Objective:
The purpose of this lab was to assess the regenerative abilities of planarians under differing environmental conditions. Planarians were cultured in both spring water and Praziquantel-containing media (PZQ) to compare the effect of PZQ on wound healing and regeneration. Incisions were also made at different sites of the body to assess the way in which planarians regenerate the missing or affected regions of their body plan. If a planarian is cut into three pieces (as shown in Figure 2 of the protocol) and cultured in spring water as a conventional regeneration assay, then I would predict each segment to reform anterior and posterior ends reflective of the original body plan. As explained in the protocol, this would be expected due to the regenerative ability of neoblasts, which are pluripotent cells that migrate to wound sites and facilitate tissue regeneration. If a planarian is similarly cut into three pieces but cultured in PZQ-containing media, then I would predict that the trunk segment would regenerate to form a two-headed specimen due to the effect of PZQ on concentration gradients within the planarian. The mechanism of PZQ is not well known, but it impacts paracrine factors (such as Wnt) which are important in establishing the anterior-posterior axis. The posterior blastema generally produces Wnts, so a double-headed regeneration would be expected if PZQ disrupts this pathway by blocking β-catenin or other such signals. If a T-cut is made on a planarian, then I would predict that the head segment might show signs of wound healing without regenerating much of the original body plan (because the cut is made so close to the head) and the trunk would regenerate two heads from the incision that split the stump into two separate halves, as shown in Figure 3C of the protocol. The regeneration of two heads on both tips of the split stump would most likely occur because the signals and concentration gradients would be identical in both, therefore resulting in separate but identical regeneration of the anterior region.

Results:
I. Observation of Normal Anatomy
The anatomy of a planarian was observed under a compound microscope at 100x magnification and the discernable anatomy was recorded in a diagram as shown in Figure 1. The shape of the specimen changed as it shortened and lengthened its body, but the general shape included an intermediate width of the anterior end (head), a slimming of the figure moving towards the posterior directly after the auricles, followed by a continual widening of the body until around the location of the pharynx, and finally a tapering off of the body at the posterior end. The observable structures, as labeled in Figure 1, were the pigment cups, auricles, anterior intestine, posterior intestine, and pharynx (which was better observed under the dissecting
II. Observation of Normal Behavior

Planarians glide as their form of movement, as opposed to swimming or crawling, because they appeared to float across the field of view without making apparent body movements that propelled them forward. They do, however, tend to occasionally lengthen and shorten their body, but this condensing and decondensing does not seem to be linked to their ability to move. The anterior end (head) often wiggled back and forth in a sniff-like fashion as they glided along, but this was not a constant occurrence and therefore did not appear to be linked to their ability to move either. Planarians do not seem to be significantly affected when flipped over because they continue to glide even when on their dorsal surface. However, they have the ability to right themselves and appeared to do so not long after being flipped (indicating that they might prefer to remain right-side up).

There was variation in the response of the planarians to stimulation based on the location
in which they were touched. When the anterior end was touched, the planarians flipped their body over by slowly twisting from their anterior to posterior through the entire length of their body. When the posterior end was touched, the planarians scrunched up their body and became more condensed for a few seconds before relaxing back to their general length. However, there was less response when stroked (as opposed to poked) as they tended to move away from the source of stroking in a calm manner. There was also little response when stroked on the side of their body because no twisting, flipping, or condensing was observed, but rather a slight movement away from the source of stimulation or no response at all.

III. Conventional Regeneration Assay

Figures 3 through 6 show the appearance of the original wounds for the head, trunk, and tail segments for planarians cultured in spring water (conventional regeneration assay). Figure 3 shows the state of incisions immediately after they were made, figure 4 shows wound healing 10 minutes after the incisions were made, figure 5 shows wound healing 48 hours later, and figure 6 shows wound healing after one week. The wounds darkened after 10 minutes, became faint after 48 hours, and were no longer visible after one week. The length of each segment increased after 48 hours and the appropriate anterior and posterior ends of the segments were visibly regenerating after one week, apart from the anterior region of the tail (because no head was visible).

Figure 3: Initial Head, Tail, and Trunk Wounds Immediately after Incisions in Conventional Regeneration Assay
IV. Regeneration Assay with Praziquantel Treatment

Figures 7 through 10 show the appearance of the original wounds for the head, trunk, and tail segments for planarians cultured in PZQ-containing media. Figure 7 shows the state of incisions immediately after they were made, figure 8 shows wound healing 10 minutes after the
incisions were made, figure 9 shows wound healing 48 hours later, and figure 10 shows wound healing after one week. The wounds darkened after 10 minutes, were still visible after 48 hours, and were no longer visible after one week. The length of each segment increased after 48 hours and the appropriate anterior and posterior ends of the segments were visibly regenerating after one week.

Figure 7: Initial Head, Tail, and Trunk Wounds Immediately after Incisions in PZQ Treatment

Figure 8: Head, Tail, and Trunk Wounds 10 Minutes after Incisions in PZQ Treatment

Figure 9: Head, Tail, and Trunk Wounds 48 Hours after Incisions in PZQ Treatment
V. Variant Regeneration Assay

Figure 11 shows the location of two incisions that were made to generate the T-Cut variant for the variant regeneration assay in which the first was made horizontally after the auricles near the anterior end, and the second was made vertically through into the anterior end of the trunk (but terminated before reaching the pharynx). Figures 12 through 15 show the appearance of the original wounds for the head and trunk (rest of the body) segments for planarians cultured in spring water and amputated with a T-cut (which was the selected incision pattern for this variant regeneration assay). Figure 11 shows the state of incisions immediately after they were made, figure 12 shows wound healing 10 minutes after the incisions were made, figure 13 shows wound healing 48 hours later, and figure 14 shows wound healing after one week. The wounds slightly darkened after 10 minutes, became fainter and less visible after 48 hours, and were still visible after one week. The length of each segment did not appear to significantly increase after 48 hours or a week and the appearance of each segment did not change much.
Figure 11: Diagram of T-Cut Incisions for Variant Regeneration Assay

Figure 12: Initial Head and Trunk Wounds Immediately after Incisions in Variant Regeneration Assay
Figure 13: Head and Trunk Wounds 10 Minutes after Incisions in Variant Regeneration Assay

Figure 14: Head and Trunk Wounds 48 Hours after Incisions in Variant Regeneration Assay

Figure 15: Head and Trunk Wounds One Week after Incisions in Variant Regeneration Assay
Discussion:

After one week, the head and trunk segments of the planarian from the conventional regenerative assay were reforming anterior and posterior ends as expected. Those two segments elongated and reformed the missing region of their body plan, but the tail segment did not regenerate an anterior region. There was in increase in length, but the lack of a newly formed head did not match the predicted outcome for this segment of the assay. This could have been due to error in the incision part of the protocol because if the incision was made too close to the posterior end, it is possible that the segment lacked sufficient signaling molecules and protein concentrations to facilitate regeneration in a normal time period. There was also hardly any movement detected from the tail segment after 48 hours, so there could have been problems with that segment of the planarian.

The PZQ segments appeared to heal faster than those of the conventional assay because in comparison, the wounds were not as dark after 10 minutes and the sites of the incision became closed off much faster. After one week, the head segment had begun to regenerate a tail (because the body tapered off to a point), the tail had begun to regenerate a head (which was almost translucent and did not have apparent auricles, but nevertheless was clearly forming), and the trunk region had begun to reform a head. This matched the expected outcome for PZQ apart from the single-headed trunk segment. It was expected that a double-headed specimen would have formed from the trunk because PZQ was expected to disrupt the anterior-posterior polarity of the fragment. The Wnt-β-catenin signaling pathway is instrumental as a mechanism for determining polarity, and this pathway would have explained the experimental observations if two heads would have formed from the trunk segment. This is because when β-catenin or Wnt1 are blocked, the posterior blastema is no longer directed to make a tail because Wnt can no longer activate β-catenin. Activation of β-catenin by Wnt results in tail formation, and thus blocking this signaling pathway should result in the formation of a head in the posterior region. As shown in Figure 10, the trunk segment only formed one head and therefore this signaling pathway does not explain the experimental observations in this case. However, the trunk segment did not necessarily form a tail because the non-head end was broadly rounded and did not taper off in a tail-like fashion.

The least successful in terms of how the results matched the expected outcome was with the variant assay because after one week, the segments did not change much in appearance. The size of the head remained the same, but this could have been expected due to the proximity of the incision to the anterior region. The incision was made much closer to the head (almost near the auricles) than the middle region between the head and the pharynx, and this could have resulted in a lack of sufficient concentration gradients to support reformation of the rest of the body. The stump of the trunk was then sliced in half with caution not to cut down to the pharynx, but the sliced stump fused back together as was seen after only 10 minutes. The results after one week were therefore not what was expected because the two fragments consisted of a head and a trunk of approximately the same size as after the incisions were made. The lack of formation of two heads in the anterior region of the trunk was most likely due to error in correctly carrying out the
protocol. The two halves of the spliced stump should have remained separated in complete isolation from touching one another for about 10 to 15 minutes after the incision was made. By making the incision and not physically preventing the halves from coming into contact with one another, they appeared to fuse back together within a matter of minutes. Error during the experimental procedure therefore influenced the results for the variant regeneration assay.

Summary:
The regenerative abilities of planarians were apparent in this experiment due to the reformation of anterior and posterior ends of cleaved segments. The quick nature of regeneration was observed by analyzing wound closure after only 10 minutes and subsequent wound healing and growth after both 48 hours and one week. The segments in PZQ-containing media appeared to heal quicker than those of the conventional assay (in spring water), but both assays produced some unexpected results. The head and trunk segments of the conventional assay produced expected results by regenerating appropriate anterior and posterior regions, but the tail did not produce expected results because it did not reform an anterior region. In the PZQ treatment, the head and tail segments produced expected results because they regenerated appropriate anterior and posterior regions, but the trunk did not produce expected results because it regenerated one head as opposed to two heads. The Wnt-β-catenin pathway therefore cannot be used to explain the experimental observations because Janus-heads did not form. The results of the variant assay were not as expected because the T-cut incision did not produce a trunk segment that regenerated two heads in the anterior region. The two segments from the variant assay rather remained similar in appearance from immediately after the incision to one week after the incision, so no new developments occurred with the segments from the variant assay. Experimental error in following the protocol most likely occurred and could have affected the results due to the precision in which the incisions must be made. Failing to keep the halves of the spliced stump isolated from each other immediately after incision most likely resulted in the fusion of the halves back together and thus the lack of two heads forming during regeneration. Also, poorly placed incisions could also have affected the growth and regeneration of the tail segment in the conventional assay and the trunk segment of the PZQ assay.