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Natural history of the critically endangered salamander *Ambystoma leorae* (Caudata: Ambystomatidae) from the Río Tonatzin, Mexico

Julio A. Lemos-Espinal,[1] Geoffrey R. Smith,[2] Aline B. Estrella Zamora,[1] Guillermo Woolrich-Piña,[3] and Raymundo Montoya Ayala[4]

[1] Laboratorio de Ecología-UBIPRO, Facultad de Estudios Superiores Iztacala, Av. Los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México, 54090, Mexico. E-mail: lemosj44@yahoo.com.mx.

[2] Department of Biology, Denison University, Granville, OH 43023, USA.

[3] Laboratorio de Zoología, División de Biología, Subdirección de Investigación y Posgrado, Instituto Tecnológico Superior de Zacapoaxtla, Carretera Acuaco Zacapoaxtla km 8, Col. Totoltepec, Zacapoaxtla, Puebla, Mexico.

[4] Laboratorio de Cómputo-UBIPRO, Facultad de Estudios Superiores Iztacala, Av. Los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México, 54090, Mexico.

Abstract

Natural history of the critically endangered salamander *Ambystoma leorae* (Caudata: Ambystomatidae) from the Río Tonatzin, Mexico. In Mexico, many species of *Ambystoma* are endangered, but unfortunately little is known about their natural history and ecology. We report on aspects of the natural history of *A. leorae,* a critically endangered endemic salamander, from the Río Tonatzin, State of México, Mexico. We observed egg masses, which were attached to vegetation, from April to June, with a mean of 7.28 eggs per egg mass. Larval *A. leorae* were found in sections of the Río Tonatzin with muddy bottoms and submerged aquatic vegetation. We observed adult and juvenile salamanders throughout the year except September, but with a peak from May to July. The use of sites along the Río Tonatzin by adult and juvenile *A. leorae* was driven primarily by vegetation type and substrate type of the site, with mud or sand substrate with grass roots and submerged aquatic vegetation being the most used site types. We found no evidence of sexual dimorphism in *A. leorae.* Our results suggest that adult and juvenile *A. leorae* use a variety of stream sites, although larvae appear to be more limited in their use of stream sites. These observations indicate that to maintain this population of *A. leorae,* the general conditions existing in the Río Tonatzin should be protected, especially the sections with muddy bottoms and aquatic vegetation where larvae, juveniles, and adults are found.

Keywords: adults, aquatic vegetation, eggs, juveniles, sexual dimorphism, substrate, water speed.

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Resumen

Historia natural de la salamandra críticamente en peligro de extinción *Ambystoma leorae* (Caudata: Ambystomatidae) del Río Tonatzin, México. En México, muchas especies de *Ambystoma* están en peligro de extinción, desafortunadamente poco se conoce de sus historias naturales y ecología. En este estudio, reportamos aspectos de la historia natural de *A. leorae,* una salamandra endémica críticamente en peligro de extinción, para la población del Río Tonatzín, estado de México, México. Observamos masas de huevos pegadas a la vegetación, desde abril hasta junio, con un promedio de 7,28 huevos por masa de huevos. Larvas de *A. leorae* fueron encontradas en secciones del Río Tonatzín con fondos lodosos y vegetación acuática sumergida. Observamos salamandras adultas y juveniles a través de todo el año, excepto en septiembre, con un pico desde mayo hasta julio. El uso de sitios a lo largo del Río Tonatzin por adultos y juveniles de *A. leorae* fue motivado principalmente por el tipo de vegetación y el tipo de sustrato del sitio, con lodo o sustrato de arena, con raices de zacate amacollado y vegetación acuática sumergida que son los tipos de sitios más utilizados. No encontramos evidencia de dimorfismo sexual en *A. leorae.* Nuestros resultados sugieren que adultos y juveniles de *A. leorae* utilizan una variedad de sitios de arroyo, aunque las larvas parecen estar más limitadas en la utilización de estos sitios. Estas observaciones indican que para mantener esta población de *A. leorae,* las condiciones generales existentes en el Río Tonatzín deben ser protegidas, especialmente las secciones con fondos lodosos y vegetación acuática donde se encuentran las larvas, juveniles y adultos.

Palabras clave: adultos, dimorfismo sexual, huevos, juveniles, sustrato, vegetación acuática, velocidad del agua.

Resumo

História natural da salamandra criticamente ameaçada *Ambystoma leorae* (Caudata: Ambystomatidae) do Rio Tonatzin, México. Muitas espécies de *Ambystoma* do México acham-se ameaçadas de extinção, e infelizmente pouco se sabe de sua história natural e ecologia. Neste estudo, fornecemos dados sobre a história natural de *A. leorae,* uma salamandra endêmica e criticamente ameaçada, para a população do Rio Tonatzín, estado do México, México. Observamos massas de ovos aderidas à vegetação de abril a junho, com uma média de 7,28 ovos por desova. Larvas de *A. leorae* foram encontradas em trechos do Rio Tonatzín com fundo lodoso e vegetação aquática submersa. Observamos salamandras adultas e juvenis ao longo de todo o ano, exceto em setembro, com pico de maio a julho. O uso de locais ao largo do Rio Tonatzin por adultos e juvenis de *A. leorae* dependeu primariamente do tipo de vegetação e do tipo de substrato, sendo lodo ou substrato de areia com raízes de gramíneas e vegetação aquática submersa os tipos de locais mais utilizados. Não encontramos evidência de dimorfismo sexual em *A. leorae.* Nossos resultados sugerem que adultos e juvenis de *A. leorae* utilizam uma grande variedade de locais de riachos, ainda que as larvas pareçam estar mais limitadas na utilização desses locais. Essas observações indicam que, para que essa população de *A. leorae* seja preservada, as condições gerais existentes no Rio Tonatzín devem ser protegidas, especialmente os trechos com fundo lodoso e vegetação aquática onde são encontradas larvas, juvenis e adultos.

Palavras-chave: adultos, dimorfismo sexual, juvenis, ovos, substrato, vegetação aquática, velocidade da água.

Introduction

In Mexico, eight of the 18 (44%) species of salamanders in the genus *Ambystoma* are critically endangered, and another three species are considered endangered by the IUCN (The IUCN Red List of Threatened Species, Version 2014.2; www.iucnredlist.org). One of the critically endangered species is *A. leorae* (Taylor, 1943). *Ambystoma leorae* has an Environmental Vulnerability Score of 15, which is within the high vulnerability range (Wilson *et al.* 2013), and is considered Threatened by the Mexican government (SEMARNAT 2010). Many populations of Mexican *Ambystoma* are subject to several anthropogenic threats, including urbanization and suburbanization, pollution, conversion to agriculture, and the introduction of fish (e.g., Lemos-Espinal *et al.* 1999, Griffiths *et al.* 2004, Contreras *et al.* 2009, Frías-Alvarez *et al.* 2010), as well as the possible threat of *Batrachochytrium dendrobatidis* Longcore, Pessier, and Nichols (Frías-Alvarez *et al.* 2008; see also Mendoza-Almeralla *et al.* 2015). Several Mexican *Ambystoma* have small, isolated populations that are prone to extinction (see Parra-Olea *et al.* 2012), including *A. leorae* (Sunny *et al.* 2014a,b). The expansion of Mexico City is of particular concern and likely affects the populations of *Ambystoma* in the natural areas surrounding it, including *A. leorae* (Monroy-Vilchis *et al.* 2015), especially given recent degradation of the forest in the vicinity (García-Romero 2002; see also Merlín-Uribe *et al.* 2013).

As with many other species of *Ambystoma* in Mexico, little is known about the natural history of *A. leorae*. *Ambystoma leorae* was originally described from near Río Frío, Mexico by Taylor (1943). *Ambystoma leorae* have been found in pools along streams with slow moving water, sand or rock substrates, cool water, and high dissolved oxygen levels (Vega-López and Alvarez-S. 1992, Sunny *et al.* 2014a, Monroy-Vilchis *et al.* 2015). The diet of *A. leorae* primarily consists of aquatic insects (Vega-López and Alvarez-S. 1992). Dyer and Brandon (1973) reported some new host records of parasitic nematodes in *A. leorae*. *Ambystoma leorae* lay a mean clutch size of 4.4 eggs (range 1–10) with eggs attached to vegetation (Sunny *et al.* 2014a). The maximum body size of *A. leorae* to date is 103 mm SVL (Lemos-Espinal and Ballinger, 1994). This species of salamander shows relatively high levels of genetic diversity (Sunny *et al.* 2014b). Additional information on *A. leorae* is needed to better understand its conservation and management. Here we report on the distribution of eggs, larvae, juveniles, and adults in the stream and body size and sexual dimorphism of *A. leorae* from the Río Tonatzin, State of México, Mexico.

Materials and Methods

Study Site

We recorded individuals of *A. leorae* in Río Tonatzin, a permanent stream located 7.5–8.8 km northeast of the town of Río Frío, municipality of Ixtapaluca, state of México, Mexico (19°22'2.9" N, 98°39'28.8" W to 19°22'18.7" N, 98°39'30.2" W; 3012 m a.s.l.). The Río Tonatzin is a small stream surrounded by a mixed forest consisting primarily of Pine (*Pinus montezumae* Lamb. and *P. hartwegii* Lindl.), Religious Fir [*Abies religiosa* (Kunth) Schltdl. & Cham.], a variety of Oak trees (mainly *Quercus rugosa* Née and *Q. laurina* Bonpl.), and Mexican Alder (*Alnus jorullensis* Kunth). The understory includes Mexican Strawberry Tree (*Arbutus glandulosa* M Martens & Gal.), Manzanita (*Arctostaphylos arguta* Zucc.), Mexican Lupine (*Lupinus elegans* Kunth), Pineapple Sage (*Salvia elegans* Vahl), Old-man Bush (*Senecio angulifolius* DC.), Toluca Bentgrass (*Agrostis tolucensis* Kunth), Fescue Grass (*Festuca* sp.), and Mühlenberg Grass (*Mühlenbergia* sp.). This portion of the stream, 2 km in length, has a gentle slope that results in a constant flow of water. Most of the stream is sinuous with a number of curves where water has eroded the stream banks producing small caves or crevices in them. The bottom of the stream consists of mud, sand, gravel, or bed rock. The width of the stream varies from 44 to 378 cm, and the depth from 14 to 134 cm. Through the year dissolved oxygen levels range from 3.01 to 6.89 ppm, and the temperature from 6.1 to 18.8°C.

Methods

We visited the Río Tonatzin monthly from October 2015 through September 2016. For safety and logistic reasons, sampling took place during the day. On each visit, we walked 2 km along the stream with the starting point for each visit shifted so that sites were not visited more than once during the study. Along this 2 km section of the stream, we surveyed 25 arbitrarily selected sites along the stream (so 25 sites per month). Each site was, on average, 75 m from the neighboring sites (generally 50 to 100 m). In an effort to sample a greater proportion of the stream across the study period, site selection for one visit was independent of site selection for other visits. Thus, some sites may have been sampled more than once across the entire study period, but specific sites were not repeatedly sampled over the course of the study, and it is unlikely specific sites were sampled more than once during the course of this study. Each site consisted of a 3 m linear section of the stream. Within each site, we carefully searched the stream section visually and by using a snake hook along the bottom of the stream and in depressions in the side of the stream to induce movement by any salamanders, thus making them obvious. We also looked under all rocks or other objects in the stream section. Using these methods to thoroughly search every site and given the small area searched at each site (< 5 m^2), we are confident that we detected most salamanders (adults, larvae, and eggs) within each site. However, since we only visited each specific site once during the course of the study we could not estimate detection probabilities (Mazerolle *et al.* 2007). Other studies have estimated detection probabilities for *Ambystoma* from 0.459 to 0.89 (Corn *et al.* 2005, Hossack and Corn 2007, Gorman *et al.* 2009, Peterman *et al.* 2013); however, all of these refer to larger pond or wetland habitats and not to streams or sites as small as our sites. Detection probabilities of non-ambystomatid salamanders in streams range from 0.39–0.96 (Jung *et al.* 2005, Kroll *et al.* 2010).

We captured salamanders with a net and measured snout-vent length (SVL; tip of snout to anterior margin of vent) and body mass of each captured individual. We determined the sex of each salamander based on the presence of swelling of the cloacal region on both sides of the tail in males and a lack of swelling in females (Brandon and Altig 1973). We released salamanders at the point of capture once measurements were completed.

We measured the width and depth of the stream of each site to the nearest cm. We measured dissolved oxygen and water temperature using a YSI model 85 meter (YSI Incorporated, Yellow Springs, Ohio). We measured water speed at the surface and in the middle of the water column using a Global Water Flow Probe Hand-held Flowmeter (Xylem Inc., White Plains, New York). We also characterized the vegetation at each site as grass roots (in crevices in the stream bank), submerged aquatic plants and algae, or no vegetation. We categorized substrate type as mud, mud with gravel, gravel, bedrock, or sand. We also recorded the microhabitat [near water surface (i.e., within 15 cm of surface), in algae, in crevice in stream bank, or on the stream bottom] of each individual salamander.

We used a generalized linear model (with binomial distribution) with presence or absence of adult juvenile *A. leorae* as the dependent variable and the abiotic stream characteristics as the independent variables. Because of the small number of sites in which we observed larvae and eggs, we did not conduct an analysis and report only statistics for the occupied sites. We compared SVL between the sexes using an ANOVA. We compared body mass between the sexes using an ANCOVA with SVL as covariate.

Statistical analyses were performed using JMP Pro 10.0.0 (SAS Institute, Cary, North Carolina). Means are given ± 1 SE.

Results

Observations of Eggs and Larvae

We observed a total of seven egg masses from April to June 2016. Two egg masses were seen in April, two in May, and three in June. Mean number of eggs per mass was 7.28 ± 0.47 (range 6–9). All egg masses were attached to vegetation. Egg masses were all found at sites with mud bottoms (*N* = 7), and in sites with submerged aquatic plants and algae (*N* = 6) or no vegetation (*N* = 1). Stream sites with egg masses were 69 ± 4.8 cm wide (*N* = 7) and 57 ± 7.4 cm deep (*N* = 7). Mean dissolved oxygen of sites with egg masses was 6.1 ± 0.3 mg/L (*N* = 7). Water temperature averaged 15.3 ± 0.8°C (*N* = 7). Surface water speed averaged 0.134 ± 0.027 m/s (*N* = 7) and water speed in the middle of the water column averaged 0.030 ± 0.006 m/s (*N* = 7).

Larval *A. leorae* were observed from May to August at three sites in May, two sites in June, and one site each in July and August. Larval *A. leorae* were only found in sites with mud bottoms (*N* = 7) and submerged aquatic vegetation and algae (*N* = 7). Individual larval *A. leorae* were found near the water surface (*N* = 25) or hidden in algae (*N* = 8). The mean stream width of sites where larval *A. leorae* were found was 121.3 ± 21.2 cm (*N* = 7). Mean depth of sites with *A. leorae* larvae was 72.6 ± 11.9 cm (*N* = 7). Dissolved oxygen levels of sites with *A. leorae* larvae was 6.49 ± 0.10 mg/L (*N* = 7). Mean temperature was 13.9 ± 1.1°C (*N* = 7). The water speed at the surface averaged 0.24 ± 0.10 m/s (*N* = 7) and at the middle of the water column averaged 0.028 ± 0.028 m/s (*N* = 7).

Observations of Adults and Juveniles

We made a total of 157 observations of individual adult and juvenile salamanders. We observed salamanders in all months of the study except September 2016 (Table 1). The number of salamanders observed was highest in May–July (Table 1).

Stream use.—The generalized linear model of the stream characteristics was significant (whole model test: χ^2 = 155.84, $p < 0.0001$). We present the results of this analysis in Table 2. Vegetation type and bottom type were the most significant terms in the model with vegetation type being the most important variable by far (i.e., greatest log worth). Dissolved oxygen, month, and substrate color were also significant. All other variables were not significant.

Below and in Table 3 we provide summaries or summary statistics for the stream characteristics we examined in the generalized linear model. We found *A. leorae* adults and juveniles on mud (*N* = 14), sand (*N* = 9), and mud and gravel (*N* = 3) substrates. We did not observe *A. leorae* on gravel or bedrock. Adult and juvenile *A. leorae* primarily used crevices in the stream bank (*N* = 10) or were near the water's surface (*N* = 12), with the stream bottom (*N* = 1) and algae (*N* = 4) used less often. If we examine the stream use of individual *A. leorae* adults and juveniles as opposed to sites, we found 40.5% (*N* = 47) of individuals on mud substrates, 30% (*N* = 35) on mud and gravel substrate, and 29.3% (*N* = 34) on sand. We observed 44.0% (*N* = 51) of individuals in grass roots, 35.3% (*N* = 41) in submerged aquatic vegetation and algae, and 20.7% (*N* = 24) in no vegetation. Most individuals were found either in crevices in the stream wall (44.8%; *N* = 52) or near the water surface (50.9%; *N* = 59), and only rarely did we find individuals on the stream bottom (1.7%; *N* = 2) or hidden in algae (2.6%; *N* = 3).

Body size and sexual dimorphism.—Male and female *A. leorae* did not differ in SVL [Males: 76.7 ± 1.6 m (*N* = 41); Females: 76.4 ± 1.4 mm (*N* = 57); ANOVA: $F_{1,96}$ = 0.028, p = 0.87]. Body mass of male and female *A. leorae* did not differ either [Males: 19.5 ± 0.98 g (*N* = 41); Females: 19.2 ± 0.86 g (*N* = 57);

Table 1. Number of adult and juvenile *Ambystoma leorae* observed along the Río Tonatzin, State of México, Mexico, in each month of the study. Number of sites is 25 for all months.

Year	**2015**						**2016**					
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
N	6	4	3	6	6	10	15	35	30	28	14	0

Table 2. Results of a generalized linear model of the presence and absence of *Ambystoma leorae* using stream characteristics.

Variable	**Log Worth**	***p***	**Parameter Estimate**
Vegetation type	26.44	< 0.0001	2.52
Bottom type	5.79	< 0.0001	-0.97
Month	2.55	0.0028	0.153
Dissolved oxygen (mg/L)	1.88	0.0132	-0.335
Substrate color	1.66	0.0219	0.73
Stream depth (cm)	0.65	0.22	-0.0091
Water speed middle of water column (m/s)	0.34	0.46	3.00
Water temperature (°C)	0.14	0.72	-0.019
Surface water speed (m/s)	0.10	0.80	-0.26
Stream width (cm)	0.04	0.90	-0.0004

ANCOVA: $F_{1,95} = 0.026$, $p = 0.87$]. Body mass increased with SVL (ANCOVA: $F_{1,95} = 1119.3$, $p < 0.0001$; Body mass = -26.4 + 0.60SVL).

Discussion

Our observations suggest that egg laying by *A. leorae* takes place from April to June. This is similar to the timing of egg laying in other Mexican *Ambystoma,* including a previous report for *A leorae*. Sunny *et al.* (2014a) reported finding eggs of *A. leorae* from February to June. *Ambystoma altamirani* Dugès, 1895 lay eggs in June (Lemos-Espinal *et al.* 2016), and *A. rosaceum* Taylor, 1941 have been recorded as laying eggs in April (Anderson 1961, Anderson and Webb 1978, Tanner 1989). These observations coincide with the wet or rainy season in central Mexico.

We observed egg masses of *A. leorae* attached to aquatic vegetation. Sunny *et al.* (2014a) also found *A. leorae* eggs attached to vegetation, as well as in “caves”. *Ambystoma altamirani* and *A. ordinarium* Taylor, 1940 also attach their eggs to vegetation (Anderson and Worthington 1971, Brandon and Altig 1973, Lemos-Espinal *et al.* 2016). *Ambystoma rivulare* (Taylor, 1940) attach their eggs to twigs and pine needles (Bille 2009), but also place their eggs under logs or rocks (Brandon and Altig 1973, Anderson and Webb 1978). However, *A. rosaceum* place their eggs exclusively under rocks or logs (Brandon and Altig 1973, Anderson and Webb 1978). Why some species of Mexican *Ambystoma* place their eggs under cover objects whereas others attach them to other structures in the water column is not clear, and represents a question that might be pursued in future research.

Table 3. Mean (± 1SE) of characteristics of stream locations along the Río Tonatzin, State of México, Mexico with and without *Ambystoma leorae* pooled across all months and years (October 2015 through September 2016).

Characteristic	With *A. leorae* (*N* = 26)	Without *A. leorae* (*N* = 274)
Stream width (cm)	128.8 ± 10.0	145.0 ± 3.2
Stream depth (cm)	69.1 ± 4.9	70.0 ± 1.3
Dissolved oxygen (mg/L)	5.50 ± 0.24	5.37 ± 0.08
Water temperature (°C)	14.0 ± 0.5	14.4 ± 0.2
Surface water speed (m/s)	0.036 ± 0.009	0.034 ± 0.003
Middle water speed (m/s)	0.003 ± 0.002	0.003 ± 0.001

We found a mean egg mass size of 7.28 eggs for *A. leorae* (range = 6–9). This is slightly higher than the mean of 4 for *A. leorae* (range = 1–10) reported by Sunny *et al.* (2014a), but these observations both suggest a relatively small egg mass for this species.

Our results suggest that the use of sites along the Río Tonatzin by *A. leorae* adults and juveniles is driven primarily by vegetation type (sites with submerged aquatic vegetation), as well as bottom type, dissolved oxygen, month, and substrate color (see Table 2). Our observations on the importance of vegetation type and substrate type for the use of sites by *A. leorae* along the Río Tonatzin is consistent with results of a study of another Mexican *Ambystoma* that compared sites with and without salamanders. *Ambystoma altamirani* used stream sites that held a greater volume of water, with higher dissolved oxygen levels, faster moving water, black substrates, and emergent vegetation than expected; and avoided sites with no vegetation and gravel and bedrock substrates (Lemos-Espinal *et al.* 2016; see also Taylor and Smith 1945, Maldonado-Koerdell 1947, Brandon and Altig 1973, Lemos-Espinal *et al.* 1999). On a broader level our results are consistent with observations of *A. leorae* and other Mexican *Ambystoma* in that they occupy narrow, slow-moving streams with oxygenated water with muddy or sandy bottoms (e.g., *A. leorae,* Vega-López and Alvarez-S. 1992, Sunny *et al.* 2014a, Monroy-Vilchis *et al.* 2015; *A. rivulare,* Brandon and Altig 1973, Bille 2009, Lemos-Espinal *et al.* 2015; *A. rosaceum,* Anderson 1961, Anderson and Webb 1978). In order to better understand the specific needs of stream-dwelling Mexican *Ambystoma,* we need more detailed examinations of their distributions along streams to have a better idea of what specific attributes of streams need to be retained to ensure the persistence of these salamanders.

We found no evidence of sexual dimorphism in the body size (SVL, body mass) of *A. leorae.* This is consistent with the findings for several other Mexican *Ambystoma: A. andersoni* Krebs and Brandon, 1984 (Krebs and Brandon 1984); *A. granulosum* Taylor, 1944 (Aguilar-Miguel *et al.* 2009); *A. lermaense* (Taylor, 1940) (Aguilar-Miguel *et al.* 2009). However, other species of Mexican *Ambystoma* are sexually dimorphic with females larger than males (*A. rosaceum;* Anderson 1961) or males larger than females (*A. altimirani;* Lemos-Espinal *et al.* 2016). It is not clear why there are differences among species of Mexican *Ambystoma* in the extent and direction of sexual dimorphism. One possible explanation that has been tentatively put forth is that sexual dimorphism may be related to the use of ponds and streams (Lemos-Espinal *et al.* 2016). However, the lack of sexual dimorphism in *A. leorae* and male-biased sexual dimorphism in *A. altimirani* (Lemos-Espinal *et al.* 2016) argues against such an explanation since both of these species are found in streams. Further study is needed to generate possible explanations for such variation.

Conclusions

Our observations indicate that *A. leorae* use a variety of sites along the Río Tonatzin, with vegetation type and substrate type being of greater importance, along with dissolved oxygen and substrate color. Larvae were only found in sections with muddy bottoms and aquatic vegetation. Therefore, in order to maintain this population of *A. leorae*, the current general conditions of the Río Tonatzin need to be maintained. Of particular importance appears to be the maintenance of fish-free streams as Lemos-Espinal *et al.* (1999) reported that no *A. leorae* were found after the introduction of fish into a stream that previously contained *A. leorae*. Populations of *A. leorae* may also be endangered by channelization of streams and the use of water by local residents (Vega-López and Alvarez-S. 1992).

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