

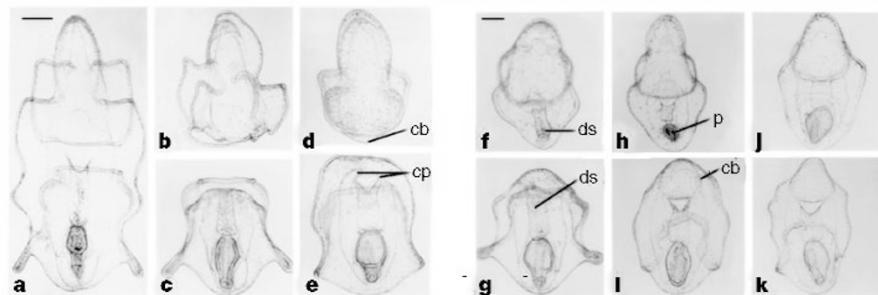
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## Regeneration in metazoan larvae

The capacity for regeneration of lost body parts requiring organogenesis has never been described for metazoan larvae. Here we show for the first time, to our knowledge, the ability of sea star larvae to rapidly and completely regenerate to form fully functional individuals following surgical bisection of the larval body. This capacity leads us to extend the developmental stage at which echinoderm larval cell fates are irreversibly determined. As planktonic larval mortality is thought to be extremely high<sup>1</sup>, this may be an adaptation to enhance recruitment into adult populations.

Larvae of the sea stars *Pisaster ochraceus* (bipinnaria and brachiolaria) and *Luidia foliolata* (bipinnaria) that had been surgically bisected across the horizontal larval axis with a scalpel, equidistant between the anterior and posterior poles, regenerated into fully functional larvae over a period of 12–14 days. No mortality occurred in any of the experimental or control larval treatments ( $n=10$ –12 larvae per treatment). The ontogeny of larval regeneration was similar in both species and is shown for *P. ochraceus* in Fig. 1.



**Figure 1** Bipinnaria larvae of *P. ochraceus* at various stages of regeneration after surgical bisection. The anterior larva is shown in the top row, and the posterior larva in the bottom row. **a**, Bipinnaria before surgical treatment. **b, c**, Day 1. Anterior (**b**) and posterior (**c**) larvae after surgical division. The gut of the posterior larva is intact enough for feeding. **d**, Day 3. **e**, Day 4. Elongation along the larval axis has taken place. **f, g**, Day 7. The anterior larva (**f**) has regenerated a functional digestive system. **h**, Day 9. **i**, Day 10. Complete regeneration of ciliary bands has occurred in both anterior and posterior larvae. **j, k**, Day 12. Both larvae have regenerated and are similar to control larvae. cb, ciliary band; cp, coelomic pouch; ds, digestive system; p, phytoplankton. **a–e**, Scale bar (in **a**), 200  $\mu$ m. **f–k**, Scale bar (in **f**), 200  $\mu$ m.

Immediately following surgical manipulation of intact larvae (Fig. 1a), both the anterior and the posterior portions of bipinnaria larvae (hereafter referred to as anterior and posterior larvae; Fig. 1b, c) exhibited swimming behaviours similar to those of control larvae. The posterior larvae consumed food, indicating that these larvae do not lose the ability to capture and ingest food particles, even though the upper portion of the oesophagus and mouth had been surgically removed.

By day 3, the anterior larvae had begun to regenerate the surgically separated post-oral ciliary bands and to elongate along the larval axis (Fig. 1d). These larvae were able to capture and transport phytoplankton to the region of the former larval mouth. By day 4, the coelom of posterior larvae had elongated and showed signs of fusing above the larval mouth (Fig. 1e). After one week, both anterior and posterior larvae completed the regeneration of the digestive organs and coelom (Fig. 1f, g). At this point in the regenerative process, anterior larvae became able to feed. Capture and ingestion of phytoplankton were observed in culture; guts of anterior larvae contained phytoplankton by day 9 (Fig. 1h). The posterior larvae completely regenerated the pre-oral ciliary bands by day 10 (Fig. 1i).

By day 12, most anterior and posterior larvae had regenerated all of their body components (Fig. 1j, k), and surgically bisected bipinnaria larvae of both sea star species were essentially indistinguishable from control larvae by day 14.

Successive bisection of a bipinnaria larva of *P. ochraceus* resulted in complete regeneration indicative of sustained regenerative capacity. Brachiolaria larvae ( $n=12$ ) showed similar chronological and morphological patterns of regeneration. In another study we bisected bipinnaria larvae of *L. foliolata* that had an adult rudiment ( $n=12$ ). By day 3, rudiment-bearing posterior larvae had undergone complete metamorphosis into juveniles, whereas anterior and control

larvae required at least three to four times longer to metamorphose into juveniles.

Planktonic larvae of sea stars<sup>2–4</sup> and brittle stars<sup>5</sup> have been shown to reproduce asexually. Although this feature of echinoderm larvae reflects the indeterminate developmental nature of deuterostomal embryonic cells, such observations provide no direct measure of the ability of larvae to regenerate lost body components. Our observations of the ability of sea star larvae to regenerate show that we need to revise ideas about the stages of development of echinoderm larvae at which cell fates are considered to be irreversibly determined<sup>6</sup>.

High estimates of larval mortality are attributed to predation<sup>7,8</sup>, abiotic factors, such as hydrodynamic shear stress<sup>9</sup>, or dispersal to inappropriate habitats<sup>10</sup>. Our results show that at least some larvae are capable of surviving and rapidly regenerating lost body parts following surgical bisection that simulates naturally induced damage. Therefore, population models that include rate functions of larval mortality in the plankton may be using mortality estimates that are unrealistically high.

It is also significant that damaged sea star larvae with an adult rudiment rapidly metamorphose to the benthic juvenile phase, thus reducing time spent exposed in the plankton and disproving hypotheses that resorption of the larval body is an energetic prerequisite to successful metamorphosis<sup>11</sup>. Regenerative capacity may be particularly important in small, abundant, planktotrophic larvae that need to spend long periods of time developing in the plankton<sup>12</sup> and which exploit chemical defences less often than large, less abundant, yolk-laden lecithotrophic larvae<sup>13,14</sup>.

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