

## Desperate planktotrophs: decreased settlement selectivity with age in competent eastern oyster *Crassostrea virginica* larvae

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Table S1. Average size and percentage of larvae with eyespots at each age in the pilot experiment. N, number of individuals sub-sampled.

Intervals represent standard deviation.

Experiment	Hours post-competency	N	Length (µm)	Height (µm)	% with eyespots
Pilot	0	22	273 ± 22	262 ± 17	54
	4	26	276 ± 16	271 ± 19	54
	8	30	287 ± 18	271 ± 25	60
	12	30	298 ± 26	287 ± 16	70

Table S2. Analysis of variance in settlement behaviors in response to settlement cue and larval age in the pilot experiment. Two-way ANOVA tests for differences in the proportion of larvae remaining on the bottom of experimental flasks and larval swimming velocities.

Factors are Cue (presence or absence of a settlement cue) and Age (larval age in hours post-competency). Significant p-values (<0.05) shown in bold.

Behavioral metric	Experiment	Factor	F	p
Proportion of larvae staying on bottom	Pilot	Cue	40.1	< <b>0.001</b>
		Age	0.47	0.650
		Cue x Age	0.53	0.605
Velocity of downward-swimming larvae	Pilot	Cue	9.15	<b>0.004</b>
		Age	1.69	0.189
		Cue x Age	2.34	0.092

Table S3. Analysis of variance of exploratory behaviors in response to settlement cue and larval age in the pilot experiment. Two-way ANOVA tests for differences in the upward swimming velocity of larvae, average gross:net distance ratio (GNDR), and proportion swimming in helices. Factors are Cue (presence or absence of a settlement cue) and Age (larval age in hours post-competency). Significant p-values (<0.05) shown in bold.

Behavioral metric	Experiment	Factor	F	p
Velocity of upward-swimming larvae	Pilot	Cue	8.59	<b>0.007</b>
		Age	0.24	0.868
		Cue x Age	6.95	<b>0.001</b>
GNDR of downward-swimming larvae	Pilot	Cue	12.7	<b>0.001</b>
		Age	1.24	0.310
		Cue x Age	4.45	<b>0.010</b>
GNDR of upward-swimming larvae	Pilot	Cue	2.78	0.108
		Age	0.200	0.895
		Cue x Age	0.64	0.595
Proportion downward-swimming larvae performing helices	Pilot	Cue	8.43	<b>0.006</b>
		Age	1.86	0.191
		Cue x Age	1.07	0.375
Proportion of upward-swimming larvae performing helices	Pilot	Cue	1.56	0.223
		Age	0.89	0.461
		Cue x Age	1.90	0.156

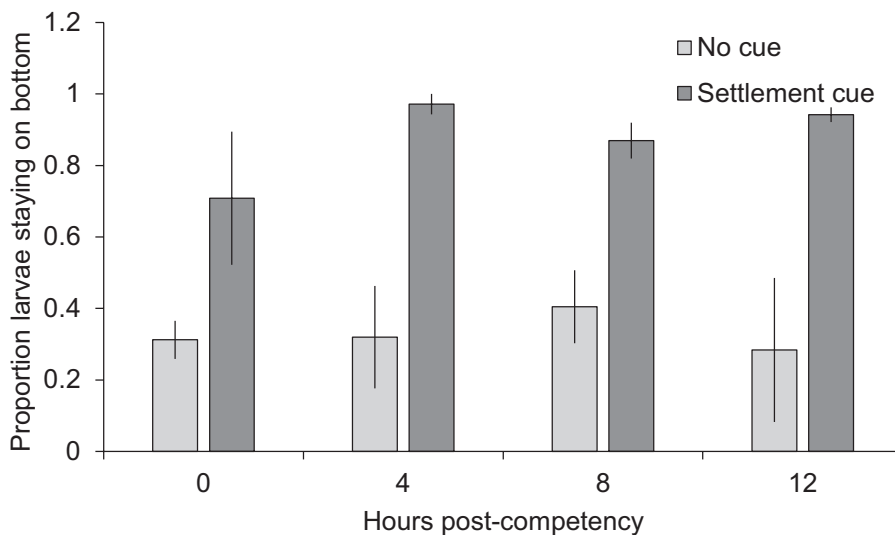


Fig. S1. Proportion of *Crassostrea virginica* larvae remaining on the bottom after swimming down as a function of larval age in the pilot experiment. Larvae were exposed to filtered seawater (no cue; light bars) or a chemical settlement cue (dark bars) in a pilot experiment. Error bars show standard error.

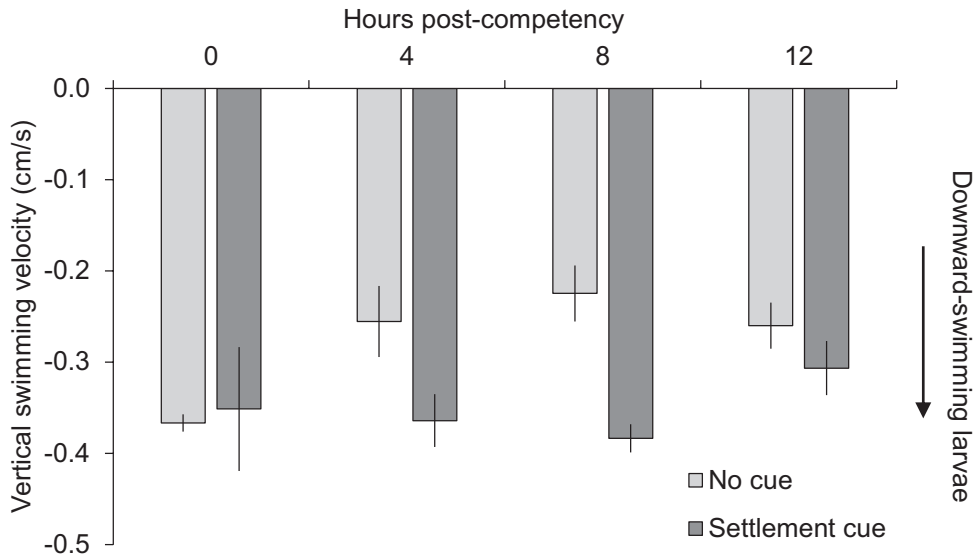


Fig. S2. Vertical swimming velocity of downward-swimming *Crassostrea virginica* larvae as a function of age in the pilot experiment. Larvae were exposed to filtered seawater (no cue; light bars) or a chemical settlement cue (dark bars) in a pilot experiment. Error bars show standard error.

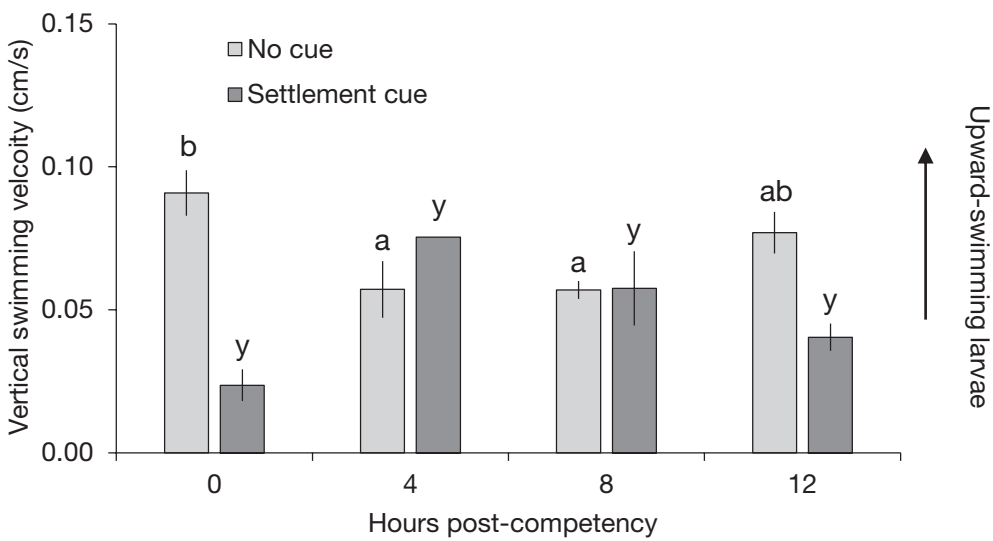


Fig. S3. Vertical swimming velocity of upward-swimming *Crassostrea virginica* larvae as a function of age in the pilot experiment. Larvae were exposed to filtered seawater (no cue; light bars) or a chemical settlement cue (dark bars) in a pilot experiment. Error bars show standard error. The missing error bar at  $t = 4$  hours indicates only one replicate had larvae swimming up off the bottom. Dissimilar letters indicate significant post hoc differences between ages, tested separately for no-cue (a, b, c) and cue (y, z) treatments.

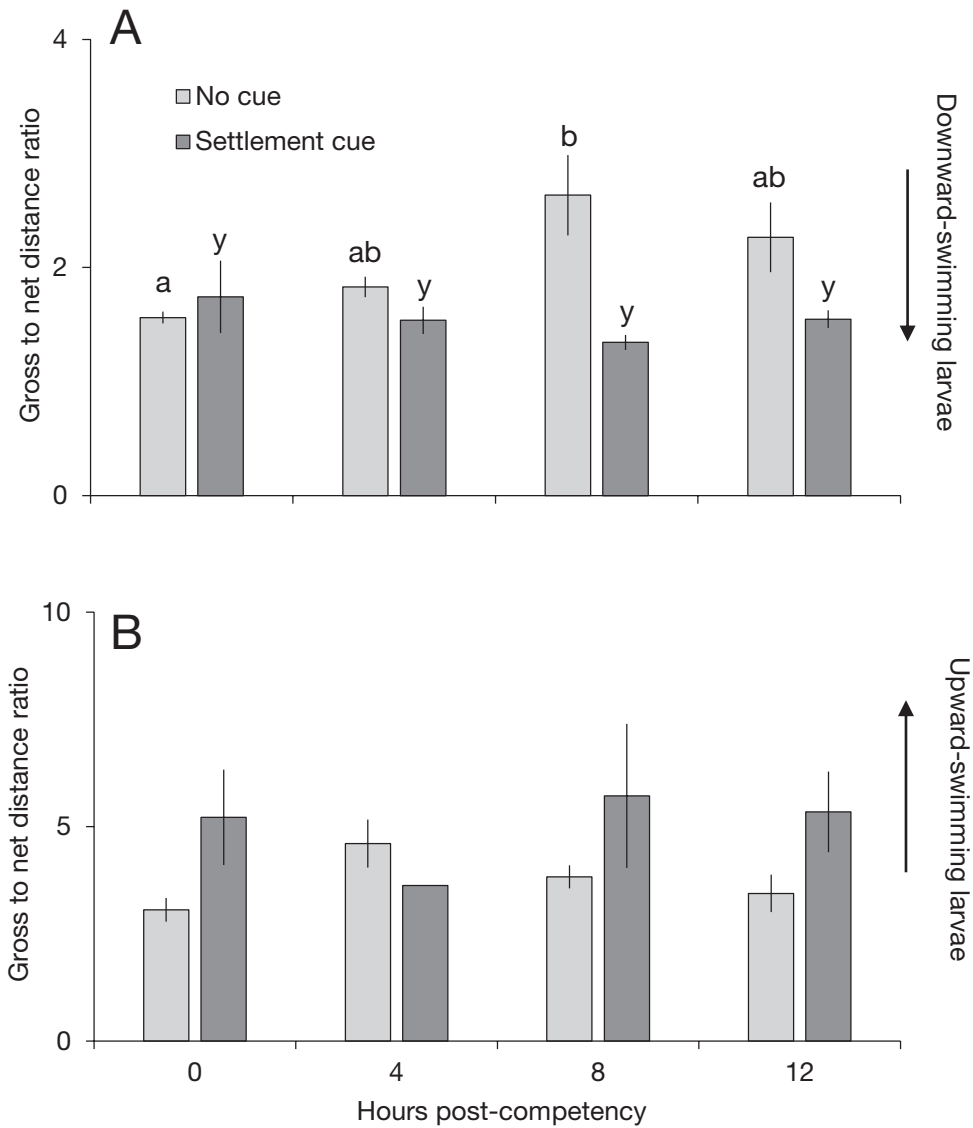


Fig. S4. Gross to net distance ratio (GNDR) of *Crassostrea virginica* larvae in the pilot experiment as a function of age for larvae swimming downward to the bottom of the flask following introduction (A) and larvae swimming back up off the bottom (B). Larvae were exposed to filtered seawater (no cue; light bars) or a chemical settlement cue (dark bars) in a pilot experiment. Error bars show standard error. The missing error bar at  $t = 4$  hours indicates only one replicate had larvae swimming up off the bottom. Dissimilar letters indicate significant post hoc differences between ages, tested separately for no-cue (a, b, c) and cue (y, z) treatments.

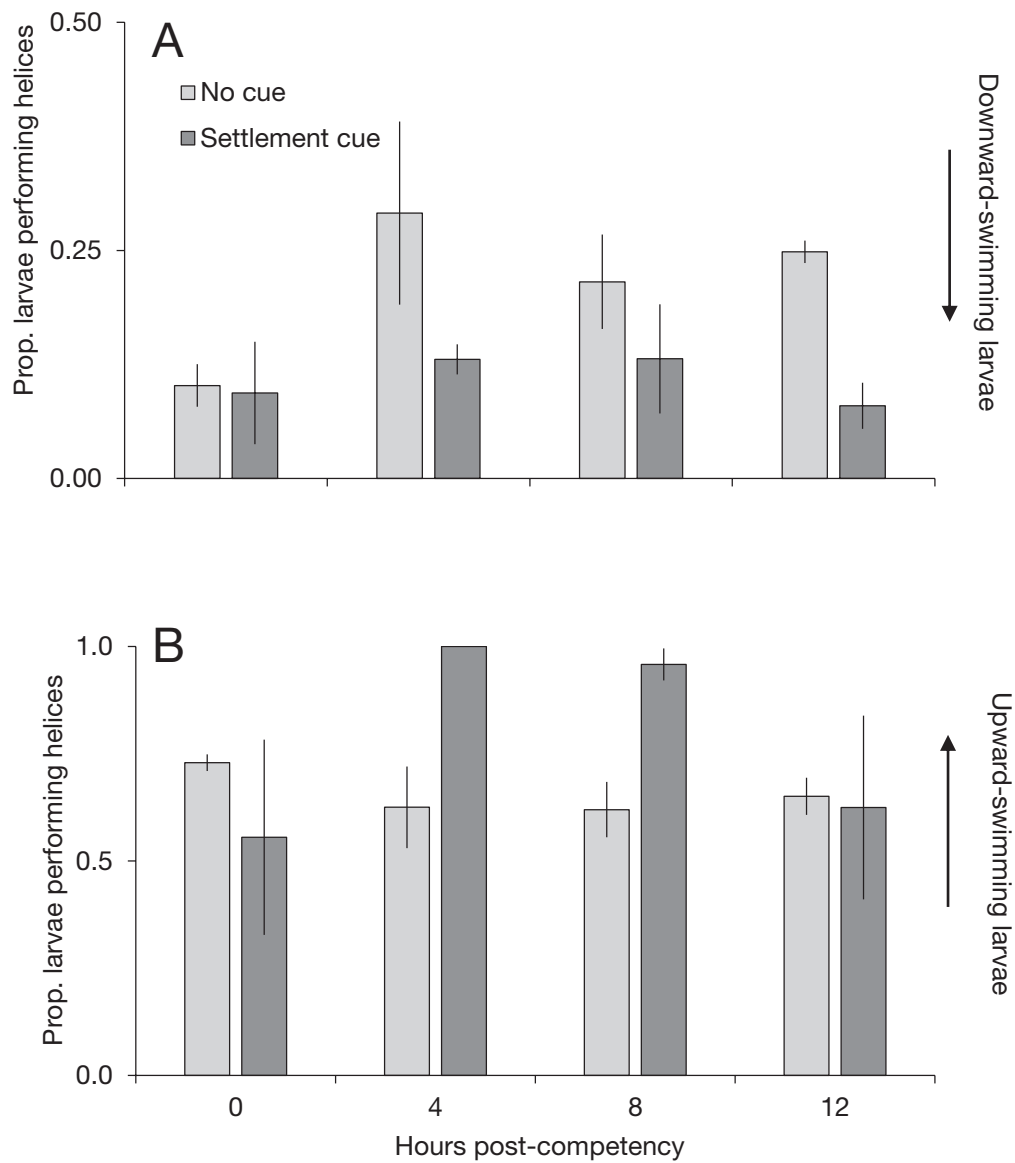


Fig. S5. Proportion of *Crassostrea virginica* larvae in the pilot experiment swimming in helices as a function of age for larvae swimming downward to the bottom of the flask following introduction (A) and larvae swimming back up off the bottom (B). Larvae were exposed to filtered seawater (no cue, light bars) or a chemical settlement cue (dark bars) in a pilot experiment. Error bars show standard error. The missing error bar at  $t = 4$  hours indicates only one replicate had larvae swimming up off the bottom.

Table S4. Non-parametric statistical results for heteroscedastic larval behavioral metrics. Mann-Whitney (M-W) and Kruskal-Wallis (K-W) tests show differences in the proportion of larvae remaining on the flask bottoms, downward swimming velocity of larvae, average gross:net distance ratio, and proportion swimming in helices. Factors are Cue (presence or absence of a settlement cue) and Age (larval age in hours post-competency). Significant p-values (<0.05) shown in bold.

<b>Behavioral metric</b>	<b>Experiment</b>	<b>Test</b>	<b>Factor</b>	<b><math>\chi^2</math> or <math>U</math></b>	<b>p</b>
Proportion larvae remaining on bottom	Pre and Post	M-W	Cue	133	< <b>0.001</b>
		K-W	Age	2.74	0.253
Velocity of downward-swimming larvae	Time Series	M-W	Cue	663	0.453
		K-W	Age	1.14	0.887
GNDR of downward-swimming larvae	Time Series	M-W	Cue	695	0.164
		K-W	Age	10.9	<b>0.027</b>
	Pre and Post	M-W	Cue	297	<b>0.007</b>
		K-W	Age	3.31	0.191
GNDR of upward-swimming larvae	Time Series	M-W	Cue	474	0.063
		K-W	Age	3.07	0.545
Proportion downward-swimming larvae performing helices	Time Series	K-W	Age	8.01	0.091
	Pre and Post	M-W	Cue	330	< <b>0.001</b>
Proportion of upward-swimming larvae performing helices	Time Series	M-W	Cue	514	0.388
		K-W	Age	2.94	0.568