



## Habitat Split and the Global Decline of Amphibians

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**Table 1.** Mean abundances,  $P$ , of motile *L. salmonis* on juvenile pink salmon and estimated parasite-induced host mortality,  $M$  (with upper and lower bounds of the 95% credible interval in parentheses), for exposed populations during infestations.

River	2002		2003		2004*		2005		2006	
	$P$	$M$	$P$	$M$	$P$	$M$	$P$	$M$	$P$	$M$
Ahta	3.4	95.21 (79.07, 98.95)	1.0	59.09 (36.87, 73.82)	0.3	23.52 (12.89, 33.10)	2.6	90.21 (69.76, 96.93)	0.4	30.06 (16.81, 41.49)
Kakweiken	3.4	95.21 (79.07, 98.95)	1.0	59.09 (36.87, 73.82)	0.3	23.52 (12.89, 33.10)	2.6	90.21 (69.76, 96.93)	0.4	30.06 (16.81, 41.49)
Viner	4.0	97.20 (84.12, 99.53)	2.2	86.00 (63.65, 94.76)	0.2	16.37 (8.79, 23.51)	2.3	87.20 (65.29, 95.41)	1.4	71.39 (47.48, 84.68)
Wakeman	4.0	97.20 (84.12, 99.53)	2.2	86.00 (63.65, 94.76)	0.2	16.37 (8.79, 23.51)	2.3	87.20 (65.29, 95.41)	1.4	71.39 (47.48, 84.68)
Kingcome	4.0	97.20 (84.12, 99.53)	2.2	86.00 (63.65, 94.76)	0.2	16.37 (8.79, 23.51)	2.3	87.20 (65.29, 95.41)	1.4	71.39 (47.48, 84.68)
Ahnuhati	2.6	90.21 (69.76, 96.93)	0.7	46.51 (27.53, 60.86)	0.2	16.37 (8.79, 23.51)	1.9	81.70 (58.27, 92.16)	0.3	23.52 (12.89, 33.10)
Lull	2.6	90.21 (69.76, 96.93)	0.7	46.51 (27.53, 60.86)	0.2	16.37 (8.79, 23.51)	1.9	81.70 (58.27, 92.16)	0.3	23.52 (12.89, 33.10)

\*These data correspond to the salmon cohort responding to the fallow treatment in 2003.

years. The time to reach sufficient temporal replication to support hierarchical mixed-effects modeling, say 10 generations (which equals 20 years), greatly exceeds the predicted time to extinction. That is, there is a major risk associated with waiting for large data sets to accumulate before implementing conservation policy. Industrial aquaculture is rapidly expanding to new species, regions, and habitats (31), which can create parasite outbreaks that contribute to the decline of ocean fisheries and ecosystems.

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## Habitat Split and the Global Decline of Amphibians

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The worldwide decline in amphibians has been attributed to several causes, especially habitat loss and disease. We identified a further factor, namely “habitat split”—defined as human-induced disconnection between habitats used by different life history stages of a species—which forces forest-associated amphibians with aquatic larvae to make risky breeding migrations between suitable aquatic and terrestrial habitats. In the Brazilian Atlantic Forest, we found that habitat split negatively affects the richness of species with aquatic larvae but not the richness of species with terrestrial development (the latter can complete their life cycle inside forest remnants). This mechanism helps to explain why species with aquatic larvae have the highest incidence of population decline. These findings reinforce the need for the conservation and restoration of riparian vegetation.

Amphibian populations are declining worldwide (1, 2). Among the factors determining the amphibian declines are habitat loss and fragmentation, which affect amphibians just as they affect any other organisms: through population isolation, inbreeding, and edge effects (3–5). Another important factor is the fungus *Batrachochytrium dendrobatidis*, a highly virulent pathogen that attacks many amphibian species and has been responsible for the decline of many populations even in undisturbed environments (6, 7). Amphibians can also be threatened by climate shifts (7), ultraviolet-B radiation (8), introduction of exotic species (9), and agrochemical contaminants (10). We inves-

tigated the role of a further factor, which we define as “habitat split.”

Amphibian species with aquatic larvae typically undergo a major ontogenetic niche shift, whereby tadpoles and adults occupy two distinct habitats (11). In pristine environments, the aquatic habitat of the tadpoles and the terrestrial habitat of the postmetamorphics grade into each other. However, in landscapes occupied by humans, land use has often resulted in a spatial separation between remnants of terrestrial habitat and breeding sites (12). Adults of species with aquatic larvae, in order to breed, are obliged to abandon forest remnants to reach water bodies, and at the end of the reproductive season, both

adults and juveniles are forced to locate and return to forest remnants (fig. S1). During this compulsory migration, they face multiple hazards that are associated with environmental conditions within the intervening matrix, such as dehydration, predation, agrochemicals, and other pollutants (10, 13). Amphibians with terrestrial development, however, should not respond to habitat split, because they may be able to successfully reproduce irrespective of the presence or absence of water bodies. In human-altered landscapes, habitat split can be expected to reduce population sizes, decrease the extent and occupancy rates of metapopulations, and affect negatively the richness of local communities.

We tested the effect of habitat split on the richness of local amphibian communities, taking into account the influence of two additional landscape properties, habitat loss and fragmentation. The study was conducted in Brazil, which harbors a rich amphibian fauna (14, 15). We focused particularly on the Atlantic Forest, one of the five most endangered biodiversity hot spots (16), which has more than 480 amphibian species (about 80% with aquatic larvae). Because less than 7% of the Atlantic Forest remains, habitat loss and fragmentation have been suggested as the main causes of the high frequency of threatened and declining amphibian species in the region (14–17). Although the Atlantic Forest riparian vegetation is in principle protected by legislation (Brazilian Forest Code 4771/65), land occupation has often been concentrated in valleys where water is readily available for agriculture, industry, and human consumption (18). This historical process has favored the occurrence of a large number of “dry fragments” that are disconnected at the landscape level from streams and other water sources (18), causing habitat split. Here, habitat split is measured as the percentage of non-natural vegetation cover, habitat fragmentation is the ratio between the perimeter of forest edge and forest area, and habitat split is the percentage of the total stream length that does not overlap with forest cover; all of these metrics are calculated on a circular landscape of 15-km diameter (19).

Across the Atlantic Forest biome, habitat split was the best predictor of the species richness of leaf-litter forest amphibians [simple linear regression:  $\beta = -0.140 \pm 0.026$  (SEM),  $F_{1,10} = 29.040$ ,  $P < 0.001$ ,  $r^2 = 0.744$ ], followed by

habitat loss ( $\beta = -0.122 \pm 0.027$ ,  $F_{1,10} = 21.321$ ,  $P = 0.001$ ,  $r^2 = 0.681$ ) and habitat fragmentation ( $\beta = -7.643 \pm 2.509$ ,  $F_{1,10} = 9.277$ ,  $P = 0.012$ ,  $r^2 = 0.481$ ). A path analysis, looking simultaneously at the effect of these three explanatory factors, showed that habitat split was the only significant factor negatively affecting the richness of leaf-litter amphibians (standardized path coefficient =  $-1.025 \pm 0.514$ ,  $P < 0.05$ ).

As expected, habitat split had a strong negative effect on the richness of species with aquatic larvae (simple linear regression:  $\beta = -0.097 \pm 0.019$ ,  $F_{1,10} = 25.134$ ,  $P = 0.001$ ,  $r^2 = 0.715$ ) and no effect on the richness of species with terrestrial development ( $\beta = -0.042 \pm 0.027$ ,  $F_{1,10} = 2.450$ ,  $P = 0.149$ ,  $r^2 = 0.197$ ; Fig. 1). Path analyses showed that habitat split was the single landscape metric affecting the richness of species with aquatic larvae (standardized path coefficient =  $-1.944 \pm 0.433$ ,  $P < 0.001$ ; Fig. 2A). In contrast, none of the three landscape metrics affected the richness of species with terrestrial development, although habitat loss exhibited the strongest path (Fig. 2B). The predominance of habitat split as the strongest factor influencing the richness of species with aquatic larvae remained unaltered after controlling, in turn, for latitude, altitude, altitudinal range, and mean annual rainfall (19).

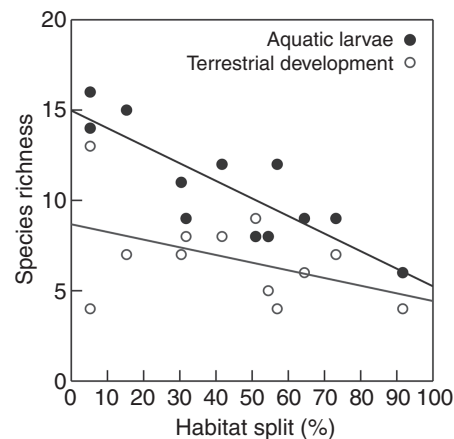
Although herpetologists have recognized the importance of small-scale connections between the habitat of immatures and that of adults (20, 21), our results suggest that the absence of these links might be a key driver of the loss of amphibian biodiversity. Habitat loss has an immediate direct effect on the size of amphibian populations by redefining the boundaries of their natural habitats (3). Habitat fragmentation acts on a longer time scale through population isolation, inbreeding, and edge effects (3–5). Habitat split is a strong force that can modify

population size, structure, and distribution in a single generation.

Habitat split should most strongly affect species with aquatic larvae. This can contribute to explaining why a disproportionate number of records of population declines are from amphibian species that have aquatic larvae, especially stream-breeders (14, 17, 22–24). Water-transmitted diseases and water pollution are also important factors producing this bias (6, 10). Our study evaluated the effect of habitat split in relation to streams, but it can also be accessed in relation to other breeding habitats, such as ponds. For instance, the occupancy of ponds by North American anurans and salamanders with aquatic larvae was affected by the distance to the nearest vegetation patch (20).

Habitat split is likely to be a major influence on other taxa that undergo ontogenetic habitat shifts, including many terrestrial invertebrates with aquatic larvae. Other vertebrates can also suffer from habitat split. The reproductive migration of a wide variety of freshwater fishes is known to be blocked or jeopardized by dams (25). Continental migration of waterbirds has been put at risk with the loss of stopover wetlands reducing the connectivity between breeding and foraging sites (26).

A wide range of conservation strategies has been proposed to halt the global decline of amphibians, including a recent international call for ex situ conservation facilities (27–29). Where pristine environments are still available, as in the Amazon, the establishment of large biological reserves comprising whole hydrographic catchments is the best in situ conservation strategy. In landscapes undergoing modification, specific laws for conserving riparian zones connecting terrestrial and aquatic habitats should be introduced or enforced. Where habitat split has already occurred, restoration programs (30) should be undertaken to reverse its negative effects on amphibian diversity.



**Fig. 1.** Effect of habitat split on species richness of leaf-litter amphibians with aquatic larvae and with terrestrial development across 12 Brazilian Atlantic Forest landscapes. Habitat split is calculated as the percentage of the total stream length that does not overlap with natural forest cover. Linear regression lines are shown.

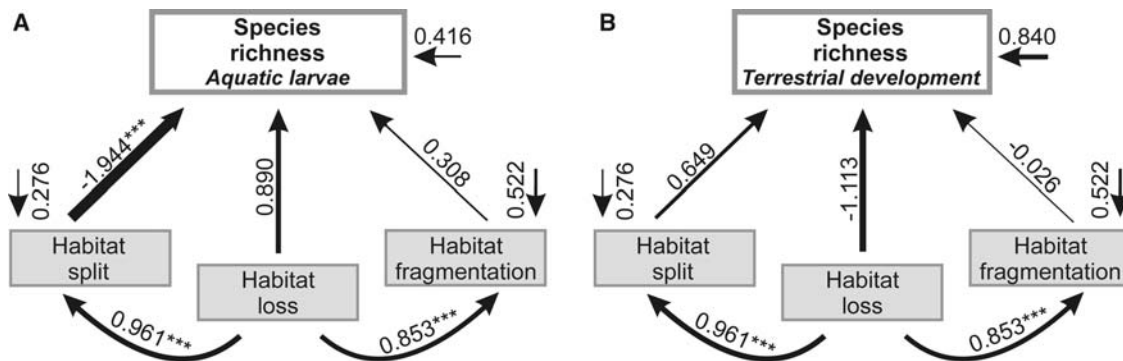
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**Fig. 2.** Path analysis models showing the relative strength of habitat split, habitat loss, and habitat fragmentation on species richness of Brazilian Atlantic Forest leaf-litter amphibians ( $N = 12$  study sites). **(A)** Species with aquatic larvae. **(B)** Species with terrestrial development. Numbers are standardized path coefficients ( $***P < 0.001$ ). The thickness of the arrows represents the relative strength of the relationship.



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Materials and Methods

Fig. S1

Table S1

References

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## Serine-7 of the RNA Polymerase II CTD Is Specifically Required for snRNA Gene Expression

Sylvain Egloff,<sup>1</sup> Dawn O'Reilly,<sup>1</sup> Rob D. Chapman,<sup>2</sup> Alice Taylor,<sup>1</sup> Katrin Tanzhaus,<sup>1</sup> Laura Pitts,<sup>1</sup> Dirk Eick,<sup>2</sup> Shona Murphy<sup>1\*</sup>

RNA polymerase II (Pol II) transcribes genes that encode proteins and noncoding small nuclear RNAs (snRNAs). The carboxyl-terminal repeat domain (CTD) of the largest subunit of mammalian RNA Pol II, comprising tandem repeats of the heptapeptide consensus Tyr<sup>1</sup>-Ser<sup>2</sup>-Pro<sup>3</sup>-Thr<sup>4</sup>-Ser<sup>5</sup>-Pro<sup>6</sup>-Ser<sup>7</sup>, is required for expression of both gene types. We show that mutation of serine-7 to alanine causes a specific defect in snRNA gene expression. We also present evidence that phosphorylation of serine-7 facilitates interaction with the snRNA gene-specific Integrator complex. These findings assign a biological function to this amino acid and highlight a gene type-specific requirement for a residue within the CTD heptapeptide, supporting the existence of a CTD code.

Human snRNA genes transcribed by Pol II, including those encoding U1 and U2 spliceosomal RNAs, have specialized promoters comprising conserved proximal and distal sequence elements (PSE and DSE) (1). Rather than polyadenylation signals, 3' box elements direct the cotranscriptional formation of the primary 3' end of transcripts (2, 3). The 3' end of these precursor snRNAs (pre-snRNAs) is further processed in the cytoplasm to yield mature nonpolyade-

nated snRNAs (2). Removal of the CTD of the large subunit of mammalian Pol II strongly affects expression of both snRNA and protein-coding genes (2–4). The CTD has a distinctive structure composed of multiple repeats containing residues that undergo reversible phosphorylation during transcription (5). For example, phosphorylation of Ser<sup>5</sup> by cyclin-dependent kinase 7 (CDK7) facilitates promoter release and RNA capping, whereas Ser<sup>2</sup> phosphorylation by CDK9 is associated with processive

elongation and 3' end processing (5, 6). No role has yet been ascribed to Ser<sup>7</sup>.

The mammalian Pol II CTD comprises 52 repeats, 25 of which deviate from the consensus at position 7. The mainly consensus repeats 1 to 25 activate snRNA 3' end processing more effectively than repeats 27 to 52, which have few serines at position 7 (2). In contrast, both halves of the CTD are equally effective in activating polyadenylation (7). We have tested the requirement for Ser<sup>7</sup> for expression of snRNA [U2-globin (U2G) (2)] and mRNA [cytomegalovirus promoter-driven heterogeneous nuclear ribonucleoprotein K (pCMV-hnRNPK) (8)] templates in human embryonic kidney (HEK) 293 cells by introducing mutations into consensus (Con) CTD repeats in an  $\alpha$ -amanitin-resistant Pol II large subunit (Rpb1) (9) (Fig. 1A and fig. S1A). The large subunit of endogenous Pol II is very sensitive to inhibition by  $\alpha$ -amanitin, facilitating complementation studies (9). A CTD with at least 25 consensus repeats [(Con)<sup>25</sup>] was used because this supports efficient production and cotran-

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