Figure S1. Anatomical landmarks on the limb and body that were used to quantify limb kinematics in walking *Ambystoma tigrinum*. The illustrated salamander was traced from a representative high-speed video frame at a time when both limbs were in stance, but not necessarily at mid-stance. Limb bones are sketched as sticks to illustrate how the digitized landmarks contributed to calculations of kinematic angles from Figure 2. Six anatomical landmarks (open circles) were tracked on lateral (shown) and dorsal views for each limb. These included: 1) the tip of the longest digit of the manus/pes, 2) metacarpophalangeal/metatarsophalangeal joint, 3) wrist/ankle, 4) elbow/knee, 5) shoulder/hip, and 6) two points along the midline of body that were dorsal to the pectoral/pelvic girdles. These landmarks were then used to calculate adduction/abduction angle (solid red curve), protraction/retraction (solid blue curve), elbow/knee angle (dashed purple curve), and wrist/ankle angle (dotted orange curve).
Figure S2. The GRF can impose moments about the joints that can differ between the forelimbs and hind limbs. The illustrated salamander was produced by combining traced still images of the body at the timings of mid-stance for both the forelimb and hind limb during a representative high-speed video trial. Although such a pose is not regularly observed during normal walking gaits in salamanders, it is illustrated here to depict the calculation of moment arms due to the GRF (see Fig. 4) at a similar time point during stance for both limbs, facilitating direct comparisons. Note that mid-stance is not the timing of the peak net GRF (Fig. 3), but is a standard depicted pose for many free body diagrams of terrestrial locomotion and is depicted here to be consistent with other studies. At mid-stance, the GRF is oriented nearly vertically for both limbs (Fig. 3), and is represented by the solid dark gray arrow. The moment arms of muscles that counter the GRF moment about the wrist/ankle ($r_{\text{wrist/ankle}}$) are depicted in the dashed red line, $r_{\text{elbow/knee}}$ are depicted in the solid blue lines, $r_{\text{protract/retract}}$ are depicted in the solid pink lines, and $r_{\text{adduct/abduct}}$ are depicted in the solid light blue lines. The curved orange arrow depicts the contribution of torsion to the bone from the GRF. Moment arms are drawn to be illustrative and are not drawn to uniform scale.
Figure S3. Vickers Hardness was greater for the humerus than the femur across all individuals of *A. tigrinum*. Although the humerus for Individual 01 was tested under slightly different loads (0.981 N vs. 0.49 N), visual inspection of Vickers Hardness illustrated that this difference in experimental design does not alter the broad patterns observed across the individuals. In addition, hardness generally remains consistent with loads within the ranged tested in this study (Zysset, 2009).
Table S1. Contrasts between hardness values within the humerus and femur of A. tigrinum

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>s.e.m.</th>
<th>z-value</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur A – Femur D</td>
<td>2.300</td>
<td>1.922</td>
<td>1.196</td>
<td>-3.397</td>
<td>7.997</td>
</tr>
<tr>
<td>Femur A – Femur P</td>
<td>0.948</td>
<td>1.742</td>
<td>0.544</td>
<td>-4.216</td>
<td>6.112</td>
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<tr>
<td>Femur A – Femur V</td>
<td>-2.513</td>
<td>1.833</td>
<td>-1.371</td>
<td>-7.945</td>
<td>2.920</td>
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<tr>
<td>Femur D – Femur P</td>
<td>-1.352</td>
<td>1.702</td>
<td>-0.794</td>
<td>-6.396</td>
<td>3.692</td>
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<tr>
<td>Femur D – Femur V</td>
<td>-4.813</td>
<td>1.554</td>
<td>-3.096</td>
<td>-9.419</td>
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<tr>
<td>Femur P – Femur V</td>
<td>-3.461</td>
<td>1.261</td>
<td>-2.744</td>
<td>-7.198</td>
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<tr>
<td>Humerus A – Humerus D</td>
<td>3.262</td>
<td>2.534</td>
<td>1.287</td>
<td>-4.247</td>
<td>10.771</td>
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<tr>
<td>Humerus A – Humerus P</td>
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<td>2.439</td>
<td>2.075</td>
<td>-2.168</td>
<td>12.289</td>
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<tr>
<td>Humerus A – Humerus V</td>
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<tr>
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<td>1.021</td>
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<td>7.018</td>
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<tr>
<td>Humerus D – Humerus V</td>
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<td>1.585</td>
<td>-3.525</td>
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<td>-0.890</td>
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<tr>
<td>Femur A – Humerus A</td>
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<td>1.136</td>
<td>1.913</td>
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<td>5.540</td>
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<tr>
<td>Femur A – Humerus D</td>
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<tr>
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<td>1.965</td>
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<tr>
<td>Femur D – Humerus V</td>
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<td>3.312</td>
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<td>1.595</td>
</tr>
<tr>
<td>Femur P – Humerus V</td>
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<td>-6.196</td>
<td>3.996</td>
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<tr>
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<td>-10.168</td>
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<td>Femur V – Humerus V</td>
<td>2.361</td>
<td>1.625</td>
<td>1.453</td>
<td>-2.455</td>
<td>7.176</td>
</tr>
</tbody>
</table>

Contrasts indicate how different the first region is to the second (e.g., Femur A is ~2.3 greater than Femur D). Based on the model: lmer(Hardness ~ Bone / Location + (1 + Location | Individual), REML = True)

Key: A = anterior, D = dorsal, P = posterior, V = ventral. Sample sizes reported in Table 7.