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Fluctuating Asymmetry and Wing Size of *Argia tinctipennis* Selys (Zygoptera: Coenagrionidae) in Relation to Riparian Forest Preservation Status

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Keywords

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Abstract

Effects of riparian vegetation removal on body size and wing fluctuating asymmetry (FA) of *Argia tinctipennis* Selys (Odonata: Coenagrionidae) were studied in the River Suiá-Miçú basin, which is part of the Xingu basin in Brazilian Amazonia. A total of 70 specimens (n=33 from preserved and n=37 from degraded areas) was measured. Five wing measures of each wing (totalizing ten measured characters) were taken. Preserved and degraded points presented non-overlapped variations of a Habitat Integrity Index, supporting the environmental differentiation between these two categories. FA increases in degraded areas approximately four times for the width between the nodus and proximal portion of the pterostigma of forewings (FW), two times for the width of the wing in the region of nodus of FW, and approximately 1.7 times for the number of postnodal cells of FW. The increase is almost five times for the width between the nodus and the proximal portion of the pterostigma of hind wings (HW), three times for the number of postnodal cells of HW, and approximately 1.6 times the width between quadrangle and nodus of HW. Individuals of preserved sites were nearly 3.3% larger than for degraded sites, based on mean hind wing length. Our results supports that the development of *A. tinctipennis* in degraded areas is affected by riparian vegetation removal and may reflect in wing FA variations. Consequently, these FA measures may be a useful tool for bioassessment using Odonata insects as a model.

Introduction

Biological species are expected to be affected by environmental disturbances in very different ways according to its intrinsic biological characteristics. Some of these differences may be attributable to its specific dependency on resource or environmental conditions which are known to affect all stages of development. For instance, developmental alterations due to harsh environmental conditions may be important indicators of the effect of disturbance at the individual and species level (Niemi & McDonald 2004, Sanseverino & Nessimian 2008). One important measure of these effects is the fluctuating asymmetry (FA), which refers to small, random deviations from a model of perfect bilateral symmetry, and is usually associated with developmental instabilities of the individual during its ontogeny (Palmer & Strobeck 1986). In species with larval stages, this instability includes causes such as resource scarcity and toxin exposition (Hardersen & Frampton 1999, Sanseverino & Nessimian 2008).

Although many studies suggests that the potential of FA may be weak (Forbes *et al* 1997, Vangestel & Lens 2011), others claim that FA is a useful biomarker of developmental stress (as reviewed by Møller 1997). There are a large number of experimental studies which evaluated increases in FA with unfavorable conditions (Chang *et al* 2007a, Hardersen &

Frampton 1999, Ho *et al* 2009, Kanegae & Lomonaco 2003). Hence, since the relationship between FA and biomonitoring is controversial, further field studies are needed to validate it as a useful tool for bioassessment (Vangestel & Lens 2011). Otherwise, FA may represent an integrative measure of the effect of stressing factors since it could reflect its effects even if they occur in different moments of the individual development (Hardersen & Frampton 1999). This could strengthen the effectiveness of bioassessment procedures, allowing a response to a broad set of stressors (Sanseverino & Nessimian 2008).

FA has been largely used in studies of environmental impact mainly in face of the increasing demand for metrics capable to indicate the accurate ecological condition of aquatic systems (Bonada *et al* 2006, Sanseverino & Nessimian 2008). As an example, larval odonates have been used in studies that evaluated the relationship between FA and the effects of insecticides on water, impact of ectoparasitism, and reproductive success (Hardersen & Frampton 1999, Leung & Forbes 1997). Indeed, alterations in odonate wing characters may decrease fitness levels by affecting flight performance and, consequently, life maintenance (Samejima & Tsubaki 2010).

The demand for better and efficient biomonitoring measurement tools increases as environmental impacts also increase in intensity and affected area (Niemi & McDonald 2004). For instance, the expansion of area devoted to agriculture in the Neotropical region create a challenge to conservation biologists for fast and efficient identification of impacts related to vegetation cover removal and other associated changes in landscape. Cerrado is one of the most affected areas in the neotropics. Cerrado is the second largest domain in Brazil, encompassing an area of nearly two million square kilometer, with high levels of environmental disturbance mainly due to extensive agriculture practice and livestock (Ratter *et al* 1997), which generate a fragmentation of continuous areas (Carvalho *et al* 2009). Removal of riparian vegetation and chemical or organic effluents in this system may lead to environmental changes in aquatic systems, affecting the availability of resources or changing the local conditions, so that they represent effective environmental stressors for many inhabitant species.

The removal of the riparian vegetation was almost often linked to other land use changes near water bodies, mostly due to the increase of agriculture areas with the consequent increase of disposal of fertilizers and agrotoxics to streams (Silva *et al* 2011). Nevertheless, vegetation removal may have contrasting effects on odonates from complete local extinction to increase in population sizes (Ferreira-Peruquetti & De Marco 2002, De Marco & Peixoto 2004).

The Coenagrionidae genus *Argia* includes species restricted to the Neotropical domain, which are associated to streams (Hornung & Pacas 2006, Dalzochio *et al* 2011, Silva *et al* 2010). As large distribution and local abundance are important properties of possible bioindicator species (Oertli 2008), *Argia tinctipennis* (Selys) is qualified as a model organism in this study since it has wild distribution in the Brazilian Cerrado and transition areas between Cerrado and the Amazonian forest (De Marco & Vianna 2005, Nobrega & de Marco 2011).

A basic ecological assumption on the use of FA as an indicator measure is that developmental instabilities affect adult morphology, which in turn may have a direct effect on how individuals respond to environmental characteristics (Jenssen *et al* 2010, Sanseverino & Nessimian 2008). Thus, we tested the FA based on wing measurements of *A. tinctipennis* would differ from adults from preserved and degraded areas (mainly affected for riparian vegetation removal). Since overall size and FA levels can strongly vary due to environmental disturbances on the larval development, we also tested for the existence of direct effects on hind wing size.

Material and Methods

Study sites

This study was conducted in a region with predominance of dry forest, in the River Suiá-Miçu (Suiá) basin, which is part of the Xingu basin (Fig 1). This basin lies at the transition area between the Cerrado and the Amazonian forest in the Central–West region of Brazil (Maeda *et al* 2008). The climate is tropical seasonal with a dry season between May and October and the rainy season between November and April (Ratter *et al* 1978).

To avoid possible seasonal or phenological effects, all individuals were captured during the dry season, September 2007. Points in preserved [“Lúcio” (CRL), “Darro” (RID) and “Suiá” (RISU 1, 2, and 3) streams] and in impacted areas [“Transição-brejo” (CRTB), “Tatu” (RISUZ), and “Piabanha” (RIPB) streams] (Fig 1) were sampled. In impacted areas, land conversion from forest to pasture and to agriculture activities was the primary disturbance factor.

The main characteristics observed to evaluate the level of disturbance were the presence and extension of riparian vegetation and presence/absence of cattle and agriculture activities. The riparian vegetation at the preserved sites was continuous from the margin to at least 50 m away from the stream. In most disturbed areas, it was possible to identify discontinuities of the surrounding riparian vegetation that indicated the presence of cattle and agriculture activities, and, as a result, a possible increased input of chemical effluents, changes in exposure to sun, decreases in oxygen availability, and/or water’s pH changes, which can influence the larval development leading to an increase in FA levels.

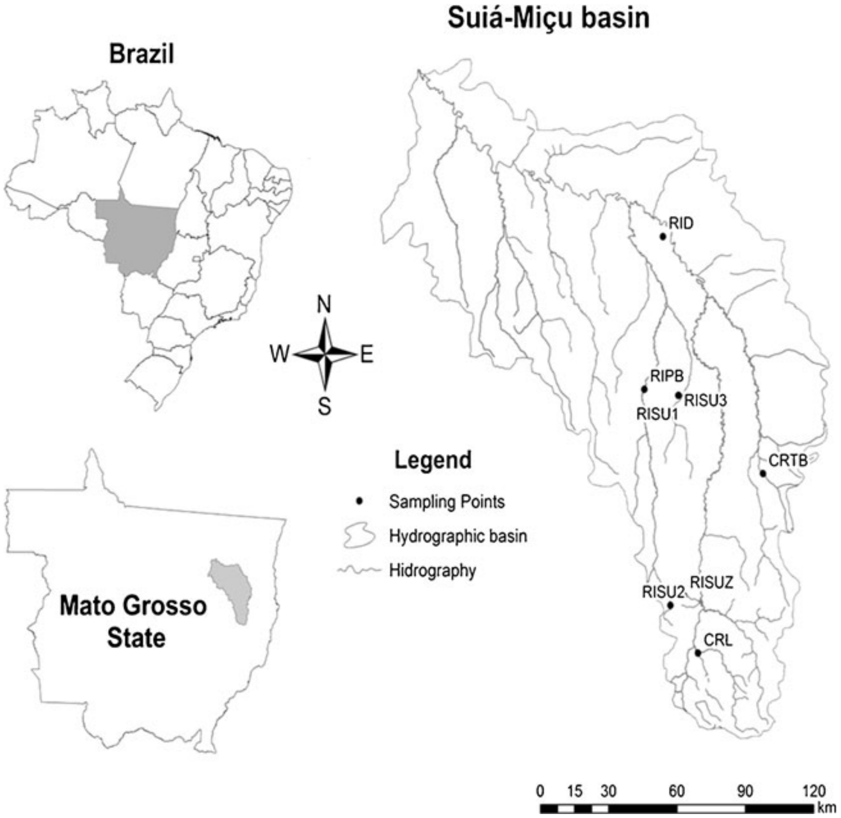

Fig 1 Location of the Suiá River basin sampling area and sampling points visited on September 2007.

The Habitat Integrity Index (HII; Nessimian *et al* 2008) was used to determine the physical integrity of the system and an additional evaluation of the preservation status. This index varies between 0 (areas extremely altered) to 1 (areas with all the features as expected in pristine areas) based on a series of parameters accessed visually, related to levels of land use, conservation of riparian vegetation, morphology of the stream channel, and its margin and streamlet bed structure. This index is based on another integrity index developed in temperate areas (Petersen 1992) but was explicitly developed to measure these features in Amazonian areas (Nessimian *et al* 2008).

The sampling of *A. tinctipennis* followed the method of scanning fixed areas (De Marco 1998), employed in other studies with this group (De Marco & Peixoto 2004, De Marco & Resende 2002, Pinto 2011, Silva *et al* 2010). The air temperature in shaded locations near the streams was measured and samplings were performed only in sunny days with air temperature higher than 19°C due to possible restrictions of dragonfly activity (De Marco & Resende 2002, May 1991). The specimens were collected with an entomological net for confirmation of identification and deposited as voucher material in the collection of the “Laboratório de Ecologia Teórica e Síntese, Universidade Federal de Goiás”. Available taxonomic identification keys were used to identify the specimens (Lencioni 2006).

Measured wing characters

Seventy specimens were captured, 33 from preserved areas: CRL=11, RID=9, and RISU 1, 2, and 3=13 and 37 from impacted areas: CRTB=13, RISUZ=11, and RIPB=13. Images of their left and right anterior and posterior wings were collected with a digital camera (Sony® cyber-shot DSC-H10, optical zoom 10×, 8.1 megapixels) mounted on a fixed tripod at 15 cm height. The acquired images were used for measuring the landmarks using the TPSDIG2 software (Rohlf 2006). FA indices were focused in the wings, since such measures tend to be not correlated with wing size, presenting normal distribution and low measurement error (Forbes *et al* 1997, Hardersen & Frampton 1999).

A set of five traits was measured in each wing (Table 1). As the sum of the variable distance between the nodus and the proximal portion of the pterostigma and distance between second antenodal and nodus (NP and SAN) represent a measure of wing length (WL), we use this metric average between sides $[(R+L)/2]$ of hind wing for the analysis of differences in total wing size in relation to preservation degrees.

FA indexes and statistical analyses

The statistical analyses and control of variation in the analysis of FA followed the methods described by Palmer & Strobeck (1986) and Palmer (1996). For each specimen and trait measured, the left side was subtracted from the right one [FA=($R-L$)]. In our protocol, we did not excluded outliers, since they are expected in such studies and may be important to the analysis, since that not is caused by trauma or injury (Hardersen 2000, Palmer 1994).

Fifteen individuals for each habitat were randomly selected and were measured three times (by two different people and with an interval of several days between measurements) to allow for the estimation of measurement errors. We used a two-way (mixed model) ANOVA with individuals as the random factor and sides as the fixed factor. This model uses mean squares to verify if the measurement errors are significantly smaller than the main factors for the measured characters. This test allowed us to verify the existence of directional asymmetry, the dependence of shape/size asymmetry of the specimens, as well as the existence and significance of nondirectional asymmetry (Palmer & Strobeck 1986).

Antisymmetry was tested with a *t* test to verify if assigned asymmetries are around mean zero, and the normality of the data was tested with a Kolmogorov–Smirnov test. Tests for correlations between absolute values of asymmetry found and wing length size were performed by Pearson's coefficient correlation. We also tested for differences in wing size and FA between habitats with a Student's *t* test for independent samples. Variance homogeneity assumption was tested using a Levene test and, when the null hypothesis was rejected, a separate variance *t* test was employed (Zar 1999).

Results

Habitat integrity, measurement error, and asymmetry patterns

The values of the HII support the differentiation between preserved and altered sites with non-overlapped ranges (from 0.39 to 0.60 in altered and 0.69 to 0.74 in preserved sites) and statistically significant differences (*t* test: t=4.721, df=6, P=0.003).

For all variables, the error mean squares were significantly smaller than for the main factors (side and individuals; Table 3). The low values of error mean square suggest that deviant values from zero derive from asymmetric variations and are not generated by measurement error. Thus, we can assume that our measures were reliable to conduct further analysis.

All measures presented normal distribution and zero mean except for NUP (Table 2). Nevertheless, we included this variable in our analyses because it is a possible "false positive" relative to the small number of asymmetry categories (Palmer 1994). Overall, we can safely assume that the asymmetry observed in *A. tinctipennis* is due to FA, excluding the existence of directional asymmetry and antisymmetry. There was no significant difference between left and right sides for all traits (Table 3), supporting the exclusion of directional asymmetry as an explanation. The high significance of interaction term (side vs. individuals) indicates the presence of nondirectional asymmetry. There was no positive correlation between the average of WL and traits measured (Table 4).

Effect of riparian removal on FA and mean wing size

There were significant differences between the level of FA of individuals from preserved and altered areas in six out of ten morphological variables evaluated (Table 5). The increase in FA level of individuals of degraded compared to preserved areas was approximately fourfold for NP_f, twofold for LN_f, 1.7-fold for NUP_f, fivefold for NP_h, threefold for NUP_h, and 1.6-fold for QN_h.

There were significant differences in the hind wing size in relation to the preservation status of sites (t=3.092, df=68, P=0.003). Wings of individuals of preserved sites were nearly 1.033-fold larger than those from degraded sites (Fig 2).

Table 1 Measured traits and abbreviations adopted in the text.

Wing trait	Abbreviation
Distance between the nodus and the proximal portion of the pterostigma (front wing)	NP_f
Distance of wing in the region of nodus (front wing)	LN_f
Number of postnodal cells (front wing)	NUP_f
Distance between second antenodal and nodus (front wing)	SAN_f
Distance between quadrangle and nodus (front wing)	QN_f
Distance between the nodus and the proximal portion of the pterostigma (hind wing)	NP_h
Distance of wing in the region of nodus (hind wing)	LN_h
Number of postnodal cells (hind wing)	NUP_h
Distance between second antenodal and nodus (hind wing)	SAN_h
Distance between quadrangle and nodus (hind wing)	QN_h

Table 2 Normality distribution using the Kolmogorov–Smirnov *d* test and the zero mean tests of $R-L$ values using a single sample *t* test ($N=30$) for all variables.

	Test for mean=0		Test for normality	
Wing characters	*t* test	*P* value	*d* (K–S test)	*P* value
NP_f	0.462	0.648	0.141	>0.200
LN_f	−0.488	0.630	0.169	>0.200
NUP_f	0.026	0.998	0.338	<0.010*
SAN_f	−1.539	0.135	0.159	>0.200
QN_f	−1.544	0.134	0.155	>0.200
NP_h	−0.923	0.363	0.201	>0.100
LN_h	−0.366	0.717	0.212	>0.150
NUP_h	0.403	0.690	0.234	<0.050
SAN_h	1.551	0.132	0.169	>0.200
QN_h	−0.852	0.401	0.175	>0.200

Please refer to Table 1 for wing character abbreviations.

K–S Kolmogorov–Smirnov.

Discussion

Our data support the hypothesis that the existing environmental changes in the degraded areas evaluated are a source of developmental stress for *A. tinctipennis*, since the level of FA observed cannot be explained by random factors. The relationship between FA and environmental stress has been already proposed (Møller 1997, Palmer & Strobeck 1992, Palmer & Strobeck 1997) and its potential use as a tool for biomonitoring was initially proposed decades ago (Palmer & Strobeck 1986), and its predictive power has been extensively evaluated (Dobrin & Corkum 1999, Hardersen 2000, Hardersen *et al* 1999, Harvey & Walsh 1993).

Table 3 Two-way ANOVA (mixed model; side×individual) for each trait.

Wing characters	Mean square (ANOVA)			
	Side	Individual	Side× individual	Error
NP_f	0.072 ns	1.107*	0.092*	<0.001
LN_f	0.002 ns	0.057*	0.015*	<0.001
NUP_f	0.200 ns	2.317*	1.234*	<0.001
SAN_f	0.063 ns	0.055*	0.026*	<0.001
QN_f	0.019 ns	0.075*	0.028*	<0.001
NP_h	0.021 ns	0.768*	0.097*	<0.001
LN_h	0.002 ns	0.043*	0.009*	<0.001
NUP_h	0.012 ns	1.959*	1.345*	<0.001
SAN_h	0.138 ns	0.073*	0.057*	<0.001
QN_h	0.131 ns	0.066*	0.054*	<0.001

Please refer to Table 1 for wing character abbreviations.

ns not significant.

*$P<0.05$, significant different ($n=30$) for all variables.

Table 4 Pearson's correlations between average ($(R+L)/2$) of NP and others traits measured, for all variables ($N=30$).

Wing characters	Pearson's *r* value	*P* value
LN_f	−0.049	0.795
NUP_f	0.038	0.841
SAN_f	0.046	0.811
QN_f	−0.444	0.014*
LN_h	−0.186	0.325
NUP_h	−0.030	0.875
SAN_h	0.013	0.944
QN_h	−0.436	0.016*

Please refer to Table 1 for wing character abbreviations.

*$P<0.05$, significant different.

A parsimonious mechanism to explain our results are based on the assumption that land use changes and more complex alterations affect the development of *A. tinctipennis* larvae, and its effects are observed on adults. Differences in late and early adults of *Xanthocnemis zealandica* (MacLachlan) (Zygoptera: Coenagrionidae) (Hardersen *et al* 1999), possibly related to larval development, could also support this interpretation. Our current understanding of larval biology of odonates suggest that temperature (Chang *et al* 2007b), feeding rate affected by food availability (Benke *et al* 2001, Dmitriew *et al* 2007) or predation pressure (Dmitriew & Rowe 2005), and direct effects of pollutants (Beketov 2004, Hardersen *et al* 1999) are the most reliable

Table 5 Comparison between absolute FA values between preserved and degraded sites.

	Absolute FA				
Trait	Preserved areas	Degraded areas	*df*	*t* test	*p*
NP_f	0.106	0.430	45	−3.482	0.001*
LN_f	0.035	0.076	61	−3.006	0.004*
NUP_f	0.636	1.054	68	−2.180	0.033*
SAN_f	0.133	0.155	68	−0.673	0.503
QN_f	0.124	0.159	68	−0.952	0.345
NP_h	0.122	0.592	47	−4.321	<0.001*
LN_h	0.064	0.091	68	−1.338	0.185
NUP_h	0.424	1.297	68	−4.767	<0.001*
SAN_h	0.098	0.128	68	−1.197	0.236
QN_h	0.096	0.151	68	−2.137	0.036*

Please refer to Table 1 for wing character abbreviations.

*$P<0.05$, significant different.

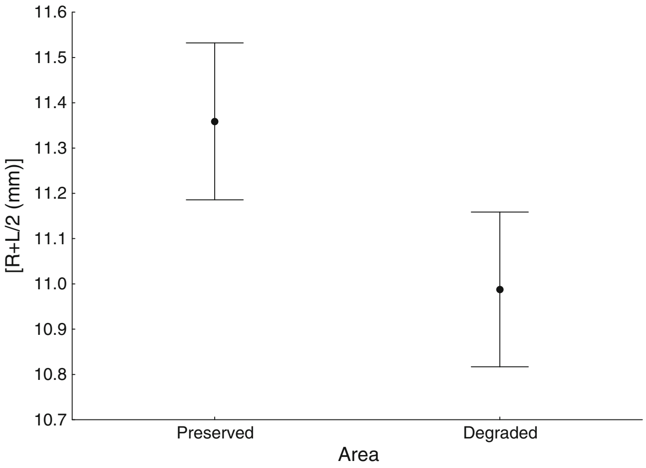

Fig 2 Mean relative size [$(R+L)/2$] of the measurements of the wing length (WL) of hind wings from *A. tinctipennis* from preserved and degraded areas. *Bars* 95% confidence interval.

stressors to explain alterations in larval development. Since the pastures in these areas have a low (if any) use of pesticides, this should be considered the least probable cause. To date, it is difficult to exclude the remaining aforementioned factors as alternative hypothesis, but it is also possible that changes in larval habitat including the presence of plants, substrate changes and luminosity (all related to the switch from forested to pasture margins) directly affect larval behavior and its success to prey and escape from predators. Effects related to changes in luminosity and microhabitat characteristics (mostly due to the increase of grass near stream margins) were also demonstrated for fish communities in similar environments (Casatti *et al* 2009).

The HII is a general descriptor of environmental changes in streams synthesizing both local (habitat description in the streams) and landscape (land use near the streams; Nessimian *et al* 2008). This metric may be useful to indicate land use alteration, but it does not necessarily assess the level of stress affecting individual species inhabiting a particular site. The advantage of FA over this habitat metric is the direct interpretation of possible ecological mechanisms affecting larval development together with its predicted effects on adult fitness. Since habitat alterations may affect individual species fitness and, consequently, its persistence in the stream, FA represents a shortcut, allowing a direct analysis of individual traits possibly interpreted as surrogate for odonate fitness (Chang *et al* 2009, 2007a).

Hardersen (2000) argued that meristic characters should be better suited to evaluate FA, at least for stressors like insecticides. Nevertheless, from six characters with significant variation among areas, four are metric and two are meristic, suggesting that both traits could be efficient to evaluate fluctuating asymmetry, at least between broad categories of environmental impact as used in this work. The choice of variables in such studies may improve with more information about developmental phases in larval odonates, which may allow to determine the best moments of the development where individual morphological traits are more susceptible to sources of stress (Hardersen 2000). Then, we expect that broad environmental changes such as those derived from deforestation and other consequences of agricultural activities may strongly affect larval development at different stages during its growth and development, and result in different traceable morphological variables. The larger number of different traits with significant FA in our study in comparison to the study of Hardersen (2000) supports this claim.

Flight performance can be affected by size or shape of wings and can reduce damselfly fitness (Samejima & Tsubaki 2010). Many of these variations in wing shape and/or length may be resultant from developmental stress (Harvey & Walsh 1993, Hoffmann *et al* 2005). We found in this study that wing size of individuals from degraded areas are significantly lower than those from preserved areas, suggesting that the development of *A. tinctipennis* in degraded areas is affected and this stress may be reflected in its wings size. Some studies have demonstrated that temperature increase could result in disruption of the developmental stability and increased levels of FA or different effects on body size (Chang *et al* 2007b, Kanegae & Lomonaco 2003, but see Taylor & Merriam 1995), at least in laboratorial conditions. This result is expected mainly because higher temperatures (as observed in areas that lack vegetation cover) allow for rapid development and eclosion, resulting in small individuals. In the same way, a rapid development is expected in areas with high presence of predators due to the rapid growth of some groups (Schaffner & Anholt 1998). Whatever is the possible path, an effect in the overall fitness of populations from disturbed systems may be expected.

In conclusion, we suggest the use of FA of *A. tinctipennis* wings as a bioindicator to monitor the effects of agricultural activities, livestock, and deforestation, especially in meristic traits (NUP). The use of FA as a biomonitoring tool provide an "easy-to-use" index of environmental stresses on biological parameters and a quick and cheap index that can be accessed to evaluate the stressors phenomena in a biological system. We also consider the use of HII as a complementary tool to increase the power of the analysis and to strengthen interpretation of local impacts.

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