

The Evolutionary Ecology of African Annual Fishes

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9.1 DISTRIBUTION AND BIOGEOGRAPHY

African annual fishes form two clades within the aplocheiloid killifishes, both belonging to the family Nothobranchiidae (Murphy and Collier, 1997; see Chapter 1). The first clade comprises *Nothobranchius*, *Pronothobranchius*, and *Fundulosoma*. The genus *Nothobranchius* currently

contains more than 60 described species that inhabit temporary pools in East African savannah habitats, ranging from the Nuba Mountains in Sudan to the northeastern part of South Africa. The closely related *Pronothobranchius* consists of four described and poorly known species recorded from a few sites. *Pronothobranchius* inhabit temporary pools in savannahs across the Sahel region between the Gambia and Central African Republic. *Fundulosoma* (a monotypic genus related to *Pronothobranchius*) is a facultatively annual species. The second clade of annual African killifishes consists of *Callopanchax* from the forested region of West Africa (with five described species from Guinea, Liberia, Sierra Leone) and *Fundulopanchax*. At least some species of *Fundulopanchax* (29 described species in total) from West Africa are facultatively annual. They produce eggs capable of surviving desiccation, but the pools where they are found do not dry out routinely, and their typical development does not involve habitat desiccation. Most information on African annual fishes comes from studies on *Nothobranchius*; this chapter primarily considers the ecology of that genus. However, whenever possible, I also report information on other annual taxa of African killifishes.

9.1.1 Habitat Types

Within the vast geographic range of *Nothobranchius*, the location of suitable habitats is extremely patchy. *Nothobranchius* pools are limited to grassland and woodland savannah, typically within alluvia of streams and rivers but sometimes completely isolated from permanent waters. For example, in the dry part of *Nothobranchius*' range in southwestern Mozambique, many pools inhabited by *N. furzeri* and *N. orthonotus* appear isolated and are filled exclusively by rainwater during intensive precipitation (Figure 9.1a and b). Such pools, however, may often be partly connected, as they represent depressions in the savannah where water drained from a larger area accumulates



Figure 9.1 *Nothobranchius* habitats. The same pool in the dry part of the gradient in southern Mozambique during wet (a) and dry (b) phases. *N. furzeri* and *N. orthonotus* were collected in the pool. (c) Extensive marsh in relatively humid coastal region of Central Mozambique, with *N. krysanovi* and *N. orthonotus* populations. (d) Remnant pool within a stream channel that is either annually colonized by three *Nothobranchius* species or supports their stable populations. (Photographs by Martin Reichard.)

after heavy rains. Populations of *Nothobranchius* can also regularly be found in isolated remnant pools within the channels of temporary streams (Figure 9.1d; e.g., Valdesalici and Wildekamp, 2005; Reichard et al., 2009; Valdesalici, 2012; Valdesalici et al., 2012). It is not clear whether these populations are stable and complete the full life cycle within the stream pools or simply comprise individuals flushed from pools adjacent to the stream channel during annual flooding and are not capable of sustaining a viable population over several generations (i.e., sink populations). Such habitats may be disproportionately targeted during sampling due to their convenient locations near fords or bridges and may not represent typical *Nothobranchius* habitats. However, at some of such sites, the same three species were recorded regularly over several years (Reichard et al., 2014). Their population genetic characteristics were comparable with those of populations in savannah pools, though their genetic diversity was relatively high (Bartáková, 2013); it is therefore possible that they represent sustainable populations but with an elevated immigration rate through connectivity.

In the humid parts of *Nothobranchius*' range, such as the coastal area of central and northern Mozambique, the shores of Lake Malawi, Zanzibar Island, and the Tanzanian coastal plains, *Nothobranchius* inhabit vast swampy areas. Likely many of these swamps are permanent, or at least connected to permanent streams, as they frequently harbor a diverse community of nonannual freshwater fishes. The occurrence of *Nothobranchius* is supported by the existence of annually desiccating areas of the grassland matrix that meet the standard requirements for the development of their eggs. After hatching, however, *Nothobranchius* in these habitats frequently coexist with nonannual teleost species and are able to sustain long-term viable populations (Valdesalici and Wildekamp, 2004; Watters et al., 2007).

The critical prerequisite of *Nothobranchius* occurrence in a particular pool is the specific composition of the substrate (Watters, 2009). Soil conditions are the primary drivers of habitat suitability for *Nothobranchius*, as the eggs can only survive the embryonic period and develop successfully on Quaternary vertisol and calcimorph soils. Specific alkaline clay minerals (smectites) are prerequisites for suitable conditions during embryonic development in desiccated pool substrates (Watters, 2009). The mud-rich layer in such pools has low permeability, enabling water to remain in the pool after the surrounding water table has receded. Without this impermeable layer, the pool rapidly desiccates (Watters, 2009). Visually, this substrate is dark brown to black, often forming a thick layer of soft mud on the bottom of the pool. Organic material aggregates in the pool in the form of dead aquatic and terrestrial vegetation but does not cover the large part of the bottom, as is typical of water bodies in forested areas with leaf litter. Despite the presence of rapidly decaying material, the water stays alkaline due to the high buffering capacity of the alkaline clay in the sediment. The water quality is also affected by the action of large mammals. Currently, throughout most of the *Nothobranchius* range, domestic cattle visit these pools, frequently urinating and defecating into the pools (Reichard et al., 2009). The pools hold water for several months after the rains and thus serve as drinking reservoirs. Historically, large African herbivores probably frequented such pools as they still do where they occur. *Nothobranchius* sometimes inhabit pools with sandy substrates (especially pools within active or former streambeds), but such pools always contain at least a small area of accumulated vertisol substrate. *Nothobranchius* never inhabit pools consisting only of orange-colored laterite soils (Reichard et al., 2009; Watters, 2009). Although these pools are very common in the African savannah, especially after heavy precipitation, they are characterized by kaoline-type clay minerals and are slightly acidic, and their substrate is not suitable for *Nothobranchius* embryo survival during the dry period. Clawed frogs (*Xenopus* spp.) and anostracan shrimps frequently occur in these pools and are rarely syntopic (co-occurring in the same pool, i.e., interacting ecologically) with *Nothobranchius* despite their broad sympatry (co-occurrence at the geographic scale). The soil type can clearly be distinguished during the dry season; vertisol mud substrates form deep cracks when desiccated, sometimes covered by a thin layer of white calcite salts, and can be readily distinguished from adjacent sandy and laterite substrates. Soil characteristics and their effect on *Nothobranchius* embryo survival have been previously reviewed (Watters, 2009).

Callopanchax occurs in slow-flowing streams and adjacent forest pools, though their presence in streams is likely secondary (Sonnenberg and Busch, 2010). They were also recorded from a rubber tree plantation, where small depressions around tree trunks formed shallow (2–5 cm) pools inhabited by *Callopanchax*, and from forest pools apparently filled exclusively by rainwater (Dinesen, 2006). *Callopanchax* pools were always shaded within secondary forest plantations, their bottoms covered by leaf litter with sandy and stony substrates. The water was acidic (pH 5.2–6.7, with pH below 6 prevalent). *Pronothobranchius* were also recorded from partly forested areas, but their typical habitat appears to be woodland savannah (Blažek et al., 2012; Valdesalici, 2013). In Niokolo Koba National Park, southeastern Senegal, the habitat of *Pronothobranchius gambiensis* was reminiscent of typical *Nothobranchius* habitats (small temporary savannah pools in sandy soils, but with a layer of black mud). At the same time, a few individuals were collected in adjacent temporary streams, draining a large flooded area into an oxbow lake of the Gambia River. However, *P. gambiensis* was never found in the oxbow lake despite frequent sampling with more than 10,000 individual fish collected over numerous visits (White et al., 2012).

9.1.2 Species Distribution and Range Size

The ranges of individual species vary greatly in size. This may be partly an artifact of incomplete knowledge about the extent of their distribution, of actual differences in species ranges, and of the different species concepts applied for the delineation of new species during recent species descriptions. Typically, however, species ranges span several river drainages. *Nothobranchius orthonotus*, whose range is relatively well studied, inhabits a vast range of at least 1200 km north-south, from the northern part of South Africa (Kwa-Zulu Natal) to southern Malawi and part of Mozambique north of the Zambezi River. In contrast, *N. guentheri* is endemic to Zanzibar Island, only 80 km along its longest axis. Zanzibar Island is also inhabited by *N. melanospilus*, a species otherwise widespread along the Tanzanian coast. Mafia Island, about 150 km south of Zanzibar, also harbors two *Nothobranchius* species, both of which are also common on the adjacent mainland (*N. korthausae* and *N. lucius*). These two islands are located on a shallow continental shelf and were largely connected to the mainland during most of the Quaternary. The third larger island in the area, Pemba, has no record of *Nothobranchius*.

Pronothobranchius ranges are geographically extensive but always contain only a few populations sparsely scattered across a large area (Figure 9.2; Valdesalici, 2013). This may be a real phenomenon wherein existing populations are relicts from a wider historical distribution during a more climatically favorable period. Alternatively, the seemingly patchy distribution may be only an artifact of poor sampling effort. It is unlikely, however, that sampling effort has been equally poor throughout the entire range of the genus. *Callopanchax* has one widely distributed species (*C. occidentalis*) across Sierra Leone, with all other species being more geographically restricted. They all occur in coastal areas drained by small streams and rivers and are not associated with major river basins (Sonnenberg and Busch, 2010).

9.1.3 Climatic Conditions

The distribution of *Nothobranchius* includes areas with either two or only a single rainy season. Two rainy seasons in the equatorial area are associated with more humid conditions than those found in areas with a single rainy season further away from the equator. However, local climatic and geomorphological conditions largely modify the effect of overall seasonality. For example, a relatively steep gradient of aridity has been described in southern Mozambique along the east-west axis. The humid coastal areas receive annual rainfall of up to 1000 mm, but the inland region in Gaza and Inhambane provinces receives only 300 mm annually. As an extension of the Mozambican gradient, the Sazale pan in Gonarezhou National Park in Zimbabwe (the type locality of *N. furzeri*

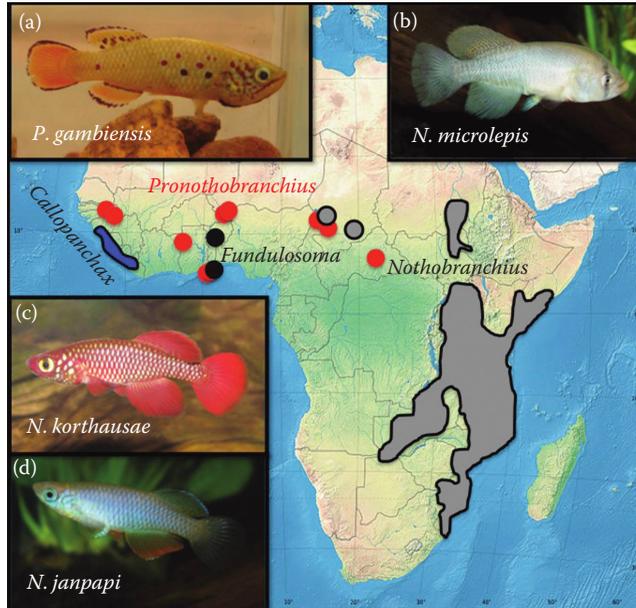


Figure 9.2 Distribution of African annual fishes and representative variation in *Nothobranchius* phenotypic variation. *Callopanchax* range represented by the blue area, *Nothobranchius* by the gray area (and circles for isolated populations), *Pronothobranchius* by red circles, and *Fundulosoma* by black circles. Note circles may represent several adjacent populations. The insets represent males of (a) *Pronothobranchius gambiensis* (Niokolo Koba National Park, Senegal), (b) a robust species from Northern clade (*N. microlepis*, population Mnazini KEN08-8), (c) an example of a coastal species phenotype (*N. korthausae*, Mafia Island TZN 08-4), and (d) a relatively delicate phenotype characteristic of the subgenus *Aphyobranchius* (*N. janpapi*, Ruvu River TZN 09-4). (Photograph (a) by Martin Reichard, photographs (b), (c), and (d) by Béla Nagy.)

at an altitude of 422 m) likely receives even less rainfall. Maybe even more importantly, rainfall is less predictable in the dry regions within this gradient, and some *Nothobranchius* pools may not be filled every year (Terzibaszi Tozzini et al., 2013). In the northern extremes of *Nothobranchius*' distribution (Sudan, Somalia, and Lake Chad region) the habitat is apparently even drier and rains perhaps even more erratic (Valdesalici, 2014). In southeastern Senegal, *P. gambiensis* was collected in only one of the five study years (Blažek et al., 2012; White et al., 2012) despite annual visits to the same sites, suggesting that in the driest regions hatching may not occur every year. However, the temporary pools were inundated only in two years during the five years of fieldwork; the other three visits were conducted some time after the rains, when the pools already desiccated (White et al., 2012).

Ambient temperature is seasonally relatively stable in the equatorial areas (25–28°C) but variable in the southern part of the distribution of *Nothobranchius*. We have recorded an annual amplitude ranging from 38°C in summer to 12°C in winter in southern Mozambique. While water has the capacity to buffer the most extreme fluctuations in air temperature, direct measurements using data loggers revealed that daily fluctuations may be similarly large, ranging from a high of 35°C in late afternoon to a low of 20°C before sunrise in a shallow savannah pool in February (Reichard et al., 2009), that is, when *Nothobranchius* in the area were approximately 6 weeks old (Polačik et al., 2011). The deeper parts of the pools (when available) are less prone to daily temperature fluctuations (Figure 9.3a and b). Over six years of fieldwork in Mozambique, we have recorded water temperature (in pools containing *Nothobranchius* at the time of measurement) from 12.8°C (July, winter when most—but not all—pools were dry) to 38.5°C (February, summer, with abundant occurrence

of *Nothobranchius*). Between February and April (i.e., the highest period of *Nothobranchius* occurrence in southern Mozambique), the water temperature rarely fell below 22°C. Even in the equatorial region, *Nothobranchius* often occur at a higher altitude (e.g., 1200 m on Lake Victoria plateau) where ambient temperature also decreases rapidly at night. In coastal areas, daily temperature fluctuation is much lower, but the open savannah habitat is generally more prone to early morning decreases and afternoon peaks in water temperature than the forested areas inhabited by other (nonannual) killifishes.

The duration of the wet season varies among regions, among pools within a region (due to variation in their size and morphology), and among years (Reichard et al., unpublished data). Long-term deployment of temperature loggers set in the upper level of the pool bottom over an entire annual cycle can identify periods of habitat desiccation through daily changes in temperature amplitude. Low amplitude indicates the presence of water, due to its buffering effect on sudden temperature changes. Abrupt changes between day and night indicate a period of desiccation (Figure 9.3c). A long-term data set showed that, at least in southern and central Mozambique, repeated pool desiccation within a season and the existence of secondary pools is relatively common (Polačik et al., 2014a). At the same time, several pools remained dry for the entire rainy season, despite evidence of *Nothobranchius* (i.e., *N. furzeri*, *N. kadleci*) presence in the preceding and following years. Similar conditions may also be common in Sudan, the northern limit of the range of *Nothobranchius*, where

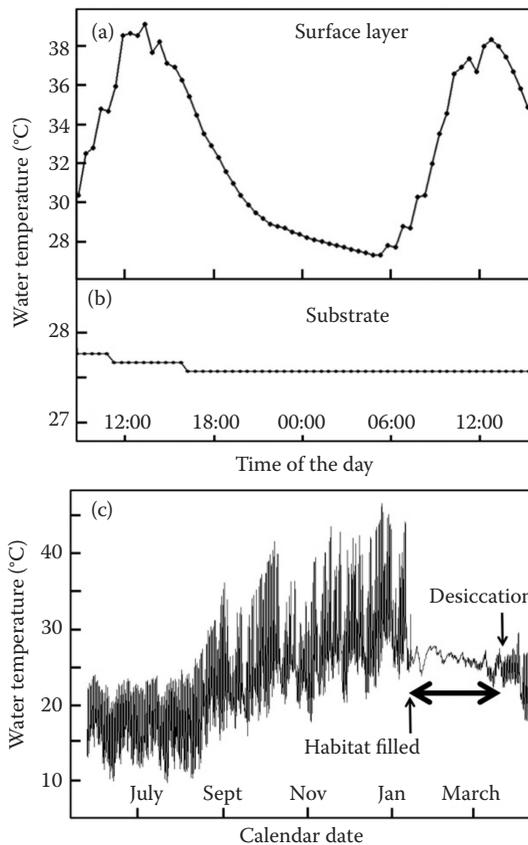


Figure 9.3 Temperature fluctuations recorded by data loggers. Daily variation in water temperature in the upper layer (a) and in the bottom (b) of the pool (water depth 80 cm, lower logger 12 cm inside the substrate) in relatively humid region in southern Mozambique. Seasonal variation in ambient temperature illustrates the duration of habitat inundation (c).

some sites known to host a population of *N. occultus* were dry for almost 10 years. However, it is not clear whether this population still persists (Valdesalici, 2014). In more humid areas of the *Nothobranchius* range, pools are likely to fill reliably every year.

9.1.4 Biogeography

The sister clade to East African *Nothobranchius* consists of *Pronothobranchius* and *Fundulosoma* in West Africa. Currently, the ancestral range of *Nothobranchius* is unknown. The species-rich area along the Tanzanian coast (where 10 different *Nothobranchius* species occur in a radius of less than 100 km) has been assumed as the origin of the genus radiation (Wildekamp, 2004). However, a recent molecular phylogeny of the genus suggested a Nilo-Sudanian origin (Dorn et al., 2014); this is compatible with its sister clade relationship to the West African taxa. This phylogeny is only partly consistent with the former division of *Nothobranchius* into subgenera and species groups (Wildekamp, 2004); furthermore, it suggests convergent evolution of characteristic phenotypic morphs in the genus. Still, these new findings need to be tested with a richer taxon sampling for molecular studies.

Four major clades of *Nothobranchius* have been clearly identified (Dorn et al., 2014). They suggest an entirely allopatric mode of diversification and, even today, their distribution shows very limited secondary overlap. The boundaries of their distribution are largely consistent with the East African Rift system. The dating of their divergence, on the basis of a molecular clock calibrated with the mutation rate of other cyprinodontiform taxa (6–11 mya, mean estimate 8.3 mya), coincides with the aridification of East Africa following its uplifting and with the establishment of savannah habitats between 8 and 5 mya. Alternative dating, using the upsurge of the Ethiopian plateau as the first vicariance event in the genus (approximately 20 mya), suggests a more ancient origin of the genus and its basal diversification in the more humid epoch of the Cenozoic. Each clade went through a period of burst diversification likely driven by climatic oscillations, and extant species are likely of Quaternary origin (Dorn et al., 2014).

Under this recent phylogenetic scenario, the basal clade (called the Northern clade) is distributed across arid areas of northeastern Africa, including Sudan, Somalia, and northeastern Kenya. The Southern clade occurs in the southern periphery of the range of *Nothobranchius*, south of the main rift valley, and the lower Zambezi River, with only a few populations recorded on the northern bank of the Zambezi and along the Shire River, corresponding to the southernmost extension of the rift fault containing Lake Malawi. Phylogeographic analyses suggest that all three species complexes forming the Southern clade colonized their range from north to south (Bartáková et al., 2015). The Northern and Southern clades, unless large extinctions have taken place, have diversified poorly (5–10 species known) compared with the other two clades, especially considering the size of their range. The Inland clade is distributed from Uganda in the north to southern Zambia across the East African plains located at an altitude of about 1000 m. The Coastal clade, the most species-rich clade, is distributed in low-altitude plains between southern Kenya and northern Mozambique (Dorn et al., 2014).

Herein, I propose that species diversity in the Coastal clade may be associated with more rapid geomorphological changes such as river captures, changes in the position of the main channel, and repeated isolation and expansion following the more frequent and dynamic occurrence of forested habitats in this more humid region. All these events create ample opportunities for allopatric diversification as a result of frequent isolation of small populations and consequent mechanisms of diversification such as genetic drift, founder effect, sexual selection, or their combination. It is in this region where the highest species richness per unit area is found.

The intraspecific population structure of *Nothobranchius*, where known, is deep. In the three species complexes of the Southern clade, the distributions of intraspecific lineages and, in some cases, allopatric sister species share similar boundaries across all three lineages. The main river

channels frequently form a barrier to dispersal, and species and intraspecific lineages are confined within areas bordered by two major rivers. In some cases, populations were able to disperse across the main channel and co-occur with other lineages in secondary sympatry. These cases appear more frequent in the lower parts of the rivers, where the topography is flat and suggestive of more frequent changes in the main channel position and overall river dynamics (Bartáková et al., 2015).

9.1.5 Dispersal and Colonization

The typical mode of *Nothobranchius* dispersal and colonization of new habitats is unknown, and different scenarios are possible. First, adult (or juvenile) fish may (actively or passively) enter different water bodies during major catastrophic floods, which irregularly connect adjacent pools (termed *flood dispersal hypothesis, FDH*). This hypothesis predicts that the dispersers will settle successfully in a suitable new habitat. Fishes are sensitive to minor changes in water velocity and are capable of navigating in desiccating flooded areas (Lucas et al., 2001), increasing the chance that adult *N. furzeri* can colonize depressions where water accumulates on a suitable substrate. Second, the eggs may be transported between savannah pools (termed *egg dispersal hypothesis, EDH*). These pools are frequented by large herbivores, including elephants, rhinoceroses, and buffalo, for drinking and mud bathing. The eggs of macroinvertebrates are known to attach on the skin of mud-bathing African megafauna (Vanschoenwinkel et al., 2011) and the same process may apply to *Nothobranchius* eggs, given that a muddy substrate is characteristic of their habitat. A similar scenario is feasible for *water-bird-related dispersal (WRD)*, where the fish eggs are entrained in the mud on birds' feet. The ornamented chorion of annual fishes may facilitate or play role in the latter two dispersal mechanisms, as suggested for Neotropical annual fishes (Loureiro and de Sá, 1996; Fava and Toledo-Piza, 2007).

These three hypotheses make different predictions about the genetic structure of *Nothobranchius* populations. In the case of the FDH, populations should be structured according to river basins (drainages), with no dispersal across major river channels. *Nothobranchius* are poor swimmers, and only passive dispersal would be possible in torrential currents, with little chance of leaving the river channel once entrained. Thus, migration would only be in a downstream direction and genetic diversity would decline with increasing altitude. Under EDH and WRD, dispersal to a higher altitude (upstream dispersal) would be possible, and boundaries between river basins would not form a significant barrier to dispersal. The two hypotheses generate different predictions for the role of river channels. Large rivers would likely wash the *Nothobranchius* eggs off large animals even during the dry season and would thus remain significant barriers to dispersal. In contrast, river channels are less likely to play a major role in structuring *Nothobranchius* populations if their eggs are dispersed by waterbirds. Importantly, these three hypotheses are not mutually exclusive, and fish may disperse via any combination of them.

Given the long history of clade divergences, it is possible that what may appear retrospectively to be dispersal across river channels may actually result from changes in river geomorphology over time (*geomorphological vicariance hypothesis, GVH*). Historical climatic changes in Africa were dramatic (Cohen et al., 2007), with large lakes desiccating and reappearing repeatedly. Similarly, the size and position of the major river channels will have altered in conjunction with changes in climate. The lower reaches on flat plains are particularly prone to changes in river channel morphology, and the majority of apparent channel crossing is expected there. Importantly, if the GVH plays a role, several sympatric annual fish species should disperse concurrently across the channel. Abrupt changes in the position of river channels are a possible explanation for a more recent intraspecific divergence via apparent channel crossing. At an interspecific level, the timing of divergence can coincide with geological events such as uplifting and other tectonic processes associated with rifting that may divide or connect river basins (Watters, 2009).

The basic outlines, predictions, and current evidence for the various dispersal hypotheses are summarized in [Table 9.1](#). The data currently available are not exclusively consistent with alternative

Table 9.1 An Overview of the Main Hypotheses Explaining the Dispersal of African Annual Fishes, Together with Their Mechanisms, Predictions, Current Evidence, and Weakness

Acronym	Hypothesis	Mechanism	Predictions for Genetic Diversity and Its Structuring	Expected Founder Effect	Evidence	Weakness
FDH	Flood dispersal hypothesis	Flood-related displacement of juvenile or adult fish	Higher at lower altitude, importance of river basins, genetic similarity higher in populations along a stream	Strong	Genetic similarity in some adjacent populations ^a	Significant dispersal to a higher altitude inferred from population genetic data ^a
EDH	Embryo dispersal hypothesis	Eggs transported in mud attached to large mammals	Isolation by distance, no role of altitude, no role of river basins, importance of river channels	Strong	Significant dispersal to a higher altitude inferred from population genetic data ^a	Tight suture zone without geographic barrier ^b
WRD	Waterbird-related dispersal hypothesis	Eggs transported on waterbirds' feet	Weak isolation by distance, no role of altitude, no role of river basins, no role of river channels	Strong	Significant dispersal to a higher altitude inferred from population genetic data ^a	Tight suture zone without geographic barrier ^b
GVH	Geomorphological vicariance hypothesis	Changes in river morphology alter the relative position of the pool	Spatially and temporally synchronous estimates of the channel crossing consistent among sympatric lineages	Weak	Synchronous apparent river crossing ^b	Cannot explain dispersal at fine scale, across pools

Note: More details are given in the main text.

^a Bartáková et al. (2013).

^b Bartáková et al. (2015).

hypotheses, and it is likely that the dispersal of African annual fishes resulted from a combination of these processes. In three species complexes in southern and central Mozambique, both river channels and river basins have played important roles in structuring *Nothobranchius* communities (Dorn et al., 2011; Bartáková et al., 2015). Strong evidence of a combination of migration to higher and lower altitudes, calculated from genetic data for *N. furzeri* (Bartáková et al., 2013), lends some support for the EDH. However, a strong suture zone between two intraspecific genetic clades without a clear geographic barrier is not compatible with the EDH (Bartáková et al., 2013). It is also notable that barriers to dispersal are frequently shared among sympatric species (Dorn et al., 2011), including apparent river channel crossing, thus supporting the GDH (Bartáková et al., 2015). In conclusion, I agree with Watters (2006) that a combination of rare adult dispersal and geomorphological processes leading to changes in river drainages appear the most likely processes involved in *Nothobranchius* dispersal at both small and large spatiotemporal scales. While some genetic data indicate upstream dispersal consistent with the EDH, additional data are needed to exclude the possibility of a false population-genetic signal indicating apparent upstream dispersal within a metapopulation.

9.2 SPECIES COEXISTENCE

9.2.1 Community Assembly

Several species of *Nothobranchius* often co-occur syntopically, in the same pool. This contrasts with *Pronothobranchius* and *Callopanchax*, which consist of strictly allopatric species. Up to five species of *Nothobranchius* have been recorded in a single pool, though coexistence of two species is most common (Wildekamp, 2004; Watters, 2006). In the most intensively researched area of *Nothobranchius* in southern Mozambique, three species (i.e., *N. furzeri*, *N. orthonotus*, and *N. pienaarri*) co-occurred in 19 cases (24%), *N. furzeri* co-occurred with *N. orthonotus* in another 19 cases (24%) and with *N. pienaarri* only in three cases (4%). *Nothobranchius furzeri* was the only species in the pool in 31 cases (39%), *N. orthonotus* in two cases (3%), and *N. pienaarri* in three cases (4%). *Nothobranchius orthonotus* and *N. pienaarri* co-occurred three times (4%). Notably, the absence of *N. furzeri* in pools with other *Nothobranchius* species was always recorded at the coastal periphery of its range, where conditions were more humid; so it is possible that these pools were actually outside the range of *N. furzeri*. The distribution of the three species was significantly nested, but the nested pattern was not evident outside the range of *N. furzeri*, where this species is replaced by its sister species and ecological vicariant, *N. kadleci*.

In the lower Rufiji River system in coastal Tanzania, five sympatric species co-occur in pools in various combinations (Watters, 2006); however, no quantitative information is available from this or any other region. There is also interannual variability in the abundance of each species, and hence the probability of capturing all species during a single survey is likewise variable, especially if the number of individuals collected is relatively low. The apparent absence of a certain species (e.g., *N. pienaarri* or *N. orthonotus* in the case of southern Mozambique) from a community in any given year may be the result of the species failure to hatch or survive to adulthood in that year or it may be that the species was present only at very low numbers. There is also variation in community structure within a year that is unlikely to result from a sampling bias (Nagy and Horváth Kis, 2010). In pools along the Tanzanian coast with up to four *Nothobranchius* species, several species disappeared from the community over a short period of 3 weeks (between January and February). This region has two rainy seasons, the long one lasting from March to May and the shorter one from October to mid-December. Sampling therefore likely coincided with the seasonal loss of habitat and, indeed, the water level in most pools had decreased and some had already dried out. However, at least one pool had a higher water level during the second visit, and cichlids were abundant in the

pool despite the disappearance of all *Nothobranchius*, indicating that the pool had been flooded from the adjacent river between the two sampling occasions. During the second visit, the number (and general abundance) of *Nothobranchius* had decreased to a single species (*N. luekei* in the case of species-rich original communities) or disappeared entirely. It is not clear whether environmental conditions had deteriorated to the level where *Nothobranchius* existence was not sustainable (high water temperature and associated factors) or whether they were largely exterminated by predation (Nagy and Horváth Kis, 2010). In at least one case, flooding from an adjacent stream was associated with *Nothobranchius* disappearance. Notwithstanding the cause of annual fishes mortality, this simple case demonstrates how inferences from a single visit to a pool can result in misleading conclusions regarding community structure and population density.

Species that are largely sympatric may nevertheless differ in their specific habitat preferences. In Uganda, sympatric *N. robustus* and *N. ugandensis* were recorded from pools that varied in their water chemistry and location (Nagy, 2010). *Nothobranchius robustus* inhabited relatively cooler (25°C or less), stagnant sections of streams with slightly acidic water, while *N. ugandensis* lived in typical *Nothobranchius* pools with alkaline water and a mean temperature of 28.5°C. The two species co-occurred only in a single pool out of 20 pools containing *Nothobranchius* (Nagy, 2010). Variation in habitat preference at a landscape scale is also apparent in the Mozambican clade. *Nothobranchius furzeri* is a dominant species within *Nothobranchius* communities in the drier region further from the coast. *Nothobranchius pienaari* may inhabit pools in this dry part of the region, but its populations are relatively scarce. In contrast, the species is more abundant in the humid coastal areas where *N. furzeri* is absent. *Nothobranchius orthonotus* is generally the least abundant species in the community, but its frequency is relatively stable across dry and humid regions (Reichard et al., 2009). Unlike the Ugandan species, the Mozambican species are largely syntopic.

Nothobranchius may regularly co-occur with nonannual fishes. While *Nothobranchius* have stable populations in annually desiccating habitats, other freshwater fishes annually colonize these habitats from adjacent permanent water bodies. Lungfishes (see below) and several small species of 'Barbus' (i.e., evolutionary diploid group of *Barbus* sensu lato) syntopic with *Nothobranchius* are frequently captured during sampling (e.g., Valdesalici and Wildekamp, 2004; Watters et al., 2007; Reichard et al., 2009; Reichard, 2010; Valdesalici, 2012; Valdesalici et al., 2012). *Clarias gariepinus* also commonly co-occurs with *Nothobranchius* (Reichard, 2010; Valdesalici et al., 2012) and is presumably their predator. Other teleosts collected with *Nothobranchius* include a mormyrid *Petrocephalus* sp. (Valdesalici et al., 2012), juvenile tilapias (Reichard, 2010), anabantids *Ctenopoma* spp., and nonannual killifishes *Aplocheilichthys* spp. (Larsen, 1999). *Pronothobranchius gambiensis* was recorded co-occurring with 'Barbus' *leonensis*, 'B.' *pobeguini*, *Clarias* sp., mormyrids *Marcusenius senegalensis* and *Petrocephalus bovei*, and *Schilbe intermedius*, though sometimes *P. gambiensis* was the only species in the habitat (M. Reichard, unpublished observation). *Callopanchax sidibeorum* co-occurred with the nonannual killifishes *Epiplatys fasciolatus* and *Sciptaphyosemion geryi* (Sonnenberg and Busch, 2010) and *C. monroviae* shared the habitat with *E. fasciolatus* and *Epiplatys dageti monroviae* (Dinesen, 2006).

Lungfishes (*Protopterus* spp.) are the only other African fish species able to have viable populations in annually desiccating habitats. *Protopterus annectens* frequently coexist with *Nothobranchius* in Mozambique (Reichard et al., 2009) and likely elsewhere. Lungfishes are larger (up to 80 cm) and have a different strategy for surviving annual desiccation from killifishes. They survive the dry period as large juveniles and adults buried in the sediment and spawn soon after the pool is inundated. *Nothobranchius*, at least as adults, are not common prey of *Protopterus*, though we detected one individual (out of approximately 10 dissected) with *Nothobranchius* in its stomach (M. Polačik, unpublished data). Lungfishes' diet consists of crabs, mollusks, and debris (Reichard et al., 2014), avoiding any niche competition with *Nothobranchius*.

9.2.2 Habitat Use

There is little quantitative evidence of habitat separation at the microhabitat scale. This may reflect the inherent difficulty of collecting data on undisturbed fish distribution within a pool because of their small size. Indirect data from sampling in southern Mozambique suggests that *Nothobranchius pienaar* may be more common in heavily vegetated shallow areas (mainly flooded grass at pool margins), whereas *N. orthonotus* may be more common in deeper, unvegetated sections of the pool, and *N. furzeri* is intermediate and occurs in vegetated areas (often associated with water lilies *Nymphaea* spp.) away from the margins of the pool (M. Reichard, R. Blažek, M. Polačik, unpublished observation). However, it is important to note that this observation is not based on a quantitative estimate. Watters (2006) also remarked that co-occurring *Nothobranchius* species inhabit different parts of the pool.

9.2.3 Morphology and Diet

Nothobranchius species have a relatively uniform body shape, and morphological differentiation among species is low. The general body shape and its variation are depicted in [Figure 9.2](#). Typical adult size ranges between 3 and 7 cm. Some species (i.e., *N. ocellatus* and *N. orthonotus*) have the dorsal and anal fin shifted posteriorly, giving the appearance of a lurking predatory fish. Indeed, their morphology is likely associated with their prevalent mode of feeding, the posterior position of the unpaired fins enabling rapid-burst swimming. A group of species, classified in the subgenus *Aphyobranchius* (Wildekamp, 2004), have a more delicate appearance, a larger anal than dorsal fin, and the base of the dorsal fin shifted posteriorly ([Figure 9.2](#)). Species differ also in the position of the mouth, being either terminal or superior (upward pointing) to various degrees. This is apparently associated with their diet and dominant mode of feeding. No *Nothobranchius* species possess the inferior (downward pointing) mouths of specialized benthic feeders.

Most *Nothobranchius* are generalized carnivores, feeding on a range of planktonic and benthic invertebrates (Polačik and Reichard, 2010; Reichard et al., 2010). *Nothobranchius ocellatus* is uniquely a large predator (body size 15 cm), and it may be piscivorous (Wildekamp, 2004). Another specialized mode of feeding has been reported for *N. microlepis*, which uses its specially adapted gill rakers to feed on small planktonic crustaceans and their nauplii (Wildekamp and Haas, 1992). *Nothobranchius jubbi*, a species with a generalized body shape, preys on larger planktonic crustaceans, *Coryxa* nymphs (Hemiptera), and mosquito larvae (Wildekamp, 1983). This was largely confirmed by a quantitative study on other species with a generalized body shape (i.e., *N. furzeri*, *N. kadleci*, *N. pienaar*, and *N. orthonotus*), with crustaceans and coarse insect larvae (Odonata, Ephemeroptera, and Coleoptera) being their primary prey (Polačik and Reichard, 2010).

Co-occurring *Nothobranchius* species demonstrate morphological differentiation (within the limits of low overall variability in the genus). In the Mozambican group, *N. pienaar* has a relatively small superior mouth, and *N. furzeri* is a robust species (5–8 cm) with a larger head and terminal mouth, while *N. orthonotus* can reach the largest body size (5–10 cm) and has a relatively large mouth. Niche separation in resource use among the three species has been confirmed *via* analysis of their gut contents and stable isotopic signatures (Polačik and Reichard, 2010; Polačik et al., 2014b). *Nothobranchius orthonotus* had the most distinct diet, which included small tadpoles and lungfish, although no teleost fishes were identified. The other two species feed predominantly on small crustaceans (Cladocera, Copepoda, Ostracoda, and Conchostraca). Mosquito larvae formed a negligible part of the diet but were also uncommon in the pools. The diet differentiation between *N. furzeri* and *N. pienaar* was more evident in their isotopic signatures than their stomach contents, demonstrating that ontogenetic changes in the diet play an important role in resource partitioning. It should be emphasized that there was considerable overlap in the diet, and differentiation among species was only evident when resource abundance and diversity was high. In resource-poor habitats, all species

consumed the same resources (Polačik and Reichard, 2010; Polačik et al., 2014b). This confirms that most *Nothobranchius* are generalized predators of small aquatic invertebrates.

9.3 POPULATION ECOLOGY

9.3.1 Population Genetic Structure

Many *Nothobranchius* populations are discrete units, with little regular (i.e., annual) dispersal between adjacent populations. In more humid regions, it is likely that populations from adjacent pools form a metapopulation, with a common exchange of individuals when the water level is high and several pools are connected within a larger floodplain. Such metapopulation dynamics can be indirectly inferred by the presence of nonannual species in a habitat. Population genetic data are consistent with this assumption. Most populations (defined as individuals inhabiting one savannah pool) are genetically distinct, that is, demonstrating significant differences in F_{ST} estimates. This includes spatially adjacent populations (Bartáková et al., 2013). In *N. furzeri*, significant genetic differentiation was found between populations located only 1.45 km ($F_{ST} = 0.015$, $P > 0.05$) and 1.25 km apart ($F_{ST} = 0.048$, $P > 0.05$) and even 0.25 km ($F_{ST} = 0.009$, $P > 0.05$) in *N. kadleci*.

In a larger-scale study across the entire range of the three Mozambican species groups, out of all possible pairwise differences among 26, 29, and 41 populations (i.e., 325–703 pairwise values for each species group), only 2–6 population pairs had nonsignificant F_{ST} estimates, indicating strong genetic structuring among most populations (Bartáková et al., 2015). It should be noted that adjacent populations (pools <5 km apart) were typically omitted from the sampling (with the exceptions described above), as the main aim of the analysis was to provide an overview of the population genetics across the range. The groups of populations with nonsignificant F_{ST} values were located either in flat plains near the coast (altitude <10 m) or in the floodplain of the same stream, despite a relatively large geographic distance (up to 75 km, populations from altitudes of 126–74 m) in the latter case. This suggests that downstream dispersal during major floods can maintain metapopulation dynamics even in savannah pools outside the alluvia of major rivers and along a relatively steep gradient. However, in this case, only unidirectional downstream dispersal is possible. There are no data on the population genetic structures of other African annual fishes.

9.3.2 Age Structure

The presence of a single cohort and hence a very simple age structure among *Nothobranchius* populations is another distinctive feature of African annual fishes. Exact birth date can be read from otoliths, that is, mineralized aragonite structures found in the inner ears of fishes, in which daily increments are deposited. In our years of research in southern Mozambique, *Nothobranchius* hatching has coincided with the arrival of heavy monsoonal precipitation. The fish typically hatch between mid-December and mid-January (Polačik et al., 2011). The onset of the rainy season in Mozambique is earlier, with rains beginning in October and peaking in December and January (Figure 9.4). It is possible that the pools are not inundated until the height of the monsoon (with rainfall of up to 80 mm per day; Figure 9.4). However, our data from temperature loggers deposited in the pools suggests the pools are frequently inundated earlier than December but then undergo rapid desiccation, usually within few days. It is not known whether such precipitation induces the hatching of some fish that will not survive until the main inundation of the pool, but so far we have no evidence that this is the case (Polačik et al., 2011). Instead, these initial rains may trigger embryos from diapause through environmental perturbation (e.g., moistened substrate, altered oxygen levels, and partial pressure) and partly dissolve the hardened substratum to enable rapid

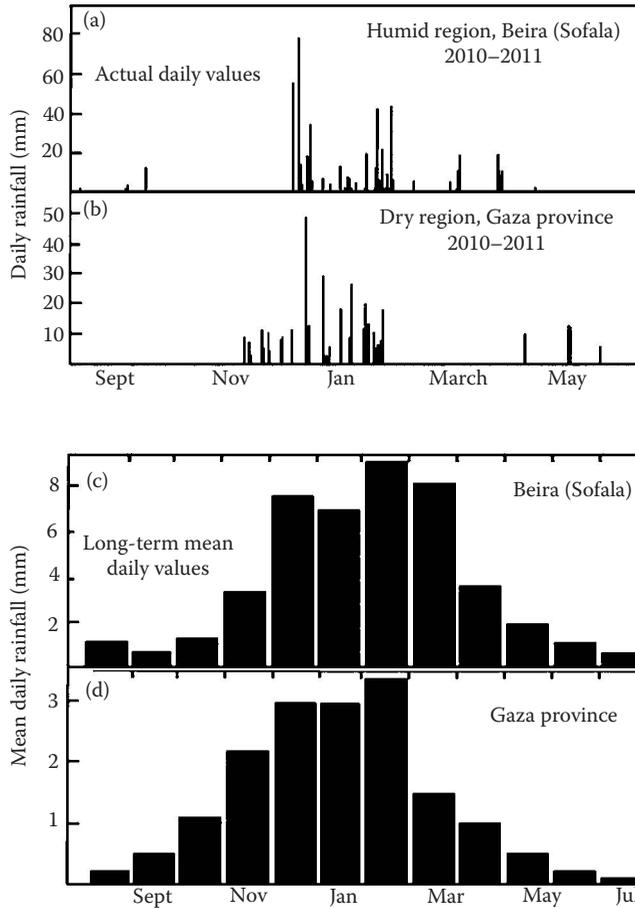


Figure 9.4 Annual precipitation dynamics in humid region (data from the city of Beira) and dry region (Mabalane and Chigubo meteorological stations) of Mozambique. Precipitation pattern expressed as actual daily record over a single annual cycle (a, b) and long-term daily mean values (c, d) illustrate that seasonal precipitation is typically clustered into a few bouts of heavy rains. (Data from National Weather Service, 2014. Climate prediction center. http://www.cpc.noaa.gov/products/fews/AFR_CLIM/GRAPHS/africa.html.)

hatching during the subsequent flooding of the habitat (Watters, 2009). Slight interindividual variation in birth dates can be ascribed to the gradual inundation of a pool, where the egg banks located in different parts of the pool are submerged at different times. The greatest age difference among fishes in the same population was 25 days, with a typical range of 10–14 days (Polačik et al., 2011). It would be extremely interesting to obtain data from different *Nothobranchius* species, particularly from the equatorial region, where two rainy seasons may effectively double the generation time if the habitats are regularly desiccated in the intervening dry seasons. This is more likely to occur in the drier equatorial regions, such as at higher altitudes. Alternatively, given a metapopulation structure, two rainy seasons may result in two overlapping age cohorts (or generations). Indeed, there is at least one observation of *N. melanospilus* from the equatorial region in Tanzania, where two size cohorts, an abundant cohort of 3- to 5-cm large fish and several larger individuals of 8–12 cm, were found coexisting in a single pool (Shidlovskiy, 2010). One population of *Callopanchax sidibeorum* was reported to consist of a single age cohort in one year but a wide size spectrum of juveniles and adults in another year (Sonnenberg and Busch, 2010).

9.3.3 Population Size, Sex Ratio, and Their Temporal Dynamics

The population size (abundance) of *Nothobranchius* varies greatly among populations but also within populations among different years. In our study sites in Mozambique, population sizes were generally congruent among years. Where annual fishes were rare, they were typically proven to be relatively rare across multiple years. In contrast, habitats with high fish abundance tended to support abundant populations across years. Obviously, there was considerable interannual variation, but this can generally be ascribed to environmental conditions and demographic stochasticity. Estimates of total population size for individual pools were made using capture-mark-recapture methods and depletion sampling. The calculated estimates were within the range of 116–216 fishes in a 1800 m² pool (density of 0.1 fishes per m²) to 1750–1956 individuals in a 875 m² pool (density of 2 fishes per m²). Many larger pools conceivably contained larger fish populations, on the order of two to three magnitudes, and there were also pools with a higher density of fishes; recapture-based population estimates in these pools were logistically impossible. These estimates clearly indicate that population sizes of *Nothobranchius* may vary by several orders of magnitude.

From a population genetic perspective, some populations departed from Hardy–Weinberg equilibrium and were likely cases of recent colonization or a severe bottleneck (Bartáková et al., 2013). Indeed, these populations were located in a narrow (15-m wide) strip between a road and railway embankment, strongly suggesting recent human influence on their structure.

Nothobranchius reach sexual maturity at 3–7 weeks after hatching, with a record successful reproduction at 17 days in *N. kadleci* and 18 days in *N. furzeri* (Blažek et al., 2013). Sexually mature individuals of *N. furzeri* approximately 3 weeks old were collected in the field (Polačik et al., 2011), and I anticipate that sexual maturity could be reliably attained earlier than in 17 days under natural conditions with abundant food. Both males and females continue growing rapidly after sexual maturity and only attain the inflexion point in their growth curve later in adulthood. The increase in age and body size is associated with an increase in fecundity, from an initial production of 2–10 eggs to hundreds of eggs produced each day when resources are abundant. In the longer term, both in the wild and in captivity, fecundity of 20–50 eggs per day is observed, at least in the group of Mozambican species (Blažek et al., 2013; Vrtílek and Reichard, 2015; Vrtílek and Reichard, unpublished data). It should be noted that *Nothobranchius* are extreme income breeders (Vrtílek and Reichard, 2015), and a positive association between female size and fecundity is only retained under analogous environmental and ration conditions (see below).

There is striking sex-ratio bias among adult *Nothobranchius* in the wild. At the same time, no such sex bias is observed in the same populations when held in captivity (Reichard et al., 2014). This clearly implies selective male mortality in the wild. Males of *N. guentheri* are more often targets of heron attacks than females under seminatural conditions (Haas, 1976). A recent study found that adult sex-ratio bias is lower at sites lacking giant predatory hemipterans (that are capable of preying on *Nothobranchius*), with clear water and abundant vegetation, and argued that complex vegetation protects males from strong predation (Reichard et al., 2014). However, the environmental predictors of sex bias varied among sympatric species, severely limiting their generalization. Another explanation for increased male mortality stems from male aggression (Reichard et al., 2014; Polačik and Podrabsky, 2015). Male–male competition is fierce in most *Nothobranchius* species and commonly results in the death of the weaker opponent, at least under the spatially restrictive conditions of captivity (Polačik and Reichard, 2011).

The sex-ratio bias becomes apparent soon after sexual maturity suggesting that male-biased mortality may start even before the onset of sexual maturity, possibly due to inherently higher frailty of males. In contrast, in replicated populations of the Neotropical annual fish *Austrolebias reicherti*, equal sex ratios across populations at the start of adulthood was found, with a decrease in male abundance later in the season (Passos et al., 2014). It is clear that male-biased mortality is

only expressed under natural conditions and therefore must be mediated by predators, interactions among males, and/or challenging environmental conditions. In Trinidadian guppies, it was shown that there are bouts of increased male-specific mortality in particular periods (in the case of guppies during flood events associated with water turbulence and turbidity) rather than the successive stable disappearance of one sex from the adult population due to its consistently higher mortality (Arendt et al., 2014). Whether a similar process is also applicable to annual fishes is unclear.

The seasonal decline in *Nothobranchius* abundance within populations (in both sexes) could provide an interesting insight into sources of their mortality. The pattern of adult mortality has important consequences for the evolution of aging. This is of great interest, because *Nothobranchius* have become a popular model in aging research (Chapter 6). There are three basic scenarios. First, there may be strong adult mortality due to predation, diseases, or intraspecific competition for scarce resources, and *Nothobranchius* populations may steadily decrease in abundance with increasing age. Second, population abundance may decline only negligibly, mainly due to accidental deaths, and most individuals die only during habitat desiccation. Third, there may be an initial decline in adult abundance due to density-dependent population effects, but little mortality afterward until habitat desiccation (Cellerino et al., in press). This distinction is not trivial, because each scenario sets different predictions for the evolution of aging and corresponding life history traits such as investment in reproduction, growth, and immunity. At present, we only know that male mortality is higher than that of females. This means that each sex has a different life expectancy. Research into the population dynamics of *Nothobranchius* fishes in the wild is needed and will provide an excellent opportunity to advance the fundamental understanding of *Nothobranchius* aging.

9.3.4 Ecology of Embryonic Development

The embryonic stage is a relatively long period of *Nothobranchius*' life, and in some species even the longest. This is a notable feature, particularly given their brief posthatching lifespan. Any evolutionary and ecologically relevant calculation of generation time should take account of the duration of the embryonic period. Embryo development, including characteristic diapauses (i.e., forms of developmental arrest) is strikingly similar between African and Neotropical annual fishes (see Chapter 2). It is notable that the presence of diapauses (and hence the ability to complete the annual life cycle) is considered an ancestral trait in killifish phylogeny (Murphy and Collier, 1997), though a recent analysis suggested that diapauses evolved in at least six killifish lineages in parallel (Furness et al., 2015).

Under standard circumstances, a fertilized *Nothobranchius* egg is deposited in the pool substrate, where it remains until the pool desiccates. The egg's chorion is covered by short, evenly dispersed filaments. Their density, length, and shape are largely species specific (Wildekamp, 2004), and they can be potentially used in species identification from a sample of eggs in the substrate. Functionally, they create a narrow space between the egg and the surrounding sediment, enabling gas exchange during development (Wildekamp, 2004). Environmental conditions change dramatically with the disappearance of the water, and the embryo survives in the egg envelope with a thickened chorion, remaining in diapause over a protracted period of drought until eventually hatching when the habitat is inundated. This simple scenario has several alternative pathways, which are considered further below.

The timespan of several weeks to months when the eggs are deposited in the sediment while water is still present in the pool is certainly much longer than the minimum time needed for completion of embryogenesis and hatching (12–30 days depending on the temperature). At the same time, gradual hatching has never been observed in *Nothobranchius*. After a brief initial development phase, the embryo may be arrested in diapause (diapause I). The presence of adult fish effectively halts embryo development beyond diapause (Inglisma et al., 1981). In the laboratory, embryos kept in aqueous aerobic media at high temperatures without the presence of adults (shallow water in Petri dishes) may omit

this diapause and continue to develop. *Nothobranchius furzeri* embryos can reach the fully developed stage at 12–18 days at high ambient temperature (28°C; Valenzano et al., 2011). Such rapid development, however, may incur significant cost to posthatching viability (R. Blažek, unpublished data).

During the standard developmental trajectory, embryos can undergo a total of three diapauses (Wourms, 1972; Chapter 2). However, all these are facultative and can be omitted, at least under laboratory conditions (Blažek et al., 2013; Polačik et al., 2014a). The duration of each diapause is not constant among embryos, even if they are full siblings fertilized on the same day and incubated under identical conditions. The variability in the timing of embryo development is enormous and certainly cannot be ascribed solely to nonhomogeneous microenvironments within incubation media. Further, there are some general trends, despite a high level of stochasticity in the process. In *N. furzeri* and the Neotropical *Austrofundulus limnaeus*, young females (more precisely females laying their first eggs) tend to produce more embryos with very rapid development; later in life, the same females (paired with the same male) tend to produce more embryos that enter diapause (Podrabsky et al., 2010; M. Polačik et al., unpublished data). This trend may be apparent even among eggs produced only a few days after the first eggs. M. Polačik et al. (unpublished data) suggest “an internal reproductive effort counter” that females use to epigenetically modify the fate of embryo development. However, environmental cues also likely play a role, and further experiments are needed to fully understand the cues females use for embryo development modification (see also Chapter 4). A tentative conclusion is that embryos enter alternative developmental trajectories and likely often do so under natural conditions.

The process is clearly of epigenetic origin, and an understanding of the mechanisms involved would be of great significance and interest for biomedical research. How is the functional expression of certain genes—and which ones—silenced or promoted by maternal epigenetic marks? Are RNAi, microRNA, histones, alternative splicing, or any other mechanisms involved? To what extent does the process remain stochastic and to what extent is it deterministic? What are the relative roles of environmental factors, epigenetic background, and their interaction? To what extent can environmental conditions affect the process of embryogenesis once it has started? These are questions that we are only beginning to learn how to answer methodologically, despite solid developmental background information (Furness et al., 2015; Chapter 2). Such a research agenda is undoubtedly one of the areas where annual fishes can provide general insights into an enigmatic biological phenomenon.

Environmental conditions certainly play a nonnegligible role in embryo development. Under natural conditions, embryos likely persist under anoxic conditions during the first weeks after the pool becomes desiccated. In an anoxic environment, all embryos are likely in diapause; any non-diapausing embryos would die. Reduced oxygen content causes the embryo to enter diapause I as early as 3 days after fertilization during the dispersed phase of development at the end of epiboly (Wourms, 1972). This diapause is rarely observed under laboratory conditions (Valenzano et al., 2011; Dolfi et al., 2014), but this may be an experimental artifact, since reduced oxygen conditions are not compatible with standard observation in aqueous media under a stereomicroscope. This indirectly suggests that this early embryogenesis may be more deterministic and dependent on environmental conditions. Still, some embryos enter diapause I even under oxygenated laboratory conditions. Given that the substrate in *Nothobranchius* habitats is likely to be mostly anoxic (Watters, 2009), most embryos should enter diapause I under standard natural conditions. Sometimes the eggs are deposited on aerobic substrates such as sand or mud soon after pool inundation (Watters, 2009) and hence may also continue their development without diapause I in the wild.

Embryos can exit diapause I after further environmental perturbation (with the potential role of epigenetic effects). Such perturbation can be related to the aerobic phase of the sediment, when air starts to penetrate desiccated sediment after several weeks or months (Watters, 2009). It takes less than 1 week to complete a phase of active development (somitogenesis, formation of neural keel, functional tubular heart) between diapause I and diapause II (Wourms, 1972). Natural conditions at this stage of development are aerobic but nonaqueous, and it is most likely that such conditions favor

entrance into diapause II (Watters, 2009). In this stage, the embryo is most resistant to environmental challenges and can persist up to several years (Podrabsky et al., 2010). Further development is likely triggered by the onset of the rainy season and saturation of the substrate. Embryos may later enter diapause III, in which they have all the structures of juvenile fish but their metabolism is considerably reduced. While this diapause is obligatory in *Austrofundulus* (Berois et al., 2014), its presence in *Nothobranchius* is unclear (Wourms, 1972; Watters, 2009; Cellerino et al., in press). Again, it may be common under natural conditions but difficult to recognize under laboratory conditions.

In summary, *Nothobranchius* embryo development is extremely variable, even within a single clutch of eggs produced by the same parents and deposited on the same substrate. A suite of environmental conditions and epigenetic effects interact to modulate embryo development and ultimately lead to an effective bet-hedging strategy ensuring that the embryos follow divergent developmental trajectories. This is an excellent adaptation to the unpredictability of precipitation and interannual variation in pool existence and duration.

9.4 ALTERNATIVE PHENOTYPES, MALE COLOR MORPHS, AND SEX DIFFERENCES

9.4.1 Alternative Phenotypes

Intriguingly, the alternative developmental trajectories that the embryo can follow (outlined above) are mirrored in its posthatching phenotype. Using a population of *N. furzeri*, Polačik et al. (2014a) provided experimental evidence that a short embryonic period (maximum of 50 days at 22°C, likely no diapause) produced phenotypes with a more rapid life history than those with a long embryonic period (200 days at 22°C, including a substantial period in diapause stages). Individuals that completed their development in 50 days were smaller at hatching but had larger residual yolk stores. The individuals with rapid embryonic development continued to grow more rapidly after hatching and exceeded the size of those individuals with long embryo development as early as six days posthatching. The individuals with rapid embryo development clearly progressed more rapidly through every life history stage. They reached sexual maturity earlier and were shorter lived, and their final body size was smaller. Such ability to produce alternative phenotypes appears unique among vertebrates. It illustrates that the slow to rapid pace-of-life pattern, known from comparisons among taxa and between populations (e.g., Bronikowski, 2008; Jones et al., 2008), can also be recognized at the intrapopulation level.

In nature, any annual fish that omit all diapauses are destined to inhabit secondary pools, emerging in the savannah following an occasional second wave of heavy precipitation after the primary pools have desiccated. There is evidence that this happens in Mozambique (Polačik et al., 2014a), and extremely rapid life history is then adaptive, allowing individuals to maximize reproductive success in secondary pools that are inherently more prone to rapid desiccation because they appear toward the end of the rainy season (Polačik et al., 2014a). Rapidly desiccating pools with relatively young fish were also reported in *N. robustus* in Uganda (Nagy, 2010) and may be more common than currently assumed. The mechanism permitting rapidly developing individuals to grow at a faster rate is unknown. It may be supported by the extra portion of yolk reserve that is found in rapidly developing embryos at hatching that promotes rapid initial growth. Alternatively, the epigenetic mechanism that caused the embryos to depart from the diapause-inclusive trajectory may also act on posthatching phenotypes and modify the expression level of growth-associated genetic pathways.

9.4.2 Sex Differences

Nothobranchius males and females differ in body size, coloration (Wildekamp, 2004), and life expectancy in the wild (Reichard et al., 2014). No differences in the diet or any other niche axis were

recorded. Intersexual differences in body size are negligible in some species (e.g., *N. orthonotus*) but reach 30% in others (e.g., *N. furzeri*, *N. kadleci*, and *N. pienaar*) (Polačik et al., 2011, 2014a). From an evolutionary perspective, larger body size in males is likely related to sexual selection and strong male hierarchy where successful competition for access to females is a major determinant of reproductive success. The most striking intersexual difference is in coloration. Females are pale, typically light brown, and in some species possess dark (e.g., *N. melanospilus* species group, *N. kadleci*, and *N. orthonotus*) or blue iridescent (e.g., *N. rachovii* species group) spots. In *N. ocellatus*, females possess a single large spot on the caudal peduncle. In *Pronothobranchius* and *Callopanchax* females are ornamented with red dots dispersed across the body and on the unpaired fins. *Nothobranchius* fishes appear to have escaped genetic linkage between male and female coloration, enabling more intensive male coloration to evolve without any selective constraint on female cryptic coloration (Sedláček et al., 2014).

9.4.3 Male Color Morphs

Males of several *Nothobranchius* and *Callopanchax* species occur in two or more color morphs. Such polymorphism is relatively widespread in African killifishes and occurs in *Fundulopanchax* and *Aphyosemion*. Typically, males are dimorphic, with red and blue or red and yellow morphs. In some cases, these color morphs may be largely sympatric and syntopic (e.g., in *N. furzeri*, *N. orthonotus*, and *N. ugandensis*), in others they appear sympatric but not syntopic despite considerable sampling effort (e.g., *N. eggersi*; Valdesalici, 2010), while no geographic sympatry is recorded in others (Wildekamp, 2004). Sometimes, color morphs represent separate, closely related species (e.g., *N. rachovii* species complex: *N. rachovii*—blue morph, *N. krysanovi*—red morph, and *N. pienaar*—black morph), and the species are indeed effectively reproductively isolated due to chromosomal rearrangements (Shidlovskiy et al., 2010). Yet in other cases, male color morphs are not discrete, and there is intergradation of colors. In *N. korthausae*, populations with pure red males, pure yellow males, and intermediate forms with a mixture of red and yellow forms have been reported (Wildekamp, 2004; Nagy, 2008; Reichard and Polačik, 2010). Likely, the apparent lack of male polymorphism in some species may be a consequence of low sampling effort in their respective ranges, and male polymorphism may be more widespread in *Nothobranchius* than currently understood. Given that species diagnosis is often based solely on male coloration, it is imperative to consider intraspecific male polymorphism during species descriptions.

It is intriguing to view male coloration as either directly adaptive or as a trait linked to some adaptive phenotypic feature. There is ample evidence from other fish taxa as well as from all other vertebrate groups, insects and other invertebrates animals, and even plants that color polymorphism is often related to traits critical to fitness (McLean and Stuart-Fox, 2014). The coexistence of two or more color morphs is then related to variation in environmental conditions or complex demographic processes (Sinervo and Lively, 1996). In other cases, color polymorphism may have evolved due to genetic drift or geographic isolation and may not be associated with adaptation (McLean and Stuart-Fox, 2014). It remains to be investigated whether male coloration in *Nothobranchius* is linked to other phenotypic traits and whether male polymorphism is adaptive.

9.5 LIFE HISTORY ADAPTATIONS

9.5.1 Rapid Growth and Sexual Maturation

The environment of *Nothobranchius* and other African annual fishes imposes strong selective pressure on a network of adaptations promoting extremely rapid life history. Rapid growth and sexual maturation is perhaps the most apparent and is exemplified in species from the drier parts of the distribution. In the south, *N. kadleci* and *N. furzeri* were reported to reach sexual maturity in captivity

as early as 17 and 18 days, respectively (Blažek et al., 2013). The youngest wild fish with an age estimate (from daily increments on otoliths) were 19 days old (Polačik et al., 2011) and possessed the coloration of sexually mature individuals. No younger fish were collected for age estimation. While histological confirmation of sexual maturity (i.e., the presence of ripe gametes) was not made in this sample, I suggest it is likely that sexual maturation in the wild can be attained even earlier than 17 days when resources are abundant. Furthermore, it is very likely that species from the northern periphery of the range of *Nothobranchius*, for example, *N. rubroreticulatus*, *N. bellemansi*, *N. occultus*, *N. microlepis*, and *N. bojiensis*, may be capable of even more rapid sexual maturation. This suggestion is based on the fact that these species face even drier and less predictable environmental conditions (Valdesalici, 2014), and consequently there would be a selective advantage in reaching sexual maturity even sooner.

The daily growth rate is faster during the second week of life, where a rate of 2.7 mm per day (constituting an increase of 23% in the body size per day) has been recorded in *N. furzeri* (Blažek et al., 2013). Under the conditions reported in Blažek et al. (2013), the steep growth continues after sexual maturation and levels off later at the age of 35 days. This underscores the extreme demands of *Nothobranchius*' life history. The rate of fish growth normally decreases upon sexual maturity (Wootton, 1990). In *Nothobranchius*, the risk of habitat desiccation provides strong pressure for early reproduction, requiring rapid growth. The resolution of the trade-off between current and future fecundity apparently results in a compromise when reproduction starts early, but there is "maturity of maturation" when fecundity increases sharply with age along with body size (Blažek et al., 2013). Females therefore initially produce small clutches of few eggs per day and only reach full fecundity when their rapid growth decelerates. Males also keep growing steadily after sexual maturity, until a break point in their growth is reached. Consequently, there are clear intersexual differences in growth rates. Males start to grow more rapidly than females from the age of approximately 2 weeks (Polačik et al., 2014a), which may be associated with the start of allocation to reproduction. Alternatively, the difference in growth rates can be related to hormonal changes, affecting juvenile development in general.

9.5.2 Immunity and Diseases

Allocation of resources to rapid growth and reproduction is disproportionately high in *Nothobranchius* fishes. Life history theory (Stearns, 1992) predicts that such allocation should come at the cost of self-maintenance. A weaker ability to cope with immune challenges is therefore predicted for annual fishes. This prediction has remained largely untested, and our current knowledge of how *Nothobranchius* cope with immune challenges is very limited. Observational evidence from captivity demonstrates that *Nothobranchius* are very susceptible to infection by *Piscinoodinium* sp. (a dinoflagellate protozoan) and *Glugea* sp. (a microsporidian). While *Piscinoodinium* infection can be effectively treated by a sudden osmotic shock that kills the parasite but leaves *Nothobranchius* fish unharmed, *Glugea* infection is untreatable, decreases host vigor and reproductive ability, and may become fatal. Fish in poor physiological condition are more susceptible to the negative effects of parasitic infections, indirectly indicating that immune response is at least partly efficient (M. Reichard, personal observation).

In the wild, *Nothobranchius* serve as an intermediate host for several species of internal parasite. Examination of adult individuals from 14 populations of *N. furzeri*, *N. kadleci*, *N. orthonotus*, and *N. pienaar* (Micháľková et al., unpublished data) yielded a relatively high diversity and abundance of endoparasites (internal parasites). Across all species, the authors identified seven species of metacercariae (larval stage of trematodes), three species of nematodes, four species of larval cestodes, and one species of adult trematode in the host digestive tracts. The only ectoparasites (external parasites) were two individual unidentified leeches. This demonstrates that *Nothobranchius* are common intermediate hosts of flukes (metacercariae), the definitive hosts being waterbirds that need to consume *Nothobranchius* to be infected.

One fluke species (*Apatemon* sp.) is of special interest, because it affects fish behavior. It has only been recorded in some savannah pools inhabited by *N. furzeri*. When present, it was located either inside the brain tissue or between the skull and brain, with an intensity of up to 10 individuals per host. Its presence can easily be predicted at the commencement of sampling, because *Nothobranchius* infected by *Apatemon* metacercariae stay near the water surface and do not escape. Instead, they frequently jump out of the water and occasionally remain exposed on the leaves of water lilies. This makes them an easy target for piscivorous birds. When imported to the lab, infected fish failed to escape in a simulated predator attack, in sharp contrast to noninfected fish. There were further behavioral and routine activity disruptions making infected fishes more susceptible to a simulated bird attack (Micháľková et al., unpublished data). Such a suite of behaviors ultimately leads to increased transmission of the parasite between its intermediate (*Nothobranchius*) and definitive (bird) hosts. Such effects of parasites on fish behavior are known from other host–parasite systems, but our understanding of the mechanisms underlying the process remains poor (reviewed in Barber et al., 2000). The major advantage that *Nothobranchius* offers is the availability of a detailed anatomical map of the *N. furzeri* brain (D'Angelo, 2013). With this aid, it may be possible to associate the location of the parasite with the brain region responsible for a particular functional response. This may help to test whether the physical location of the parasite is important in inducing the changes in host behavior, for instance *via* mechanical stimulation. Alternatively, host behavior may be altered *via* secretion of a chemical substance, making the precise position of the parasite within the host brain less important.

Investment in self-maintenance also includes the ability to cope with internal damage and retain homeostasis, maintaining organ and cellular function and dealing with metabolic waste products. A high incidence of neoplasias (liver tumors in particular) has been reported for several *Nothobranchius* species (Di Cicco et al., 2011), despite being generally rare in teleosts. Other damage at the organ level includes various types of lesions in the kidney, liver, and heart (Di Cicco et al., 2011). These signatures of internal damage are generally associated with aging and are discussed in detail in Chapter 6.

9.5.3 Phenotypic Plasticity

The single overarching feature of *Nothobranchius*' life history is its extremely high level of phenotypic plasticity. This is adaptive, as variation in environmental conditions and population density may be enormous, exposing the same genotypes to a potentially wide range of conditions. The flexibility of the genotypes to produce phenotypic traits according to the prevailing environmental conditions (i.e., strong gene by environment interaction) enables fine-tuning of individual traits to achieve optimal values across conditions.

Some phenotypic traits are irreversible and respond to the conditions prior to their expression. Sexual maturation is the simplest example. Sexual maturity can be achieved in less than 3 weeks when abiotic conditions (e.g., ambient temperature) are optimal and resources (e.g., food ration) plentiful (Blažek et al., 2013). In the same species, and conceivably in the same genotype, time to sexual maturity may be doubled and reached as late as in 5–6 weeks posthatching (Graf et al., 2010), when population density is high, food ration low, or environmental conditions suboptimal. There is also variation in sexual maturity (and growth rate) among individuals due to social interactions, likely modulated by stress hormones in subordinate individuals. In captivity, dominant individuals often inhibit the growth of subordinate individuals, and removal of the dominant individual is followed by rapid growth of the fish that resumes dominant rank.

Other phenotypic traits remain flexible throughout life. Most notably, in *N. furzeri*, fecundity responds strongly to ration manipulation. Actual fecundity was tightly associated with actual ration, and females undergoing changes in ration level tracked current conditions (Vrτίlek and Reichard, 2015). Surprisingly, there was no apparent cost of compensatory growth on female fecundity, at least in a time

span of 6 weeks. Females fully compensated for their smaller body size (and mass) after 6 weeks on a high ration and produced the same number of eggs as females fed a high ration throughout their lives (Vrtflek and Reichard, 2015). The fecundity of *N. furzeri* females was predicted to vary among populations across an aridity gradient, with a higher allocation to reproduction in populations from the drier parts of the range (where life expectancy is lower due to shorter habitat existence). However, this prediction was not confirmed in wild populations (Vrtflek and Reichard, unpublished data). The two most likely explanations are (1) interannual variation overrides any adaptive value of differential allocation between populations by habitat and (2) high importance of resource availability due to population density leads to high flexibility in fecundity traits overriding any interpopulation differences. Laboratory experiments in which females from several populations are housed in common environmental conditions may illuminate interpopulation differences in fecundity. These experiments are being undertaken.

Egg size is variable across *Nothobranchius* species. In *N. janpapi*, the egg diameter is only 0.65 mm; in *N. furzeri*, it is typically 1.2 mm; and in *N. ocellatus*, it is 2.5–3 mm (Larsen, 1999; Wildekamp, 2004; Vrtflek and Reichard, 2015). Egg size is related to the size of adult fishes rather than environmental conditions and may retain a phylogenetic signal, with closely related species having similar egg sizes. The size of the egg is apparently unrelated to its ability to survive habitat desiccation, at least at an interspecific level. Species with the largest (*N. ocellatus*) and smallest egg sizes (*N. luekei*, only 25% of the egg diameter of *N. ocellatus*) coexist in the same pools. Despite this, there is significant variation in egg size at the interpopulation level in *N. furzeri*. Wild females from populations in the drier part of the range (with a shorter life expectancy) had smaller eggs than females from populations in the humid part of the range (Vrtflek and Reichard, unpublished data). This variation is perhaps associated with the trade-off between egg size and number rather than being directly adaptive.

Egg size is also flexible within a population. Captive *N. furzeri* females receiving a low ration (i.e., under less favorable conditions) produced relatively larger eggs, at the expense of their number (Vrtflek and Reichard, 2015). This is in accordance with adaptive maternal effects but was not expected in annual fishes with unpredictable interannual variability in conditions. Whether this variability reflects *Nothobranchius*' phylogenetic history (retained ancestral trait) or indeed represents an adaptation is unclear.

9.6 SUMMARY AND CONCLUSIONS

Most information on the ecology of African annual fishes comes from research on the genus *Nothobranchius*. There is a mix of reports from collecting trips by hobbyists and aid workers (often themselves non-professional scientists), scientific field expeditions, and experimental work in the laboratory. All three avenues have provided unique insights and are important sources of our current understanding of *Nothobranchius* ecology. Given the popularity of collecting *Nothobranchius* populations in the wild, there is a considerable scope for involvement in citizen science by sharing data on habitat conditions, community assembly, and population parameters (species co-occurrence, sex ratio, body size), and by accumulating samples for phylogenetic and phylogeographic analyses. Such collaboration between scientists and hobbyists may prove instrumental in acquiring a comprehensive data set and considerably advance our knowledge of annual fishes ecology and evolution.

I hope that our ongoing research on the Southern clade of *Nothobranchius* (*sensu* Dorn et al., 2014) will ultimately be extended to include a larger geographic area. Most of our current inferences relate to this clade of annual fishes and to *N. furzeri* in particular. This is because this species has become a model for the biology of aging and several related disciplines (Chapter 6). The methodological resources developed for this species have made it relatively easy to utilize state-of-the-art tools for research on other annual fish species.

The most characteristic features of African annual fishes ecology are linked to their habitat. *Nothobranchius* have rapid life history and short lifespan, both in the wild and in captivity.

Nothobranchius kadleci and *N. furzeri* have shown the most rapid sexual maturation and generation time of any vertebrate species recorded (Blažek et al., 2013). One captive population of *N. furzeri* (GRZ strain) was reported to have a median lifespan of 9 weeks and a maximum lifespan as short as 12 weeks (Valdesalici and Cellerino, 2003). While this has subsequently been shown to be particularly short even for *N. furzeri*, a median lifespan in a range of 20–43 weeks and maximum lifespan of 25–67 weeks (Terzibasi et al., 2008; Terzibasi Tozzini et al., 2013; Polačik et al., 2014a) are still remarkable and enable many studies requiring the lifetime observation of individuals to be completed within conveniently short time scales.

There is a paucity of information on many aspects of the ecology of *Nothobranchius* in the wild. Even basic data such as the sources of mortality, competition, dispersal, habitat use within the pool, reproductive behavior, and mating patterns are based on circumstantial evidence at best. A study visiting multiple wild populations throughout the annual cycle would be instrumental in revealing how African annual fishes respond to annual fluctuations in habitat conditions and how populations cope with the extreme environmental conditions of savannah pools.

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