



THE DISTRIBUTION OF JAPANESE GIANT SALAMANDERS (*ANDRIAS JAPONICAS*) IN TRIBUTARY STREAMS IN HYOGO PREFECTURE, JAPAN

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Introduction

Background on the Japanese giant salamander (*Andrias japonicas*):

- It is one of the largest amphibians (grows up to 150 cm), endemic to central and western Japan, and listed as “vulnerable species” by the Japanese Ministry of Environment.
- It externally fertilizes and breeds in August and September [2,4].
- It can be found in nesting cavities along the sides of riverbeds or under rock slabs [1].
- Little is known about how it utilizes small tributaries or what their distributions in these streams may be. Research suggests these streams may serve as microhabitats for larval development [3].

To examine this, we collected and analyzed water samples from three small tributaries for environmental DNA (eDNA), DNA left in stream



Figure 1. Wild *A. japonicas* being measured.

Hypotheses

- 1) Salamander eDNA will be found in all tributaries.
- 2) Due to differences in stream length and width, there will be a difference in overall eDNA concentration between the three streams.
- 3) eDNA concentration will decrease with increasing distance from the main stem.

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References

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Methods

We examined three tributary streams around Ikunocho Kurogawa, Hyogo Prefecture, Japan in summer 2018.

- We started collection at the base of the stream, and hiked upstream until the water dried.
- Water samples: N=8 (Stream A), N=21 (B), N=22 (C)



Figure 2. (left) Sample collection by Hundermark (L) and Bjordahl (R).

Figure 3. (right) One of the Stream B collection sites.

- Filtered water samples and extracted eDNA from each
- Ran quantitative PCR to estimate eDNA concentrations
- Ran ANCOVA and Pearson’s correlation tests

Results I

eDNA was found across all sample streams. Stream A had the highest concentrations at the 2nd and 8th sample sites, B at the 4th, 5th, and 10th sites, and C at the 8th and 15th sites. Stream C also had the highest average concentration at 0.02ng/L.

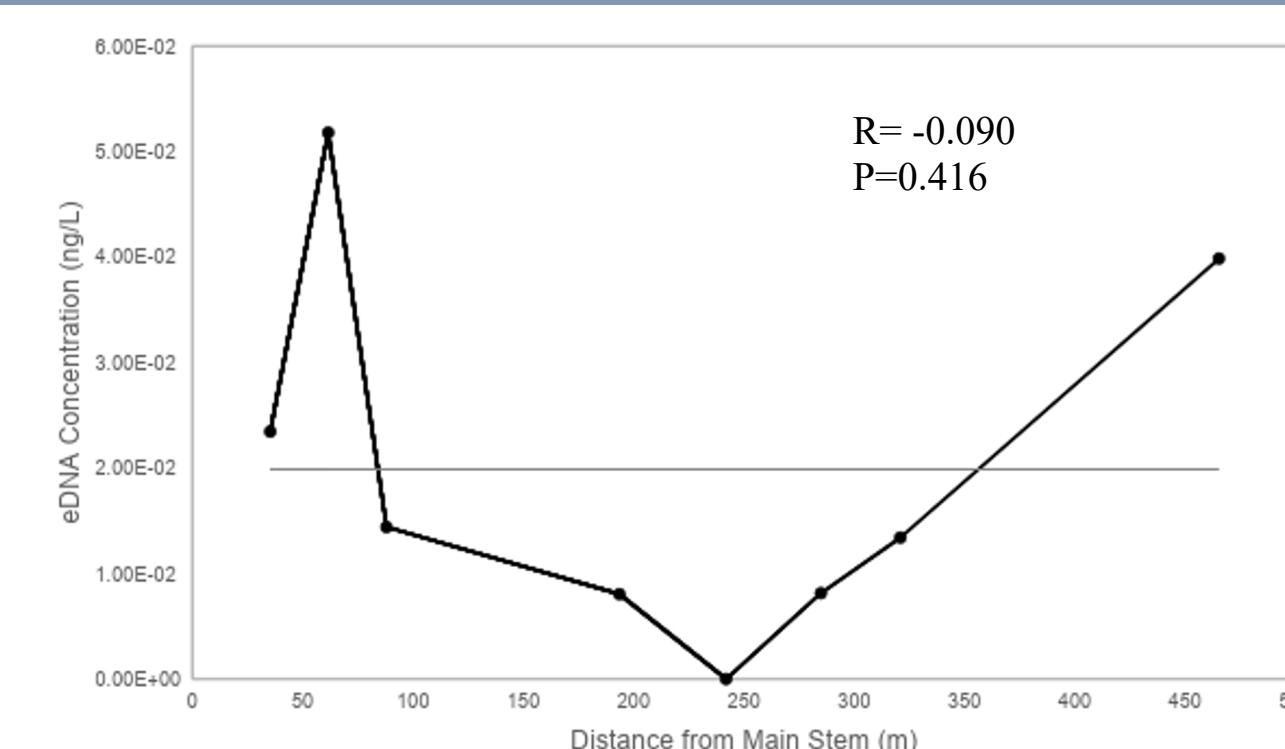


Figure 4. Stream A eDNA concentrations for each site and the average plotted against distance from the main stem. R indicates Pearson correlation with statistical significance

Results II

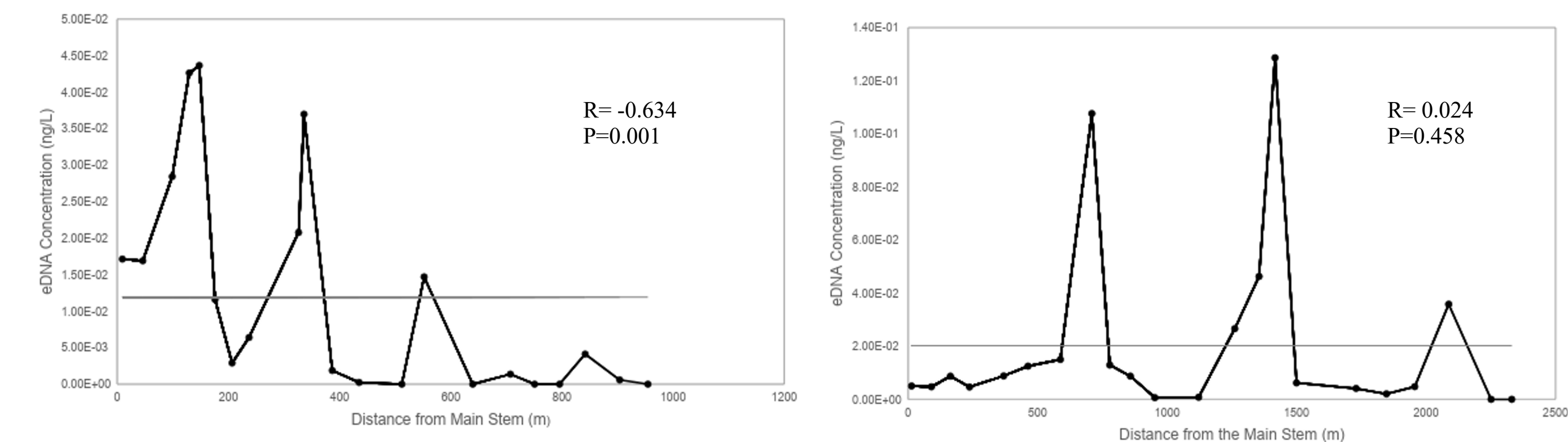


Figure 5. (left) Stream B eDNA concentrations for each site and the average plotted against distance from the main stem.

Figure 6. (right) Stream C eDNA concentrations for each site and the average plotted against distance from the main stem.

The results of our Pearson’s correlation tests only showed a significant correlation between eDNA concentration and stream distance in Stream B ($R = -0.634$, $P = 0.001$).

The results of our ANCOVA showed F values of 0.243 and 0.761 and P values of 0.624 and 0.473 for stream distance and stream difference respectively. There is thus no significant difference between the three streams’ eDNA concentrations or no overall effect of stream distance on eDNA concentration,

Conclusions

Hypothesis 1: There were eDNA results for each of our tributaries, suggesting salamanders are utilizing all of these streams.

Hypothesis 2: Our analyses show no significant difference in eDNA between the three sites.

Hypothesis 3: With the exception of Stream B, our analyses showed no correlation between eDNA and distance from the main stem, suggesting salamanders are utilizing all available habitat.

- Combined, the presence of salamanders in each stream mostly regardless of distance demonstrates the importance of these habitats for larval and juvenile salamanders (no salamanders were observed during sampling)
- Our results demonstrate tributary importance for future conservation.

Future studies: manually searching for salamanders in each tributary, to verify our results and understand unusual spiking patterns.