Regeneration in worm larvae, Family Spionidae

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Abstract

Metazoan lineages exhibit a wide range of regenerative capabilities in their adult forms, however, less is known about larval regeneration. Our study evaluated whether larvae of Spionid worms were capable of regeneration. We chose Spionids due to their abundance in the near-shore waters of Friday Harbor Laboratories (WA) and because information is available on their regenerative abilities as adults. We sought to answer the following questions: (1) do larvae survive after bisection? (2) do larvae exhibit wound healing and regeneration of the lost tissues at cut regions of the anterior and posterior body segments? To do this, we collected 78 larval Spionid worms, photographed each one and grouped them into tentative larval stage and species groups. We then bisected and monitored worms for survival and signs of regeneration. Overall, anterior body parts had higher survival than posterior body parts and regeneration was observed in both worm sections. However, we were unable to detect patterns in regeneration due to uneven sampling size per group. In order to better understand the difference between groups, surviving larvae will be barcoded to determine actual species. Future experiments should be extended to observe until complete regeneration occurs. Studies in regeneration, such as this one, are important as it can reveal evolutionary relationships among polychaetes, further our understanding of developmental processes in marine worm larvae, and understand survival under sublethal predation.

Introduction

A wide range of regenerative capabilities exist within metazoan lineages (Alvarado, 2000). Regeneration is defined as the ability of organisms to restore tissues or organs after an injury or body part loss (Hentschel & Harper, 2006; Vickery et al., 2001). While healing capabilities are common, reconstruction of organs and body parts is restricted to a few groups of animals (Muller et al., 2003). Echinoderms, Cnidarians, and Platyhelminthes are the most common lineages in which adult regeneration has been studied (Cary et al., 2019). Few studies, however, have investigated regeneration at the larval stage.

The majority of larval regeneration literature has focused on echinoderms. Both sand dollars and sea star species have been observed to regenerate after bisection (Bosch et al., 1989; Vickery & McClintock, 1998; Vickery et al., 2002). On a broader scale, ophiuroids, crinoids, asteroids, holothuroidea, and echinoids have all displayed varying levels of larval regeneration (Carnevali 2006) and are known to clone (Allen et al., 2018). Similar to echinoderms, annelids have demonstrated remarkable regeneration capabilities in the adult form (David & Williams, 2012). However, less is known about annelid regeneration at the larval stage. In our study we focused on regeneration in planktonic annelid larvae from the family Spionidae found abundantly in near-shore waters of Friday Harbor, WA.

We chose the Spionids due to their regenerative abilities for adults (Blake & Arnofsky, 1999). Spionids, specifically, are also known to reproduce asexually by fission or budding in 13 species (David & Williams, 2012; Vickery et al., 2001). Asexual reproduction in Spionids can occur either by architomy (fragmentation and individual regeneration of posterior and anterior ends) or paratomy (budding from the parents to create a new individual) (David & Williams, 2012). Because we know that regeneration occurs, either when body parts are lost or through asexual reproduction at the adult stage, we hypothesized that larval stages may employ these same regenerative mechanisms (Vickery & McClintock, 1998). To determine whether Spionid larvae are capable of regeneration we sought to answer the following questions: (1) Do larvae survive after bisection?; (2) Do larvae exhibit regeneration in the form of tissue addition to the wound site of cut regions of the anterior and posterior body fragments?

Methods

Field Collection

We collected Spionid larvae via plankton tows off the Friday Harbor Laboratories dock during high tide in August, 2019. Following collection, plankton samples were brought back to the lab and larvae were sorted using a dissecting microscope. Seventy-eight Spionid larvae were placed individually into wells of 6-well plates containing filtered sea water (1 micron). These larvae were then split into two Cohorts. Cohort 1 was checked every 4 days (Cohort 1, n=18), and Cohort 2 was checked every 3 days (Cohort 2, n=60). Plates were held in a flow-through seawater table to maintain larvae at ambient seawater temperature. Water changes were conducted every two days.

Larval Manipulation and Imaging

For species identification and initial measurements, individual worm were pipetted onto microscope slides, fitted with a cover slip, and photographed the day of collection. Following initial imaging, worms were bisected at approximately midway along their bodies under a dissecting microscope severing them with a thin metal probe (Figure 1). After an 18-hour recovery period, worms were imaged for the first time and then imaged every 4 (Cohort 1) or 3 days (Cohort 2). All photos were taken using a compound microscope (Nikon Microphot-FXA, Meridian Instrument Company, Inc.) at 4X magnification with a video camera (Point Grey Grasshopper Express GX-FW-28S5) and Astro IIDC imaging program.

Image Analysis and Regeneration Metrics

Initial photos were used to categorize worms into eight groups (A-H) by using morphological characteristics (e.g. length, coloration, segments, shape, palp lengths, length of setae, parapodia, etc.; see Figure 1). These groups reflect different possible species and developmental stages (e.g. larval stages and early juveniles). Worm lengths were measured using ImageJ segmented lines drawn from the tip of the head to the tip of the tail and using each segment on the worm as a line segment (Figure 2). Metrics used to determine regeneration were: percent survival, proportion of larvae exhibiting growth on the anterior body half, proportion of larvae exhibiting growth on the posterior body half, and proportion exhibiting both. We classified regeneration as the addition of new segments and tissue added to cut area. Healing of the wound was not considered regeneration.

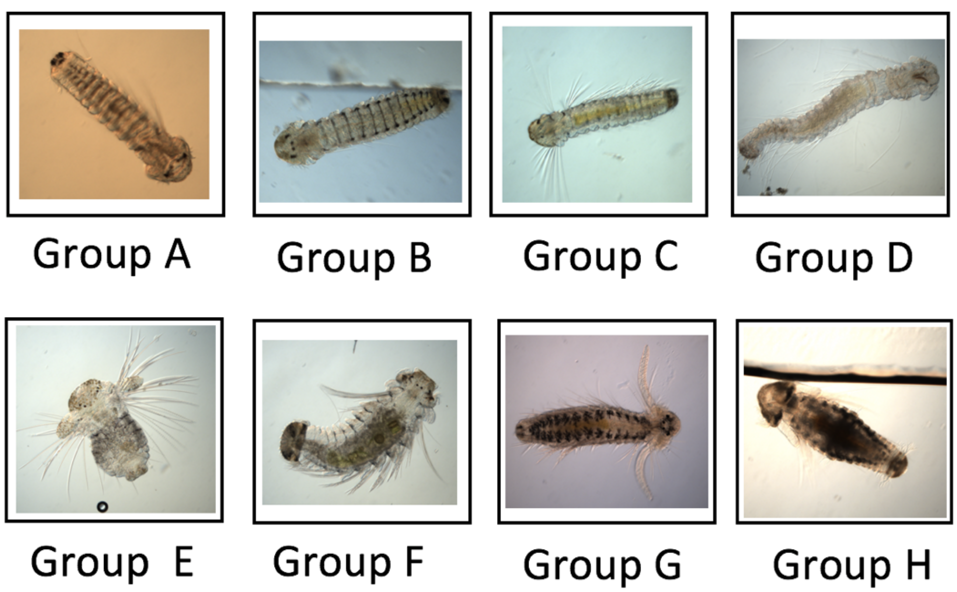

Figure 1. Eight different groups (A-H) were characterized based on morphological features.

Results

We began the project with 78 experimental worm larvae, however due to human error, 4 larvae in Cohort 1 and 25 larvae in Cohort 2 were lost. This left us with 49 worm larvae to examine survival and regeneration.

Survival

Cohort 1 was monitored every 4 days, for a total of 8 days. A total of 10 out of 15 larvae (66.7%) of Cohort 1 worms still had at least one surviving body region at the end of 8 days. Four days after bisection, 78.6% of anterior parts and 78.6% of posterior parts were alive (Figure 3). After 8 days, 72.7% anterior body parts and 63.6% posterior body parts of the surviving larvae from day 4 were alive at day 8. To test if survival of the body type (anterior and posterior) is related to the days since cutting, we performed a Pearson's Chi-Squared test of independence. There was no significant difference (p=0.6501, X-square=0.20578) which means these two variables are independent. This suggests that the number surviving, whether anterior or posterior, was not affected by day after cutting.

Figure 2. Example of measuring a worm using ImageJ's segmented lines and the halfway mark at which worms were bisected.

In Cohort 2, for 31 out of 42 worms (73.8%) had at least 1 body region still alive at the in of the six-day experimental period. Cohort 2 was monitored every 3 days. Three days after bisection, 46.7% of anterior parts and 42% of posterior parts were alive (Figure 4). After 6 days, 89.3% of previous surviving anterior parts and 76% of previous surviving posterior parts were alive. Again, we performed a Pearson's Chi-Squared test of independence. There was no significant difference (p=0.9384, X-square=0.0059753) which means these two variables are independent, suggesting the number of worms surviving was not affected by day after cutting.

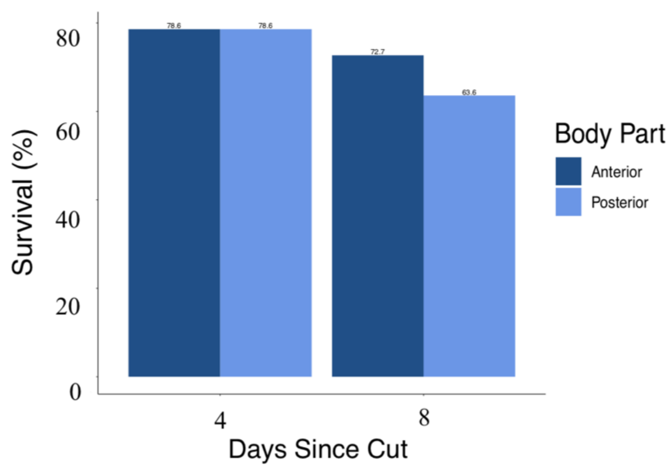

Figure 3. Percent survival of posterior and anterior body parts across experimental period for Cohort 1. Survival was assessed at 4 and 8 days after cutting.

Survival from day of bisection to last day of monitoring varied between worm groups (Figure 5). A similar pattern is seen among groups where anterior body regions tended to survive better than the posterior body regions.

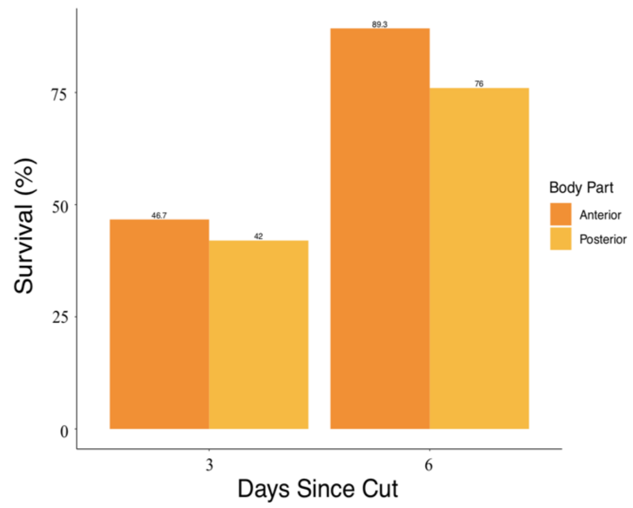

Figure 4. Percent survival of posterior and anterior body parts across experimental period for Cohort 2. Survival was assessed at 3 and 6 days after cutting.

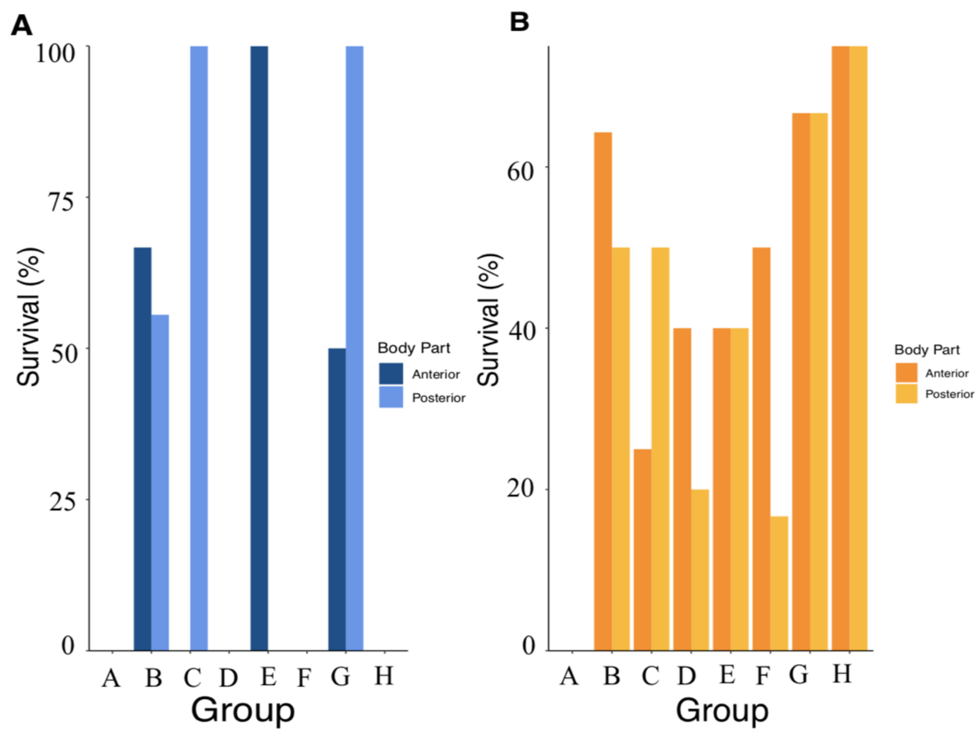

Figure 5. Percent survival of posterior and anterior body parts across experimental period for Cohort 2. Survival was assessed at 3 and 6 days after cutting.

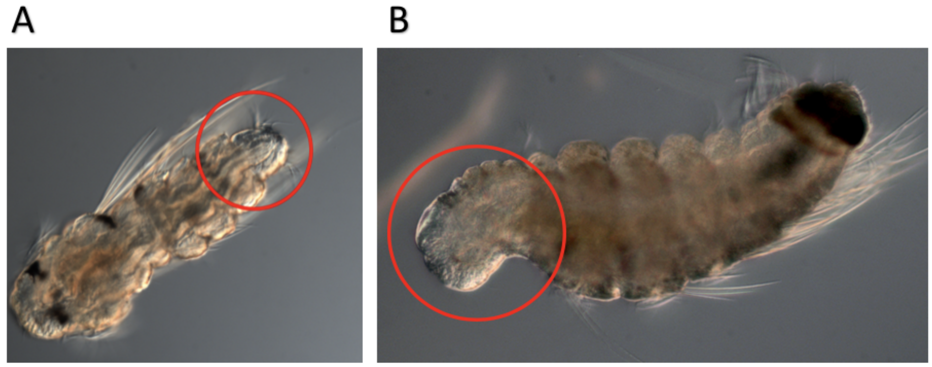

Figure 6. (A) Regeneration of the posterior portion of the body on the anterior cut site. New segment and cilia are observed. (B) Regeneration of the anterior portion of the body on the posterior cut site.

Regeneration

Of the 42 worms that survived until the end of the experiment, 16 exhibited some form of regeneration (Figure 6). In Cohort 1 (n=10), five worms showed regeneration. Two showed only posterior regeneration of the anterior body half, while the remaining three showed regeneration in both body segments. In Cohort 2 (n=32), eleven worms showed regeneration. Three showed only posterior regeneration of the anterior body half, 4 showed anterior regeneration of the posterior body half, and 4 showed both.

We observed varying levels of regeneration among worms. For those that regenerated the posterior portion of the body, some showed only to have growing tissue, while others had more resolved body parts such as segments and a ciliary band that appears to replace the former telotroch. Worms that were in the process of regenerating the anterior portion of the body also showed varying levels of regeneration. Some worms showed only tissue growth, while others demonstrated more resolved segments and the formation of a gut.

In analyzing regeneration with regard to group, results were confounded due to the varying number of larvae within each group. Because groups varied greatly in the amount of worm larvae, we were unable to compare them equally. A general observation was that in Cohort 2 groups B and C had greater regeneration at both segments than other groups (Figure 7). We were unable to make group comparisons in Cohort 1, because most of the worm larvae belonged to worms to only group B(n=6), and all the remaining groups had two or less larvae.

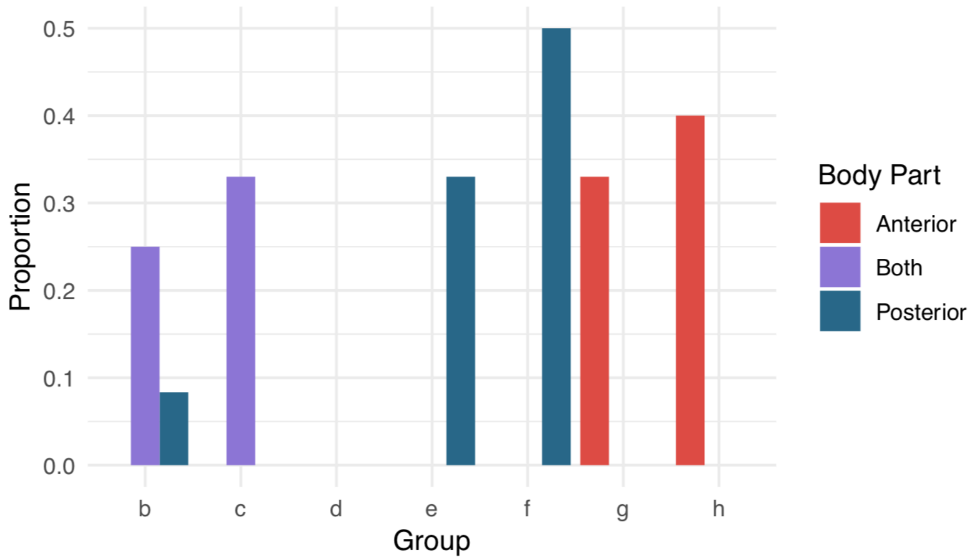

Figure 7. Proportion of worms demonstrating growth at the anterior, posterior, or both ends for groups in Cohort 2. Groups varied in the original total number of worms, B (n=11), C (n=3), D (n=5), E (n=6), F (n=4), G (n=4), H (n=6).

Discussion

Overall, worm survival (excluding those killed during manipulations) was high (>65%) for both cohorts. In general, anterior body parts survived better than posterior parts. Survival varied between groups, but it's unclear why. Identification of groups by barcoding several worms from each may permit distinguishing which species regenerate. Although little is known about the process of regeneration in larvae, some developmental studies have been performed on adult annelid and Polychaete worms (Giani et al., 2011). Posterior regeneration in adults follows a similar pathway as normal growth by first healing the wound, creating a growth zone, and then adding segments sequentially (Whitford & Williams, 2016). Anterior regeneration, however, is more complicated. First, the wound site is healed, followed by the creation and growth of a blastema, a regenerative bud where motile cells gather and proliferate, which can take up to 2 days in Spionids (Giani et al., 2011; Whitford & Williams, 2016). Growth then occurs by epimorphosis which is the de-differentiation of somatic cells to an undifferentiated state and re-differentiated to create new organs by mitotic divisions (Kozin et al., 2010; Skold et al., 2009). These same processes may occur in larvae, and may make it more difficult for new anterior body parts to regenerate from a posterior body. It is possible that anterior body parts survived better as they could perform similar wound healing, while posterior segments would require more energy to regenerate entire heads.

Regeneration was observed in experimental worms. However, due to the differences in survival and number of larvae per group, it was difficult to detect any pattern in regeneration. Because worm groups were determined only by observation, differences in regenerative capabilities could not clearly be attributed to specific species or larval stages. To make this more clear, 2 larvae from each group will be barcoded in order to more properly categorize larvae.

Most worms, regenerated body parts became visible only during the last sampling day of the experiment, so future experiments should run for a longer period of time to allow for observation of the complete body regeneration (i.e. the head on the anterior portion of the body with functional structures). However, it seems that worms can potentially regenerate within 9 days at least in certain groups. Interestingly, 3 days following the termination of the experiment we observed that some larvae had begun to develop functional structures such as a mouth and palps. This would support our suggestion for running future experiment for a longer period of time.

Regeneration among closely related species is important to study, as it can reveal evolutionary relationships among closely related species (Blake & Arnofsky, 1999) as well as further our understanding of developmental processes in marine worm larvae (Vikery et al., 2001). Additionally, these mechanisms in the larval stage could lead to higher survival in the face of sublethal predation or physical disturbance (David & Williams, 2012) as well as increase the total number of larvae available to metamorphose (Vickery & McClintock, 1998). Furthermore, it is an important process to understand in the context of asexual reproduction (David & Williams, 2012). Thus, larval worm regeneration should be studied more thoroughly with longer experimental periods, a focus on development, and known species groups.

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