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Supporting Information for
A predatory gastrula leads to symbiosis-independent settlement in Aiptasia

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Supporting Information Text

Detailed Materials and Methods

Organism culture. Aiptasia were cultured and induced to spawn as described (1) with the following modifications: animals were kept at 25°C in artificial seawater (ASW) made with Tropic Marin Pro Reef prepared in MilliQ water. Adult polyps were fed twice weekly with freshly hatched *Artemia salina* brine shrimp nauplii, followed by water exchange 2-5h after feeding and weekly cleaning. Aiptasia larvae were collected and filtered as previously described (1). *Tisbe* copepods (planktino.de) were kept at a density of 20-30 animals per ml in ASW under aeration at 22-24°C. Cultures were dosed three times weekly with a nutrient solution (7.5% w/v in water) consisting of spirulina and spinach powders and baker's yeast or Rotiwonder powder (planktino.de). *Tisbe* cultures were cleaned via ASW replacement while collecting adults in a 100 µm plankton sieve, and nauplii were further separated with a 60 µm plankton sieve. Clonal and axenic Symbiodiniaceae strains *Breviolum minutum* SSB01 (28S LSU identification at GenBank Accession MK692539) and *Symbiodinium linuchae* SSA01 (GenBank MK692538) were cultured as described (2-5).

Aiptasia larvae feeding. Larvae were kept in 24-well plates with one larva per well in 1 ml ASW. Where applicable, larvae at 2 dpf were infected with the Symbiodiniaceae strains at a final concentration of 100,000 algal cells per ml. After 24h of infection, larvae hosting at least one algal cell were kept in the experiment. Larvae were fed with 5-10 freshly hatched *Tisbe* nauplii (6) daily or every three days starting at 2 dpf, and not eaten *Tisbe* were removed the following day. Larvae were transferred to fresh plates after 2-3 feedings. Starting at 8 dpf for daily fed larvae, and 15 dpf for larvae fed every three days, larvae were additionally fed with 3-5 freshly hatched Artemia nauplii, and not eaten Artemia were removed the day after feeding. Settlement was documented daily. To test heat-killed food, larvae were fed with 5 live or 5 heat-killed (70°C for 10 min) *Tisbe* nauplii daily from 2 dpf to 9 dpf.

Inhibition of nematocyst discharge. 50 larvae (unfed, 4 dpf) were transferred to each well of a 12-well plate. The [2.2]paracyclophane compound 1 previously described to inhibit nematocyst discharge in *Hydra* (7) was added to the wells in triplicates at the indicated concentrations. The solvent DMSO was used in the control condition and the larvae were incubated for 30 min at 25°C. Then 10 artemia were added to each well and after 1h the fraction of killed artemia per well was recorded.

Staining, fixation, and imaging. For DIC and epifluorescence microscopy, larvae were stained with Hoechst 33258 for 1h before fixation where applicable. Fixation was conducted 30-70min or 24h after feeding to document *Tisbe* uptake or larval growth, respectively, in 3.7% formaldehyde or 4% paraformaldehyde in ASW for 30min at RT, followed by 3 washes in 0.05% Triton X-100 in PBS. After a final wash in PBS, larvae were embedded in glycerol on glass slides with glass coverslips. For live imaging of autofluorescence, animals were anesthetized in 40 mM chloral hydrate in ASW (larvae) or 180 mM $MgCl_2$ (polyps) on glass slides with glass coverslips. Elongated larvae and top-view polyps were imaged live in ASW. For live videos, larvae and *Tisbe* nauplii were kept in 1 mm or 4 mm diameter wells made of Silicone Isolators™ Material (Grace Bio-Labs) in ASW on glass slides with a glass coverslip. Larvae, *Tisbe* and Artemia nauplii were imaged with a Nikon Eclipse 80i microscope using Differential Interference Contrast (DIC) or Fluorescence with a Nikon 10X Plan Apo dry lens or a Nikon Plan Fluor 20X dry lens and a DS-Fi3 camera (Nikon Instruments). Oral views of polyps were captured using a Nikon SMZ25 with a SHR Plan Apo 0.5X nosepiece and a DS-Ri2 camera. Videos were imaged using a Nikon Eclipse 80i microscope using Differential Interference Contrast (DIC) with a Nikon 10X Plan Apo dry lens and a DS-Fi3 camera (Nikon Instruments) or with an iPhone 13 mini through LabCam® from iDu Optics® (New York, NY, USA).

Confocal microscopy. For confocal microscopy (Fig. 1B), larvae were fixed at 2 dpf in 3.7% formaldehyde and 1X PHEM (60 mM PIPES, 25 mM 4-HEPES, 10 mM EGTA and 2 mM $MgCl_2$,

pH 6.9) in FASW for 30min, followed by permeabilization in 20% DMSO and 0.1% Triton-X100 in PBS for 30min. Larvae were then stained with Hoechst 33258 and Phalloidin Atto 565 for 4h, washed thrice in 0.05% Tween20 in PBS, and mounted in 100% glycerol. Samples were imaged on a Leica SP8 laser scanning confocal at 63X magnification. For visualization of nematocyst autofluorescence, the detector for the 488 nm laser was set to reflection, and 3.5 µm stacks (7 z-planes) were recorded. Images were prepared using averaged Z-projections in ImageJ (8).

Scanning electron microscopy. For scanning electron microscopy, larvae were fixed in 2.5% glutaraldehyde in PBS for 2 h at 4°C, rinsed in 0.1 M cacodylate buffer pH 7.2, and post-fixed in 1% Osmium tetroxide in 0.1 M cacodylate buffer for 1h at room temperature. Samples were rinsed in water and dehydrated in steps through a series of 50 to 100% acetone. Larvae were critical-point dried in a Leica CPD300 (Leica Microsystems, Vienna, Austria) and mounted on stubs with carbon adhesive discs. Finally, the larvae were sputter coated with 10 nm gold/palladium and imaged with a Leo 1530 scanning electron microscope (Zeiss, Oberkochen, Germany).

Induction of Aiptasia F1 spawning. F1 primary polyps were kept in groups of 15 in the same conditions as adult Aiptasia polyps above, and were fed twice weekly with Artemia nauplii for 5 months before exposure to spawn induction cues (7). Polyps were then kept individually and the identity of spawned gametes was documented.
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Movie S1 (separate file). Hunting and feeding of Aiptasia I. Gastrula-like Aiptasia larvae, 2 dpf, hunting on *Tisbe* nauplii (Interference contrast, real time).

Movie S2 (separate file). Hunting and feeding of Aiptasia II. Feeding and uptake of a Tisbe nauplius by an Aiptasia larva, 2 dpf (Interference contrast recording of ~60 minutes, time lapse factor 20X).

Movie S3 (separate file). Settlement of Aiptasia planulae. Fed larvae of Aiptasia, 15 dpf, slow down swimming and elongate before they finally contract and adhere on the substrate to metamorphose into a primary polyp (interference contrast, total recording time ~6 minutes, time lapse factor 2X and real time).

SI References

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