The following supplements accompany the article

Early metamorphosis is costly and avoided by young, but physiologically competent, marine larvae

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SUPPLEMENT 1. The effect of larval development on the position of *Centrostephanus rodgersii* and *Tripneustes gratilla* larvae in the water column in response to light and dark: Methods and Results.

Methods

Marine larvae are known to undertake diel vertical migration, and this may be affected by larval development or the ability of larvae to metamorphose (e.g. Tapia & Pineda 2007). To assess the effect of light on the position of early and advanced *T. gratilla* and *C. rodgersii* larvae in the water column, larvae were exposed to either darkness (0 μmol.m^{-2}.sec^{-1}) or bright light (95 – 100 × 10 μmol.m^{-2}.sec^{-1}, Schott KL1500 electronic light source). *T. gratilla* and *C. rodgersii* larvae were microscopically sorted into 'early' (rudiment, no pedicellaria) and 'advanced' (one or more pedicellaria) groups.

There were ten replicates per treatment. Replicates consisted of a 2 L plastic container (185 H × 120 Ø mm), containing a total of 1.75 L filtered sea water (FSW, 1.0 μm). The bottom and the sides of the containers were painted black, except for a 5 cm vertical strip to observe the larvae. Actively swimming larvae were selected from 10 L samples of the 300 L larval cultures that were left for 10 min to allow non-swimming larvae to settle out, sorted and added to each replicate (20 – 30 in total) via pipette. Assays were conducted in a temperature controlled laboratory (25°C *T. gratilla*, 23°C *C. rodgersii*) between 13:00 – 19:00 h, corresponding to when the 300 L larval cultures were exposed to light. The position of larvae in the water column was recorded after one hour using six equally spaced horizontal sections marked on each container.

Statistical Analysis

Differences between treatments in the proportion of larvae in the six sections of the containers could not be analysed directly as sections were not independent and violated the assumptions for ANOVA, i.e. larvae 'chose' one section out of the six sections (plus the bottom) that were available simultaneously. Therefore we used the section number (see Fig. S1) of the median larvae in each replicate for analysis. Thus low median values were indicative that larvae were concentrated in the uppermost sections of the containers and high median values were indicative of a spread of larvae throughout the water column (Table S1).

The median values were compared (light vs. dark, and early vs. advanced larvae) by two-way permutational analysis of variance (PERMANOVA, Anderson, 2001) using Primer 6 (Primer-E, Plymouth) with PERMANOVA$^+$ extension (v.6.1.11) software. The assumptions for ANOVA for the median values were not violated as each replicate was independent. The proportion of larvae (%) on the bottom of containers (i.e. ceased swimming) between light and dark treatments for early and advanced larvae were also compared using PERMANOVA.

Results

There was no difference in the number of larvae on the bottom of the containers between light and dark treatments for early (light – 1.4% ± 0.6% , dark – 2.6% ± 1.1 SE, $F_{1, 19} = 1.0485$, $P = 0.3236$) and advanced (light – 7.4% ± 2.8 SE, dark – 2.2% ± 1.5 SE, $F_{1, 19} = 2.7763$, $P = 0.1177$) *T. gratilla*, or early (light – 46.5% ±

4.5 SE, dark – 41.5% ± 3.1 SE, $F_{1, 19} = 0.9165$, P = 0.3522) and advanced (light – 37.5% ± 3.7 SE, dark – 33.8% ± 4.2 SE, $F_{1, 15} = 0.4532$, P = 0.5126) *C. rodgersii* larvae.

The position of *T. gratilla* larvae in the water column in response to light was dependent on a significant interaction between light and development (Fig. S1, Tables S1 & S2). Early larvae exposed to light migrated down into the water column, whilst those in the dark congregated at the surface (Fig. S1, Tables S1 & S2). There was no difference in the median values between advanced larvae exposed to light and dark (Tables S1 & S2), however there were fewer larvae swimming in the uppermost section in the light (96.8%) compared to the dark (75.9%) (Fig. S1).

Swimming behaviour of *C. rodgersii* larvae was affected by light, but not development (Table S2). Early and advanced larvae exposed to light migrated down into the water column, whilst those in the dark congregated at the surface (Fig. S1, Tables S1 & S2).

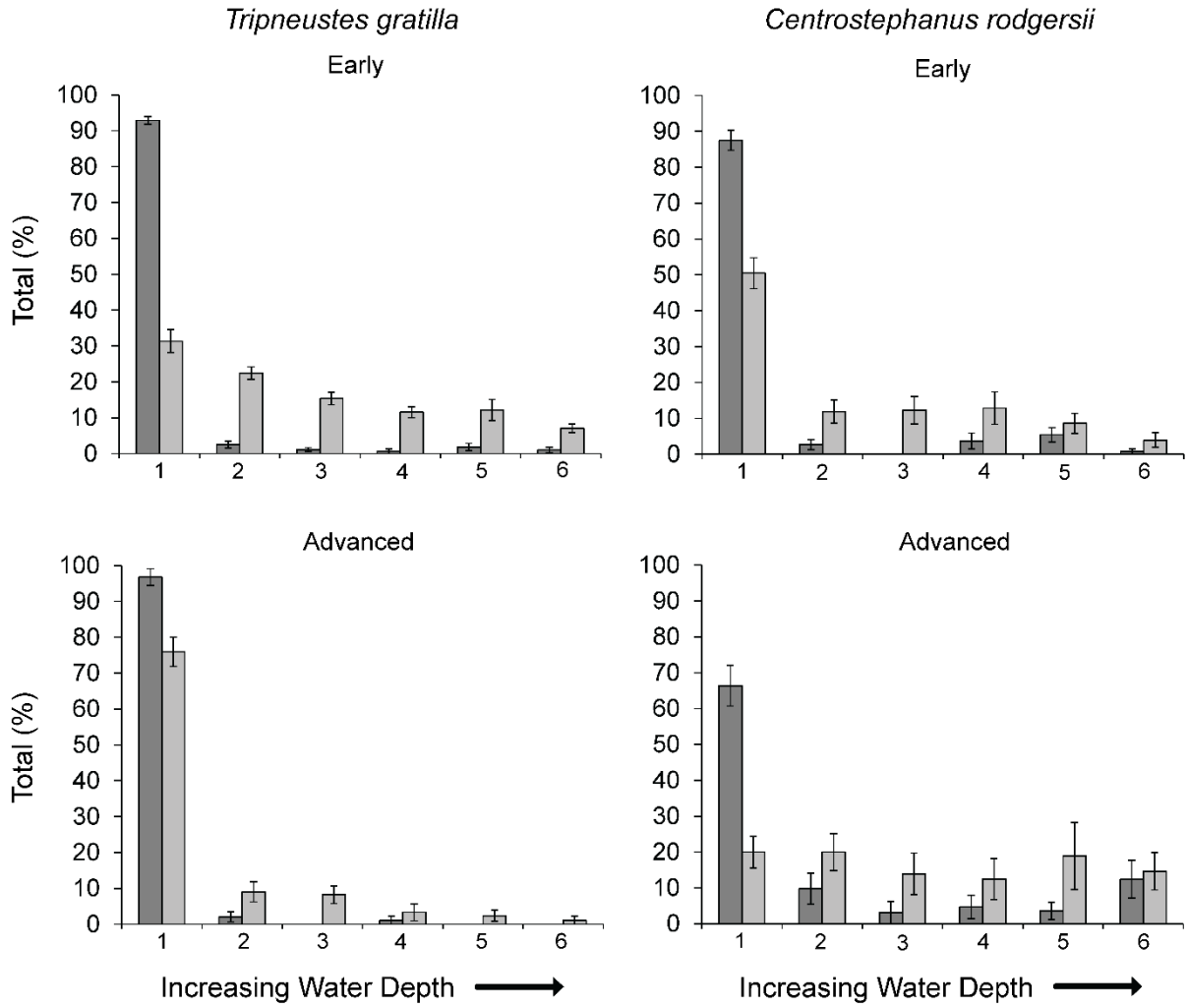

FIGURE S1: The effect of light (light bars) and dark (dark bars) on the position of early and advanced *Tripneustes gratilla* and *Centrostephanus rodgersii* larvae in the water column. Early larvae possessed a rudiment only, advanced larvae were more developed and possessed pedicellaria or tube feet extending from the vestibule. *X* axis numbers refer to six equal vertical layers used to determine the position of larvae in the water column. Data are means ± S.E., n = 8 or 10.

TABLE S1: Median position of actively swimming early and advanced *Tripneustes gratilla* and *Centrostephanus rodgersii* larvae in six vertical sections of containers exposed to light and dark (see Fig. S1). Data are means ± S.E.

	Treatment		Median	S.E.
T. gratilla	Light	Early	1.5 [A]	0.2
	Dark	Early	1.0 [B]	0.0
	Light	Advanced	1.0 [B]	0.0
	Dark	Advanced	1.0 [B]	0.0
C. rodgersii	Light	Early	4.2	0.6
	Dark	Early	1.8	0.7
	Light	Advanced	3.8	0.5
	Dark	Advanced	2.0	0.8

T. gratilla: Superscript letters denote significant difference between treatments (Table A1.2, followed by post-hoc pair-wise tests).

TABLE S2: PERMANOVA analyses examining the swimming behaviour of early and advanced *Tripneustes gratilla* and *Centrostephanus rodgersii* larvae in response to light and dark.

	Source	df	MS	*F*	*P*
T. gratilla	Larval Stage	1	5.63	45.00	**0.0001**
	Light	1	5.63	45.00	**0.0001**
	Light × Larval Stage	1	5.63	45.00	**0.0001**
	Residual	36	0.13		
C. rodgersii	Larval Stage	1	0.03	7.33E-3	0.9115
	Light	1	40.85	10.64	**0.0030**
	Light × Larval Stage	1	0.38	0.10	0.7508
	Residual	32	3.84		

Significant differences ($P < 0.05$) are in bold; df, degrees of freedom, MS, mean square

SUPPLEMENT 2. PERMANOVA tables.

Table S3: Repeated measures analysis examining the effect of larval development on settlement of *Tripneustes gratilla* in response to *Corallina officinalis* after 6, 18 and 48 h.

Source	**df**	**MS**	***F***	***P***
Larval Stage	3	3.99E4	168.30	**0.0001**
Time	2	351.23	18.31	**0.0001**
Replicate (Larval Stage)	36	237.04	12.36	**0.0001**
Larval Stage × Treatment	6	71.58	3.73	**0.0035**
Residual	72	19.18		

Significant differences (P < 0.05) are in bold; df, degrees of freedom, MS, mean square

Table S4: PERMANOVA analysis examining the effect of larval development on settlement of *Centrostephanus rodgersii* in response to *Sargassum linearifolium*, *Corallina officinalis* and histamine after 72 h.

Source	**df**	**MS**	***F***	***P***
Larval Stage	2	1.30E4	106.1	**0.0001**
Treatment	6	1.66E4	135.75	**0.0001**
Larval Stage × Treatment	12	2.44E3	19.91	**0.0001**
Residual	126	122.65		

Significant differences (P < 0.05) are in bold; df, degrees of freedom, MS, mean square

Table S5: PERMANOVA analyses examining the effect of larval development on possession of spines, test diameter (TD) and occurrence of abnormalities after 72 h for *Centrostephanus rodgersii* induced to settle by *Corallina officinalis* and 100 μM histamine.

	Source	**df**	**MS**	***F***	***P***
Spines	Larval Stage	1	6.91E3	25.75	**0.0001**
	Treatment	1	1.13E3	4.21	0.0516
	Treatment × Larval Stage	1	469.75	1.75	0.2037
	Residual	24	268.2		
TD	Larval Stage	1	2.88E-2	30.68	**0.0001**
	Treatment	1	4.83E-4	0.51	0.4744
	Treatment × Larval Stage	1	3.12E-4	0.33	0.5652
	Residual	22	9.39E-4		
Abnormalities	Larval Stage	1	2.95E3	12.25	**0.0017**
	Treatment	1	7.86E-2	3.26E-4	0.9861
	Treatment × Larval Stage	1	18.36	7.62E-2	0.7799
	Residual	22	240.99		

Significant differences (P < 0.05) are in bold; df, degrees of freedom, MS, mean square

Table S6: PERMANOVA analyses examining the effect of larval development on the response of (a) *Tripneustes gratilla* larvae to seawater conditioned with *Corallina officinalis*, (b) *Centrostephanus rodgersii* larvae to 1, 10 and 100 μM histamine, and (c) *T. gratilla* and (d) *C. rodgersii* to seawater samples collected from sand and seaweed dominated habitats, while swimming in the water column.

		Source	df	MS	*F*	*P*
a	*T. gratilla*	Larval Stage	1	1.03E4	99.42	**0.0001**
		Treatment	4	2.44E3	23.50	**0.0001**
		Treatment × Larval Stage	4	2.36E3	22.80	**0.0001**
		Residual	59	103.67		
b	*C. rodgersii*	Larval Stage	1	1.94E4	234.40	**0.0001**
		Treatment	2	6.09E3	73.48	**0.0001**
		Treatment × Larval Stage	2	2.73E3	32.98	**0.0001**
		Residual	24	82.90		
c	*T. gratilla*	Larval Stage	1	7.03E3	34.01	**0.0198**
		Treatment	1	1.40E3	6.78	**0.0001**
		Treatment × Larval Stage	1	3.50E3	16.90	**0.0009**
		Residual	21	206.77		
d	*C. rodgersii*	Larval Stage	1	3.44E3	24.80	**0.0004**
		Treatment	1	3.62E3	26.14	**0.0002**
		Treatment × Larval Stage	1	3.00E3	21.67	**0.0005**
		Residual	16	138.6		

Significant differences ($P < 0.05$) are in bold; df, degrees of freedom, MS, mean square