



# A predatory gastrula leads to symbiosis-independent settlement in *Aiptasia*

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The planula larvae of the sea anemone *Aiptasia* have so far not been reported to complete their life cycle by undergoing metamorphosis into adult forms. This has been a major obstacle in their use as a model for coral–dinoflagellate endosymbiosis. Here, we show that *Aiptasia* larvae actively feed on crustacean nauplii, displaying a preference for live prey. This feeding behavior relies on functional stinging cells, indicative of complex neuronal control. Regular feeding leads to significant size increase, morphological changes, and efficient settlement around 14 d postfertilization. Surprisingly, the presence of dinoflagellate endosymbionts does not affect larval growth or settlement dynamics but is crucial for sexual reproduction. Our findings finally close *Aiptasia*'s life cycle and highlight the functional nature of its larvae, as in Haeckel's *Gastrea* postulate, yet reveal its active carnivory, thus contributing to our understanding of early metazoan evolution.

endosymbiosis | gastrulation | settlement | nutrition | basal metazoan evolution

Coral reefs are biodiversity hotspots threatened by global climate change, including by coral bleaching, a loss of the symbiotic relationship that corals form with algae during their larval/juvenile stage (1). Anthozoan larval growth and settlement as a prerequisite for symbiont uptake has so far been elusive due to the difficulty of establishing settlement under laboratory conditions. While triggers for cnidarian larval settlement are chemical or other environmental cues, we focused on the dual nutrition sources of diet and algal symbionts in the sea anemone *Aiptasia* (*Exaiptasia diaphana*), a model for cnidarian endosymbiosis (Fig. 1*A*) (2, 3). Similar to coral and sea anemone larvae (4), *Aiptasia* planulae readily ingest animal homogenates or symbiotic algal cells, but these have not resulted in growth or settlement (5). Here, we demonstrate that *Aiptasia* larvae actively feed on live nauplii of the copepod *Tisbe*, leading to substantial growth, settlement, and metamorphosis. Endosymbionts are not necessary for these processes, but crucial for gametogenesis. Our findings highlight the predatory nature of these late gastrulae/early planulae, shedding light on the early evolution of (eu)metazoans and providing a breakthrough in the *Aiptasia* model system.

## Results

We attempted feeding *Aiptasia* planulae with the copepod *Tisbe* (6). Surprisingly, we found that even planulae at the gastrula stage, 2 d postfertilization (dpf) (Fig. 1*B*), were able to hunt *Tisbe* nauplii (Fig. 1 *C*, *D*, and *F* and [Video S1](#)), caught by the planulae's nematocysts (Fig. 1 *B* and *D–F*) and ingested into the gastric cavity (Fig. 1 *D* and *F* and [Video S2](#)). Larvae fed with *Tisbe* nauplii grew continuously in size, followed by eventual metamorphosis and settlement (Fig. 2 *A–E*). In contrast, unfed larvae developed as described (5), i.e., size and endoderm thickness shrank over time (Fig. 2 *B–D*). Live prey were preferred to heat-killed nauplii (Fig. 2*D*), and after 8 d of daily feeding, planulae also hunted and ingested *Artemia*. Inhibition of nematocyst discharge by the [2.2] paracyclophane compound 1 (7) prevented prey capture and led to increased prey survival (Fig. 1*E*).

After ~14 d of daily feeding, planulae behavior began to markedly change as a precedent to settlement, exhibiting slower swimming, cycles of lengthening and contraction, and substrate exploration with the apical tuft (Fig. 2*E* and [Video S3](#)); concurrently, autofluorescence appeared at the aboral end (Fig. 2*F*). Settlement and metamorphosis occurred between 13 and 20 dpf (Fig. 2*A*), when planulae attached to the substrate at the aboral end, flattened, and displayed eight tentacle primordia (Fig. 2*E* and [Video S3](#)). With feeding every third day, settlement onset was delayed, yet the kinetics and final settlement efficiencies were strikingly similar, suggesting a size tipping point past which settlement was nearly always triggered (Fig. 2*A*).

We then investigated whether symbionts affected settlement dynamics. Because the parental lines harbor either *Symbiodinium limuchae* alone (male “CC7”) or in combination

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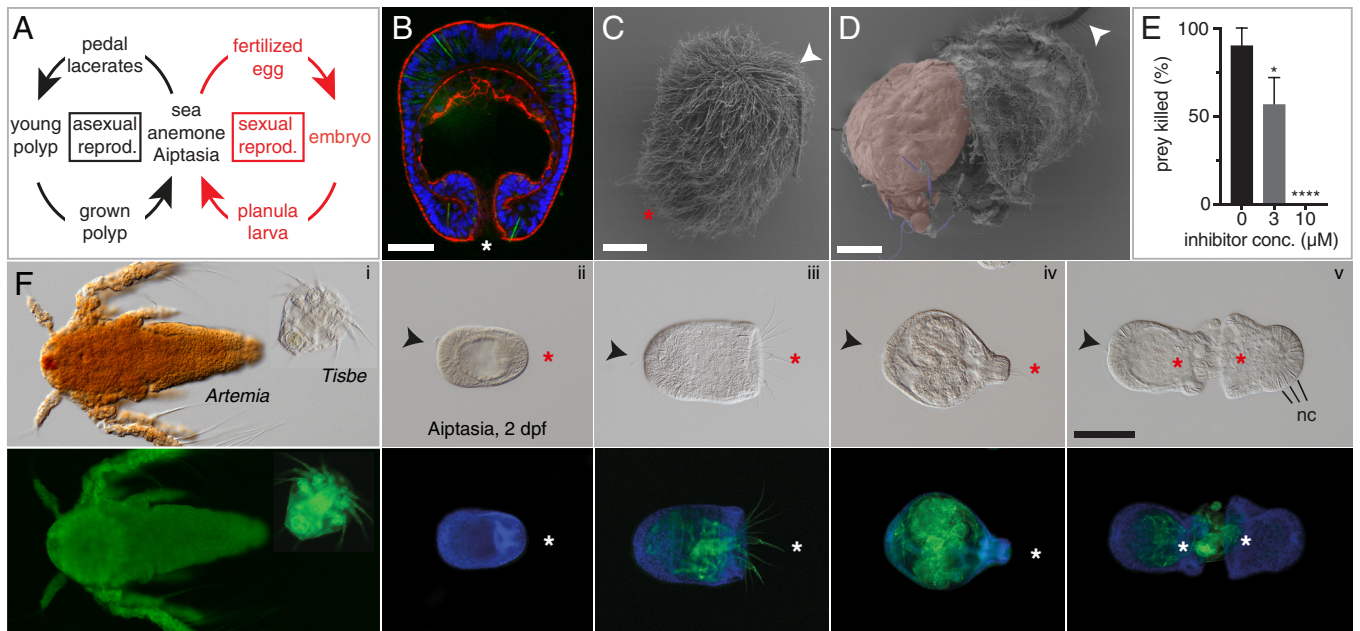
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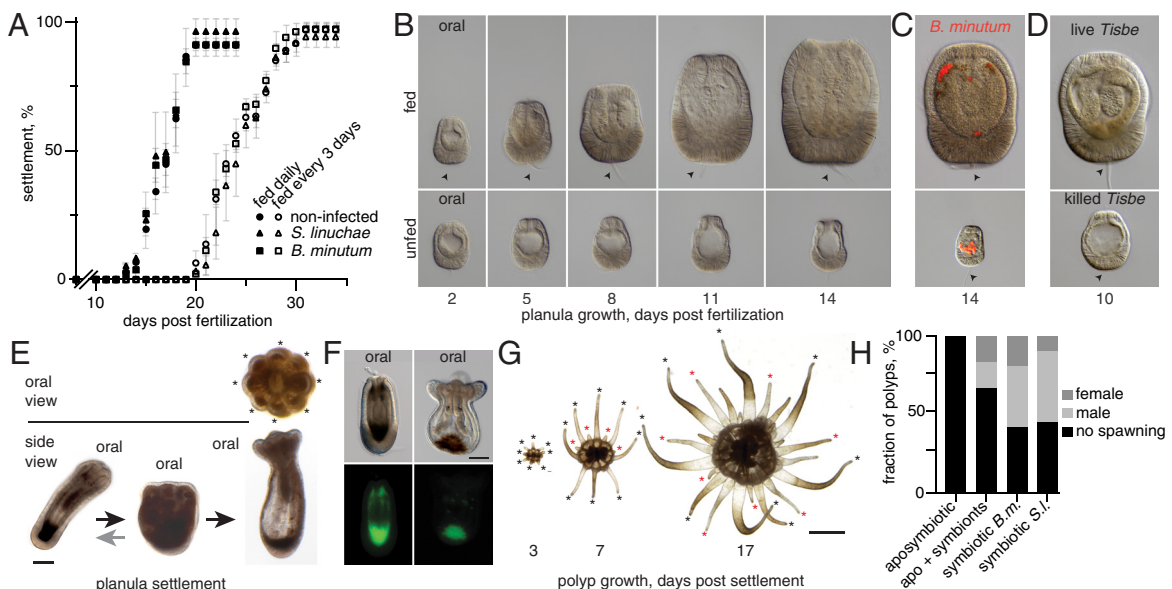


**Fig. 1.** Carnivorous larvae of *Aiptasia*. (A) Life cycle of *Aiptasia*. (B) Representative image of a late gastrula/early planula 2 dpf (also in F). Blue, Hoechst; red, phalloidin; green, autofluorescence of nematocysts. The blastopore corresponds to the mouth. (C and D) Scanning electron micrographs of planulae (3 dpf) with cilia (C); ingestion of a *Tisbe* nauplius (red) penetrated by nematocysts (purple) (D). (E) Impaired nematocyst activity by a small molecule inhibitor (fraction of killed prey is mean  $\pm$  SD from three independent experiments, Student's *t*-test). (F) *Aiptasia* larvae 2 dpf and prey under DIC (Upper row) and fluorescence (Lower row) microscopy. Blue, Hoechst; green, autofluorescence of chitin. *Tisbe* but not *Artemia* nauplii (i) can be ingested by young planulae (ii). *Tisbe* nauplii are predated by one (iii and iv) or multiple larvae (v) and ingested (iii and iv) [Scale bars, 25  $\mu$ m (B–D), 100  $\mu$ m (F)]. B–D and F: Asterisks, mouth; arrows, aboral apical tuft; nc, nematocysts.

with *Breviolum minutum* (female “F003”), we infected *Aiptasia* larvae at 2 dpf with either strain under the two feeding regimes (Fig. 2 A and C) (SI Appendix). All larvae displayed indistinguishable settlement dynamics: Only larvae fed with nauplii grew and eventually settled, regardless of symbiotic status (Fig. 2 A–C).

After settlement, *Aiptasia* primary polyps (F1 generations) developed to reproductive maturity (Fig. 2 E–H) and spawned,

giving rise to F2 generations. Most primary polyps displayed radially symmetric tentacle primordia, then bilateral symmetry in the next tentacle wave, before eventual sixfold symmetry (Fig. 2 E and G). Growth patterns were identical in apo- and symbiotic primary polyps when fed at least twice weekly with *Artemia* nauplii, including asexual reproduction (Fig. 1A) starting 14 d postsettlement (dps). In contrast, sexual reproduction (6 mo postsettlement),



**Fig. 2.** *Aiptasia* larval and polyp development. (A) Settlement of fed planulae hosting either Symbiodiniaceae symbiont strain (mean  $\pm$  SD of four replicate experiments with 72 larvae per condition, Student's *t*-test). No unfed larvae underwent metamorphosis or settlement in any tested condition. (B–D) Planula growth in different feeding regimes. (B) Growth with (Upper row) or without (Lower row) daily feeding over 14 dpf. (C) Fed with addition of symbiont strain *Breviolum minutum*. (D) Fed with live (Upper row) or heat-killed (Lower row) *Tisbe* after 10 dpf. (E) Planula settlement and metamorphosis. Note cycling between elongated and laterally flattened forms before settlement. Arrows, aboral apical tuft. (F) Autofluorescence in live planulae appears 5–8 dpf and gets brightest around settlement. (G) Tentacle formation in growing polyps 3–17 dps. Black asterisks, symmetric first; red asterisks, asymmetric second tentacle wave (H). Sexual reproduction (spawning) in grown primary polyps without symbionts (“aposymbiotic”), aposymbiotic polyps infected with the Symbiodiniaceae strains (“apo + symbionts”), or polyps from planulae with symbionts (“symbiotic” in A) [Scale bars, 50  $\mu$ m (B), 100  $\mu$ m (E and F), and 1,000  $\mu$ m (G)].

relied on symbiotic state: over half of symbiotic polyps hosting either algal strain spawned within two induction cycles (41 of 73), whereas no aposymbiotic polyps spawned (0 of 18). Critically, aposymbiotic polyps infected with symbionts recovered the ability to spawn (4 of 12) (Fig. 2H). Finally, crossing of primary polyps (F2 generation) and back-crossing with parental lines in both combinations produced viable larvae capable of settlement.

## Discussion

Our data demonstrate that the predatory gastrula is a crucial step in the metamorphosis and settlement of *Aiptasia* planula larvae. This finding has significant implications for various fields, including coral–algae symbiosis, embryology, and ecotoxicology. It opens the door to functional genetics and manipulation, allowing the establishment of stable transgenic lines.

Intriguingly, our results reveal that algal symbionts do not appear to affect the growth and settlement of *Aiptasia* planulae, neither through signaling nor nutritional contribution. The importance of diet outweighs the role of symbiosis during primary polyp growth, development, and early asexual reproduction. However, in adult polyps, the nutritional balance may shift toward the primacy of symbiosis. Our observations indicate a striking reliance on symbiosis for sexual reproduction in *Aiptasia*, similar to the *Hydra/Chlorella* symbiosis (2).

The carnivorous nature of *Aiptasia* larvae is remarkable, as they possess the ability to hunt live food as early as 2 dpf, during the late gastrula/early planula stage. This is in contrast to most scleractinian corals, which develop into planulae after 3 or 4 dpf and settle spontaneously or under the influence of environmental chemical cues (4). The quick development and voracious appetite of *Aiptasia* are consistent with its lack of lipid-rich yolk in comparison to other cnidarians. This predatory lifestyle likely provides an advantage in heavily human-impacted eutrophic environments where *Aiptasia* is commonly found.

While symbiosis eventually becomes critical in *Aiptasia*'s ontogeny, the observed autofluorescence in planulae is consistent with the “beacon” hypothesis of symbiont attraction in juvenile corals (8). However, it is possible that the predatory lifestyle was also an ancestral feature of cnidarian gastrulae. Ancestral anthozoans primarily consisted of taxa with solitary and nonsymbiotic polyps (9), and the yolk-rich embryos of derived cnidarians like hydrozoans

might be an adaptation to a benthic lifestyle (10). Thus, although sea anemones are not strictly basal cnidarians, *Aiptasia*'s predatory, yolk-poor planulae that result from gastrulation by invagination (the ancestral mode) may represent a maintenance of (or return to) deeply conserved traits shared by Anthozoa.

The predatory gastrula of *Aiptasia*, which is carnivorous rather than the hypothetical filter-feeding gastrula of Haeckel (11), carries significant implications for the evolution of early emerging metazoans. The presence of specialized stinging cells used for prey capture in cnidarians, which is reflected by extrusive organelles in protist eukaryotes (12), ctenophores (13, 14), and planarians (15), suggests that predation played a crucial role in the early origin of metazoans from unicellular eukaryotes, although further analyses into cell types with more taxa are needed. The predatory lifestyle may have been a major driving force for early metazoan evolution and the development of organized nervous systems. Recent evidence points to ctenophores as the sister clade to all metazoans (16). A secondary loss of the predatory life style and extrusive organelles in sponges would support this hypothesis and suggest a shared origin of both toxin-producing cells with extrusive functions and nervous systems between ctenophores, cnidarians, and bilaterians.

## Materials and Methods

*Aiptasia* and axenic Symbiodiniaceae (*B. minutum* strain SSB01, *S. linuchae* strain SSA01) were cultured as described (5). *Aiptasia* planulae were fed with *Tisbe* nauplii (6) and after sufficient growth also with *Artemia*. Metamorphosed F1 polyps were induced to spawn after 6 mo. Nematocyst discharge in starved 4 dpf planulae was inhibited for 30 min with “compound 1” [2.2] paracyclophane in DMSO (7). Imaging with epifluorescence, confocal, and scanning electron microscopy followed standard methods. For details, see *SI Appendix*.

**Data, Materials, and Software Availability.** All study data are included in the article and/or [supporting information](#).

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