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Review

Trends in Stream Biodiversity Research since the River Continuum Concept

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Abstract: Lotic environments contain a disproportionate amount of biodiversity given their relatively small proportion of the worldwide landscape. We conducted a systematic literature search of research directed towards understanding factors that influence biodiversity in lotic habitats, published in 31 major ecological and freshwater science journals from 1981 to 2014. Our goal was to characterize emergent themes in research successes and identify important areas in need of study. We show an overwhelming taxonomic bias favoring studies of macroinvertebrates and fish, and a paucity in studies of other important groups such as bacteria and fungi. While most studies assessed habitat variables that affect diversity at a local scale, there has been a recent push to investigate regional drivers of beta and gamma diversity. Several factors were consistently found to be important drivers of diversity including local habitat type, hydrologic variables, disturbance, and stream morphometry. Others such as nutrients and chemical variables showed mixed support. Species interactions, dispersal, and evolutionary processes were rarely considered but show promise as fruitful areas for future study. We suggest that researchers should give increased attention to diversity drivers at different scales as well as take advantage of new molecular techniques to address questions regarding organismal diversity in streams.

Keywords: stream ecology; river; microbial diversity; biodiversity; review; metacommunity

1. Introduction

Understanding the processes and mechanisms that underpin distributions of biodiversity has been a core objective of ecological research since the advent of ecology [1,2]. Historically the quest for this understanding was largely a pursuit of basic knowledge, but curiosity has transformed into necessity as the applied value of biodiversity has become increasingly apparent. Contemporary paradigms such as biodiversity-ecosystem function [3], diversity-stability relationships [4], diversity-productivity relationships [5,6], ecosystem services [7,8], host dilution effects of diseases [9], and many others emphasize that a better understanding of biodiversity is vital for conservation, economics, and human health and welfare [10,11]. As a result, the drive to understand the causes and consequences of biodiversity patterns is stronger than ever in the wake of this expanding motivation that now includes economic concerns and global health implications.

Lotic ecosystems contribute to global biodiversity at a rate that is greatly disproportionate to their physical extent, and rank highly among the more threatened ecosystems on the planet [10,12–14]. Diverse stream and river communities provide obvious services to people including recreation, potable water and food, but also facilitate nutrient cycles and energy flows that connect biological systems from continental interiors to the oceans [15]. Unfortunately, lotic communities are particularly susceptible to anthropogenic destruction as a result of land use change, species introductions, pollution, and physical alterations to stream morphologies such as channelization and dams [10,16]. Thus a comprehensive understanding of the processes that shape and maintain stream biodiversity is critical to maintain valuable ecosystem services in the Anthropocene [17].

The challenge to understand the causes and consequences of biodiversity in rivers and streams has been embraced by many researchers. However, uncovering the natural and anthropogenic forces that shape diversity in riverine systems presents a unique set of challenges for theorists and empiricists [18]. Streams and rivers are speciose and trophically complex, and the most frequently studied communities in these systems—benthic invertebrate communities—are among the most complex and diverse, which contributes to the preponderance of stream studies utilizing macroinvertebrates [18,19]. Lotic ecosystems are also subjected to frequent, often intense, hydrologic disturbances that are both natural and anthropogenic in nature [20,21]. Stream communities are highly variable in space and time and the drivers of this variability are numerous and often interact [22–26]. Additionally, the characteristic dendritic and hierarchical structure of stream networks add complex spatial properties to regional community structure that push the limits of available theory and analytical tools to uncover underlying processes [27,28]. Adding to this complexity is the growing appreciation that multi-scale approaches are often necessary for a workable understanding of the assembly and maintenance of local biodiversity because connections among localities create emergent patterns that transcend local processes [29–32]. A growing number of empirical and theoretical studies reiterate the concept that understanding local biodiversity frequently requires an understanding of processes that operate at regional scales (e.g., [29,33–38]).

Despite these challenges—or perhaps inspired by them—stream ecology has produced a rich body of both empirical and theoretical work describing the patterns of biodiversity in streams, and the processes that underlie those patterns. However, to the best of our knowledge, there has not been a contemporary review of biodiversity research in stream systems. Therefore, our goal is to provide a broad assessment of the questions, approaches, successes, and current opportunities for biodiversity research in streams and rivers. We highlight areas of research that have been well-resolved and, perhaps more importantly, reveal areas of research that are ripe for further exploration. The connectedness of diverse stream and river communities and its relevance for understanding the biotic as well as functional processes has been captured in a number of concepts that aim to predict patterns in river systems [39–42]. As the River Continuum Concept (RCC) was the first paper that attempted to provide a testable hypothesis on the ecosystem changes observed along a river continuum, we therefore used this landmark paper as our starting point and conducted a systematic literature search of research directed towards understanding biodiversity in lotic habitats published in 31 major ecological and freshwater science journals from 1 January 1981 to 1 October 2014. Although the RCC did not directly comment on biodiversity per se, its emphasis on predictable and quantifiable change along a longitudinal river gradient has inspired generations of studies of biodiversity in lotic ecosystems.

Specific goals and questions

(1) *What are the major questions that investigators asked with regard to diversity in lotic systems?* We considered a number of aspects that defined research questions including the focal organism(s), the measured predictors of biodiversity, whether studies were explicitly motivated by investigating anthropogenic impacts on diversity, the way in which diversity was measured, and whether questions explicitly dealt with the relationship between biodiversity and evolution. We also considered the major underlying theories or themes motivating the research.

(2) *At what spatial scales are researchers investigating biodiversity?* We estimated both grain size and extent of studies, as well as whether investigations were explicitly designed as multi-scale studies. Additionally, we examined keywords that are indicative of multi-scale research.

(3) *What proportion of studies had an experimental component?* We were particularly interested in how many studies approached the question of biodiversity using experiments to investigate mechanisms.

(4) *What factors influence biodiversity in lotic systems according to published studies?* Can our current compilation of research on stream biodiversity suggest which commonly considered factors most strongly influence biodiversity patterns?

(5) *How have approaches for studying biodiversity in stream systems changed through time?* For all of the previous questions, we were also interested in temporal shifts in approaches, questions, or results that signify adaptive responses of stream ecologists to emerging theory and available analytical and observational tools.

2. Experimental Section

Methods

To conduct an objective review of studies on biodiversity in lotic ecosystems since the RCC, we searched the Web of Science™ (http://apps.webofknowledge.com/) using the search terms described in Table 1 and produced a list of 690 candidate articles. We further refined the list by excluding articles based on two criteria. First, we excluded studies that did not examine biodiversity in the active stream or river channel. For example, we did not include studies targeting floodplains, riparian wetlands, or with riparian organisms as focal species. Second, we excluded any studies that did not establish a specific response/predictor relationship between at least one metric of biodiversity and at least one predictor variable. We did not require that the relationship be stated explicitly and included implied relationships via reported correlation or inclusion in statistical models. Finally, several studies were excluded because we could not gather sufficient information regarding observed diversity or its causal factors from the published work. We then created a semi-quantitative description of the scope and predictors used to explain biodiversity patterns for each study by populating a database (see Supplementary Materials) using the fields outlined in Table 2 to describe each article. The categories in Table 2 were not necessarily mutually exclusive. For example, studies frequently measured multiple predictors of biodiversity, and many studies that performed experiments also incorporated surveys. We did not perform any statistical operations on the summary data since, to the best of our knowledge, the dataset represents a census of the literature based on our established criteria. For the purposes of this review, α-diversity was defined as local diversity, γ-diversity as diversity with a region, and β-diversity as the turnover between localities. To assess temporal trends in research foci, we binned all papers into 6-year intervals. Binning clearly illustrated long-term temporal trends in research themes while simultaneously smoothing across outlying observations in any particular year.

Table 1. Search criteria for review of stream and river biodiversity literature using the Web of Science™.

Field	Search Term
Title	Stream * OR river *
Topic	biodiversity OR diversity OR richness
Year	1981–2014
Journal	Proceedings of the National Academy of Sciences Nature Science Proceedings of the Royal Society B PeerJ PLOS One PLOS Biology Ecology Letters Trends in Ecology and Evoloution (TREE) Annual Review of Ecology, Evolution, and Systematics Ecology Ecological Applications Ecological Monographs Frontiers in Ecology and Environment Ecosphere Oikos Oecologia The American Naturalist Journal of Ecology Journal of Animal Ecology Ecography Conservation Ecology Conservation Biology

Table 1. *Cont.*

Field	Search Term
Journal	Ecological Entomology Freshwater Science Freshwater Biology Limnology and Oceanography Canadian Journal of Fisheries and Aquatic Science J. of Aquatic Sci. Archiv für Hydrobiologie Hydrobiologia

Table 2. Descriptors used to summarize studies of biodiversity in streams and rivers in literature review (Supplementary Materials).

Field Type	Field	Field Value	Field Description
Study descriptors	Author	Text (e.g., "Allan")	Author surname(s)
	Year	Numeric (e.g., 1990)	Publication year
	Response	Text (e.g., alpha, beta, gamma)	Metrics used to quantify stream or river biodiversity and used as response variables in the study
	Grain Size (m)	>0	Approximate size of the unit of inference
	Extent (km)	>0	Approximate study extent (*i.e.*, furthest distance separating two observations)
	Survey?	Binary	Was the study a survey?
	Experiment?	Binary	Was the study an experiment?
	Evolution?	Binary	Did the study address evolution as a driver of biodiversity?
	Multiscale?	Binary	Did the study explore multiscale issues in biodiversity (e.g., local *vs.* regional controls)?
	Anthropogenic?	Binary	Did the study focus on human influences over biodiversity patterns (e.g., climate change, mining)?
	Metacommunity?	Binary	Did the study mention the term "metacommunity"?
	At least 1 of the "Big 4" metacommunity paradigms mentioned?	Binary	Did the study refer to any of the four metacommunity paradigms described by Leibold *et al.* (2004)?
	Organism?	Text (e.g., "macroinvertebrate")	Type of organisms for which biodiversity was quantified

Table 2. *Cont.*

Field Type	Field	Field Value	Field Description
Predictors of biodiversity	Theme	Text	A brief description of the study theme
	Chemical	{0, 1, 2} *	Water chemistry variables (e.g., pH, conductivity, non-nutrient solute concentrations)
	Disturbance	{0, 1, 2} *	Was disturbance quantified to predict biodiversity? (e.g., heavy metal concentrations caused by mining operations)
	Hydrologic	{0, 1, 2} *	Measures of local hydrology (e.g., discharge, hydroperiod)
	Land use/cover	{0, 1, 2} *	Land cover variables (e.g., land use categories quantified using GIS)
	Local Habitat	{0, 1, 2} *	Physical reach scale variables (e.g., temperature, substrate complexity)
	Macro-scale stream morphology	{0, 1, 2} *	Broad scale descriptions of stream, river, or catchment (e.g., stream order, catchment size)
	Nutrients	{0, 1, 2} *	Nutrient concentrations or availability (e.g., N or P concentrations, nutrient diffusing substrates)
	Species interactions-INTRAguild	{0, 1, 2} *	Were intra-guild biotic interactions invoked to describe biodiversity (e.g., competitive exclusion)?
	Species interactions-INTERguild	{0, 1, 2} *	Were inter-guild biotic interactions invoked to describe biodiversity (e.g., food availability, predation)
	Other	{0, 1, 2} *	Other predictors of biodiversity not described above

Note: * Fields representing predictor variables were scores using a 0, 1, or 2; where a score of 0 indicates no information was reported; a score of 1 indicates information was reported, but not found to be an important predictor of biodiversity; and 2 indicates information was reported and the variable was an important predictor of biodiversity.

3. Results and Discussion

We retained and evaluated 326 papers from our original literature search. For a complete list of the papers we retained see Supplementary Documentation. While there was a diverse range of focal organisms in biodiversity research in lotic systems overall, macroinvertebrates were overwhelmingly the most frequently investigated group (Figure 1). There was also a strong focus on fish (19%), and plants represented 12% of studies. Cumulatively, these 3 general taxonomic groups represented 94% of the total studies reviewed. While some studies investigated fungi, protists, rotifers, bacteria, and amphibians, they represented a slim minority of studies. We did not observe any trends in the choice of study organism as a function of time.

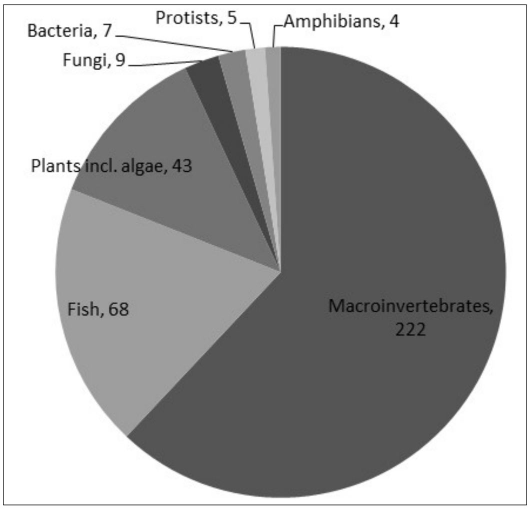

Figure 1. Study organisms represented by stream biodiversity publications over the last 34 years.

We found varying amounts of attention and support for commonly measured predictors of biodiversity. Local habitat was the most commonly reported predictor variable as well as the most frequent significant predictor of biodiversity (Figure 2). Hydrologic variables, macro-scale stream morphology, and disturbance were the next most commonly reported predictor variables. For most predictors, the large majority of studies considering that predictor found a significant effect, with the exceptions of chemical and nutrient variables (Figure 2).

Investigations into the relative numbers of papers examining natural or anthropogenic drivers of diversity showed that natural drivers of diversity were more common. Sixty-nine percent of papers examined natural drivers of diversity (Figure 3). In contrast, 31% of papers examined how anthropogenic aspects of streams can influence biodiversity.

Stream diversity studies were largely focused on explaining local-scale diversity (α-diversity), but recent trends suggest in increased attention towards regional diversity (γ-diversity) and species turnover (β-diversity). α-diversity was by far the most common measure of biodiversity with 87% of all published studies measuring or explaining diversity at a single location (Figure 3a). The frequency of studies focusing on α-diversity remained relatively constant through the 34-year period with 97%–100% of studies in each time period focusing on α (Figure 3b). Ten percent of studies measured β-diversity, while only 3% investigated γ-diversity. Proportions of investigations measuring β-diversity have increased markedly over the last 15 years with 10%–15% of published studies including some measure of β during the last 15 years while only one study explicitly investigated β prior to 1999 (Figure 3c). Similarly, the focus on γ-diversity has increased over the last 20 years, rising from no published studies in the late 1980s and early 1990s to 7% of papers in the most recent 2009–2014 time period (Figure 3d).

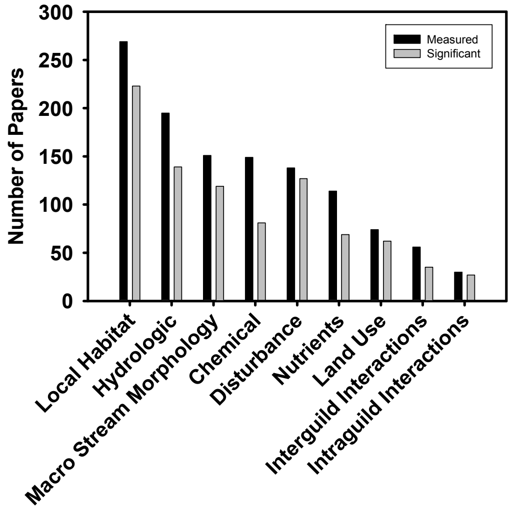

Figure 2. Dark bars represent the number of studies in which each predictor variable was measured or manipulated; while grey bars represent the number of times a predictor was found to have an effect on biodiversity.

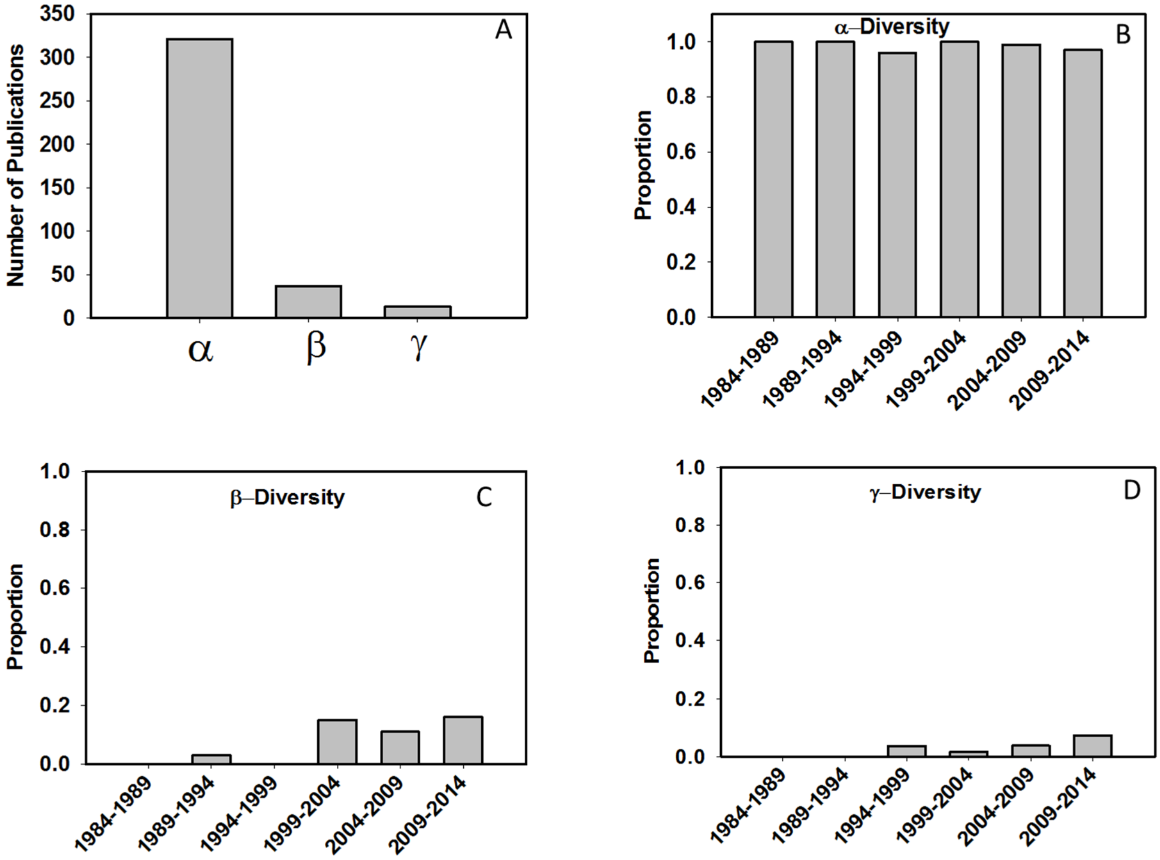

Figure 3. (**A**) The number of publications in which α, β, and γ diversity were the focal points of studies; (**B–D**) The proportion of papers as a function of time that examined (**B**) α-diversity; (**C**) β-diversity; and (**D**) γ-diversity.

Evolutionary studies of stream biodiversity have remained relatively rare throughout the past decades, but showed a punctuated decline throughout the 1990s and subsequent resurgence in recent years. Studies in which evolution was invoked as a causative agent to explain patterns of lotic diversity made up approximately 12% of papers in the mid to late 1980s and dropped to less than 4% in the early 2000s and rose again to around 11% in recent years (Figure 4).

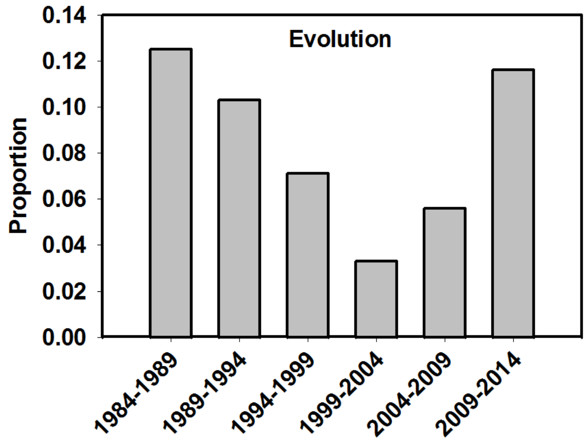

Figure 4. Proportion of studies utilizing evolution to explain biodiversity.

Papers utilizing a multiscale approach to explain stream biodiversity have steadily increased from around 12% to slightly over 40% over the 34 year time period we examined (Figure 5a). As evidence of this growing emphasis on multiscale phenomena, studies invoking metacommunity theory have increased to 10% in the most recent 2009–2014 time period. The preceding time periods contained only one publication each that mentioned "metacommunity" (Figure 5b). Usage of the four metacommunity paradigms (*i.e.*, species sorting, mass effects, patch dynamics, and neutral theory) has been steadily increasing over the 20 years (Figure 5c). There was also a pronounced period in the early 1990s in which the patch dynamics paradigm was the focus of several studies.

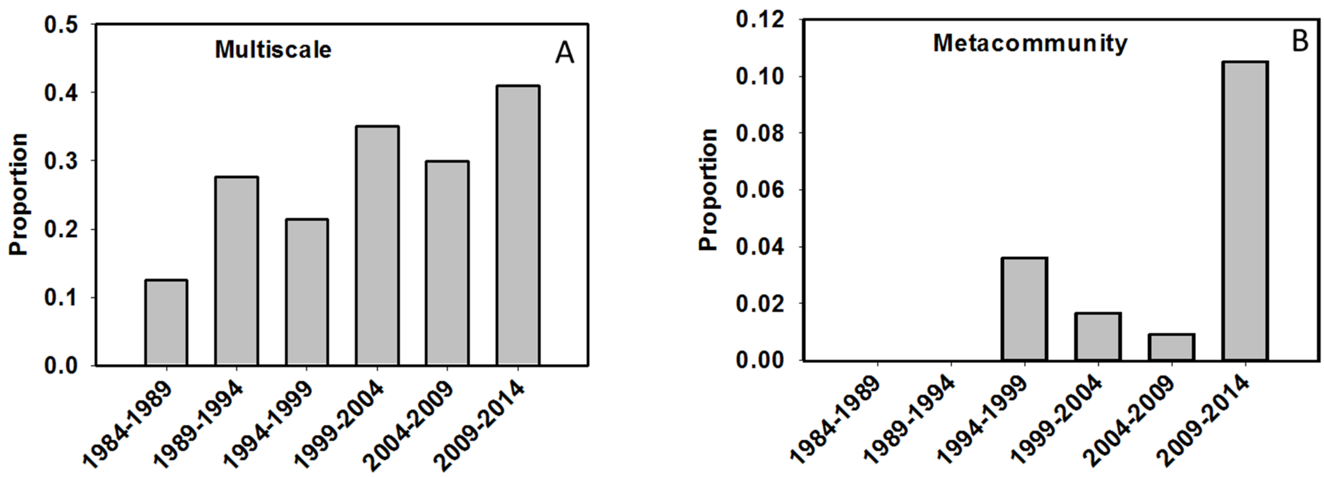

Figure 5. *Cont.*

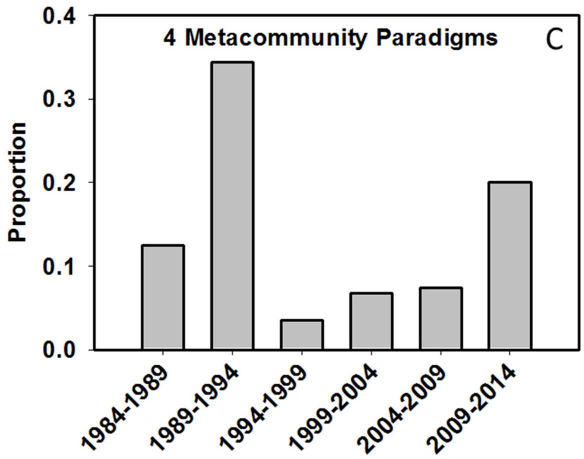

Figure 5. (**a**) Published studies examining diversity at multiple scales; (**b**) Studies specifically mentioning metacommunity theory; (**c**) Published studies specifically mentioning one of the four major metacommunity paradigms (mass effects, patch dynamics, neutral theory, and species sorting).

Human disturbances including mining activity, land use changes, pollution, and dams were the most common theme with regards to investigations of stream biodiversity (Figure 6). Habitat type and associated manipulations of habitat was the second most common theme, followed by natural disturbances like floods and droughts. Dispersal of organisms and connectivity within stream networks was a theme in over 20% of studies, while species interactions, trophic studies, bioassessment and investigations of habitat template frameworks constituted a minority of studies.

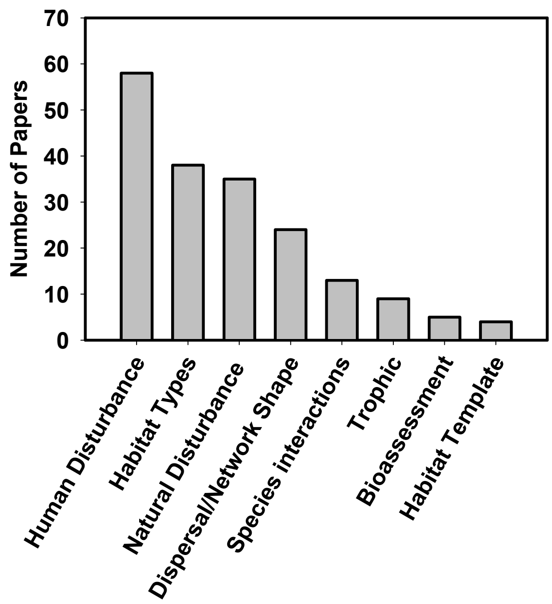

Figure 6. The number of papers investigating a particular theme regarding biodiversity in streams.

Surveys made up 80% of investigations with 16% being experiments and 4% utilizing surveys in conjunction with experiments. Four percent of studies performed surveys in addition to experiments. No trends over time were observed for the proportions of survey-based and experimental studies.

The extent of studies has remained fairly constant over the 34-year period with the exception of a sharp increase in the last five years. Grain size, *i.e.*, the level of inference, increased sharply from the mid-1980s to the late-1990s, after which it has remained fairly constant at a median size of 25–30 m (Figure 7).

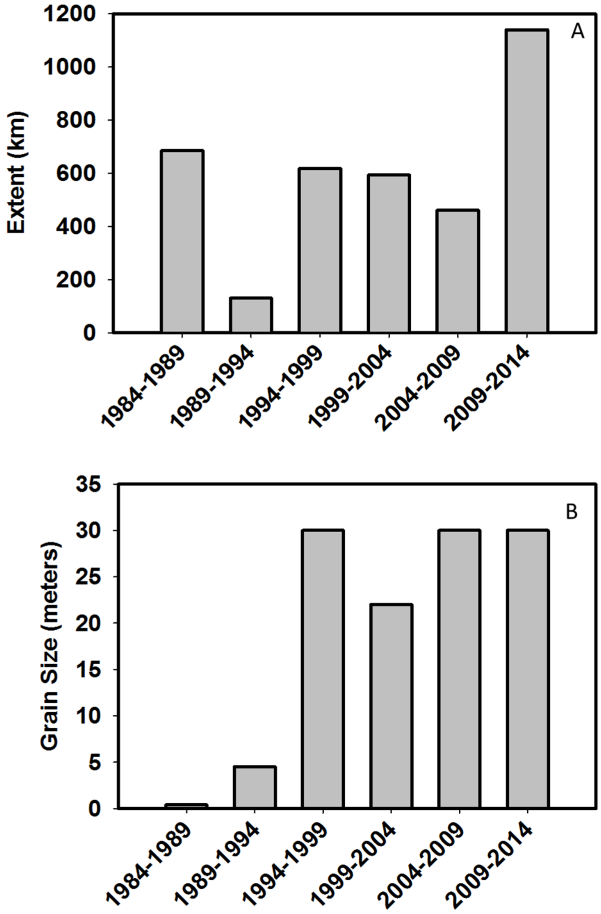

Figure 7. The median (**A**) extent and (**B**) grain size of published studies in each time period.

4. Discussion

Our review of published studies investigating lotic biodiversity since the publication of the River Continuum Concept (RCC) [39] in 1980 revealed an enormous diversity of approaches and questions. Despite wide variation, certain patterns and temporal trends emerged from our investigation. We found that most studies of lotic diversity can be typified as a survey of the alpha diversity of benthic macroinvertebrates, conducted at the reach level (20–40 m), and with a focus on how local habitat and hydrology affected biodiversity. However, there was significant variation around that central theme and, perhaps more significantly, many of those general themes have changed through time. Highlighting these trends can serve multiple purposes: They are both a history lesson, revealing where stream ecology has been, and a potential guide for the future, suggesting places that stream ecology could, and perhaps should go. To these ends, we interpreted the results of our review as responses to the 5 questions we posed in the Introduction.

4.1. What are the Major Questions that Investigators Asked with Regard to Diversity in Lotic Systems?

Some of the trends from this review will come as no surprise to most stream ecologists, and perhaps none is less surprising than the overwhelming focus on benthic macroinvertebrates with a secondary, but still strong focus on fish. Heino, *et al*. [43] found a similar result. Both of these taxa represent significant applied interests that motivate many studies; both taxa are conspicuous, fish are food and sport, and benthic invertebrates are indicators of water quality. Additionally, the overwhelming focus on benthic invertebrates is likely related to several qualities that recommend them as study organisms. They are diverse and numerous, yet they are macroscopic, require only modest equipment for Order or Family level identification, and even casual observation reveals a wealth of interesting natural history. Many aspects of their habitat occur on the scale of a few meters, allowing meaningful studies to be conducted in relatively small areas. Additionally, their study usually requires little administrative paperwork at most institutions, unlike fish, which often require significant effort in the form of animal care and use protocols.

However, one result with regard to focal organisms that we found surprising was the lack of a noticeable temporal trend, particularly with respect to microorganisms. Investigating the biodiversity of bacteria, archaea, and fungi has become practical and affordable with the rapid advancement of sequencing technology, and studies of these organisms have become a prominent and growing part of ecological literature over the past decade [44]. Furthermore, bacteria and fungi are critical components of stream ecosystems that are absolutely necessary to nutrient cycles and form the base of most lotic food webs. However, we did not see a recent increase of microbial studies in our review, suggesting that for some reason, the study of microorganisms in lotic systems has not followed this same trajectory as in ecology as a whole.

There were some interesting trends regarding the major predictors of biodiversity used by lotic biodiversity studies (Figure 2). The results suggest that overwhelmingly researchers attempt to explain biodiversity using abiotic parameters rather than biotic parameters. The top four most frequently examined categories of predictors—local habitat, hydrologic variables, macro-scale stream morphology, and chemical parameters—are all unquestionably abiotic in nature. Of the next three most common predictors, disturbance and land-use could be loosely interpreted as biotic given the frequent human element and accompanying variation in riparian and watershed vegetation, but only in the most inclusive and indirect of definitions. Of the categories we tracked, the only predictors explicitly devoted to biotic interactions were the two least often employed predictors. Again, this trend *appears* to differ markedly from the general discipline of ecology in which biotic interactions play a very prominent role in biodiversity studies. For instance, studies of community ecology and biodiversity outside of streams have had a decades-long fixation with competition and predator/prey interactions [45]; however, we cannot definitively make this conclusion since we have not performed a similar investigation for ecology as a whole. What is clear is that stream ecologists have tended to shy away from investigating biotic drivers of diversity.

A comparison between significant predictors and measured predictors revealed two additional interesting results (Figure 2). The first point of interest is the strong congruence between measured parameters and significant parameters; generally if a study reported measuring a predictor, then that predictor was highly likely to show a significant effect on biodiversity. For some predictors, like disturbance, the effect rate was over 90%. There are multiple potential explanations for this high level of congruence. One possible explanation is a bias in what researchers report in publications. There is a well-documented bias towards positive results in general [46], and it is likely that researchers also have a tendency to omit predictors that were considered non-significant. However, a more positive potential explanation is that stream ecologists are simply good scientists. The first step in the scientific method is observation, whether those observations come from our own personal field observations or from respecting previously published results, and the goal of those observations is the design of studies that are likely to produce results. So perhaps the high congruence between measured and significant predictors is a mark of effectively applying the scientific method? In reality, both explanations likely play a role. A second, more subtle point of interest in the comparison of measured *vs.* significant predictors is that not all predictors have the same rate of success. In particular, chemical and nutrients had low rates of predictive success relative to other predictors. The explanation for the pattern in these predictors seems straightforward: they are easily measured parameters that are often estimated as a matter of course in stream studies, often without respect to a particular prediction or hypothesis, so it is not surprising that they are frequently not good predictors of biodiversity. This observation also indirectly supports the Good Science explanation for high congruence between measured and significant for many parameters posed above.

One somewhat alarming result was that very few studies employed evolution in explanations of lotic biodiversity (Figure 4). Dobzhansky famously stated, “Nothing in biology makes sense except in the light of evolution”, yet less than 10% of studies appealed to our most foundational of biological sciences with regard to its potential implications for biodiversity. We should add that our threshold to give a study credit for the “evolution” category was not high. The focus of the study did not have to specifically be evolutionary biology; the study had only to invoke evolutionary explanations for results in order to be counted in this category. Nevertheless, few studies surpassed even this low bar. The reasons for this result are likely several and complex. One explanation is that stream ecologists are interested in processes that operate at ecological, not evolutionary time scales. Another contributing factor is that there has long been a divide between evolutionary biologists and ecologists, which has resulted in general evolution-phobia among many ecologists who see evolutionary biology as foreign territory, but other practical reasons are also likely motivators [47]. With regard to conducting actual evolutionary research in lotic systems, the disturbance prone stream environment combined with multi-year life cycles makes long term experiments difficult, and while evolution can certainly be detected on the time-scale of a three-year grant in fast reproducing bacteria and protists, evolution of non-microbes is unlikely to be detected on a limited time-scale, perhaps making lotic systems unappealing for testing evolutionary theory. However, with regard to the last point, we would argue that many aspects of the lotic environment actually recommend its use as an evolutionary study system, particularly with respect to the influences of disturbance regimes and dispersal within river networks [48,49].

4.2. At What Spatial Scales are Researchers Investigating Biodiversity?

Scale is an ever-present issue in ecology, argued by some to be the central problem or question of ecology [50], and several investigators have urged an increased focus on scale in ecology in general [51], but also specifically in stream ecology ([52]). Our investigation addressed the question of scale in 4 ways: the physical scale at which biodiversity was actually measured (*i.e.*, grain and extent), the conceptual way in which biodiversity was measured (*i.e.*, local diversity, regional diversity or turnover), whether a study was explicitly designed as a multi-scale study, and by searching for particular keywords in studies that indicated a multi-scale approach.

The grain size of studies investigating stream biodiversity has remained relatively constant over the last 20 years, with median grain ranging from 20–30 m, approximately the length of an average riffle or pool (Figure 7b). However, the average extent of a study has shown a large increase in the last five years, nearly doubling after over 15 years of relatively constant extent. This increase is primarily the result of many studies that investigate biodiversity at continental or multi-continental scales. The emergence of these sorts of studies suggests that researchers have recognized the utility of studies of greater spatial extent and/or studies of greater spatial extent became more feasible. Very possibly we are beginning to reap the rewards of global connectivity via online databases and greater electronic connectivity in the form of increased data sharing and collaborations, allowing the type of coordination and communication necessary to investigate biodiversity at these enormous spatial extents.

With regard to the choice of scale at which to investigate biodiversity, α-diversity, or diversity of a single locale, was by far the most utilized scale with 87% of studies examining alpha diversity and most studies focusing only on α-diversity (Figure 3). Several types of metrics were commonly employed at this scale, including species richness and various metrics of diversity (e.g., Shannon) and evenness (e.g., Simpson). Relatively few studies investigated β- or γ-diversity (*i.e.*, turnover and regional diversity respectively). However, one particularly interesting result was a dramatic increase in studies including β and γ during recent years. The proportion of studies incorporating these larger-scale measures is still low; a maximum of 16% for β and 7.5% for γ, but the increase is noteworthy. We saw a similar trend with regard to studies that were explicitly designed as multi-scale, with an increase from 11% in the ’84–’89 interval to nearly 40% in the most recent 5 years (Figure 5a). This dramatic increase in interest in multi-scale patterns closely coincides with a general movement in ecology to incorporate processes at multiple spatial scales. In particular, the Metacommunity Concept—a framework that incorporates both local scale processes (e.g., species interactions and environmental filtering) and the regional-scale processes driven by the dispersal of organisms—has come to dominate much of the thinking of community ecologists within the general discipline of ecology [53–57], and specifically in stream ecology [18,29,33,37,48,58–64].

The rise in influence of metacommunity theory is obvious from the increase in use of language associated with metacommunity ecology. Appearance of the specific terms “metacommunity” or “metacommunities” has increased dramatically in the last five years, with currently over 10% of studies referencing metacommunity theory, whereas in the previous time interval (2004–2009) less than 1% of studies referenced metacommunity theory (special recognition to Chris Taylor who, in 1997, appears to be the first stream ecologist to reference the term “metacommunity” [65], seven years prior to the publication of Leibold *et al.* 2004, the seminar paper on metacommunity ecology) (Figure 5b). Likewise, references to the four paradigms associated with metacommunity theory has generally increased since 1994 (Figure 5c). However, an exception to the general trend is that in the 1989–1994 interval, use of the term “patch dynamics” was extremely common, appearing in 35% of studies on biodiversity although at the time, patch dynamics meant something slightly different that its most common current use. This result was actually not surprising, and when the same data are examined on a finer temporal scale, examining every citation year individually, a similar spike can be observed in the early 2000s with regard to neutral theory. These temporal trends occur because the four paradigms commonly associated with metacommunity theory all pre-date the metacommunity concept and were not formalized as part of the metacommunity concept, despite many misleading references in current literature. The four paradigms were simply grouped together as historical paradigms that all fall under the purview of unified metacommunity theory [57].

What proportion of studies had an experimental component? The ratio of experiments to survey-based investigations of stream biodiversity is low with only 20% of total studies incorporating an experimental component. The high number of survey-based investigations on stream biodiversity is likely the result of a number of factors. First, surveys are often easier to perform than experiments. Surveys in streams typically involve collecting organisms, identifying them, and measuring one or more predictor variables (Figure 2). In contrast, by definition experiments involve modifying some aspect of a stream or organism and frequently attempting to maintain that modification through time. The disturbance prone nature of streams makes field experiments particularly vulnerable to the whims of nature or demonic intrusion to follow the parlance of Hurlbert [66]. We doubt there is a single experimental stream ecologist who has not been the unfortunate victim of a flood that destroyed a stream experiment. In the quest for both increased experimental control and reductions in destroyed experiments, stream ecologists have come up with creative workarounds. For example, many examples of researchers performing experiments in artificial stream channels exist but as with any human made attempt to replicate nature, artificial stream channels have their own difficulties and shortcomings. Artificial stream channels are often limited to use during certain times of the years since they often easily freeze up or get too hot and they are expensive to construct.

5. Conclusions

The study of diversity in lotic systems has had many successes, but much remains to be discovered. We close by briefly summarizing topics that have been largely settled and point to those in need of work. Studies of macroinvertebrate and fish diversity are plentiful, while other groups, perhaps most notably microbial taxa, have received relatively little attention. Given the long-known importance of microbial communities to in-stream nutrient and energy dynamics, and the recent increase in the accessibility and affordability of molecular-based tools for the study of microbial communities, studies of lotic microbial diversity remains as an understudied field ripe for future investigation. We also found that studies of stream diversity have been predominantly local-centric, and many local factors like local habitat, stream morphometry, hydrologic variables, and disturbance regimes have been overwhelmingly demonstrated as important to local lotic biodiversity. However other commonly measured factors show only mixed support including chemical variables and nutrient availabilities. Still others, most notably intra- and inter-specific interactions, are infrequently considered, but typically found to be significant when considered. Though studies of regional patterns of lotic biodiversity are still relatively scarce, recent and encouraging trends suggest growing interest in regional drivers and the use of multi-scale theoretical and conceptual frameworks that echo seminal works from more general fields of study. Additionally, there was a surprising lack of evolution as either a point of study or as an explanation for biodiversity patterns in the material that we surveyed.

Based on our results we suggest the following as critical areas in need of study: (1) Studies that utilize affordable and rapidly developing molecular tools to investigate patterns of lotic microbial diversity and their underlying mechanisms; (2) multiscale biodiversity studies that embrace recently developed theoretical frameworks and spatial analytical tools to incorporate regional dispersal processes with local environmental factors to explain patterns of stream biodiversity; (3) increased focus on biotic interactions; and (4) studies that utilize the increasingly affordable and accessible sequencing technologies to incorporate evolutionary and dispersal history to explain contemporary patterns of biodiversity. Streams are inherently dynamic and fluid environments and understanding their diverse inhabitants demands the same qualities from stream researchers. We hope this review will stimulate thoughtful introspection among stream ecologists to move beyond what is known and utilize recent technological and theoretical advances to explore the most productive unknown areas of our field.

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Author Contributions

Brett Tornwall, James Skelton, Eric Sokol and Bryan L. Brown conceived and designed the presented literature study. Brett Tornwall, James Skelton, Eric Sokol and Bryan L. Brown collected and analyzed data, and all authors contributed to data interpretation and manuscript preparation.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1424-2818/7/1/016/s1.

Conflicts of Interest

The authors declare no conflict of interest.

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