ECOLOGY

Major shifts in biogeographic regions of freshwater fishes as evidence of the Anthropocene epoch

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Animals and plants worldwide are structured in global biogeographic regions, which were shaped by major geologic forces during Earth history. Recently, humans have changed the course of events by multiplying global pathways of introduction for nonindigenous species and propagating local species extirpations. Here, we report on how introductions and extirpations have changed the distributions of freshwater fishes worldwide and how it affected their natural biogeographic regions. We found major shifts in natural regions, with the emergence of an intercontinental region arising from the fusion of multiple faunas, which we named Pan-Anthropocenian Global North and East Asia (PAGNEA). The PAGNEA region is evocative of the Pangea supercontinent, as flows of introductions show that dispersal has become possible again across multiple continents, suggesting that human activities have superseded natural geological forces. Our results constitute evidence on the expected modification of biostratigraphic boundaries based on freshwater fish, which are abundant in the fossil record, thereby supporting the concept of the Anthropocene epoch.

INTRODUCTION

Virtually all species, before humans, could not disperse across the entirety of Earth (*1*). The distribution of all organisms was determined by intrinsic dispersal abilities and extrinsic barriers. These barriers can be physical, such as mountains and oceans, or environmental, such as climatic conditions. Their permeability changes slowly over time [from thousands to millions of years for climatic and physical barriers, respectively (*2*)]. Consequently, throughout the history of the Earth, barriers have led organisms to evolve and diversify separately in isolated regions, called biogeographic regions, each composed of a unique set of endemic lineages (*3*). These regions have been studied in biogeography for the past three centuries (*4*–*7*). Global biogeographic regions have been defined for plants (*4*, *8*), birds, mammals, amphibians and reptiles (*3*, *5*, *9*, *10*), microbes (*11*), and more recently, for freshwater fishes (*12*). Although these organisms have distinct dispersal abilities, they all show prominently similar biogeographic regions, which seems to reflect the shared history of diversification conditioned by geographical, geological, and climatic history (*6*, *13*, *14*).

However, these universal rules of natural dispersal limitation have changed recently in the history of the Earth due to the creation of global pathways of transportation by humans. These pathways led to massive intentional and nonintentional species introductions worldwide (*15*). The drastic increase in species dispersal worldwide has altered the composition of biogeographic regions through the process of biotic homogenization, an increased similarity of regions over time caused by the replacement of native species with nonindigenous species (*16*) and by regional species extinctions (hereafter, extirpations) due to anthropogenic pressures (*17*). This biotic homogenization process may in turn lead to the redefinition of the limits of historical biogeographic regions, as shown by pioneering works on terrestrial gastropods (*18*), amphibians, birds, and mammals (*19*). Evidence on human-induced alterations of global biogeographic regions has far-reaching implications, suggesting that global changes induced by humans may have superseded the geological drivers which generated these regions over millions of years (*20*).

Here, we report on how anthropogenic species introductions and extirpations have affected the global biogeographic regions of strictly freshwater fishes. Freshwater fishes are here defined as fishes that live in fresh or brackish waters but cannot tolerate high salinities and thus cannot disperse via the marine environment (i.e., excluding marine and amphidromous families of fish) (*12*). Strictly freshwater fishes have very limited dispersal abilities compared to other vertebrates, because they can generally not disperse over land or seas, and are constrained to drainage basins (i.e., watersheds) (*21*). As a consequence, their native biogeographic regions have been shown to have extreme degrees of endemism [99.24% of freshwater fish species are endemic to a single region (*12*)]. Freshwater fish regions are organized in a complex biogeographic hierarchy of spatial units. The largest regions have the highest degree of endemism, reflecting barriers impermeable to dispersal, and smaller units nested within the larger ones display a lower degree of endemism, reflecting increasingly permeable barriers (*12*). Yet, freshwater fishes have a long history of intentional and nonintentional introductions in all regions of the globe (*22*, *23*), which makes them one of the most introduced groups worldwide (*15*, *24*). Despite this observation, a previous study, using 39,704 records from 1054 river basins to assess whether introductions of nonindigenous species and extinctions increased the taxonomic similarity of freshwater fish faunas worldwide (i.e., taxonomic homogenization), had concluded that the current level of

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homogenization was still low (*25*). However, over recent years, documentation and compilation of data on freshwater fish introductions have greatly increased, notably with the release of the most comprehensive global database on freshwater fish occurrences, which tripled the number of records (110,565) and the number of river basins (3119) included (*26*). This database compiled both introductions and extirpations of freshwater fishes, making it possible to explore whether biogeographic regions of freshwater fishes have been altered by humans. Furthermore, introduced freshwater fishes have been described as specific markers of the Anthropocene, which is the geological epoch starting in the mid-20th century reflecting the substantial impact of human activities on the planet (*27*). Introduced freshwater fish can be markers because their fossil remains are likely to be abundant in future palaeontological records (*28*). Therefore, the investigation of current changes in biogeographic regions of freshwater fish species is an indicator of what geologists call "Anthropocene assemblage zones," i.e., zones with co-occurring fossilizable species representing unique assemblages with respect to the fossils of previous epochs (*28*).

Here, we investigate how anthropogenic introductions of nonindigenous species and extirpations of native species have altered the natural biogeographic regions of strictly freshwater fishes. From the global database of freshwater fish occurrence, we extracted four pools of species for our analyses: natural (i.e., native occurrences without introductions or extirpations), Anthropocene (i.e., present-day distribution of species, native occurrences plus anthropogenic introductions, and extirpations), natural plus introduction only, and natural plus extirpation only. First, we explore how introductions and extirpations together have altered the natural endemism rates in drainage basins within fish biogeographic regions (*12*), endemism defined here as the natural occurrence in a single region. Drainage basins are the spatial units upon which biogeographic regions are built; therefore, we expect any substantial changes in the endemism rates of drainage basins to impact the delineation of biogeographic regions. Second, we explore how the spatial definition of biogeographic regions has changed following human-induced distribution changes. To do this, we use a hierarchical clustering approach to identify regions with a unique species composition, on the basis of the distribution of species in drainage basins (*10*). Third, we explore the relative importance of introductions and extirpations in altering biogeographic regions. Fourth, we specifically investigate the fluxes of introductions (origins and destinations) and the characteristics of introduced species. Last, we provide a map of selected drainage basins with characteristics that may result in a fossil record of the changes we documented here.

RESULTS

Endemism of freshwater fishes has decreased in drainage basins

We first explored how introductions and extinctions changed the endemism in the established natural biogeographical regions (*12*) at the granularity of drainage basins. We found that, despite differences among regions, there was an important and significant decrease in the percentage of endemism in all regions between the Natural and Anthropocene pools (Fig. 1 and statistics in table S1). The most important decreases were observed for the Palearctic, Nearctic, and Sino-Oriental, followed by the Australian, Neotropical, and the Ethiopian regions.

Natural biogeographic regions of freshwater fishes shifted to Anthropocene regions

We identified the major biogeographical regions (i.e., first three hierarchical levels of the clustering algorithm) for our four pools of species. For the natural pool of freshwater fish there was a nested hierarchy of two supercontinental regions, six major regions, and numerous large subregions (Fig. 2, A to C) as previously identified (*12*).

For the Anthropocene pool (i.e., present-day distributions, including effects of introductions and extirpations) we found regions notably different from the natural ones (Fig. 2, D to F). First, we did not detect the two natural supercontinental regions. Instead, we found, at the first clustering level, a large intercontinental region (Fig. 2D) encompassing the Nearctic, the Palearctic, part of the Sino-Oriental, and most of the Australian region, including New Zealand. We named this intercontinental region the Pan-Anthropocenian Global North and East Asia (PAGNEA) region, because it spreads over all continents of the Anthropocene epoch (Pan-Anthropocenian), and its delineation is centralized around countries of the Global North and East Asia (North America, Europe, Australia, New Zealand, Japan, South Korea, and China). In addition to the PAGNEA region, there were three other regions at the first level: the Neotropical and Ethiopian regions, which were marginally altered compared to their natural counterparts, and the Neo-Oriental region, which is a subset of the former Sino-Oriental region merged with the Australian region. At the second clustering level, we found highly heterogeneous regions (Fig. 2E), ranging from major regions (Palearctic and Nearctic) to subregions (subregions in the Neotropical, Ethiopian, and Neo-Oriental regions). Intriguingly, at the third level, we did not detect biogeographic structure for most regions of the world (i.e., most drainage basins were each a cluster of their own or grouped in clusters of small surfaces), except for the Nearctic and Neotropical regions where subregions emerged (Fig. 2F), which are distinct from the natural subregions (Fig. 2C).

Analyzing the hierarchy of bioregions with radial trees gives additional insights into the observed changes. Natural bioregions had a complex structure with multiple levels (i.e., multiple circles in Fig. 3A). The majority of drainage basins show at least four levels of clustering, illustrating the spatial nestedness of endemic species in distinct regions. In contrast, Anthropocene regions were simpler than natural regions in their structure (Fig. 3B). Most clusters in the PAGNEA region displayed a simple structure with two levels of clustering (two circles only in Fig. 3B), which evidences regions of basins with similar faunal composition. The other three regions in the Anthropocene pool also have a simple structure with only two to three nested levels of clustering.

When we analyzed the pool of species accounting only for extirpations, we found regions identical to the natural ones (Fig. 3C and fig. S1, A to C). Likewise, when we analyzed the pool accounting only for introductions, we found regions similar to the Anthropocene regions (Fig. 3D and fig. S1, D to F), at the exception of the subregional division of the Neotropical region (fig. S1D). Further analyses based on the areas of clusters and on the structure of biogeographical networks resulted in similar conclusions regarding the comparison of the four pools (see Supplementary Text and figs. S2 to S6). Overall, these results suggest that introductions of nonindigenous species are the prominent driver of change in natural

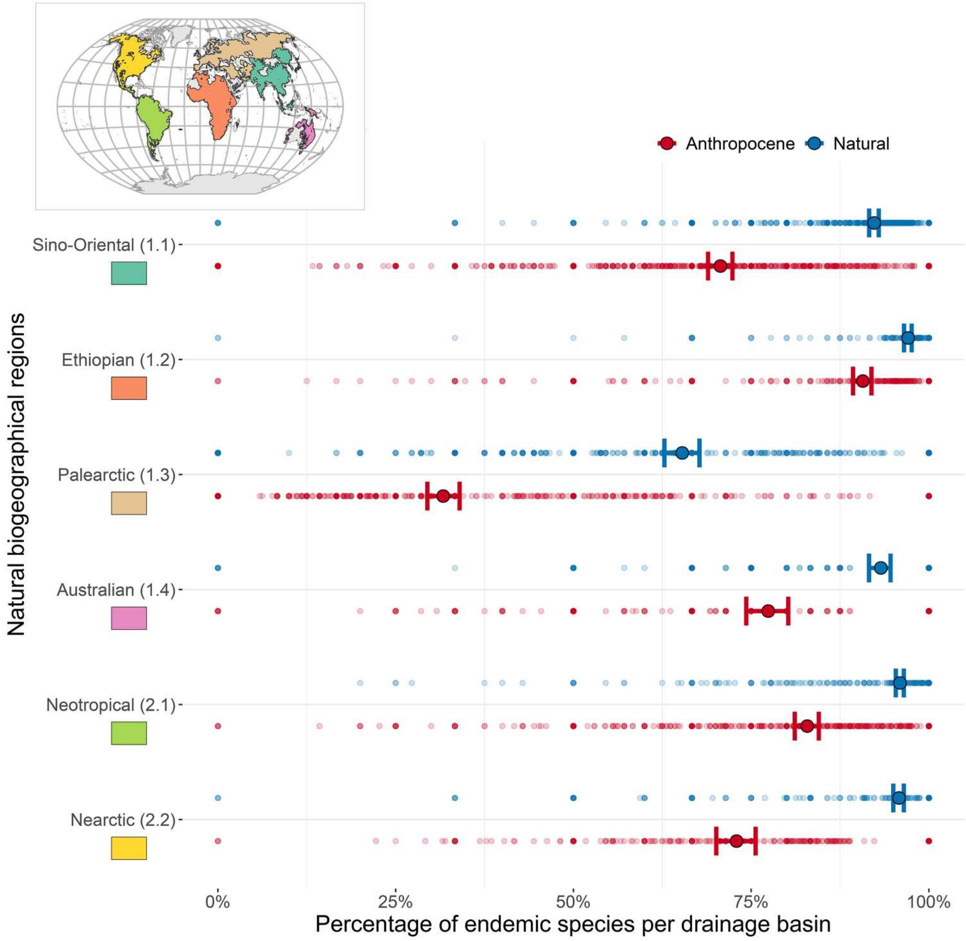

Fig. 1. Change in the percentage of endemic species per drainage basin for each natural biogeographic region between the Natural and Anthropocene datasets. The map of natural biogeographic regions is illustrated as a small inset on the top left corner. Small points represent individual drainage basins. Large points circled in black represent the marginal effects predicted by the generalized linear mixed model testing if there is a significant change in the percentage of endemism at the granularity of basins between natural and Anthropocene pools. Thick colored lines present 95% confidence intervals around the marginal effects. On the *y* axis, colors indicate correspondence with the inset map, and numbers in parentheses indicate the cluster number code identified by Map Equation. Only freshwater species were considered (i.e., excluding species dispersing via marine pathways).

biogeographic regions; hence, in the next section of results, we will focus specifically on biogeographic patterns of species introductions.

The shift from natural to Anthropocene regions can be explained by patterns of freshwater fish introductions

Since introductions appear to account for most of the observed changes, we specifically mapped the fluxes of introduction between natural regions to explain the bioregionalization shifts between natural and Anthropocene species pools (Fig. 4). Foremost, we found that 83.3% of the introduced species originated from three regions (the Nearctic, the Palearctic, and the Sino-Oriental regions), and were mostly introduced within those regions. We also observed that, although almost no introduced species originated from the Australian region, this last region has received 10% of the total species introductions, mostly from the Nearctic, the Palearctic, and the Sino-Oriental regions. We also found that most introductions occurred within regions, rather than between regions, especially in the Nearctic and Palearctic regions (Fig. 4).

These fluxes of introduction illustrate the 6227 reported introductions of fishes in drainage basins. In total, 453 species were introduced at least once (4% of all strictly freshwater fishes) among which 88 were introduced to more than 10 basins and 13 to more than 100 basins (Fig. 5). We found that the pool of introduced species is disproportionately composed of naturally widespread species (see Supplementary Text, figs. S3 and S4, and table S2). Twenty percent of nonrare species (i.e., natural occurrence above 10) have been introduced; 48.7% of widespread species (i.e., natural occurrence over 50) have been introduced, and 100% of super-widespread species (natural occurrence over 200) have been introduced (table S3).

As expected, we found that those species that were introduced many times and in multiple regions were the ones contributing most to reshaping biogeographic regions (i.e., species with a large dilution value; Fig. 5). Introduced species originated from almost all regions, but the majority originated from the major natural regions that merged into the PAGNEA region of the Anthropocene (Nearctic, Palearctic, and Sino-Oriental regions). We found that most

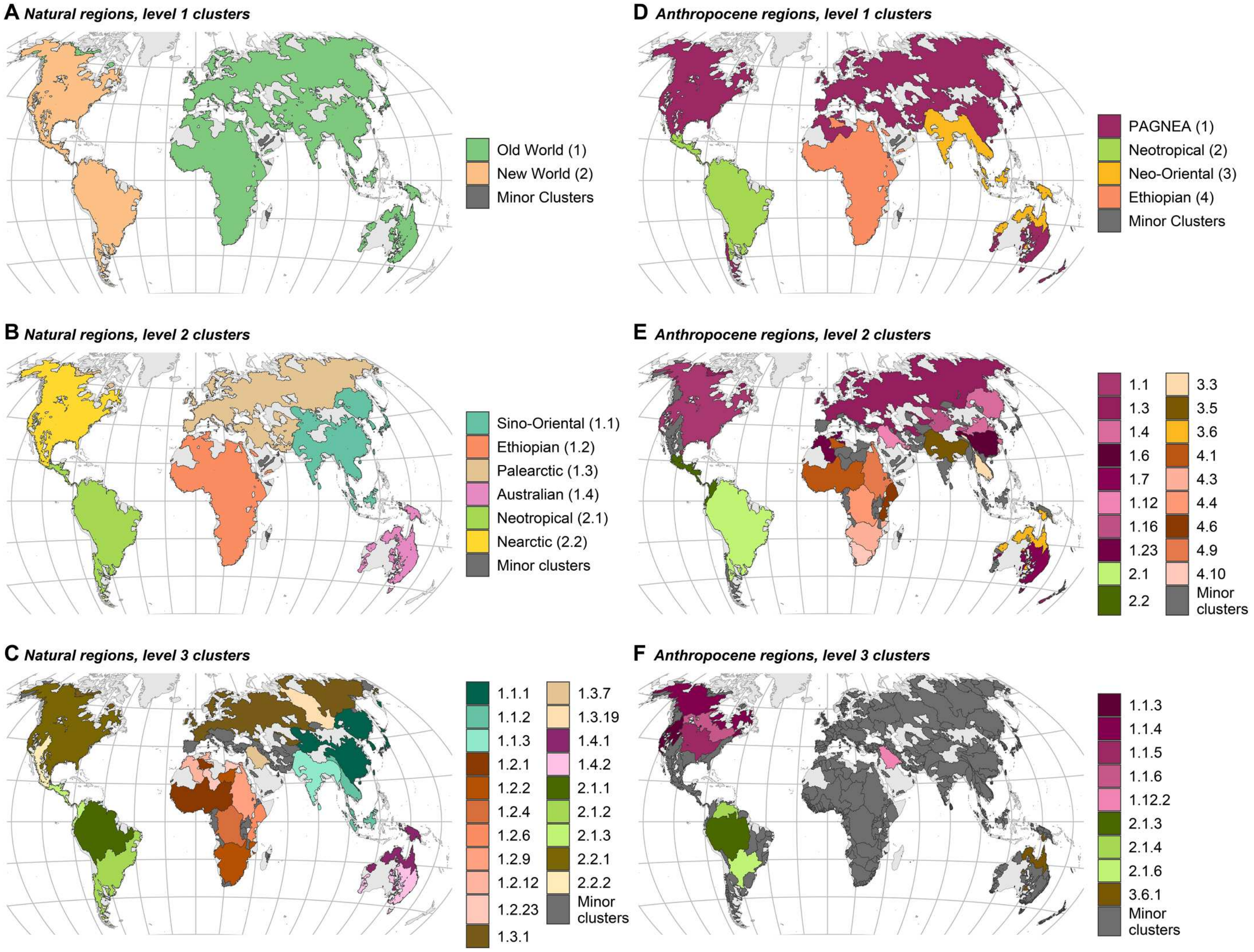

Fig. 2. Maps of natural and Anthropocene biogeographic regions of freshwater fish species. Natural regions are in (**A** to **C**) and Anthropocene regions are in (**D** to **F**), showing the first three hierarchical levels of the bioregionalization. Numbers in parentheses [(A), (B), and (D)] indicate cluster codes. Only freshwater species were considered (i.e., excluding species dispersing via marine pathways).

introduced species were introduced in nonnative areas of their native regions (286 species). However, almost all top introduced species (over 100 introductions) were mostly introduced outside their native regions (Supplementary Text and table S4).

The shift in biogeographic regions is likely to be noticeable in fossil records of basins across the world

We illustrated six drainage basins of the world that have rivers with multiple channels that diverge and rejoin the main stem and produce lacustrine and perilacustrine environments. These environments have characteristics [low hydrodynamism and high sedimentation rates (*29*–*31*)] that facilitate the occurrence of fossil records from which shifts in communities can be observed (Fig. 6) (*32*–*36*). For each of these basins, several of the top-introduced species occur in abundance, which we expect to be noticeable in future fossil records because we found evidence of identifiable fossil remains for all of these species in the literature, although sometimes only at the genus level [e.g., European carp (*37*), goldfish (*38*), mosquitofishes and guppy (*39*, *40*), tilapias (*41*–*43*), largemouth bass (*40*, *44*), grass carp (*38*, *45*), European perch (*37*, *38*), pumpkinseed (*46*), and silver carp (*47*)].

DISCUSSION

Here, we have shown how the delineation of biogeographic regions of freshwater fishes has been altered by human-induced introductions and extirpations. The natural distribution of many species has been changed, resulting in substantial losses of endemism, which in turn altered and simplified the complex hierarchy of natural biogeographic regions to the point that multiple historical regions are no longer detectable with our methods. An intercontinental region, the PAGNEA region has emerged from the fusion of the Nearctic, the Neotropical, the Sino-Oriental, and Australian faunas and extends to all continents. The PAGNEA region is evocative of Pangea, which was the last supercontinent to have existed on Earth. Species dispersal was possible across Pangea because of the lack of oceanic

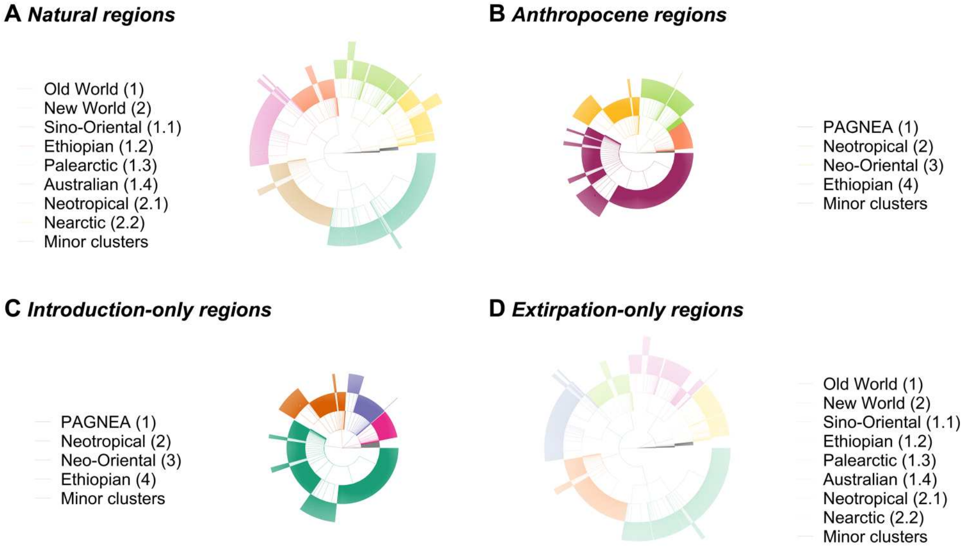

Fig. 3. Radial trees representing the bioregionalization structure of each species pool. (**A**) Natural pool, (**B**) Anthropocene pool, (**C**) introductions only pool, and (**D**) extirpations only pool. Each tip of these trees is a drainage basin. Branches of the tree represent clusters of basins with homogeneous species composition. Each circle, starting from the center outward, represents a hierarchical level of the bioregionalization, the more numerous the circles, the more complex the biogeographic structure. Note that for the natural (A) and extirpations only (D) pools, the colors of "Old World" and "New World" refer only to innermost branches of the radial trees.

barriers. In the Anthropocene, species dispersal across continents is possible again because of the pathways of transportation and introduction created by human societies, which resulted in the emergence of the PAGNEA region.

Timing of shift from natural to Anthropocene regions

The biogeography of freshwater fish species has always been driven by the geological history of the Earth. For example, the two supercontinental regions and the six major regions of freshwater fishes had almost completely distinct faunas, with 99.24% species endemic to their native region (*12*). Major continental masses have been isolated since the Paleogene Period, which ended 23 million years (Ma) ago (*48*), and only a very limited number of dispersal events happened during or after the Neogene (−23 to −2.5 Ma ago) (*12*), which illustrates the importance of barriers to freshwater fish dispersal. Likewise, within regions, many subregions with a high degree of endemism naturally occurred because of regional patterns of diversification linked to historical climate change, paleoconnectivity, and dispersal events (*12*, *49*–*53*). We showed here that this natural biogeographic structure has been redefined by human species introductions.

Our Anthropocene results represent the present-day status of freshwater fish biogeographic regions, and these changes occurred rapidly, as evidenced by the temporal trends in introductions (*54*). Although the onset of global introductions started in the 19th century, the number of introductions and diversity of introduced species remained low until the mid-20th century (*54*). An abrupt change was identified in 1947, with a marked acceleration in introductions worldwide, and a globalization of the origins and destinations of introduced species (*54*). This mid-20th century abrupt change in patterns of introductions likely marks the cut-off date for the alterations of natural biogeographical regions we described here. This mid-20th century start date aligns well with other markers of global change and thus with the base of the Anthropocene as defined by the Anthropocene Working Group (*27*, *55*, *56*). We showed here that human activities have superseded in a few decades the natural geological forces which generated biogeographical regions over millions of years (*51*), which is a compelling illustration that humans are globally overwhelming the great forces of nature (*57*).

Biogeography of freshwater fishes in the Anthropocene epoch

The biogeographical redefinition we identified suggests that multiple regions have been merged together into the PAGNEA region. The limits of this intercontinental region can be explained by both the characteristics of introduced species and the fluxes of introductions among regions. First, even if only 4% of the total number of species were introduced [a proportion identical to plants (*58*)], these introduced species are disproportionately composed of naturally widespread species. Widespread species form the basis of biogeographical regions, because their distributions overlap in specific regions constrained by major barriers. Any substantial change to their distribution erases the natural boundaries and generates overlaps among formerly disconnected basins, eventually altering the definition of biogeographical regions.

Second, the PAGNEA region is well explained by the fluxes of introduction, which result from inter-country socioeconomic activities that act as pathways of introduction (*59*), such as aquaculture, ornamental trade, stocking for fisheries, and biological control (*24*). This is well illustrated by the fact that the PAGNEA region is composed by the countries that contribute the most to international trade (*59*). Climate similarity between donor and recipient regions may also play a role in redefining the boundaries of major regions (*18*), but this effect may also be confounded with the fact that countries with highest international trade are generally located in temperate areas. However, we did not observe in freshwater fishes the same alterations to regions that are recorded for

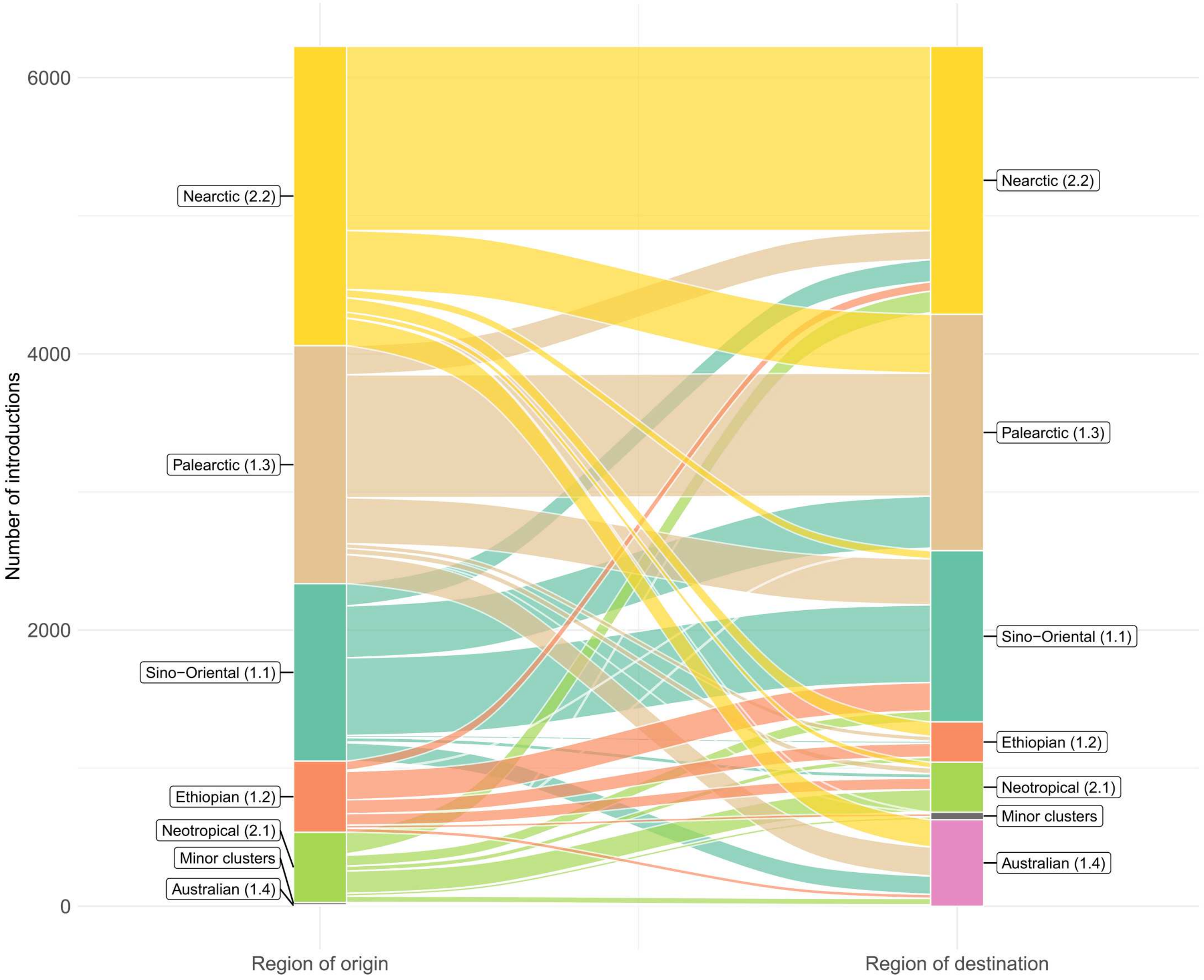

Fig. 4. Alluvial diagram of the fluxes of reported introductions of freshwater fish species among natural biogeographical regions. Regions are depicted in Fig. 2B. The left part indicates the region of origin (each box is proportional to the number of egressions) and the right part indicates the region of destination (each box is proportional to the number of introductions). The stream fields in the middle are proportional to the number of introductions from each region of origin to each region of destination. Only freshwater species were considered (i.e., excluding species dispersing via marine pathways).

amphibians, birds, and mammals, where the change was the merging of the New World supercontinental region with the Australian region (*19*). The main reason for this difference is likely not resulting from introductions, but rather from extinctions, because the results for amphibians, birds, and mammals used global scenarios of extinctions with hundreds of extinct species (*19*), whereas we used reported observations of extirpations, which makes a major difference in magnitude.

Not all regions merged into the PAGNEA region. An Anthropocene region that we called "Neo-Oriental" emerged from the fusion of the south-eastern part of the Sino-Oriental region and the northern part of the Australian region. Regional introductions prevailed in this area over global introductions, most likely facilitated by similar climatic conditions. Formerly, only three species were found in both the Sino-Oriental and Australian regions, suggesting that continental shelves were a barrier to dispersal (*12*). However, introductions increased the number of shared species by 10-fold, reaching 30 species, one-third of the number of native species in the Australian region. This substantial change of the Australian fauna resulted in its inclusion into the tropical Neo-Oriental region rather than in the more temperate PAGNEA region. Last, the Neotropical and Ethiopian regions remained relatively unaltered, which can probably be explained by a lower propagule pressure due to limited international trade (mostly export) and their extremely high species richness making them more robust to bioregion alterations, compared to other regions. However, our data on introductions for these regions is probably incomplete, illustrative of regional biases in the knowledge of introductions (*15*).

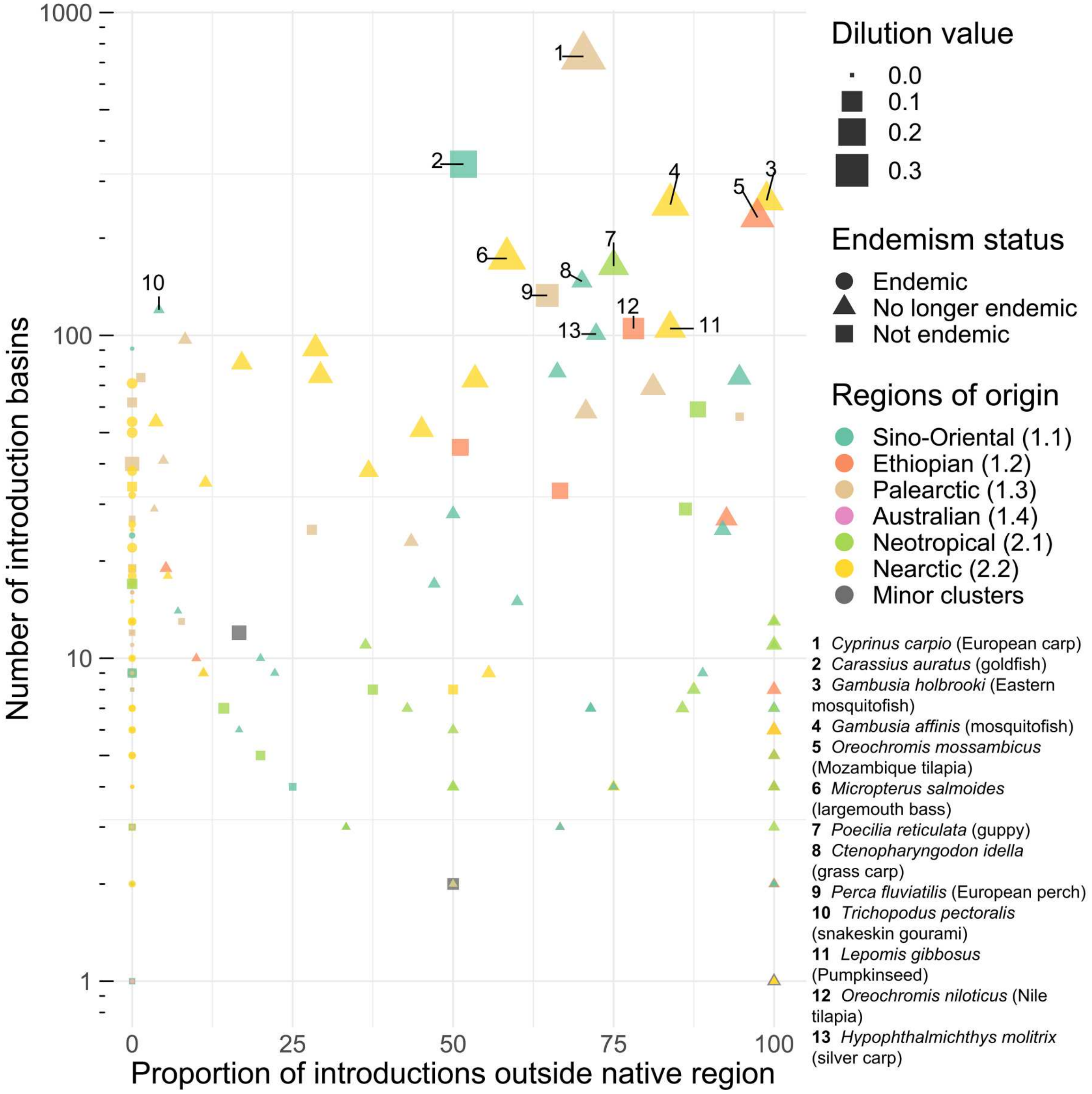

Fig. 5. Characteristics of all introduced species of freshwater fishes. Only freshwater species were considered (i.e., excluding species dispersing via marine pathways). For each species, *y* and *x* axes indicate the total number of basins where the species was introduced and the proportion of basins of introduction outside the native region of the species, respectively. The color indicates the region of origin of the species. The shape of each point indicates the status of endemism of each species. Endemism is here defined with respect to natural regions: A species is considered endemic if it is present in only one natural region. Circles indicate species endemic even after introduction (i.e., endemic to their natural region in both natural and Anthropocene pools); triangles indicate species no longer endemic after introduction (i.e., endemic in natural but not in Anthropocene pools); and squares indicate species that were not naturally endemic (i.e., nonendemic in natural and Anthropocene pools). The size of each point indicates the dilution value, i.e., the degree to which a species is reducing the likelihood that a cluster is detected. The name of the 13 species, which have been introduced in more than 100 basins, are indicated in the legend, and the full list of introduced species with their characteristics is provided in table S4.

The redefinition of global regions is only the most visible aspect of the biogeographic changes, because we also found that those natural subregions were no longer delineated in the Anthropocene, except in the least altered Neotropical and Ethiopian regions. Intriguingly, we could still detect the Palearctic and Nearctic regions as subregions of PAGNEA (Fig. 2E), but not their subregions. This can be explained by the high number of within-region introductions, which strengthened the delineation of these regions while diluting their subregions. Within-region introductions happened through the same pathways as between regions (i.e., aquaculture, ornamental trade, stocking for fisheries, and biological control) but also because of two other pathways, the increase of interconnected waterways and the release of bait for angling (*24*).

Consequences of the major role of introductions and the unresolved role of extirpations

Our results suggest that the main driver reshaping the bioregions appears to be species introductions, while the role of extirpations

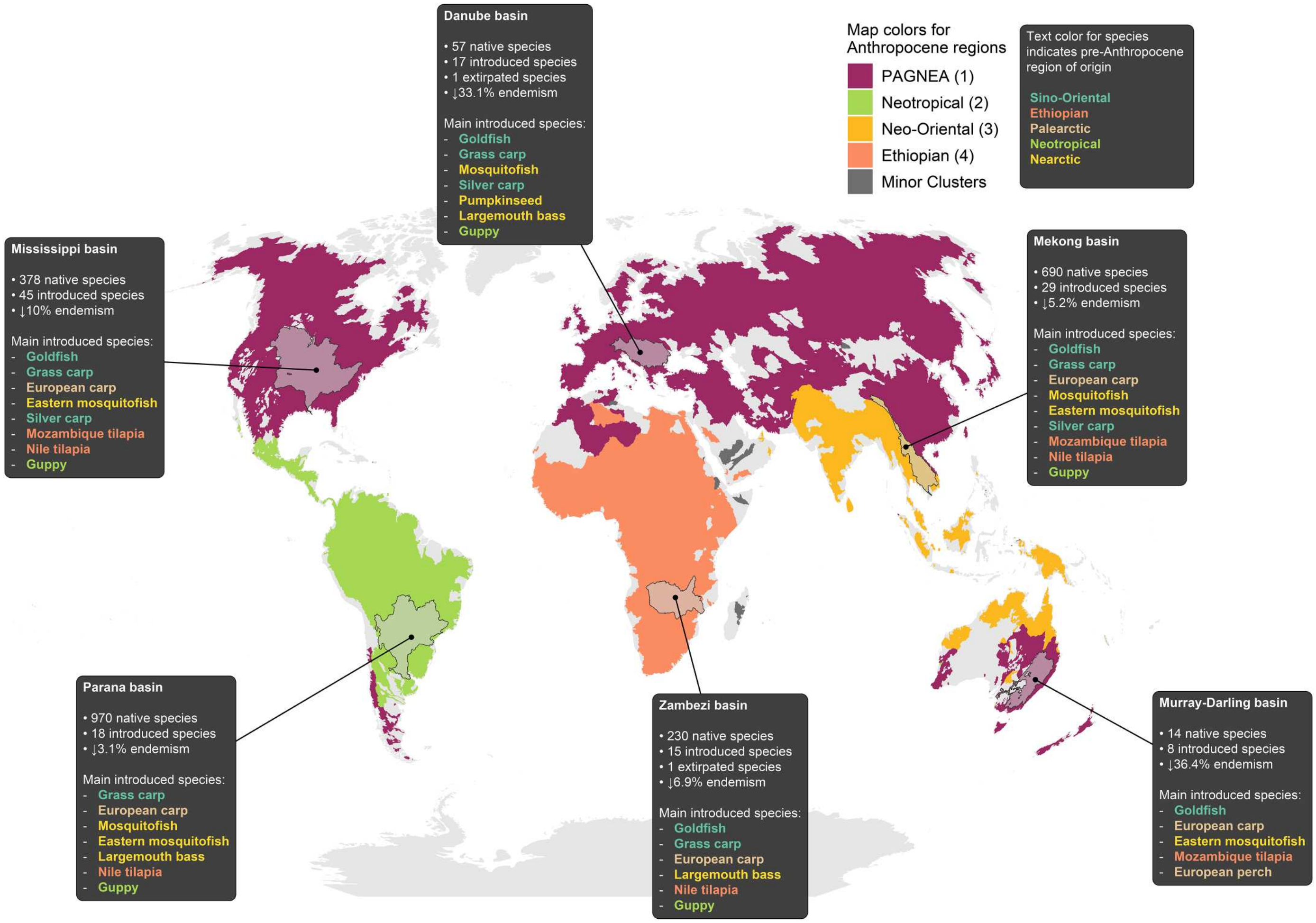

Fig. 6. Examples of drainage basins with potential for a fossil record of changes. This map illustrates six major drainage basins that have characteristics likely to facilitate the occurrence of a fossil record. For each drainage basin, several characteristics are indicated: number of native species, number of introduced species, number of extirpated species (if any), and the change in endemism following introductions. In addition, we list the main introduced species as an illustration of the changes to be expected in the fossil record. Species scientific names: European carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), Eastern mosquitofish (*Gambusia holbrooki*), mosquitofish (*Gambusia affinis*), Mozambique tilapia (*Oreochromis mossambicus*), largemouth bass (*Micropterus salmoides*), guppy (*P. reticulata*), grass carp (*C. idella*), European perch (*Perca fluviatilis*), pumpkinseed (*Lepomis gibbosus*), Nile tilapia (*Oreochromis niloticus*), and silver carp (*Hypophthalmichthys molitrix*).

seems minor. Introductions conclusively play a major role, with some species such as the European carp *Cyprinus carpio* and the goldfish *Carassius auratus* having been introduced in more than 300 basins. This result corroborates and extends previous results on a major, yet rarely quantified, global effect of introductions on global biogeographical regions (*18*, *19*). The effect of species introductions has been widely investigated under the prism of biotic homogenization (*60*), mostly focused on the ecological consequences, such as functional homogenization, on describing the increased similarity among regions (*25*, *61*, *62*) or on the short- and mid-term evolutionary consequences (*63*). However, changes in biogeographic boundaries and their significance remain an area or research that ought to be developed, especially given that bioregions are also being altered by other drivers such as climate change.

Furthermore, the alteration of biogeographic regions by introductions has major implications for the fields of ecology and evolution. Biogeographic regions are a widespread unit of analysis in these fields, under the assumption that they represent sets of species with a shared diversification and colonization history (*13*, *64*). Yet, we argue here that the current distribution of biodiversity is now departing from this assumption because of the human transgression of natural barriers. This issue has been acknowledged for a long time, but the scale and magnitude of change we highlighted here suggest that it may be more important and pervasive than previously thought. We believe that the implications extend beyond freshwater fishes and are particularly worrisome for taxa or areas with major knowledge shortfalls such as the Wallacean (i.e., lack of distribution data) or Linnaean shortfalls (i.e., undescribed species), for which violations of the assumptions behind biogeographic regions may be unnoticed, leading to biased conclusions or interpretations. We consider that this discussion should serve as, if not a trigger, at least a reminder that even scientific assumptions should adapt to global change, questioning whether the reference unit should be natural regions or Anthropocene regions.

With respect to extirpations, their minor role compared to introductions is expected given the difference in magnitude between species extirpations (70 documented extirpations) and introductions (6227 documented introductions) in our dataset. We hypothesize that the role of extirpations is unresolved, because our data on extirpations may be incomplete or biased due to the difficulty to ascertain and report extirpations in comparison to introductions (*65*). This limit could be addressed in the future by exploring scenarios of extinctions (*19*); however, such an exploration may be difficult to implement, because the red list for freshwater fishes is not as comprehensive as those for mammals, birds, and amphibians.

Outlook: Biogeography of the Anthropocene

Our major finding was unequivocally the identification of original regions of freshwater fishes for the Anthropocene. The Anthropocene is the concept of a geological epoch characterized by the global impacts of human activities on Earth, which is not yet formally defined by the International Union of Geological Sciences (*27*). For the Anthropocene to be formally defined as a unit of the Geological Time Scale, the stratigraphic boundary between the Holocene and the Anthropocene must be demonstrated by accumulating evidence (*27*, *56*, *66*). One of the lines of evidence discussed by geologists is biotic change, as an indicator of biostratigraphic boundaries, i.e., boundaries detectable in the fossil record (*55*, *56*, *67*). Our results illustrate the likely changes in future paleontological records that geologists use to identify assemblage zones (i.e., the body of strata characterized by an assemblage of fossils that distinguishes it from adjacent strata (*28*). However, besides cranial and postcranial fossil elements that can give a relatively good taxonomic identification at the genus or species level [e.g., (*35*)], bones, scales, teeth, or otoliths that are the most abundant fish elements found in sediments (*32*, *37*–*47*, *68*) can be difficult to identify (*33*). In this case, sedimentary ancient DNA could be a powerful proxy in the near future to track biodiversity shifts (*69*–*71*), especially for recent shifts such as those described here. We can expect the fossil record of freshwater fishes to shift from pre-anthropogenic assemblage zones, which were entirely distinct due to their extreme isolation for millions of years (*12*), to Anthropocene assemblage zones, which span multiple continents. These shifts in the fossil record are likely to occur in drainage basins prone to the accumulation of fossils as illustrated in Fig. 6. In these basins, our results suggest that fossil records of Anthropocene assemblage zones may become dominated by the widely introduced species such as the European carp (*C. carpio*), the grass carp (*Ctenopharyngodon idella*), mosquito fishes (*Gambusia* species), tilapias (*Oreochromis* species), the largemouth bass (*Micropterus salmoides*), goldfish (*C. auratus*), and guppys (*Poecilia reticulata*), all of which are known to occur in fossil records (*37*–*47*). Most of these species were endemic to a single biogeographic region before their anthropogenic introduction; hence, the change in the fossil record will likely be noticeable. Furthermore, all these species are recognized as invasive alien species negatively affecting native ecosystems, oftentimes found in high abundances, dominating invaded communities (*72*), although not all introduced species may thrive in high numbers in the presence of predators [e.g., goldfish, (*73*)]. Beyond these invasive species, many of the other introduced species are also likely to occur in abundance in areas of introductions due to their association with human activities generating a high propagule pressure, such as aquaculture, ornamental trade, biological control, or stocking for fisheries (*24*). Consequently, our results provide evidence on the expected modification of biostratigraphic boundaries, supporting the concept of the Anthropocene.

Ultimately, mapping the biogeography of the Anthropocene leads us to think beyond the short- and mid-term ecological and evolutionary impacts of biotic homogenization (*60*) and realize the very long-term evolutionary consequences of our actions (*74*). Natural biogeographic regions carry the evolutionary imprint of the appearance and disappearance of dispersal barriers over millions of years. Let us realize that, millions of years from now, the future biogeographic regions will carry the evolutionary imprint of lineages, which once had intercontinental distributions resulting from the breakage of biogeographical barriers during the Anthropocene epoch.

MATERIALS AND METHODS

Distribution data

We used the global database on freshwater fish species occurrence in drainage basins (*26*), version updated in August 2019 (see Data and materials availability statement to access it). This version comprises 110,565 occurrence records for 14,953 species in 3119 drainage basins of the world, covering more than 80% of the terrestrial surface. A drainage basin is defined as the area of land where precipitation collects and drains off into a common outlet that could be either a lake (endorheic basin) or the ocean (exorheic basin). Occurrence records correspond to samplings and collection data from sources published between 1960 and 2019. Species names in the database were validated according to FishBase (*75*) and the Catalogue of Fishes (*76*), and occurrence records were screened by the team developing the database (*26*). We applied the same filters as in (*12*), to define strictly freshwater fishes. To summarize, we used drainage basins as geographical units and species level information. We excluded species that could disperse through marine waters, and retained only families having fewer than 10% of their species occurring in marine waters. The resulting dataset included 66,715 records on 11,333 species in 2771 basins.

All records have descriptors detailing the native, introduced, and extirpated status of each species within each drainage basin of the world (*26*). Tedesco *et al.* (*26*) defined an introduction as an established nonnative occurrence and an extirpation as the documented disappearance of a species from a drainage basin. Occurrences were confidently classified as "introduced" if the species introduction happened after around 1850. Before that, there is insufficient literature on introductions to distinguish native from introduced occurrences. As a consequence, older species introductions may have been misclassified as native occurrences in our dataset. Likewise, extirpations before 1850 may have been missed because of the sparse literature available before this date. With the "introduced" and "extirpated" information, we extracted four pools of species from the database (Table 1): (i) the natural pool, which includes only the native distribution ranges of species and corresponds to the pool of species used to establish global regions of freshwater fishes (*12*) with marginal differences due to database updates; (ii) the Anthropocene pool, which describes the present-day state of freshwater fish biogeography by considering both introductions and extirpations; (iii) the extirpation-only pool, which contains the natural pool altered only by extirpations; (iv) the introduction-only pool, which contains the natural pool altered only by introductions (Table 1).

Table 1. Description of the four pools of species extracted from the global database on freshwater fish occurrences used for our analyses. Each pool was constructed by considering or not the events of introductions and/or extirpation in each drainage basin from the global dataset. Two species do not have native occurrences because they have recorded introductions outside of their native range, but are native from drainage basins not included in the freshwater fish database (*26*).

	Species pool			
	Natural	**Anthropocene**	**Extirpation only**	**Introduction only**
Native occurrences	Considered	Considered	Considered	Considered
Introduced occurrences	Not considered	Considered	Not considered	Considered
Extinct occurrences	Not considered	Considered	Considered	Not considered
Number of species	11,331	11,318	11,316	11,333
Total number of basins with records	2588	2771	2588	2771
Number of records	60,488	66,645	60,418	66,715

Changes in endemism

To understand how natural bioregions have been altered by introductions and extirpations, we analyzed the changes in endemism between natural and Anthropocene pools, using drainage basins as the spatial grain. We defined endemism on the basis of natural regions here, i.e., a species is endemic to a region if its distribution is entirely contained in its native region and nowhere else. We tested whether the percentage of endemism at the granularity of basins significantly changed between natural and Anthropocene pools using a generalized linear mixed effect model with binomial family distribution, which is appropriate when the response variable is a percentage. We included as fixed effects the native region (to account for differences in endemism among regions), the species pool (native or Anthropocene) and the interaction between species pool and native region to test for the differences in endemism between native and Anthropocene pools for each native region. Last, we included the basin identity as a random effect, to account for random differences among drainage basins (e.g., difference in species richness).

Delineation of biogeographic regions

To delineate biogeographic regions, we applied a hierarchical clustering method (called bioregionalization procedure), which consists in identifying regions with a unique species composition, on the basis of the distribution of species in drainage basins. We applied the same bioregionalization procedure as in (*12*). This method consists in creating a network that connects specific sites (drainage basins in our case), with the species that were known to live there. We then apply a clustering algorithm upon the network to delineate the regions. Because this network contains two types of nodes, sites and species, it is called a bipartite network (*10*). Following this definition, site-site and species-species links are not allowed in this type of analysis. An important feature of bipartite network analyses is that because they keep track of species identity throughout the whole analysis, they enable capturing the co-occurrence of species and identifying patterns of clustering among sites that would not be apparent if we only looked at the similarity among sites.

We chose to apply here the clustering algorithm called "Map Equation" which delineates groups of nodes in networks that are more strongly connected to each other than to other nodes in the network (*77*). In other words, this algorithm seeks to delineate clusters of sites sharing a common set of species, with as little overlap among clusters as possible. This corresponds well to the definition of biogeographic regions, i.e., regions of distinct assemblages of endemic taxa. In addition, the Map Equation algorithm identifies clusters hierarchically, which describes the spatial nestedness of endemism in species distributions, from clusters at coarse levels (corresponding to large areas with the highest degree of endemism) to fine levels (corresponding to small areas with the lowest degree of endemism, nested within larger clusters). Because of these features, Map Equation has been recommended for bioregionalization analyses (*10*) and has been shown to outperform other clustering algorithms to detect regions of freshwater fishes (*12*). We used the Map Equation community detection algorithm version 2.6.0 (*77*) with 1000 trials to find an optimal solution. The step-by-step code to reproduce the analysis is available in the biogeonetworks R package (*78*).

We constructed four biogeographic networks with our four pools (Table 1), and applied the Map Equation algorithm to each network. The outputs of Map Equation can be conceptualized as a tree with multiple levels, where the largest clusters are found at level 1. These first-level clusters have the highest degree of endemism. Each cluster at level 1 can be subdivided into smaller clusters at level 2, with a lower degree of endemism, and so on until no more biogeographic structure is detected. At the maximum level of each branch, the leaves of the tree are always nodes (i.e., species or basins). Here, we mapped specifically the first three clustering levels of our different bioregionalizations. These levels 1, 2, and 3, respectively, corresponded to supercontinental regions, major regions, and subregions of freshwater fish species in Leroy *et al.* (*12*). To compare changes among pools, we used the biogeographic structures identified by Map Equation to produce radial trees of each bioregionalization with the R package ggtree (*79*), in order. In addition, we provide visualizations of the four networks constructed with Gephi v0.10.0 (*80*); we spatialized all networks with the Force Atlas 2 algorithm under default settings.

Because the algorithm identifies clusters mainly on the basis of endemism, the first levels of the bioregionalization often detect several marginal clusters of minimal size, such as areas with extremely low species richness of endemic species, typically islands. Since the focus here is on the major biogeographic regions, we defined a threshold area of 1,000,000km^2 to distinguish major biogeographic regions from minor clusters. Such a threshold is large enough to ensure that minor clusters are not identified as major regions at coarse levels where the difference in area between clusters is generally extreme. At finer levels (e.g., subregions), this threshold is less useful because the distribution of area per cluster is generally continuous. To illustrate the number of major and minor clusters identified by the algorithms for each pool, we created plots of number of clusters ordered by their area for the first three levels (fig. S2). We only mapped clusters (groups of basins) in these plots, setting aside individual basins.

Biogeographic patterns of species introductions

To understand the differences in bioregionalization between species pools with respect to introductions, we mapped the flows of introductions within and between natural biogeographic regions with an alluvial diagram, using the ggalluvial R package (*81*). We mapped onto this diagram the introductions of fish species from their region of origin to the region(s) where they were introduced.

Then, for each introduced species, we calculated three metrics to describe their introduction patterns: (i) the total number of basins where the species was introduced; (ii) from this total number of introduction basins, the percentage of basins outside the native region of the species; and (iii) their dilution value, which describes the degree to which a species expands its range beyond its native region, thereby contributing to weakening the definition of its cluster (*19*). The dilution value of species i is calculated as follows: $\mathrm{DilVal}_i = A_i \times (1 - F_i)$ where A_i and F_i are the affinity and the fidelity of species i to its native region, respectively. The affinity is calculated as $A_i = R_i/Z$, where R_i is the range of species i in its native region, and Z is the total area of its native region. The fidelity is calculated as $F_i = R_i/D_i$, where D_i is the total range of species i.

Examples of drainage basins with potential for a fossil record of changes

We mapped areas likely to register a fossil record of the biogeographical changes we documented. The decay and fossilization of freshwater fish is influenced by a complex interplay of different factors, such as the hydrodynamism, the chemical characteristics of water bodies and substrates, and tectonic activities (*32*), which make it difficult to accurately predict where fossil records of present-day distributions are likely to occur. Because our study is at the granularity of drainage basins, we focused on major characteristics of drainage basins that make them more likely to contain fossil deposits. Specifically, anabranching rivers, i.e., rivers with multiple channels that diverge and rejoin the main stem, promote the occurrence of lacustrine and perilacustrine environments such as oxbow lakes and swamps (*29*–*31*). These lacustrine and perilacustrine environments are characterized by a low hydrodynamism and high sedimentation rates (*29*–*31*), which, in turn, are known to promote the occurrence of fossil records (*32*–*36*). Hence, to map examples of drainage basins with potential for a fossil record of the changes we documented here, we proceeded in two steps. First, we selected a list of 33 basins with major anabranching rivers worldwide (*29*–*31*) producing lakes and perilacustrine environments in abundance (*82*, *83*). Second, we filtered this list to illustrate only one basin per native region, we selected the basin with the highest number of introduced species as the best candidate to carry the imprint of introductions in the fossil record. Third, we searched the literature to find evidence that all the mentioned species can indeed occur in the fossil record. We produced all analyses in R version 4.1.0. We used the Winkel-Tripel projection for maps.

Supplementary Materials

This PDF file includes:

Supplementary Text
Figs. S1 to S8
Tables S1 to S3

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