Contrasting species- and trait-specific effects of deer wallows on life-history performance in two amphibians

Naru Matsuura[1CO], Hisanori Okamiya[2CO], Akira Terui[3], Masayuki K. Sakata[4], Hitoshi Araki[4], Osamu Kishida[5]

[CO] Contributed equally to this work with: Naru Matsuura, Hisanori Okamiya

[1] Graduate School of Environmental Sciences, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

[2] Museum of Natural and Environmental History, Shizuoka, Shizuoka 422-8017, Japan

[3] University of North Carolina at Greensboro, Dept Biol, Greensboro NC 27410, USA

[4] Agriculture Graduate School of Agriculture Research Faculty of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan

[5] Wakayama Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Kozagawa, Higashimuro, Wakayama 649-4563, Japan

Corresponding author:

Osamu Kishida

Email: kishida@fsc.hokudai.ac.jp

Electronic Supplementary Material

Supplementary Figures

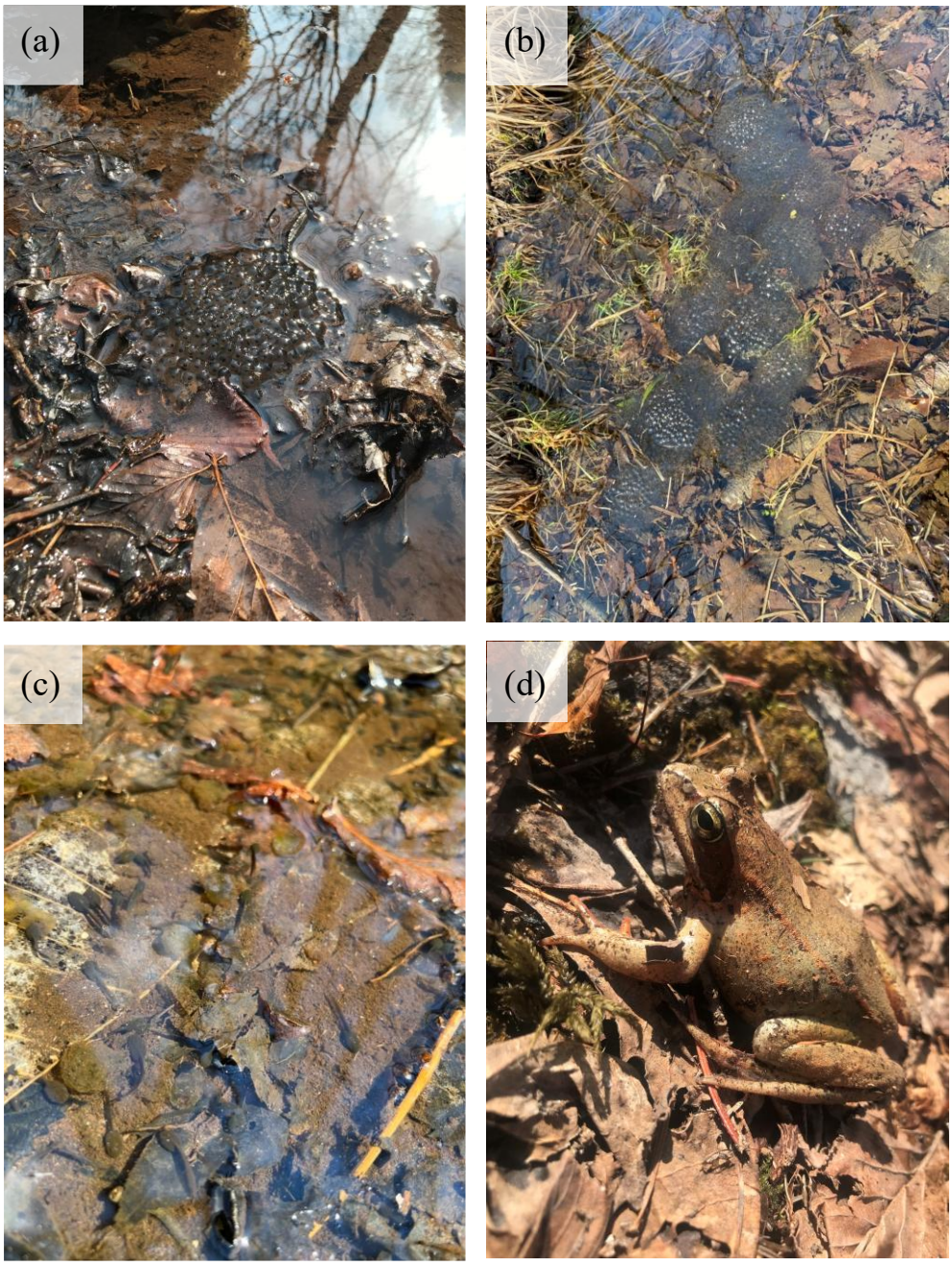


Fig. S1 Representative life stages of *Rana pirica*. (a) Egg mass, (b) cluster of egg masses, (c) larvae, and (d) adult (Photo credit: (a, c, d) Naru Matsuura, (b) Hisanori Okamiya).

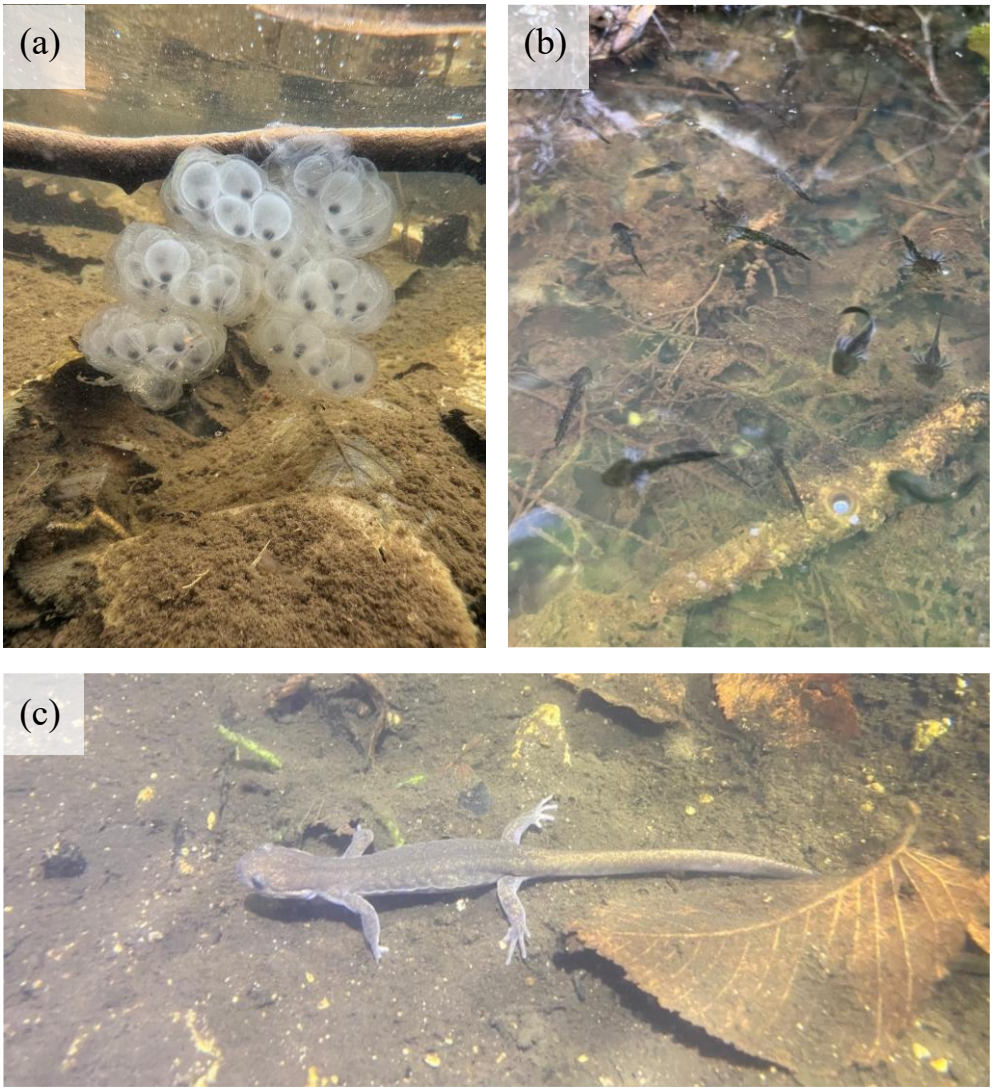


Fig. S2 Representative life stages of *Hynobius retardatus*. (a) A pair of egg sacs, (b) larvae, and (c) adult (Photo credits: (a) Naru Matsuura, (b, c) Hisanori Okamiya).

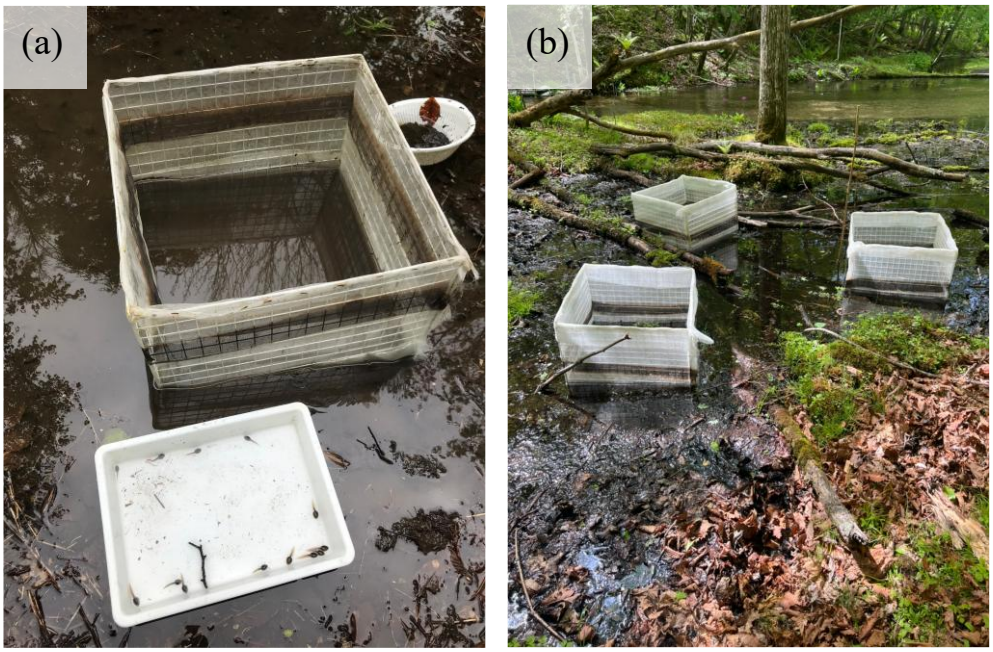


Fig. S3 Quadrat method used in the capture survey. (a) A single quadrat used for sampling, with captured *R. pirica* larvae. (b) Example of multiple quadrats placed within a waterbody (Photo credits: (a) Naru Matsuura, (b) Hisanori Okamiya).

Supplementary Methods

Methods S1 Identification of wallows

Surveys to identify wallows were conducted on 16, 17, and 19 October 2021. During this period, when sika deer actively wallow, leaf litter and vegetation are displaced, exposing bare ground. Thus, wallows can be easily distinguished from ponds. A site was classified as a wallow when more than half of the depression area showed soil disturbance attributable to deer.

Methods S2 Environmental Measurements

Because water levels fluctuate substantially immediately after snowmelt, water surface area and water depth were measured on 11 April, several days after complete snowmelt. Water surface area was estimated using a tape measure by recording the long and short axes and approximating its shape as an ellipse or a circle; surface area was then calculated using π multiplied by the long and short axes. Central water depth was measured at five locations near the center of each waterbody, around the intersection of the long and short axes, with measurement points spaced approximately 20 cm apart, and the mean depth was calculated.

Stream connectivity was determined visually based on whether surface water exchange with the stream was observed; waterbodies with visible water flow were classified as connected, whereas those without were classified as isolated. Although stream connectivity occasionally varied during the survey period due to flooding or drying events, cases in which isolated waterbodies became connected to the stream were rarely observed; therefore, stream connectivity was treated as a fixed in subsequent

analyses. For both species, a small number of oviposition sites could not be measured for these variables because oviposition occurred later in the survey season; these sites were treated as missing values in subsequent analyses (4 waterbodies for *R. pirica*; one waterbody for *H. retardatus,* see Table S2).

To quantify water temperature and water chemistry (pH, electrical conductivity (EC), and dissolved oxygen (DO)), we conducted repeated surveys of each waterbody on four occasions (1–2 April, 11 April, 26 April, and 22 May), spanning the period from oviposition to the mid-larval stage for both species. To minimize the influence of within-day variation associated with survey timing, the order of surveys was alternated among occasions: sites were surveyed sequentially from upstream to downstream on one occasion and from downstream to upstream on the next. Water temperature, pH, and electrical conductivity were measured using a portable water quality meter (HI98129N and HI98130N), and dissolved oxygen was measured using a dissolved oxygen meter (Seven2Go™ DO meter).

The dragonfly naiad density was estimated from individuals captured during the *R. pirica* survey conducted on 3–5 June. We counted dragonfly naiads (Corduliidae, Libellulidae, and Aeshnidae) larger than 10 mm and calculated their density based on quadrat area (for quadrat method) or total waterbody area (for total-capture method).

Methods S3 Detailed methods of capture survey

For the quadrat method, 1-mm mesh quadrats (0.6 × 0.6 × 0.6 m; Figure S3) were pressed into the substrate to prevent organisms from escaping. One to five quadrats were placed per waterbody in proportion to its size (Figure S3), and all individuals within each quadrat were carefully captured with a net.

For *R. pirica*, larvae tend to aggregate along the edges of waterbodies. Areas with high edge density were treated separately from other areas, and the total larval abundance for the entire waterbody was estimated by combining these area-specific estimates.

We used either the quadrat or total-capture method depending on waterbody size and shape. To assess whether survey method may have influenced density estimates, we conducted supplementary analyses. For *R. pirica*, we fitted a linear model of log-transformed larval density including survey method (quadrat or total-capture), habitat type (pond or wallow), their interaction, and log-transformed waterbody size in June. Habitat type and size were included to account for potential confounding. Neither the main effect of survey method nor its interaction with habitat type was statistically significant. Variance inflation factors indicated no evidence of problematic multicollinearity among predictors (all VIFs < 5).

For *H. retardatus*, the total-capture method was used in two ponds and seven wallows, whereas the quadrat method was used in nine ponds and no wallows, limiting our ability to assess whether method effects on larval estimate differed between habitats. Nevertheless, survey method is unlikely to explain the lower larval survival in wallows. Survival was estimated as zero in four of six wallows and one of nine ponds, and intensive searches lasting at least 30 minutes at zero-survival sites failed to detect any larvae. These observations suggest that the frequent zero-survival estimates in wallows reflected mortality rather than underestimation of abundance by the total-capture method, although survey-method effects could not be fully excluded statistically.

Methods S4 Supplementary details of pre-hatching FAMD axes

Among the four survey occasions of water temperature and chemistry (1–2 April, 11 April, 26 April, and 22 May), we defined the pre-hatching period as 1–2 and 11 April for *R. pirica* and 11 and 26 April for *H. retardatus*. These intervals broadly covered the period from the onset of oviposition to hatching in each species. For analyses of hatching success, we used FAMD axes derived from environmental data during the pre-hatching period. In contrast, analyses of post-hatching environmental drivers—including larval survival probability in both species, and larval size, metamorphic size, and days until metamorphosis in *R. pirica*—were based on FAMD axes calculated from all four survey occasions. Dragonfly naiad density was excluded from the pre-hatching FAMD but included in the FAMD based on all four survey occasions. Interpretation of the latter FAMD axes follows the descriptions provided in the main text.

Detailed interpretations of the pre-hatching FAMD axes are as follows. For *R. pirica*, the first three FAMD axes explained 31.2%, 23.3%, and 13.7% of the environmental variation, respectively. Higher Dim1 values indicate sites with higher pH and DO, greater stream connectivity, and spring presence. Higher Dim2 values indicate sites with larger water surface area, greater depth, and higher DO. Higher Dim3 values indicate sites with higher pH and greater desiccation risk. For *H. retardatus*, the first three FAMD axes explained 30.9%, 23.6%, and 13.9% of the environmental variation, respectively. Higher Dim1 values indicate sites with higher pH, DO, and EC, greater stream connectivity, and spring presence. Higher Dim2 values indicate sites with larger water surface area, greater depth, greater desiccation risk, and lower water temperature. Higher Dim3 values indicate sites with higher pH, EC, and water temperature, greater desiccation risk, and spring absence.

For each species, we fitted separate linear models with the pre-hatching FAMD axis score as the response variable and habitat type as a categorical predictor. Only Dim2 for *R. pirica* differed significantly between habitat types, with lower scores in wallows than in ponds (estimate = −1.34; 95% CI = −2.31 to −0.36; $P = 0.009$). No significant differences were detected for the other axes in *R. pirica* or for any axis in *H. retardatus*.

Supplementary Video

Online Resource 2 Wallowing behavior of the Ezo sika deer (*Cervus nippon yesoensis*). During the breeding season, adult males paw and thrash the ground violently with their antlers or forefeet, followed by lying down and rubbing their bodies against the ground (Video credit: Naru Matsuura).
https://www.dropbox.com/scl/fo/r46raicvf7ixqqulmiq7n/h?rlkey=s4ux25og2xhuio0v3v678z5wj&dl=0

Supplementary Tables

Table S1 Regression model formulations to assess the effects of habitat type on survival and growth (Question 2) and to identify the factors driving these differences (Question 3).

Table S2 Environmental data at each site.

Table S3 Life-history data of two amphibians at each site.