

Settlement of a marine tube worm as a function of current velocity: Interacting effects of hydrodynamics and behavior¹

Joseph R. Pawlik

Department of Biological Sciences and Center for Marine Science Research, University of North Carolina, Wilmington 28403-3297

Cheryl Ann Butman

Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Abstract

Settlement experiments were conducted with larvae of *Phragmatopoma lapidosa californica* (a reef-building sabellariid polychaete) in turbulent flume flows (near-surface velocities of 5, 10, 15, 20, 25, 30, and 35 cm s⁻¹) over a hydrodynamically smooth bed. Boundary shear velocities spanned the critical shear velocity for initiation of particle motion and for suspended-load transport of passive larval mimics. Larvae were allowed one pass over a sediment array with two treatments: tube sand, a natural inducer of metamorphosis, and noninductive sand. Delivery of larvae to the array was the result of interactions between the flow regime and larval behavior. At intermediate flows (15, 20, and 25 cm s⁻¹), where numbers of metamorphosed juveniles and total animals (larvae + juveniles) in the array were maximal, larvae tumbled along the flume bottom, as did the passive larval mimics. At slower flows, larvae actively left the bottom and swam into the water, passing over the array. At the fastest flows, hydrodynamics alone may have reduced settlement because larvae, like the mimics, were eroded from the bed and carried as suspended load over the array or because enhanced turbulent mixing distributed larvae more evenly in the water, thus reducing their concentration close to the bed. Once delivered to the substratum, behavioral responses to chemical cues were ultimately responsible for metamorphosis; in all flows, >96% of metamorphosed juveniles were in the tube sand, whereas most unmetamorphosed larvae were in noninductive sand.

Explanations for the observed patterns of distribution and abundance of benthic marine organisms have shifted in the past decade from those invoking postrecruitment mortality (disturbance, predation, competition) to those in-

volving prerecruitment phenomena (e.g. Keough 1983; Sutherland 1990). In recent discussions of settlement processes, the relative importance of physical and biological factors has been widely debated (e.g. Woodin 1986; Butman 1987; Pawlik 1992). There is, however, an emerging consensus that both biological and physical processes can play important roles in determining the ultimate distribution of settled larvae, and the current challenge is to delimit precisely when and where biology or physics tend to dominate. Toward this end, we recently began studying the settlement of a reef-building polychaete worm, *Phragmatopoma lapidosa californica*, under well-defined flow conditions in a laboratory flume (Pawlik et al. 1991).

P. l. californica constructs tubes of agglutinated sand grains on hard substrata in the intertidal and subtidal of southern California. Site-specific settlement of the planktonic larvae on adult tubes results in the formation of aggregations and reefs (see Pawlik and Faulkner 1986). Larvae of *P. l. californica* are particularly well suited for flume experiments for

¹ Dedicated to the memory of Douglas P. Wilson, D.Sc., F.I. Biol., Hon. F.R.P.S., 1902–1991.

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several reasons. Larvae are easily reared to maturity and delay settlement indefinitely in the absence of the proper substratum (Pawlik 1986). Settlement occurs almost exclusively on the sand of adult worm tubes (Jensen and Morse 1984; Pawlik 1986). Tube sand can be reliably obtained by "farming" anterior tube segments of adult worm colonies. Larvae are large enough (500–700 μm) to be observed macroscopically in the flume. Settlement results in an immediate and specific morphological change (loss of the provisional chaetae) so that larvae can be distinguished from metamorphosed juveniles in preserved samples.

In our first set of experiments (Pawlik et al. 1991), larvae were offered five choices of substrata at two flow speeds. In both flows, metamorphosed juveniles largely were restricted to the sand treatments that had induced settlement in single-choice, still-water experiments (Pawlik 1986, 1988). The unexpected result, however, was that significantly more larvae and juveniles were present in arrays in the fast flow. This result occurred even though the total distance travelled by the water mass in the flume was similar at both velocities. In addition, larval behavior was strikingly different in the two flows. In fast flow, larvae bounced and rolled over the flume bottom and presumably were cumulatively delivered to the array during multiple passes around the flume. In slow flow, many of the larvae were observed near the water surface or swimming in the water column; these larvae passed over and were never delivered to the array.

The two flow velocities chosen for our initial experiments fell on either side of the critical shear velocity required to initiate motion (u_{*crit}) of passive larval mimics (plastic spheres with fall velocities within the range of downward swimming speeds of competent larvae of *P. l. californica*). Boundary shear velocities (u_*) in these flume experiments were within the range of those for typical tidal flows in coastal embayments (Butman 1986). The vertically averaged mean flow speeds were of the same order as long-term averages for the southern California shelf (Winant and Bratkovich 1981), where reefs made by *P. l. californica* occur in the shallow subtidal. Much faster flows than those tested are frequent, however, over short time scales due to local wind events (e.g. Winant 1980) or to local effects of shoreline mor-

phology and bathymetry (e.g. Geyer and Signell 1990). Moreover, our flume experiments were not designed to simulate the very strong oscillatory flows experienced by intertidal reefs built by this species.

Our objective was to investigate larval settlement responses of *P. l. californica* over a wider range of steady, unidirectional flows than tested previously and to include shear velocities exceeding the threshold for suspended-load transport (u_{*susp}) of passive larval mimics. Previous results (Pawlik et al. 1991) indicated that behavioral responses of larvae resulted in low settlement when $u_* < u_{*crit}$ (of passive larval mimics) and that settlement was enhanced under bedload-transport conditions. In the present study, we hypothesized that when $u_* > u_{*susp}$ (of passive larval mimics), settlement should decrease due to hydrodynamical processes alone. For example, if larvae acted like the mimics in fast flow, they would be unable to attach because of high boundary shear stress and would be distributed more evenly in the water column by enhanced turbulent mixing, perhaps reducing their probability of encountering the bottom.

The experimental design of this study was refined and simplified in three important respects relative to previous experiments (Pawlik et al. 1991). First, experiments were conducted in a much larger flume specifically designed to generate highly one-dimensional flows for surface velocities of $\sim 5\text{--}100\text{ cm s}^{-1}$ (Trowbridge et al. 1989). Second, larvae were allowed only a single pass over the array and were captured at the end of the channel so they would not recirculate. This modification eliminated any uncertainty regarding the time required for larval response, because cumulative settlement on multiple passes over the array could not occur. Third, the sediment array was simplified to a 2×2 latin square design with two sediment treatments, tube sand (the naturally occurring substratum that induces settlement) and filmed sand (a noninductive control).

Methods

Larval culture—Colonies of *P. l. californica* were collected in September 1990 in the rocky intertidal at the foot of Ladera Street on Point Loma (San Diego, California) and transported to Woods Hole Oceanographic Institution

(Massachusetts), where they were maintained in aquaria supplied with 18°C flowing seawater. Five batches of larvae were raised from adults spawned on 26 September and 10, 22, 26, and 30 October 1990. Adult worms spawned when removed from their tubes (see Pawlik 1986, 1988); gametes from several females and males were used for each spawning.

Larvae were cultured as described by Pawlik (1986, 1988) with minor modifications. Swimming trochophores were separated from unfertilized gametes and transferred to gallon (~4 liters) jars filled with 3 liters of 0.45- μm filtered seawater containing a 1:1 mixture of the diatom *Phaeodactylum tricornutum* and the flagellate *Pavlova lutheri* at $\sim 10^5$ cells ml^{-1} . The contents of each culture jar were gently swirled by a slow stream of bubbles from the tip of a pipet attached to an airline. Cultures were maintained in a 20°C constant-temperature room under fluorescent lamps set at 14:10 L/D. Jars were cleaned and media changed every other day. Under these culture conditions, >80% of the larvae became competent to metamorphose after 20–25 d (see Pawlik 1986, 1988). Five batches of larvae were used for the experiments, with each batch used over the course of 3 d or less. Experiments began when the larvae were 37-, 30-, 26-, 28-, and 33-d old, respectively (see dates of spawning, above).

Experimental conditions—Experiments were carried out in the 17-meter flume (see Butman and Chapman 1989; Trowbridge et al. 1989), a recirculating, temperature-controlled, seawater flume located in the Coastal Research Laboratory of Woods Hole Oceanographic Institution. Flow through the flume channel (0.6 m wide and 17.3 m long from the flow straightener to the weir; filled to 12 cm with 1- μm filtered seawater for these experiments) was driven by a centrifugal pump. Seawater in the flume was kept at 20°C by thermostatically controlled heat exchangers.

Each flow was tested in a separate flume run with larvae from each of five batches. For each run, 2,000 larvae were introduced to the flume by pouring them into a funnel and through a 23-cm length of Tygon tubing (0.7-cm i.d.) that emerged from the upstream flow straightener (a vertical baffle composed of cells $1.2 \times 1.2 \times 7.5$ cm long) so that the tube opening was 1.5 cm downstream from the baffle and 3 cm

above the center of the flume bottom. From this point, larvae travelled 12.75 m over the polyvinylchloride bottom before reaching the acrylic plate containing the sediment array. The four square depressions of the 2×2 array, recessed into the bottom, were 5 cm on a side, 0.3 cm deep, with a 0.3 cm space between adjoining squares. Each depression held 9 ± 1 g of sand (Ottawa sand, 20–30 mesh, Fisher Scientific) when filled to the level of the bottom (see description of sand treatments, below). The array was centered in the flume with one side (row 1, see below) facing the flow. A vertical weir was located 4.65 m downstream from the array. Larvae were captured in a 100- μm -mesh plankton net placed over the channel terminus, downstream from the weir.

Each experiment was run for 3 h, then the pump was turned off, the channel allowed to drain slowly, and the contents of each of the four depressions removed by suction and preserved in a solution of 70% ethanol dosed with Rose Bengal stain. After 1 h or more, each of the four samples was rinsed in tapwater, partitioned on 100- and 300- μm sieves, spread on a Petri dish under a dissecting scope at $15\times$ magnification, and the numbers of metamorphosed juveniles and unmetamorphosed larvae counted. As previously mentioned, larvae of *P. l. californica* retain their provisional chaetae and are easily distinguished from juveniles.

The number of larvae remaining in the flume channel at the end of an experiment was determined for the last three of the five batches of larvae. For each flume run, after the channel had drained and the array contents were removed, the plankton net was cleaned and replaced, and residual water in the channel (e.g. trapped in the seams between the false bottom panels and the spaces between the panels and channel walls) was rinsed into the net by running the pump at high speed with the weir fully open. The contents of the net were preserved and processed as for larvae in the array.

Variables—Larval settlement response as a function of flow speed and u_* was of primary interest in these experiments. Near-surface (10 cm above the bottom) velocities of 5, 10, 15, 20, 25, and 30 cm s^{-1} were tested with each of the five batches of larvae; two of the five batches were also tested at 35 cm s^{-1} , but these data were not included in the statistical analyses. For each batch of larvae, the order of the

six flows tested was randomized (35 cm s⁻¹ flow excluded). For each flow, the flume was allowed to equilibrate for at least 15 min prior to the start of an experiment. During each experiment, velocity was monitored 10 cm above the center of the upstream edge of the array with a two-axis, forward-scatter, laser-Doppler velocimeter (LDV) to ensure similarity in flow regime among repeated experiments. In addition, detailed vertical profiles of mean horizontal velocity were made for each flow in subsequent runs without larvae but with the same flume settings used in the settlement experiments.

Boundary shear velocity was calculated from the velocity profiles with a semi-empirical expression for the mean velocity in a steady, open-channel flow above a smooth bottom, as described by Trowbridge et al. (1989) and Butman and Grassle (1992). Resulting values of u_* were 0.26, 0.47, 0.66, 0.86, 1.03, 1.22, and 1.42 cm s⁻¹ for the seven flows. Boundary shear velocity was calculated with this “full profile” technique rather than the “log-layer method” (e.g. Gross and Nowell 1983) because of uncertainty in the precise location of the log layer. In a comparison of methods used to estimate u_* conducted in the 17-meter flume, Trowbridge et al. (1989) found that the technique used here produced more reasonable (and lower) estimates of u_* and smaller error bars when compared with the log-layer method; it also produced similar values to the “Reynolds stress” technique with less associated uncertainty. For comparison with the present study, u_* was recalculated for both flows tested by Pawlik et al. (1991) with the full profile technique. This analysis resulted in smaller values of u_* (0.26 cm s⁻¹ for the slow flow and 0.64 cm s⁻¹ for the fast flow) than those reported by Pawlik et al. (1991), where the log-layer method was used to calculate u_* .

To estimate u_{*crit} and u_{*susp} , we assessed whether passive larval mimics moved as bedload or suspended load in each flow. The larval mimics were polystyrene spheres (Polysciences No. 17805, diam = 425 ± 10.1 μm, density = 1.05 g cm⁻³) with a mean Stokes' fall velocity of 0.27 cm s⁻¹ (30‰, 20°C seawater), which is within the range of downward swim speeds of competent larvae of *P. l. californica* (Pawlik et al. 1991). During flume runs without larvae, but with the same settings used in the

settlement experiments, spheres were pipetted onto the flume bottom and their movement observed macroscopically. The spheres remained motionless on the bed at $u_* = 0.26$ cm s⁻¹. Bedload transport was observed at $u_* = 0.47$ cm s⁻¹ and spheres rolled along the bottom at $u_* = 0.66$ and 0.86 cm s⁻¹. At $u_* = 1.03$ cm s⁻¹, however, a few spheres were observed to make brief excursions into the water column and then fell back down to the bed. As u_* increased further, the number of spheres leaving the bed increased, as did the vertical and horizontal transport distances of spheres during their water-column excursions. On the basis of these qualitative observations, 0.26 cm s⁻¹ < u_{*crit} < 0.46 cm s⁻¹ and u_{*susp} > 1.03 cm s⁻¹. Note that our definition of u_{*susp} differs from the traditional definition based on concentration profiles near the bed. We relegate any vertical transport of the spheres off the bottom to suspended-load transport.

The two sand treatments used in the sediment array were tube sand and filmed sand. Tube sand was a potent inducer of settlement of *P. l. californica* in experiments conducted in both still-water (Pawlik 1986) and flow (Pawlik et al. 1991), whereas filmed sand did not induce settlement. Tube sand was prepared by allowing adult worms in aquaria to build anterior sections of their tubes with clean Ottawa sand (baked at 550°C for 6 h) over several days, removing the newly built anterior sections, freeze-drying them, and breaking them up into individual sand grains (see Pawlik 1986). Filmed sand was made by putting clean Ottawa sand in shallow dishes in aquaria containing adult worms that were building tubes. Filmed sand thereby developed an organic-microbial film but was not manipulated by adult worms. Filmed sand was freeze-dried and treated in the same manner as tube sand. For each flume run, two array depressions, arranged diagonally, were filled with each sand type. The sand type in each diagonal was determined randomly.

Three additional variables were evaluated in the ANOVA model (described below): batch, row, and column. Methods for obtaining the five batches of larvae used in the experiments are described above. Settlement patterns as a function of row and column position within the 2 × 2 array were evaluated because, in previous studies with larger arrays, row and

column effects sometimes explained a significant portion of the variance (Pawlik et al. 1991; our unpubl. data). Rows were oriented perpendicular to the flow direction, with the first row at the leading edge (upstream), and columns were numbered left to right, looking downstream.

Statistical analyses—The settlement experiments were conducted as a split plots design with flow effect as the whole plot, the five larval batches as replications of the whole plot, and sand treatment, row, and column effects as subplots in a latin square design (Cochran and Cox 1957; Hicks 1973). The ANOVA model for the analysis was $y = \mu + \text{flow} + \text{batch} + (\text{flow} \times \text{batch}) + \text{treatment} + (\text{treatment} \times \text{flow}) + (\text{treatment} \times \text{batch}) + (\text{treatment} \times \text{flow} \times \text{batch}) + \text{row} + (\text{row} \times \text{flow}) + (\text{row} \times \text{batch}) + \text{column} + (\text{column} \times \text{flow}) + (\text{column} \times \text{batch}) + \text{error}$. All interaction terms not in the model were assumed to be nonsignificant. The three-way interactions of (flow \times batch \times row) and (flow \times batch \times column) were assumed to be zero (these interactions would appear to have little biological relevance) and were used for the error term to test the batch main effect and the interaction terms containing batch. The ANOVA model was used to test total animals (larvae + juveniles) and metamorphosed juveniles separately, and the analysis assumed no interactions between them. Tukey's HSD multiple-comparisons tests were performed to determine which of the means were different when main effects were statistically significant at $P \leq 0.05$.

The a priori hypotheses for these experiments were that there is a range of values of u_* for which settlement would be maximized and that there would be less settlement at lower and higher values of u_* . Total animals (larvae + juveniles) and juveniles that occurred in the array as a function of flow were therefore tested for a linear and a quadratic fit with an orthogonal contrast analysis (e.g. Sokal and Rohlf 1981).

The null hypothesis of no difference in the number of larvae trapped in the upstream portion of the flume and rinsed into the net at the end of each flow experiment (for the last three larval batches only) was tested with a randomized blocks design ANOVA with flow as the fixed main effect and larval batch as the blocking factor (i.e. a random effect). The (flow \times

batch) interaction was assumed to be nonsignificant.

For all statistical analyses, a $\log(x + 1)$ transformation was performed on the data to homogenize variances.

An assumption for all the ANOVAs performed here is that there is no spatial autocorrelation of total animals or juveniles that settled in the treatments in the 2×2 array. Spatial autocorrelation in total animals or juveniles could arise in two ways: autocorrelation between replicates within a treatment (i.e. between cells along the diagonal) and autocorrelation between the two treatments (i.e. between two adjacent cells in the array). Such spatial autocorrelation in larvae has been tested statistically in other flume flow experiments on larval sediment selection with a 5×5 , two-treatment, checkerboard array and was never significant in a direction that would bias the results (e.g. Butman and Grassle 1992; Grassle et al. 1992a). These statistical tests could not be done in the present study because of insufficient degrees of freedom with a 2×2 array. Given the lack of spatial autocorrelation in previous studies and the consistency of the results reported herein and by Pawlik et al. (1991), we suggest that it is unlikely that spatial autocorrelation of total animals or juveniles biased the outcome (i.e. obscured or enhanced treatment effects) of this study.

Results

The mean number of total animals (larvae + juveniles) and juveniles of *P. l. californica* in the sediment array varied as a function of flow speed, as predicted; numbers were fewer in the array at relatively high and low flows and were greatest at intermediate flows (Fig. 1). The flow effect was significant for both total animals and juveniles (Table 1). An a priori orthogonal contrast analysis of this flow effect (Fig. 1) revealed that a quadratic fit to the curves was significant for both total animals ($F_{1,20} = 156.5$, $P < 0.0001$) and juveniles ($F_{1,20} = 327.5$, $P < 0.0001$), whereas a linear fit was not significant for total animals ($F_{1,20} = 0.98$, $P < 0.331$) but was significant for juveniles ($F_{1,20} = 13.6$, $P < 0.0014$). This analysis suggests that there is a predictive, parabolic relationship between total animals or juveniles in the array and the flow.

The batch effect was also significant for total

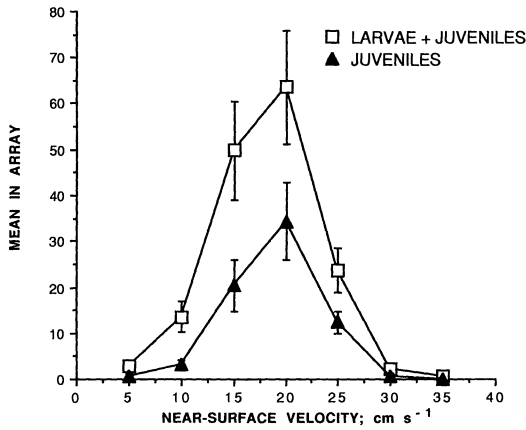


Fig. 1. Mean number (\pm SE) of total animals and juveniles in the 2×2 array for seven velocity regimes tested with five batches of larvae, hence, $N = 5$, except $N = 2$ for 35 cm s^{-1} .

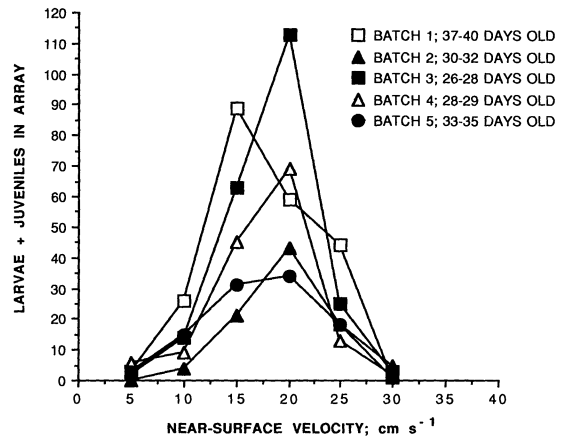


Fig. 2. Number of total animals in the 2×2 array for each of five batches of larvae.

animals and juveniles. Of the significant interactions (Table 1), (flow \times batch) effect is the most interesting biologically because it indicates the extent to which the results can be generalized across batches of larvae of different age. This interaction resulted because the total number of animals in the array was greatest at 20 cm s^{-1} for four batches but was greatest at 15 cm s^{-1} for the fifth batch (37–40-d-old larvae) (Fig. 2). Furthermore, although the number of total animals in the array varied among batches, there was no obvious relationship between number in the array and larval age. In fact, the trend of smaller numbers of total animals and juveniles in the arrays at the slowest

and fastest flows tested and larger numbers at intermediate flows occurred for all batches.

The treatment effect was significant for juveniles only, but the (flow \times treatment) interaction was significant for both total animals and juveniles (Table 1). Because of this significant interaction, Tukey's multiple-comparisons tests of treatment effects were analyzed separately for each flow, and flow effects were analyzed separately for each treatment. For total animals, the (batch \times treatment) effect was significant, but the (treatment \times batch \times flow) interaction was not significant, indicating that the differences between batches were consistent within a given flow.

Considering treatment effects for each flow,

Table 1. Results of ANOVA for the model. Asterisks: **— $P \leq 0.01$; ***— $P \leq 0.001$.

Source of variation	df	Total animals		Juveniles	
		SS	F-value	SS	F-value
Flow	5	89.54	29.58***	30.90	34.76***
Batch	4	5.52	6.23***	6.20	7.04***
Treatment	1	0.53	0.62	36.76	87.74***
Row	1	16.82	78.49***	6.06	20.23*
Column	1	0.43	3.98	0.25	5.29
Flow \times batch	20	12.11	2.73**	3.56	0.69
Flow \times treatment	5	5.47	12.37***	20.25	46.33***
Flow \times row	5	4.10	3.70**	2.11	1.92
Flow \times column	5	0.94	0.85	0.24	0.22
Batch \times treatment	4	3.44	3.88**	1.68	1.90
Batch \times row	4	0.86	0.97	1.20	1.36
Batch \times column	4	0.43	0.48	0.19	0.22
Flow \times batch \times treatment	20	1.77	0.40	1.75	0.40
Error	40	8.86		8.80	

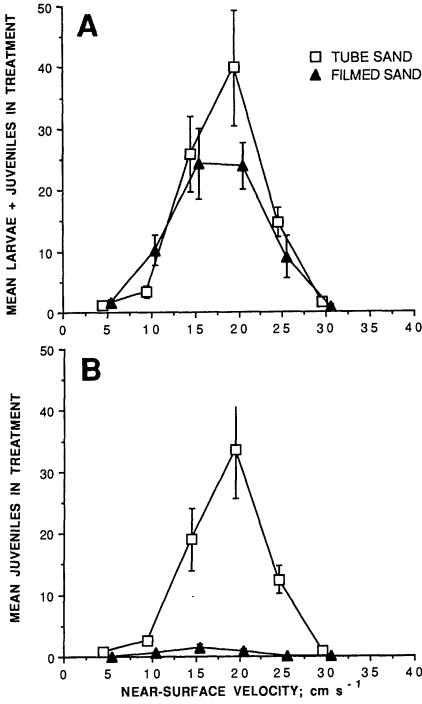


Fig. 3. Mean number (\pm SE) of total animals (A) and juveniles (B) in tube sand vs. filmed sand. Data points are slightly offset along the x -axis to prevent overlap of error bars.

significantly greater numbers of total animals were present in filmed sand than in tube sand for the 10 cm s^{-1} flow; for all other flows, the treatment effect was not significant (Fig. 3A). Significantly greater numbers of juveniles were present in tube sand than filmed sand for the 15, 20, and 25 cm s^{-1} flows, and there was no difference between treatments in the 5, 10, and 30 cm s^{-1} flows (Fig. 3B). Juveniles were found almost exclusively in tube sand (Fig. 3B): in fact, summed over all flume runs, only 14 of 360 juveniles (<4.0%) were in the filmed sand treatment, and only 96 of 430 larvae (\sim 22%) were in the tube sand treatment.

The analysis of flow effects for each treatment (Table 2) is even more revealing. Significant differences between flows were identical for total animals and juveniles in tube sand. There were significantly fewer numbers of total animals and juveniles in tube sand at 5, 10, and 30 cm s^{-1} flows than at 15, 20, and 25 cm s^{-1} flows. In addition, numbers of total animals and juveniles were significantly great-

Table 2. Results of Tukey's HSD multiple-comparisons tests of the flow effect for each treatment. Means are ordered from greatest to least, and horizontal bars are drawn below means that are not significantly different at $P \leq 0.05$.

	Near-surface velocity (cm s^{-1})					
Total animals						
Tube sand	20	15	25	10	30	5
Filmed sand	20	15	10	25	5	30
Juveniles						
Tube sand	20	15	25	10	30	5
Filmed sand	15	20	10	5	25	30

er in tube sand at the 20 cm s^{-1} flow than at the 5, 10, 25, and 30 cm s^{-1} flows. In contrast, for the filmed sand, there was no flow effect for juveniles. For total animals, the significant flow effect in filmed sand can be summarized as follows: 20, 15 > 10, 25 > 5, 30 cm s^{-1} . Our interpretation of these results is that total animals in filmed sand as a function of flow reflects passive larval delivery to the array. Across all flows, larvae tend not to metamorphose in filmed sand, however, as reflected in the low numbers of juveniles in this treatment (Fig. 3B) and the lack of a significant flow effect (Table 2). Larvae initially delivered to tube sand undergo metamorphosis as indicated by the similar flow effects for total animals and juveniles in this treatment.

The row effect was significant for both total animals and juveniles, and the (flow \times row) interaction was significant for total animals (Table 1). Tukey's multiple-comparisons tests were performed to determine which rows differed. There was a significantly greater mean number of total animals in row 1 than row 2 of the array for the 25 cm s^{-1} flow only (Fig. 4). Row 1 contained significantly greater numbers of juveniles than row 2 at all flow speeds tested.

The mean number of larvae trapped in the upstream portion of the flume and rinsed into the net in flume runs 3, 4, and 5 was greater at flow speeds 5, 10, 15, and 20 cm s^{-1} than at higher flow speeds (Table 3), but the mean number of larvae trapped did not differ sig-

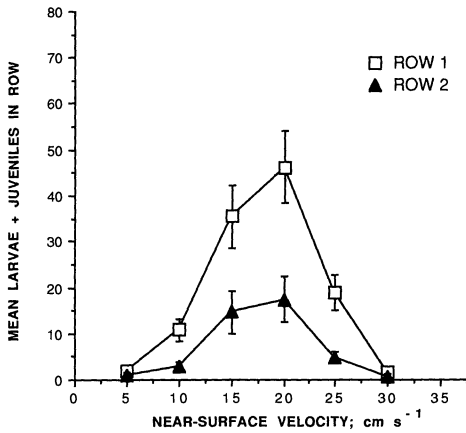


Fig. 4. Mean number (\pm SE) of total animals in each of the two rows of the 2×2 array. Row 1 was upstream from row 2.

nificantly between flows ($F_{5,10} = 2.793$, $P = 0.078$). The variance among batches in the number of larvae trapped also was not significant ($F_{2,10} = 0.878$, $P = 0.513$).

Discussion

Marine invertebrate larvae have long been recognized to respond to a host of environmental factors (e.g. Wilson 1937, 1968; Thorson 1950; Scheltema 1974). Vertical movements of larvae in response to light and gravity may alter larval distributions in the water column, ultimately affecting patterns of settlement (Grosberg 1982; Sulkin 1984). Field studies suggest that behavioral responses at the time of substratum-selection may be important for many species (e.g. Strathmann and Branscomb 1979; Woodin 1985; Raimondi 1991), and short-term flume experiments of initial settlement confirm the importance of larval behavior at settlement in flow (e.g. Butman et al. 1988; Grassle and Butman 1989; Mullineaux and Butman 1991). Yet recent recruitment models generally consider only variability in the physical transport of passive particles when predicting variability in larval supply and settlement (e.g. Roughgarden et al. 1988). Our results suggest that larval behavior may be important in determining settlement sites for some species, not only during selection of a substratum once they have reached the seafloor but also during transport in near-bed flows.

Table 3. Number of larvae caught in net after the flume was drained and flushed.

Velocity (cm s ⁻¹)	Flume run			Mean	SD
	3	4	5		
5	18	9	88	38.33	35.31
10	98	13	2	37.67	42.90
15	106	22	18	48.67	40.57
20	41	44	20	35.00	6.00
25	10	1	4	5.00	6.00
30	0	3	5	2.67	2.05
35	—	12	13	12.50	0.50

As in our previous study (Pawlik et al. 1991), *P. l. californica* metamorphosed almost solely on substrata that had previously been shown to induce metamorphosis in still-water, non-choice experiments (Pawlik 1986, 1988). We reduced the choices from five (Pawlik et al. 1991) to two in the present single-pass experiments to focus on the magnitude of settlement and selectivity over a wider range of flows. The difference in the treatment effect for juveniles (highly significant) and for total animals (larvae + juveniles; not significant) (Table 1) suggests that larvae were delivered by the flow to the treatment substrata and then chose whether to metamorphose. In our previous study, larvae could leave noninductive substrata and travel around the recirculating flume to pass over the array again; the extent of this cumulative effect on the final distribution of larvae and juveniles in inductive vs. noninductive sediments is evident in comparisons of experiments run at 15 cm s⁻¹ for 2 h vs. 20 h (figures 1A and B of Pawlik et al. 1991). Therefore, substratum selection for *P. l. californica* is ultimately a behavioral response to the substratum after larvae are delivered to the seabed by the flow.

The flow regime and larval response to near-bed flow greatly affected delivery of larvae of *P. l. californica* to the array in this study. At flows that resulted in maximal delivery of larvae to the array (near-surface velocities of 15, 20, and 25 cm s⁻¹), larvae were observed tumbling end over end, often adhering briefly by their tentacles, as they progressed down the flume. Larval mimics similarly rolled along the flume bottom as bedload in these flows. At slower flows (5 and 10 cm s⁻¹), larvae were observed swimming haphazardly on the flume

bottom near the point where they were released into the flume. Over the course of an hour, however, these larvae were observed to leave the bottom and swim toward the water surface where they would largely bypass the array as they were transported downstream. It occurred to us after running experiments with the first two batches of larvae that one alternative explanation for the small numbers of total animals in the array at the slower flows tested was that larvae might move into small cracks between and along the sides of the bottom panels and therefore remain in the upstream portion of the flume without passing over the array. We tested this hypothesis in the remaining flume runs by vigorously rinsing the drained flume after each run and collecting these trapped larvae; there were no significant differences in the mean number of larvae retained at the six velocities tested (see Table 3 and results).

At the two fastest flows tested (30 and 35 cm s⁻¹), larvae of *P. l. californica* were observed in the water column above the array. Because the mimics could be eroded from the bed and carried in suspension, at least for a time, in these flows, we hypothesize that the boundary shear stresses at these flows may physically prevent larvae from attaching. Previous flume studies have, for example, delineated the limiting boundary shear stress at which barnacle larvae can attach to a smooth, solid surface (Eckman et al. 1991). Flume studies have also demonstrated that barnacle larvae actively choose to settle in regions of relatively low shear stress along a plate, even though the highest shear stresses on the plate were not sufficient to dislodge the larvae (Mullineaux and Butman 1991). Thus, it is also possible that larvae of *P. l. californica* behaviorally avoided settlement in these relatively high shear-stress regimes, but the rationale for this behavior is unclear given the distribution of this species in fast-flow environments in the field.

Two alternative explanations for low settlement at the fastest flows tested involve the possibility of decreased larval supply to the bed. First, because the stronger flows enhanced turbulent mixing, larvae may have been distributed more evenly (and, on average, up higher) in the water column, reducing the con-

centration of larvae near the bottom. It is unclear whether this would result in a net reduction in the probability that a larva would encounter the bed (relative to slower flows) because, by definition, enhanced mixing also results in an enhanced encounter rate of a given water parcel (and the passive particles within it) with the bottom. Second, because larvae were allowed only one pass over the sediment array, as flow speed increases the time an individual larva spends over the sediment array decreases, thus decreasing the probability that it will encounter the sediment. Although this may have been partially responsible for the low settlement at the fastest flows, the percentage decrease in time spent over the array is not sufficient to account for the magnitude of the difference in settlement between the fastest and intermediate flow speeds.

We propose that larvae of *P. l. californica* behaviorally avoid settling in relatively slow flows and settle in the highest flows for which attachment and substratum exploration is physically possible. Active avoidance of slow-flow regions is reasonable for an organism that both filter feeds and scavenges coarse sand from the water for tube building; flow regions with relatively high horizontal fluid flux would increase the probability of an adequate supply of food and sand to these sedentary animals. The upper limit of flows in which larvae can settle is likely highly conservative, as determined in this flume study of turbulent flows over hydrodynamically smooth beds, because in the field, complex topography and bed roughness may result in local regions of slower flow that would permit attachment. Moreover, settlement under oscillatory flow conditions experienced by intertidal populations of this species cannot be inferred from these steady, unidirectional flow experiments.

There is mounting evidence that larvae of a variety of invertebrate species are capable of exploiting aspects of the near-bed flow regime to enhance their probability of settling in an appropriate adult habitat and that flow conditions determine to some extent whether active selection can take place. Larvae of the barnacle *Balanus amphitrite*, for example, initially contacted a substratum passively and then explored it actively; exploration occurred, however, only in the direction of flow at the

plate surface (Mullineaux and Butman 1991). Likewise, larvae of the deposit-feeding, infaunal polychaete *Capitella* sp. 1 appeared to select a favorable adult habitat by swimming up and down as they were carried by the flow, either accepting or rejecting sediments encountered at touchdown sites (Butman et al. 1988; Butman and Grassle 1992; Grassle et al. 1992a). Furthermore, larvae of *Capitella* sp. 1 were highly susceptible to passive transport by even relatively weak secondary flows in a laboratory flume. Larvae of the infaunal bivalve *Mulinia lateralis* appeared to be more selective in flow than still water because they are such weak swimmers that flow enhanced the probability of contact with a favorable substratum (Grassle et al. 1992b). Similarly, Jonsson et al. (1991) demonstrated that the cockle *Cerastoderma edule* became concentrated in the viscous sublayer in relatively fast, turbulent flows over a hydrodynamically smooth bed, suggesting that this species may encounter the substratum more frequently (i.e. with more opportunity for sediment selection) at faster flows. Our studies of *P. l. californica* may be the first, however, in which larvae have been observed to actively reject entire flow regimes (i.e. as opposed to rejection of very small-scale flow features, as shown by Mullineaux and Butman 1991). These results suggest that it may be possible to define an optimal range of hydrodynamic conditions for settlement of an invertebrate larva that would depend on both larval behavior and physics and that would likely take critical life-history characteristics of the species into account.

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