Predator-Induced Tail Coloration Toward Diverse Dragonfly Nymphs in Tadpoles of the East Japan Tree Frog (*Dryophytes leopardus)*

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Running head: **Predator-induced tail coloration in tadpoles of *Dryophytes leopardus***

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Abstract

Anuran larvae can change their coloration and morphology to avoid predation. Particularly, some tadpoles in the genus *Dryophytes* in the Americas develop deep tail fins and bright orange tail coloration in response to predators such as dragonfly nymphs. These conspicuous tails are hypothesized to attract predator attacks, protecting vital body parts from fatal injuries. However, it remains unclear whether the extent of the induced phenotypic changes differs between various predators with distinct feeding patterns and habitats. To explore this, we reared tadpoles of the East Japan tree frog, *Dryophytes leopardus*, with four dragonfly species, water bug, water scorpion, or newt, and measured phenotypic changes, especially focusing on tail coloration. We found that the aeshnid *Anax nigrofasciatus*, a sit-and-wait predator that climbs on aquatic vegetation, induced bright orange and broad tails of tadpoles. Other odonate species that forage in and on the substrate also elicited tail coloration or deeper larval tails, but it took more time to induce the tadpoles' response compared to *A. nigrofasciatus*. In contrast, non-odonate predators did not induce color and morphological changes. This study provides the first evidence of predator-induced tail coloration in an Asian species of *Dryophytes*, which appears to be a defensive response specifically to dragonfly nymphs, as visually adept predators. Moreover, the differences in the extent and timing of the phenotypic changes among odonate species suggest that anti-predator phenotypes are modulated based on relative predation risks, considering the predators' foraging patterns and visual capabilities, thereby allowing tadpoles to minimize physiological costs and unintended risks associated with the induced traits.

The utilization of prey's body color and shape is highly dependent on its context, including the sensory capabilities of predators and the background condition (e.g., Espanha et al., 2016; Barnett et al., 2018). Especially in aquatic habitats, substantial variation exists in background conditions and the composition and abundance of predators across different localities, making it difficult for prey to achieve a uniform predation avoidance ability across their range (Van Buskirk, 2002; McIntyre et al., 2004). Therefore, such environmental uncertainty has generally been believed to favor plastic rather than constitutive anti-predator defenses (Agrawal and Karban, 1999; McIntyre et al., 2004).

Anuran larvae, or tadpoles, have served for decades as a model system for investigating induced defense mechanisms, as well as predator avoidance strategies and trade-offs mediated by plastic traits (Kasumovic, 2013; Kruger and Morin, 2020). They often rely on chemical cues to evaluate predation risk through continually released predator-borne cues and digestion-released, prey-borne cues, and then exhibit predator-induced defense mechanisms (Brönmark and Hansson, 2000; Hettyey et al., 2010, 2015). Predator-induced plastic responses in tadpoles can be categorized into four primary domains: behavioral, morphological, physiological, and chemical defenses. Behavioral responses encompass changes in overall activity levels (e.g., Lawler, 1989; Takahara et al., 2012a; Reuben and Touchon, 2021), increased burst swimming speed (Takahara et al., 2008), and spatial avoidance of predators (e.g., Laurila et al., 1998; Nunes et al., 2013). Morphological changes for predator avoidance have been well documented in tadpoles of various species (Relyea, 2001; Van Buskirk, 2002; Richter-Boix et al. 2007). For instance, tadpoles undergo changes in body size (i.e., correlated with growth rate and metamorphosis timing), body shape such as the development of deeper tail fins (Lardner, 1998, 2000), and coloration (McCollum and Van Buskirk, 1996; Touchon and Warkentin, 2008). Behavioral and morphological responses are sometimes co-expressed (Hossie et al., 2017). Physiological responses include changes in

respiration rate and gut evacuation rate (Steiner, 2007; Steiner and Van Buskirk, 2009; Costello and Michel, 2013). These may arise due to trade-offs in predator avoidance (Relyea and Auld, 2004) or as responses to partially offset the costs of lower energy intake resulting from evasion (Barry and Syal, 2013). Additionally, tadpoles of certain groups, particularly toads, evade predation through toxin production (Bókony et al., 2016; Hettyey et al., 2019).

The types and intensities of induced responses in tadpoles often differ according to predator types (Relyea, 2001; Teplitsky et al., 2004, 2005; Hettyey et al., 2011). For instance, Kishida and Nishimura (2005) revealed that tadpoles of *Rana pirica* developed deeper tail fins in response to dragonfly nymphs, whereas in the presence of salamander larvae, they exhibited not only deeper tails but also bulging heads and bodies. Tadpoles expressing these predator-specific traits should have a survival advantage over those with mismatched or non-induced phenotypes when faced with predation. Conversely, expressing such defense traits incurs energetic costs and can increase vulnerability to non-focal predators (Touchon and Warkentin, 2008; Innes-Gold et al., 2019). Due to these costs, defense traits are expected to remain plastic rather than fixed, and their types and intensities can vary depending on predator species, sometimes exhibiting predator-specific responses.

Dragonfly nymphs, as one of the major predators of tadpoles, are known to induce morphological responses characterized by deep tail fins across many anuran species (Relyea and Werner, 2000; Van Buskirk, 2002). This induced broad tail has been repeatedly hypothesized to improve the swimming performance of tadpoles and facilitate their escape from predators (Smith and Van Buskirk, 1995; McCollum and Leimberger, 1997; Van Buskirk and McCollum, 2000a). However, experimental evidence of this benefit is lacking (Van Buskirk et al., 2003; Johnson et al., 2008). Moreover, a few tree frog species in the genus *Dryophytes* respond to odonate predators by not only exhibiting deeper tails but also turning their entire tails bright orange (McCollum and Van Buskirk, 1996; Van Buskirk and

McCollum, 1999; LaFiandra and Babbitt, 2004). Thus, as another function, the induced deep and brightly colored fin is thought to serve as a lure, diverting predator attacks away from the more vulnerable head and body regions (Van Buskirk et al., 2003, 2004; Hawlena et al., 2006; Johnson et al., 2008). Given that tail fins tear easily (Doherty et al., 1998) and that tadpoles can maintain their swimming performance even after partial tail loss (Hoff and Wassersug, 2000; Van Buskirk and McCollim, 2000b), the tail is considered expendable relative to other body parts. Because dragonfly nymphs are predominantly visual predators, exhibiting the ability to discern both the shape and color of immobile as well as moving prey (Bybee et al., 2012; Espanha et al., 2016), color and shape changes in tadpoles are likely to be effective in avoiding predation by odonates.

Among dragonfly nymphs, several ecological types have been identified based on microhabitat usage and foraging behavior (Pritchard, 1965; Corbet, 1999), including climbers (e.g., Green darner, *Anax junius*), moving among aquatic vegetation and ambushing prey on plants; sprawlers (e.g., Globe skimmer, *Pantala flavescens*; Sherratt and Harvey, 1989), inhabiting pond bottoms and swimming through the water column to attack; and burrowers (e.g., Golden-ringed dragonfly, *Cordulegaster boltonii*), burrowing in mud or bottom debris and often lying in wait for prey with only the eyes protruding. The reliance on vision for foraging varies among these types (Pritchard, 1966; Corbet, 1999). For tadpoles, the frequency of encounters with and relative predation risk of these predators differ among habitats and seasons. These differential predation risks likely determine the cost−benefit balance of induced traits, thereby suggesting that tadpoles may exhibit different defense responses. However, prior experimental studies have primarily focused on a single dragonfly species and have not examined how tadpole responses may vary among odonate (and other) species.

The East Japan tree frog, *Dryophytes leopardus*, which was recently recognized as distinct from *Dryophytes japonicus*, is a prevalent species with a wide distribution encompassing northeastern Japan (central to eastern Honshu and Hokkaido), as well as regions outside Japan including Sakhalin Island (Shimada et al., 2025). Tadpoles of this species inhabit various environments such as rice paddies, marshes, ponds, puddles, and other ephemeral water bodies (Matsui and Maeda, 2018). Tadpoles, including this species, serve as important prey for numerous aquatic predators, including dragonfly nymphs, water scorpions, and red-bellied newts (Ohba and Nakatsuji, 2006; Hirai et al., 2020; Kita et al., 2020; Ozaki and Nishikawa, 2023). Morphological responses to predators have also been documented in this species, with dragonfly nymphs inducing deep tail fins, while chemical cues from goldfish prompt the development of shallow fins (Takahara et al., 2012b). The occurrence of orange tail coloration has also been briefly reported, although it has been attributed to ingested algae rather than predator-induced plasticity (Doi et al., 2005; Yuasa, 2006, 2007). Despite being rarely documented, tadpoles exhibiting brightly colored tails were frequently observed at our study site in Kyoto, central Japan (Noda, personal observation).

To clarify the cause and function of the orange tail coloration in tadpoles of *D. leopardus*, we conducted laboratory experiments with the tadpoles and several predator species. These experiments aimed to achieve two primary objectives. First, we investigated whether the tail coloration of tadpoles is induced by dragonfly nymphs, and if so, whether the intensity and speed of this response vary among different odonate species with different ecological characteristics. Simultaneously, we examined changes in body shape that are known to be plastically induced by predators. The second objective was to assess whether tadpoles exhibit color/morphological responses to predators characterized by different foraging styles compared to odonates. We used water bugs (*Appasus major*), water scorpions (*Laccotrephes japonensis*), and newts (*Cynops pyrrhogaster*) as non-odonate predators. Water

bugs and scorpions capture prey by enclosing them with their frontal legs, and newts swallow prey whole. Accordingly, predation avoidance strategies involving tail coloration, which are based on diverting attacks toward a specific body part, are expected to be less effective against these predators than against dragonfly nymphs. Therefore, tadpoles may exhibit weakened or no color/morphological responses to minimize the costs associated with developing plastic traits. In both experiments, tadpoles were reared with a single predator species, and their morphological traits of interest were compared with those of tadpoles raised in predator-free tanks. By analyzing the extent of tail coloration in tadpoles exposed to diverse predators, we revealed that tadpoles modulate their induced responses according to the risks posed by predators with different foraging types and sensory capabilities.

MATERIALS AND METHODS

Two series of laboratory experiments were carried out: Experiment I involved the comparison of induction of color/morphological changes in tadpoles by four different dragonfly species as predators, and Experiment II employed three non-odonate predators.

Experiment I.—Seven mating pairs of *Dryophytes leopardus* were captured in the paddy fields at Kyoto University (35°01'N, 135°47'E) on June 9 and 10, 2022. Following capture, mating pairs were housed overnight in plastic cages (19 × 19 × 14 cm; 6 cm water depth), and eggs were obtained the following day. The eggs hatched in about 2–3 days, and after hatching, tadpoles were separately kept in plastic containers (43 × 31 × 16 cm; 6 cm water depth), as those from the same clutch were kept together in a single container. A room temperature of 20°C was maintained until the experiments began, and tadpoles were fed powdered food (Kyorin Co., Ltd.) every two days. In this experiment, four species of dragonfly nymphs were

161 used as predators taking into account their foraging styles: *Anax nigrofasciatus* (Black-striped
162 lesser emperor; climber), *Gynacantha japonica* (Blue-spotted dusk-hawker; climber),
163 *Orthetrum albistylum speciosum* (White-tailed skimmer; burrower), and *Pantala flavescens*
164 (Wandering glider; sprawler). Nymphs of *A. nigrofasciatus* collected in Sakura City, Chiba
165 Prefecture, and those of *G. japonica* collected in Higashihiroshima City, Hiroshima
166 Prefecture, were obtained from an online vendor, and *O. a. speciosum* and *P. flavescens* were
167 collected from the same paddy field as the mating frog pairs. The dragonfly nymphs were
168 maintained individually in plastic trays (23 × 17 × 8 cm) and fed tadpoles of *D. leopardus*
169 every two days until the beginning of the induction experiment.

170 The induction experiment commenced on July 12, 2022. Each experimental tank
171 housed 21 tadpoles, with three individuals randomly selected from each of the seven egg
172 clutches to randomize genetic effects in each tank. Tadpoles in a tank were assigned to one of
173 five treatments (Ax group with *A. nigrofasciatus*; Gy group with *G. japonica*; Or group with
174 *O. a. speciosum*; Pf group with *P. flavescens*; and the control Ct1 group) with eight replicates
175 per treatment. Each experimental tank (31 × 18 × 24 cm; 10 cm water depth) was equipped
176 with a transparent isolation case (17 × 9 × 10 cm) with approximately 5 mm diameter holes
177 spaced about 5 mm apart, where one dragonfly nymph was introduced. This setup allowed
178 visual and chemical-mediated interaction between the predator and tadpoles without physical
179 contact. The tanks were aerated and maintained under a 14-h light/10-h dark photoperiod at a
180 room temperature of 22°C, with half of the water in all tanks changed every three days. Air
181 was continuously supplied to the isolation case within each tank through an aquarium air
182 stone. Tanks designated for the Ct1 group lacked predators and utilized an empty isolation
183 case. Tadpoles in the experimental tanks were fed every two days with commercial food for
184 Axolotl, *Ambystoma mexicanum* (Kyorin Co., Ltd.), while dragonfly nymphs received two
185 tadpoles as food every two days.

186

187 ***Experiment II.***—The design of Experiment II mirrored that of Experiment I, with
188 adjustments made for sampling dates, the interval between hatching and the experiment's
189 commencement, and the selection of predators. For this experiment, three mating pairs of *D.*
190 *leopardus* were captured from the paddy fields at Kyoto University on May 18 and 20, 2022.
191 Eggs were collected and tadpoles from clutches were reared in the same way as in Experiment
192 I until the experiments began. Three predator species were chosen based on their foraging
193 style and prey capture behavior: Ferocious water bug, *Appasus major* (sit-and-wait predator
194 that seizes prey with its frontal legs), water scorpion, *Laccotrephes japonensis* (sit-and-wait
195 predator that grasps prey with its frontal legs), and Japanese fire belly newt, *Cynops*
196 *pyrrhogaste*r (active tracker that swallows prey whole). Aquatic insects, collected in
197 Higashihiroshima City, were purchased from the online vendor, while red-bellied newts were
198 collected from irrigation ditches in Kyoto City (35°05'N 135°47'E). These predators were
199 individually housed in plastic trays and fed with tadpoles every two days.
200 The induction experiment began on June 18, 2022. Each experimental tank housed
201 21 tadpoles, with seven individuals randomly selected from each of the three egg clutches.
202 Tadpoles in each tank were allocated to one of four treatments (Be group with *A. major*; Ws
203 group with *L. japonensis*; Fn group with *C. pyrrhogaster*; and the control Ct2 group) with
204 eight replicates per treatment. One predator was introduced into an isolation case in each tank.
205 Other experimental setups, i.e., tank design, room temperature, light condition, and feeding
206 frequency, were kept the same as in Experiment I.
207

208 ***Measuring Coloration and Morphological Responses.***—All tadpoles were photographed two
209 and three weeks after the start of the experiment using a Canon® EOS RP mirrorless camera.
210 Individual tadpoles were placed in a narrow acrylic aquarium (20.5 × 4.5 × 13.5 cm) with a

211 ruler and color chart in the background. To minimize the effect of posture variation among
212 individuals, multiple photographs were taken per tadpole, and the image most suitable for
213 measurement was selected. Each trait was measured once per individual. The distance
214 between the aquarium and the camera was kept constant for all individuals. All photos were
215 taken with a F/16 aperture, a 1/20th second exposure time, and ISO 3200. Consistency in
216 artificial lighting conditions was maintained for individuals photographed on the same day,
217 with the white balance manually set and standardized at 4600 K. Some individuals were
218 excluded from the analysis due to poor nutritional condition or significant tail loss during
219 rearing. The exclusion criteria included: (1) head and body length less than 6 mm, (2) severe
220 tail damage or loss, and (3) inability to maintain posture in water.

221 Tail fin parts, defined as all tail areas excluding the central tail muscle, were trimmed
222 manually using the image measuring software, Natsumushi version 1.12.2 (Tanahashi and
223 Fukatsu, 2018; https://sites.google.com/site/mtahashilucanid/program/natsumushi) and
224 selected for color measurement. The color of the tail was quantified using the average of
225 CIELAB values (CIE 1976) calculated in Adobe Photoshop 25.5.1 (Adobe Inc.). In the
226 CIELAB color space, color was represented using three components: the lightness component
227 L* and the color components a* and b*. The L* component ranged from black to white
228 between 0–100, while a* and b* components ranged from green to red and blue to yellow,
229 respectively, on a scale of −128 to 127. As Photoshop displays images in 8-bit format, these
230 values were rescaled to range from 0 to 255. Therefore, L* was multiplied by 100/255 and a*
231 and b* were adjusted by subtracting 128, respectively, to facilitate analysis (Fig. 1). Due to
232 incorrect white balance, photographs taken at the second week of Experiment II were
233 excluded from the analysis of tail coloration. During the third week of Experiment II,
234 photographs of 11 individuals from the Ct2 group were also taken with an incorrect white
235 balance, so the color tone was later corrected using Photoshop. We used the “Match Color”

function in the “Image Adjustments” menu, referencing one of the other photographs from the Ct2 group that was taken under the same conditions, with the white balance properly set to 4600 K. Additionally, morphological measurements were conducted based on the photographs using Natsumushi. Morphological traits included tadpole body length (BL), tail length (TAL), and tail depth (TD) were measured using the scale within each image.

Statistical Analyses.—All statistical analyses were conducted in R version 4.3.2 (R Core Team, 2023). Linear mixed models (LMMs) were employed based on the measured traits, using the lmerTest package (Kuznetsova et al., 2017) to estimate differences between the control group and the other treatments. The experimental tank was treated as a random factor in the LMMs. When color components (e.g., a* and b*) were utilized as the response variables, the treatment (predator) was used as the explanatory variable in the models to compare the treatment groups to the control group. In the same way, when body length (BL) was utilized as the response variables, the treatment was used as the explanatory variable in the models. On the other hand, in analyses for TAL and TD as the response variable, four models were constructed for each trait. These models included: the model with only BL as the explanatory variable, the model with only the treatment as the explanatory variable, the model with both BL and treatment as explanatory variables, and the model incorporating BL, treatment, and their interactions. The final model was determined via backward elimination, by applying likelihood ratio (LR) tests. The significance level was set at 5%. If a significant difference was found between the former and reduced models, the former model was adopted as the final model for further analysis.

After constructing final models, we evaluated the overall effect of predator treatment by performing ANOVA via the anova function in the lmerTest package. Subsequently, comparisons between each treatment group and the control were performed using Dunnett’s

test with estimated marginal means calculated by the emmeans package (Lenth, 2025). Marginal means were derived under the asymptotic framework. The significance level was set at 5%, with treatment groups considered significantly different from the control group if the *P* values were below this threshold. Orange tail coloration and increased tail depth were reported as predator-induced traits in previous studies (McCollum and Van Buskirk, 1996; Touchon and Warkentin, 2008), so we decided to focus primarily on b* values and tail depth. To evaluate the covariation between tail coloration and morphology at the third week of the experiment, we also conducted Pearson's correlation tests between residuals of b* values and tail depth (TD), after removing the effects of body length (BL) via linear regression. This analysis was performed separately for each predator treatment that showed a significant difference from the control group in either trait.

RESULTS

Coloration.—Experiment I showed that predator presence induced changes in tadpole tail coloration. At the second week, the overall effects of predator treatment were significant for both a* and b* values (a*: $F = 11.41$, $P < 0.001$; b*: $F = 12.05$, $P < 0.001$). The response was strongest with *Anax nigrofasciatus* (Ax group), where tadpoles developed significantly more orange tails than controls, as indicated by a consistent decrease in a* and increase in b* values (Dunnett's test, $P < 0.05$; Table 1; Fig. 2). At the third week, a significant overall effect of predator treatment remained only in b* values ($F = 11.31$, $P < 0.001$), not in a* values ($F = 2.18$, $P = 0.096$). Nevertheless, tadpoles in the Ax group still showed significantly lower a* values ($P = 0.028$) and higher b* values ($P < 0.001$) than controls. A few individuals in the Ax group exhibited particularly high values in both a* and b*, corresponding to intensely vivid orange tails. These data points likely represent the upper range of predator-induced color

286 expression, rather than artifacts. A weaker, yet still significant, effect was observed with
287 tadpoles exposed to *Orthetrum albistylum speciosum* (Or group), which showed elevated b*
288 values compared to the control ($P = 0.045$). In the tadpoles with *Pantala flavescens* (Pf
289 group), a similar trend was noted, but the differences from the control group were not
290 statistically significant ($P = 0.143$ in b* value). In contrast to the results of Experiment I,
291 overall treatment effects in Experiment II were not significant (a*: $F = 0.49$, $P = 0.689$; b*: F
292 $= 1.84$, $P = 0.166$), and no group showed significant differences from the Ct2 group in the a*
293 or b* values at the third week (Table 2; Fig. 3).

294

295 ***Morphological Responses.***—In Experiment I, predators had minimal effects on body length
296 (BL). The overall effect of predator treatment on BL was not statistically significant at either
297 week (Week 2: $F = 2.35$, $P = 0.075$; Week 3: $F = 2.35$, $P = 0.074$), although a weak trend was
298 observed. Tadpoles exposed to *P. flavescens* (Pf) developed slightly larger bodies than
299 controls at the second week (Dunnett's test, $P = 0.048$), but this difference was no longer
300 significant at the third week (Table 3; Fig. 4). Similarly, although predator treatment had a
301 significant overall effect on tail length, its effects were limited to certain groups or time
302 points. When tail length was used as the response variable, models with body length and
303 treatment as explanatory variables were selected as the final models at both the second and
304 third weeks after the start of the experiment. In both models, tail length showed a strong
305 positive association with body length ($P < 0.001$), indicating that larger tadpoles tended to
306 have longer tails. In these models, predator treatment had a significant overall effect at both
307 the second week ($F = 6.76$, $P < 0.001$) and the third week ($F = 3.28$, $P = 0.023$). Independent
308 of BL, tadpoles in the Pf and *A. nigrofasciatus* (Ax) groups had shorter tails than controls at
309 the second week ($P < 0.001$), but not at the third week. In contrast, tadpoles exposed to

310 *Gynacantha japonica* (Gy group) showed reduced tail length only at the third week (P =
311 0.005).

312 The impact of predator treatment on tail depth was more pronounced. When tail
313 depth was used as the response variable, BL and treatment were chosen as explanatory
314 variables at both the second and third weeks after the start of the experiment. In both models,
315 tail depth also showed a strong positive association with BL ($P < 0.001$), and the effects of
316 predator treatments were interpreted independently of body size. Predator treatment had a
317 strong overall effect on tail depth at both the second week ($F = 4.86$, $P = 0.003$) and the third
318 week ($F = 9.60$, $P < 0.001$). Post-hoc tests revealed that tadpoles in the Ax group developed
319 deeper tails than controls at both time points ($P < 0.01$). Moreover, tadpoles in the Pf group
320 also developed deeper tail fins than controls at the third week ($P = 0.022$). A similar trend was
321 observed in the *O. albistylum* (Or) group, although this difference did not reach statistical
322 significance ($P = 0.113$).

323 In Experiment II, predators had minimal effects not only on BL, but also on tail
324 morphology. Predator treatment had no significant effect on BL (Week 2: $F = 1.82$, $P = 0.168$;
325 Week 3: $F = 2.18$, $P = 0.118$). For tail length, models including both BL and treatment as
326 explanatory variables were chosen at the second week. In this model, tail length showed a
327 strong positive association with BL ($P < 0.001$), and predator treatment had a significant
328 overall effect ($F = 7.43$, $P < 0.001$). Post-hoc tests revealed that a significant reduction in tail
329 length was observed in the Ferocious water bug (Be) group ($P < 0.001$; Fig. 5). At the third
330 week, however, models with only BL were selected, indicating that the effect of the treatment
331 on tail length appeared limited. Similarly, for tail depth, models with only BL were selected in
332 both weeks, and tail depth was positively associated with body length ($P < 0.001$). Post-hoc
333 tests also confirmed that tail depth was largely unaffected, with no significant differences

observed. Thus, tadpoles in all non-odonate predator treatments were not induced to develop significantly deeper tail fins compared to the control group.

The orange coloration and deeper tail morphology tended to be co-expressed within individuals. Significant, though weak, positive correlations between b* values and tail depth were detected in all three odonate predator treatments that induced changes in either trait by the third week (Pf: $r = 0.393$, $P < 0.001$; Ax: $r = 0.294$, $P = 0.003$; Or: $r = 0.277$, $P = 0.011$).

DISCUSSION

Tail Coloration in Asian Species of Dryophytes.—Our experiments revealed that tadpoles of *Dryophytes leopardus* developed induced changes in tail coloration as well as tail shape in response to dragonfly nymphs. Although tail coloration has rarely been documented in this species or in its close relative, *Dryophytes japonicus*, its occurrence has been reported from several locations in Japan, including Hokkaido, Shiga, Hyogo, Hiroshima, Shimane, Kouchi and Ehime prefectures (Doi et al., 2005), as well as from our collection site in Kyoto (Noda, personal observation). The phenotypes induced by *Anax nigrofasciatus*, i.e., deep tail fins and bright orange tails, closely resemble those previously observed in other species in *Dryophytes* in the New World, Cope's Gray Treefrog (*Dryophytes chrysoscelis*; McCollum and Van Buskirk, 1996), Pine Woods Treefrog (*Dryophytes femoralis*; LaFiandra and Babbitt, 2004), and Gray Treefrog (*Dryophytes versicolor*; Van Buskirk and McCollum, 1999). Thus, our findings represent the first documented case of a predator-induced tail coloration phenotype in Asian species of *Dryophytes*.

Our discoveries of the plasticity in the tail coloration in the Asian species of *Dryophytes* suggests that the trait is symplesiomorphically shared between the North American and ancestral Asian lineages, and has been inherited by present-day Asian species,

359 including *D. leopardus*. An ancestral stock of *Dryophytes* is inferred to have migrated from
360 North America to East Asia across the Bering Land Bridge during the Miocene (Duellman et
361 al. 2016; Dufresnes et al., 2016). Present-day Asian species of *Dryophytes* are monophyletic,
362 having diversified from this ancestral stock. It is plausible that tail coloration has persisted as
363 a phenotypic plasticity in both North American and East Asian species of *Dryophytes* due to
364 the consistent importance of dragonfly nymphs as predators.

365 It remains unknown whether tail coloration can be induced in the three other Asian
366 species, *Dryophytes flaviventris*, *Dryophytes immaculates*, and *Dryophytes suweonensis* (see
367 Borzée et al., 2020). In North America, Pine Barrens Treefrog, *Dryophytes andersonii* are
368 induced to have a darker tail, not an orange tail, by *Anax junius*, which induces orange tails in
369 other species of *Dryophytes* (Kruger and Morin, 2020). Furthermore, Squirrel Treefrog,
370 *Dryophytes squirellus* exhibits no morphological changes in response to odonate species
371 (McCoy and Bolker 2008; Baker, 2021). These cases indicate that the plastic responses in
372 *Dryophytes* have diversified, and some may have lost the ability to develop orange tails.
373 Future studies are needed to elucidate the interspecific variation in plastic responses to
374 predation risk and the evolutionary causes underlying this variation.
375

376 ***Differences in the Extent of Coloration by Predators.***—Our experiments demonstrated that
377 the extent and timing of induced responses varied depending on the dragonfly species reared
378 with tadpoles. Two weeks after the start of the experiment, nymphs of *A. nigrofasciatus*
379 induced both deep fin and tail coloration in tadpoles. Additionally, by the third week, similar
380 but weaker responses were observed in the other predator treatments: *Pantala flavescens* also
381 induced deep tail and *Orthetrum albistylum speciosum* induced tail coloration. Furthermore,
382 although weak, some tadpole individuals exposed to *P. flavescens* showed tail coloration, and
383 those exposed to *O. albistylum speciosum* also tended to have deeper tails. These results

384 indicate that the defense traits (coloration and deep tail) were induced by the nymphs of *A.*
385 *nigrofasciatus* more rapidly and strongly, but similar responses were also elicited by the other
386 two predator species. Furthermore, the significant positive correlation observed between the
387 b* value and tail depth suggests that these traits tended to be co-expressed in a coordinated
388 manner. These findings support the idea that the expression of these anti-predator defenses is
389 governed by a shared, integrated strategy, whose intensity varies depending on predator
390 identity.

391 The differences in the degree and rate of responses induced by different dragonfly
392 species may reflect varying levels of predation risk for tadpoles posed by each species. Plastic
393 phenotypes with significant tail coloration should result in trade-offs because of energetic
394 costs associated with synthesizing pigmentation (Grether et al., 2001) or costs due to the
395 increased vulnerability to mismatched predators. Consequently, tadpoles are expected to
396 minimize these costs by developing effective defense traits for each predator as much as
397 possible based on the perceived predation risk (Touchon and Warkentin, 2008; Innes-Gold et
398 al., 2019). The predation risk for tadpoles would be different among dragonfly species
399 according to (1) the foraging style of dragonfly nymphs, (2) their color vision, and (3) the
400 total amount of intake related to their body size and occurrence period.

401 Firstly, the four dragonfly species used in our experiment exhibit different foraging
402 styles. According to the classification by Corbet (1999), *A. nigrofasciatus* and *G. japonica* are
403 climbers, sit-and-wait predators on aquatic vegetation, while *P. flavescens* is a sprawler,
404 foraging while moving along the bottom, and *O. albistylum speciosum* is a burrower, residing
405 within the substrate and ambushing prey. For sit-and-wait predators, there is often a time lag
406 between identifying prey and launching an attack, during which they can choose which part of
407 tadpole's body to target. Therefore, the lure effect of tadpole tails is likely to attract ambush
408 predators more effectively than those that actively chase their prey. In addition, as *A.*

409 *nigrofasciatus* prefers habitats abundant in floating-leaved plants (Ozono et al., 2021), it can
410 move freely along the plant structures from roots anchored at the bottom to leaves extending
411 above the water surface. As a result, the risk of predation on tadpoles could be present
412 throughout the entire water column, from the bottom to the surface, making behavioral
413 responses like moving to a specific depth insufficient as a means of avoiding predation.
414 Conversely, *P. flavescens* and *O. albistylum speciosum* primarily forage at the bottom, so
415 tadpoles may evade these predators by relocating to higher water layers. However, such
416 avoidance behavior often leads to a decrease in growth rate due to reduced foraging efficiency
417 (Teplitsky et al., 2003); i.e., for tadpoles that primarily feed on detritus, avoiding the bottom
418 layer may result in malnutrition. The combination of moderate morphological responses with
419 behavioral responses (the "double defense" strategy; Dewitt and Hucko, 1999) may help
420 mitigate the impact of malnutrition from behavioral changes while minimizing the costs of
421 defensive traits.
422 Secondly, differences in eyesight, particularly color vision among dragonfly species,
423 may influence the extent of the tail coloration changes in the tadpoles. Pritchard (1966) noted
424 a correlation between foraging habit and compound eye size in dragonfly nymphs, with
425 climbers typically possessing larger compound eyes compared to sprawlers and burrowers. He
426 also stated that the climbers, such as species in *Anax*, rely heavily on vision for prey
427 detection, whereas other foraging types depend more on tactile stimuli. Hence, it is plausible
428 that the lure effect of the brightly colored tails is more effective for climbers, which are more
429 visually oriented. Furthermore, Futahashi et al. (2015) investigated the genetic basis of color
430 vision in dragonflies and redocumented the number of opsin genes and the characteristic
431 spatiotemporal expression patterns in the nymphs of various species, including those of the
432 burrower *O. albistylim* and the climber *A. parthenope*, a species closely related to *A.*
433 *nigrofasciatus* used in our experiment. They found that dragonflies possess a greater number

434 of opsin genes than other insects, and *A. parthenope* possesses and expresses a greater number
435 of opsin genes, particularly those sensitive to long-wavelength light. Although the functions
436 of these diverse genes are not fully understood, this suggest that species of *Anax* may have
437 enhanced abilities to perceive reddish colors, potentially enabling tadpoles with bright orange
438 tails to more effectively avoid predation by using their tails as lures. Thus, the larval
439 antipredator defense of *D. leopardus*, which involves tail coloration, may have been driven by
440 the sensory systems of predatory dragonfly nymphs, and subsequently evolved specifically
441 against color-sensitive predators, represented by dragonfly nymphs of *Anax*.
442 Thirdly, the variation in the degree of induced responses may reflect the relative
443 predation risks associated with body sizes or occurrence periods of respective odonate
444 predators. In nature, it is generally difficult to determine which predators exert higher
445 predation pressure on tadpoles, and few studies have investigated such differences. However,
446 during the rearing process, we observed that the nymphs of *A. nigrofasciatus* exhibited higher
447 food demands and displayed more frequent attacks on tadpoles compared to other species.
448 Additionally, they are approximately twice the size of the nymphs of *P. flavescens* and *O.*
449 *albistylum speciosum* (see Ishida et al., 1988), suggesting that size-related predation pressures
450 could influence the necessity and effectiveness of tadpole morphological responses. However,
451 tadpoles did not exhibit significant responses to *G. japonica*, which is similar in size to *A.*
452 *nigrofasciatus* (see Ishida et al., 1988). This indicates that tadpoles likely respond not only to
453 size-related predation pressure but also to other characteristics of dragonfly species, such as
454 foraging style or habitat overlap, thereby modulating the extent of induced responses. Since
455 the habitat of nymphal *G. japonica* overlaps with that of tadpoles of *D. leopardus* (Nakagawa
456 et al., 2004), the lack of an apparent induced response may be due to the limited period in
457 which they co-occur. This species grows more rapidly compared to other species in Aeshnidae
458 in Japan, occurring in aquatic environments only from May to August (Ozono et al., 2021).

459 Considering that the nearly-metamorphosing nymphs cease foraging, the period during which
460 this species poses a predation threat to *D. leopardus* is even shorter. This brief coexistence
461 period suggests that this dragonfly species may not be an important predator for the tadpoles.
462 In addition to the tail coloration and depth we focused on, a tendency toward larger
463 body lengths and shorter tail lengths compared to those in the control group was observed,
464 although not statistically significant in most treatment groups. These changes may represent
465 general responses to predator treatments, as noted in previous studies. The increase in body
466 length could be attributed to enhanced availability of food resources resulting from leftovers
467 and feces of caged predators (e.g., McIntyre et al., 2004; Richardson, 2006). Similarly, shorter
468 tail lengths in the presence of predators have been reported in several frog species (Laurila et
469 al., 2002; LaFiandra and Babbitt, 2004).
470
471 ***Responses Toward Non-Odonate Predators.***—Tadpoles of *D. leopardus* showed neither
472 conspicuous tail coloration nor deep fin when exposed to newts or aquatic insects other than
473 dragonfly nymphs in our experiment, likely because the costs of expressing these defenses
474 outweighed their benefits for avoiding predation. Although Experiment II did not include a
475 positive control (i.e., treatment with dragonfly nymphs, such as *A. nigrofasciatus*), we believe
476 that the absence of color induction was unlikely caused by experimental failure, because
477 preliminary experiments demonstrated the high reproducibility of the induced responses when
478 using dragonfly nymphs. Therefore, our results suggest that changes in body color and tail
479 depth are not strongly induced by these predators and may not serve as effective predation
480 avoidance strategies against them. An important point in understanding why tadpoles did not
481 show tail coloration is that defensive traits come with physiological costs and may increase
482 predation pressure from non-focal predators. If changes in tail color and shape do not

483 significantly contribute to non-odonate predator avoidance, tadpole should minimize these
484 disadvantages by refraining from developing these antipredator defenses.

485 Given that the non-odonate predators do not target specific body parts of their prey
486 during attacks, the strategy of using colored deep tails as lures may be ineffective and provide
487 only limited benefits against them. For instance, diving beetles, water bugs, and water
488 scorpions capture prey by grasping the entire body with their arms, so the tails of tadpoles
489 should not serve as lures for them. Additionally, they use tactile, visual, and olfactory senses
490 for locating prey (Babbitt and Jordan, 1996; Kehr and Gómez, 2009; Mogali et al., 2019;
491 Nowińska and Brożek, 2019), and possess well-developed organs for detecting auditory and
492 mechanical signals (Yager, 1999; Cocroft and Rodríguez, 2005). Previous studies have
493 reported predator-induced changes in larval morphology in response to water bugs (Benard,
494 2006; McCoy, 2007; Michel, 2012), but few have reported effects on larval tail coloration
495 (McIntyre et al., 2004; Touchon and Warkentin, 2011). Similarly, newts and salamanders, both
496 as larvae and adults, are active foragers that swallow their prey whole like fish (Wilson et al.,
497 2005; Kishida and Nishimura, 2007). Consequently, the tails of the tadpoles are unlikely to
498 function as a lure for these predators. Newts tend to rely on olfactory cues as well as vision
499 for food recognition (Copeland, 1913; Lindquist and Bachmann, 1982; Schwarz et al., 2020).
500 Few studies have reported that tadpoles exhibit induced defensive traits in response to these
501 predators, and none have documented induced changes in body coloration (Kishida and
502 Nishimura, 2004; Hettyey et al., 2011; Urban et al., 2017). These facts support the hypothesis
503 that the brightly colored tails of *D. leopardus* do not function as lures against the non-odonate
504 predators, and that, as a result, tadpoles do not express costly defensive traits in response to
505 them.

506 The lack of induction of defensive phenotypes may also be attributed to
507 physiological costs associated with developing tail coloration, although the magnitude of such

508 costs in tadpoles remains poorly understood. Since most animals are unable to biosynthesize
509 carotenoids, the pigments thought to be causing the bright orange coloration, they metabolize
510 and accumulate these pigments by absorbing them from their diet (Bertrand et al., 2006;
511 Thibaudeau and Altig, 2012). Thus, the allocation costs of expressing coloration by
512 transporting these pigments may not be as high as commonly assumed. On the other hand, the
513 potential for increased vulnerability to non-focal predators may also interfere with the
514 expression of defensive traits (Innes-Gold et al., 2019). This disadvantage may sometimes
515 outweigh the energetic costs of expressing a defensive phenotype. Carfagno et al. (2011)
516 found that tadpoles of *Acris* exhibit darker a tail spot, which is considered a defensive trait
517 against dragonfly nymphs, even in the absence of predators. However, the presence of fish
518 predators induces the loss of this spot. This suggests that the cost of expressing defensive
519 traits may be lower than typically expected, and the disadvantage of increased vulnerability to
520 mismatched predators may prevent these phenotypes from being constitutively expressed.
521 Similarly, in tadpoles of *D. leopardus*, the elevated risk of predation from non-odonate
522 predators, considered non-focal predators, due to expressing bright tail coloration, rather than
523 its energetic cost, could explain why they did not exhibit deep and brightly-colored tail in
524 response to these predators. These defense traits against dragonfly nymphs may not only be
525 ineffective in preventing predation by non-odonate predators but could also increase the
526 potential vulnerability to them. For example, the deepened tail may increase the vibrations
527 produced during swimming, thereby heightening predation pressure on tadpoles by tactile or
528 olfactory predators (i.e., water bugs, water scorpions, and newts). Additionally, for other
529 predators such as fish, tail coloration may make the tadpoles more vulnerable by increasing
530 their visibility. However, further experiments are warranted to assess and compare the
531 effectiveness of these defense traits toward each predator, including evaluating how tadpole
532 coloration influence their survival rate in the presence of various aquatic predators.

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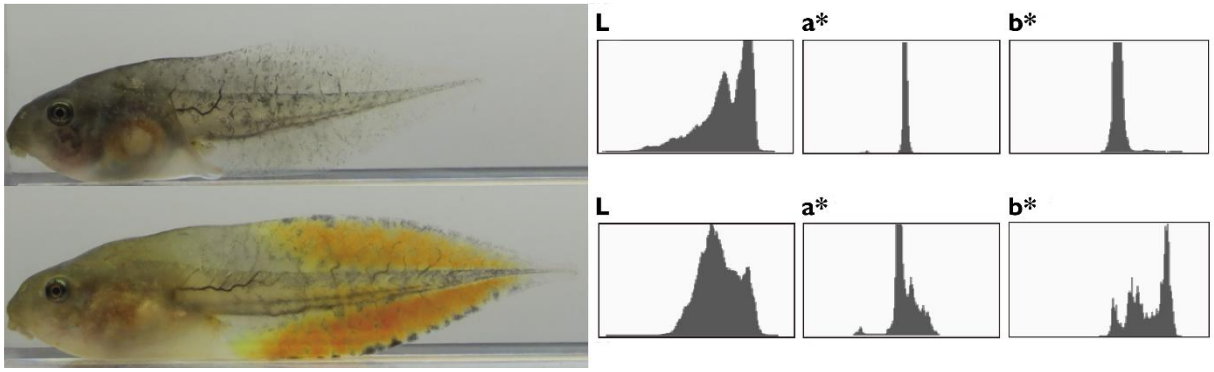

Fig. 1. Photographs of tadpoles of *Dryophytes leopardus* reared without predator (top) and with *Anax nigrofasciatus* (bottom) and their corresponding Photoshop Lab color histograms. The mean values of tail coloration are L = 61.2, a* = -2.5, and b* = 7.96 (without predator), and L = 57.96, a* = 1.75, and b* = 35.51 (with *A. nigrofasciatus*), respectively.

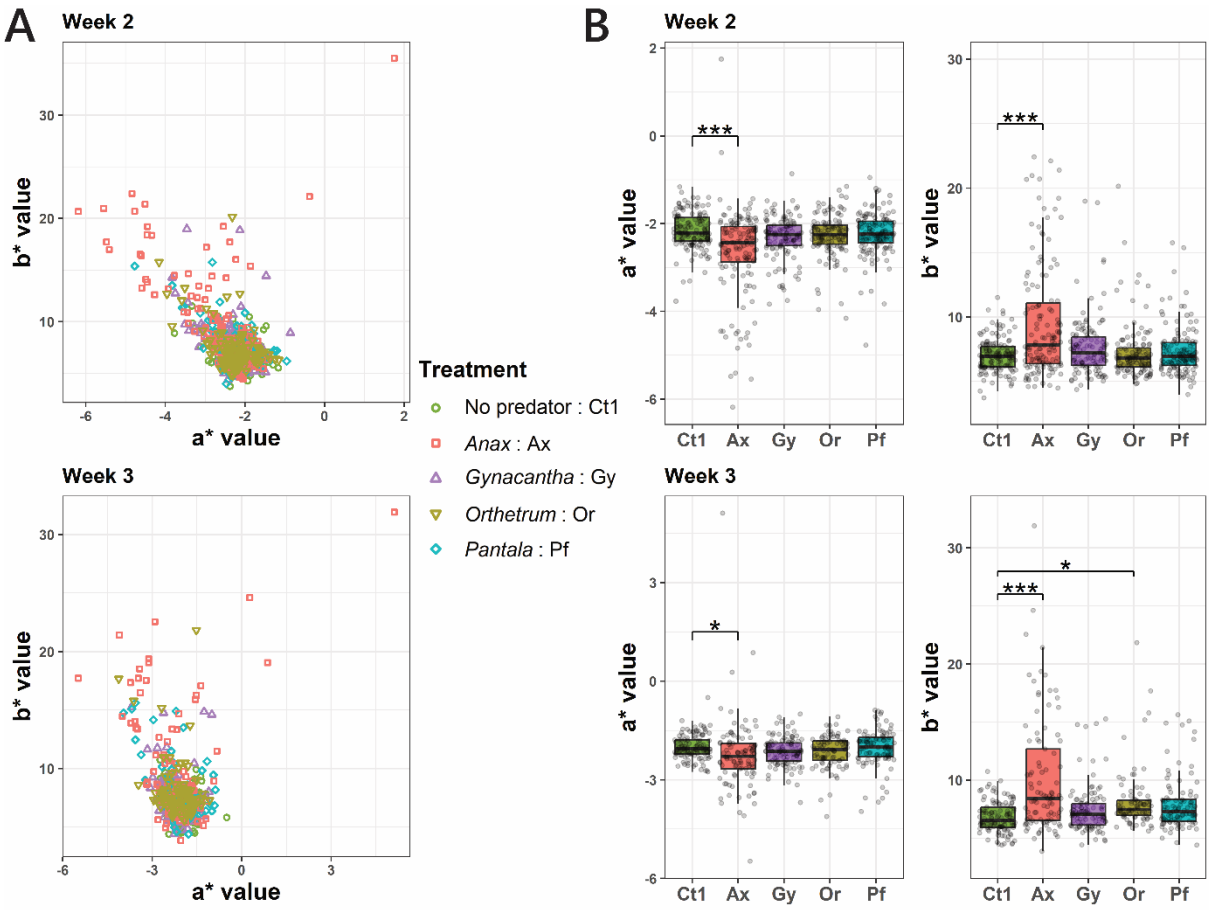

Fig. 2. (A) Scatterplots of tail coloration parameters (a* and b* values) of tadpoles two weeks (top) and three weeks (bottom) after the start of Experiment I, using different dragonfly species. Tadpoles were reared in the presence of no predators (the control group; Ct1), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). (B) Box-and-whisker plots displaying the range of the tail color traits (a* and b* values) two weeks (top) and three weeks (bottom) after the start of Experiment I. The asterisks in the figure indicate that there were significant differences compared to the control group, which were led by Dunnett's test (see Materials and Methods). *$P < 0.05$, ***$P < 0.001$.

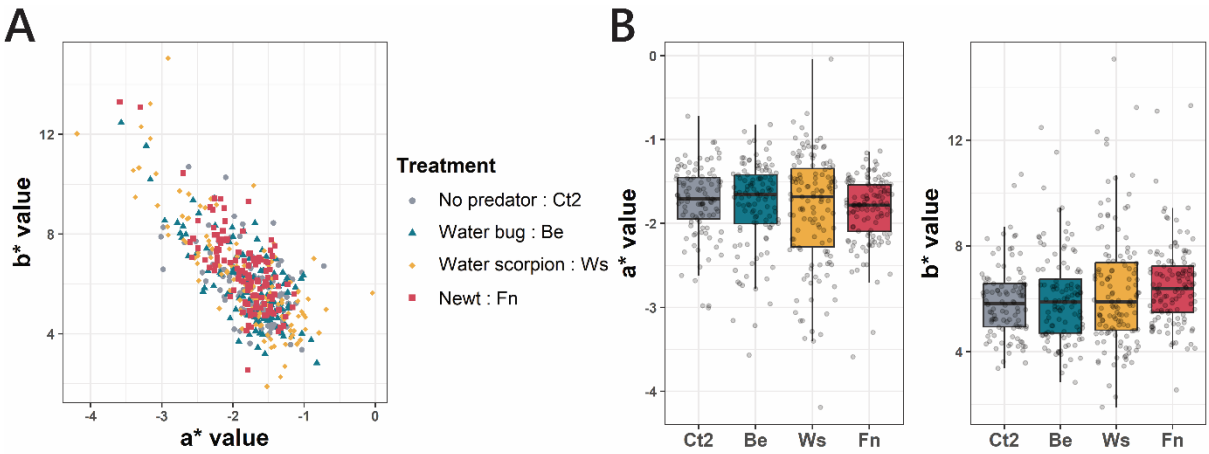

Fig. 3. (A) Scatterplots of tail coloration parameters (a* and b* values) of tadpoles three weeks (bottom) after the start of Experiment II, using non-odonate species. Tadpoles were reared in the presence of no predators (the control group; Ct2), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). (B) Box-and-whisker plots displaying the range of the tail color traits (a* and b* values) three weeks after the start of Experiment II.

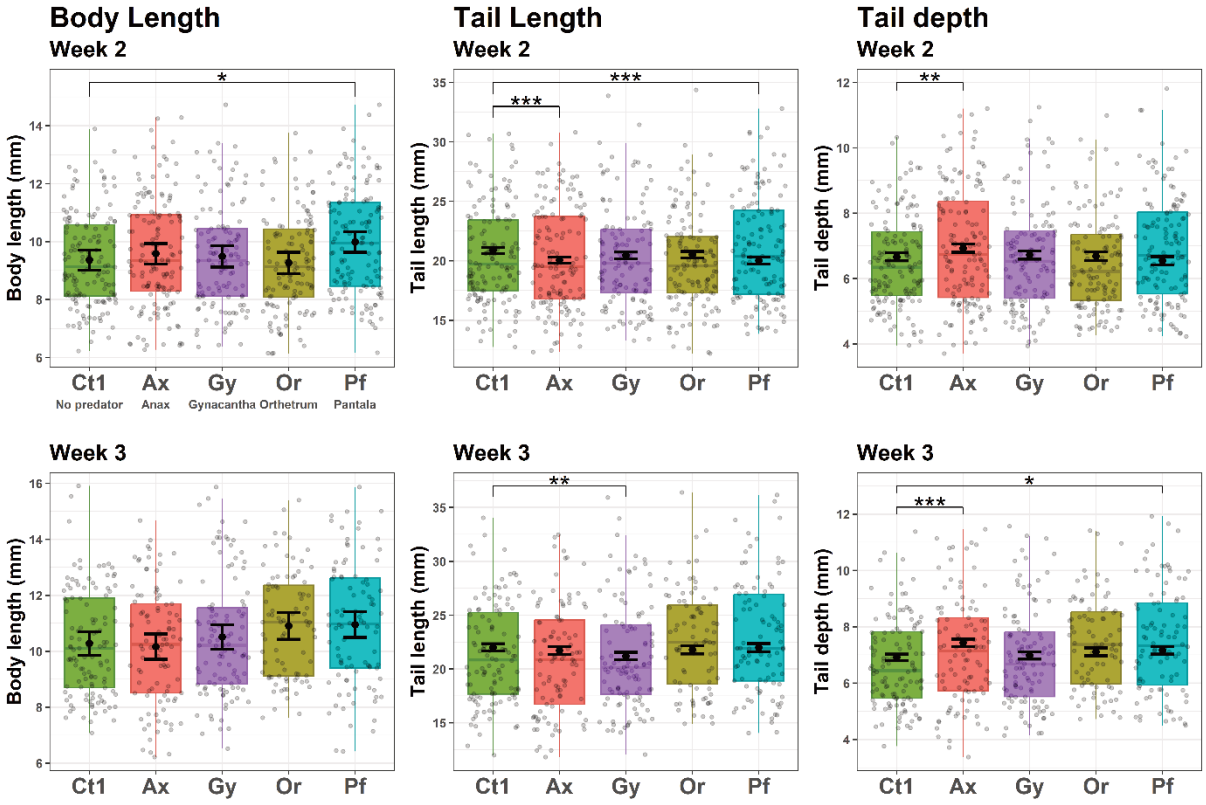

Fig. 4. Box-and-whisker plots showing the distribution of tadpole morphological traits, BL (body length), TAL (tail length), and TD (tail depth) in Experiment I, using different dragonfly species. Tadpoles were reared in the presence of no predators (the control group; Ct1), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). Boxplots represent the raw data distributions, while black points and error bars indicate estimated marginal means and 95% confidence intervals derived from linear mixed-effects models (see Materials and Methods). The asterisks in the figure indicate that there were significant differences compared to the control group, which were led by Dunnett's test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

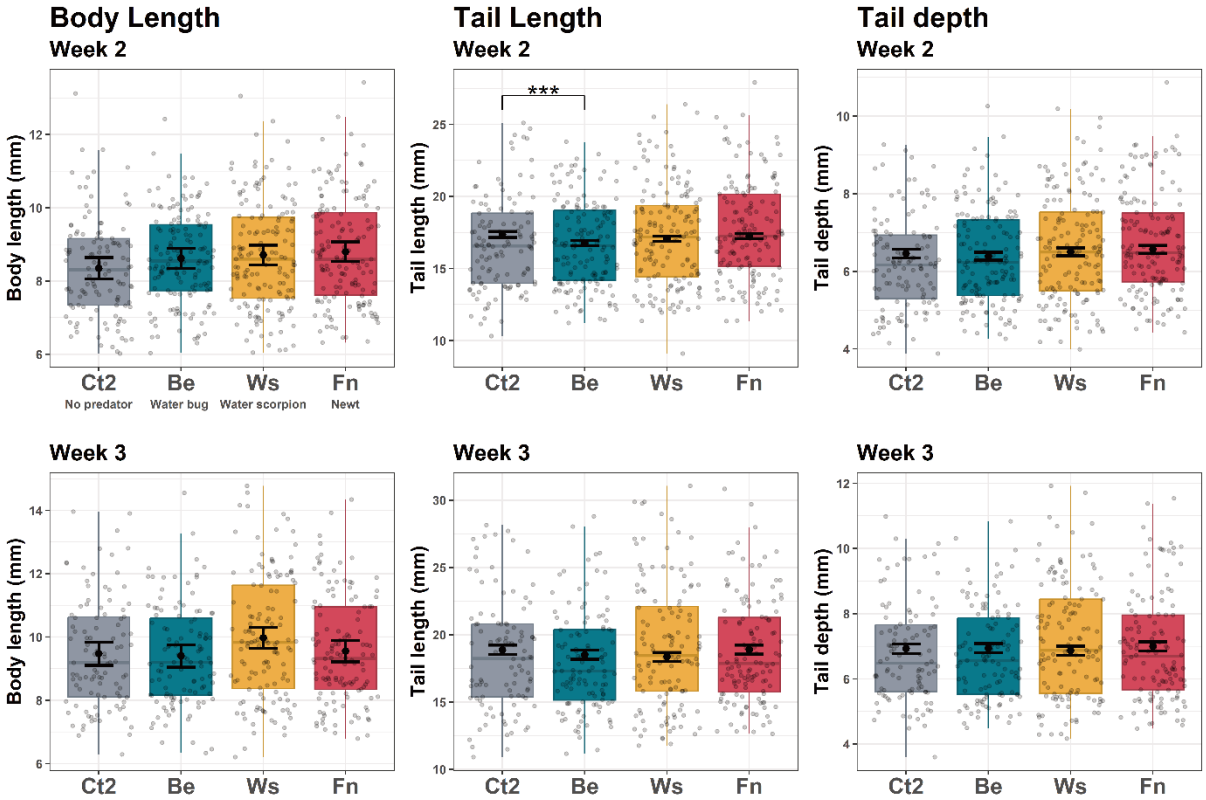

Fig. 5. Box-and-whisker plots showing the distribution of tadpole morphological traits, BL (body length), TAL (tail length), and TD (tail depth) in Experiment II, using non-odonate predators. Tadpoles were reared in the presence of no predators (the control group; Ct2), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). Boxplots represent the raw data distributions, while black points and error bars indicate estimated marginal means and 95% confidence intervals derived from linear mixed-effects models (see Materials and Methods). The asterisk in the figure indicates a significant difference compared to the control group based on Dunnett's test. ***P <0.001.

850 **Table 1.** Summary of model results for tail coloration (a* and b* values) measured at Week 2
851 and Week 3 after the start of Experiment I. Tadpoles were reared in the presence of no
852 predators (the control group), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy),
853 *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). Fixed effects include the
854 corresponding estimates (Est.), standard errors (SE), degrees of freedom (df), t-values (*t*), and
855 model P-values (P_m). Post hoc comparisons were performed using Dunnett's test to compare
856 each predator treatment to the control group (P_D). Variance (Var.) and standard deviation
857 (SD) for the random effect (Tank) and residuals are also shown. The control group served as
858 the reference level in all models.

a* value

Week 2

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–2.158	0.052	32.153	–41.753	<0.001	
Ax	–0.462	0.074	33.245	–6.256	<0.001	<0.001
Gy	–0.157	0.076	37.975	–2.062	0.046	0.129
Or	–0.112	0.076	37.689	–1.462	0.152	0.392
Pf	–0.074	0.075	34.351	–0.991	0.329	0.689

Random Effect	Var.	SD
Tank	<0.001	0.009
Residual	0.378	0.615

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–1.998	0.063	25.632	–31.725	<0.001	
Ax	–0.247	0.093	26.952	–2.659	0.013	0.028
Gy	–0.141	0.091	27.399	–1.558	0.131	0.338
Or	–0.145	0.097	32.740	–1.495	0.145	0.373
Pf	–0.035	0.094	26.841	–0.369	0.715	0.966

Random Effect	Var.	SD
Tank	0.002	0.043
Residual	0.429	0.655

b* value

Week 2

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	7.048	0.296	31.896	23.786	<0.001	
Ax	2.568	0.422	32.707	6.083	<0.001	<0.001
Gy	0.620	0.431	35.535	1.439	0.159	0.405
Or	0.219	0.431	35.478	0.507	0.615	0.929
Pf	0.299	0.425	33.461	0.705	0.486	0.849

Random Effect	Var.	SD
Tank	0.269	0.519
Residual	7.679	2.771

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	6.798	0.363	28.806	18.712	<0.001	
Ax	3.398	0.532	31.019	6.391	<0.001	<0.001
Gy	0.658	0.520	30.095	1.266	0.215	0.513
Or	1.355	0.543	34.275	2.494	0.018	0.045
Pf	1.083	0.538	29.914	2.012	0.053	0.143

Random Effect	Var.	SD
Tank	0.500	0.707
Residual	7.967	2.823

859

860

Table 2. Summary of model results for tail coloration (a* and b* values) measured at Week 3 after the start of Experiment II. Tadpoles were reared in the presence of no predators (the control group), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). Fixed effects include the corresponding estimates (Est.), standard errors (SE), degrees of freedom (df), t-values (*t*), and model P-values (P_m). Post hoc comparisons were performed using Dunnett's test to compare each predator treatment to the control group (P_D). Variance (Var.) and standard deviation (SD) for the random effect (Tank) and residuals are also shown. The control group served as the reference level in all models.

a* value

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–1.739	0.067	32.760	–25.970	<0.001	
Be	–0.031	0.094	31.019	–0.336	0.739	0.954
Ws	–0.097	0.092	29.311	–1.053	0.301	0.578
Fn	–0.086	0.092	29.619	–0.933	0.358	0.655

Random Effect	Var.	SD
Tank	0.015	0.123
Residual	0.258	0.508

b* value

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	5.871	0.216	28.429	27.198	<0.001	
Be	0.117	0.301	26.657	0.387	0.702	0.938
Ws	0.452	0.295	25.105	1.532	0.138	0.294
Fn	0.605	0.296	25.383	2.043	0.052	0.108

Random Effect	Var.	SD
Tank	0.131	0.363
Residual	3.026	1.740

882 **Table 3.** Summary of model results for morphological traits (body length, tail length, and tail
883 depth) measured at Week 2 and Week 3 after the start of Experiment I. Tadpoles were reared
884 in the presence of no predators (the control group), *Anax nigrofasciatus* (Ax), *Gynacantha*
885 *japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). Fixed effects
886 include the corresponding estimates (Est.), standard errors (SE), degrees of freedom (df), t-
887 values (t), and model P-values (P_m). Post hoc comparisons were performed using Dunnett's
888 test to compare each predator treatment to the control group (P_D). Variance (Var.) and standard
889 deviation (SD) for the random effect (Tank) and residuals are also shown. The control group
890 served as the reference level in all models.

891

Body length

Week 2

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	9.365	0.176	29.472	53.098	<0.001	
Ax	0.221	0.251	30.283	0.879	0.386	0.757
Gy	0.128	0.257	33.237	0.499	0.621	0.932
Or	–0.098	0.257	33.152	–0.381	0.706	0.964
Pf	0.626	0.253	31.049	2.472	0.019	0.048

Random Effect	Var.	SD
Tank	0.077	0.277
Residual	3.047	1.746

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	10.283	0.214	29.421	47.990	<0.001	
Ax	–0.116	0.316	31.356	–0.367	0.716	0.967
Gy	0.233	0.308	31.190	0.757	0.455	0.824
Or	0.627	0.326	36.584	1.921	0.063	0.174
Pf	0.673	0.319	30.756	2.111	0.043	0.116

Random Effect	Var.	SD
Tank	0.082	0.288
Residual	4.089	2.022

Tail length

Week 2

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–0.410	0.352	441.321	–1.165	0.245	
Body length	2.230	0.035	639.859	64.157	<0.001	
Ax	–0.824	0.191	34.547	–4.315	<0.001	<0.001
Gy	–0.436	0.197	39.064	–2.214	0.033	0.091
Or	–0.376	0.197	38.784	–1.910	0.063	0.178
Pf	–0.860	0.194	36.388	–4.439	<0.001	<0.001

Random Effect	Var.	SD
Tank	0.006	0.079
Residual	2.416	1.554

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–2.121	0.409	316.445	–5.182	<0.001	
Body length	2.287	0.036	488.929	63.557	<0.001	
Ax	–0.307	0.257	29.473	–1.192	0.243	0.560
Gy	–0.810	0.251	29.271	–3.224	0.003	0.005
Or	–0.224	0.266	34.751	–0.842	0.405	0.778
Pf	–0.020	0.261	29.368	–0.078	0.938	0.999

Random Effect	Var.	SD
Tank	0.064	0.253
Residual	2.586	1.608

Tail depth

Week 2

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–1.229	0.141	373.721	–8.733	<0.001	
Body length	0.827	0.014	642.972	61.130	<0.001	
Ax	0.266	0.088	31.452	3.029	0.005	0.009
Gy	0.061	0.090	34.327	0.683	0.499	0.860
Or	0.025	0.090	34.238	0.280	0.781	0.983
Pf	–0.114	0.089	32.718	–1.288	0.207	0.499

Random Effect	Var.	SD
Tank	0.010	0.100
Residual	0.358	0.598

Week 3

Effect	Est.	SE	df	*t*	P_m	P_D
(Intercept)	–1.545	0.154	328.542	–10.038	<0.001	
Body length	0.801	0.014	488.464	58.376	<0.001	
Ax	0.519	0.091	27.792	5.723	<0.001	<0.001
Gy	0.068	0.089	28.102	0.763	0.452	0.821
Or	0.200	0.095	34.000	2.119	0.042	0.113
Pf	0.253	0.092	28.144	2.751	0.010	0.022

Random Effect	Var.	SD
Tank	0.004	0.061
Residual	0.380	0.617