turbulence exposure (0–5.9 W kg⁻¹) on settlement by using logistic regressions. Turbulence intensity (W kg⁻¹) was treated as a categorical factor when we examined only two turbulence intensities (0 and 5.9 W kg⁻¹) and as a continuous factor in the one case in which we examined three turbulence intensities (0, 2.2, and 5.9 W kg⁻¹; Monterey 2015 experiment). Both dpf (Orcas-Moclips, see below) and replicate exposure trials (all experiments) were treated as random effects in the models. We used the least squares means calculated from the models to first estimate the differences between turbulence-exposed and control trials for each cross. We subsequently employed a Z test accompanied by a Tukey honest significant difference method to account for multiple comparisons in *post hoc* comparisons (Monterey 2015 experiment). We assessed the effect of turbulence on proportion of larvae on the bottom by a logistic regression. For all data, we assessed normality of residuals using Shapiro tests and *q-q* plots.

For the Moclips-Orcas settlement data, we conducted a mixed effects logistic regression, using a Markov chain Monte Carlo simulation within JAGS (Just Another Gibbs Sampler, Plummer, 2017). Our model included turbulence as a fixed factor and replications and dpf (we conducted settlement trials at 11 and 12 dpf) as random effects. For the population analysis, we included the three different categories of crosses (Orcas, Moclips, and hybrids) as a fixed factor, with males and females as random effects; for differences within females or males, we included them as random effects for the respective

analysis. We allowed the intercepts to vary with our random effects, and when examining the differences within females or males, we also allowed the effect of turbulence intensity to vary by individual parents. We then sampled the posterior distribution to see whether the differences in turbulence response between parents fell outside of zero in a 95% credible interval; this enabled us to compare whether the effects of turbulence intensity differ from parent to parent.

Our priors for each parameter’s mean are normally distributed, with a mean of 0 and precision of 0.001. For each parameter’s variance value, our prior was a truncated Cauchy distribution with a scale of 2.5, as suggested in Gelman *et al.* (2008). We ran the analyses with 3 chains, a burn-in iteration of 10,000 and for 50,000 iterations, and checked the trace plots for all analyses to ensure that the chains were mixing and that convergence had been reached.

For the graphs showing “proportion elevated settlement,” we calculated this value for each turbulence-exposure trial (i.e., exposure in the Taylor-Couette cell), using the proportion of larvae that settled in that turbulence trial (P1) and the proportion of larvae that settled in controls (no turbulence; P2) as follows:

$$\frac{P1 - P2}{1 - P2}$$

Note that we used these values in certain figures solely for ease of comprehension; the only statistical analysis in which we used elevated proportion settled was the Monterey experiment.

Because the Monterey experiment only had two males and two females, we were able to conduct a simpler analysis. First, we calculated elevated proportion settled as outlined above for each replicate trial (i.e., we matched individual unexposed control trials with corresponding 2.2 and 5.9 W kg⁻¹ exposure trials, thus calculating elevated proportion settled for each cross and replicate separately). Then, using a generalized linear mixed effects model, we conducted a logistic regression with males, females, turbulence intensities (2.2 and 5.9 W kg⁻¹), and their interaction terms as fixed effects; we treated replication and jar of larvae as random intercepts. We subsequently reduced the complexity of the model based on an Akaike Information Criteria score (AIC score; Akaike, 1978), ultimately settling on the model that had the lowest AIC score, as we describe in the Results.

When examining how turbulence intensities impact the proportion of larvae on bottom, we also conducted a logistic regression using a generalized linear mixed effects model. We used males, females, and source population (Moclips, Orcas, or hybrids) as fixed effects in three separate regressions and included replications and dpf as random intercepts in all three regressions. We also added males and females as random intercepts when we examined differences among the three categories of population crosses. Using planned contrasts and Bon-

ferroni corrections, we examined whether individual males or females had offspring that were significantly different in their knocked-down response from the other crosses.

We conducted all statistical analyses using R (ver. 3.4.2, R Foundation for Statistical Computing, Vienna). We used JAGS and the R2jags package for the Bayesian analyses and the lme4 package for the generalized linear mixed effects model; we conducted *post hoc* tests using the lsmeans and multcomp packages.

Results

Variation in settlement responses among sand dollar cohorts and populations

Our prior results indicated that our different rearing episodes (cohorts) of *Dendraster excentricus* derived from geographically separated populations (Fig. 1) demonstrated marked variability in the propensity with which their advanced larvae responded to fluid turbulence. We illustrate a subset of these observations in Figure 2A: dose responses of larvae from three different populations to increasing levels of fluid turbulence, where we examined the induction of competence to settle following a brief turbulence exposure. Larvae deriving from adults collected off Bodega Head in 2012 showed a very robust response to turbulence, with an apparent steep rise in the response at $\sim 1 \text{ W kg}^{-1}$. By contrast, larvae deriving from Monterey adults collected in 2014 appeared to show a more

gradual rise in responsiveness with increasing turbulence. Finally, a cohort of larva derived from adults collected in Orcas in 2016 showed the most tepid response of the three populations shown—this despite their being tested on a day where their controls (0 W kg^{-1}) had a much higher propensity to settle than in the Monterey and Bodega Head larvae.

In addition to apparent batch-to-batch variation in the turbulence-activation threshold for the settlement response (Hodin *et al.*, 2015), we also occasionally observed entire cohorts of larvae that showed very low responsiveness to turbulence. We show one such example in Figure 2B. Larvae derived from Seattle adults collected in 2015 and Orcas adults collected in 2016 (and fertilized in May 2017) showed very modest increases with turbulence intensity in the settlement response (*cf.*, the Bodega Bay larvae in Fig. 2A), with only a 20%–30% increase in proportion settled in the 5.9 W kg^{-1} treatment *versus* the 0 W kg^{-1} controls (Turbulence: $Z = 2.564$, $P = 0.01$; Intercept [Seattle]: $Z = -0.544$, $P > 0.5$; Intercept [Orcas]: $Z = -0.525$, $P > 0.5$, Population \times Turbulence: $Z = -0.62$, $P = 0.535$).

The results in Figure 2A appear consistent with the hypothesis that response to turbulence correlates with the likely exposure of larvae to fluid turbulence in the wild as they are approaching their respective habitats (see Fig. 1). Bodega Head is an extremely high-intensity environment characterized by strong swell and crashing waves. Monterey is also high energy, though that particular sand bed is partially protected by a pier.

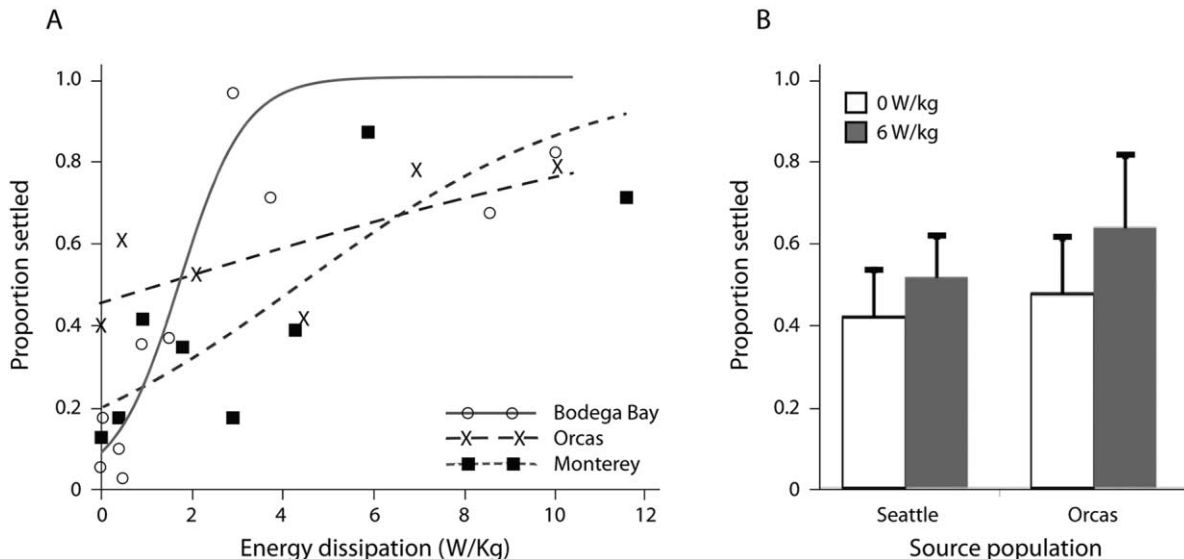


Figure 2. Examples of apparent among-population variation in the settlement response following turbulence exposure in *Dendraster excentricus*. We here illustrate specific examples of experiments merely to give a sense of the apparent among-population variation. Because we performed these experiments at different times in different locations, we do not draw explicit conclusions about this variation. (A) Dose response of settlement over a range of turbulence intensities. The curves shown are logistic functions fitted to the data; we show them here for convenience only. (B) An example of two experiments showing low turbulence responsiveness in Salish Sea sand dollars. Each data point in (A) represents a single exposure trial; thus, there are no error bars in that panel. Error bars in (B) are ± 1 SD.

By contrast, Orcas and Seattle are sheltered sites within the Salish Sea. The larval responses to turbulence thus appear to parallel the energy gradient: Bodega Head larvae seem to show the most robust turbulence induction, followed by Monterey, with seemingly weaker responses in larvae from Orcas and Seattle. Still, it is difficult to make explicit comparisons between these different batches of larvae. In addition to differences in the source population, these different larval cohorts were reared in different locations at different times, with numerous possible sources of variation with respect to rearing conditions. Therefore, we set out to design experiments to explicitly test for within-population genetic variation in the response of sand dollar larvae to turbulence. We also set up a pilot study to begin to examine whether populations of sand dollars that differ in the energetic exposure of their native sand dollar beds might have offspring that differ in a consistent manner in their response to turbulence at settlement.

Within-population variation in turbulence induction of larval settlement

To examine whether *D. excentricus* larvae show within-population variation for a behavioral response to turbulence, we set up reciprocal single male-female crosses between two males and two females collected in Monterey. We reared two replicate larval cultures from each of the four crosses to the precompetent stage and then conducted a series of replicate trials exposing batches of larvae to one of three turbulence intensities (0, 2.2, or 6.0 W kg^{-1}), followed by exposure to 40 mmol L^{-1} excess KCl in MFSW to assess competence and settlement. We analyzed these data as elevated proportion settled *versus* controls in each trial, as outlined in the Materials and Methods.

Our initial statistical model for these data included males, females, turbulence intensity, and the interaction terms as fixed effects. In this initial analysis, we detected no statistical difference among the offspring of the two females (F1 and F2 in Fig. 3), regardless of father ($Z = 0.216$, $P = 0.829$). By contrast, we found that offspring of one of the two males (M2 in Fig. 3) showed borderline evidence for higher rates of elevated settlement following turbulence exposure than those of the other male (M1), regardless of mother ($Z = 1.908$, $P = 0.056$). We saw no evidence for any interaction effects ($P > 0.5$ in all cases).

Because of the absence of any detectable interaction effects in this initial analysis (“full model”), we simplified the model, dropping all of these nonsignificant interaction terms; this improved the model fit (AIC score 157.4 *vs.* 163.9 for the full model). In this reduced model, we still saw no evidence for a statistical difference among the offspring of the two females ($Z = 1.560$, $P = 0.119$), and we now detected a clear difference between the offspring of M1 and M2 across turbulence exposures. Specifically, the offspring of M2 showed a consistently more robust induction of competence at both 2.2 and

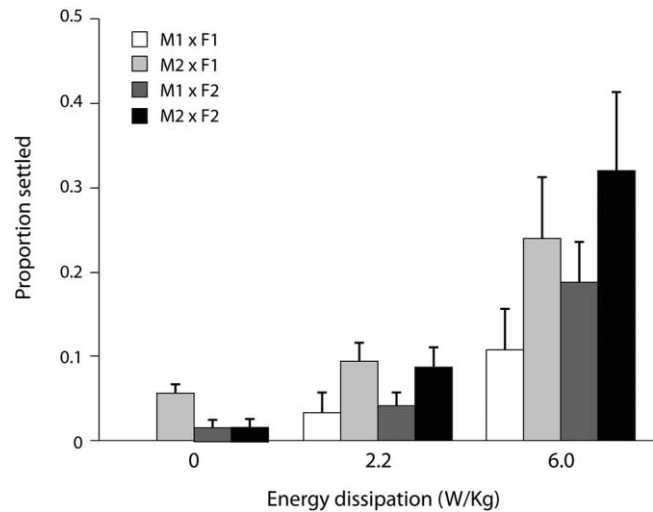


Figure 3. Monterey sand dollars (*Dendraster excentricus*) showed within-population variation in their behavioral response to turbulence, likely genetic. Both crosses involving one of the fathers (M2) showed a more robust settlement response to turbulence than those involving the other father (M1), regardless of the mother ($Z = 4.180$, $P < 0.001$). The offspring of the two mothers (F1, F2) did not differ from one another ($Z = 1.560$, $P = 0.119$). $N = 1675$ larvae, 2 replicate larval cultures per cross, 2–3 independent turbulence exposures per replicate. Error bars are ± 1 SD. Note that no M1 \times F1 larvae settled in any of the 0 W kg^{-1} control trials, hence the absence of error bars associated with those data.

6.0 W kg^{-1} when compared to the offspring of M1 ($Z = 4.180$, $P < 0.001$; Fig. 3).

Settlement response to turbulence in sand dollars from contrasting populations

To begin to examine the hypothesis that habitat energy correlates with the larval response to turbulence, we collected adults from an outer coast and a protected locale in Washington State—Moclips and Orcas, respectively (see Fig. 1)—and set up a series of within- and between-population crosses using two males and two females from each locale. Note that due to the small numbers of unique Moclips, Orcas, and hybrid crosses (three or four crosses each, two crosses each involving unique males and females), our design is insufficient to detect interpopulation variation *sensu stricto*. Instead, our goal was to treat this as a pilot study to see whether we detected trends indicating consistent and dramatic differences in the response of the offspring from sand dollars from these two contrasted populations.

For ease of following the individual mothers and fathers, we here assign names to the parents, with the first initial of the name matching the first initial of the population. Thus, Mian and Mindy were the Moclips females, Ophelia and Olivia were the Orcas females, Messi and Mork were the Moclips males, and Oscar and Otto were the Orcas males. We then reared all larvae in parallel to the precompetent stage and conducted turbulence and settlement experiments as before, ex-

cept we exposed larvae to only one of two turbulence treatments: 0 and 5.9 W kg^{-1} .

We first grouped our data by father ($N = 4$ males, 11 crosses total [Messi \times Olivia cross dropped due to too small a proportion of remaining precompetent larvae to be able to detect a response by treatment], 4 replicate exposure trials per cross, 88 total exposure trials), using a Bayesian analysis to compare the turbulence response of the offspring of each male to that of the other 3 males, regardless of mother (Figs. 4A, A1). We detected pairwise differences among the following males: both Oscar (98.1% credible interval) and Otto (98.5% credible interval) were significantly more responsive to turbulence than Mork. Next, we grouped the data by mother, comparing the turbulence response of the offspring of each female to those of the other three females, regardless of father (Fig. 4B). Among females ($N = 4$ females, 11 crosses total [see above], 4 replicate exposure trials per cross, 88 total exposure trials), we detected the following pairwise differences: the offspring of Ophelia showed consistently more robust responses to turbulence than the offspring of all of the other females (Mian: 99.8% credible interval; Mindy: 97.8% credible interval; Olivia: 99.4% credible interval), and the offspring of Mindy were generally more responsive than the offspring of Mian (94.2% credible interval).

We also grouped our data by source population to look for any hint that larvae derived from the exposed coast population (Moclips) responded more to turbulence exposure with respect to proportion settled (Fig. 4C). In fact, our data show initial support for the opposite conclusion: offspring that had two Orcas parents happened to respond more to turbulence, on average, than offspring that had two Moclips parents (99.9% credible interval). We also detected borderline support for a greater response in offspring that had two Orcas parents when compared to the hybrid offspring (94.0% credible interval), as well as support for a greater response in hybrid offspring when compared to those with two Moclips parents (90.2% credible interval). Again, due to limited replication at the level of parent, we cannot confidently extend these trends to the populations as a whole.

In this experiment, we monitored an additional behavior associated with settlement: the proportion of larvae on the bottom of the dish (and hence in contact with a potential substrate inducer of settlement) following turbulence exposure (Fig. A2). As with the settlement data itself, we looked for evidence of differences among males and females and among the Orcas, Moclips, and hybrid crosses in the proportion of larvae on the bottom after turbulence treatment. In this case we detected a difference in the offspring of only one con-

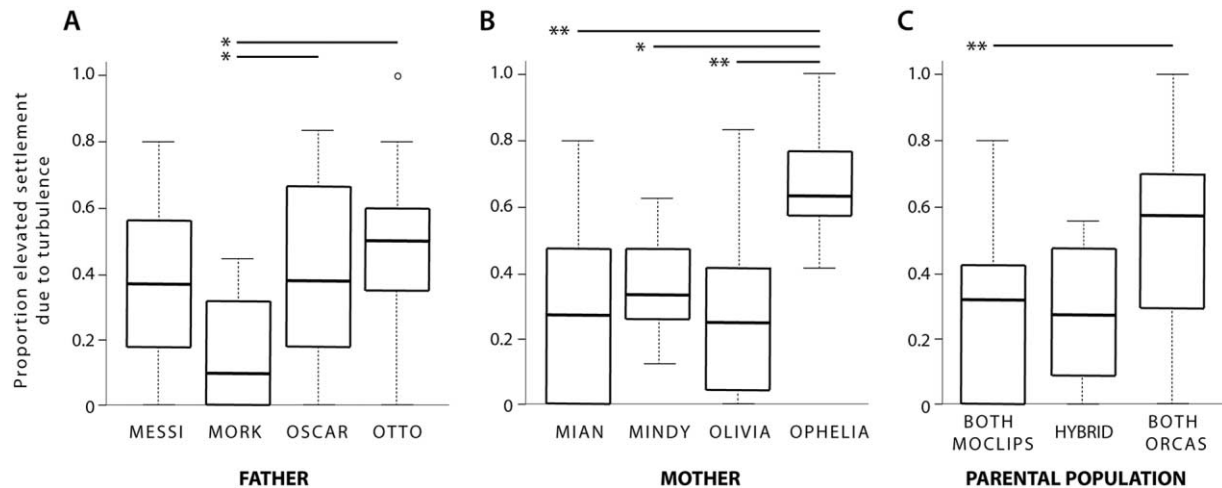


Figure 4. Within- and among-population variation in the behavioral response to turbulence in *Dendraster excentricus*. For convenience, we named the individual fathers and mothers with the first letter of the name matching the population: names beginning with *O* were from Orcas; names beginning with *M* were from Moclips. In these standard box plots, we show the proportion of larvae that settled in response to 5.9 W kg^{-1} turbulence as “elevated settlement” with respect to no-turbulence controls. The lines over the graphs connect statistically different sets of larvae, with asterisks indicating pairs of sets of larvae with a >95% likelihood that the true difference between individuals is not zero (* >95%, ** >99%, *** >99.9%). (A) Crosses grouped by father. (B) Crosses grouped by mother. (C) Crosses grouped by population (Moclips \times Moclips, Orcas \times Moclips hybrids, or Moclips \times Moclips). Note that due to the limited number of individual parents from each population in our design (four Moclips and four Orcas parents), we cannot conclude that the trends in (C) are reflective of the populations as a whole. Note also that for the statistical analyses, we compared proportion settled in the control (0 W kg^{-1}) and turbulence exposure (5.9 W kg^{-1}) treatments for each cross, rather than using this “elevated settlement” value *per se*. We show the elevated settlement data here because we find them intuitively easier to understand than the settlement differentials used in our analysis.

trasted pair of females: Olivia's offspring were knocked down less by turbulence exposure than the offspring of Ophelia ($Z = -3.188$, $P < 0.05$; Fig. A2B). We did not detect any such differences among the offspring of any of the males ($P > 0.2$ for all contrasts; Fig. A2A), nor did we detect any overall differences among the crosses grouped by source population (Moclips, Orcas, and hybrids) in the propensity with which the larvae were knocked down by turbulence exposure ($P > 0.9$ for all contrasts; Fig. A2C).

Larvae on bottom following turbulence exposure are more likely to settle

Using a separate cohort of larvae from Orcas parents (reared at 13–15 °C in this case), we investigated whether the larvae that are knocked down (*i.e.*, that are on the bottom of the dish) following turbulence exposure are more likely to settle than the larvae still swimming. Following 3 minutes of $\sim 5.1 \text{ W kg}^{-1}$ turbulence exposure and recovery of larvae, we immediately transferred all larvae into 40 mmol L^{-1} excess KCl in seawater; but for each exposure trial we separated the larvae that were on the bottom 4 minutes after exposure *versus* those swimming at that time. About twice as many larvae that were on the bottom following exposure settled when compared to those larvae swimming following the same exposure: of 69 total larvae tested (in 4 replicate turbulence exposures), $71.7\% \pm 20.6\%$ (SD) of the knocked-down larvae settled, compared to $36.5\% \pm 22.1\%$ of those that were still swimming following exposure ($Z = -2.596$, $P < 0.01$).

Discussion

Settlement is a critical and generally irreversible process in the life history of many benthic marine organisms. In animals, larvae that are competent to settle are known to assess environmental characteristics of the benthos before making their decision to commit to the shift in habitat. Most studies on the environmental characteristics or “cues” that larvae use to assess potential settlement habitat have focused on olfactory features that might indicate the presence of conspecifics or a favored food source (reviewed by Hadfield and Paul, 2001). Other studies have revealed situations in which light, substrate texture, hydrodynamics, or sound are likewise assessed by settling larvae (for a review, see Hodin *et al.*, 2018a). Recently, we have elucidated an additional environmental feature that echinoid (sea urchin and sand dollar) larvae appear to use as a contributory indicator of approach to their shoreline habitat: an increase in fluid turbulence (Gaylord *et al.*, 2013; Hodin *et al.*, 2015). Here we show evidence for within-species genetic variation in fluid turbulence responsiveness of larvae of the Pacific sand dollar (*Dendraster excentricus*), a species whose range of habitats varies from wave-impacted to calm and protected shores (Mooi, 1997). This finding provides support for a new category of individual variation in settlement-

associated behaviors (Nanninga and Berumen, 2014), specifically relating to larval response to turbulence at the crucial and irreversible settlement transition. We consider the implications in further detail below.

Genetic variation in echinoids with dispersing larvae

Benthic marine invertebrates typically have limited dispersal potential as adults. By contrast, their larvae can disperse for days to months or longer in the plankton, and some are even known to cross ocean basins (Scheltema, 1971). In those taxa with broad geographic ranges and lengthy dispersal periods, there tends to be little genetic differentiation among populations, whereas species with short dispersal periods (such as those with non-feeding larvae) tend to show higher levels of genetic differentiation among populations (Cowen and Sponaugle, 2009; but see, *e.g.*, Richardson *et al.*, 2016).

Dendraster excentricus has a wide geographic range in the Northeast Pacific, from Alaska to Baja, and a minimum larval period of approximately 3 weeks at 14 °C. Populations of adults are patchily distributed across this range, with sediment type being the likely limiting factor for adult survival (Mooi, 1997). Interestingly, *D. excentricus* populations are found across a substantial gradient of energy exposure, from shallow subtidal populations on wave-battered coasts to sheltered populations that experience little swell (Nakamura, 1995). Pacific sand dollars are also found across a substantial depth range, from the mid-high intertidal zone down to 90 m (Mooi, 1997). Despite this habitat diversity, there is little evidence for genetic differentiation in the mitochondrial DNA sequences for 16S rRNA among *D. excentricus* populations as distant as Southern California and Vancouver Island (D. Eernisse, California State University Fullerton, pers. comm.); this pattern is reminiscent of that for many echinoids with feeding larvae (*e.g.*, Debenham *et al.*, 2000; Duran *et al.*, 2004; Banks *et al.*, 2007).

Despite what is typically a dearth of robust population structure, echinoids do tend to harbor substantial degrees of within-population genetic variation and maintain high allele frequencies at certain loci (*e.g.*, genes thought to be important for reproduction, such as *bindin*; Lessios and Zigler, 2012). For example, by most estimates, within-species variation in the purple sea urchin, *Strongylocentrotus purpuratus* (*e.g.*, Garfield *et al.*, 2012; Pespeni and Palumbi, 2013), is greater than the scope of genetic variation between chimps and humans (*e.g.*, Prüfer *et al.*, 2012).

Apparent genetic variation in turbulence induction of settlement

Here we provide evidence for individual variation in a settlement-associated behavior: the propensity with which turbulence induces competence in Pacific sand dollar larvae. As in previous work (Gaylord *et al.*, 2013; Hodin *et al.*, 2015), we assessed this behavior by exposing nominally precompe-

tent larvae to three minutes of a high intensity of fluid turbulence and comparing the subsequent response of these larvae and unexposed controls to a chemical inducer of settlement. In the current study, we looked for differences in this behavioral response to turbulence in offspring of a number of individual male-female crosses.

Based on previous work (Hodin *et al.*, 2015), we suspected that a shallow subtidal population of *D. excentricus* in Monterey, California (Fig. 1), harbored genetic variation for the way turbulence induces competence to settle. As reported here, we found strong evidence that the offspring of one particular Monterey male sand dollar (M2, regardless of female) showed a consistently more robust competence induction by turbulence compared to the offspring of the other Monterey male (M1; see Fig. 3). Because we here infer genetic variation in the absence of any genetic data *per se*, this finding was fortuitous, because it allows us to exclude maternal effects as an explanation for the differences among these particular crosses.

These data alone do not prove that the variation that we observed is genetic in nature *sensu stricto*. Paternal effects, while considered relatively uncommon compared to maternal effects, are likely an under-appreciated contributor to phenotypic variation (Crean and Bonduriansky, 2014). Nevertheless, because sand dollars (like most marine invertebrates) have external fertilization and no parental care, opportunities for non-genetic paternal contributions to embryonic and larval development are limited to substances or organelles in the sperm itself (such as the centriole) and paternal imprinting (*via, e.g.*, methylation patterns; see, *e.g.*, McBirney *et al.*, 2017), of which there have been no reports to date in echinoids (sand dollars and urchins). As such, it seems likely that the differences we observed were instead due to differences in genotype and thus indicate heritable variation for the larval response to turbulence at settlement in this nearshore sand dollar species.

In a second experiment (crossing Moclips- and Orcas-derived sand dollars; see Figs. 1, 4), we detected moderate support for differences in the behavioral response to turbulence among the offspring of certain males. These results lend additional support to a conclusion of individual genetic variation in this settlement behavior. In this latter experiment, we also detected substantial individual variation among the offspring of certain females, both within and between the two populations tested (see Fig. 4). In this case, we cannot readily distinguish between genetic and maternal effects (or a combination of both) as an explanation for the differences in the behavioral responses of the offspring of certain females. For reasons that we explain below, we suspect maternal effects as driving some or all of these responses among females.

Evidence for between-population variation in the behavioral response to turbulence?

Moclips is an outer coast population in Washington that is routinely exposed to extreme wave energy (and thus highly

turbulent waters), whereas Orcas adults are from a much lower-energy locale in the Salish Sea. We thus designed the Moclips-Orcas experiment as a pilot test of the hypothesis—motivated by the suggestive data shown in Figure 2—that sand dollars from higher-energy habitats (such as Moclips) would produce larvae that respond more robustly to turbulence exposure at settlement, as compared to larvae derived from adults collected in more protected locales (such as Orcas).

We propose four hypotheses for how turbulence responses might vary with the mean exposure of the population to turbulent waters.

H1. Sand dollar larvae with parents from high wave-energy (“exposed”) locales show a more robust response to turbulence than sand dollar larvae deriving from more protected locales, because turbulence-responsive larvae would be more likely to settle in the exposed locales, thus increasing the proportion of adults deriving from larvae having that phenotype.

H2. Sand dollar larvae with parents from protected locales show a more robust response to a given level of turbulence than sand dollar larvae with parents from more exposed locales, because larvae settling in these locales may have a lower response threshold to turbulence than larvae settling in exposed locales.

H3. There is population-level genetic variation in larval responses to turbulence, and this variation swamps any among-population differences.

H4. Any differences that we observe among populations are due to non-genetic maternal or paternal effects and not genetic variation *per se*.

Surprisingly, our analysis of these limited data sets showed that our particular Orcas × Orcas crosses (protected) tended to show a more robust induction of competence in response to turbulence than the Moclips × Moclips crosses (exposed; see Fig. 4C); it is thus contrary to our initial prediction. Note that our design—with only two males and two females from each population in a series of pairwise crosses—lacked the statistical power to be able to conclude anything definitive about these two populations and hence distinguish among H1–H4. Indeed, the differences between the population of origin that we detected in our analysis (Figs. 4C, A1C) are almost entirely due to the very robust turbulence-primed settlement responses of a single Orcas female, *Ophelia*, regardless of the father (see Figs. 4B, A1B). In other words, here again we are seeing evidence for individual (parent)-based variation in the larval response to turbulence.

As such, the Orcas and Moclips results that we present here (Figs. 4C, A1C, A2C) should only be considered a first pass at an analysis of population-level variation in the settlement responses in sand dollars. Additional studies contrasting exposed-coast and sheltered *D. excentricus* populations across their extensive geographic range, and with more parents per population studied, seem warranted in order to evaluate our four hypotheses.

Little evidence for variation in another behavioral response to turbulence

In the Moclips-Orcas experiment, we also monitored an additional larval response to turbulence associated with settlement: the larvae sink to and remain on the substrate for several minutes following turbulence exposure. Unlike the settlement response itself, we detected minimal evidence for differences in the scope of this “knockdown” response among the offspring of different parents, and we did not see differences between our Moclips and Orcas crosses as a whole.

In a separate experiment, we did establish a connection between the knockdown effect and subsequent settlement: larvae that were knocked down settled in substantially higher numbers than those that were not knocked down by the exposure. Note that our observations here of a knockdown phenotype resulting from turbulence exposure are distinct from previously reported changes in mollusc larval swimming responses within turbulent flows (Fuchs *et al.*, 2004, 2010, 2013, 2015a, b; Wheeler *et al.*, 2015).

One interpretation of our reported behavioral effects of turbulence on echinoid larvae is as follows. The physiological nature of the knockdown effect might be considered a more or less generic behavioral response: the extreme turbulence exposure likely causes a temporary cessation of ciliary activity, and the larva simply sinks. By contrast, the effect on settlement (*via* the induction of competence) itself seems more specific in nature, in the sense that in our trials, the settlement behavior manifests after the turbulence exposure ceases. Furthermore, unlike the knockdown response, which lasts for less than 30 minutes before the larvae recover, the induction of competence by turbulence persists for at least 24 hours after the turbulence exposure (MCF, JH, GN, and BG, unpubl. data). Finally, while many crosses yielded offspring that responded only tepidly with respect to turbulence induction of settlement itself, those same crosses nevertheless showed a strong behavioral knockdown response (Fig. A2).

Evolutionary implications

If indeed the knockdown response to turbulence is more or less generic, while the settlement response itself is more specific in nature, we can envision the following scenario for the evolution of turbulence responsiveness in late-stage echinoid larvae. The knockdown effect may be akin to a preadaptation (*sensu* Gould, 1984) for the evolutionary acquisition of turbulence-induced settlement. Knockdown behavior in response to turbulence would tend to carry the larvae to the substrate when they approach the nearshore, thus exposing them to substrate-associated chemical or physical cues. Then, the subsequent evolutionary acquisition of the more specific turbulence-settlement response would involve a physiological pathway where, for example, turbulence would induce the sensitization of the larvae to those aforementioned surface-associated cues.

As mentioned previously, our differing results regarding individual variation in the knockdown response *versus* the settlement behavior is consistent with this evolutionary scenario. Nevertheless, a more precise test of this hypothesis would involve a strict comparative approach among echinoids. Specifically, we would predict that the turbulence-knockdown effect is more evolutionarily widespread, with the more specific turbulence-settlement behaviors being found in taxa nested within clades sharing the turbulence-knockdown behavior (Fig. 5).

Furthermore, our observations of individual variation in the turbulence-settlement behavior suggest a plausible pathway for evolutionary vicariance. Imagine an ancestral population that is more or less obligatorily subtidal, with very occasional observations of scattered individuals found intertidally. Then an allele arises in that population that promotes the turbulence-settlement behavior. One possibility is that those larvae carrying that allele would be more likely to settle in response to

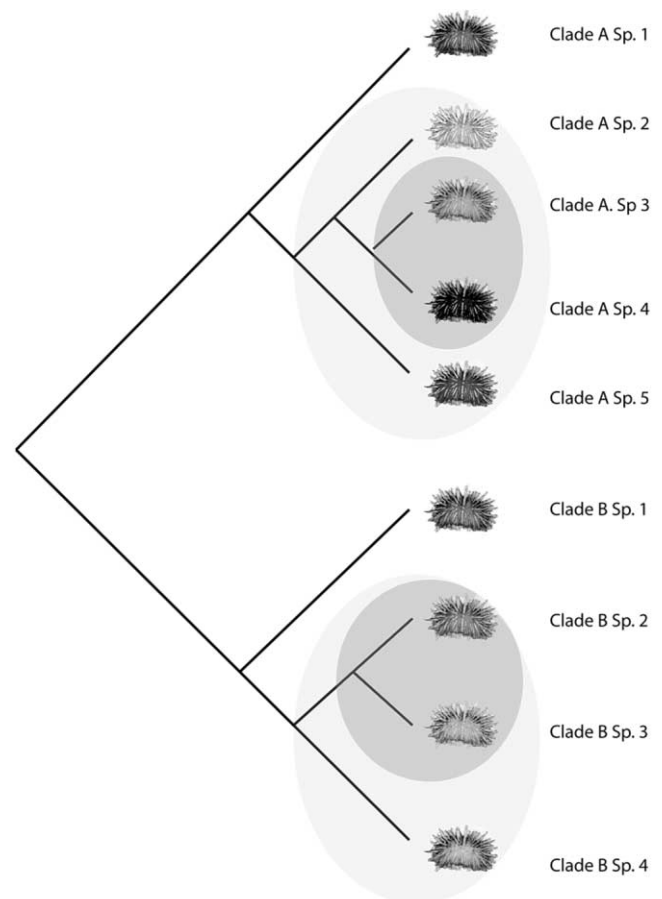


Figure 5. Hypothetical phylogeny of nine related sea urchin species from two clades (A, B). The light gray shading (larger ellipses) indicates species exhibiting the turbulence-knockdown response. The darker gray shading (smaller ellipses) indicates species exhibiting the turbulence-settlement response. Results like these would tend to support the hypothesized scenario that the turbulence-knockdown behavior is a preadaptation for the evolution of the more specific turbulence-settlement response.

turbulence and thus in the intertidal zone. In the short run, such a situation could manifest as limited allelic variation within cohorts of settling larvae when compared to the population as a whole (the Sweepstakes Reproductive Success hypothesis, *sensu* Hedgecock, 1994), or more generally as genetic variation among recruits resulting from heritable differences in habitat selection (see Johnson and Black, 1984). Subsequent generations deriving from the intertidal individuals would presumably be enriched for the turbulence-settlement allele, thus setting up nascent allopatry and the plausible divergence of the subtidal and intertidal populations as separate species. Indeed, despite the commonly cited “onshore-offshore” pattern of nearshore innovations spreading to deep habitats (Jablonski *et al.*, 1983), there is also accumulating paleontological evidence across many invertebrates for the reverse pattern of offshore origins for onshore taxa, including echinoderms (*e.g.*, Lindner *et al.*, 2008; Tomašových *et al.*, 2014; Bribiesca-Contreras *et al.*, 2017).

Echinoids—with their extreme habitat diversity from intertidal to the deep sea, from exposed coasts to protected shores, and from the poles to the tropics—offer abundant opportunities for testing these types of evolutionary hypotheses. The rapid growth in genomics resources available for echinoids will further assist in our evaluations of the molecular and physiological bases of the responses that we have studied herein and in a population genetics context as well. Lastly, the rich fossil record of echinoids will continue to inform upon evolutionary hypotheses and broader taxonomic trends.

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Appendix

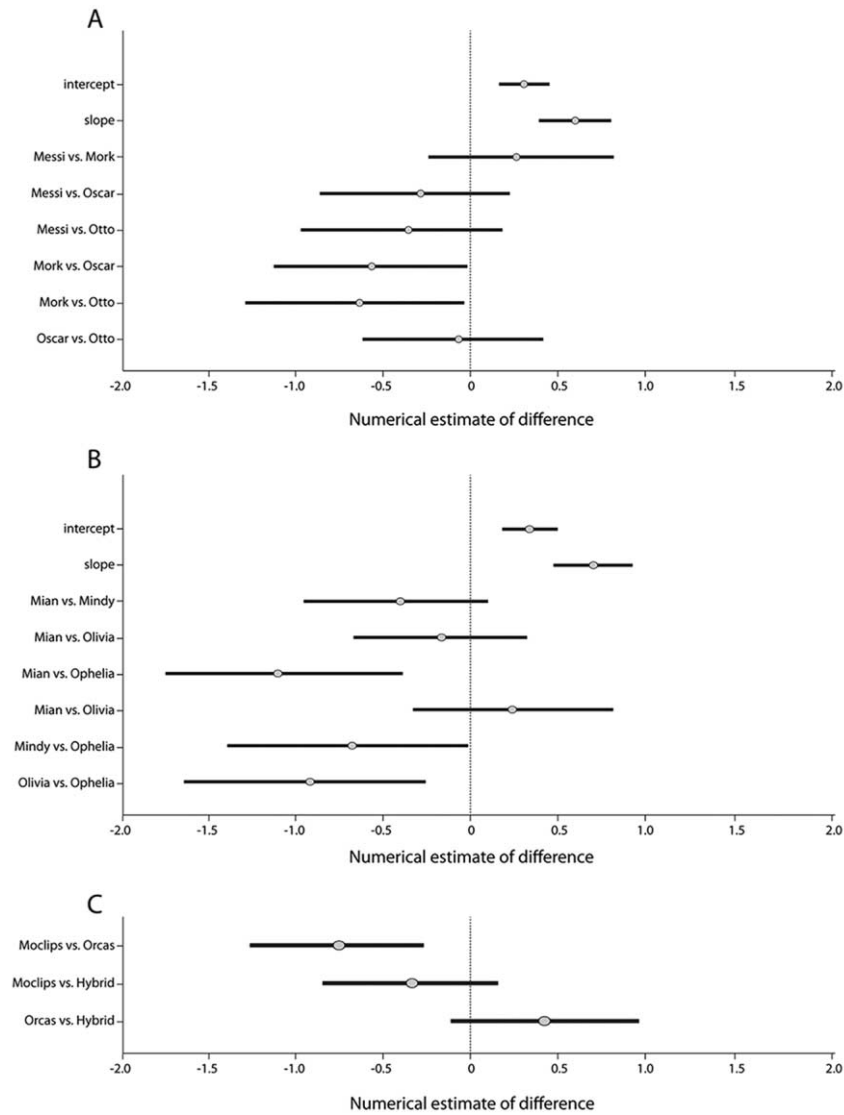


Figure A1. Individual contrasts comparing the settlement responses of different sets of Moclips, Orcas, and hybrid *Dendraster excentricus* offspring to turbulence. For convenience, we named the individual fathers and mothers with the first letter of the name matching the population: names beginning with *O* were from Orcas; names beginning with *M* were from Moclips. (A) Crosses grouped by father. (B) Crosses grouped by mother. (C) Crosses grouped by population (Orcas \times Orcas, Moclips \times Moclips, or Orcas \times Moclips). The *x*-axis shows the numerical estimate either of the overall differences among the members of each group (*i.e.*, slope and intercept) or between the individual parents or populations (as shown on the *y*-axis). The mean differences are shown by the open circles; the horizontal solid lines show the corresponding 95% confidence intervals. If the 95% confidence interval ranges do not intersect with zero (*i.e.*, if they do not overlap with the dashed vertical lines), then we can consider those contrasts to be significantly different from one another with $>95\%$ confidence. We thus detected (A) differences between the four males, and specifically between Mork and both Oscar and Otto; (B) differences between the four females, and specifically between Ophelia and each of the other three females; and (C) differences between the offspring of two Orcas parents and two Moclips parents.

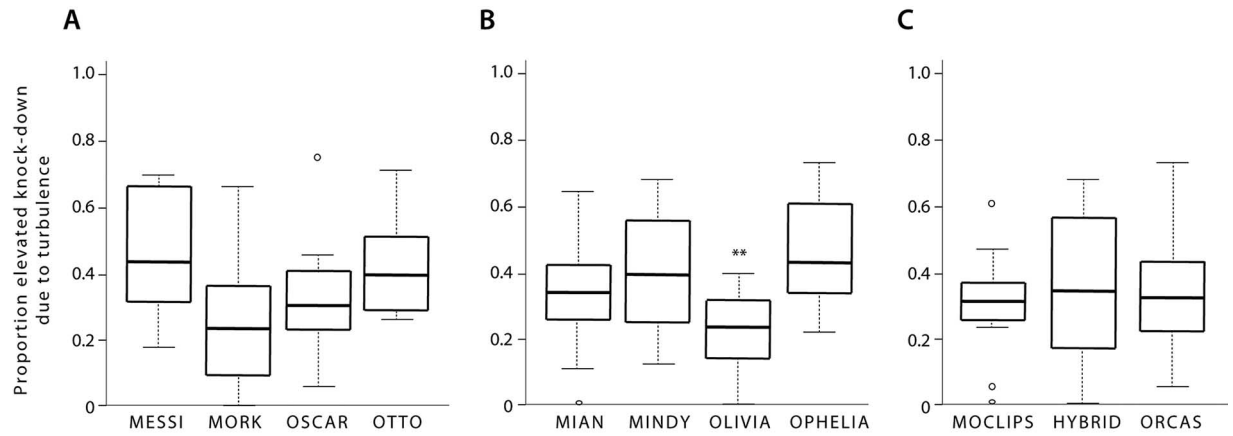


Figure A2. Limited evidence for variation within or between populations in the knockdown effect by turbulence in *Dendraster excentricus*. Standard box plots showing (A) crosses grouped by father, (B) crosses grouped by mother, and (C) crosses grouped by population (Orcas \times Orcas, Moclips \times Moclips, or Orcas \times Moclips). For convenience, we named the individual fathers and mothers with the first letter of the name matching the population: names beginning with *O* were from Orcas; names beginning with *M* were from Moclips. Of all of the groups of offspring, only those of Olivia were significantly different in their propensity to be knocked down by turbulence exposure: Olivia's offspring were knocked down less by turbulence than those of the other three mothers (asterisks in B; $Z = -3.267$, $P < 0.01$).