

## Supplementary materials



**Figure S1.** Representative images of the new breeding sites of *Ichthyosaura alpestris inexpectata*: Piano di Zanche in April (**a**) and September (**b**) 2022; Pantano Lungo in April (**c**) and July (**d**) 2022.

**Table S1.** Water physical-chemical parameters measured in the study ponds. Mean ( $\pm$ SD) of electric conductivity = EC, pH, total dissolved solids = TDS, oxygen percentage saturation = O<sub>2</sub> %, dissolved oxygen = DO recorded in (s) summer and (a) autumn is shown.

| Parameter        | Site name      |               |               |               |               |                   |               |               |               |               |               |               |               |               |
|------------------|----------------|---------------|---------------|---------------|---------------|-------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                  | FO             |               | DU            |               | TR            |                   | TRIF          |               | FA            |               | PZ            |               | PL            |               |
|                  | s              | a             | s             | a             | s             | a                 | s             | a             | s             | a             | s             | a             | s             | a             |
| T (C°)           | 25.7<br>± 0.6  | 13.7<br>± 0.2 | 26.1<br>± 0.2 | 18.8<br>± 0.2 | 24.7<br>± 0.6 | 12.8<br>± 0.2     | 23.5<br>± 0.5 | 12.6<br>± 0.2 | 26.1<br>± 0.3 | 18.8<br>± 0.3 | 19.2<br>± 0.4 | 13.2<br>± 0.4 | 15.9<br>± 0.4 | 12.6<br>± 0.4 |
| EC (μS/cm)       | 84.3<br>± 0.6  | 78.3<br>± 1.5 | 58 ±<br>1     | 40.3<br>± 0.6 | 78.3<br>± 0.6 | 72.7<br>± 0.6     | 78 ±<br>1     | 80.7<br>± 0.6 | 81 ±<br>2     | 82.5<br>± 0.6 | 88.8<br>± 0.6 | 85.2<br>± 0.3 | 81.7<br>± 1.1 | 98.3<br>± 0.6 |
| pH               | 6.3 ±<br>0.06. | 6.6 ±<br>0.05 | 7.2 ±<br>0.1  | 6 ±<br>0.05   | 6.7 ±<br>0.1  | 5.9 ±<br>0.02     | 6.8 ±<br>0.05 | 6 ±<br>0.05   | 6.5 ±<br>0.06 | 6.2 ±<br>0.1  | 6.1 ±<br>0.05 | 6.5 ±<br>0.1  | 6.9 ±<br>0.05 | 6.6 ±<br>0.09 |
| TDS (ppm)        | 43.1<br>± 0.5  | 38.8<br>± 0.6 | 31.2<br>± 0.5 | 25.4<br>± 0.4 | 39 ±<br>0.5   | 36.3<br>± 0.6     | 39.2<br>± 1   | 46.3<br>± 0.5 | 40 ±<br>0.5   | 42 ±<br>1     | 45.4<br>± 0.4 | 48 ±<br>0.6   | 40.2<br>± 0.2 | 50.4<br>± 0.2 |
| O <sub>2</sub> % | 65 ±<br>1      | 72 ±<br>1     | 70.1<br>± 0.5 | 75 ±<br>0.6   | 68 ±<br>1     | 78 ±<br>0.5       | 65.2<br>± 0.2 | 70 ±<br>0.7   | 61.2<br>± 0.2 | 68 ±<br>0.5   | 46 ±<br>0.3   | 46 ±<br>0.2   | 58.1<br>± 0.2 | 65 ±<br>0.5   |
| DO (mg/L)        | 8.8 ±<br>0.06  | 9.8 ±<br>0.02 | 8.8 ±<br>0.01 | 8.7 ±<br>0.3  | 7.8 ±<br>0.6  | 11.3<br>±<br>0.03 | 5 ±<br>0.02   | 8.9 ±<br>0.01 | 8.5 ±<br>0.05 | 8.9 ±<br>0.05 | 5.2 ±<br>0.05 | 5.4 ±<br>0.02 | 6.8 ±<br>0.01 | 6.9 ±<br>0.03 |

**Table S2.** Taxonomic list and specimens abundance (number of individuals m<sup>-2</sup>) relative to the total of 43 Families, 15 Orders, and 7 Classes of macroinvertebrates collected in the two sampling campaigns involving the four selected monitoring sites. June = J, October = O, TRIF = Trifoglietti inferiore, PL = Pantano lungo, PZ = Piano di Zanche, FA = Fossa Armando.

| Class       | Order            | Family            | TRIF |      | PL   |      | PZ   |      | FA   |     |
|-------------|------------------|-------------------|------|------|------|------|------|------|------|-----|
|             |                  |                   | J    | O    | J    | O    | J    | O    | J    | O   |
| Arachnida   | Hydrachnida      | Hydrachnidae      | 11   | -    | -    | -    | 5    | 5    | 16   | 6   |
| Bivalvia    | Eulamellibranchi | Sphaeriidae       | 219  | 475  | 149  | 421  | -    | 133  | 80   | -   |
| Crustacea   | Isopoda          | Asellidae         | 144  | 112  | -    | 80   | -    | -    | 144  | 16  |
| Gordioidea  | Gordioidea       | Gordiidae         | -    | -    | 5    | -    | -    | 11   | -    | -   |
| Hirudinea   | Hirudinida       | Haemopidae        | 27   | 11   | -    | -    | -    | -    | 48   | -   |
|             |                  | Capniidae         | -    | -    | 85   | 16   | -    | -    | -    | -   |
|             | Plecoptera       | Leuctridae        | -    | -    | 112  | 16   | -    | -    | -    | -   |
|             |                  | Nemouridae        | -    | -    | 21   | 21   | -    | -    | -    | -   |
|             | Ephemeroptera    | Baetidae          | 90   | 165  | 37   | -    | -    | -    | 160  | -   |
|             |                  | Heptageniidae     | -    | -    | -    | 11   | -    | -    | -    | -   |
|             |                  | Helicopsychidae   | -    | -    | 5    | -    | -    | -    | -    | -   |
|             |                  | Hydropsychidae    | -    | -    | 27   | -    | -    | -    | -    | -   |
|             |                  | Lepidostomatidae  | -    | -    | -    | -    | 37   | 32   | -    | -   |
|             | Trichoptera      | Limnephilidae     | 27   | 272  | 240  | 133  | 27   | 314  | -    | 139 |
|             |                  | Odontoceridae     | -    | -    | 112  | 75   | 32   | 112  | -    | 85  |
|             |                  | Phryganeidae      | -    | -    | -    | -    | 11   | 11   | 27   | 27  |
|             |                  | Sericostomatidae  | 283  | 192  | 91   | 43   | 373  | 608  | 442  | -   |
|             |                  | Ceratopogonidae   | 37   | -    | 37   | -    | -    | -    | 21   | 16  |
|             |                  | Chironomidae      | 619  | 405  | 715  | 459  | 229  | 53   | 885  | -   |
|             | Diptera          | Culicidae         | -    | -    | -    | -    | 155  | 133  | -    | -   |
|             |                  | Dixidae           | -    | -    | 32   | 5    | -    | -    | -    | -   |
|             |                  | Limoniidae        | -    | -    | 16   | 21   | -    | -    | 5    | -   |
| Insecta     |                  | Simuliidae        | -    | -    | 96   | -    | -    | -    | -    | -   |
|             |                  | Tabanidae         | -    | -    | 5    | -    | -    | -    | 5    | 59  |
|             |                  | Aeschnidae        | 43   | 32   | 5    | 27   | -    | -    | -    | -   |
|             | Odonata          | Calopterygidae    | -    | 5    | -    | 5    | 11   | -    | -    | 11  |
|             |                  | Coenagrionidae    | -    | 37   | -    | -    | -    | -    | -    | -   |
|             |                  | Cordulegasteridae | -    | -    | 3    | 21   | -    | -    | -    | -   |
|             |                  | Libellulidae      | 5    | 11   | -    | 5    | -    | -    | -    | 5   |
|             |                  | Dryopidae         | 5    | -    | -    | -    | -    | -    | 21   | -   |
|             |                  | Dytiscidae        | -    | 21   | -    | 53   | -    | -    | 48   | 48  |
|             | Coleoptera       | Elminthidae       | -    | -    | -    | -    | -    | -    | -    | 11  |
|             |                  | Gyrinidae         | 5    | -    | -    | -    | -    | -    | 5    | -   |
|             |                  | Haliplidae        | -    | 11   | 5    | 48   | -    | -    | -    | 11  |
|             |                  | Scirtidae         | -    | -    | 37   | 59   | 283  | 251  | -    | -   |
|             |                  | Hydraenidae       | -    | 11   | -    | -    | 11   | 16   | -    | 11  |
|             |                  | Hydrophilidae     | -    | -    | -    | -    | -    | -    | 5    | -   |
|             | Heteroptera      | Nepidae           | -    | -    | -    | 5    | -    | -    | -    | -   |
|             |                  | Notonectidae      | 5    | -    | -    | 5    | -    | -    | 43   | -   |
|             |                  | Veliidae          | -    | -    | 5    | 5    | -    | -    | -    | -   |
| Oligochaeta | Opisthopora      | Lumbricidae       | -    | 5    | 21   | 21   | -    | -    | 5    | 5   |
|             | Lumbriculida     | Lumbriculidae     | 11   | 11   | -    | 37   | -    | -    | -    | 229 |
|             | Tubificida       | Naididae          | 27   | 16   | 5    | 16   | -    | -    | 11   | -   |
|             | <b>Total</b>     |                   | 1558 | 1792 | 1866 | 1597 | 1174 | 1679 | 1971 | 743 |

**Table S3.** Morphometric parameters (SVL: Snout vent length; SMI: Scaled mass index) of *Ichthyosaura alpestris inexpectata* from the study sites (TRIF: Trifoglieti inferiore; FA: Fosso Armando; PZ: Piano di Zanche; PL: Pantano Lungo).

| Site | Sex | N  | Body mass (g) |           | SVL (mm)     |         | Total length (mm) |          | SMI         |             |
|------|-----|----|---------------|-----------|--------------|---------|-------------------|----------|-------------|-------------|
|      |     |    | mean ± SD     | range     | mean ± SD    | Range   | mean ± SD         | range    | mean ± SD   | range       |
| TRIF | M   | 24 | 2.4 ± 0.49    | 1.6 - 3.3 | 47 ± 4.13    | 40 - 57 | 81.29 ± 5.4       | 71 - 90  | 2.41 ± 0.44 | 1.46 - 3    |
|      | F   | 18 | 4.14 ± 0.94   | 2.5 - 5.7 | 53.5 ± 3.20  | 48 - 60 | 93.5 ± 5.43       | 85 - 103 | 4.14 ± 0.76 | 2.41 - 5.45 |
| FA   | M   | 11 | 2.66 ± 0.32   | 2.1 - 3.1 | 45.94 ± 2.93 | 43 - 51 | 77.91 ± 3.96      | 73 - 86  | 2.72 ± 0.35 | 2.07 - 3.35 |
|      | F   | 4  | 4.57 ± 0.74   | 3.9 - 5.4 | 54.5 ± 3.32  | 50 - 57 | 93 ± 5.48         | 85 - 97  | 4.6 ± 0.77  | 3.47 - 5.12 |
| PZ   | M   | 5  | 3.54 ± 0.24   | 3.2 - 3.8 | 48.8 ± 1.09  | 48 - 50 | 83.8 ± 2.17       | 80 - 85  | 3.54 ± 0.16 | 3.37 - 3.79 |
|      | F   | 4  | 4.87 ± 1.22   | 3.8 - 6.5 | 53.25 ± 2.75 | 50 - 56 | 94.25 ± 6.75      | 87 - 100 | 4.85 ± 0.67 | 4.04 - 5.59 |
| PL   | M   | 16 | 2.71 ± 0.41   | 2.2 - 3.6 | 47.19 ± 3.54 | 40 - 54 | 81.06 ± 6.27      | 70 - 93  | 2.71 ± 0.34 | 2.3 - 3.35  |
|      | F   | 42 | 4.42 ± 1.24   | 2.2 - 7   | 52.74 ± 4.82 | 40 - 60 | 95.55 ± 5.68      | 85 - 105 | 4.35 ± 0.73 | 3.17 - 6.19 |