

Ecological Experimenters:

9/2/99

For Sept. 8's meeting please read the following

Science articles/letters:

269: 313-315

270: 561-564

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If you are interested, you can read the letters and articles by Blaustein, Shuter, et al. to get a more complete picture of the controversies.



# When Rigor Meets Reality

Ecological experiments have become quite good at isolating causes and effects. But there's a debate brewing over whether these results reveal anything about the natural world

When ecologist Andrew Blaustein linked vanishing amphibians to disappearing ozone last year, he made a splash in the popular press. Alarming field studies showing big drops in frog and salamander populations all over the world have had scientists scrambling for explanations. One controversial idea was that a thinner ozone layer—produced by global atmospheric changes—was the culprit. And Blaustein and his colleagues at Oregon State University had what looked like dramatic experimental data suggesting the amphibians are suffering from higher levels of ultraviolet radiation—a result of thinner ozone. The scientists had placed UV filters over some frog and salamander eggs and left other eggs uncovered. Survival rates in the uncovered eggs were markedly lower. And media attention suddenly became higher. *The New York Times* editorialized that “the Oregon team has provided suggestive evidence that wildlife is affected by the thinning ozone layer. Those vanishing frogs are telling us something.”

But some of Blaustein's colleagues aren't sure what that something is. “The study was very poorly grounded in long-term, quantitative field data,” says Joseph Bernardo, an ecologist at the University of Texas. The Oregon team failed to investigate whether UV levels had actually risen over the last 10 years—the period in which, according to Blaustein, amphibians have become more difficult to find—nor did they test other possible explanations for frog egg mortality, Bernardo says. For instance, a fungus known to be spreading through some frog populations in the Northwest could have killed enough of the eggs to provide a more mundane solution to the mystery of the attenuated amphibians.

Although Blaustein insists that “we've been doing natural history on these animals since 1979,” and “we studied UV because we can't find any other reason why they are dy-



Ecologists use many tactics in their attempts to understand how organisms relate to one another and to their surroundings. In the News section of this special issue on ecology, our lead story deals with one of those tactics, experimentation, and an emerging debate over how ecologists design these tests of the natural world. This is followed by stories on how small organisms have large influences, two hugely ambitious conservation plans, and the value of a biological survey in Hawaii.

Articles, beginning on page 324, start with an exploration of the value of large- and small-scale manipulations of ecosystems. Other topics discussed include strategies for assessing climate-driven effects on ecosystems, world biodiversity, the impact of human population growth, land restoration programs, public opinion on the environment, and the effects of environmental discontinuities and synergisms.

ing,” Bernardo is not impressed. The “inferential chain to what's going on in nature” is weak in this work, he says.

And there are too many such experiments being done, he and others charge. For 3 decades, ecologists have been replacing assumptions about natural systems with testable theories and rigorous statistical analyses, says William Resetarits, an ecologist for the Illinois Natural History Survey. While this effort has been key to the field's progress, Resetarits says it's gone a bit too far, and experiments often reduce nature to oversimplified caricatures that have little to do with the real world. “Experiments can do something for ecology that no other approach can do: establish cause and effect. But they don't tell you what questions to ask, or

whether you are testing your questions appropriately,” Resetarits says.

Now, says Bernardo, “there is a little bit of a backlash from people like me, younger folks

who are fed up with that kind of artificiality.” And at the annual meeting of the American Society of Zoologists in St. Louis in January, in a 2-day symposium called “The State of Experimental Ecology,” these new experimentalists held an organizational rally of sorts. They argued that only by combining careful experimental design with long periods spent observing ecosystems and their inhabitants—what field researchers call “muddy-boots biology”—can ecologists come up with truly meaningful results. “We wanted to provide a framework for the next paradigm in experimental ecology,” says Resetarits.

## Ecology's evolution

This budding revisionist movement is a reaction to what, 30 years ago, was a revisionist trend of its own: controlled lab and field manipulations. Now comprising at least 60% of the studies published in ecology's three major journals, according to a 1994 survey, such research was rare throughout most of the discipline's history, says Robert Holt, a community ecologist at the University of Kansas Museum of Natural History. “People would observe patterns in nature consistent with their theories, then conclude that this proved the theories right,” Holt says. Beginning in the 1960s, however, “ecology went through a very critical phase where it was realized that in order to actually nail down that a particular process is taking place, you have to go out and kick the system.”

Trailblazing investigations published by ecologists Joseph Connell in 1961 and Robert Paine in 1966 did much to convince their colleagues of the power of experiment. By removing, enclosing, or transplanting small populations of the barnacle *Balanus balanoides* along the intertidal zone of the rocky Scottish coastline, Connell proved that the distribution of another barnacle species, *Chthamalus stellatus*, was regulated mainly by competition with *Balanus*. Paine, by contrast, was able to show that the removal of a “keystone” carnivore, the starfish *Pisaster ochraceus*, from patches of Washington shoreline allowed its favorite prey, the mussel *Mytilus californianus*, to edge out most other local invertebrates, drastically altering local species diversity (see p. 316). Although ecologists had long suspected the importance of mechanisms like competition and predation in shaping species distribution, never before had these



**Natural design?** Experiments to test evolutionary theories, such as ecologist Dolph Schluter's artificial fish pond (above), have some wondering if the tests are too artificial.

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forces been so explicitly demonstrated.

These and similar experiments spawned "an incredible maturation and intellectual momentum" in ecology, says David Tilman, director of the University of Minnesota's Cedar Creek Long Term Ecological Research area. "In the intervening 3 decades, ecology has gone from not even considering the possibility of being able to predict patterns in nature to having an understanding of some broad general principles," Tilman says. Contemporary ecologists conduct experimental manipulations in nearly every accessible habitat and on every practical scale, from Rutgers University ecologist Peter Morin's laboratory investigations of food webs among bottled algae and bacteria to Tilman's own studies of changing species diversity within dozens of square-meter plots, each seeded with up to 54 local plant species, on the Minnesota prairie.

And ecology journals, full of differential equations and multiple regression analyses, are growing more and more difficult to distinguish from their counterparts in "hard" sciences like geophysics or applied mathematics. "The push toward experimentation beginning in the 1960s was the result of 'physics envy,'" says Resetarits. "We wanted to be a hard science."

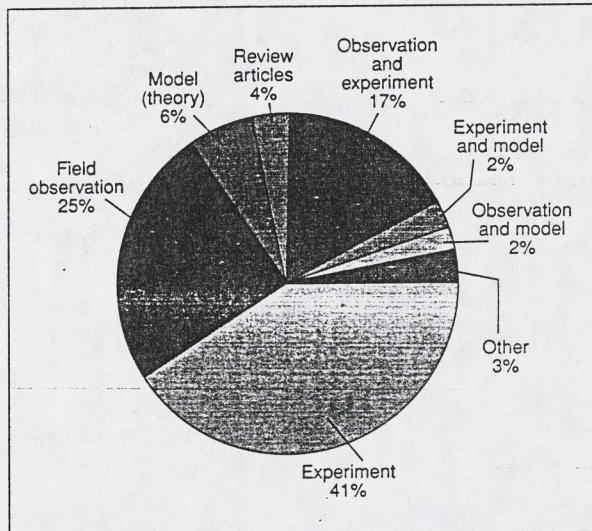
#### A disconnect with nature

But this effort to transform ecology into an experimental science has had a downside, say critics. "Now that we've infused people with the need for rigor, we've perhaps drawn them a bit too far from the roots of ecology," says Resetarits. Authors can have difficulty persuading journal editors to include tables of field observations germane to their experiments, he says. And unlike Connell's and Paine's pioneering field experiments, which were based on prolonged observation of local population dynamics, Bernardo argues that many experiments conducted by today's ecologists evidence no such intimacy with nature. "The problem is that ecologists threw out the proverbial baby with the bath water," Holt says.

One instance of this estrangement, Bernardo and other critics say, was the Oregon team's work on amphibians and UV radiation. Blaustein and co-workers found that frog and salamander eggs inside UV-protected enclosures had a much greater chance of developing into tadpoles than those in unfiltered enclosures. They also discovered that eggs from a frog species with high natural levels of photolyase, an enzyme that repairs UV damage to DNA, survived better in all the enclosures than did those with lower photolyase levels. Human activity, they concluded, may be depleting Earth's ozone layer faster than many amphibian spe-

cies can evolve new defense mechanisms against UV radiation [*Proceedings of the National Academy of Sciences* 91, 1791 (1994)].

Bernardo, however, dismisses Blaustein's study as a "science fair experiment" whose central variable, UV radiation, was chosen with no strong grounding in local field conditions. "Has UV influence over those lakes changed over the same period that frog egg mortality has changed? He has no data," Bernardo states. "Suppose Blaustein had decided to manipulate temperature instead of UV—then the story he's weaving in the press would have been that global warming is



**Hard science.** Experiments have taken on a dominant role in ecology, indicated by this breakdown of observational techniques used in studies published in the journals *Ecology*, *Oecologia*, and *Oikos*, from 1987 to 1991.

causing [the amphibian decline]."

David Reznick, an ecologist at the University of California, Riverside, adds that some amphibian populations—such as one Central American tree frog species that inhabits dense foliage—are declining even though they live beneath UV radiation's reach, indicating that some other mechanism must be at work. Says Reznick, "These global patterns don't lend themselves to a single easy explanation."

Blaustein agrees with this last point, saying "UV is definitely not a universal explanation for amphibian declines," and adds that new experiments are already under way to test for a possible synergism between UV radiation and a fungal disease now spreading quickly through amphibian populations in the Oregon Cascades. But while he admits there are no data showing that UV incidence has increased at the team's field sites, he notes "there are absolutely no long-term data on UV anywhere, let alone in our area ... so that can be a criticism of any UV study." Further, he says his team searched hard for other environmental changes that might be harming amphibians, such as acid rain,

heavy metals and other pollutants, and habitat destruction, but found nothing. "What we've seen in about 15 different field sites is that the eggs that are dying are right out in the open," where they are most exposed to solar UV, Blaustein says.

Bruce Menge, a community ecologist also at Oregon State University, calls the lack of long-term UV data to back up Blaustein's findings irrelevant. "If we followed [Bernardo's] arguments, we wouldn't do much of anything relevant to these pressing problems" like ozone thinning, Menge says. Blaustein is "an outstanding naturalist" who "doesn't go out and do experiments without having a natural-history basis to do them," Menge adds.

#### Designing reality

Whatever the truth of the amphibian puzzle, being disconnected from nature isn't the only factor that can throw off an ecological experiment, the revisionists say. Many studies are also undermined by basic flaws in their design. It's on these grounds that Bernardo, Resetarits, and University of Pennsylvania ecologist Arthur Dunham have attacked an influential study of "character displacement" published in *Science* last year. Character displacement is the theory that competition for an ecological niche can force species that initially have similar characteristics to evolve in slightly different directions—in effect to keep out of one another's way. In the study, Dolph Schluter, an ecologist at the University of British Columbia in Vancouver, filled both halves of each of two divided artificial ponds with "generalist" three-spine stickleback fish (*Gasterosteus aculeatus* complex) that feed both high and low in the water columns of their native glacial lakes (*Science*, 4 November 1994, p. 798). To one half of the pond, he added a second stickleback species that feeds exclusively on plankton near the water's surface.

After 3 months, Schluter began recording the generalists' growth. Fish in the untreated halves of the two ponds grew normally. But in the presence of the top-feeders, he found, the quickest growing generalists were those whose mouths and gill shapes most resembled those of a third, bottom-feeding type of stickleback. Schluter's conclusion: Natural selection was starting to favor the generalists with more bottom-feeding capabilities. If the trend had been allowed to continue through subsequent generations, the initial generalist characteristics would have been displaced because they are heritable.

Says Resetarits, "It's a sexy result, and it's gotten a lot of play, but it's a very bad experiment." Resetarits was so skeptical of the results that he, Bernardo, and Dunham chal-





lenged them in a recent Technical Comment (*Science*, 19 May, p. 1065). The experiment's fatal flaw, they say, was that Schluter failed to control for the possibility that plain overcrowding in the treated halves of the two ponds—rather than the specific presence of the top-feeders—gave the bottom-feeding generalists a growth advantage over their competitors. One simple way to establish such a control, says Resetarits, would have been to add an equal number of generalists to the untreated halves of the ponds, thus keeping the sticklebacks' densities in the two halves equal.

In addition, the critics point out, Schluter artificially increased the frequency of extreme characteristics among the generalist sticklebacks by using hybrid fish with genes from both top- and bottom-feeding species. As a result the generalists were swimming in a far richer gene pool, so to speak, than could be drawn on by individuals in a natural lake.

Schluter responds that extreme phenotypes are so rare in nature that his experiment could not have been done within a reasonable research budget without priming the genetic pump. "If you wanted to [test character displacement] with purely natural variation in those same traits, you would need a much larger sample size and a greater number of ponds," says Schluter. "It's doable in principle, but in practice it would be very daunting."

He acknowledges that his experiment did not strictly rule out density as a contributor to morphological changes. He says he chose the design described in the *Science* paper over the alternative Resetarits outlines because the alternative design would not have yielded any information about selection pressures.

In this, Schluter has allies. "I strongly respect the call for ecological realism in the design and conduct of field experiments. But Bernardo and his colleagues have argued the hard line a little too strongly," says Peter Grant, an evolutionary ecologist at Princeton University. "Not only does the stickleback experiment demonstrate a phenotype-specific effect of a competitor on individual growth rates of another—in a manner expected from the hypothesis of character displacement—but it is solidly grounded in 30 years' worth of knowledge of the animals in nature."

Bernardo, however, says the gospel of good experimental design can never be reinforced too strongly, as "there are still plenty of young [ecologists] doing mindless, stupid experiments." Too many researchers, Bernardo and Resetarits say, fail to identify explicitly the biological questions they are trying to address or to translate these ques-

tions into a set of precise, statistical tests that unambiguously distinguish between alternative hypotheses. In addition, they say, too many ecologists let their interpretations stray beyond the theory being tested or the natural system under investigation.

Such experiments carry risks that go beyond ecology, says Dunham. "When you overgeneralize your results—particularly when there is a need for applied ecological principles in conservation and biodiversity protection—then you run the risk of having bad science accepted by resource managers, with potentially disastrous results," he says.

The remedy, says Bernardo, is to "allow more complexity and multiple causality to enter into our designs." The revisionists point to ongoing field studies by James H. Brown, an ecologist at the University of New Mexico, as an example of experimental ecology done right. On two dozen quarter-hectare plots in the Chihuahuan desert of southeastern Arizona (a hectare is 2.47 acres), Brown and colleagues have spent the last 18 years manipulating one factor after another in an attempt to explain predator-prey relationships and species composition among seed-eating rodents, ants, and seed-producing plants (*Science*, 10 February, p. 880). At times Brown has fenced out certain ant spe-

periments: computer simulations and lab-based ecosystems. John Lawton, an ecologist at the U.K.'s Imperial College, has used a terrarium-like enclosure called the Ecotron to measure plant productivity and carbon dioxide uptake as functions of species diversity. He advocates such "controlled environment facilities" as "halfway houses between the simplicity of mathematical models and the full complexity of the field" (see p. 316 and Article by Lawton on p. 328). Adds Rutgers' Morin, "There are some ecologists who put down [lab experiments] because we have abstracted things so much. Our response is that if you don't start with a simple system, you won't understand what's going on anyway."

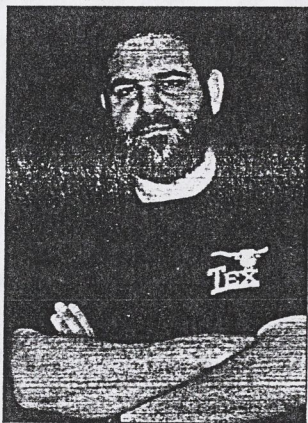
Other ecologists say critics like Dunham, Bernardo, and Resetarits sometimes make too much of the occasional flaws in published experiments. "It's possible to do anything badly," says Nelson Hairston, an emeritus ecologist at the University of North Carolina, Chapel Hill, and author of the 1989 volume *Ecological Experiments*.

But many ecologists not in the thick of the debate, such as Minnesota's Tilman, say participants in the St. Louis symposium are prescribing a necessary antidote to the excesses of experimentalism. Continuing generational change will reinforce this message, he believes. "Twenty or 30 years ago, most ecologists were either theorists or experimentalists or natural historians. But as younger generations are drawn in, an increasing number of individuals are acquiring skills in all three disciplines." Tilman says this has fostered "a trend in the whole field ... toward the realization that ecology will advance most rapidly through a balanced combination."

That advance won't be easy, notes Gary Polis, a community ecologist at Vanderbilt University in Tennessee. Understanding the natural variability in conditions at most field sites and detecting subtle, infrequent, or hidden ecological processes takes studies much longer than the usual timescale of ecological experiments. Restoring natural history to ecological experiments will also mean broadening their spatial scales, Polis says, because many natural processes like mobility, dispersal, and species interactions can create patterns visible only from a macroperspective.

All that will take money, and although funding is scarcer than ever, many ecologists think it's worth the effort to try. "I think we're at a very early, embryological stage in the ontogeny of ecology," says Polis. "There are lots of really neat questions out there for the picking. It's just a question of recognizing them." And asking them in the proper manner.

—Wade Roush



**Hard questions.** Ecologists Joseph Bernardo (left) and William Resetarits (right) have challenged some of their colleagues' methods. Says Bernardo: "There are still plenty of young [ecologists] doing mindless, stupid experiments."

ties to study the effects of decreased competition; at other times he has fenced out certain rodent species to study resulting changes in grass cover and cascading effects on other species. Says Bernardo: "The experiments have been tedious, costly, and difficult, but very realistic."

#### The limits of description

Many researchers believe, however, that Bernardo and his fellow critics are setting unrealistic standards. They argue that complex problems like the ecological effects of global environmental change will never be untangled without help from the most reductionist of



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# LETTERS



## One world?

Ecologists discuss a News article in our special section "Frontiers in biology: Ecology" (21 July, pp. 313-360) and subsequent letters (1 Sept., p. 1201). While 24 letter writers describe one dispute as a "minor squabble," the views expressed in other letters belie this description.

## The Role of Experiments in Ecology

We thank *Science* for giving ecology coverage in the "Frontiers in Biology: Ecology" special section (21 July, pp. 313-360). It was unfortunate that the lead News article by Wade Roush, "When rigor meets reality," highlights a minor squabble that stemmed from the remarks of one postdoctoral researcher. We encourage the editors and reporters of *Science* to continue coming to ecological meetings so that they can broaden their knowledge and expand their coverage of the substantive issues. Ecology is a true frontier, being perhaps the most complex system that science has ever tried to understand. Increasingly, ecologists are combining experiments, observations, and theory to expand the temporal and spatial scale of our inferences. We are strongly motivated by the pressing need for answers to major questions of direct relevance to the long-term sustainability and habitability of Earth.

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Roush's article portrays the American Society of Zoologists' symposium "The State of Experimental Ecology" as an "organizational rally of sorts" for the "new experimentalists" and as part of a "revisionist movement" advocating a return to more "muddy-boots biology." As co-organizer of the symposium, I strongly disagree with this portrayal. Although the coverage given to this symposium is appreciated, the article confers a negative tone on the proceedings and does not convey the scope and goals of the symposium. I also disagree with the article's presentation of the important issues in experimental ecology.

The symposium brought together experimental ecologists representing the broad array of experimental approaches used in ecology, from laboratory microcosms to manipulation of entire ecosystems, in order to illustrate the myriad ways in which experiments are applied to ecological questions. The symposium specifically emphasized the value of a plurality of experimental approaches; it was definitely not about attacking other ecologists or "challeng[ing] . . . colleagues' methods" (nor were my own discussions with Roush). It was experimental ecologists critiquing themselves to move experimental ecology forward on all fronts, from better designs, to better links between experiments and theory, to more realism in experiments designed to explore specific natural systems. It was also a forum in which to discuss the limitations and obstacles to applying experiments to specific ecological systems and questions. Our only agenda was to reinforce the importance of experiments and experimental rigor in un-



derstanding ecological processes and to stress the need to continually improve our application of experimental methodology and achieve better integration between experiments, theory, and natural history. Our goal was to ensure that the rate of progress in the application of experimental methods to complex ecological problems continues to accelerate. It is unfortunate that the article did not capture the energy and positive tone of the symposium, and missed the real story of experimental ecology: the tremendous progress in ecological understanding achieved through experimentation.

Similarly, the article depicts my personal views in ways that I would not and so vaguely ascribes opinions that I have subsequently been criticized, in print and elsewhere, for statements I did not make and opinions I do not hold. I presumably criticized "experiments [that] often reduce nature to oversimplified caricatures that have little to do with the real world." That certainly does not reflect my view, as much of my work makes use of mesocosms (1), and I firmly believe that such simplified systems instruct us about the real world. Subsequent letters (1 Sept., pp. 1201-1203) criticize me for attacking Andrew Blaustein. I was not quoted regarding his work, as I had, in fact, refused to discuss it.

The article's negative tone was amplified by exclusion of positive statements or by their paraphrasing into negative, critical statements. I have been critical (2) of Dolph Schluter's recent experiment (3) and agreed to discuss it because the paper was published and criticisms rendered in *Science*. However, my repeated caveat that criticisms were limited to the specific experiment and that Schluter's other work on character displacement is compelling was not included. Even a positive prescription for experimental ecology penned (with Joseph Bernardo) at the request of *Science* was paraphrased into a series of negative statements on what experimental ecologists "fail" to do, and then linked with another quote that neither should have been made nor printed.

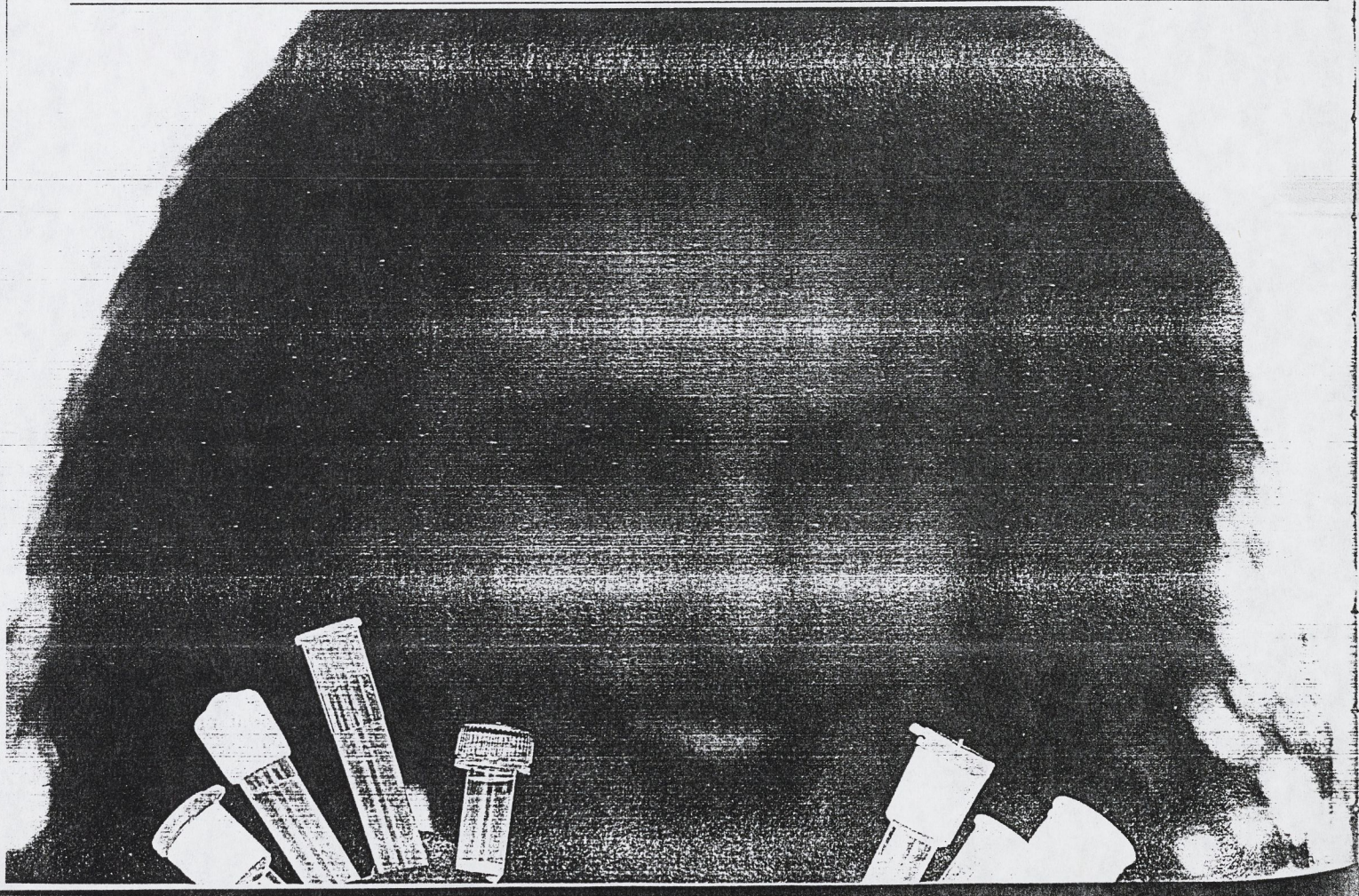
There was an interesting article to be written about the tremendous strides made in ecology through experimentation and the many directions experimental ecology is taking under several generations of experimental ecologists. Indeed, many of the important figures in the evolution of experimental ecology were interviewed, many more than were represented in the article. Why, then, were these strides and directions not made the focus of the article? The rationale given by *Science's* News editors was that these topics were simply "not engaging." I disagree.

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#### References

1. W. J. Resetarits Jr., *Ecology* 72, 1782 (1991); *Oikos* 73, 188 (1995).
2. J. Bernardo, W. J. Resetarits Jr., A. E. Dunham, *Science* 268, 1065 (1995).
3. D. Schluter, *ibid.* 266, 798 (1994).

My purpose in criticizing high-profile ecological experiments (1) is to stimulate reasonable debate about the fair extent of inferences that scientists make from their experimental results. This general aim is reflected in my efforts to co-organize a symposium whose goal was to offer constructive insights to improve the future practice of experimentation in ecological and evolutionary research. It is also reflected in my efforts to ensure the accuracy of *Science's* article, which I understood was to be about the role of experiments in contemporary ecological research, the focus of the symposium. To this end, I gave Roush our symposium proposal that detailed its diverse goals and a list of names and addresses of all of the symposium participants (many of whom he interviewed). I also spent more than 6 hours in three





interviews over several weeks expanding on these themes. Roush's article inaccurately represented the symposium and the spirit of our conversations. My criticisms span a variety of issues in the use of experimentation in ecological inference, ranging from problems of confounded designs and unnatural experimental conditions (1), to difficulties with the choice of experimental variables and treatment levels that affect interpretation, and over-generalization (comments I made in Roush's article). I concur with Reznick (Letters, 1 Sept., p. 1202) that such issues are complex and deserving of careful discussion.

Neither my criticisms, nor our symposium, had much to do with young naturalists leading a rebellion against experimentation, or a call for a return to "natural history." Thus, I took exception to a draft of Roush's article that told a story of young naturalists revolting against the approaches of their older, experimentalist mentors. The draft included quotes from esteemed experimentalists—some of whom I had cited as instrumental to the development of experimental ecology—which were clearly at odds with my supposed views. I called Roush to respond to his draft. I told that it inaccurately represented the sym-

posium and our views, and that there was, in fact, no generational controversy about the role of experiments in ecology. I asked that he revise the piece to reflect the issues we had discussed and that he remove an introductory vignette that highlighted a nonexperimentalist's views that were extreme and, hence, did not fairly represent the symposium. Barring this, I insisted that references to the symposium and our quotes be removed from the piece, because the story that he said he was authoring was about broader issues surrounding experimentation in ecological research, not about resurging interest in natural history, a bias retained in the published article.

Further, it is disturbing that Roush ignored many constructive remarks I made in multiple interviews and that he chose to highlight—in a highly contrived, negative paragraph that distorted other statements we had made in an explicitly constructive way—part of a statement I made in an off-the-record conversation (not in one of the three interviews). My comment came at the end of a frustrating, 72-minute conversation (initiated by me) in which I tried to convince an unwavering Roush of the inaccuracy of his draft. I made an unfortunate, blunt statement emphasizing that there are

both older, seminal experimentalists who rooted their studies in natural history and many young ecologists who do experiments without the benefit of same, that is, that controversy between young naturalists and old experimentalists was imagined. I then contacted Roush's editor.

After I conveyed these concerns to the editor, the introductory vignette was deleted, and additional emphasis was to have been placed on other issues (experimental design, multiple causality, and so forth) discussed in the symposium. I suggested that a historical synopsis of ecology as a discipline would be a logical replacement introduction, but the editor dismissed this as "not engaging." *Science's* interest in provoking controversy rather than in telling a factual story about experimental ecologists of all ages and career stages taking a hard look at experimentation in our discipline—while ignoring indications from me and other ecologists that the story was inaccurate—is at best, regrettable. Curiously, the editor refused my repeated requests to review the final version of the article. This is particularly disconcerting in light of assurances to me by Roush and his editor that *Science's* motivation was to publish an accurate piece and their repeated thanks for my efforts to ensure this goal. Such an article would have

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been informative and easy to write, given the diversity of ecologists with whom Roush spoke and our symposium proposal that provided the necessary background. It is unfortunate that the article took such a narrow view both in topic and in highlighting my comments, particularly since it was the lead article in a special issue devoted to ecology.

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*Response:* We invited Bernardo and other knowledgeable ecologists to comment on our article and we made changes based on their comments. As Bernardo points out, we even removed a vignette about a researcher with whom Bernardo disagreed. It was not appropriate, however, to shape the entire article to reflect Bernardo's views, which his letter makes clear was his intent.

Bernardo and Resetarits say that we ignored their efforts to focus the article on experimental design. Yet the article high-

lights their own comments and those of other scientists on some of the very issues—such as multiple causality and inference—they raise in their letters. And although they object to our portrayal of the roots of the debate, it was supported by other researchers, some of whom were quoted by name in the article. No one told Resetarits that the strides made in ecological experimentation were “not engaging”; indeed, the article included a long section describing those strides.

It is unfortunate that Bernardo now seeks to distance himself from one of his many “blunt statements” by saying it was made off the record. At no point in our discussions, including the interview he initiated, did Bernardo request that we not quote him.

We regret that the idea of researchers seeking value in myriad experimental approaches did not come across more clearly in the article. We agree with Power *et al.* that ecology is a rich and important field and intend to continue our coverage of it. Our intent in this article was certainly not to provoke controversy, as Bernardo asserts. As these letters, and letters we published on 1 September, indicate, ample controversy already exists.

—Joshua Fischman, *Deputy News Editor*

#### AIDS Intervention in Uganda

Rachel Nowak, in her News article “Testing AIDS interventions: When is the price too high?” (8 Sept., p. 1334), suggests that our study in Rakai District, Uganda, which uses intensive control of sexually transmitted diseases (STDs) through mass treatment as a means of preventing HIV (human immunodeficiency virus) transmission, “run counter to internationally accepted guidelines.” The basis for this statement is that the international guidelines recommend that should the therapy prove efficacious, it should “be made reasonably available to the inhabitants of the host community country,” and Nowak writes that “If the intervention works, most Africans may not be able to afford the drugs.”

Drug costs are a relevant issue, but many of those used in the Rakai study are cheap, readily available in Uganda, and appropriate to the Ugandan context. The drugs, Azithromycin and Ciprofloxacin, were selected for their high rates of effectiveness against key STDs and their ease of administration, and their prices have been falling in the United States. Azithromycin now costs approximately \$9.50 per course of treatment, which is comparable to other recommended prescription reg-

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# LETTERS

## Western toad at high noon?

Field experiments show that ultraviolet (UV) light can damage amphibian eggs. Field observations show a decline in many populations of frogs and toads, such as this threatened toad *Bufo boreas*. But to what degree is solar radiation a factor in the decline? The latter, more complex, question is of a global scale. Many letters praising the UV light experiments of Andrew Blaustein and his colleagues were received in response to the 21 July special section "Frontiers in Biology: Ecology." How the media, including *Science*, report on such experimental findings, and what kind of studies should be done next, are more problematic for these writers.



WILLIAM LEONARD

## Ecological Research

Our recent work showing that ultraviolet (UV) radiation can contribute to amphibian egg mortality is criticized by Joseph Bernardo and William Resetarits in a news article for, as Bernardo says, being "very poorly grounded in long-term, quantitative field data" ("When rigor meets reality," by Wade Roush, in a special section: *Frontiers in Biology: Ecology*; 21 July, p. 313). These criticisms are unfounded. We have collected and published data (including yearly egg mortality estimates) on the ecology of northwestern amphibians for 15 years. Moreover, we have about 40 years of background data on northwestern amphibians from Robert Storm and his numerous students.

With this natural history basis, we became concerned in the mid-1980s when we observed unprecedented mortality of amphibian eggs in the Cascade Range. After systematically analyzing pond water for pollutants, acidification, and many other factors, we found only one factor associated with egg mortality—a pathogenic fungus (1). Bernardo ignores relevant issues when he presents the fungus as an alternative to UV for high egg mortality without acknowledging that I proposed this explanation (1). We also noted that dying eggs were laid in shallow, open water, an observation consistent with the view that mortality is related to UV radiation. Thus, after 8 years of observing dying eggs, conducting preliminary experiments, and after ruling out many potential mortality factors, we designed field experiments to test the hypothesis that amphibian embryos are sensitive to ambient UV-B radiation.

hypothesis that amphibian embryos are sensitive to ambient UV-B radiation.

Eggs of several species were placed in enclosures in a randomized block design at natural oviposition sites. This design allows experimental and control treatments to be conducted simultaneously, side by side, after randomly assigning enclosures to positions along the shore. Each block had three treatments (not just filtered and unfiltered treatments, as stated by Roush): enclosures (i) open to natural sunlight including UV-B, (ii) covered with a UV-B blocking filter, or (iii) covered with a filter that transmitted UV-B (a control for placing a filter over eggs). Each block was replicated four times. To ensure that our results were not unique to a specific site, each species was tested at two sites. Experiments were conducted in both 1993 and 1994. Our published papers (1, 2), those in press, and those in review suggest that in certain species both UV radiation and the fungus contribute to egg mortality, and that is all we have stated in our papers. We do not know how continued egg mortality will affect amphibians at the population level. But we do know that our experiments had the potential to invalidate the view that UV radiation contributes to egg mortality. We have not claimed that UV radiation is the single worldwide cause of amphibian population declines, as is implied in the news article. We have repeatedly stated that habitat destruction is the main cause for the declines (3–5); that they do not lend themselves to single explanations is a point that we have made in several papers (3–5). However, this statement



is mistakenly attributed to David Reznick, apparently because Reznick paraphrased one of my papers (5) on amphibian declines to Roush (6). It is unfortunate that Bernardo and Resetarits appear not to have read our papers carefully and have criticized us for what some of the popular press has said about our work.

Instead of being poorly grounded in long-term field data, as Bernardo alleges, we believe that our work demonstrates how long-term observations point the direction toward relevant, realistic experiments.

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I wish to express my concern over the quote attributed to me in the article by Roush. The quote (which gives the incorrect im-

pression that I am critical of Blaustein's work) was actually derived from Blaustein's own writings (1). Blaustein is at the forefront of the worldwide investigations into all the potential causes of amphibian decline, including UV radiation. In view of his clear statement of likely multiple causes of the amphibian decline, I interpreted Blaustein's experiment as a test of the plausibility of UV radiation as one of those possible causes. The fact that the experiment was performed without the benefit of prior long-term data indicating an increase in UV radiation should not be a concern because, in a rapidly changing world, it is impossible to foresee what the important changes might be. Rather than criticize the work for not being motivated by such data, I instead view it as contributing to the motivation for collecting such data in the future.

More generally, it is ironic that Roush featured criticism of two such fine papers. Both Dolph Schluter (2) and Blaustein were working on systems for which there are abundant ecological data. Both took these prior observations into account when designing and executing their experiments. Both studies represent novel approaches to a problem and produced interesting results that should be of interest to a general, critical audience such as *Science's* reader-

ship. Both studies incorporated complexities that merit some open debate, so it is not unreasonable that one of them has been discussed in *Science's* Technical Comments section (3); however, the tone of Roush's news article in no way represents the subtleties of this kind of work or the costs and benefits of alternative experimental approaches to a problem, such as the role of density or the use of hybrids in Schluter's work. In my opinion, Schluter made the right decisions. For all of these reasons, I feel that Roush's article presents an inaccurate, destructive view of the scientific process.

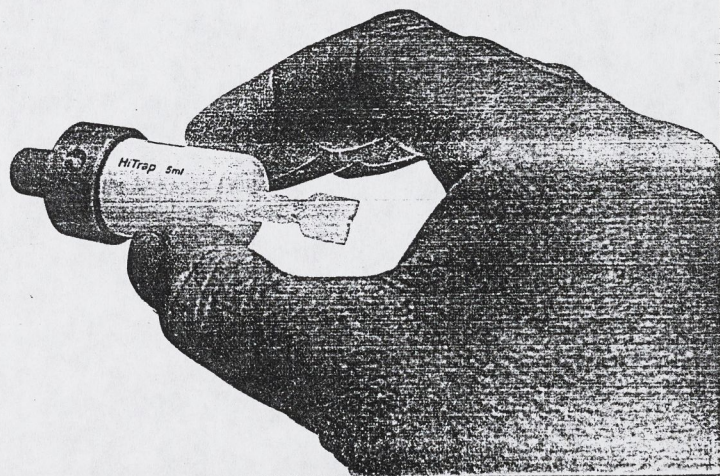
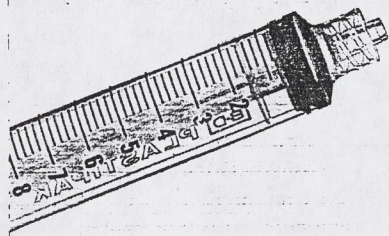
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I am appalled and dismayed by the views attributed to Bernardo and Resetarits in the article by Roush. Experiments in ecology, as in all branches of biology, must be well grounded in an understanding of the natu-

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ral world, but to attack Blaustein for not having followed this principle is absurd. The declines in amphibian populations that have recently been observed in many parts of the world are disturbing to many biologists, and increased UV radiation resulting from ozone depletion is an obvious candidate as a cause of at least some of these declines. Blaustein's experiments were a simple, well-designed, and carefully carried out test of this hypothesis, and they yielded strong and persuasive results in its support; they should be judged on their merits as experiments, and it is for the biological community to evaluate their wider significance. They do not solve the mystery of the declines, and Blaustein has never claimed that they do; they do, however, open up important new areas of investigation. Blaustein's decision to study the effects of UV radiation on amphibian eggs may have been a largely intuitive one, but where would science be if researchers ignored their intuition?

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Who would have anticipated 10 years ago that collecting long-term data on UV might be important now? The point of Blaustein's research is that UV exposure *does* affect amphibian egg survival and that changes in UV radiation *have the potential* to contribute to declines in some populations.

Most ecologists recognize that the two approaches to studying ecology are not in opposition, but are complementary. Long-term field experiments of the type advocated by Bernardo and Resetarits have the advantage of retaining some of the complexity of natural systems. Disadvantages include (i) a lack of control of factors that may affect the population under study; (ii) little replication of results; and, in many cases, (iii) little power to prove or disprove inferences about causality. Laboratory or controlled field experiments have the advantage of larger numbers of replicate studies, greater statistical power, and more power to reveal causality. The primary sacrifice made in the latter approach is the elimination of possibly relevant factors.

I agree with Bernardo and Resetarits on the general point that it is critical to articulate biological hypotheses and to collect precise experimental or observational data that distinguish among alternative causes,

although I suspect that most ecologists would agree that this should be standard operating procedure.

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Response: Some of the experiments discussed in my news article—studies by Andrew Blaustein and colleagues and by Dolph Schluter—had generated discussion and debate among ecologists well before I wrote about them. The article reflected that debate. It also allowed the scientists to refute the critiques; for instance, Blaustein's initial point in his letter, that he had 15 years of data on his study population, is also made by him in the news article.

The criticisms of Blaustein's work conveyed in the news story focused on a specific paper [A. R. Blaustein *et al.*, *Proc. Nat. Acad. Sci. U.S.A.* 91, 1791 (1994)]. That paper did not include the qualification that a pathogenic fungus might be another source of egg mortality, nor did it contain any reference to the 1991 paper in *Biological Conservation* that Blaustein cites in his letter above. Nevertheless, the news article should have acknowledged that Blaustein himself had raised the fungal hypothesis elsewhere.

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26. A highly conserved region was identified by comparison of secA sequences from *P. lutherii*, *Anthi- amnion spp.*, *E. coli*, and *B. subtilis*. This region of the *P. lutherii* chloroplast secA gene, from base pair 1043 to 1458, was amplified by polymerase chain reaction with primer 5'-GCTCCACCATATGAAA-ATCGCCGAGATGAAGACAGG-3' containing an in-frame Nde I site and reverse primer 5'-GGAA-TGTTTCAAGCTTTCGGGAGATTATTAGTGG-3' containing a Hind III site with pMAQ805 (7) as template and was cloned into an appropriately digested pET24b (Novagen) to allow in-frame fusion with the His-6 tag. The resulting clone was introduced into BL21(λDE3) and the protein expressed and purified from inclusion bodies as described (10). Antibody to the SDS-denatured peptide was prepared in rabbits by Cocalico Biologicals (Reamstown, PA). Protein assays were performed with bovine serum albumin as standard as described [M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976)]. Immunoblotting was conducted as described [L. Payan and K. Cline, *J. Cell Biol.* **112**, 603 (1991)].
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## Experimental Evidence That Competition Promotes Divergence in Adaptive Radiation

Dolph Schluter

Interspecific competition driving divergence in adaptive radiation has not previously been tested experimentally. Natural selection on a morphologically variable species of stickleback fish was contrasted in the presence and absence of a close relative. Selection was nondirectional when the target species was alone, whereas addition of the second species favored individuals most different from it morphologically and ecologically. Disproportionately severe competition between similar phenotypes indicates frequency-dependent selection, verifying a crucial element of theory of competition and character divergence. The findings help resolve outstanding debates on the ecological causes of diversification and the evolutionary consequences of competitive interactions.

The ecological causes of adaptive radiation are poorly understood. Especially contentious is the issue of whether rates and patterns of speciation and morphological divergence have been greatly affected by resource competition between species (1). This debate mirrors a long-standing issue in ecological research: whether differences between coexisting species are commonly the outcome of ecological character displacement (evolutionary change resulting from interspecific competition) (2-4). Conflicting views have been difficult to resolve because evidence is scarce and entirely correlative. I addressed the problem experimentally by measuring the strength of divergent natural selection between closely related, morphologically similar species.

Threespine sticklebacks (*Gasterosteus aculeatus* complex) inhabiting small lakes of coastal British Columbia, Canada, were used for the study. The collection of species diversified very recently, mainly at the end of the Pleistocene (≤13,000 years ago) (4, 5). Earlier work suggested that coexisting pairs of species were character-displaced (4): One species (the "benthic") feeds on benthic invertebrates in the littoral zone

and is large and deep-bodied with few, short gill rakers and a wide gape; the other species (the "limnetic") feeds on plankton, is small and slender, and has long, numerous gill rakers and a narrow gape. Species occurring alone in lakes are intermediate in body form and exploit both benthic and plankton habitats. These morphological differences are strongly associated with feeding efficiency and growth rate in the two habitats (6, 7). The pattern is replicated over several watersheds and is a general characteristic of radiations of fish taxa that inhabit low-diversity post-Pleistocene lakes (8).

The experiment was carried out in summer 1993 in two divided 23 m by 23 m ponds on the University of British Columbia campus (9). The solitary species from Cranby Lake, Texada Island, was the target of the experiment; it is morphologically intermediate between benthic and limnetic species (4). The limnetic species from near by Paxton Lake was the potential competitor. This species is morphologically and ecologically most similar to one extreme of the range of phenotypes in the Cranby species (4) (Table 1). The goal of the experiment was to test the prediction from theory that individuals at this extreme should suffer disproportionately when the limnetic species is added, generating natural selection

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tion in favor of phenotypes at the opposite extreme (3, 10). The combination of morphological forms used in this experiment (that is, limnetic plus intermediate) recreates those thought to have been initially present 10,000 to 13,000 years ago in lakes colonized twice by ancestral forms (4, 5).

The study took place within a single generation. Selection was assessed by comparing growth and survival of different Cranby phenotypes in the presence and absence of the limnetic species. Growth rate in fish is highly correlated with food

intake (7) and is closely linked to fitness through its effects on overwinter size and survival (11), time of breeding (12), and fecundity (7, 13). The most critical comparison was that between extreme phenotypes of the Cranby population; yet individuals at these extremes are inevitably rare in nature. I used interspecific hybridization to increase the frequency of these individuals and, therefore, the sensitivity of the test. Hybridization is a valid manipulation because all previous crosses between closely related freshwater sticklebacks have not revealed

any intrinsic reduction in offspring viability (14).  $F_1$  hybrids were raised from artificial crosses between the Cranby species and the Paxton benthic species ( $C \times B$ ), and between the Cranby and the Paxton limnetic species ( $C \times L$ ). The target experimental population was a mixture of equal numbers of  $C \times B$ ,  $C \times L$ , and offspring from crosses between Cranby individuals ( $C \times C$ ).

A potential problem when comparing fitness of phenotypes between treatments was that growth and development of several trophic traits are affected by diet (15). For this reason I instead used two hard parts of the anatomy, gill raker number and body armor, as markers for trophic phenotype. Growth of these traits is unaffected by diet (15). Each Cranby cross is distinct from the other two in these markers, and a combination of markers yields an index that substitutes for underlying differences in trophic morphology (Table 1). All analyses were carried out on this index. This step is conservative, because the patterns reported were stronger when plastic traits such as gape width were used directly.

A paired design was used in which 1800 individuals of the Cranby experimental population were introduced to both sides of each pond. Then 1200 limnetic individuals were added to one randomly chosen side of each pond (16). Addition of limnetic fish to one side caused the treatments to differ in the total density of fish and in the combined frequencies of different phenotypes present. However, theory is explicit about which phenotypes in the target species should be most affected by the addition. If the prediction is upheld, then it may be concluded that selection was frequency-dependent as required by theory (3, 10).

The experiment was run for 3 months (17). All remaining fish were removed, stained, and preserved. A random sample of 60 to 70 Cranby individuals was taken from each half pond for measurement. Because fish were introduced at a very small size, growth rate (millimeters per 90 days) was calculated as  $90 \times (\text{final body length}) / (\text{duration of experiment})$ .

Mean growth rate in the target population inversely matched total fish density within and between ponds (Table 2), indicating that competition was density-dependent [one-tailed  $F$  test on independent contrasts (18),  $r = 0.95$ ,  $F(1,2) = 17.5$ ,  $P = 0.026$ ]. Different Cranby phenotypes used different pond resources: Individuals most like the limnetic species in morphology were also most similar to it ecologically. This pattern further justifies the prediction that phenotypes most like the limnetic species should be most greatly affected by its addition.

As predicted, the presence of the limnetic species altered natural selection in the target species (Table 3 and Fig. 1). Different

**Table 1.** Mean attributes of populations and species. Measurements are means from 120 laboratory-raised fish ( $\pm$ SE). Offspring from the three crosses  $C \times B$ ,  $C \times C$ , and  $C \times L$  make up the experimental Cranby population. Trophic morphology is a composite shape variable (first principal component) based on size-adjusted ln-transformed measurements of body length, body depth, gape width, and number and length of gill rakers, calculated as described in (4). Fish having a negative value are relatively deep-bodied with a wide gape and few, short rakers; fish having a positive value are more slender and narrow-gaped with long, numerous rakers. The gill raker number is counted on the long arm of the first gill arch. The armor is a composite variable (first principal component calculated from the correlation matrix) of the number of plates and the size-adjusted ln-transformed lengths of the pectoral spines and pelvic girdle. Traits were size-adjusted with residuals of regressions of each trait on standard length. All the armor traits contributed positively and approximately equally to the combined variable. The morphological index is a linear combination (first principal component) of armor and gill raker number. Negative values indicate fish with low armor and few gill rakers; positive values indicate high armor and numerous gill rakers.

Trait	Cranby			Paxton limnetic
	$C \times B$	$C \times C$	$C \times L$	
Trophic morphology	-1.02 (0.50)	0.00 (0.52)	0.53 (0.46)	1.04 (0.40)
Gill raker number	13.8 (0.8)	13.8 (0.6)	15.3 (0.9)	17.0 (0.9)
Armor	-1.66 (0.75)	0.27 (0.50)	0.27 (0.49)	0.49 (0.49)
Morphological index	-0.97 (0.75)	0.00 (0.37)	0.75 (0.73)	1.79 (0.48)

**Table 2.** Pond conditions at the end of the experiment. The pond was treated as a fixed effect in the statistical tests, and degrees of freedom reflect the number of fish sampled rather than the number of ponds. This was done to describe conditions in the individual ponds, not to test experimental effects (which is carried out in the text). The number of fish is the total number surviving to the end of the experiment. Initial numbers of fish were 3000 on the experimental side, of which 40% were limnetics (1200) and 1800 were on the control side (all Cranby). The mean growth rate ( $\pm$ SE) is based on 60 to 70 randomly sampled Cranby individuals per half pond. Two-way analysis of variance (ANOVA) revealed that overall mean growth rate (ln-transformed) differed between treatments [ $F(1,260) = 8.37$ ,  $P = 0.004$ ] but not between ponds [ $F(1,260) = 0.28$ ,  $P = 0.602$ ]. The magnitude of the difference between treatment sides also varied between ponds [interaction  $F(1,260) = 13.92$ ,  $P < 0.001$ ]. Correcting for three simultaneous tests with the sequential Bonferroni method (21) did not alter these conclusions. The diet fraction is the number of cladocerans and copepods in the stomach as a proportion of all prey items (remaining items were predominantly benthic ostracods and amphipods). This fraction was measured on 10 to 15 individuals sampled from the lower third of the frequency distribution of phenotypes, and on 10 to 15 individuals from the upper third (presumably mainly  $C \times B$  and  $C \times L$  crosses, respectively). Means are averages of individual fractions. The diet fraction in limnetic fish was 0.99 in both ponds ( $n = 30$ ). Three-way ANOVA showed that diet fraction differed between the two morphological extremes of the Cranby population [ $F(1,101) = 23.8$ ,  $P < 0.001$ ], between treatments [ $F(1,101) = 3.06$ ,  $P = 0.041$ ], and between ponds [ $F(1,101) = 24.0$ ,  $P < 0.001$ ]. The data suggest that a diet shift between treatment and control sides was limited to pond 2, but the interaction between pond and treatment was not significant, after correcting for four simultaneous tests (21).

Treatment	Number of fish	Percent limnetics	Cranby mean growth rate (mm/90 days)	Diet fraction ( $C \times L$ , $C \times B$ )
Pond 1				
Experiment	1058	33	48.0 (0.7)	0.89, 0.68
Control	751		48.4 (0.7)	0.95, 0.63
Pond 2				
Experiment	1136	40	46.2 (0.8)	0.55, 0.24
Control	403		50.7 (0.8)	0.71, 0.58



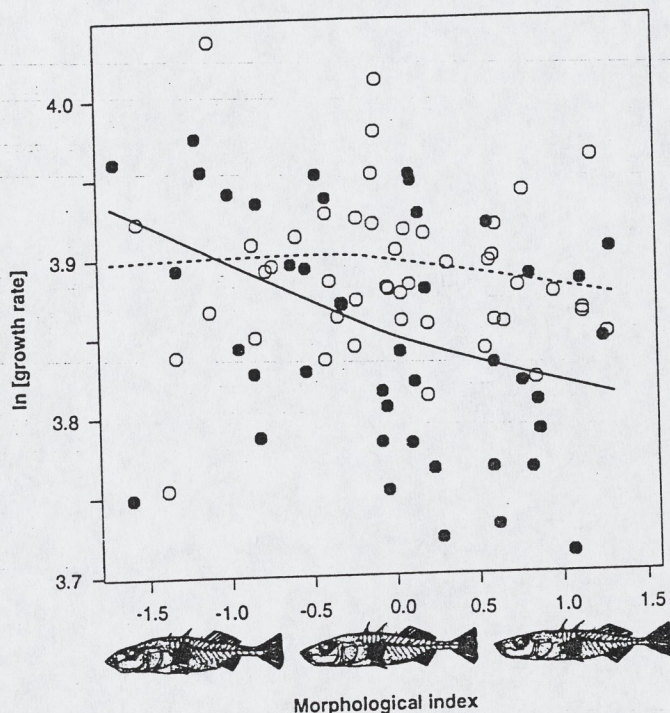
Cranby phenotypes grew at similar rates on the control sides of the ponds. This is in contrast to the treatment sides, where Cranby individuals closest to the limnetic species in morphology suffered a substantial growth depression (one-tailed paired *t* test on regression slopes; *df* = 1, *P* = 0.016). This effect on the Cranby species diminished gradually (rather than suddenly) with increased morphological distance away from the limnetic competitor. Intensity of

**Table 3.** Experimental results. The growth differential is calculated from the slopes of linear regressions of  $\ln[\text{growth rate}]$  on the morphological index. Significance levels refer to tests of the null hypothesis that the slope is zero. No significant curvilinearity was detected with quadratic regression. Estimates were similar when robust methods less sensitive to outliers were used instead (22). Significance levels were confirmed with the unsmoothed bootstrap method recommended in (23). Only the growth differential in pond 2, experimental side, is significant after correcting for four tests of slope with the sequential Bonferroni method (21). The survival differential is the difference between treatments (experiment minus control) in mean value of the morphological index at the end of the experiment. Significance levels are from two-sample *t* tests between pond sides, with individual fish as replicates (see Table 2 for justification).

Pond	Growth differential		Survival differential
	Experiment	Control	
1	-0.025**	-0.004	0.03
2	-0.050***	-0.006	-0.18*

\**P* ≤ 0.10. \*\**P* ≤ 0.05. \*\*\**P* ≤ 0.01.

**Fig. 1.** Growth rate of Cranby phenotypes in the presence (filled symbols, solid line) and absence (open symbols, dotted line) of the limnetic species. The morphological index reflects the gradation of forms within the experimental population, from more benthic on the left (deep body, wide gape, and short gill rakers) to more limnetic on the right (slender body, narrow gape, and long gill rakers). Drawings slightly exaggerate differences in profile among the three cross types (from left to right: C × B, C × C, and C × L). Original growth measurements are millimeters per 90 days. Data from both ponds are combined; growth rates within each treatment were pooled to the same mean. Each curve is a cubic spline (24), a nonparametric regression function that may assume any shape the data warrant. For clarity, each symbol is an average of three adjacent points.



directional selection away from the limnetic species was correlated with final limnetic density [one-tailed test of independent contrasts,  $r = 0.96$ ,  $F(1,2) = 25.6$ ,  $P = 0.018$ ]. Individuals morphologically closest to the limnetic species also tended to have reduced survival in one of two ponds (Table 3), but no overall experimental effect was identified (one-tailed paired *t* test on survival differentials; *df* = 1,  $P = 0.31$ ).

These findings constitute experimental evidence that resource competition promotes morphological diversification in a radiating lineage. They support the view developed from ecological, genetic, and biogeographic data (that is, indirect evidence) that competition played a large role in the rapid diversification of sticklebacks (4–8). The current morphological differences between sympatric species are consistent with the selection intensities recorded herein and with the presumed duration of sympatry: If total fitness is proportional to growth rate, then a persistent  $\ln[\text{growth}]$  differential of  $-0.025$  (Table 3) is sufficiently strong to produce the observed difference between limnetic and benthic species in about 500 years (or generations) (19). Detection of frequency-dependent selection on trophic traits also confirms a crucial element of mathematical theory of competition and character divergence (3, 10). Such selection also provides a simple mechanism for adaptive peak shifts and has been implicated in the process of speciation itself (20). Finally, the results emphasize the ex-

perimental advantages of studying adaptive radiations in their early stages, when species diversities are low and intermediate stages can be re-created.

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- Ponds had a sandy bottom sloping gradually (3:1) to a maximum depth of 3 m. They were constructed in 1991, seeded with plants and invertebrates from Paxton Lake on Texada Island, and left standing for 2 years. Sampling indicated that benthic invertebrates and plankton were abundant before fish introduction. Most natural predators were also present (insects and piscivorous birds, but not predatory fish). Each pond was divided into two equal sections with a plastic sheet immediately before the experiment. A 2 m by 2 m mesh window (100  $\mu\text{m}$ ) allowed exchange of water but not prey between sides.
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## Hydroxyurea as an Inhibitor of Human Immunodeficiency Virus-Type 1 Replication

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Hydroxyurea, a drug widely used in therapy of several human diseases, inhibits deoxynucleotide synthesis—and, consequently, DNA synthesis—by blocking the cellular enzyme ribonucleotide reductase. Hydroxyurea inhibits human immunodeficiency virus-type 1 (HIV-1) DNA synthesis in activated peripheral blood lymphocytes by decreasing the amount of intracellular deoxynucleotides, thus suggesting that this drug has an antiviral effect. Hydroxyurea has now been shown to block HIV-1 replication in acutely infected primary human lymphocytes (quiescent and activated) and macrophages, as well as in blood cells infected *in vivo* obtained from individuals with acquired immunodeficiency syndrome (AIDS). The antiviral effect was achieved at nontoxic doses of hydroxyurea, lower than those currently used in human therapy. Combination of hydroxyurea with the nucleoside analog didanosine (2',3'-dideoxyinosine, or ddI) generated a synergistic inhibitory effect without increasing toxicity. In some instances, inhibition of HIV-1 by hydroxyurea was irreversible, even several weeks after suspension of drug treatment. The indirect inhibition of HIV-1 by hydroxyurea is not expected to generate high rates of escape mutants. Hydroxyurea therefore appears to be a possible candidate for AIDS therapy.

Further attempts to design drugs for therapy of AIDS are necessary (1). Despite their differences in structure, antiviral activity, and pharmacokinetic properties, ddI, zidovudine (azidothymidine, or AZT), noncompetitive HIV-1 reverse transcriptase inhibitors, and HIV-1 protease inhibitors (2) share a common feature: They directly target viral proteins. As an alternative approach, we have suggested targeting one or more cellular components (3). The rationale for this strategy is to avoid triggering the onset of viral escape mutants as a result of direct selective pressure against viral proteins. Another rationale is to achieve specific antiviral effects of the drug with low or no toxic effects on the cell.

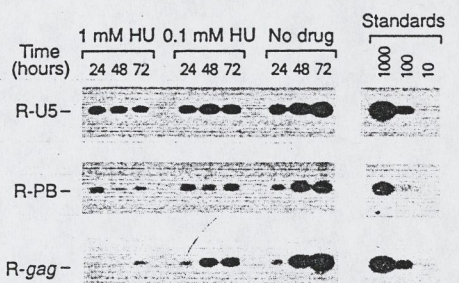
Hydroxyurea has been widely used over the last 30 years for the treatment of human malignancies, especially chronic myelogenous leukemia and other myeloproliferative syndromes (4). More recently, hydroxyurea has been proposed for the treatment of sickle cell anemia (5). High doses of hydroxyurea

are commonly used in leukemia treatment (4). Oral administration of the drug at a dose of 500 mg/m<sup>2</sup> every 4 hours generated plasma peak concentrations ranging from 0.5 to 2.5 mM and trough concentrations of 0.2 to 0.5 mM (6). Hydroxyurea is a free radical quencher and inhibits the cellular enzyme ribonucleotide reductase [a rate-limiting enzyme in the synthesis of deoxynucleoside triphosphates (dNTPs)]. We have shown that, by decreasing the intracellular pool of dNTPs, hydroxyurea inhibits HIV-1 DNA synthesis, resulting in the generation not

only of decreased amounts of viral DNA, but also mainly incomplete chains (3). Goulaoui *et al.* (7), with Moloney murine leukemia virus, confirmed that inhibition of reverse transcription by hydroxyurea depends on the intracellular nucleotide pool (rather than on the precise arrest of the host cell cycle). High single doses of hydroxyurea delay HIV-1 spread *in vitro* (8). Furthermore, by decreasing the amount of cellular dNTPs, hydroxyurea was expected to increase the uptake and metabolism of nucleoside analogs, such as ddI or AZT, and consequently to enhance the effect of these compounds, hopefully in a synergistic manner. We now demonstrate that low, subtoxic doses of hydroxyurea, alone or in combination with AZT or ddI, block HIV-1 replication.

Because nonstimulated lymphocytes are not productively infected by HIV-1, but only allow viral entry and reverse transcription (3, 9), we assessed HIV-1 infection in peripheral blood mononuclear cells (PBMCs) by monitoring HIV-1 DNA synthesis (3, 10). Compared to untreated cells, HIV-1 DNA synthesis was slower and less efficient, and the final DNA was mostly incomplete, in hydroxyurea-treated quiescent lymphocytes (Fig. 1). A similar phenomenon has been described for activated lymphocytes (3). The effect of hydroxyurea was dose-dependent, especially for the synthesis of the full-length minus strand DNA (R-gag, the longer DNA synthesis product analyzed in our experiments). The block of DNA synthesis was almost complete at 1 mM. The DNA shown in Fig. 1 mainly represents DNA carried by the incoming virions (3, 10); the amount of DNA did not vary during the time course and remained mostly incomplete. More elongation was observed at 0.1 mM, although at much lower levels compared to the untreated control. No cytotoxic effects were observed at the drug concentrations used in these experiments (11) because quiescent cells do not undergo genomic DNA synthesis.

**Fig. 1.** Time course of inhibition of HIV-1 DNA synthesis by hydroxyurea in quiescent PBMCs. PBMCs were isolated from healthy donors and infected after 2 days with the HIV-1 strain HTLV-III<sub>B</sub> (18) at a multiplicity of infection of 1 in the absence of cell stimulation. After 2 hours at 37°C, the cells were washed, and fresh medium containing hydroxyurea (HU) at the indicated concentrations was added. Cells were harvested after 24, 48, and 72 hours and analyzed by quantitative polymerase chain reaction (PCR). Primers were used as described (3) to amplify different regions of the HIV-1 genome: R-U5 [between the R and U5 regions of the long terminal repeat (LTR)], R-PB (between the R region of the LTR and the primer binding site), and R-gag (between the R region of the LTR and the gag gene). After 30 cycles of PCR amplification and subsequent electrophoresis on 2% agarose, the samples were blotted on a nylon membrane and hybridized with a <sup>32</sup>P-labeled oligonucleotide as described (3). Quantitation of HIV-1 DNA during PCR amplification was achieved by comparison with a standard curve of serial dilutions of pHXB2(Rip7) plasmid DNA (19). The numbers above the lanes labeled "Standards" indicate the number of plasmid copies.



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## Criteria for Testing Character Displacement

Dolph Schluter (1) purports to demonstrate that competition among populations of the threespine stickleback leads to character divergence, based on an experiment in two divided ponds. Both halves of each pond were stocked with individuals from a benthic stickleback species, and individuals of a second, limnetic species were then added to a randomly chosen side of each pond. Thus, the half-pond is the experimental unit, and there are two replications of the comparison between treated and control halves. The responses of interest are based on the growth rates of individuals of the benthic species, in the presence and absence of the limnetic species.

A key part of Schluter's evidence that "the presence of the limnetic species altered natural selection in the target species" is in table 3 of his report, which shows "growth differentials" (slopes of linear regressions of log growth rate versus a morphological index reflecting the continuum between benthic and limnetic characteristics) in the four half-ponds. If competition from the limnetic species is most severe for individuals of the target species having limnetic characteristics, then the growth differentials in the experimental half-ponds should be more negative than those in the control half-ponds. However, the difference between slopes in the experimental and control halves, accounting for the pairing within pond, is not statistically significant (paired *t* test on the four slopes in table 3 of the report;  $t^* = -2.826$ ,  $df = 1$ , two-tailed test,  $P = 0.22$ ). Schluter reports an apparently erroneous *P* value of 0.016 for a one-tailed test (which implies that with a two-tailed test,  $P = 0.032$ ).

Schluter further reports a significant correlation between growth differentials and the final densities of limnetic fish in the half-ponds. His method, involving regression of three independent contrasts of the four slopes in table 3 of his report against the corresponding contrasts of the four measured densities of limnetic fish, is flawed because one of the response contrasts depends on the magnitude of the pond effect and has a variance different from that of the other two contrasts (2). In any event, the most direct test of the association between the limnetic fish treatment and the growth differentials in table 3 of the report remains the nonsignificant paired *t* test.

The same inappropriate methodology (2) is used to support an association between mean growth rates and the total numbers of fish in the four half-ponds (table

2 of the report by Schluter). A direct test of the effect of the experimental manipulation (which led to the different fish densities) on mean growth rates shows no evidence for an effect of the treatment (paired *t* test on the four rates in table 2;  $t^* = -1.1951$ ,  $df = 1$ , two-tailed test,  $P = 0.44$ ).

Another line of evidence that the presence of limnetic fish affects growth rates is figure 1 in the report by Schluter, which is a plot of transformations of log growth rate versus morphological index. This plot appears to show a decreasing trend for fish in the presence of the limnetic species and no trend for fish in the absence of the limnetic species. My statistical modeling of the points shown in the graph does not indicate different slopes for the experimental and control groups (3), but, even if it did, this would not justify the conclusion that the presence of the limnetic species was driving the difference. Without information on the pond-to-pond variation in the slopes of such regression lines, which is lost in the pooled data of figure 1, we cannot judge whether or not the limnetic fish treatment is causing additional variability.

The trends in Schluter's data are consistent with effects of competition from the limnetic species, but the key statistical comparisons of experimental units do not support his conclusion that "resource competition promotes morphological diversification in a radiating lineage." The lack of statistical significance does not necessarily mean that competition is unimportant, as only large effects would be detectable in an experiment with two replicates.

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2. Let *Y* represent the response (for example, growth differential, or mean growth rate) and *X* represent the explanatory variable (for example, density of limnetic fish, or total fish density), with subscripts denoting half-ponds as follows: 1 = experimental side of pond 1; 2 = control side of pond 1; 3 = experimental side of pond 2; and 4 = control side of pond 2. Suppose  $\mu$  is the mean response in pond 1 with  $X = 0$ ;  $\alpha$  is the effect of pond 2;  $\beta X$  is the effect of *X* units of the explanatory variable; and the  $\epsilon$ 's are random errors. Then we can model the responses in the four half-ponds as follows:

$$Y_1 = \mu + \beta X_1 + \epsilon_1 \quad Y_3 = \mu + \alpha + \beta X_3 + \epsilon_3$$

$$Y_2 = \mu + \beta X_2 + \epsilon_2 \quad Y_4 = \mu + \alpha + \beta X_4 + \epsilon_4$$

The three independent contrasts used by Schluter (personal communication) have expectations  $E(Y_1 - Y_2) = \beta(X_1 - X_2)$ ;  $E(Y_3 - Y_4) = \beta(X_3 - X_4)$ ; and

$$E\left(\frac{Y_3 + Y_4}{2} - \frac{Y_1 + Y_2}{2}\right) = \alpha + \beta\left(\frac{X_3 + X_4}{2} - \frac{X_1 + X_2}{2}\right)$$

When these response contrasts are regressed against the corresponding contrasts in *X* (that is,

$$X_1 - X_2, X_3 - X_4, \text{ and } \frac{X_3 + X_4}{2} - \frac{X_1 + X_2}{2}),$$

the resulting slope will provide a biased estimate of  $\beta$  because of the dependence of the third response contrast on the pond effect,  $\alpha$ . Furthermore, the variance of the third response contrast is different from that of the other two contrasts, violating an assumption of the regression model. If  $\text{Var } \epsilon_i = \sigma^2$  for all *i*, and we assume a non-zero correlation,  $\rho$ , between error terms from the same pond (that is,  $\text{Cor}(\epsilon_1, \epsilon_2) = \text{Cor}(\epsilon_3, \epsilon_4) = \rho$ ), then  $\text{Var}(Y_1 - Y_2) = \text{Var}(Y_3 - Y_4) = 2\sigma^2(1 - \rho)$ , and

$$\text{Var}\left(\frac{Y_3 + Y_4}{2} - \frac{Y_1 + Y_2}{2}\right) = \sigma^2(1 + \rho)$$

A possible modification of Schluter's test is to fit a regression line through the origin using just the first two contrasts, which have equal variance, and expectations not involving  $\alpha$ . This approach does not find a significant association between *Y* contrasts and *X* contrasts for either the analysis of growth differentials by limnetic fish density ( $\beta = -0.0001$ ,  $df = 1$ , two-tailed  $P = 0.13$ ) or the analysis of mean growth rate by total fish density ( $\beta = -0.0054$ ,  $df = 1$ , two-tailed  $P = 0.20$ ).

3. Let GROWTH = log growth rate, MI = morphological index, and COMP = indicator for presence (1) or absence (0) of limnetic fish. Then the "full" regression model fit to 88 points read off figure 1 in the report by Schluter is: GROWTH = 3.8887 - 0.0406 COMP - 0.0013 MI - 0.0307 COMP·MI. The coefficient for the interaction term, COMP·MI, is nonsignificant ( $P = 0.087$ ), which indicates that we cannot reject the hypothesis that the slope of GROWTH versus MI is the same in the presence and in the absence of limnetic fish. As each symbol in figure 1 of the report is an "average of three adjacent points," and "growth rates within each treatment were pooled to the same mean," we cannot be sure how an analysis of the full set of untransformed data would come out.

14 December 1994; accepted 25 January 1995

The study by Schluter (1) has problems in its design, conduct, and analysis; at the request of the editors, we limit our comment to the first two areas. The first problem concerns experimental design. The treatment in which the putative competitor was introduced confounds two factors: presence of "heterospecific" individuals and a 1.7-fold increase in total fish density. Both additive and replacement designs are appropriate for competition experiments, but each is used to test distinct hypotheses (2, 3). The additive design used by Schluter would be appropriate were he simply attempting to detect competition between his two "species," that is, to determine whether the two "species" have a joint carrying capacity (or density function). Evidence of character displacement requires a distinct effect: that heterospecific competitors differentially affect some subset of the population relative to intraspecific competition. If addition of heterospecifics produces an identical effect to that of adding the same number of conspecifics, then character displacement, as classically defined, should not be invoked.



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Given this criterion, experimental tests for character displacement require treatments in which total densities are held constant to determine whether inter- and intraspecific competition are equivalent from the perspective of the focal "species." Lacking such controls, treatment effects cannot be attributed uniquely and unambiguously to interspecific competition as distinct from increased fish density (4). The appropriate null hypothesis that must be rejected here is that the two "species" are equal competitors with regard to all phenotypes of the focal "species." The appropriate minimal test is a replacement design holding densities in the control (single "species") and competition (two "species") treatments constant, and testing for competitive equivalence (3) between the limnetic and different segments of the phenotypic distribution of the focal "species." Confounding is a serious, common error in ecological experiments (5), and while the specific type of confounding present in this study is appropriate in testing certain hypotheses regarding competition, it constitutes a fatal flaw in a test for character displacement. Thus, Schluter has not unambiguously established interspecific competition as the cause of the purported differential effect on phenotypes of the focal "species" because the appropriate hypothesis-specific control was not included.

We see two difficulties in the conduct of the experiment concerning extrapolation back to nature. The first concerns the use of hybrids. Schluter argues (1, p. 799), "Hybridization is a valid manipulation because all previous crosses between closely related freshwater sticklebacks have not revealed any intrinsic reduction in offspring viability." This may be true, but other problems arise from the use of hybrids. First, the pool of fish on which effects of interspecific competition was assayed was assembled using equal numbers of offspring from three experimental crosses (1), and hybrid fish were created using the limnetic species (the species used as a putative competitor), as one parental type. Because the limnetic species contributed half the genes to the L × C hybrids in which the greatest effect of competition was expected, the question essentially asked is: Do the limnetics have a greater effect on their own hybrid-genetic progeny than on genetically unrelated individuals? This is not an illuminating question in the context of character displacement: Because of this relatedness, Schluter is not studying an interspecific competitive interaction, which is fundamental to the hypothesis that interspecific competition drives character displacement. Second, there is both morphological and genetic correspondence between these L × C hy-

brids and the limnetic competitor, that is, genotypes and morphology are confounded. Hence, the particular hybrids (L × C) most likely to compete with the limnetics are also most genetically similar to limnetics. Further, the use of hybrids introduces the potential for heterotic genetic effects (6) in responses of hybrids to the limnetic morph that are not genetically accessible to individuals of a single species in nature. Hence, one cannot argue that any effect observed in the experiment would be evidenced by fish in nature. Although the experiment is internally consistent in that the putative control also consisted of hybrids, the responses of the experimental fish cannot be unambiguously attributed to either their morphological phenotypes or to their artificial hybrid genetic constitution. That is, any resulting selection differential may have little to do with morphological similarity. Experimental expansion of a phenotypic distribution for the purpose of analyzing selection is creative, but is interpretable only if the novel phenotypes so produced are not confounded with other, unmanipulated parts of the phenotype, or with changes in genetic constitution (7).

The second difficulty arises from the relationship of experimental to natural densities and the background conditions of the experimental ponds. Densities in the experiment were not matched to natural densities. Rather, "Densities were set such that growth rate of pond fish would equal that in the wild, as judged from an earlier pilot experiment . . ." (1, p. 800). This seems reasonable, except that experimental ponds were fish-free for 2 years before the experiment, thus accumulating far greater invertebrate biomass and attaining far different species composition (8) than would be expected in natural ponds containing sticklebacks. Experimental densities far above natural densities would be required to achieve natural growth rates, thus increasing encounter rates and the potential for interference competition. Also, resource competition (the presumed mechanism driving character displacement) would have occurred against an artificial resource background.

It is tempting to accept the results of provocative, high-profile experiments at face value (9). Although we strongly support using experiments to validate mechanisms hypothesized from comparative data (10), such experiments must comply with rigorous standards of design, conduct, and analysis. Because of deficiencies on all three points, this is not a landmark demonstration of character displacement.

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Response: Murtaugh mentions three concerns about my statistical analyses (1), which I address in turn.

1) The paired comparison of growth differentials was incorrect.

This criticism is accurate. The growth differentials were properly listed, but the results of the paired *t* test should have read as follows:  $t = -2.826$ ,  $df = 1$ ,  $P = 0.11$ ; one-tailed test. Complementary analyses suggest that the treatment effect was nevertheless real. Growth differentials in the presence of the competitor were significantly negative in both ponds, but were near zero in the competitor's absence (1). The slopes differed significantly between treatment and control sides [Fisher combined probability test (2);  $\chi^2 = 9.49$ ,  $df = 4$ ,  $P = 0.050$ ]. The steepness of the growth differential increased with increasing competitor density. Spearman rank correlations between growth rate and morphological index also differed between treatments (one-tailed paired *t* test;  $t = -6.708$ ,  $df = 1$ ,  $P = 0.047$ ). Two ponds (replicates) can withstand only so much statistical analysis; but the important point is that the growth patterns are in the predicted direction and that



despite low power most analyses detect a difference.

2) The regressions based on independent contrasts were flawed.

This concern is unwarranted, as all possible corrections lead to the same answer. The criticism is based on the fact that the variance of the between-pond contrast is not the same as that of the two within-pond contrasts. However, adjusting for heterogeneity in variance by standardizing the contrasts (3) has no effect on the results, which is why I neither standardized nor elaborated on the method in my report. At one extreme is the possibility that ponds do not differ (except in ways caused by the experiment), in which case the between-pond contrast has one-half the variance of the other contrasts. When the contrasts were standardized accordingly, the correlation between growth differential and competitor density ( $r = -0.97$ ;  $P = 0.018$ ; one-tailed test) was nearly identical to that originally reported (1). At the other extreme is the possibility of a large pond effect. For example, if the variance of the between-pond contrast is 10 times that of the other two contrasts, standardizing yields a result not much different from the first ( $r = 0.98$ ;  $P = 0.012$ ; one-tailed test). The same was true when the method of independent contrasts was used to compare growth rate with total fish density ( $r = 0.94$ ;  $P = 0.029$  and  $r = 0.95$ ,  $P = 0.024$ ; one-tailed tests). Consequently, the method is justified and the original results hold.

3) The data plotted in figure 1 of my report do not show a significant treatment effect.

The purpose of this figure was solely to depict the shape of the relationship between growth rate and the morphological index. The figure does not contain data that would allow a valid statistical test of treatment effect. The points in the figure were averages of trios of observations, and combine data from both ponds. Murtaugh's analysis is pseudoreplicated because it assumes that individual observations within ponds are independent, an unlikely situation. However, were independence a valid assumption, the actual data would still support a significant treatment effect on growth differentials [one-tailed test of heterogeneous slopes;  $F(1,260) = 2.916$ ,  $P = 0.044$ ].

Bernardo *et al.* list two general design concerns. Both were raised in my report (1) where little space was available for details.

1) A different experimental design should have been used.

My design, hereafter called (A), had two treatments: a control, in which the target species occurred alone; and an experimental, in which the target and a competitor species (the limnetic) were present. The starting density of the target was constant. The advantage of (A) is that any change in natural selection on the target species can be traced to the presence of the competitor species. Its weakness is that (A) cannot rule out the possibility that changes in selection were solely the result of an increased density of fish. A reasonable expectation is that increased density alone would effect all phenotypes of the target equally, which did not happen. Moreover, details of habitat use corroborate the frequency-dependent prediction: limnetics specialized on plankton, and the more planktivorous phenotypes of the target population suffered the brunt of their presence. In contrast, the "density-only" hypothesis predicts that the planktivorous phenotypes would suffer disproportionately even if the competitor species avoids plankton. A mechanism for this is difficult to envision.

Bernardo *et al.* suggest a second design (B) in which the total density of fish in both treatments is constant. This is accomplished by replacing individuals of the target species by the same number of competitors (limnetics) in the experimental treatment. The strength of this design is that any difference between treatments in natural selection can be attributed to a change in the frequency of different phenotypes overall. Its weakness is that the effects may be a result of a lower population density of the target species rather than of the presence of the competitor species. Design (B) therefore does not test whether presence of the competitor species is the cause of a treatment effect. Hence it is an inferior design.

A third two-treatment design (C) uses two competitor species. A plankton specialist (the limnetic) is added to one treatment, and a specialist on benthos (the benthic species) is added to the other. This has the advantage of varying phenotype frequency while keeping constant both the total density of fish and the density of the target species. I used (A) rather than (C) for two reasons. First, I wanted a baseline measurement of selection on the target species alone (that is, a control). Second, I wished to test specifically whether the differences between the modern limnetic and benthic species are the result of ecological character displacement (4). This was best achieved by using the phenotypes most likely to have been those present in lakes when sympatry

between their ancestors was first established (1).

2) The experimental conditions were not natural.

A concern was that the limnetic competitor was genetically related (through hybridization) to one component of the target population. However, genetic similarity between limnetics and part of the target population was intentional (I am interested to hear how similarity in a heritable suite of ecologically relevant traits might be achieved without genes in common). In figure 1 of my report, I looked for visual evidence that effects of the added competitor were limited to the limnetic-hybrid component, but found none. Other possible effects of hybridization are common to both treatments and are therefore controlled for. Additional design concerns are minor, but surround a deeper question: Are ponds identical to natural lakes? They are not. Nevertheless, ponds are similar to lakes in enough ways that have allowed many important ideas in ecology to be tested using them. But it is essential that a hypothesis such as character displacement be tested with a combination of methods that includes comparisons of wild populations (4).

In sum, the experimental results greatly bolster earlier conclusions based on comparative studies (4) that competition was an important force in the diversification of sticklebacks. They do not constitute final proof of the evolutionary significance of competition in general, which awaits further experimental study especially of other systems.

A final correction: In the summary of my report in that issue's "This Week in Science" (p. 709), the phrase "and the first generation of offspring clearly showed an increased divergence between the two types" was incorrect. My experiment was conducted wholly within a generation and involved no evolutionary change between generations.

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## Devaluation of non-experiments in the current ecological paradigm

In design and analysis texts, as well as in graduate course lectures, a distinction is frequently made between 2 design types: experimental and non-experimental (i.e., observational, comparative, and quasi-experimental). In an experimental study, the researcher controls outside factors and applies treatments to experimental units. If done correctly, these procedures allow the researcher to make a valid inference of causation. In contrast, treatments in non-experimental studies are imposed prior to initiating research and do not allow the researcher to infer causation. Rather, non-experimental designs describe correlation. This inability to infer causation limits non-experimental studies in a way that has led to the current devaluation of this type of study in the present ecological paradigm. This devaluation is resulting in reduced funding and reduced publication possibilities for such studies. Consequently, fewer researchers are willing to take on non-experimental projects. We feel that this situation is unfortunate because non-experimental studies contribute to the development of our scientific knowledge in ways that experimental studies do not.

Experiments are less suitable than non-experiments for addressing questions of large-scale phenomena. Many of the applied questions that researchers are addressing today focus on large-scale issues. Ecologists are becoming increasingly aware of the importance of scale in designing studies and interpreting results (Wiens et al. 1986). Researchers must take into account the scale that is relevant to the organisms under study. If large scales are necessary (e.g., when studying avian migration), adequate replication or control for extraneous environmental factors is often impossible. This is particularly true because biological systems are open. Experiments attempt to control outside factors and to manipulate local factors; however, if the controlling factors of a system are regional factors, manipulating local factors in an experiment may be irrelevant (J. A. Wiens, pers. commun.).

Experiments are also less suitable than non-experiments for gaining knowledge of natural history. Wildlife ecology is the process of looking for patterns in nature. These patterns are often correlative and not causative. Accurate natural histories of organisms provide the conditions for finding new patterns and developing theories from these patterns.

Most ecologists would agree that research questions and research design should not be dictated by the biases of a paradigm but by the phenomena un-

der study. However, despite this agreement, 3 issues prevent many researchers from investigating questions that would require a non-experimental design: funding, publication, and job opportunities. Presently, a research proposal that does not incorporate controlled experiments is unlikely to be awarded grants from funding agencies. Moreover, academic journals are currently showing a bias toward publishing experimental research rather than non-experimental. The inability to publish non-experimental research seriously diminishes an individual's job opportunities. Finally, because of these 3 sociological issues, the current paradigm tends to stifle creative development of research questions. We, along with many others, believe that creativity is an important component of the scientific process.

Research should not be limited by a paradigm that places undue emphasis on experiments. Non-experimental studies need to be recognized for their own strengths, their applicability, and their flexibility to address a wider range of questions.—*Liza Graham, Amy A. Yackel-Adams, and Eric A. Odell, Department of Fishery and Wildlife Biology and Graduate Degree Program of Ecology, Colorado State University, Fort Collins, CO 80523.*

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### *The debate continues...* Students comment on proposed legislation

#### Teaming With Wildlife and the Conservation and Reinvestment Act of 1998

I support the Conservation and Reinvestment Act of 1998 (CARA: H.R. 4717). I acknowledge there are weaknesses, but feel the positives outweigh the negatives when you analyze the legislation collectively. I believe the proactive approach to wildlife conservation and the emphasis on nongame species in Title 3 will make CARA landmark legislation, if enacted.

The similarities of Teaming With Wildlife (TWW) to the enormously successful Federal Aid in Wildlife Restoration Act of 1957, Federal Aid in Sport Fish Restoration Act of 1950, and the Wallop-Breaux Act of 1984 would almost guarantee equal success. The



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PREDICTIVE ECOLOGY -  
A NATURAL HISTORY FANTASY

In the following essay I give reasons for rejecting the view that there is a scientific method which if rigorously applied will lead to reliable, proven knowledge in wildlife and fishery biology. I refer particularly to sermons which have appeared sporadically in recent decades in most of the journals about wildlife and fishery research and related ecological topics. I call these papers sermons because of their overtones of morality and their hope and trust in intellectual salvation through method. Their authors rightfully recognize that research in wildlife topics has been largely inconclusive and management based on it has often been less than effective. Their mistake is blaming incorrect "scientific method" for these shortcomings. Our problems are more profound and less subject to correction than these essay-sermons would have us believe. The inconclusiveness of our research lies in the nature of our subject matter and not in our scientific (logical) method. To contradict Shakespeare, our faults lie in the stars at least as much as in ourselves.

I have not cited the specific papers with which I disagree. I make this omission to defer urgent defensive response and allow an interval sufficient for those who disagree with my views to read some new sources (several weeks, several years?). Bruised egos shooting from the hip will not help the profession gain maturity and will make dull reading. Those who argue for the scientific method of DesCarte, Newton or even Mill (Flew 1984) have failed to keep abreast of current mainstream controversy on the subject. Anyone planning to reform the scientific logic of the wildlife



profession should first read at least a half dozen significant mainstream authors who have published books in the last 2 decades. I readily admit that this is a formidable task but it is a necessary one. The chief insight to be gained by doing so is that these people are arguing about something very different than the discredited scientific method still propounded in introductions of many textbooks in biology, geology, statistics, chemistry, physics, etc. The controversy now rages over the meaning and reality of explanatory theories and the degree to which logic or evidence can support a theory if ever. No one denies man's ability to produce impressive technologies but there is legitimate controversy about the logical meaning of these feats. More on this later.

Despite a perpetual debate on scientific logic no single most effective method for discovering the "Truth" has emerged and few are even suggested. The core of all this "wheel spinning" is the logical impossibility of material proof. No matter how impressive the evidence, things could be otherwise. All predictions from experience (inductions) are fallible. Just because we have no alternative to induction doesn't mean that convincing evidence equals proven truth (see induction, Hume, Pierce and related topics in Flew 1984). All criteria grading the reliability of evidence are subjective. Despite illusions about the meaning of probability there is no logical connection between the past and the future. How can there be? For those who propose the hypothetico-deductive model, consider that the testing part is pure fallible induction despite contrary claims. As for the deductive aspect of this paradigm of



scientific morality, all current mainstream logicians recognize that deduction is a tautology, a circular symbol game or a kind of grammar (see logic, deduction and tautology in Flew 1984). When we attach meanings to a logical symbol as in z means a shaggy dog we must ask how shaggy. We thus re-introduce subjectivity because of the ambiguity and fuzzy criteria of our classifications of nature. The attempt to correct subjectivity always leads to definitions and exemplars which are only codified subjectivity or convention (see translation in Flew 1984).

I refrain from specific citations of original philosophical sources in the text because I believe it is unfair, pedantic and pompous to introduce an entire unfamiliar literature to readers trained in a different tradition without serious preparation. Citations must have a fair chance of being checked or they are propaganda. I do, however, cite entries in one understandable and concise source which I found to be very candid and accessible: A Dictionary of Philosophy by Antony Flew (2nd edition). Most dictionaries or encyclopedias of philosophy are certain, stuffy and protective of their subject matter; Flew's isn't. His should be called "A Critical Dictionary of Philosophy" for fair criticism as well as supporting opinion are given. One of its other virtues is that it only costs about \$10.00

In addition to refraining from citing original philosophical sources I equally reject a recommended reading list. Each persons preferred readings are unique for him and perhaps partly accidental. I browsed the shelves in the philosophy of science and probability sections of a few



university libraries and serious book stores. The popular chain book stores usually don't stock technical philosophy books. Libraries of many state colleges and universities offering degrees in wildlife, environmental and natural history subjects are often inadequate in philosophical books too. The most efficient procedure for me was to find the most recent accessions that were at least partially understandable (I rarely understand entire books on philosophy of science at first reading if ever), and "raid" their literature citations for other recent books to look for, and so on.

The indirect writing style of all philosophy books contrasts sharply with the direct, sparse style of scientific journals and gave me some initial difficulties. Figures of speech (litotes, metaphor, simile, irony, sarcasm) abound. Philosophical journals were largely opaque to me, too technical. I rapidly read and reread 2 or 3 books at a time (I don't study them) and again reread confusing ones after getting clues to what was meant from other books. I have profitably read some books a half dozen times in a few years. I never try to pin down "facts" but rather try to understand controversies. I withhold commitment to any particular view indefinitely and keep seeking the latest views to contrast with earlier ones. I am wary of "how to" science manuals and textbooks introductions on scientific method. Some are honest but most are propaganda. Most text authors, professors and students can't tolerate uncertainty but in such toleration lies our only claim to being scientists.



I will relent a bit from my book list stance to list the following mainstream authors on philosophy and history of science and philosophy of probability who, with a few exceptions, have published since 1985 as well as earlier. These at least give an idea where some of my views came from: Harold Brown, Bas Van Fraassen, Ian Hacking, Edward Lepin, Paul Feyerabend, Imre Lakotas, Michael Ruse, R. P. Thompson, David Hull, John Watkins, James Bourke, Roy Weatherford, Thomas Kuhn and Larry Laudan. Some will prefer others. For summaries of not so recent authors including authors of some classics I again suggest Flew (1984). A minimum list of entries to be read might include Duhem, Hume, Keynes, Mach, Nietsche, Peirce, Popper and Whewell. Newton, DesCarte, Bacon, Kant and Mill are historically interesting but have been largely discredited.

In addition to the summaries of opinions of significant authors other selected entries can provide a critical and concise but not quite current introduction to the ongoing controversies on science and probability. I list these as follows: abstraction, acceptance, affirming, etc., analytic and synthetic, a priori and a posteriori, atomic uniformity, Bayes theorem, begging the question, Bernoulli's theorem, Bertrand's paradoxes (2), confirmation, corroboration, decision theory, empiricism, fallibilism, gamblers fallacy, Hume's fork, hypothetico-deductive, implication and entailment, induction, instrumentalism, language game, limited independent variety, linguistic philosophy, logic, logical positivism, necessary and contingent truth, necessary and sufficient conditions, probability theory, philosophy of science,



statistics, uncertainty principle, uniformity of nature, valid and invalid, verifiability, vindication. While Flew's dictionary of philosophy may seem somewhat superficial it can hardly be accused of gross errors or editorial slant. Too many people were involved in its preparation.

Whenever I criticize scientific method in social conversations someone usually jumps to the defense of science by pointing to our impressive technologies. Examples often given include the "atom bomb," nuclear generation of power, silicon chips, quantum mechanics, various vaccines, genetic engineering, and beer and wine making if the group is imbibing. Seldom mentioned are 5 day weather predictions, 1 day earthquake predictions, 1 hour tornado warnings, the course of epidemics, 3 month economic forecasts, 1 hour football predictions, or 6 month forecasts of next fall's pheasant population density. The common quality of the 1st group of impressive examples is that they are all technologies and that of the 2nd group of unimpressive examples is that they are not. Industrial technologies are merely controlled redundant experiments. Theories often guide a pilot controlled experiment but are never proven in the process (see Duhem and related entries in Flew 1984). Many successful controlled experiments were not related to coherent theories but rather were the result of just fooling around. For many applied scientists, scientific method means only tightly controlled experiments in which the number of possible events and effects is severely limited so as to seem to exclude all ambiguity about "apparent" causes. The successful application of a controlled experiment to the larger world demands control of that



larger world to the same degree as the experiment was controlled. This is what we call industry. Both the initial experiments and industry are technologies. A controlled ecological experiment, however, is an oxymoron. We may "isolate" and protect biological interactions in the laboratory, but then they are removed from the virtual infinity of mutual relations which is ecology. Biological predictions may be greatly improved in the laboratory but to what natural "industry" do they apply?

A few "wild" phenomena seem to be regular and predictable but these are very rare among "wild" systems. If the movements of the planets and stars were considered on a "fast forward" cosmic time scale instead of the scale of our brief human history the cosmos becomes chaos. If we considered ecosystem change second by second it would look very predictable. A physicist or molecular biologist may talk of a failed experiment because they have specific expectations and protocols with highly defined experimental subjects. Unexpected outcomes are usually dismissed as being caused by an error in experimental procedure and not in the core of the theory. One can usually find an error in a system in which he controls most elements. The poor wildlife biologist or ecologist or for that matter the meteorologist, epidemiologist, tectonic specialist, economist or Las Vegas odds-maker must accept anything that happens in his chunk of chaos and can never dismiss outcomes. None of these practitioners can know all the elements relevant to their realms of interest and control the realms to fit the experiments like engineers do. Nature is charmingly perverse and that's why it is more interesting and



important than engineering.

Much conviction based on statistics in applied ecology is nonsense. The historical debates about what probability really is and how to use it have never been settled. Most introductory texts on statistics sweep all of this under the rug and proceed with unsupported arcane and recondite methodology. There are a number of ongoing old but germane debates about crucial issues in probability theory (see several entries in Flew 1984) which remove most credibility from its application. We invoke limit theory where it has no clear relation to the small samples and changing probabilities we deal with. We claim to understand the future distribution and magnitude characteristics of nature from the always extinct realm sampled. There is no present only past. We invoke the metaphysical "uniformity of nature" shibboleth which we apply over our generalities to other realms in time and space. Even when our research results seem very precise they always could be different in the future and elsewhere and usually are. The most frequent statistical culprit is correlation analysis. Ehrenburg (1975) clearly shoots down correlation and regression analysis. Beside not telling much that we can't tell from graphs it is usually interpreted in a misleading way. I refer to the optimistic misinterpretation of the coefficient of determination ( $r^2$ ) (Ehrenburg again). This statistic tells how much of the sum of squares (or mean square) has been explained and is not the field biologist's idea of variability in raw measurements. An  $r^2$  of 0.75 ( $r = 0.87$ ) means that approximately 1/2 of the range or spread of the covariable to be



"predicted" is not explained by the "predictor" covariable. An  $r^2$  this high is rare in normally distributed samples of 10 or more of anything wild. In most studies published an  $r^2$  of 0.6 is considered excellent and yet this means that almost 2/3 of the spread of the "predicted" thing is due to causes the author is ignorant of. The formula for getting back to original measurements is:

$$1 - \sqrt{1 - r^2}.$$

I do not mean to say that all wildlife and fishery research and popular spin-offs are worthless. At the very least it is as interesting as most novels and just as justified for its special but large readership. Applied ecologists are just as smart as rocket scientists and molecular biologists. Past research effectively describes the entities and phenomena we think exist, gives them names, locates them in space and records their kinds of associations even if only weakly predictive. We manipulate a large body of hard won but now dimly appreciated natural history in planning and carrying out our research. Such difficultly gained commonplaces guide us but knowing these things doesn't give us any more population predictive power than intelligent amateur naturalists possess.

Physiology and individual behavior are somewhat more precise forms of knowledge than ecology and population dynamics. They are in a sense highly controlled natural mass experiments in which most individual cases are savagely rejected by nature as errors or deviations. All salmon return to the native stream except the 99% or so eliminated in natures



quality control. Who knows how diverse a "species" could be if nature just wasn't so critical of her own experimental methodology?

In a more specific way we can and do make useful predictions of non-occurrence or low populations of plants and animals by noting that at least one necessary condition for survival is absent or minimal. Habitats we assess as favorable all stand the risk of losing at least one attribute necessary (see necessary and sufficient, Flew 1984) for existence of a species. To predict that there will be large healthy populations requires predictions of a virtual infinity of necessary habitat attributes which in aggregate would be sufficient. Lose one necessary attribute and the habitat is no longer adequate despite the continuing presence of all the other necessary attributes. One bad ice storm in March can dim next season's pheasant prospects in otherwise ideal habitat. Negative predictions are always much less "iffy" than positive predictions because they can be legitimately based on the continuing or very recent absence of only one necessary habitat feature. In this vein we could salvage useful information from some weak and rejected correlations in which the predicted correlate was always low when the predictor correlate was low despite a poor correlation at higher values. This is of course the typical tipped funnel shaped distribution we often find in data from nature.

A few wildlife practitioners have recognized the chaotic nature of our enterprise. Aldo Leopold frequently commented on our arrogance. Buck, Thoits and Rose recognized the "butterfly effect" as early as 1970



but they didn't call it that. Chaos is the name of the game (Glieck 1987). The weather people are slowly coming around to the view that an extremely detailed, almost real time, running analysis of atmospheric conditions is necessary to cope with chaos (Ross 1990). Yesterday's data doesn't do it for even today's site specific forecast (for my favorite stream perhaps). This suggests the way out of some of our problems in wildlife management and research. The problem is that the computer to do this hasn't been produced yet. If and when it is, it should yield new ways to do last minute site specific population and habitat surveillance (data acquisition and analysis). Canny wildlife managers realize that wild creature populations fluctuate widely or disappear for many more reasons than conventional computer models can ever include. Unpredictable, unusual and even bizarre events that cause great changes in wildlife populations are common though each event is unique. You can't play the stock market with last week's quotations. If we are unable to improve at least our near future predictions in nature's chaos I suggest that we at least admit our ineffectiveness and practice the virtue of humorous candor.

There will always be practitioners who will make recommendations on how to precisely counter "environmental impacts" (their jargon not mine) of our insatiable demands for comfort, mobility, energy and growing markets. They are part of the problem, however, not the solution. If we are not sincere enough to investigate our assumptions and logic of science, let's enjoy our weakly effective wildlife research for what it is



without carping erroneous sermons.

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# THE ECOLOGICAL BASIS OF MANAGING FLOW REGIMES FOR RIVER FISH

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## 1. Introduction

About one out of every three species or subspecies of North American freshwater fish is endangered, threatened, or deserving of special concern (Williams et al., 1989, cited in Allan and Flecker, 1993). In the U.S., 34 % of fish species are as classed as rare to extinct, whilst 20 % of the world's freshwater fish can be conservatively classed as extinct or in serious decline (page 59 in Naiman et al., 1995). If evolutionary units (populations that maintain their identity at appropriate temporal and spatial scales, within a unique evolutionary lineage; Nielsen, 1995) are considered instead of species, these figures would be much higher. Consider for instance, the multitude of life-history forms in Pacific salmon species (what we usually call "salmon runs").

Allan and Flecker (1993) propose that the following six factors are of critical importance for biodiversity in lotic environments: habitat loss and degradation, spread of exotic species, overexploitation, secondary extinctions, chemical and organic pollution, and climate change. In their study of North American freshwater fish extinctions, Miller et al. (1989; cited in Allan and Flecker, 1993) found that habitat loss and species introductions were the main culprits, involved in 73 and 68 % of all cases, respectively. It is interesting to note that altered flow régimes usually lead directly to habitat loss, and can indirectly affect native species by favouring the dispersal of exotics.

Fish habitat in rivers and streams is altered, lost, or degraded by many different factors, such as land-use changes (e.g., disforestation for agriculture). Here, we will focus on the effects of altered flow régimes due to dams, diversions, and channelisation works, which appear to have impacted river fish more than any other human activity.

## 2. Common impacts of hydraulic works

Large-scale hydraulic works cause a suite of impacts on the hydrology, morphology, and ecology of a river system (for reviews on this subject, see Petts, 1984; Brookes, 1988; Gore and Petts, 1989; Collier et al., 1996). Two general effects have been found worldwide (Stanford et al., 1996):

i.) Habitat diversity is substantially reduced: Flow and sediment régimes are altered, so that the fluvial processes that create heterogeneous channel and habitat patches are changed. The longitudinal connectivity is interrupted by barriers and by dewatering.



Seasonal flow variability is reduced, but discharges can fluctuate at shorter time-scales. The natural temperature régime is lost. Channelisation procedures and constant flows disconnect the wetted channel from its floodplain, altering baseflow/groundwater interactions, degrading riparian habitats, impeding seasonal floodplain inundation, and creating an homogeneous wetted channel. The lack of flooding allows woody vegetation to encroach upon the once-active channel, and the riparian zone becomes then less diverse. Summarising, these projects create discontinuities along all three spatial dimensions of a river system, and homogenise channel and floodplain habitat, to the detriment of native biota.

ii.) Native diversity decreases whilst exotic species proliferate: The altered hydrologic, sediment, and temperature régimes do not provide adequate conditions for most native species, adapted to the natural régimes. On the other hand, homogenisation of habitats allows exotics to compete better (or to compete at all). For example, some desert fish are adapted to extreme flow and temperature régimes, where no exotic generalist species could survive (Minckley and Meffe, 1987), but if the flow is regulated by a dam, then the non-native can outcompete the native species (Edwards, 1978).

Of course, the right course of action would be to restore the natural flow régime (Poff et al., 1997), but this is difficult, if not impossible for some types of hydraulic works. The changes in the natural hydrologic régime depend very much on the nature and operation of the project. The most common are: constant subtraction or addition of flow due to diversions, decreased peak flows and increased low-flows due to regulating dams, and fluctuating flows due to hydropeaking.

### **3. Some fish adaptations to natural flow régimes**

Some species have evolved quite specialised strategies for spawning during flooding stages, for example, over flooded meadow grasses or in the inundation forest ( see chapter 8 in Petts, 1984). Other species depend on the flooded forest for food (Goulding, 1980).

Salmonid species have evolved to local hydrologic conditions, so that spawning and emergence are timed to minimise entrainment of eggs and larvae by floods. This could explain the failure or success of particular trout introductions around the world. The lack of high, flushing flows can result in sedimentation of spawning beds, but ill-timed flooding can scour the bed material, entraining eggs or larvae. Fluctuating flows can result in severe mortality due to stranding of individuals, even those of larger sizes.

Discharge also acts as a migration stimulus for many anadromous and potamodromous fishes. Sometimes though, "creating" floods by releasing water from dams does not elicit movement, so that factors other than flow must be involved (Trépanier et al., 1996).



#### **4. Controlling factors of fish distribution and abundance**

Orth (1987) proposes that river and fish ecology should be taken into account when developing and applying instream flow-habitat models. He reviews the ecological factors that control fish populations in streams. These are energy source (food), water quality, temperature régime, physical habitat structure, flow régime, and biotic interactions. It is important to realise that these are definitely not independent !

Another way of looking at the impacts of river regulation on fish is through the hierarchical framework of causation proposed by Petts (1984). This further illustrates the complexity of the river system, even before the lateral and vertical dimensions of the fluvial system are considered (Petts and Amoros, 1996).

#### **5. Crash course in instream flows methods**

Jowett (1997) reviews the different types of instream flow methodologies. These can be somewhat arbitrarily classified as hydrologic (or historic) methods, that only consider historic hydrologic records; hydraulic methods, that only look at the hydraulic characteristics (depth, width, wetted perimeter, etc) of stream cross-sections; and habitat methods, that attempt to estimate the availability of physical habitat for a given life-stage of a given species.

#### **6. Can we prescribe flow regimes for river fish?**

There have been numerous criticisms of instream flow methods, including "state of the art" habitat methods such as PHABSIM (Orth, 1987; Castleberry et al., 1996). Indeed, it has been found for the vast majority of cases, that habitat availability is not correlated, or is even negatively correlated with fish abundance.

A big concern is the choice of "target species". How can we determine an adequate flow régime for the whole community? And for the whole ecosystem?

Moyle et al. (1998) used qualitative models to determine an instream flow régime for Putah Creek, and did manage to convince at least one judge, without needing elaborate computer models of fish habitat. Their strategy was based on requesting four different components of the flow régime: sufficient water to keep a continuous flow to the mouth of the creek, seasonally enhanced flows for spawning and rearing of native fishes, habitat maintenance flows every three to five years (to improve habitat and reduce exotics), and seasonally enhanced flows for anadromous salmonids. How different would this be from the optimal condition, the natural flow régime?

Do you think it is possible to compromise between conservation of native species and maintenance of recreational fisheries?



How important are biotic influences on fish community structure? When could one expect them to become important or prevail?

Can we prescribe instream flow régimes for fish communities?

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# **The Ecological Basis of River Restoration**

## **1. River Ecology for Hydraulic Engineers**

Claudio I. Meier<sup>1</sup>, Student Member, ASCE

### ***Abstract***

Knowledge of the basic concepts of river ecology is fundamental to understanding the nature of human impacts on river systems and articulating sound restoration strategies. This article reviews the fundamental elements and processes that structure fluvial ecosystems: the physical setting, living organisms, and inputs and flow of energy. It also highlights those factors that maintain a high heterogeneity of habitats and ecological connectivity, resulting in higher biological diversity and productivity.

### ***Introduction***

The case for river restoration should be self-evident, considering the widespread degradation of river systems due to dams, pollution, over-exploitation of species, water diversions, intensive land-use, channelisation and floodplain development, introduction of exotic species, etc. Because of these and other anthropogenic changes, a large proportion of freshwater organisms is extinct or imperilled (Karr, 1996), and the ecological, recreational, and aesthetic value of many running waters has been reduced. As a response to this damage, river restoration (renaturalisation, rehabilitation) plans are proposed and carried out, in the hope of revitalising fluvial ecosystems.

However, most projects focus on particular organisms or on single physical characteristics of streams, and have relatively narrow goals, without due regard for ecosystem-wide processes and overall biodiversity. For example, procedures as diverse as enhancing physical habitat for trout or stabilising river banks are routinely referred to as "river restoration". Even though these activities could, sometimes, be part of a successful restoration project, they should not be confused with the concept itself.

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Thus, there is no clear agreement on what river restoration means, or what its objectives should be. I propose that there is a serious need for defining "river restoration" based on a holistic consideration of a river as an ecosystem.

In this article, I present fundamental concepts of river ecology that are needed to understand the different types of impact on river ecosystems and to articulate a coherent, ecologically-based definition of river restoration. A companion paper (Meier, 1998) deals with these impacts and attempts to define river restoration from an ecological perspective. Both papers are addressed to an audience of hydraulic engineers and hydrologists, because these professionals are usually charged with managing rivers, and should also be involved in any river restoration interdisciplinary team. Readers are referred to Jeffries and Mills (1995) for a basic introduction to stream ecology. Allan (1995) provides an exhaustive review of this interesting discipline.

### *The natural river ecosystem*

A river ecosystem consists of many interacting organisms of different species (the biota) living in a physical setting (the abiotic environment). These organisms need an energy source (e.g., food to stay alive, grow, and reproduce) and a place to live in the physical environment (a habitat). They also interact with each other (biotic interactions), for example through predation (either as prey or predator) and competition (fighting for limiting resources, such as space or food).

### *The physical environment*

A river environment is the result of physical, chemical, and biological processes occurring in the catchment basin over a range of time-scales. Climatic factors (mainly precipitation and temperature régimes), acting over the basin's geology, determine the landscape, the character of the soil, and the type of vegetational cover, or absence thereof (Morisawa, 1985). In turn, all of these control the discharge régime (hydrology) of the river, together with its inorganic sediment load (silt, sand, gravel, etc.), organic sediment detritus (leaves, twigs, large woody debris, etc.) commonly referred to as particulate organic matter (POM), dissolved matter fluxes (solutes), and stream temperature régime.

In alluvial streams, the overall channel morphology is determined by the discharge and sediment load. In any given reach, the hydraulics of the flow, and the load entering the reach interact with bank and bed materials and with the riparian vegetation. The resulting morphology of the reach (width, depth, slope, channel pattern, etc.) is a balance between erosional and depositional processes (Morisawa, 1985).

In unaltered river systems, these processes create a complex environment which is highly heterogeneous, both spatially and temporally. This changing mosaic of channel and floodplain structures provides habitat for many different species of plants and animals, both aquatic and riparian, whose life-cycles have evolved in response to the highly dynamic and heterogeneous environment (Stanford et al., 1996).



Because different species, and life-stages of a given species, have different environmental requirements, any change in the physical environment or its dynamics can result in changes in the composition of the biological community. For example, many insect species are eradicated downstream of large reservoirs, because of deep releases that result in unnaturally warm waters during winter. The current view (Stanford et al., 1996) is that the community structure (the distribution and abundance of animals and plants) of flood-prone rivers is mainly determined by physical processes, not biotic interactions.

### *The diversity of organisms*

Streams and rivers contain a high diversity of plant, invertebrate, and vertebrate species. Most plant diversity is in the diatoms and other microscopic algae that grow in a thin layer around wetted surfaces exposed to sunlight, such as cobbles in the stream bed, or the stems and leaves of aquatic plants. This growth is generically known as periphyton; it causes the stones at the bottom of any clear-water river to be slippery. There are hundreds of species of these microalgae in a stream (Allan and Flecker, 1993).

Aquatic macrophytes, the larger plants of running waters, can be flowering plants (such as watercress and reeds), mosses and liverworts (bryophytes), or large algal species (e.g., mats of filamentous algae). Headwater streams may contain only mosses, with diversity increasing downstream, as slow water habitats become more common. If conditions are adequate, there can be up to 20 macrophyte species in a reach (Allan and Flecker, 1993).

Invertebrate diversity is made up of aquatic insects (e.g., the mayflies, stoneflies, and caddisflies dear to the fly fisherman), various worms, crustaceans (such as scuds), clams, and snails. Minute worms and midges usually make up most of the many hundreds, or even thousands of invertebrate species that one can find in a stream reach. Most of these species live on or in the stream bed, and are thus generically known as the benthos.

There are three main food categories for freshwater invertebrates: detritus (particulate organic matter, or POM), periphyton, and prey (other invertebrates or fish fry). Most species have evolved feeding adaptations to exploit only one type of food resource, and can thus be classified into functional feeding groups: shredders feed on coarse detritus (CPOM, which is mainly vegetal litter imported from the riparian zone); collectors feed on fine POM (arbitrarily defined as organic particles less than 1 mm in size), either by filtering the water (as clams and net-building insect larvae do) or gathering from surface deposits; scrapers, also called grazers, feed on periphyton; predators such as dragonfly larvae are carnivores that hunt for their prey.

Classifying species into functional feeding groups can help in predicting the impacts of alterations, because most anthropogenic changes in rivers alter the dynamics of food resource inputs in relatively predictable ways. For example, the deposition of washload behind a dam changes a naturally turbid river into a clear-water stream, allowing for periphyton production at the stream bottom. This increases the food source for scraping (grazing) species, commonly resulting in a higher production of invertebrates and fish.



Fishes are the charismatic megafauna of freshwaters because of their economic, recreational and aesthetic value. They are indicators of the health of a river. The majority of fishes eat invertebrates or other fish, and are thus at the top of the stream foodweb.

Because of the nature of river networks, freshwater fishes become segregated in drainage basins, giving rise to genetically distinct populations or races with evolutionary time. In the case of anadromous (sea-going) fish, a river can have different runs, i.e., populations that can be distinguished by the timing of their spawning run from the ocean. For example, the Columbia River had three main groups of returning chinook salmon: spring, summer, and fall-run fish, in a host of local races, adapted to each of the different tributaries. Most of these went extinct when large dams were built on the main stem.

Many animal and plant species exhibit this type of variability in different ways. Thus, conserving biodiversity implies far more than just saving a given species from extinction. Sub-species and local populations with distinct adaptations must also be considered.

### *The energy sources*

The food for all organisms is organic matter containing stored chemical energy that ultimately comes from the sun. It can enter the aquatic ecosystem along two different pathways. Some of it is produced in the stream itself, as periphyton, macrophytes, and other plants convert dissolved inorganic nutrients into organic matter, thus storing sunlight. This plant material photosynthesized in-situ is known as autochthonous organic matter, and most of its biomass is in the periphyton layer surrounding the stream bed.

The allochthonous organic matter is material photosynthesized in the surrounding terrestrial or riparian ecosystems, that is subsequently imported into the aquatic system. Dead leaves, twigs, boughs, etc. represent allochthonous organic matter inputs.

The river continuum concept (see Allan, 1995, pp. 276-281), is a theory that explains changes in energy inputs and community structure along the longitudinal direction of temperate streams. Figure 1 shows a generalised river stem, with stream order and width increasing from its headwaters to its mouth. The upper reaches are light-limited because of shading by riparian vegetation, so that production in the stream is small as compared to import of terrestrial organic matter (litter). Shredders and collectors, that feed on coarse and fine POM, respectively, dominate the community of invertebrates.

Mid-sized rivers are usually shallow, clear, and wide enough for riparian shading to be reduced, favouring in-situ photosynthesis. The importance of terrestrial inputs of CPOM decreases, and now fine POM is imported from upstream reaches. As a result, invertebrate functional groups are dominated by grazing and collecting species.

Further downstream, large rivers tend to become too deep and turbid to sustain photosynthesis. Most organic matter is FPOM that has drifted from upstream, so that the majority of benthic species are collectors, filtering the water or gathering from deposits.



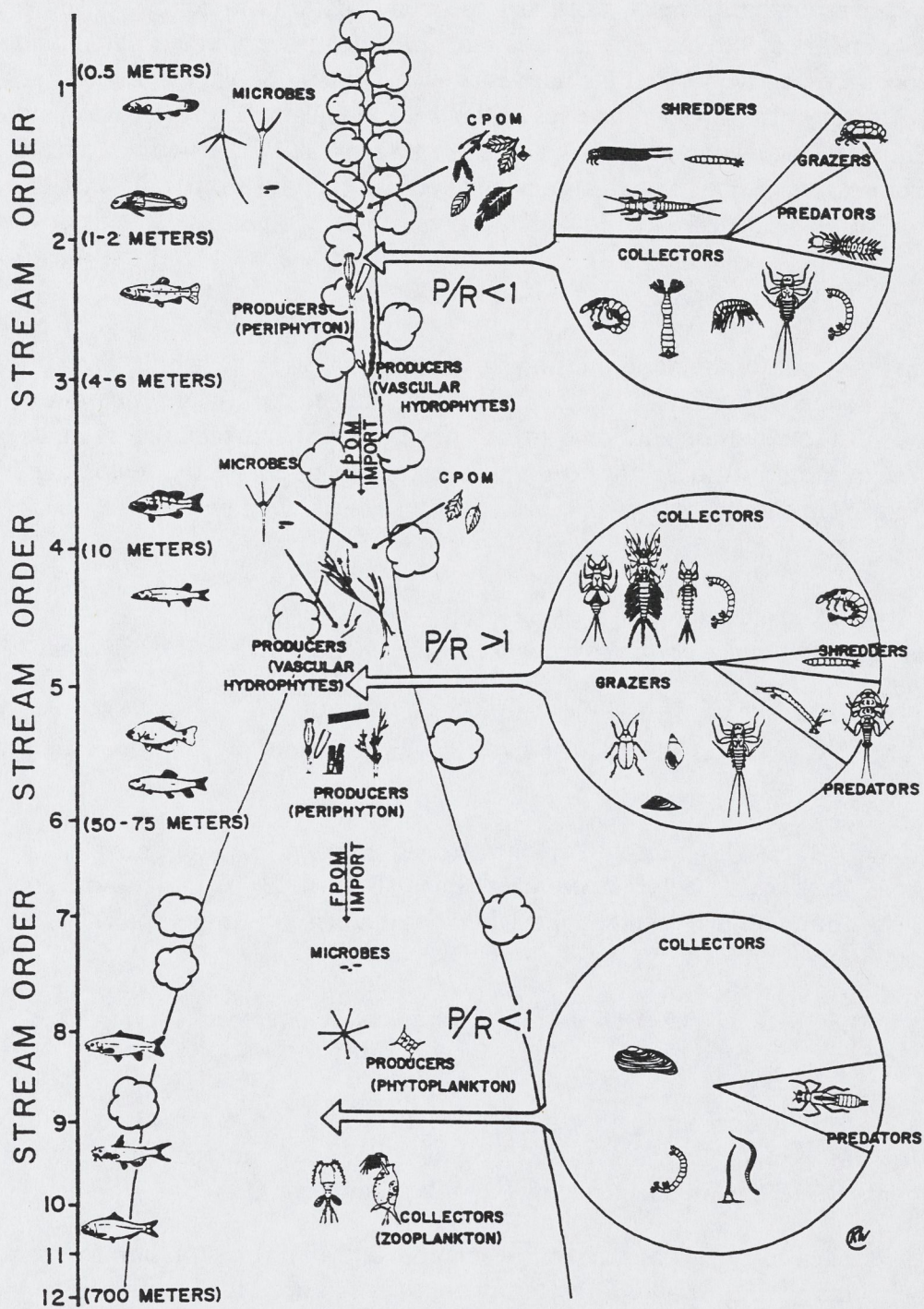


Figure 1. Pictorial representation of the river continuum (from Cummins, 1975)



### *Connections within the fluvial system*

A river ecosystem encompasses much more than the wetted part of the flowing channel. It has diffuse boundaries with the terrestrial and groundwater systems (the riparian and hyporheic zones, respectively), and includes bars, side-arms, floodplain lakes, and all other features created by fluvial processes within the floodplain. These channel and floodplain features change with time. Thus, a river ecosystem can be considered to have three spatial dimensions (longitudinal, lateral, and vertical) that are temporally variable (Stanford et al., 1996). It is essential to maintain connectivity along these dimensions.

### *Summary*

Biological diversity and productivity in running waters are a result of habitat diversity and ecological connectivity. These are created and maintained by fluvial processes of erosion and deposition, that depend on a stream's hydrological and sediment regimes. Changes in the dynamics of these physical processes, or loss of connectivity along any of the three spatial dimensions of a river, will lead to changes in the aquatic community.

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## **The Ecological Basis of River Restoration**

### **2. Defining Restoration from an Ecological Perspective**

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#### ***Abstract***

The main effects of regulation, channelisation, and diversion works on river ecosystems are a decrease in habitat diversity, and a shift from native to exotic biodiversity. To restore a river is to attempt to bring it back to high levels of ecological integrity - conditions as close as possible to those that unimpaired rivers would have had in the region, taking into account the prevailing socioeconomic, political, and technological constraints. When a river is highly managed, this goal is unrealistic; the objective should then be ecological health - that the river ecosystem provides services to society in a sustainable fashion, without degradation for future use, performing all of its functions properly, with minimal outside care. If the natural flow and water quality régimes are restored, and the channel is reconnected to its floodplain, a river left to itself will regain a high ecological integrity, without need for further intervention

#### ***Introduction***

What does it mean, to restore a river ? Is it necessary to first stabilise degrading reaches in order to restore a stream ? What if the degradation is due to "natural" reasons ? Can a river be restored if we establish some fixed, "optimal" channel shape, without allowing for migration ? What happens if we cannot allow any lateral mobility ? Do we afford sufficient protection to the aquatic biota when setting minimum instream flows ? What occurs to all of the other species when we create habitat for adult trout ? Should river restoration protect charismatic species such as salmon, or whole ecosystems ? ....

These questions are answered in many different, and frequently opposing ways, depending on the objectives and constraints of a particular restoration project, and on

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one's professional background. Thus, there are disagreements and misconceptions regarding the meaning and objectives of river restoration. I propose that river restoration should be defined from the perspective of ecology, the integrative natural science.

This paper is a first step in this direction: an attempt to offer a widely applicable definition of river restoration, on ecological grounds. A companion paper (Meier, 1998) set the stage by introducing the fundamental notions and language of river ecology. Based on this, we present here the most common anthropogenic impacts on river ecosystems. Then, we introduce the important concepts of ecological integrity and ecological health, and go on to propose a definition of river restoration based on them.

A good introduction to the subject of river restoration is the respective chapter in the book by the National Research Council (1992). Calow and Petts (1994), Boon et al. (1992), and Harper and Ferguson (1995) are compilations of contributed chapters that cover in detail most aspects of river management and restoration. Petts and Amoros (1996) provide an integrative vision of ecological change in river systems

### *Impacts on river ecosystems*

Very good restoration results can be had by simply removing the impact-causing factors, without the need for further manipulation. Thus, it is important to understand the most common types of effects on river ecosystems. We are concerned here with the environmental impacts of channelisation (training), regulation, and diversion works. These alter flow and sediment régimes and break off the ecological connectivity along a river's spatial dimensions, decreasing the ability of the fluvial system to sustain the natural biodiversity. The effects of other anthropogenic changes, such as point pollution and overfishing, are usually reversible (with the exception of extinction), and the mitigation strategies are obvious. It must be noted that definitive solutions are hard or even impossible to achieve in the cases of diffuse (non-point) pollution and invasion by exotic species (e.g., the introduction of the parasitic sea lamprey in the Great Lakes).

The effects of dams, diversions, and channelisation works show some striking generalities worldwide (Stanford et al., 1996; Poff et al., 1997; Brookes, 1988; Petts, 1984):

1. *Habitat diversity is substantially reduced.* Flow and sediment régimes are drastically affected, so that the fluvial dynamics that create heterogeneous channel and floodplain habitat patches are altered. The longitudinal connectivity is interrupted by dam barriers. Seasonal flow variability is reduced, but daily discharges can be highly variable. The natural temperature régime is lost. Channelisation procedures disconnect the wetted channel from its floodplain, altering baseflow/groundwater interactions, degrading riparian habitats, impeding seasonal floodplain inundation and creating an homogeneous wetted channel. Dewatering severs the longitudinal dimension and can cause high mortality of aquatic organisms through stranding. The lack of flooding allows vegetation to encroach upon the channel and the riparian zone becomes then less diverse.



In summary, hydraulic works create discontinuities along all of the river's spatial dimensions, and homogenize channel and floodplain habitat conditions, to the detriment of the native biota.

2. *Native diversity decreases while exotic species proliferate.* The altered hydrologic, sediment, and temperature régimes do not provide adequate environmental conditions for most native species. On the other hand, the homogenisation of habitats allows exotics to compete better. For example, some desert fish species are adapted to extreme flow and temperature régimes. They fare well where no exotic species could survive, but if a dam regulates flow conditions, then the non-natives can invade and outcompete the native species, driving them to extinction.

It must be noted that in some cases, productivity can be enhanced by the changes, for example when a highly variable flow régime is regulated into a constant discharge year-round. In this case, a handful of species can reach large population numbers, but this is always matched by a decrease in diversity, due to the extirpation of many other, rarer species, that depended on the temporal variability of the flows, and the associated spatial variability of the habitat, for their survival.

The environmental and ecological impacts of hydraulic works are predictable, in some cases only in a qualitative fashion. Certain impacts can be mitigated if the right design and operation procedures are adopted (see Gore and Petts, 1989, for a review of regulated river management). For example, selective multi-depth withdrawal structures can alleviate water quality problems downstream of dams. Difficult societal decisions can be involved, as is the case when a complete flow régime, including extremes of floodplain inundation and low-flows periods, has to be determined (Poff et al., 1997), or when one wants to allow lateral migration of a river.

### ***Ecological integrity and ecological health***

Many restoration projects are not driven by a general goal, but by narrow "professional-discipline" approaches that place a heavy emphasis on structural solutions and quantification, with little regard for the principles of river ecology. The objectives can be very specific indeed: increasing low-flow habitat for adult brown trout, stabilising slumping banks, setting a minimum instream-flow for a dewatered reach, etc. Certainly, any or all of these procedures could be needed in a particular project, but care must be taken not to confuse the end with the tools.

The general objective of any restoration project should be to improve the ecological conditions in a river, an outstandingly complex natural system. The tools used in this endeavour should be based on sound ecological principles.

What does this vague phrase mean, "improving ecological conditions"? It has often been restated as enhancing productivity and/or biodiversity. It should be clear that greater productivity is a human value that should not be put on natural systems: a coastal



wetland has far higher productivity, but is not better than a mountain meadow or a desert patch; a river with moderate sewage pollution is more productive than one with clean water but we still prefer the latter.

*Biological diversity* (biodiversity) is "the variety and variability among living organisms and the ecological complexes in which they occur...it encompasses different ecosystems, species, genes, and their relative abundances" (OTA, 1987; cited in Angermeier and Karr, 1994). The term is commonly used to mean species diversity, the list of biological species at a location. This is incorrect, as the definition makes it clear that both higher and lower levels of organisation are involved (as was shown in the example about the chinook salmon, in part 1. of this article).

"Enhancing biodiversity" has intuitive appeal. Still, it is not an adequate objective for ecological restoration. If it were, we could argue that a safari park in Nebraska is an ecosystem in "good conditions", or we could attempt to "restore" a pristine ephemeral arroyo by managing for year-round flow and then introducing a lot of exotic species.

Evidently, we cannot pretend to improve a pristine system; it is by definition the best that we can hope for. Also, we feel that giraffes have nothing to do in the Great Plains of North America. This implies that ideas of "naturalness" and "sense of place" are involved in our objective; these are explicit in Karr's (1996) definition of *ecological integrity*:

*"..it refers to the capacity to support and maintain a balanced, integrated, adaptive ecosystem, having the full range of elements (genes, species, assemblages) and processes expected in the natural habitat of a region"*

Ecological integrity reflects the unimpaired, original conditions of an area. It can be understood as an ecosystem's wholeness, including the presence of all appropriate elements and the occurrence of all processes. It refers to conditions under little or no influence from human actions; an ecosystem with high integrity reflects natural evolutionary and biogeographic processes (Angermeier and Karr, 1994). For example, most national parks can be considered to have high ecological integrity.

To assess ecological integrity, one needs to select a benchmark state against which other states can be compared, and a variety of measurable ecological indicators. For example, native biodiversity is an important indicator of ecological integrity. Once a restoration goal (a benchmark state) has been selected, the degree of success can be appraised by comparing measured values of the indicators with values for the benchmark.

The objective of restoring a river to levels of ecological integrity close to the original can be impossible to reach due to economic, social, political or technological constraints. Then, one has to settle for lower levels of integrity. As is the case in Europe, some rivers have been modified for such a long time, or so intensively, that little or nothing "natural" remains about them. In other cases, the system will be continuously managed, e.g., as a series of hydropower reservoirs. At these sites, ecological integrity cannot be the



restoration goal, but one can strive for *ecological health*, defined by Karr (1996) as follows:

*"An ecosystem is healthy when it performs all of its functions normally and properly; it is resilient, able to recover from many stresses, and requires minimal outside care. Ecological health describes the goal for conditions at a site that is managed or otherwise intensively used. Healthy use of a site should not degrade it for future use, or degrade areas beyond the site."*

### **River restoration**

The Oxford Encyclopedic English Dictionary (1991) gives some of the following entries under the word *restore*: "... bring back or attempt to bring back to the original state ... bring back to health ... bring back to a former condition ..". All of these agree quite well with the following proposed definition of river restoration:

*"To restore a river is to attempt to bring it back to as high a level of ecological integrity as possible, taking into account the prevailing socioeconomic, political, and technological constraints. In highly managed rivers, the objective should be to maintain a healthy ecosystem, that is able to meet societal needs in a sustainable manner."*

This definition is ecologically-based; it is site-specific because ecological integrity has to be defined locally; it is culture-specific because the constraints will be different everywhere, as will be the chosen benchmark, reflecting the local values about river ecosystems (this is called normative restoration by Stanford et al., 1996). It allows for incremental approaches, because ecological integrity is not one state, but is defined on a continuous scale. It also allows one to answer all of the questions from the introduction, provided that the specific location and constraints are known.

For example, let us consider the hypothetical case of a once-meandering stream that was straightened and channelised a long time ago. Assume that discharge and water quality are relatively unaltered. We know that a meandering river naturally migrates both laterally and down the valley (Morisawa, 1985), creating a variety of features, such as oxbow lakes, scrolls, bars, etc., that provide habitat for many organisms. If we rebuild the meander morphology but fix the stream's plan form by rip-raping or stabilising the banks in any other form, we will not attain high levels of ecological integrity, but it will be certainly better than a straight trapezoidal canal. It might be that the high price of local land is an insurmountable constraint to allow the river to meander freely. In such conditions, if the stated goals of the restoration project were met (in terms of ecological indicators), this example would be a success.

It is important to stress that, if the natural flow and water quality régimes are restored, and the channel is allowed to reconnect with its floodplain, a river left to itself will regain much of its ecological integrity (Poff et al., 1997; Stanford et al., 1996)



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## BOOK REVIEWS

**Applied River Morphology.** By Dave Rosgen. *Wildland Hydrology, Pagosa Springs, Colorado.* 1996. 380 pages. \$89.95.

Dave Rosgen has probably spent more time with his feet in moving water than any other practicing hydrologist or geomorphologist, and he has drawn on this experience to write *Applied River Morphology*. His channel classification scheme, which is the centerpiece of this book, is already widely used. He has pioneered the restoration of channels to achieve more natural, stable, and habitat-friendly configurations. Hundreds of hydrologists and fishery biologists, particularly from management agencies, have taken his short courses and are already familiar with this material. Stream managers will find this book a useful addition to their bookshelves, which will presumably include more process-oriented geomorphic texts. Fishery biologists will use it as a useful starting point to communicate with hydrologists and geomorphologists.

Rosgen's contribution starts with the well-known principle that channel form is constrained by geology, physiography, and climate, and over short time scales, it adjusts to annual and seasonal variations in runoff and the volume and caliber of sediment supplied from the basin. Certain repeatable channel types characterize similar geomorphic settings, and within this range, substantial disturbances can cause a channel to change to a type that is not typical of its setting under stable watershed conditions. Therefore, stable channels in the same geomorphic settings serve as examples (1) to evaluate deviations in form and process of unstable channels and (2) to design restoration.

This book is now the standard reference for Rosgen's classification. Previous versions have been published, but here the classification is updated, richly illustrated, and explained in full. Rosgen's classification is already widely used, particularly by resource agencies and consultants who actively manage wildland channels and watersheds. To practice in this field today, it is useful to know the Rosgen classification whether you agree with it or not. It provides a frame of reference and appreciation of the variety of natural channel forms, the probable geomorphic settings under which they are likely to be found, and an introduction to their behavior.

The classification is hierarchical. Each successive level requires more time spent in the field to gather information: classification at level I is done mainly

from aerial photos and maps but requires field verification; the greater detail in level II requires field measurements of channel dimensions; level III evaluates channel condition; and level IV suggests measurements of streamflow and sediment transport to support more in-depth analysis of channel problems. At advanced levels (III and IV), the classification becomes less of a classification and more of a structured evaluation of form and process. Thus the classification is integrated into analyses leading to management strategies, including channel restoration.

The classification is taxonomic. Channels necessarily fall into standard ranges of objective, quantitative criteria (e.g., stream gradient, width : depth ratio). There is a slot for every channel, and the classification has proliferated over the years as new channel types have been identified. It covers the full range of channel types and spans Rosgen's experience from around the world, although readers from the Pacific Northwest and other wood-rich environments will find the treatment of large woody debris sketchy. Stream types in level II are based on six variables. Consistently observed combinations of characteristics provide the break points used to stratify the continuum of channel morphologies into discrete, recognizable units. However, in some cases, a variable may fall outside of the range that corresponds to the stream type that is indicated by values of the remaining variables. Although the genetic implications (channel-forming processes) are related to these types, Rosgen's classification differs from more genetic classifications used by geomorphologists (particularly in research) that are based on sedimentologic features, such as presence-absence and types of bars in channels. A taxonomy does not bring everything you need to know to manage a species. Similarly, a channel classification by itself does not provide an understanding of channel processes. Rosgen brings additional information to aid in this understanding, but a treatise on the fluvial geomorphology of stream channels is beyond the scope of this book.

The classification (particularly levels I and II) is well explained and illustrated. However, advanced levels depart from classification and delve into channel processes. Space limits this discussion, and someone starting an analysis of a channel problem may find some explanations brief or vague; scientists may find some of the science misrepresented or outdated; and field practitioners may favor other

methods than those presented here. Thus, the classification works most comprehensively at the coarsest scale (levels I and II) and less so as it progresses through more advanced levels. Rosgen is aware of these limitations and urges the reader to use his guidelines and indices to identify red flags and better define the problem before seeking other literature and aid from other professionals (like geomorphologists!). However, there are not enough references, particularly from recent literature, to adequately help readers find supporting information. Unfortunately, I cannot recommend a book to complement the classification framework provided by Rosgen because geomorphology texts are presently either outdated or too specialized.

Nevertheless, there is some useful information and practical advice in the later chapters that result from three decades of managing fluvial systems and interacting with a variety of professionals (including fishery biologists). The best models of channels with desirable features (stability, habitat) to use when confronting an altered channel are those most similar in valley form, sediment, and gradient in the immediate area. It takes field work to adequately use these examples. Prescribed or desired habitat guidelines such as width : depth ratios and pool spacings are not achievable in all geomorphic settings, even under natural, stable conditions; these attributes vary by channel type. Rosgen is not offering a catalog of designer streams with building instructions. Similarly, a central theme and a source of some useful discussions and tables is the variation of appropriate interpretations of variables, indices, and management strategies with channel type. For example, in an extensive table covering all channel types, he predicts what methods of channel restoration are likely to be appropriate and durable in each channel type (while cautioning that a successful treatment depends on correctly identifying the conditions desired and the watershed processes that influence channel stability).

Although Rosgen is famous for his classification, readers should take note that his approach to channel management is not channel-centric. If a channel is adjusted to water and sediment delivered by the watershed, what is left to do in terms of in-channel restoration? Rosgen reiterates that channel evaluation, protection, and restoration start at the cause of channel change, which is usually found in the watershed. Many channel problems arise from watershed disturbance, and solutions often lie in healing hillslopes. Rosgen provided an example of watershed analysis more than a decade before it became standard procedure. In-channel remedies would

most likely be appropriate for former and engineered channels, mechanical streambanks (e.g., from grazing), and where the channel and valley bottom are the major source of destabilizing sediment. There are pitfalls if restoration acolytes do not see the full scope of messages contained in the example, stable channel types follow shed conditions, but stable channel types under unstable conditions may lead back to channel instability.

I hope that fishery biologists will breach some disciplinary boundaries and professionals managing watersheds and rivers, just as a geomorphologist would, to manage a fish population on the basis of a accessible book, the fishery biologist should seek professional help in managing the physical stream channels.

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**Molecular Systematics of Fishes.** By James D. Kocher and Carol A. Stepien. *San Diego, California.* 1997. \$79.95.

Many scientific books today are collections of papers from multiple authors. The parent effort made to develop coherent chapters. In this volume, Kocher and Stepien made a concerted effort to develop a coherent throughout. This volume surveys the state of molecular methods to address relationships from the level of population genetics to the highest branches of the piscine family tree. The 17 chapters (total of 38 authors) provide reviews of years of effort on an interesting group of fishes. Each chapter provides a thorough discussion of the problem, approaches to a solution, a level of progress permitted in most journal articles. Some are stronger than others, but reading this book will give the reader ample opportunity to see how various authors have reasoned solutions to their particular problem. Those of us who are interested in the field of systematics will find excellent details of principles and practices of employing molecular genetic data.