

# Supplementary Materials: Connections among land use, water quality, biodiversity of aquatic invertebrates, and fish behavior in Amazon rivers

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**Table S1.** Comparison between 1985 and 2020 of land use classes and vegetation cover in the study area, in the municipality of São Félix do Xingu, Pará state, Brazil.

| STUDY AREA                   |                         |          |                         |          |
|------------------------------|-------------------------|----------|-------------------------|----------|
| Class                        | 1985 (km <sup>2</sup> ) | 1985 (%) | 2020 (km <sup>2</sup> ) | 2020 (%) |
| Forest                       | 16,997.3                | 94.3     | 7860.7                  | 43.61    |
| Non-forest natural formation | 183.5                   | 1.02     | 145.3                   | 0.81     |
| Farming                      | 521.1                   | 2.89     | 9578.7                  | 53.15    |
| Non-vegetated area           | 7.9                     | 0.04     | 122.6                   | 0.68     |
| Water                        | 314.4                   | 1.74     | 316.3                   | 1.75     |
| Total                        | 18,024.2                | 100      | 18,023.6                | 100.0    |

**Table S2.** Comparison between 1985 and 2020 of land use classes and vegetation cover at the P1XR site, in the municipality of São Félix do Xingu, Pará state, Brazil.

| Class                        | P1XR 1985 (km <sup>2</sup> ) | P1XR 1985 (%) | P1XR 2020 (km <sup>2</sup> ) | P1XR 2020 (%) |
|------------------------------|------------------------------|---------------|------------------------------|---------------|
| Forest                       | 24.7                         | 88.9          | 16.3                         | 57.6          |
| Non-forest natural formation | 1.4                          | 5.0           | 1.2                          | 4.2           |
| Farming                      | 1.6                          | 5.6           | 10.7                         | 37.7          |
| Non-vegetated area           | 0.1                          | 0.5           | 0.2                          | 0.6           |
| Water                        |                              |               |                              |               |
| Total                        | 27.8                         | 100           | 28.3                         | 100           |

**Table S3.** Comparison between 1985 and 2020 of land use classes and vegetation cover at the P2FR site, in the municipality of São Félix do Xingu, Pará state, Brazil.

| Class                        | P2FR 1985 (km <sup>2</sup> ) | P2FR 1985 (%) | P2FR 2020 (km <sup>2</sup> ) | P2FR 2020 (%) |
|------------------------------|------------------------------|---------------|------------------------------|---------------|
| Forest                       | 23.2                         | 86.5          | 16.8                         | 60.8          |
| Non-forest natural formation | 1.1                          | 4.0           | 1.0                          | 3.6           |
| Farming                      | 2.2                          | 8.2           | 9.4                          | 33.9          |
| Non-vegetated area           | 0.4                          | 1.4           | 0.5                          | 1.7           |
| Water                        |                              |               |                              |               |
| Total                        | 26.8                         | 100           | 27.7                         | 100           |

**Table S4.** Comparison between 1985 and 2020 of land use classes and vegetation cover at the P3XFR site, in the municipality of São Félix do Xingu, Pará state, Brazil.

| Class                        | P3XFR 1985 (km <sup>2</sup> ) | P3XFR 1985 (%) | P3XFR 2020 (km <sup>2</sup> ) | P3XFR 2020 (%) |
|------------------------------|-------------------------------|----------------|-------------------------------|----------------|
| Forest                       | 26.9                          | 91.8           | 19.0                          | 63.9           |
| Non-forest natural formation | 0.9                           | 2.9            | 0.8                           | 2.6            |
| Farming                      | 1.5                           | 5.1            | 9.9                           | 33.3           |
| Non-vegetated area           | 0.0                           | 0.1            | 0.0                           | 0.1            |
| Water                        |                               |                |                               |                |
| Total                        | 29.3                          | 100            | 29.8                          | 100            |

**Table S5.** Concentrations of Mn and Zn (μg.L<sup>-1</sup>) in water samples from P1XR and P2FR.

| Replicates | P1XR |       | P2FR |       |
|------------|------|-------|------|-------|
|            | Mn   | Zn    | Mn   | Zn    |
| #1         | 0.0  | 21.0  | 5.0  | 9.0   |
| #2         | 2.0  | 10.0  | 4.0  | 66.0  |
| #3         | 0.0  | 15.0  | 3.0  | 20.0  |
| Average    | 0.67 | 15.33 | 4.00 | 31.67 |
| SD         | 1.15 | 5.51  | 1.00 | 30.24 |

\*The other metals (Cd, Cu, Fe, and Pb) were not detected.

**Table S6.** Concentrations of total solids (mg.L<sup>-1</sup>) in water samples from P1XR and P2FR.

| Replicates | P1XR  | P2FR   |
|------------|-------|--------|
| #1         | 25.39 | 163.11 |
| #2         | 25.06 | 142.00 |
| #3         | 25.30 | 138.60 |
| Average    | 25.25 | 147.90 |
| SD         | 0.17  | 13.28  |

**Table S7.** Turbidity (UT) of water samples from P1XR and P2FR.

| Replicates | P1XR | P2FR   |
|------------|------|--------|
| #1         | 3.48 | 117.33 |
| #2         | 3.16 | 117.67 |
| #3         | 3.4  | 114.33 |
| Average    | 3.35 | 116.44 |
| SD         | 0.17 | 1.84   |

**Table S8.** Principal component analysis (based on the correlation matrix).

| PC | Eigenvalue | % variance |
|----|------------|------------|
| 1  | 6.49       | 81.06      |
| 2  | 0.98       | 12.24      |
| 3  | 0.50       | 6.26       |

**Table S9.** Principal component analysis loadings, considering the following variables: Mn, Zn, avoidance (AVO), total solids (TS), turbidity (TB), area with forest (FOR), aquatic invertebrate abundance (ABU), and aquatic invertebrate diversity (DIV).

|     | Axis 1 | Axis 2 | Axis 3 |
|-----|--------|--------|--------|
| AVO | 0.38   | 0.11   | -0.20  |
| TS  | -0.39  | -0.06  | 0.08   |
| TB  | -0.39  | 0.04   | 0.10   |
| Mn  | 0.25   | 0.51   | 0.82   |
| Zn  | -0.17  | 0.85   | -0.48  |
| FOR | -0.39  | 0.03   | 0.12   |
| ABU | 0.39   | -0.03  | -0.12  |
| DIV | 0.39   | -0.03  | -0.12  |

**Table S10.** Numbers of zooplankton collected, according to family (morphotypes), showing the abundance and diversity of the organisms.

| Family (morphotypes) | Number of organisms |      |       |
|----------------------|---------------------|------|-------|
|                      | P1XR                | P2FR | P3XFR |
| 1                    | 336                 | 174  | 268   |
| 2                    | 2                   | 1    | 0     |
| 3                    | 371                 | 41   | 29    |
| 4                    | 425                 | 20   | 1     |
| 5                    | 22                  | 9    | 1     |
| 6                    | 0                   | 3    | 1     |
| 7                    | 0                   | 2    | 0     |
| 8                    | 0                   | 1    | 0     |
| 9                    | 1                   | 3    | 1     |
| 10                   | 4                   | 3    | 1     |
| 11                   | 1                   | 0    | 0     |
| 12                   | 0                   | 1    | 0     |
| 13                   | 14                  | 1    | 0     |
| 14                   | 1                   | 1    | 0     |
| 15                   | 1                   | 0    | 0     |
| 16                   | 0                   | 1    | 0     |
| 17                   | 0                   | 1    | 2     |
| 18                   | 2                   | 0    | 1     |
| 19                   | 0                   | 0    | 1     |
| 20                   | 0                   | 0    | 4     |
| 21                   | 0                   | 0    | 2     |
| 22                   | 0                   | 0    | 1     |
| 23                   | 1                   | 0    | 3     |
| 24                   | 3                   | 0    | 7     |
| 25                   | 0                   | 0    | 1     |
| 26                   | 0                   | 0    | 1     |
| 27                   | 2                   | 0    | 2     |
| 28                   | 0                   | 0    | 1     |
| 29                   | 17                  | 0    | 1     |
| 30                   | 0                   | 0    | 1     |
| 31                   | 3                   | 0    | 0     |

|           |      |     |     |
|-----------|------|-----|-----|
| 32        | 44   | 0   | 0   |
| 33        | 2    | 0   | 0   |
| 34        | 31   | 0   | 0   |
| 35        | 2    | 0   | 0   |
| 36        | 1    | 0   | 0   |
| 37        | 3    | 0   | 0   |
| 38        | 1    | 0   | 0   |
| 39        | 1    | 0   | 0   |
| 40        | 3    | 0   | 0   |
| 41        | 1    | 0   | 0   |
| 42        | 11   | 0   | 0   |
| 43        | 10   | 0   | 0   |
| 44        | 5    | 0   | 0   |
| 45        | 1    | 0   | 0   |
| 46        | 2    | 0   | 0   |
| 47        | 2    | 0   | 0   |
| 48        | 4    | 0   | 0   |
| 49        | 1    | 0   | 0   |
| 50        | 1    | 0   | 0   |
| 51        | 1    | 0   | 0   |
| 52        | 1    | 0   | 0   |
| 53        | 11   | 0   | 0   |
| 54        | 1    | 0   | 0   |
| 55        | 1    | 0   | 0   |
| 56        | 1    | 0   | 0   |
| 57        | 1    | 0   | 0   |
| 58        | 4    | 0   | 0   |
| 59        | 1    | 0   | 0   |
| 60        | 3    | 0   | 0   |
| 61        | 3    | 0   | 0   |
| 62        | 1    | 0   | 0   |
| Abundance | 1361 | 262 | 330 |
| Diversity | 48   | 15  | 21  |

**Table S11.** Distribution of *A. bimaculatus* in the control test with well water.

| Replicates | Chambers |     |     |    |     |     |
|------------|----------|-----|-----|----|-----|-----|
|            | C1       | C2  | C3  | C4 | C5  | C6  |
| #1         | 4        | 3   | 1   | 3  | 4   | 3   |
| #2         | 2        | 2   | 3   | 4  | 3   | 4   |
| #3         | 2        | 3   | 6   | 2  | 2   | 2   |
| #4         | 2        | 3   | 5   | 3  | 2   | 3   |
| Mean       | 2        | 2.7 | 4.7 | 3  | 2.3 | 3.3 |
| SD         | 0        | 0.6 | 1.5 | 1  | 0.6 | 0.6 |

**Table S12.** ANOVA applied to the distribution of the organisms in the control test.

|                | <b>Sum of squares</b> | <b>df</b> | <b>Mean square</b> | <b>F</b> | <b>p (same)</b> | <b>F critical</b> |
|----------------|-----------------------|-----------|--------------------|----------|-----------------|-------------------|
| Between groups | 4                     | 5         | 0.8                | 0.6      | 0.700596        | 2.772853          |
| Within groups  | 24                    | 18        | 1.333333           |          |                 |                   |
| Total          | 28                    | 23        |                    |          |                 |                   |

**Table S13.** Distribution of *A. bimaculatus* in the test with water from P1XR, P3XFR, and P2FR.

| Replicates | <b>Chambers</b> |     |       |     |      |     |
|------------|-----------------|-----|-------|-----|------|-----|
|            | C1              | C2  | C3    | C4  | C5   | C6  |
|            | P1XR            |     | P3XFR |     | P2FR |     |
| #1         | 6               | 4   | 3     | 1   | 3    | 1   |
| #2         | 6               | 4   | 3     | 2   | 2    | 1   |
| #3         | 5               | 4   | 2     | 1   | 3    | 3   |
| #4         | 4               | 5   | 2     | 1   | 4    | 2   |
| Mean       | 5.0             | 4.3 | 2.3   | 1.3 | 3.0  | 2.0 |
| SD         | 1.0             | 0.6 | 0.6   | 0.6 | 1.0  | 1.0 |

**Table S14.** One-way ANOVA applied to the distribution of the organisms in the test with water from P1XR, P3XFR, and P2FR.

|                 | <b>Sum of squares</b> | <b>df</b> | <b>Mean square</b> | <b>F</b> | <b>p (same)</b> |
|-----------------|-----------------------|-----------|--------------------|----------|-----------------|
| Between groups: | 37.75                 | 2         | 18.875             | 21.72    | 0.00000771      |
| Within groups:  | 18.25                 | 21        | 0.869              |          |                 |

**Table S15.** Tukey's test pairwise comparisons ( $p < 0.05$ ) for the distribution of the organisms in the test with water from P1XR (C1 and C2), P3XFR (C3 and C4), and P2FR (C5 and C6) in the avoidance system.

|             | <b>diff</b> | <b>lwr</b> | <b>upr</b> | <b>p adj</b> |
|-------------|-------------|------------|------------|--------------|
| P2FR-P1XR   | -2.375      | -3.549872  | -1.2001279 | 0.0001365    |
| P3XFR-P1XR  | -2.875      | -4.049872  | -1.7001279 | 0.0000117    |
| P3XFR- P2FR | -0.500      | -1.674872  | 0.6748721  | 0.5409110    |