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Declining Amphibian Populations: The Problem of Separating Human Impacts from Natural Fluctuations

JOSEPH H. K. PECHMANN,* DAVID E. SCOTT, RAYMOND D. SEMLITSCH, JANALEE P. CALDWELL, LAURIE J. VITT, J. WHITFIELD GIBBONS

Reports of declining amphibian populations in many parts of the world are numerous, but supporting long-term census data are generally unavailable. Census data from 1979 to 1990 for three salamander species and one frog species at a breeding pond in South Carolina showed fluctuations of substantial magnitude in both the size of breeding populations and in recruitment of juveniles. Breeding population sizes exhibited no overall trend in three species and increased in the fourth. Recent droughts account satisfactorily for an increase in recruitment failures. These data illustrate that to distinguish between natural population fluctuations and declines with anthropogenic causes may require long-term studies.

EVALUATION OF THE REPORTED DECLINES of amphibian populations, some possibly to extinction (*1*), has been hampered by the dearth of long-term census data on amphibians. Conclusions of National Research Council workshop participants about the status of amphibian populations (*1*) were based primarily on anecdotal observations. These observations have convinced many that there is a general decline worldwide, although not all species and regions appear to be affected (*1, 2*). In many individual cases, however, it may be difficult to distinguish declines resulting from human activities from natural population fluctuations without long-term data on the natural variation in both real and apparent (catchable) population sizes (*2*).

We have monitored amphibian populations at one ephemeral pond, Rainbow Bay, continuously for the past 12 years, the period during which most of the reported declines have occurred (*1*). Although data from one site cannot be extrapolated to other sites, Rainbow Bay nonetheless provides an important test site for the amphibian decline question because of the extensive data available.

Rainbow Bay is a Carolina bay (*3, 4*) located on the U.S. Department of Energy's 780-km^2 Savannah River Site (*4*) in the upper coastal plain sandhills region in South Carolina. The pond is approximately 1 ha with a maximum water depth of 1.04 m and usually fills during the winter and dries each spring or summer (*5*). Rainbow Bay and the adjacent terrestrial habitats were protected from most human impacts during our 12-year study, but were altered in the past (*6*). Anthropogenic factors have been implicated in many of the reported declines and extinctions of amphibian populations, yet others have occurred in protected, seemingly pristine areas (*1*). Thus, Rainbow Bay's current protected status does not make it an exception with respect to its potential for amphibian declines.

Amphibians migrating to and from the pond have been censused since 21 September 1978 with the use of a terrestrial drift fence with pitfall traps that completely surrounds the pond (*7*). Traps are checked daily, and data to 31 August 1990 are reported here. Upon capture, all amphibians were identified, marked by clipping toes, and released on the opposite side of the fence from where captured.

Five species of salamanders and 11 species of frogs and toads are known to have bred at Rainbow Bay (*5*). We report data on *Ambystoma opacum* (marbled salamander), *A. talpoideum* (mole salamander), *A. tigrinum tigrinum* (eastern tiger salamander), and *Pseudacris ornata* (ornate chorus frog). These species were chosen because demographic interpretation of the drift-fence data is most straightforward for them (*8*). The four are primarily terrestrial and fossorial except for the aquatic larval stage (*9*). Reproductive *A. opacum* migrate to breeding ponds from September to November, whereas breeding migrations of the other three species occur primarily from November to March. Adults spend a few days to weeks at the pond before returning to terrestrial habitats (*10*). Juveniles metamorphose and emigrate from the pond during the following spring and summer. Age at first reproduction varies considerably, but some individuals of all four species reproduce at 1 year of age (*11, 12, 13*).

These species usually return to their natal pond to breed, that is, they are philopatric (*13, 14*). Four smaller breeding sites occur within 1 km of Rainbow Bay, and low rates of dispersal connect populations of these species to form metapopulations (*15*). Immigration and emigration are usually minor components of the population dynamics of these philopatric species, but may be important in long-term persistence (*15*).

Because individuals of the four species cannot trespass the drift fence, this technique provides a nearly complete census of breeding adults and juvenile recruits. Terrestrial immatures and adults that skip breeding are not censused, however. Breeding populations had approximately 1 to 1 or male-biased sex ratios each year; therefore only data for females are presented. We tested for evidence of a decline in numbers of breeding females or of metamorphosing juveniles.

Female breeding population sizes fluctuated over three orders of magnitude among years, and juvenile recruitment over five (Fig. 1). Each species was common in some years but uncommon or absent in others. Year-to-year variation and short-term trends make it difficult to discern long-term trends. Breeding populations declined during some time periods, but increased during others (Fig. 1). Fluctuations in breeding population sizes were not significantly correlated among species (*16*).

Breeding population sizes vary more than adult population sizes. Adults migrate to ponds only during warm night rains within their breeding season and may skip breeding in years of low rainfall (*13, 17*). For example, breeding populations of *A. talpoideum*, *A. tigrinum*, and *P. ornata* were reduced in

J. H. K. Pechmann, Savannah River Ecology Laboratory, University of Georgia, P.O. Drawer E, Aiken, SC 29802, and Department of Zoology, Duke University, Durham, NC 27706.
D. E. Scott and J. W. Gibbons, Savannah River Ecology Laboratory, University of Georgia, P.O. Drawer E, Aiken, SC 29802.
R. D. Semlitsch, Zoologisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland.
L. J. Vitt and J. P. Caldwell, Oklahoma Museum of Natural History and Department of Zoology, University of Oklahoma, Norman, OK 73019.

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*To whom all correspondence should be addressed.

the driest years (1981, 1985, 1988, and 1989) (Figs. 1 and 2A) in comparison with years that immediately preceded or followed them, except for *P. ornata* in 1980 (Fig. 1) (*11*). We used breeding season rainfall as a covariate to remove rainfall-related variance and tested for partial rank correlations of female breeding population sizes with year, that is, for trends through time (Fig. 1). The only significant partial correlation with year was for *A. opacum*, and this correlation was positive. No females of *A. opacum* were present during the first 2 years, and only two during the third, but 594 females bred in 1990 (Fig. 1C). There was a statistically significant correlation between the number of breeding females and rainfall for *A. talpoideum* and *A. tigrinum* (Figs. 1 and 2A).

Juvenile recruitment of all species was episodic; thousands metamorphosed in some years, few or none in others (Fig. 1). Recruitment per female was significantly positively correlated among species for four of six pairwise comparisons (*18*). Successful recruitment characterized the first 6 years of the study, whereas recruitment failures were far more frequent from 1985 to 1990. From 1979 to 1984, one complete recruitment failure occurred for *P. ornata* (*11*), with two nearly complete failures for *A. tigrinum* and one for *A. talpoideum*. In contrast, during the last 6 years, *A. talpoideum* and *A. tigrinum* had no recruitment in 5 years, and *A. opacum* and *P. ornata* had none in 3 years.

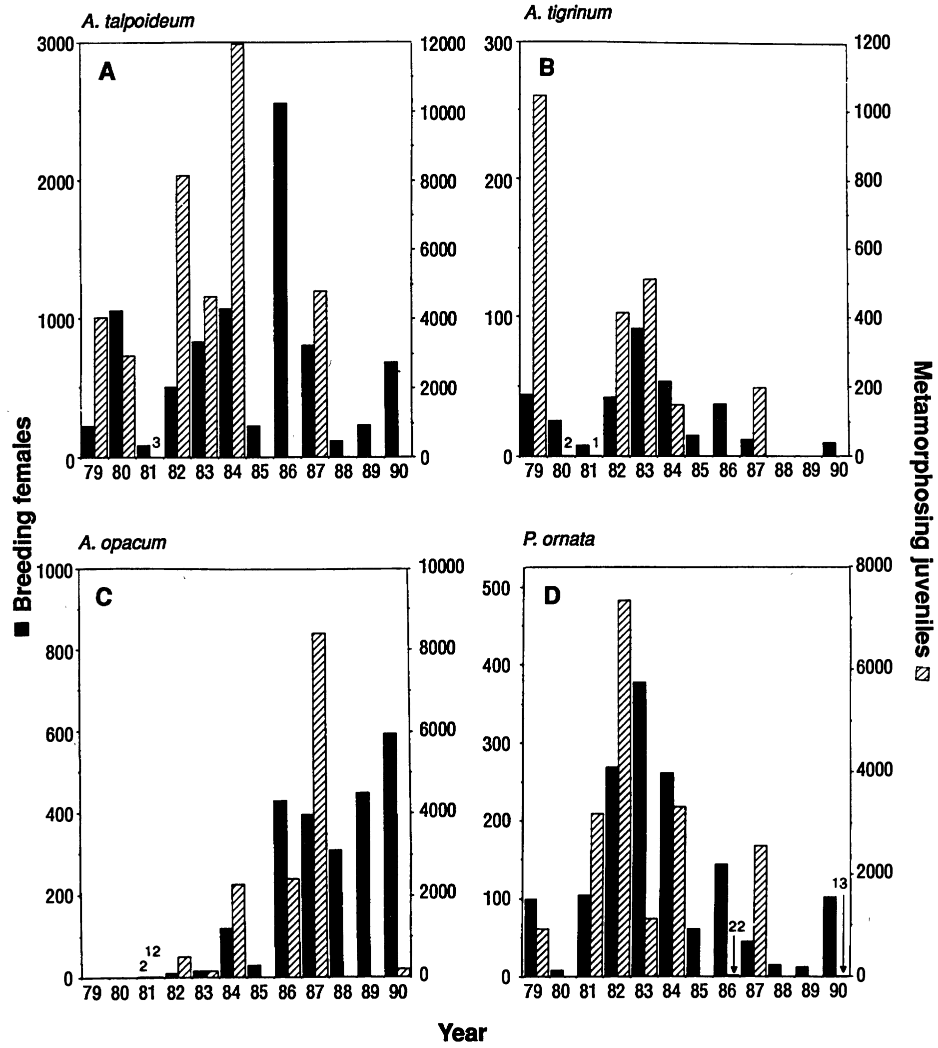

Fig. 1. Female breeding population sizes (solid bars) and numbers of metamorphosing juveniles (crosshatched bars) at Rainbow Bay each year. Females that entered the pond from September to December were counted with the following calendar year because they contributed to the following year's cohort of juveniles. Kendall's partial rank correlation between the number of breeding females and year, correcting for breeding season rainfall (Fig. 2A), was calculated to test for population trends over time: *A. opacum*, $\tau_b = 0.85$, $P < 0.002$; *A. talpoideum*, $\tau_b = 0.17$, $P = 0.46$; *A. tigrinum*, $\tau_b = -0.40$, $P = 0.12$; *P. ornata*, $\tau_b = -0.16$, $P = 0.47$; P values were calculated from the quantile estimates of Maghsoodloo (*35*), $n = 11$ for *A. opacum*, $n = 12$ for others. Data for 1979 were eliminated for *A. opacum* because rainfall data were incomplete. Kendall's rank correlations between the number of breeding females and breeding season rainfall were: *A. opacum*, $\tau_b = -0.16$, $P = 0.48$; *A. talpoideum*, $\tau_b = 0.52$, $P = 0.02$; *A. tigrinum*, $\tau_b = 0.47$, $P = 0.03$; *P. ornata*, $\tau_b = 0.27$, $P = 0.22$; $n = 11$ for *A. opacum*, $n = 12$ for others. Year and breeding season rainfall were not significantly correlated; Kendall's rank correlation: *A. opacum*, $\tau_b = -0.24$, $P = 0.31$, $n = 11$; other species (Fig. 2A), $\tau_b = -0.21$, $P = 0.34$, $n = 12$.

Drought was largely responsible for these recruitment failures. Except in 1980, failures occurred in the 6 years in which Rainbow Bay held water for the fewest number of days, five of which were in the last 6 years (Figs. 1 and 2A). In 1985 and 1989, the pond dried before any larvae had reached the minimum size for metamorphosis (*19*), and in 1988 the pond never filled. Evaluation of partial correlations between juvenile recruitment and year, to assess trends through time after correcting for pond hydroperiod and the number of breeding females, was precluded by correlations among the predictor variables (*20*). Consequently, we calculated simple rank correlations between per capita recruitment and year for each species. Only *A. talpoideum* showed a significant correlation, which was negative, indicating a decline (*22*). These simple correlations are not very informative, however, because of the confounding correlations and the large number of zero recruitment years, which had tie ranks.

Pond hydroperiod was positively correlated with the breeding population sizes of *A. talpoideum*, *A. tigrinum*, and *P. ornata* (*23*). These correlations suggest one reason that these populations have persisted through frequent drought-related recruitment failures. Breeding can be costly in terms of decreased adult survival (*11, 13, 24*). In dry years, females risk the mortality associated with breeding, yet all larvae perish from early pond drying. Selection may favor a tendency to breed in wet years, with rainfall serving as one predictive cue related to pond hydroperiod. Female breeding population sizes of *A. talpoideum* and *A. tigrinum* were correlated with breeding season rainfall (Figs. 1 and 2A), which in turn was correlated with hydroperiod (Fig. 2A). In addition to the potential for selection, lack of rainfall may reduce opportunities to migrate to breeding sites (*17*) and to forage, decreasing energy stores available for egg production.

We conclude that there have been no declines in these four populations at Rainbow Bay that cannot readily be explained as natural fluctuations related in part to drought. Although one climate model predicts that increases in atmospheric greenhouse gases will result in decreased rainfall in the southeastern United States (*25*), we are not aware of any evidence that the droughts during our study had an anthropogenic cause. Data from a nearby site show that similar dry periods have occurred in the past, notably in the 1930s (Fig. 2B).

The fluctuations in our study populations were not controlled only by rainfall, as predation, competition, disturbance (including drought), and other factors may also influence the dynamics of amphibian populations (*26, 27*). For example, larvae are more likely to attain the minimum size for metamorphosis before a pond dries if larval densities are low or are reduced by predation (*27*). Intraspecific density dependence alone may cause wild or even chaotic population fluctuations in amphibians because of their high intrinsic rate of increase and the time lag between recruitment and maturity (*28*). Population dynamics can be affected by factors in both the aquatic and terrestrial stages of the life cycle, but little is known about factors affecting the terrestrial stage of pond-breeding amphibians (*29*).

Our data illustrate some cautionary tales for evaluating declines in amphibian populations. Many short-term or two-point subsets of our data easily might have been interpreted as human-caused declines, whereas those same data were interpreted as natural fluctuations in the context of the complete data set. For example, the 30-fold decrease from 1983 to 1989 in the number of breeding female *P. ornata* appears different by itself than following the general increase from 1980 to 1983 (Fig. 1D). Large populations may be more likely to be noticed or used by researchers. Anecdotal data therefore may be biased toward observing peak populations that eventually will decline, rather than the reverse.

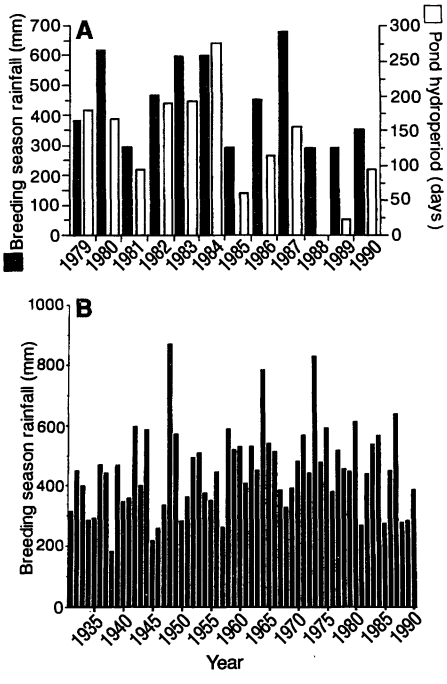

Fig. 2. (**A**) Rainfall at Rainbow Bay during the November to March breeding migration season of *A. talpoideum*, *A. tigrinum*, and *P. ornata* (solid bars), with November and December included in the following calendar year, and the number of days Rainbow Bay contained standing water each calendar year until first drying (open bars). Rainfall and pond hydroperiod were significantly correlated; Kendall's rank correlation: $\tau_b = 0.70$, $P = 0.002$. (**B**) Rainfall at Blackville, South Carolina, located 35 km east-northeast of Rainbow Bay, from November to March as in (A), 1931 to 1990 (*34*). [Compiled from data provided by NOAA, National Climatic Data Center, Asheville, North Carolina]

Alternatively, one easily might mistake a true human-caused population decline as a natural fluctuation, or natural fluctuations might mask a decline. For example, if an unknown human impact had reduced juvenile recruitment at Rainbow Bay during the last 6 years, we might not have detected it because of the drought-related decrease and high variance among years. The persistence of populations despite frequent natural recruitment failures does not necessarily imply that they would persist in the event of similar human-caused mortality. Also, natural fluctuations and anthropogenic effects acting together could result in local extinction more easily than either alone. Habitat fragmentation may make populations less resilient to natural downturns, for example.

The observation that animal population sizes, and especially juvenile recruitment, can fluctuate by orders of magnitude is not new. The extent to which amphibian populations can fluctuate has not been well documented, however. Hairston (*30*) concluded that fluctuations in salamander numbers are minor compared to other groups of animals. This conclusion may have resulted from the fact that researchers have not followed a variety of salamander populations for a sufficient time. Hairston cited Rainbow Bay data for *A. tigrinum* for 1979 to 1982 (*24*) and for *A. talpoideum* from 1979 to 1984 (*21*). The additional data reported here increase the variation in breeding female *A. tigrinum* from a factor of 5.5- to 90-fold, and that in breeding female *A. talpoideum* from 12.4- to 30-fold. Only part of this variation is in actual population sizes, because adults can skip breeding years. If the annual variation in our data were due primarily to adults that skipped breeding then our data would show that a putative decline could represent nothing more than a "catchability" artifact. Many amphibian species can be observed easily only at their breeding site, so this problem of interpretation may be a common one.

Fluctuations in breeding population sizes at Rainbow Bay were not synchronous among species. Elsewhere, declining and stable species have been observed to co-occur and sometimes are related phylogenetically (*1*). Together these observations suggest that the use of "indicator species" to assess amphibian declines must be done carefully. In contrast, per capita recruitment was generally synchronous among species at Rainbow Bay; recruitment increased in wet years. This suggests that population increases or decreases may represent natural fluctuations even when several species show similar trends.

Ambystoma opacum was not present at Rainbow Bay during the first 2 years of the study (Fig. 1C). The regrowth of forests around Rainbow Bay during the last 37 years (*6*) may have permitted recent colonization or recolonization by *A. opacum*, a woodland species. Alternatively, *A. opacum* occurred at Rainbow Bay in the recent past, but reached a nadir as our study began, similar to the pattern of *A. tigrinum* in 1988 and 1989. At the extreme, local extinction may have occurred at this nadir, necessitating recolonization from another pond.

Harte and Hoffman (*31*) have provided some of the few published data on the amphibian decline available for comparison with our results. A Colorado population of *A. tigrinum nebulosum* was censused from 1982 to 1988. During this period, the adult population declined while juvenile recruitment was episodic. These data bear a striking resemblance to our data for the eastern subspecies during the same time period (Fig. 1B). Harte and Hoffman noted that their census data could be indicative of either natural fluctuations or egg mortality resulting from anthropogenic acidification of ponds during snow melt, and they present experimental evidence for the latter hypothesis (*31*).

The pH of Rainbow Bay was not measured until 1987; 1987 to 1991 pH measurements varied from 5.3 to 6.1 (*32*), which is within the range that Harte and Hoffman observed for egg mortality in *A. t. nebulosum.* It is unlikely, however, that the population dynamics of *A. t. tigrinum* at Rainbow Bay were related to changes in pH. Carolina bays are naturally acidic (median pH = 4.6, *n* = 49 sites), in part because of dissolved organic acids, and there has been no long-term decrease in pH at two Savannah River Site bays sampled several times during the time period of our study (*4, 33*). We have not observed high mortality or pH-related developmental abnormalities described by Harte and Hoffman in *A. t. tigrinum* eggs at ponds with pH values similar to that at Rainbow Bay. There is also no seasonal snow melt to cause episodic acidification at Rainbow Bay.

We conclude that there is no evidence that the declines in amphibian populations observed in other locations have occurred in populations at Rainbow Bay. Factors responsible for amphibian declines or extinctions elsewhere may not have affected this relatively protected site. Understanding of the causes of declines may be enhanced as much by a clear determination of which populations are not affected as which ones are. Our data support previous admonitions that it may be difficult to distinguish natural population fluctuations from human-caused declines (*2*) and underscore the role of long-term surveys at numerous sites to separate the many confounding factors (*1, 34*).

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7. The 440-m drift fence is constructed of aluminum flashing, 50 cm high, buried 10 to 15 cm in well-packed soil. The pitfall traps are 40-liter buckets located every 10 m on each side of the fence. See J. W. Gibbons and R. D. Semlitsch [*Brimleyana* 7, 1 (1981)] for discussion of this technique.
8. Data are less easily interpreted for the other species because of trespass across the drift fence, apparent lack of pond philopatry, failure of adults or metamorphosed juveniles to leave the pond basin and therefore be censused at the fence, or a combination of these factors.
9. Terrestrial home ranges of *A. talpoideum* are located 12 to 280 m from their breeding pond [R. D. Semlitsch, *Can. J. Zool.* **59**, 315 (1981)] and those of *A. opacum*, 0 to 450 m [P. K. Williams, thesis, Indiana University, Bloomington, IN (1973)]. There are no paedomorphs at Rainbow Bay because the pond dries annually.
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23. Kendall's rank correlation, $n = 12$: *A. talpoideum*, $\tau_b = 0.45$, $P = 0.04$; *A. opacum*, $\tau_b = -0.26$, $P = 0.24$; *A. tigrinum*, $\tau_b = 0.78$, $P = 0.0005$; *P. ornata*, $\tau_b = 0.45$, $P = 0.04$.
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A Polypeptide from Tomato Leaves Induces Wound-Inducible Proteinase Inhibitor Proteins

GREGORY PEARCE, DANIEL STRYDOM, SCOTT JOHNSON, CLARENCE A. RYAN*

Defensive genes in plants can be activated by several different types of nonpeptide signaling molecules. An endogenous polypeptide, consisting of 18 amino acids, was isolated from tomato leaves and was able at very low concentrations to induce the synthesis of two wound-inducible proteinase inhibitor proteins when supplied to young tomato plants. The sequence of the polypeptide was determined, and an identical polypeptide was synthesized that possessed full inducing activity. These data establish that a polypeptide factor can initiate signal transduction to regulate the synthesis of defensive proteins in plant tissues.

WOUNDING OF PLANT TISSUE BY predator attacks stimulates the release of signals that activate proteinase inhibitors in both local and distant tissues (*1*). Molecules that may regulate proteinase inhibitor I and II genes in both wounded and unwounded leaves of potato and tomato plants are thought to include small pectic fragments of plant cells walls (*2, 3*), chitin and chitosan fragments from fungal cell walls (*4*), abscisic acid (*5*), auxin (*6*), salicylic acid (*7*), and methyl jasmonate (*8*). The active pectic fragments, α-1,4-galacturonic acid oligomers with degrees of polymerization (DP) of about 20 uronide units, were originally called the proteinase inhibitor inducing factor, (PIIF) (*9*). Oligogalacturonides, which elicit the synthesis of antibiotic phytoalexins in plant cells near the sites of infections (*10, 11*), were initially considered to be primary candidates as systemic signals for the wound response. However, the pectin-degrading enzymes that would be required to release plant cell wall fragments in response to wounding are not

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G. Pearce, S. Johnson, C. A. Ryan, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164.
D. Strydom, Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, MA 20015.

*To whom correspondence should be addressed.