Female polymorphism and colour variability in *Argia oculata* (Coenagrionidae: Zygoptera)

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Body colouration frequently possesses a communicative function, particularly in species with colour polymorphism and developed visual systems as odonates, and also affects the conspicuousness of animals in relation to the background. Therefore, these factors can influence colour evolution and its development. The ecology and ethology of *Argia oculata* (Hagen in Selys, 1865) were studied in three populations with different degrees of habitat alteration. Three different sources of colour variability were found: female polymorphism, female ontogenetic change and daily change in both sexes. All males observed presented the same blue thoracic colouration pattern, without ontogenetic changes. Some of them (6–48%, variable between populations) showed a darkening of the thorax colouration in the afternoon. The frequency of androchrome females (male-like thoracic colouration) showed a negative relationship with human habitat disturbance. These females changed their body colour from bluish to brownish at old ages. Gynochrome females started their adult life with sky-blue thorax, which turned to olive green and finally to brown with sexual maturation. One gynochrome female returned to the immature colouration in late hours of the day. Colour changes were apparently not associated to sunlight intensity or temperature. Behavioural observations suggest that colour variability in females might contribute to reduce or avoid male harassment. We contextualise our findings in the *Argia* literature and propose physiological and evolutionary mechanisms for their explanation.

**Keywords:** variability; recapture; mimicry; fitness; phenotype

**Introduction**

Body colouration plays an important role in the interactions within and between species with developed visual systems. The costs of colour production and maintenance are compensated by their function as a signal [1]. In addition, body colours could be affected by environmental conditions like pollution [2], altitude [3], temperature [4] and/or have a homeostatic function [5]. In an evolutionary perspective, the origin and maintenance of body colour patterns could also have evolved by sexual selection pressures, frequently associated with alternative behaviours or strategies [6,7].

Dragonflies and damselflies possess one of the most advanced visual systems among insects [8]. The capability to perceive a wide range of the visual spectrum and polarised light as well as the eye differentiation in specialised ommatidia regions, allow them an efficient recognition for flying prey and conspecifics [8,9]. A rich diversity of wing and body colour patterns is present within and between species in the order Odonata. Colour polymorphism constitutes a common phenotypic variation in odonates likely produced by sexual selection forces [10]. Frequently, colour patterns are associated to behaviours that reinforce the differences between colour morphs (e.g. [11]). Studies about colour polymorphism in odonates were principally focused in coenagrionids and calopterygids of the northern hemisphere (e.g. [12–14]), while few studies have analysed this variability in the South American tropics [15,16], where biodiversity studies are prioritary [17].

In contrast to other taxa (e.g. cephalopods; [18]), daily colour changes in hexapodes are usually slow and irreversible [19]. However, some insects like the genus *Carausius*, possess centrally controled polychromatic chromatophores which allow a rapid colour change in response to environmental stimuli and diurnal rhythms (see [19,20]). About 30 species of odonates show physiological colour change, some of them characterised by a dark phase overnigth and/or low temperatures and a light phase during the day and/or high temperatures (see [19,21–23]). The change from the dark phase towards light colours occurs rapidly and independly of the nervous system control in *Austrolestes annulosus*, while the opposite (and more slow change; 9 h) is produced in the same species by the liberation of chromatophorothopins from the terminal and ventral ganglia after temperature stimulation [20]. The response of the chromatophores towards the hormones depends on the time of the day (circadian rhythm).

It has been suggested that odonate polymorphic species are less vulnerable to changes in the environment and/or in the riparian habitat in which they dwell [24].
However, changes in the vegetation that involve habitat modification could determine fitness alterations in species with colouration-based strategies due to the spectrum change of the background (see [25]). In southern Ecuador, rivers and streams are surrounded by lowland semi-deciduous tropical forests, with a variable degree of anthropic habitat disturbance. The aims of this study were therefore to test for possible relationships between habitat disturbance and the diversity of thoracic colourations. We selected *Argia oculata* due to its high abundance in the studied area and to the colour diversity of the genus (colour polymorphism and daily colour change [21,22,26,27]). Therefore, we studied the ecology and ethology of this species in a gradient of disturbance of lowland tropical streams.

**Methods**

**Study area**

Observations were performed for 158 h between 19th June and 10th July 2016. The study was performed by sampling three streams of second order with different degrees of habitat modification inside and at the proximities of the Buenaventura Biological Reserve (Jocotoco Foundation; Piñas, El Oro, Ecuador). Transects were performed along 100–300 m covering an average of five metres around the stream. In addition, complementary transects were performed along path, rivers and streams within the reserve to record the diversity of odonates of this area. The three studied streams possessed similar physical properties (width: 1.5–2.1 m; depth: 10–25 cm; and flow speed: 0.25–0.5 m/s). The substrate was composed by sand and rocks, frequently interrupted by small waterfalls. Among other attributes, these streams differed in the management or the area due to their location inside or outside the reserve. The stream located inside the reserve (thereafter, protected population; 3° 39′ 24.6852″ S, 79° 46′ 18.318″ W; 456 m of altitude) possessed natural riparian vegetation and dense native trees that intercepted the direct sunlight over most of the stream. The stream belongs to the Arenillas watershed [28] and ends in the Río Arenillas, the principal river of the reserve parallel to the 585 road (Saracay–Piñas). The weather was generally cloudier and wetter inside the reserve. The stream located in the limit of the reserve (thereafter, mixed population; 3° 39′ 10.962″ S, 79° 48′ 1.782″ W; 307 m) possessed native riparian vegetation and dense native trees that intercepted the direct sunlight over most of the stream. The stream flows over pastureland and a teak plantation (*Tectona grandis*). The sunlight arrived more directly to the surrounding areas of the stream, which ends in the other edge of the same above-mentioned river. The last stream was located outside the reserve in Moromoro village (thereafter, modified population; 3° 40′ 45.1992″ S, 79° 44′ 14.1288″ W; 880 m). The selected stream ends in Moromoro River and flows through sugar cane plantations (*Saccharum officinarum*). Large areas were illuminated directly by the sun in this stream. The protected population was at 3.23 km from the mixed population and at 4.57 km from the modified population. The other two populations were 7.61 km apart.

**Population frequencies**

A mark-recapture study was performed to determine frequencies, dispersion and survivorship of both sexes and to follow colour patterns over time. Individuals were captured with an aerial entomological net, marked in the right hindwing with a unique code number and photographed. Recaptures were performed by naked eye or by collecting the individuals. As in Bick and Bick [29], we did not study colour changes in tenerals due to their susceptibility to damage by handling. The age of the specimens was determined assessing their body brightness, the wing hardening and the abdominal thickness in females. Tandems were interrupted when marking due to the necessity to obtain a large number of marked individuals to analyse the colour proportions. This fact probably affected the number of matings observed.

The estimation of survival (Phi) and recapture (p) probabilities was done by fitting Cormack–Jolly–Seber (CJS) models via numerical maximum likelihood techniques in MARK 8.0 [30]. Each population was analysed separately because observations were performed in different days and no movements between them were recorded. Sex (g), time (t) and their interaction were included as factors of the models. The goodness of fit of the saturated model was estimated by means of the programme RELEASE from within MARK. We could calculate TEST 2 (similar catchability of marked individuals), which was not significant, but not TEST 3 (similar survivorship for animals alive at a given occasion, on the next sampling day) due to the low recapture rate. The extra-multinomial variance factor (c-hat) was estimated from the saturated model, by dividing the value of the deviance of this model by the mean deviance estimated from the bootstrap procedure in MARK, and was always lower than 1, so that no correction was necessary. Our a priori interest was to estimate survivorship of both sexes. Models were ordered by AICc [31].

Female colour polymorphism in odonates is a widespread phenomenon [32], which includes one male-like coloured morph (androchrome) and one or more cryptic morphs (gynochromes). In the case of *A. oculata*, we considered as androchrome those females closely resembling male colouration, and as gynochrome the remaining females. The male colour change was time- and population-dependent. Therefore, to estimate the frequency of individuals that showed colour change we only considered the individuals which were observed after the first individual that changed its colouration on a given day.

The identification of the *Argia* species was performed by an expert taxonomist that examined representative
vouchers of our collected samples. Morph frequencies were compared between the protected population and the populations outside the reserve (mixed and modified) by a Chi-square test. Differences in the frequency of individuals between populations and wheatear conditions were analysed by ANOVA test. Differences in the presence of individuals at the stream were analysed by general linear mixed models, adding the hour of observation as predictor variable, sexes and populations as fixed factors, and individuals and days as random factors. Statistics were performed using GenStat software [33] and R version 3.3.2 [34]. Over the text, values are given as the mean ± SE (N).

Results

**Population ecology**

*Argia oculata* constituted the most abundant species (92%) of the genus among the five observed species (including *A. fulgida*, *A. indicatrix*, *A. transalata* and *A. variegata*). Of *A. oculata*, 563 individuals were marked and released (380 males and 183 females; Table 1). Only 111 individuals were recaptured once (92 males and 19 females), 28 were recaptured twice (26 males and two females) and three males were recaptured in three different days. No individuals were recaptured out of their original population, not even in other streams situated at distances around 100 m. Using the AIC criterion, the best model in the protected population was Phi(g) p(t), Phi(t) p(t) in the mixed and Phi(.) p(g) in the modified habitat (see Supplementary Table for details of model selection). Estimates of survivorship using a model with sex dependent survival (Table 2) suggest that males had lower mortality than females in all populations, but in the case of the mixed population, none of the most supported models allowed estimating survival by sex. Therefore, parameter estimates for this population have to be treated with caution.

The sex ratio was highly biased towards males in all populations, especially in the mixed population (Table 1). Considering all places studied, males doubled the female abundance (total sex ratio = 2.08). However, the sex-ratio was biased to females (0.29 males per female) along paths inside the reserve. Androchrome females were infrequent in all studied populations, but they had a higher representation inside the reserve (18%) than outside (mixed and modified population, 3%; contingency $\chi^2$ with Yates correction = 4.151, $p = 0.042$). Males were more abundant outside the reserve, especially in the mixed population (Table 1).

**Colours**

All individuals presented black stripes on the mesepisternum, along the humeral suture (between mesepisternum and mesepimeron) and along the metapleural suture (between metepisternum and metepimeron) (Figure 1). However, the remaining parts of the pterothorax showed different colourations across ages, the time of the day and between female morphs. All males observed presented the same thoracic colouration pattern, without ontogenetic changes. Males showed a different blue range of colouration in each thoracic segment (Figure 1(a)). However, some males showed a time and population-dependent change of their colouration. In the mixed population, 48 ± 18% (13) of males observed after 16:00 h changed their body colouration (colour changes were observed only during two of the six days of observation). In the modified population, 6 ± 3% (48) of males changed their colouration after midday (changes observed in two out of the four days of observation). In these males, all pterothoracic segments (but especially the mesepisternum and mesepimeron) became brownish (Figure 1(b)). A similar darkening occurred in the colouration of the last abdominal segments (Figure 2(a)–(b)). However, no colour changes were observed in males after the cited hours in the rest of the sampled days.

Two colour morphs were identified in females. The androchrome morph presented male-like thoracic colouration (Figure 1(c)), but with the same abdominal colour pattern as gynochrome females (Figure 2(c)–(d)). Old androchromes presented brownish thoraces with some remains of bluish colours (Figure 1(d)). Although bluish intensity varied among individuals, no colour changes were observed in this morph.

Gynochrome females showed age-related colour changes. When immature, these females presented a skyblue thorax and abdomen (Figures 1(e) and 2(c)). This kind of females were frequently found far from the streams and never in tandem. With sexual maturatión, body colouration became olive green (Figure 1(f)). Individuals with intermediate colouration were also

| Table 1. Proportions of captured individuals for each population inside (Reserve) and outside Buenaventura reserve. |
|---------------------------------|-----------|-----------|-----------|-----------|-----------|
|                                | Preserved | Mixed     | Modified  | Total reserve | Total outside |
| Sex ratio (♂/♀)                | 1.75      | 2.81      | 1.69      | 1.90        | 2.22       |
| Males                          | 70 (20)   | 132 (44)  | 88 (28)   | 160         | 220        |
| Androchromes                   | 6 (1)     | 2 (0)     | 1 (0)     | 15          | 3          |
| Gynochromes                    | 38 (2)    | 45 (8)    | 51 (8)    | 69          | 96         |
| Observation (h:min)            | 26:21     | 31:34     | 25:18     | 95:09*      | 63:07      |

* includes walking time between points of observation due to the marking of specimens between the studied areas (46 h 42 min).
Note: Individuals recaptured at least once are indicated in parentheses.
observed, as well as daily thoracic colour change. The female daily colour change was only observed in one female in two separate days, in which she showed olive colouration at 13:22 and 11:30 h and sky blue colouration at 17:00 and 14:30 h, respectively. Five and three females respectively were observed with olive colouration (no change) after the colour change of this female. Old females were completely brown (Figure 1(g)) and did not present light coloured areas in the dorsal region of the last abdominal segments (Figure 2(d)).

Table 2. Estimates of survival and recapture parameters from CJS models with MARK software.

| Population | Model       | Delta AICc | Parameter                | Estimate | SE  |
|------------|-------------|------------|--------------------------|----------|-----|
| Preserved  | $\phi(t)$ $p(t)$ | 0          | Phi males                | 0.902    | 0.02|
|            |             |            | Phi females              | 0.797    | 0.05|
|            |             |            | $p^*$                    | 0.431    | 0.12|
| Mixed      | $\phi(g) p(.)$ | 42.784     | Phi males                | 0.868    | 0.04|
|            |             |            | Phi females              | 0.840    | 0.06|
|            |             |            | $p^*$                    | 0.201    | 0.05|
| Modified   | $\phi(g) p(.)$ | 4.0126     | Phi males                | 0.981    | 0.05|
|            |             |            | Phi females              | 0.933    | 0.06|
|            |             |            | $p^*$                    | 0.206    | 0.06|

*Only one estimate of $p$ was possible due to low recapture rates.

Notes: Phi: survivorship probability; g: sex; p: recapture probability; t: time; (.) constant. Delta AIC indicates how far is the model compared to the best model, with 0 as maximum support (first model) and a larger number indicating lower support.

Figure 1. Thoracic colour variability in *A. oculata*. (A): male common colouration; (B): male after 16:00 h; (C): androchrome; (D): old androchrome; (E): immature gynochrome; (F): mature gynochrome; (G): old gynochrome.
Individuals did not change their colour pattern in the morning, when temperatures decreased below 21 °C. Individuals showed pruinosity in the metapleural suture (Figure 1).

**Ethology**

The number of individuals observed per hour depended of the weather conditions ($F_{1,8} = 18.05$, $p = 0.003$) but did not differ between populations ($F_{2,8} = 3.90$, $p = 0.066$) and between the interaction of both factors (Population × Weather: $F_{2,8} = 1.72$, $p = 0.240$). However, a tendency was recorded for an increase in the number of individuals found during cloudy days in more open habitats (higher human disturbance; protected = 2.29 individuals/h; mixed = 4.57 individuals/h; modified = 6.48 individuals/h). Individuals did not differ in the use of the water mass between sexes, populations and their interaction ($p > 0.05$). However, males arrived on average almost 1.5 h later to the stream in the protected population (10:53 h) and disappeared almost three hours earlier (14:07 h) than in the mixed population (09:35–16:46 h). In the modified population, males appeared at the stream at an intermediate range of hours. Males perched at around 2–5 m of height at the beginning of the morning but also occupied lower perches later. Females showed a similar departure pattern than males in all populations but they presented a common temporal window to arrive to the water (around 11:30 h).

Three copulations were observed (one androchrome female and two gynochromes) but only two were completely recorded. Since pairs were detected already in tandem, the intra-male sperm translocation could not be recorded. Mating sequence included the stage I (23.02 min ± 7.42, $N = 3$), stage II (2.28 min ± 0.31, $N = 3$) and stage III (10.81 min ± 3.19, $N = 2$) of Coenagrionidae [35]. Pumping was continuous in the stages I and II and absent in stage III. The two matings recorded lasted 18.3 (androchrome) and 42.4 min (gynochrome).

After mating, individuals flew in tandem and suffered disturbance by single males. Male harassment was also frequent towards females released after marking. Oviposition was performed in tandem, but single females were also seen ovipositing at any time of the day. Tandem oviposition lasted from 23 min to 1.5 h ($N = 2$). Sperm ejection was observed in one gynochrome female in post-copula tandem, just after mating.

**Discussion**

We found female dimorphism and daily colour changes in both sexes of *Argia oculata*. Although both kinds of colour variability were confirmed, the frequency of occurrence was low and only observed in certain days. The high number of individuals captured indicates that *A. oculata* was a common species in the Buenventura’s surroundings, and the absence of marked individuals far from their population suggests that they dispersed very little (as has been reported for *Argia plana*, [36]; *Argia moesta*, [37]; and *Argia vivida*, [38]). Our estimates from the recapture histories of marked individuals indicate a higher survival for males, a finding in agreement with most previous studies in Coenagrionidae [39]. We found differences in the survival and recapture rates among the studied populations suggesting local effects that might be related to the degree of shading of the studied streams (see below).

Sex-ratio differences between streams and forest paths do not necessarily imply a biased sex-ratio in the whole population. In other *Argia* species, larval sex-ratio

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**Figure 2. Abdominal colour variability in *A. oculata*. (A): male bright phase in a dorsal view; (B): male dark phase in a lateral view; (C): young female in a dorsal view; (D): mature female in a lateral view.**
was found to be balanced [36], and this might also apply to our populations. However, the operational sex-ratio (i.e. the sex-ratio of the reproductive adults) was clearly male-biased at streams. This fact produces high male-male competition, exacerbated by a persistent and frequent site-tenacious aggressive behaviour, also observed in the studied populations, that explains the low reproductive success of most males [36,40,41]. In addition, Conrad and Pritchard [42] suggested that males could practise female-control instead of resource-control in habitats with high abundance of oviposition areas. Accordingly to the reduced time that females were present at the stream in comparison with males, we can conclude that females avoided visiting the streams for most of the time. This fact was especially observed during their immature stage (A. moesta individuals remain 14 days in the paths before returning to the water mass [37]). However, females mate in every visit (at least in A. plana, [36]. In contrast, the sex ratio of A. moesta at the stream was found highly biased towards females in two seasons, and both sexes achieved more than one mating per day [37]. The mating rate of different Argia species seems therefore highly variable.

Our data suggest a lower proportion of androchrome females outside the reserve. If this pattern is confirmed in future studies, it would mean that human alteration of habitats might reduce the evolutionary potential in this polymorphic species (see [24]. The genus Argia constitutes an indicator of environmental quality [43–46] and the analysis of its intraspecific variability could enrich these assessments [47,48]. Due to their colour and behavioural similarities with males, androchromes might avoid male harassment and therefore be favoured if male density increases [49,50] under a negative frequency-dependent selection (e.g. [51]. Nevertheless, we did not find more androchromes in the mixed population, where the sex ratio was more biased towards males. The type of vegetation, the shade [52] or the weather could affect the contrast of the androchrome colouration with the background, which in turn determines differences in the ability of males to detect colour morphs (see [25]. Furthermore, the diminution of vegetation cover in disturbed environments might allow an increase of activity of individuals due to the higher reception of solar radiation in cloudy days or in early and lately hours. In four Megalagrion species, it has been shown that female dimorphism varies according to the exposure to the sun [3]. Highly exposed populations present red females while low exposed populations present green females. Both morphs are mixed in intermediate populations. These authors argue that the red colouration could possess immune or UV protection functions. They suggest that the polymorphism of these species have a polyphyletic origin and is caused by ecological selection. Furthermore, they argue that this explanation may not be suitable for non-territorial polymorphic species with high male harassment.

Argia is one of the most speciose genera among odonates and also one of the most difficult to identify at the species level (112 species; [53], partially due to its colour variability. Colour polymorphism is known in 27 species of the genus [26,27], while daily colour change is known in at least ten species [21,22]. May [22] described a change of colour under laboratory conditions of eight species of Argia: A. apicalis, A. bipunctulata, A. extra-nea, A. moesta, A. oculata, A. sedula, A. tibialis, Argia sp. All of them presented body colour changes after being exposed to low temperatures during the night, except for the males of A. moesta. The test was not performed with females of four of these species (including A. oculata). In contrast to May [22], Bick and Bick [29] observed in Argia apicalis that the colour change does not follow a consistent pattern, does not occur in all individuals and it is not dependent of temperature, humidity, weather, geography, genetics, age or changes after mating. We found conspicuous colour variability in A. oculata, similar to the pattern described for A. apicalis (daily colour change for both sexes and two female morphs; [29]. As in this species, colour changes during the day in A. oculata were not temperature-dependent, the process found in A. vivida [21]. Conrad and Pritchard [21] did not observe colour change when they placed individuals in a mesh cage ‘in the sun on a warm day’ but they observed the change when individuals were preserved overnight at low temperatures. May [22] reported a colour change in A. oculata males after an exposition to temperatures between three and 25 °C during night. He argued that environmental factors could obscure the interpretation of field results about colour changes. However, colouration of coenagrionids is a complex phenomenon, as illustrated by the development of melanism under laboratory conditions in some species [54, see 55]. In our observations, males presented the same colour pattern during all day (even if they were directly exposed to the sunlight), but some of them changed their colour in the afternoon. Surprisingly, collected individuals did not show colour changes between the time of capture and their preservation (hours later). Therefore, it seems that the Argia species possess different mechanisms to change their colour. One possible explanation for this process might be the dependence of circadian rhythms [20,56]. Besides the effect of the light, circadian rhythms also depend of other environmental factors. For instance, the tidal and the semilunar rhythms modify the daily colour change promoted by the diurnal rhythm in Uca pugnax (Arthropoda: Malacostraca) [57]. The decrease of temperature probably releases the darkening hormone (see [20] in A. oculata [22] but its effect might be obscured by time, the habitat condition and the differences among individuals in their physiology (e.g. hormone concentrations, chromatophores stimulation) as well as their behaviour (e.g. solar intake, light exposition), as revealed by the finding of some individuals with mixed colouration (also see [23]. The combination of temperature and circadian rhythms effects could also explain the differences obtained between Bick and Bick [29] and May [22] in A. apicalis. We did not observe the opposite change of colouration (from dark to light
colours) probably due to its rapidity, provoked by different migration paths for pigment granules [23] or because it is directly affected by temperature without hormonal release [20]. In two species of the genus *Diphelebia* and two of the genus *Austrolestes* [19,56] the daily colour change is dependent of the temperature but is also affected by the exposition to light. Veron [19] concluded that chromatophores may be light sensitive or sufficiently insulated to become heated independently of the rest of the body. This could explain differences in the time of the colour change in *A. oculata* males from different populations.

Although we did not follow focal individuals to assess their behavioural patterns within the day, our observations agree with the described use of the stream by males of *A. vivida* [21,42]. In this species, males perform longer matings in the morning (grasping females out of the stream) than in the afternoon, when males grasped females released by other males after oviposition. This behavioural pattern agrees with the observed distribution of males through the day, perching in upper vegetation at the beginning of the morning (a possible strategy to intercept females arriving from trees) but switching to lower perches later (perhaps to be closer to the areas of oviposition). We hypothesise that the time-dependent thoracic colour change that males and gynochromes suffered could be influenced by mimicry processes (see [58]). In one hand, mature gynochromes could mimic the immature colouration later on the day. Although they might become more conspicuous (due to the male preference for reflecting colourations; [29]), this body colour could also signalise their unavailability to mate as has been suggested in other coenagrionids [59]. On the other hand, at the end of the day, some males showed the same colouration as old androchromes. Perhaps this darker colouration decreases their conspicuousness (brown colouration showed the lowest reflectance in *A. vivida*; [21]).

Unfortunately, the formation of the pre-copula tandem was not observed but, following the observations of male agonism and the tandem formation in other *Argia* species in the same area, we suggest that males grasp females in the air without courtship (in agreement with [40,60]), especially when they fly between the upper parts of the forest towards the water course. The sperm translocation should be performed immediately after the female capture [29,40,60,61]. Our observations of copula and oviposition duration agree with the common behaviour of the genus [29,40,42,60].

To conclude, our results indicate that *A. oculata* shows several sources of variation in colouration. Males concentrate in the oviposition areas, actively harass conspecifics, guard females during copula, retain females during oviposition, and may change their thoracic colouration towards cryptic colours at the end of the day. On the other hand, females avoid places with the highest concentrations of males (females are common in forest paths and clearings), try to oviposit alone, are able to eject sperm, show a specific thoracic colouration that could signal immaturity, thoracic colour polymorphism with an androchrome morph, and a possible daily thoracic colour change towards the immature colouration. Future studies should analyse male preferences towards morphs and colour phases as well as female fitness in the light of both phenomena.

**Geolocation information**

Protected population: 3° 39’ 24.6852″ S, 79° 46’ 18.318″ W; mixed population: 3° 39’ 10.962″ S, 79° 48’ 1.782″ W; modified population: 3° 40’ 45.1992″ S, 79° 44’ 14.1288″ W).

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**Supplemental data**

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