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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

EFFECT OF THE AMPUTATION SITE ON REGENERATIVE CAPACITY AND SURVIVAL OF *EISENIA FETIDA* (SAVIGNY, 1826) "CALIFORNIAN RED WORM"

EFECTO DEL SITIO DE AMPUTACIÓN EN LA CAPACIDAD REGENERATIVA Y SUPERVIVENCIA DE *EISENIA FETIDA* (SAVIGNY, 1826) "LOMBRIZ DE TIERRA CALIFORNIANA"

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ABSTRACT

This study aimed to associate the regeneration capacity of *Eisenia fetida* (Savigny, 1826) with the amputation site. Amputations were performed on different body segments and the survival rates and regeneration lengths of the anterior and posterior sections were examined. 60 individuals were collected, from which 16 juvenile individuals were selected, which were amputated at anterior segments 32 and 90. The development of the anterior and posterior regions was evaluated over 28 days, monitoring the regeneration process of the segments and weighing the individuals. Regeneration was completed in 21 days and was only successful in the anterior region of individuals cut at segment A90; in the other segments, only wound healing was observed. Survival was 100% and 62.5% only for the anterior regions of groups A90 and A32, respectively, suggesting that the number of amputated posterior segments is a key factor for regeneration in *E. fetida* individuals.

Keywords: *Eisenia fetida* – regeneration – segments A90 and A32 – survival

RESUMEN

El objetivo de este estudio fue asociar la capacidad de regeneración de *Eisenia fetida* (Savigny, 1826) con el sitio de amputación. Se realizaron amputaciones en diferentes segmentos del cuerpo y se examinaron las tasas de supervivencia y longitudes de regeneración de las secciones anterior y posterior. Se recolectaron 60 individuos, de los cuales se seleccionaron

16 individuos juveniles, que fueron amputados en los segmentos anteriores 32 y 90. Se evaluó el desarrollo de las regiones anterior y posterior durante un periodo de 28 días, monitoreando el proceso de regeneración de los segmentos y pesaje de los individuos. La regeneración se completó en 21 días y solo fue exitosa en la región anterior de los individuos cortados en el segmento A90; en los otros segmentos solo se observó la cicatrización de la herida. La supervivencia fue del 100% y 62,5% solo para las regiones anteriores de los grupos A90 y A32, respectivamente, esto sugiere que la cantidad de segmentos posteriores amputados es un factor clave para la regeneración en los individuos de *E. fetida*.

Palabras clave: *Eisenia fetida* – regeneración – segmentos A90 y A32 – supervivencia

INTRODUCTION

Eisenia fetida (Savigny, 1826), known as "Californian red worm" or "earthworm", is a triploblastic invertebrate of the phylum Annelida (LaDouceur, 2021). This species is considered important due to its ecological role in soil formation and decomposition of organic matter (Al-Maliki *et al.,* 2021). Additionally, *E. fetida* has the capacity to regenerate damaged segments (Yuan *et al.*, 2024), and it is easily cultured and handled withal; these characteristics make these species an ideal model for studying regeneration (Singh *et al.*, 2020).

Annelids are characterized for regenerating by epimorphosis, causing cell de-differentiation, proliferation and formation of a blastema; also, it is often accompanied with morphallaxis to renew the pre-existing tissues (Kostyuchenko & Kozin, 2021). Studies have shown that the regenerative capacity of lumbricid earthworms can be influenced by temperature (Jesna & Livingstone, 2023) or number of body segments remaining along the body region end (Subarna *et al.*, 2020). Since earthworms are frequently injured by predators and cultivators, understanding how the regeneration capacities and the survival of the injured individuals might be affected is crucial.

The main aims of the present study were to: (i) determine the effect of the number of segments amputated in the regeneration capacity; (ii) to determine the differences in regenerative capacity between the anterior and posterior regions; (iii) to analyze the survival rates of earthworms by the region and number of segments amputated. The study was carried out using the weight of the individuals as a regeneration indicator.

MATERIALS AND METHODS

Sample collection and selection

A total of 60 individuals were collected from a composting establishment and housed in a polystyrene box (20 x 20 x 50 cm) with 10 holes along its longest sides (Siddique *et al.*, 2005). Their diet consisted of sweet fruits like bananas and apples, while they were exposed to a 12-hour photoperiod.

16 individuals were selected based on the following criterion: be in the juvenile stage, characterized by the absence of clitellum, and fall within the weight range of 0.23 -0.43 g. Subsequently, they were distributed into two groups based on the site of amputation: Group 1, consisting of 8 individuals that were cut at the 32nd segment (counting since the first segment from the anterior region) and Group 2, comprised of 8 individuals that were cut at the 90th segment (Table 1). In fact, both 32nd and 90th segments represent approximately 1/4 and 3/4 of the total body segments respectively, and were chosen to analyze the differences in regenerative capacity between the number of segments amputated by anterior and posterior regions (Figure 1). The control group had no amputations with the aim of discarding the influence of external factors such as the soil, food and temperature, on the regeneration process and survival.

After selection, the earthworms were transferred to cardboard containers filled with 75 g of soil and 22.5 ml of sterilized distilled water. Moreover, small ventilation holes were punctured into each container to, finally, be sealed with cardboard lids.

Table 1. Amputation treatment and number of segments amputated according to the body region of *Eisenia fetida*.

Body region	Treatment	Number of body segments amputated	Number of body segments observed
Anterior	A32a	76	32
	A90a	18	90
Posterior	A32p	32	76
	A90p	90	18

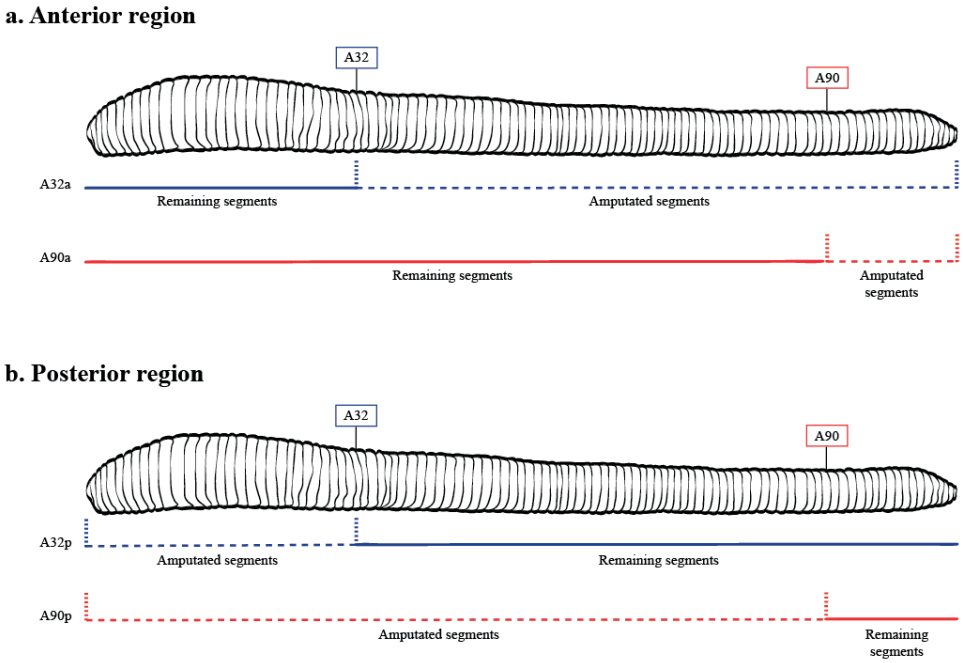


Figure 1. Amputation site on *Eisenia fetida* according to the treatment.

Segment amputation procedure

The individuals were placed in Petri dishes (100 mm x 15 mm), where distilled water was sprayed on them in order to remove any soil residues. Afterwards, they were anesthetized by immersion in carbonated water for 1 minute (Bodó *et al*, 2021). Using a stereoscope at 10X, segments were counted starting from the anterior part of the body. The amputation was performed using a sterile scalpel according to the treatment they were going to receive and, immediately thereafter, two drops of NaCl 0,9 % saline solution were applied to the amputation. The specimens were placed into the cardboard containers.

Regarding monitoring, using a digital scale, the weight was recorded, both anteriorly and posteriorly, before and after the amputation over a period of four weeks. In addition, both amputated regions were observed under a stereoscope. The regeneration process was evaluated across three stages: healing of the amputation, with daily observations until its complete completion; blastema formation, with observations every two days; and segmentation appearance, observed weekly.

Statistical analysis

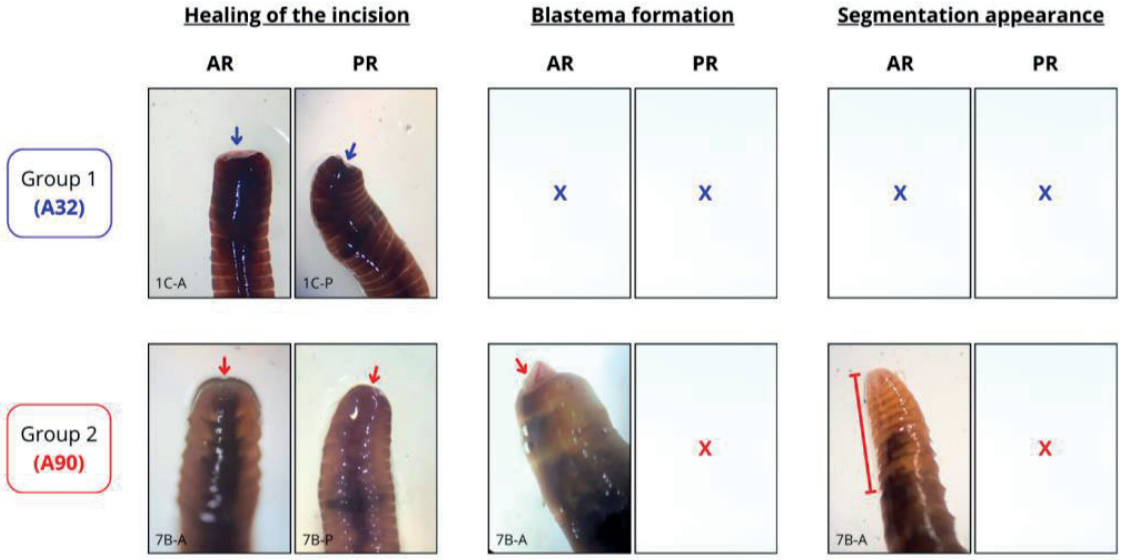
The data were analyzed using the programming language Python (3.12.2 version, Python Software Foundation) and the data library Matplotlib (3.8.3 version, The Matplotlib development team). In order to compare the regeneration of the anterior and posterior regions by the number of segments amputated, a linear regression graphic was conducted. Furthermore, the survival rates of earthworms by the region and number of segments

amputated were analyzed through a Kaplan-Meier survival curve.

Ethic aspects: *Eisenia fetida* individuals were acquired in establishments dedicated to vermiculture. It is worth highlighting that the lack of information regarding vermiculture and the applications that it can generate, opens spaces for new experiences regarding the management of worms; and therefore contributes to the reduction of environmental contamination with waste generated in human settlements.

RESULTS

Regeneration stages



Anterior region (AR). Posterior region (PR). "X" signifies that none of the individuals progressed through the stage.

Figure 2. Regeneration process of *Eisenia fetida* by treatments and body regions.

The regeneration process comprised 3 stages: healing of the amputation, characterized by the apparition of a translucent layer above the amputation site; blastema formation, marked by the growth of a bud lacking pigmentation; and segmentation, where the bud acquired coloration and division to form the segments removed. Nonetheless, the only group that went through all those stages was the A90 anterior region; although, the A32 and A90 posterior region could complete the healing of the wound (Figure 2).

Regeneration capacity by treatments and regions

Table 2. Weight of anterior and posterior regions of *Eisenia fetida* during the 28-day period.

Treatment	Region of the body	Days after the amputation (daa)				
		0	7	14	21	28
A32	Anterior	0,10	0,12	0,12	0,12	0,12
	Posterior	0,19	0,19	0,19	0,19	0,19
A90	Anterior	0,22	0,49	0,62	0,60	0,60
	Posterior	0,09	0,09	0,09	0,09	0,09

Weight measurement of the anterior and posterior regions of *E. fetida* under two different treatments (A32 and A90) is presented; the data is recorded at five time points: Day 0 (immediately after amputation), Day 7, Day 14, Day 21, and Day 28 (Table 2).

For A32 treatment, the anterior and posterior regions show minimal weight change, remaining constant at 0.12 g and 0.19 g, respectively; indicating no regeneration of the amputated segments. In contrast, for A90 treatment, the anterior region exhibits considerable growth with weight increasing from 0.22 g (Day 0) to 0.60 g (Day 28), suggesting regenerative response; however, the posterior region remains unchanged at a constant weight of 0.09 g (Table 2).

Survival rates

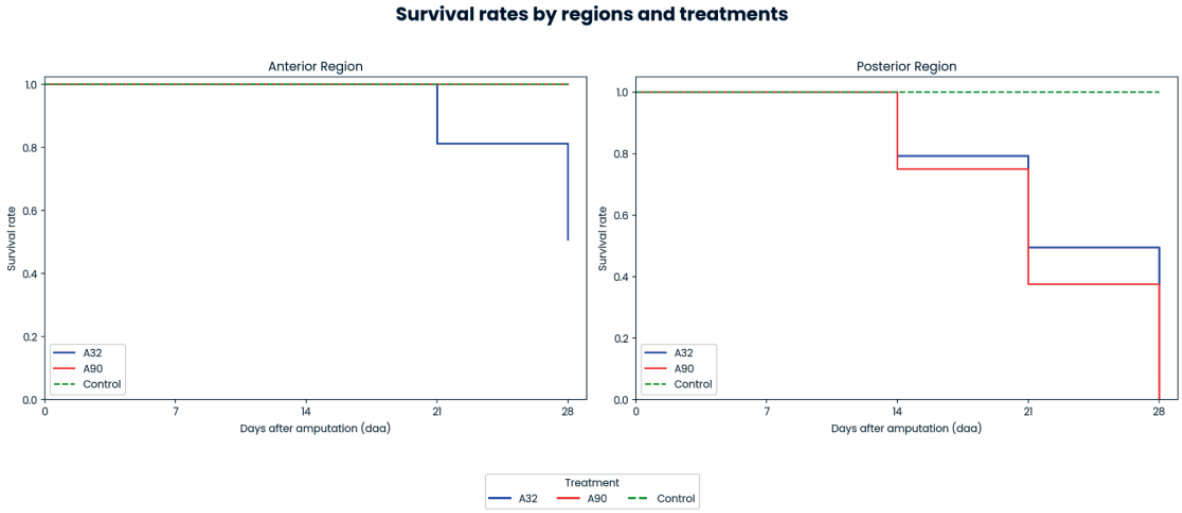


Figure 3. Survival rate of the anterior and posterior regions by the number of segments amputated of *Eisenia fetida,* based on the experimentation period.

The anterior region of the individuals cut in the segment A90 presented a survival rate of 100% during the 28 day-trial. As for the anterior region of those belonging to group A32, in the third week, the death of three individuals was recorded; however, the number of deaths did not increase and this group completed the trial with a survival rate of 62.5% (Figure 3).

The A90 posterior region group experienced an abrupt decrease in the survival rate: by the second week, more than half of the individuals (75%) had died and by the third week none of them were alive. The posterior region of group A32 also reached a survival rate of 0%, but by the fourth week. In summary, the anterior regions had better survival, regardless of the cut site. On the other hand, the posterior regions did reach 100% mortality, showing that the more anterior segments left, the higher the survival rate (Figure 3).

DISCUSSION

Regeneration stages

Our study reports that the amputation healed, the blastema and the segmentation appeared 7 and 21 days after the amputation (daa), respectively; albeit, it was only seen in the A90 anterior region group. Previous experiments conducted on *Eisenia* have reported that the blastema appears 3 daa while the regeneration had completed within 30 - 35 daa (Shao *et al.,* 2020; Singh *et al.,* 2022). This result likewise aligns with findings by Patel *et al.* (2020), who indicated a robust regeneration of posterior segments.

Regeneration capacity by treatments and regions

This study evinces disparities in regeneration capacities by anterior and posterior regions. In the anterior region, our study showed that specimens with ¼ of the total body segments amputated (A90) were able to regenerate completely within 4 weeks, evidenced by the observation of the 3 stages and the increase of weight (Table 2);

although, individuals with ¾ of the total body segments amputated (A32) did not achieve complete regeneration. As in ours, the study of Xiao *et al.* (2011) demonstrated that the posterior regeneration -or, the capacity to regenerate the tail segments- was greater than the anterior, this occurred in both groups, individuals with ¼ and ¾ of the segments amputated.

Nevertheless, in the posterior region, neither the A32 nor A90 groups completed their regeneration, showing any difference despite having 32 and 90 segments amputated respectively. Contrary to our results, Subarna *et al.* (2020) reported that *E. fetida* regenerates anterior segments -anterior regeneration- at a faster rate and greater numbers. However, Paul *et al*. (2022) observed that in another clitellate, *Eudrilus eugeniae* (Kinberg, 1866), the earthworm segments could regenerate successfully independently of its region only if they were attached to the clitellum; hence, this could be a new theme to explore in future research.

Our results may suggest that the regeneration capacity in *E. fetida* does not depend only on the number of segments amputated, instead in the region. It means, the number of posterior segments amputated is a key factor in the regeneration of *E. fetida*.

Survival rates

All the anterior regions of the individuals cut in the anterior segment ninety (A90) survived during the 28 days of evaluation. These results are similar to those obtained by Xiao *et al*. (2011), who also selected juvenile individuals and after the same period of time, reported a survival rate of 95%. Furthermore, they found that the remaining segments died 15 days after cutting, which coincides with our results. Regarding the cut in the 32nd anterior segment (A32), they reported that 28 days after the cut, the anterior region had a survival of 62.5%, in our case 65% were still alive. Finally, they obtained that the posterior region, after 28 days, had a survival of 82%, in contrast, in our trial, the survival percentage was 0%.

Based on what was obtained, it can be stated that there is a direct relationship between the amputation site and survival. In this case, *E. fetida* had a higher survival rate when the number of posterior segments amputated was lower.

Author contributions: CRediT (Contributor Roles Taxonomy)

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Validation: PCR, MPZ, NPS, HGF
Visualization: PCR, MPZ, NPS, HGF
Writing – original draft: PCR, MPZ, NPS, HGF
Writing – review & editing: PCR, MPZ, NPS, HGF

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