Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors

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Amphibians

Amphi- means ‘dual’ or ‘on both sides’; -bian is from bios, meaning life.

Amphibian thus refers to the dual life cycle of most amphibians, called a complex life cycle.
Why should we care about amphibians?

- Integral parts of many ecosystems
  - Cascading effects?
- Warning signals of environmental health
  - Complex life cycles = double jeopardy
  - Permeable membranes
- Moral/ethical arguments
Industrial Pollution
Agricultural Pollution
Amphibian Deformities

Pollutants, UV-B, or Parasites?
How can we assess threatened amphibian populations before declines or deformities take place?

**Developmental Stability**
Developmental Stability (DS)

- DS is one component of an organism’s ability to withstand environmental and genetic disturbances during development.
- Previously used as a stress indicator in numerous species.
- Few studies have compared DS in amphibians, particularly in regard to stress.
Asymmetry in Bilateral Organisms
Population Asymmetry (PA) can be used to evaluate DS

- PA is population-level differences between the left and right sides of paired bilateral characters.
- Populations that are more developmentally stable have lower population asymmetry.
- Environmental stressors lead to decreased DS and thus greater PA.
Research Questions

- Is amphibian PA correlated with anthropogenic (contaminant levels, land use, water chemistry) or natural (density) stressors?
- Is PA applicable across amphibian species and life history stages?
<table>
<thead>
<tr>
<th>Species</th>
<th>Larval habitat</th>
<th>Adult habitat</th>
<th>Breeding habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullfrog</td>
<td>Aquatic</td>
<td>Semi-terrestrial</td>
<td>Aquatic</td>
</tr>
<tr>
<td>Leopard frog</td>
<td>Aquatic</td>
<td>Semi-terrestrial</td>
<td>Aquatic</td>
</tr>
<tr>
<td>Eastern newt</td>
<td>Aquatic</td>
<td>Aquatic</td>
<td>Aquatic</td>
</tr>
<tr>
<td>Spotted Salamander</td>
<td>Aquatic</td>
<td>Terrestrial</td>
<td>Aquatic</td>
</tr>
<tr>
<td>Slimy Salamander</td>
<td>Terrestrial</td>
<td>Terrestrial</td>
<td>Terrestrial</td>
</tr>
</tbody>
</table>
Study Organisms

*Rana catesbeiana* (bullfrog) tadpoles.
*Notophthalmus viridescens*

(Eastern newt) males.
**Ambystoma maculatum**

*(spotted salamander)* males.
Tadpole asymmetry was greater in agricultural than forested sites.

\[ t = 3.50, \quad p = 0.01 \]

\[ \alpha = 0.00625 \]

\[ \text{log transformed} \]
Tadpole asymmetry increased with anthropogenic disturbance.

EN (F_{2,6} = 5.6, p = 0.04)

EHL (F_{2,6} = 10.0, p = 0.01)
Male newt asymmetry increased with pH.

\[ Y = -1.501 + 0.271 \times X; \quad R^2 = 0.493 \]

\[ R^2 = 0.49 \]
HCB

Polychlorinated Biphenyl (PCB)

DDT

Chlordane
<table>
<thead>
<tr>
<th>Sediment</th>
<th>Number of Analytes</th>
<th>Accuracy Assessment: Z-Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides</td>
<td>11</td>
<td>&lt;2</td>
</tr>
<tr>
<td>PCB Congeners</td>
<td>0</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Sediment</td>
<td>20</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Fish</td>
<td>1</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Pesticides</td>
<td>19</td>
<td>&lt;2</td>
</tr>
<tr>
<td>PCB Congeners</td>
<td>1</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Fish</td>
<td>19</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>&gt;3</td>
</tr>
</tbody>
</table>

Z-scores: <2 = satisfactory; >3 = unsatisfactory. (NIST's Accuracy Assessments are: Satisfactory, Questionable and Unsatisfactory).
Amphibian contamination varied by population and species.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Species</th>
<th>Life Stage</th>
<th>Total PCBs</th>
<th>Total DDTs</th>
<th>HCB</th>
<th>Chlordane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shuman #2 (Def)</td>
<td>Bullfrog</td>
<td>Larva</td>
<td>22.43</td>
<td>3.36</td>
<td>0.11</td>
<td>2.20</td>
</tr>
<tr>
<td>Bishop #1</td>
<td>Bullfrog</td>
<td>Larva</td>
<td>10.36</td>
<td>1.98</td>
<td>0.75</td>
<td>2.21</td>
</tr>
<tr>
<td>M. Morgan #3</td>
<td>Bullfrog</td>
<td>Larva</td>
<td>8.70</td>
<td>5.05</td>
<td>0.77</td>
<td>2.28</td>
</tr>
<tr>
<td>Bishop #4</td>
<td>Bullfrog</td>
<td>Larva</td>
<td>9.99</td>
<td>4.02</td>
<td>1.40</td>
<td>2.53</td>
</tr>
<tr>
<td>Tower LBL (4)</td>
<td>Bullfrog</td>
<td>Larva</td>
<td>11.30</td>
<td>6.43</td>
<td>BDL</td>
<td>1.38</td>
</tr>
<tr>
<td>Elk &amp; Bison (1M)</td>
<td>Spotted Salamander</td>
<td>Adult Male</td>
<td>24.56</td>
<td>6.88</td>
<td>3.98</td>
<td>33.96</td>
</tr>
<tr>
<td>Site 68/80</td>
<td>Spotted Salamander</td>
<td>Adult Male</td>
<td>13.61</td>
<td>9.70</td>
<td>3.47</td>
<td>1.13</td>
</tr>
<tr>
<td>Star Camp 2 (1M)</td>
<td>Spotted Salamander</td>
<td>Adult Male</td>
<td>18.72</td>
<td>BDL</td>
<td>1.80</td>
<td>1.21</td>
</tr>
<tr>
<td>LBL-142 (4M)</td>
<td>Eastern Newt</td>
<td>Adult Male</td>
<td>20.73</td>
<td>6.07</td>
<td>0.49</td>
<td>1.20</td>
</tr>
<tr>
<td>LBL-220 (6M)</td>
<td>Eastern Newt</td>
<td>Adult Male</td>
<td>33.40</td>
<td>6.80</td>
<td>0.78</td>
<td>3.14</td>
</tr>
</tbody>
</table>
HCB levels varied significantly across species.

\[ F_{2,5} = 21.0, \ p < 0.004 \]
Is PA related to contaminant concentration in bullfrog tadpoles?

<table>
<thead>
<tr>
<th>Population</th>
<th>Total PCBs (ng/g wet wt)</th>
<th>EHL FA 7 (mpu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan</td>
<td>8.70</td>
<td>0.020</td>
</tr>
<tr>
<td>Bishop</td>
<td>9.90-10.36</td>
<td>0.034</td>
</tr>
<tr>
<td>Shuman</td>
<td>22.43</td>
<td>???*</td>
</tr>
</tbody>
</table>

*major deformities
Is PA related to contaminant concentration in eastern newts?

<table>
<thead>
<tr>
<th>Population</th>
<th>Total PCBs (ng/g wet wt)</th>
<th>FA 11 (pixels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBL-142</td>
<td>20.73</td>
<td>39.4</td>
</tr>
<tr>
<td>LBL-220</td>
<td>33.40</td>
<td>41.1</td>
</tr>
</tbody>
</table>

*major deformities*
Conclusions

- Amphibian asymmetry was *correlated* with anthropogenic disturbance.

- Data suggest that contamination levels might also be related to asymmetry and phenodeviants.
Implications

- Developmental stability in amphibians may provide an early warning of environmental stressors affecting humans.
Consequences of Tissue Contamination in Humans

- Reproductive failure
- Developmental problems
- Hepatic damage
- Respiratory and cardiovascular problems
- Immune system suppression
- Cancer
Implications

- Developmental stability may act as a biological indicator for monitoring and restoring amphibian populations.
Bufo boreas

Colorado Gap Analysis Project
Western Toad (Bufo boreas boreas)
Current and Future Research

- Other life stages and species
- Further contaminant analysis
- Age effects (B. Kobylarz MS)
- GIS/Remote Sensing (J. Boynton MS)
- Experiments
  - Causative factors
  - Effects of metamorphosis
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