Assessing functional changes in the limbs of the blue-spotted salamander (*Ambystoma laterale*) during limb regeneration.

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INTRODUCTION:

The capacity to quickly adapt to a variety of environmental and physiological changes is intrinsically linked with survival and reproductive success. Although this plasticity is demonstrated by all metazoans, those that can regenerate lost or injured body parts are particularly remarkable. Notably, salamanders that loose their limbs can quickly adjust their locomotion and behaviour to accommodate their new morphology[1]. From a control perspective, this ability is unmatched in the world of robotics and is of interest to engineers building machines robust to damage or mechanical failure[2].

Although previous literature has explored many paths concerning limb regeneration[3], the changes in kinematics and force production encountered during this period have yet to be evaluated in great detail. This study aims to broaden the knowledge on salamander locomotory plasticity by evaluating changes in limb kinematics and force production during different phases of limb regeneration.

- **Research question**: How will the forelimb function change when the hindlimbs are lost?
- **H1**: Salamanders will swim on land (fold limbs backwards).
- **H2:** Forelimbs will produce significantly more thrust.

REGENERATION STAGES

The hind limbs of three Blue Spotted Salamanders *(Ambystoma laterale)* were surgically removed and allowed to naturally regenerate. Regeneration was divided into 4 stages, pre-amputation, early, middle and late stage.

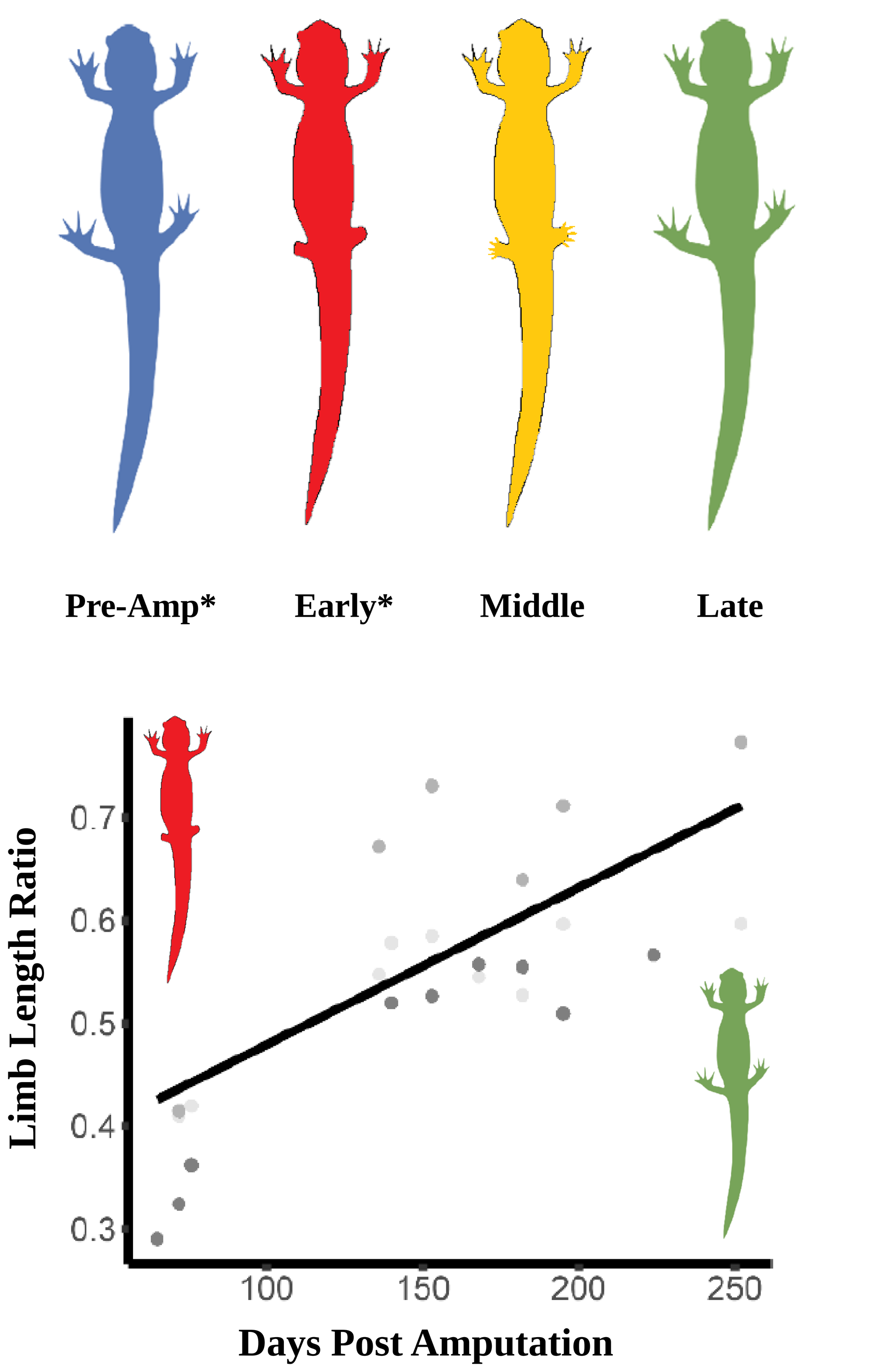

Figure 1 - Diagram of limb regeneration process. Limb regeneration was separated into 3 distinct stages; early, middle and late. During the early stage, limb buds are the only visible structure. During the middle phase, short toes are visible. During the late phase, the limb length and morphology closely mimics (although not perfectly) those found in the pre amputated limbs. (Figure extracted from Donatelli et al. submitted, 2020, Frontiers.)[2]

METHODOLOGY

Following a month-long recovery period, terrestrial stepping trials were held on a weekly or bi-weekly basis. Salamanders were filmed walking and trotting across a six array force plate. A PVC shelter was placed at the end of the track to entice the animal forward. One lateral camera and one top down camera with a 45 degree mirror captured three orthogonal views of the filming arena.

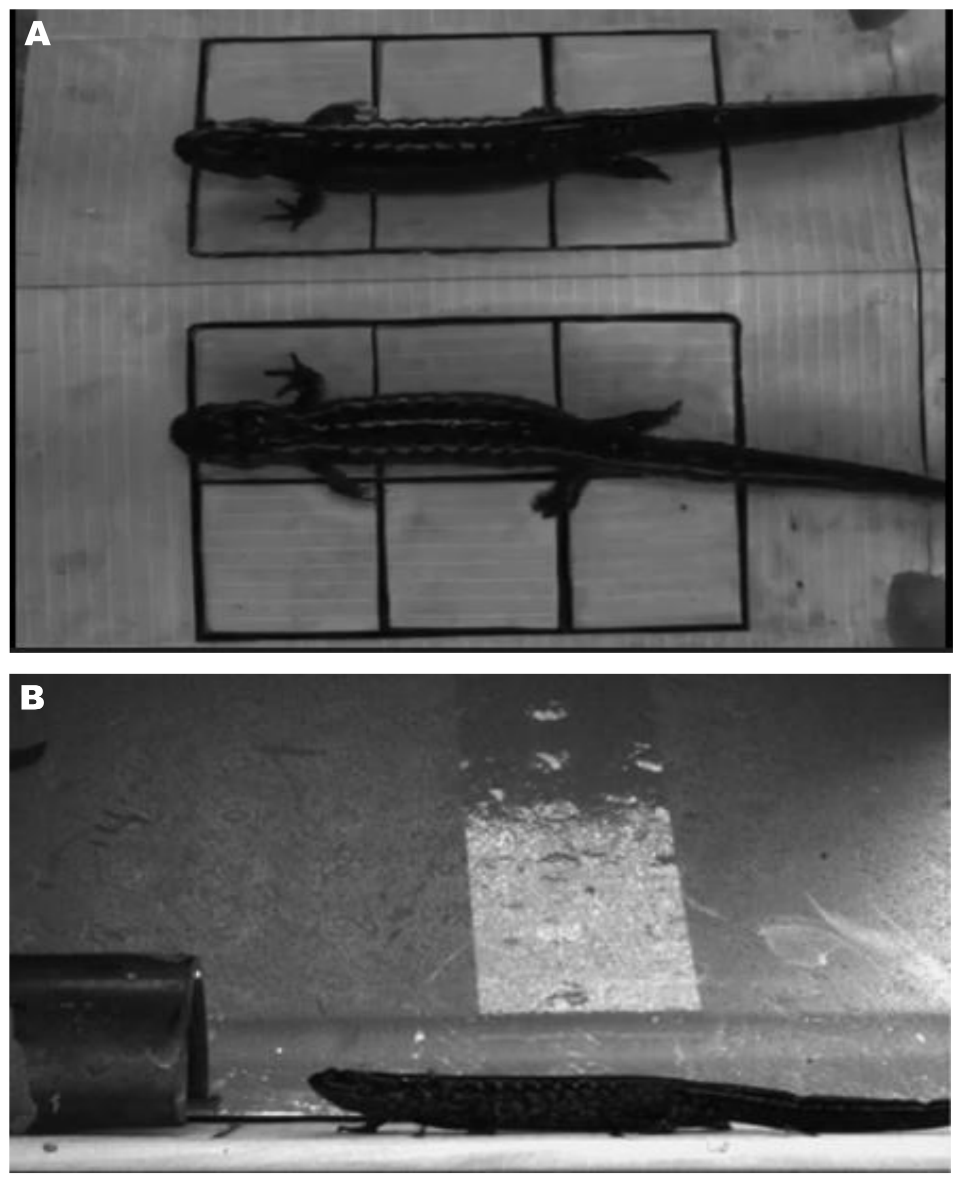

Figure 2 - Experimental setup consisting of two highspeed cameras (Fastec IL5, 250fps), a walking platform, a six-array force plate, a hide and a mirror. The cameras were used in conjunction with a mirror (placed at 45°) to create 3 separate viewing angles; dorsal (A), mirror (A) and lateral (B).

METHODOLOGY – KINEMATIC VARIABLES

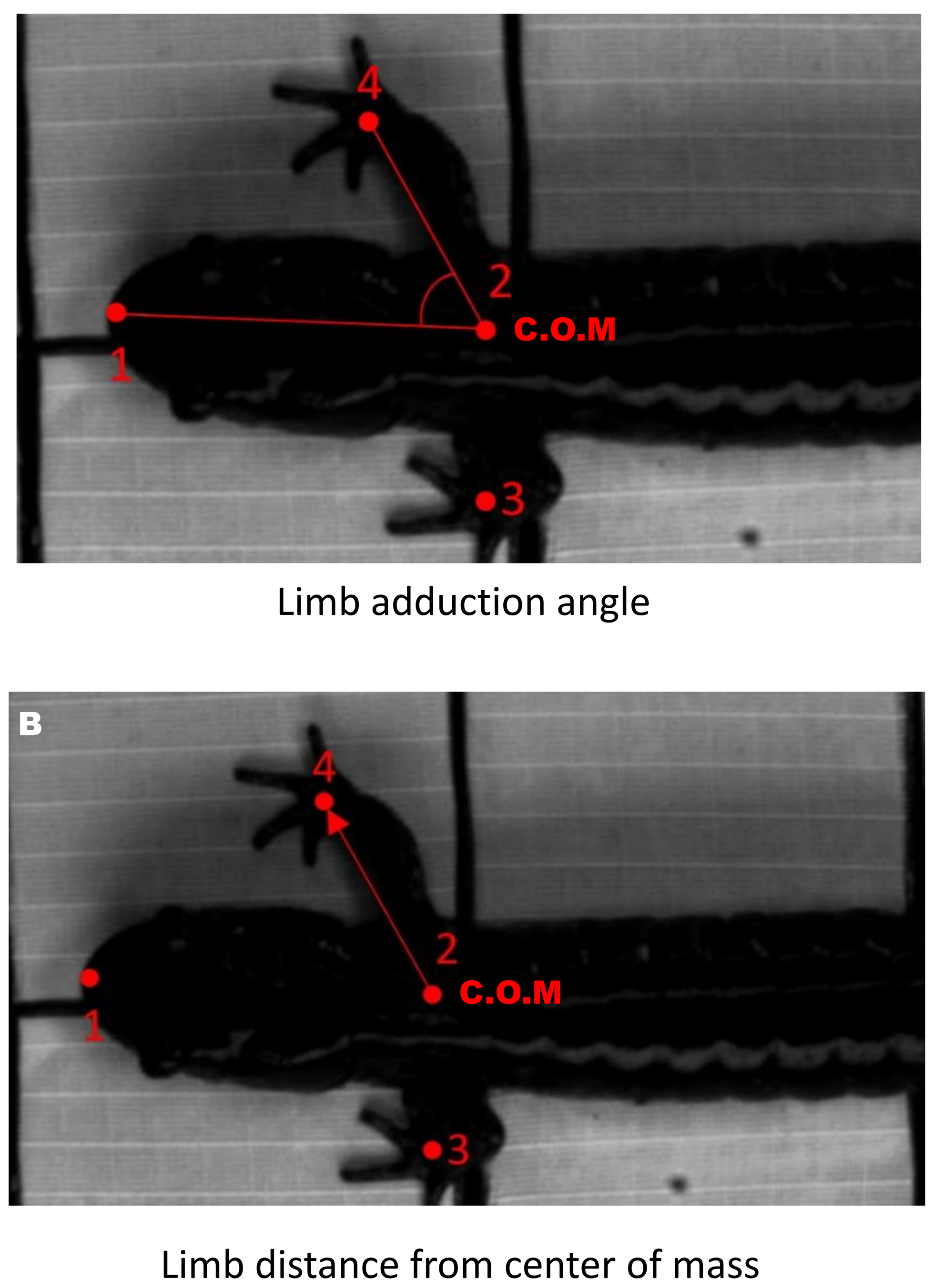

Figure 3 – Examples of angles and distance. (A) shows how minimum and maximum and limb adduction angle was calculated (B) shows how maximum and minimum distance (of the limb) from the center of mass was calculated. C.O.M is the center of mass. Point (1) is the tip of the snout, (2) is the middle of the pectoral girdle, (3) and (4) are located in the middle of the left and right manus (respectively).

RESULTS

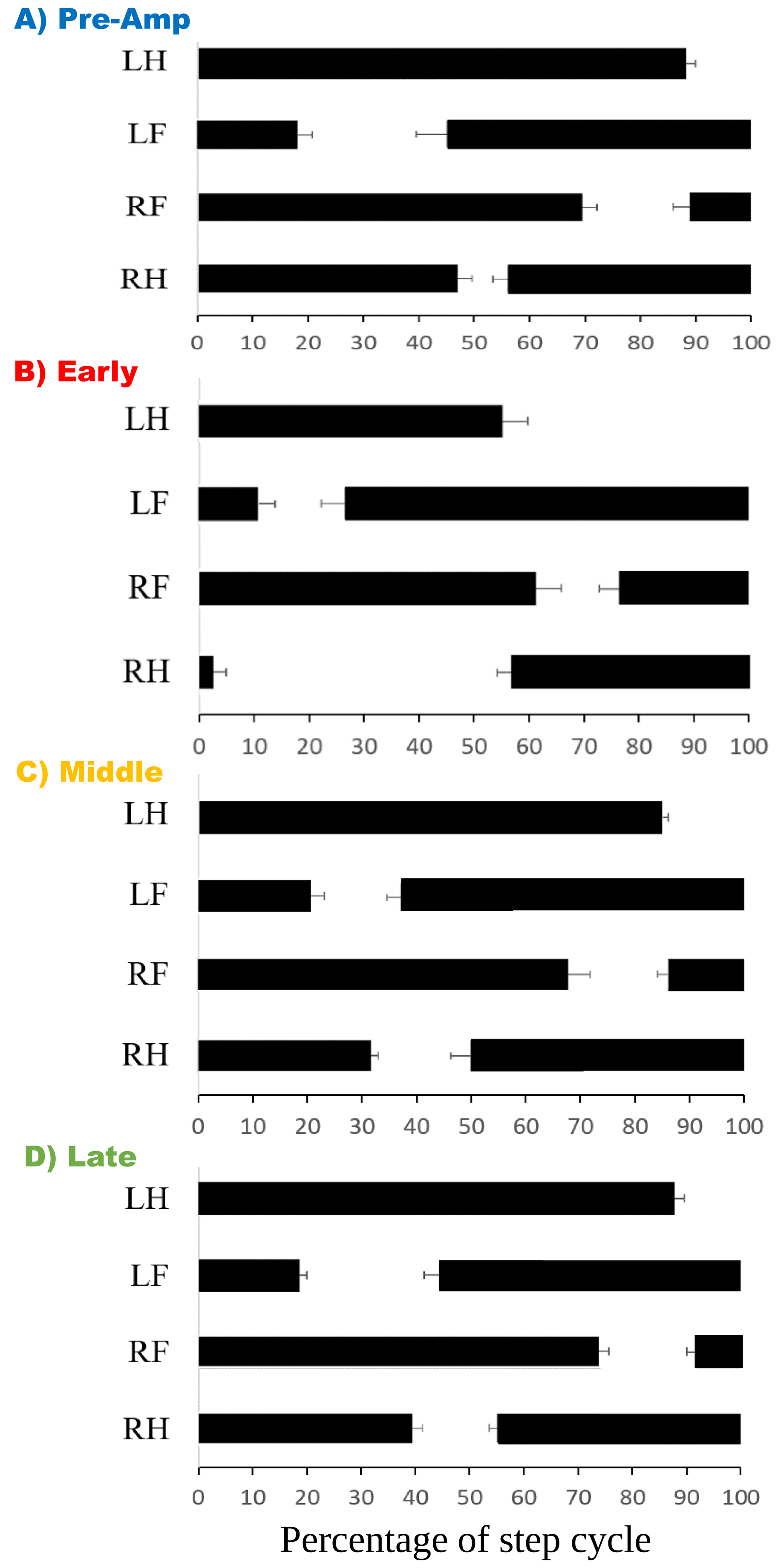

Figure 4 – Hildebrand-style gaits diagrams of *Ambystoma laterale* during various stages of regeneration. (A) Pre-amputation, (B) early stage, (C) middle stage (D) late stage. LH, left hindfoot; LF, left forefoot; RF, right forefoot; RH, right hindfoot. Thick black bars represent time where the limb is in the stance phase, with the ends of these bars representing mean footfall or liftoff times for each step. Small bars indicate standard error of the mean. (N= 4 step cycles (SC) for middle stage, N=5 SC for all other stages)

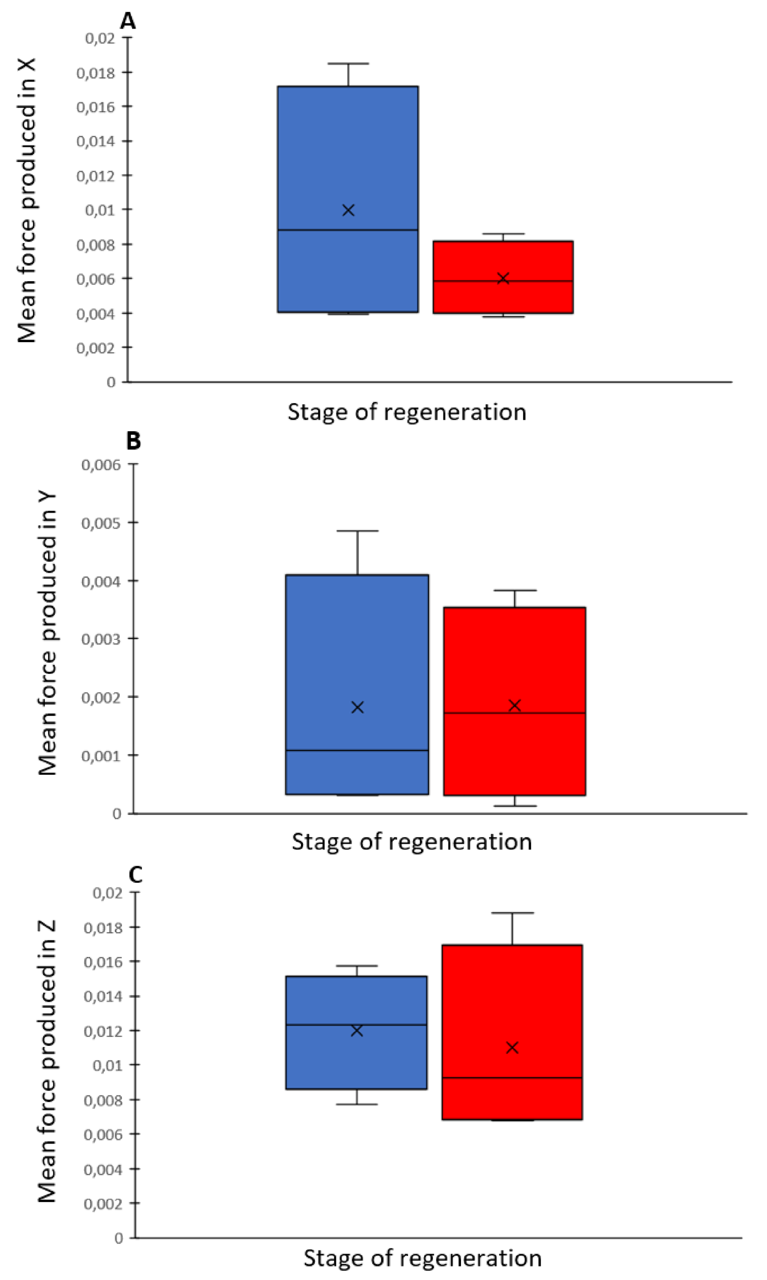

Figure 5 – Ground reaction forces produced by forelimbs of *Ambystoma laterale* before amputation (blue) and early regeneration (red). (A) mean forces in the anteroposterior axis, (B) mean forces in the mediolateral axis, (C) mean forces in the vertical axis. Force values for each ideal step were used to plot the boxplot. Boxplots show median, mean (X), interquartile ranges and maximum and minimum values. Stage of regeneration did not have a significant effect on mean force production.

RESULTS

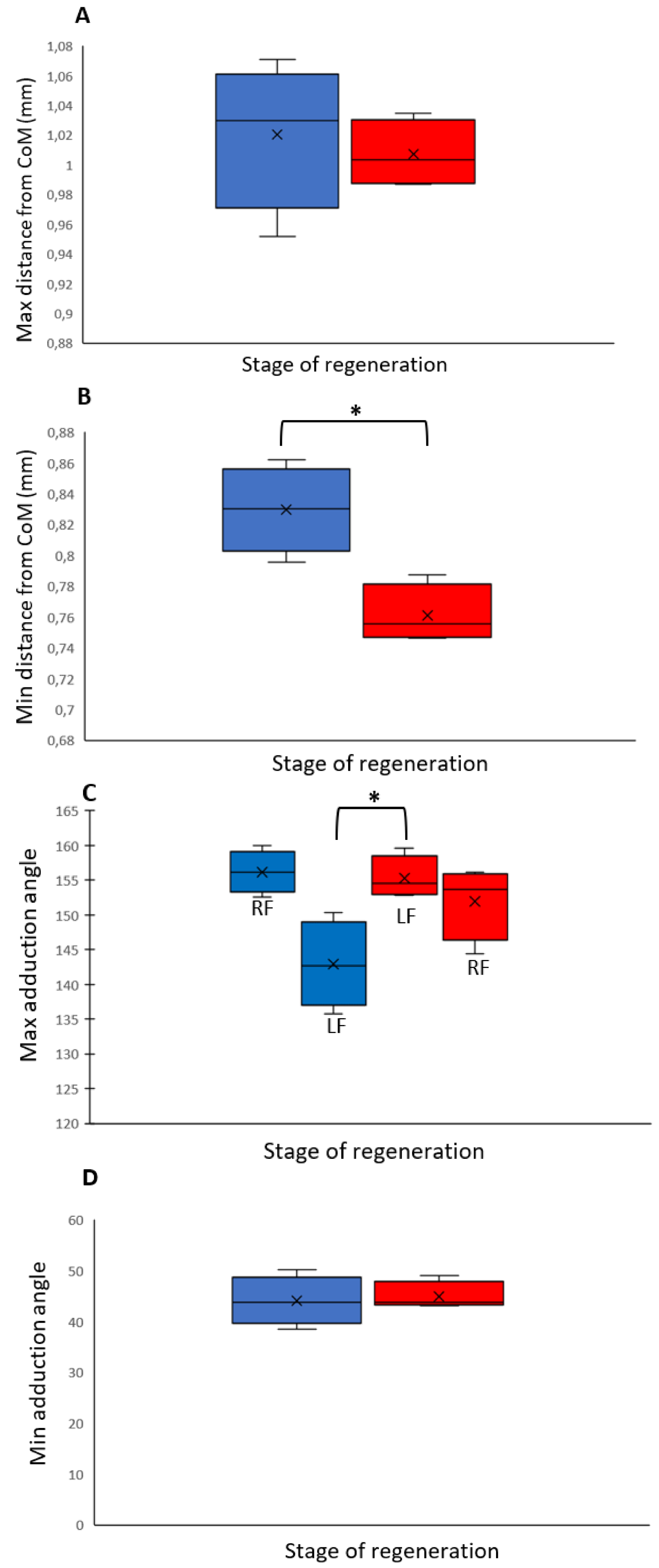

Figure 6 – Forelimb kinematic variables of *Ambystoma laterale* before amputation (blue) and during early regeneration (red). (A) Maximum distance from the center of mass (COM), (B) minimum distance from the COM, (C) maximum forelimb adduction angle, (D) minimum forelimb adduction angle. Mean values of a given trial were used to plot each boxplot with the exception of (C) in which mean values for LF and RF were separated. For (C) AIC values indicated that including "limb" as an additional fixed effect improved the model's fit. Boxplots show median, mean (X), interquartile ranges and maximum and minimum values. Forelimb Distance values were corrected for differences in forelimb length between animals. Stage of regeneration had a significant effect on min distance from COM and maximum adduction angle (only LF).

CONCLUSION & NEXT STEPS

This study demonstrates that coordination and timing of the limbs fluctuates during subsequent stages of hindlimb regeneration, with the greatest differences seen in the hindlimb duty factors and timing of salamanders in the early stage of regeneration. Furthermore, although forelimbs in the early stage of regeneration were planted closer to the body and increased their stance phases, significant changes in force production (when compared to pre-amputated specimens) were not observed.

Next steps include:

- Repeating this data to confirm results
- Evaluating the kinematics and force production of the Hindlimbs.

REFERENCES:

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