



Annual fish biology and survival strategy in nature. A working hypothesis

Summary

Annual fishes inhabit isolated bodies of freshwater in Africa and South America that dry up seasonally. The species survive extinction by laying drought-resistant embryos which undergo various phases of reversible developmental arrests or diapauses throughout their ontogeny. A hypothesis regarding their survival strategy in nature is presented here based on the current state of knowledge regarding its biology and the environmental factors which regulate the onset and the termination of diapause.

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INTRODUCTION

Environments characterized by adverse climatic cycles provide one of the strongest selective pressures for organisms to develop adaptive mechanisms to escape extinction. The isolated waterholes and swamplands of the tropics typify habitats often exposed to periodic drought. While most fishes are decimated by such an ecological catastrophe, a “resourceful” few still managed to survive by taking advantage of a variety of ingenious adaptations. The lungfish, *Protopterus* solves this predicament by burrowing, like an earthworm, 3 feet under the mud. Coiled up snugly at the base of its burrow, the air-breathing lungfish awaits the rainy season in tranquil hibernation. Other more energetic critters such as the mud skipper, *Periophthalmus*, or the climbing perch, *Anabas*, move out of the water in search of food or better quarters in nearby ponds.

Less celebrated, but even more fascinating is the strategy of a unique group of freshwater teleosts known collectively as annual fishes. During the dry season all the adult fishes are decimated by lack of food and high water temperatures even before the isolated pools have totally evaporated (Turner 1964). The survival of the species becomes dependent entirely upon fertilized embryos embedded in the muddy bottom (Peters 1963, 1965, Wourms 1964). Yet, despite recurrent drought, some even lasting many years, these fishes are able to maintain permanent populations within a given region.

They belong to the subfamily Rivulinae (Family: Cyprinodontidae) and are noted among aquarists for the exquisite coloration of the males. Little was known about annual fishes until the turn of the century. The iridescent coloration of the Argentine pearlfish, *Cynolebias belottii*, was so sought after that those avid collectors searched the pampas for specimens to bring home. These dedicated aquarists and amateur ichthyologists must have been the first to note the curious absence of this fish during the summer months. Soon after its importation to Germany in 1906, it became evident that they can live only six to eight months in captivity even under the very best of care known at the time, though they can now live much longer under more advanced care. The term annual fish was coined much later by the noted ichthyologist, George Myers (1952), since their life cycle appears to be completed within a single year.

How do these fishes manage to survive in such precarious habitat? For decades this question has stimulated the curiosity and the imagination of many biologists and aquarium hobbyists alike. Though many more questions remain unanswered, the increasing body of information point to an intricate mechanism that assures their survival.

Unable to breathe air like *Protopterus* or ‘stroll out’ of the pond like the mudskipper, the adult and embryonic populations of the annual fishes have evolved an amazing repertoire of adaptive responses to escape extinction.

HABITAT

Various species of annual fishes are found in the savannah and forested regions of Africa and South America (Figure 1). Scheel (1972) speculated that annual rivulins may have evolved during the dry spells of the Permian Age before the two continents drifted apart. The most prominent feature of their habitat is the erratic nature of the climactic cycles. Being dependent upon the intensity of the rainy season, the existence of the water-filled habitat may range from only several months (Wourms 1964, Thomerson 1971). Specimens of these fishes have also been collected in permanent bodies of fresh water or even in habitats exposed to yearlong drought (Boschi 1953, Piennar 1968).

Evaporative water loss exposes the fish populations to drastic changes in water chemistry (Geisler 1959, Jubb 1967, Haas 1976). Predation by other fishes is rare unless the habitat becomes accidentally connected to nearby streams. Because of small size, people inhabiting the region rarely use them for food. However, wading birds, such as egrets, herons and hammerheads, prey extensively on annual fishes. The transitory pools support a wide variety of invertebrates, such as ostracod crustaceans, insect larvae, and pupae. Examination of the stomach contents of fishes caught in the wild showed their preference for insect larvae (Bailey 1972).

ADULT ADAPTATIONS

Because of excellent color visions, wading birds make easier prey of the brightly colored males. The cryptic colorations of the females make them a less likely target. From the standpoint of Darwin’s theory, the frequent elimination of the brightly colored males should select for less striking colors. The behavioral studies by Richard Haas (1976a,b) have shed some light into this paradox. The pools are very turbid. Watering places, in particular, are frequently stirred up by large terrestrial animals. Vivid coloration enables the female to easily locate the males even at very high turbidity.

The aggressive displays are similar to other freshwater fishes and are described in detail elsewhere by Ewing (1975) and Haas (1976). Because receptive females prefer to mate with the aggressive, brightly colored males, the selection for vivid colors overshadow the detrimental effects of avian predation. Dominant males actively interfere with the spawning advances of submissive males. However, once eliminated from the pool, the

role of the dominant male is immediately taken over by the others of lower rank. Since spawning is promiscuous, only a small number of males are actually needed for mating. The selective feeding on the aggressive brightly colored males relieves the excessive aggressive interactions --- an energetically unproductive use of the limited time available to the adult populations.

Courtship, territoriality and pair formation are common in most freshwater fishes. These traits are luxuries which annual fishes can not afford in their short-lived habitats. Their absence in the behavioral repertoire ensures that all available energy is channeled into two important pre-occupation – feeding and reproduction.

Most African annuals deposit their eggs at the surface of the mud while South American forms bury their eggs much deeper to avoid the cold surface temperatures during the winter months. Spawning in annual fishes is not subject to the season and occurs daily until death. Egg production varies from species to species and is subject to food availability. The rapid growth to sexual maturity in less than four weeks is considered the fastest growth rate ever recorded for fishes under natural conditions (Haas, 1976).

EMBRYONIC ADAPTATIONS

Life is short and time is precious to these fishes. All the behavioral and physical characteristics of the adults are designed to maximize the production of viable embryos. The ultimate survival of their species rests upon the ability of the thousands of embryos embedded in the bottom substrate to survive the impending ecological catastrophe.

The pattern of embryonic development is atypical of that found in other fishes because of the presence of diapause or a reversible developmental arrest (Peters 1963, Wourms 1972a). The term diapause, a Greek word meaning to rest, applies to a state of existence characterized by an abrupt, non-injurious cessation of developmental progression. Unable to escape through space, the embryos escapes through time by entering a state of suspended animation. This type of developmental interruption is also demonstrated by other life forms facing similar environmental problems. Instances of diapause have been extensively documented in the seeds of desert plants or the eggs, pupae, and larvae of certain insects (Lees, 1955). Normal development resumes only when environmental conditions become conducive again for the survival of the species.

The annual fish egg has a tough, thick chorion which enables it to withstand physical damage while embedded in the mud. The outer surface is covered by Velcro-like projections or microvilli (see Fig. 1) that allow it to attach to other particulate matter on the substrate.

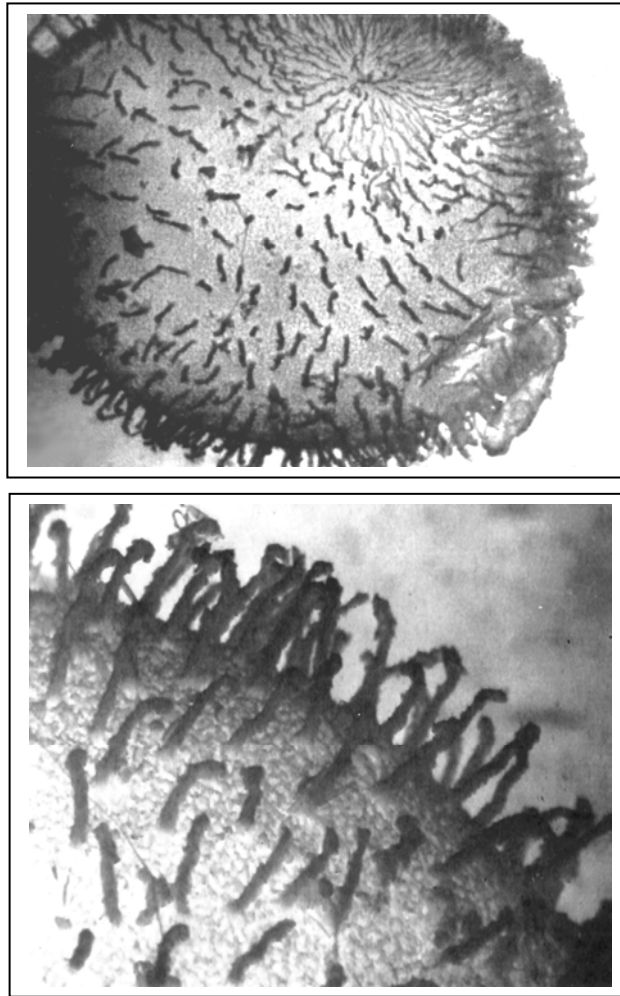


Figure 1. Photo on top shows the surface of the embryo with the micro-villi protruding from the surface. The photo below is a closer view of the embryonic surface in more detail.

The developmental pattern of the annual fish embryo has been described in detail by Wourms (1972a) and is described here only briefly. The early developmental events are similar to other teleosts until the onset of epiboly. The mass of deep blastomeres migrate throughout the surface of the yolk between the periblast and the enveloping layer. The cellular extensions of the amoeboid cells contribute to the arrangement of the blastomeres in rows. The regular spacing during the early epiboly gives way to a randomized pattern

during the dispersion phase (Lesseps et al 1975). Diapause I may intervene at stage 20 when all of the amoeboid blastomeres are completely and randomly distributed throughout the yolk surface as shown in Fig. 1. The termination of this arrested stage is signaled by the re-aggregation of the previously dispersed cells into a consolidated mass.

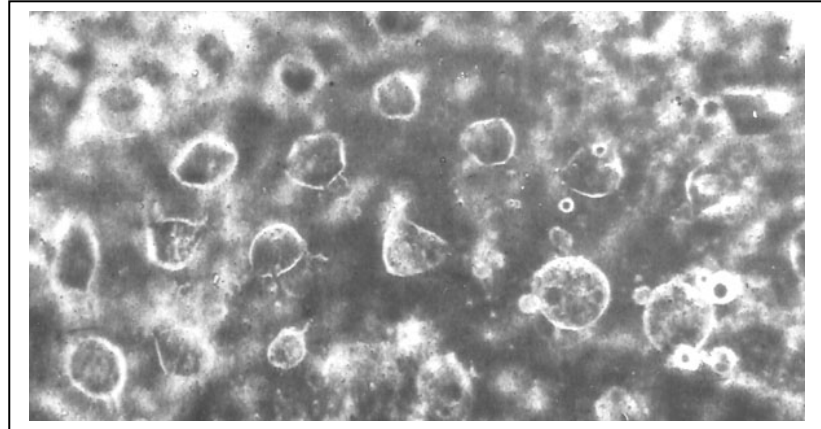


Figure 2. High magnification photograph of the amoeboid blastomeres as they reach even distribution throughout the surface of the egg yolk that signals entry into Diapause I. From Wourms, 1972a.

Diapause II occurs during embryogenesis. Morphologically, the embryo is characterized by the presence of 38 somites. The optic lobes are distinct and the heart appears as a non-contractile tube. Diapause may continue for many months without any deleterious effects. Rhythmic heart contractions mark the termination of this arrest.

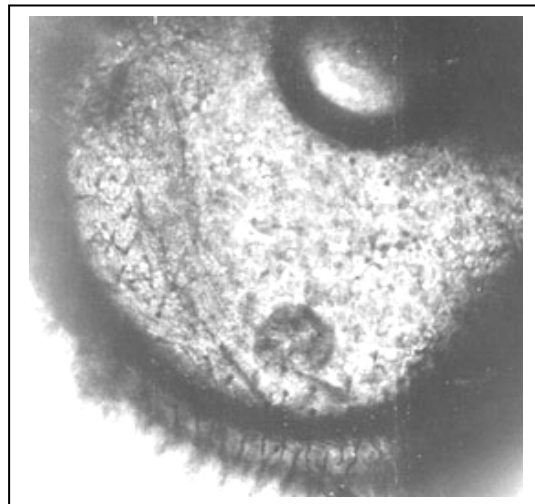


Figure 3. N. guentheri embryo in Diapause II.

Diapause III takes place at the late pre-hatching stage. All the embryonic organs are formed and the embryo is fully capable of undergoing the hatching process. Diapause occurs at this stage when cardiac activity ceases and yolk mobilization is no longer

apparent. Hatching terminates this arrested stage. In other species of annual fishes, Diapause III does not occur. A modification probably akin to the ‘delayed-hatching phenomenon’ reported by Harrington (1959) in *Fundulus confluentus*, has been observed. For example *Nothobranchius guentheri* may undergo Diapause I and II, but remain in the delayed-hatch stage for at least 100 days without any marked reduction in metabolic activity (Matias 1979). Upon depletion of the yolk reserves, the embryos are either forced to hatch or die without hatching.



Fig. 4. *N. guentheri* embryos approaching the last stage prior to entry into Diapause III.

REGULATORY FACTORS IN DIAPAUSE INDUCTION AND TERMINATION

The early experiments by Peters (1963) showed that oxygen tension is the primary environmental cue which stimulates the onset of Diapause I. It has been observed that in the presence of adult fishes under aquarium conditions, the fertilized embryos in the bottom substrate remain in the first arrest for many months although oxygen tension was well within the range that can support their continued development. Data published by Inglis and his colleagues (1981) demonstrated that Diapause I and II may also be induced by adult-produced inhibitory substance(s) in the water. In the presence of adults, the embryos were prevented from escaping out of developmental arrest. Reversible developmental arrest was limited exclusively to stages at which normal diapause takes place. However, diapause may also be induced in the presence of other non-annual adult fishes in the aquarium. This suggests that other “factors” might represent compounds generally found in teleosts and may be products or chemicals released actively, by-products or wastes.

The stimulus provided by daily photoperiod changes serves an important role in the control of Diapause II. The effects of light:dark (LD) cycles first became evident in a breeding population of *N. guentheri* maintained at 25°C and ambient photoperiods (Markofsky and Matias 1977, Markofsky et al 1979). For a number of years, a population of *N. guentheri* were maintained in a room with the window not covered so that ambient light comes normally came through. The laboratory was situated in New York City (latitude 40° N, longitude 75° W) where there is defined seasonal changes in day length. The original intention of maintaining *N. guentheri* populations was to collect sufficient number of eggs to produce adults for aging studies. However, it was a difficult task at the time to have synchronous populations since the embryos can undertake various developmental arrest periods. It was until all the eggs collections were matched with the dates of collections that a seasonal cycle became apparent. Fig. 5 shows that the highest percentage of embryos entering Diapause II coincided with the winter season when there was reduced day length. An inverse relationship between diapause incidence and season was consistently observed for several years, with the highest incidence occurring in the winter and lowest during the summer.

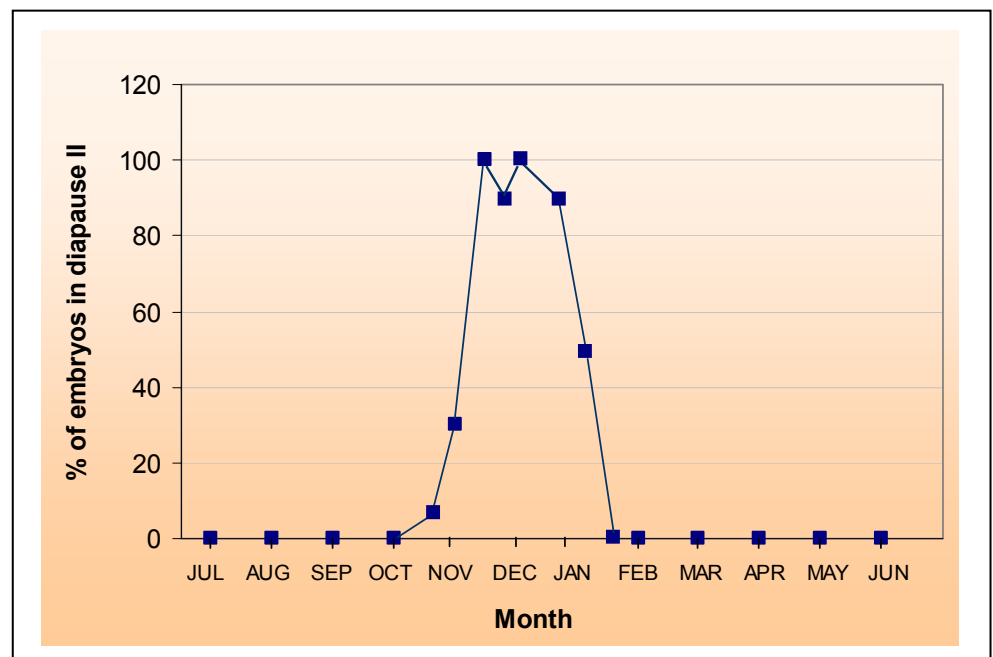


Figure 5. Relationship between the date of the spawning period and the incidence of Diapause II in a laboratory population of *N. guentheri* maintained in New York City under constant temperature (25°C) and ambient light conditions.

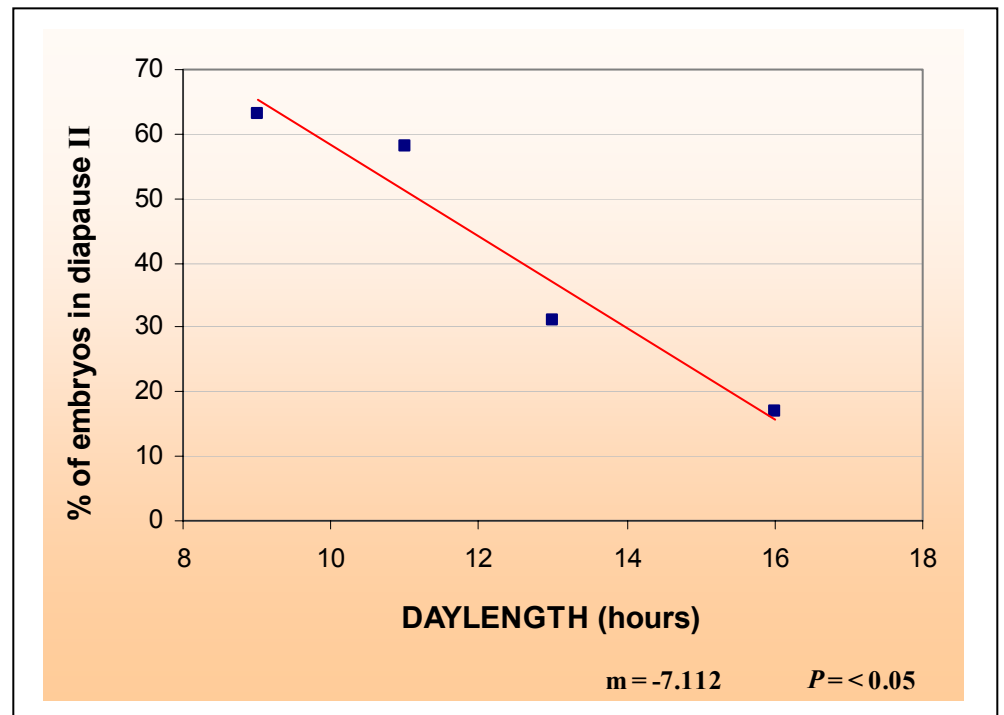


Figure 6. The incidence of Diapause II in embryos of *N. guentheri* spawned by adults kept in various artificial lighting conditions.

Studies using artificial Light-Dark (LD) regimens demonstrated that short-day photoperiods (<12 h light per day) stimulate the onset of Diapause II while long-day cycles (> 12 h light per day) inhibit its induction (Fig. 6). Since the LD cycle did not directly affect embryonic development, it was concluded that the seasonal induction of Diapause II was dependent upon the photoperiod experienced by the maternal generation (Markofsky and Matias 1982). Diapause I, II and delayed hatch stages are not influenced by the photoperiodic changes.

Low temperatures (16-22°C) extend the duration of the first arrest, probably through the retardation of the dispersion re-aggregation phase of development. In contrast, a prolonged stay in Diapause II can be induced even in embryos produced by long-day adapted females as long as the incubation temperatures remain low. Increasing the temperature causes the rapid termination of the arrest and resumption of organogenesis. In embryos spawned by short-day adapted females, low temperatures enhance the duration of Diapause II while higher incubation temperatures (23-32°C) tend to shorten its duration (Markofsky and Matias 1977).

The experimental termination of annual fish diapause by conditions that is inimical to normal development parallels that observed in insects. Short-term exposures of early stage embryos to temperatures ranging from 4°C down to subzero results in rapid development to pre-hatching stage without the entry into Diapause II (Matias and Markofsky 1978). Other injurious conditions, such as exposure to ammonium hydroxide also produced similar inhibitory effects (Matias 1983). However, it is important to note that the exposure of embryos already at Diapause II to extreme conditions not only failed to break the arrest period but also delayed its termination. A similar phenomenon was encountered when the effect of partial drying was examined. Although desiccation by itself does not stimulate the onset of Diapause II, it enhances the length of time the embryos may remain arrested (Matias 1982).

Similar to Diapause I, PO₂ tension occupies a key role in the regulation of Diapause III and of the delayed-hatch embryos. Hatching is stimulated by reduced oxygen levels while arrested hatching is induced at higher levels. The factor(s) which mediate the effect of reduced oxygen level is unknown although data by Schoots, *et al* (1983) indicated that the control of hatching in teleosts might involve the dopaminergic system.

Even under constant laboratory conditions, each individual female produces population of eggs which consists of subgroups having different developmental histories. Subpopulations may consist of eggs which totally bypass diapause or remain at developmental arrest for viable durations of time. The relationship between this variability and the erratic nature of the habitat has been the subject of speculation. Wourms (1972c) postulated that the ontogeny of annual fishes has been designed to permit “the repeated loss of individual eggs under conditions which may initiate hatching but do not allow for maturation and successful reproduction”. The variability of the developmental schedules guarantees that at least one of these subgroups will manage to hatch and repopulate the habitat.

RESISTANCE TO EXTREME CONDITIONS

It is generally known that the susceptibility of the teleost embryo to stress is determined by the developmental stage at which it takes place. Early studies (e.g., Stockard 1921, Hayes 1949) showed that there are points in developmental time which are least susceptible to external insults. Further amplification of this intrinsic insensitivity to stressful conditions occurs upon entry into the diapause state.

Large fluctuations in temperature and the partial drying of the habitat are the greatest environmental stresses which these embryos experience in nature. The protective effect of diapause against extreme temperatures is manifested only in lower temperatures (Markofsky and Matias, 1972b). At higher temperatures, there is little difference in the survival capacity of diapause and non-diapause embryos. The sensitivity of these embryos to partial desiccation is also stage dependent with highest resistance again shown by Diapause II embryos (Matias 1982). The resistance to desiccation by diapausing embryos has also been documented in *Austrofundulus limnaeus* (Podrasky et al, 2001)

Further protection from mechanical and chemical damage is provided by the thick chorion which encapsulates the embryo. When the susceptibility of this protective capsule was challenged by exposure to a proteolytic enzyme, it was also stage-specific with highest resistances occurring at stages at which diapauses normally occur (Matias, 1984). Induction of diapause prior to exposure to damage increased the resistance of the chorion to external insults.

One of the most prominent features of the annual fish egg is the presence of a large oil or lipid droplet that formed from the coalescence of tiny droplets after fertilization. The droplet remains constant in size until hatching, unless hatching is delayed. During the delayed hatching process, which can take as long as 90 days, the yolk and the lipid droplet gradually disappear. Biochemical analysis of this lipid droplet by Brind and his colleagues (Brind et al, 1982) showed that the lipid droplet comprise 7% triacylglycerol and 14% fatty acids as major components. The authors suggested that the lipid droplet likely served as an emergency energy source when hatching is delayed due to unfavorable conditions.

Since inter-diapausal development time occurs for only several days and since the embryonic development schedules are normally asynchronous, only a tiny proportion of the embryonic population is actually subjected to the environmental extremes at any given time. This developmental asynchrony, coupled with the resistance imparted by the onset of diapause, ensures that only a small fraction of the embryonic population is destroyed by the prevailing catastrophic event.

THE SURVIVAL STRATEGY

Because *N. guentheri* has been used extensively as a model system for the study of annual fish biology, sufficient information is now available to permit the formulation of a detailed hypothesis regarding their survival in nature. Early theories about the survival mechanism of annual fishes in the wild have been too simplified to account for their permanency in ephemeral bodies of freshwater.

N. guentheri is a native to the isolated ponds of Tanzania and Kenya, and has never been observed in permanent bodies of water. A detailed field study was undertaken by Bailey (1972) in the Ilonga pond near Kilosa in Tanzania. From the available climatic data for this region and from what is currently known about their developmental biology, a hypothesis on the series of biophysical events which control annual fish cycle may be constructed for any given year.

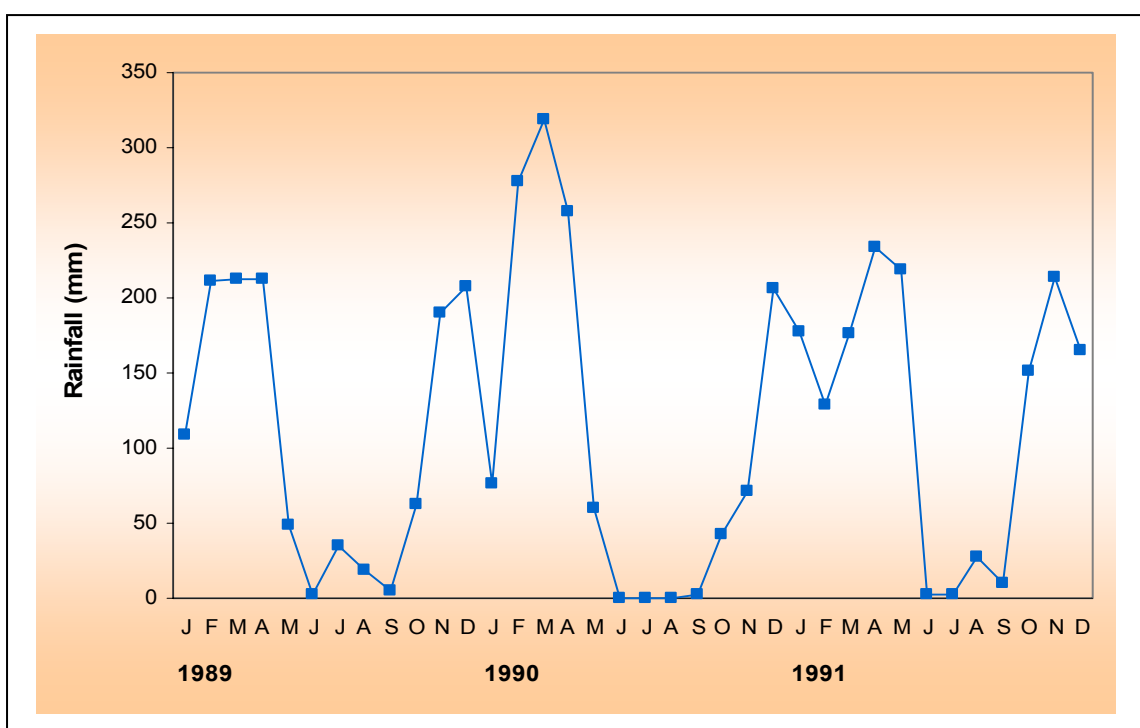


Figure 7. Annual rainfall pattern in the Kasakela region of Tanzania (Station 942909) (Source #3: Directorate of Meteorology, Tanzania)

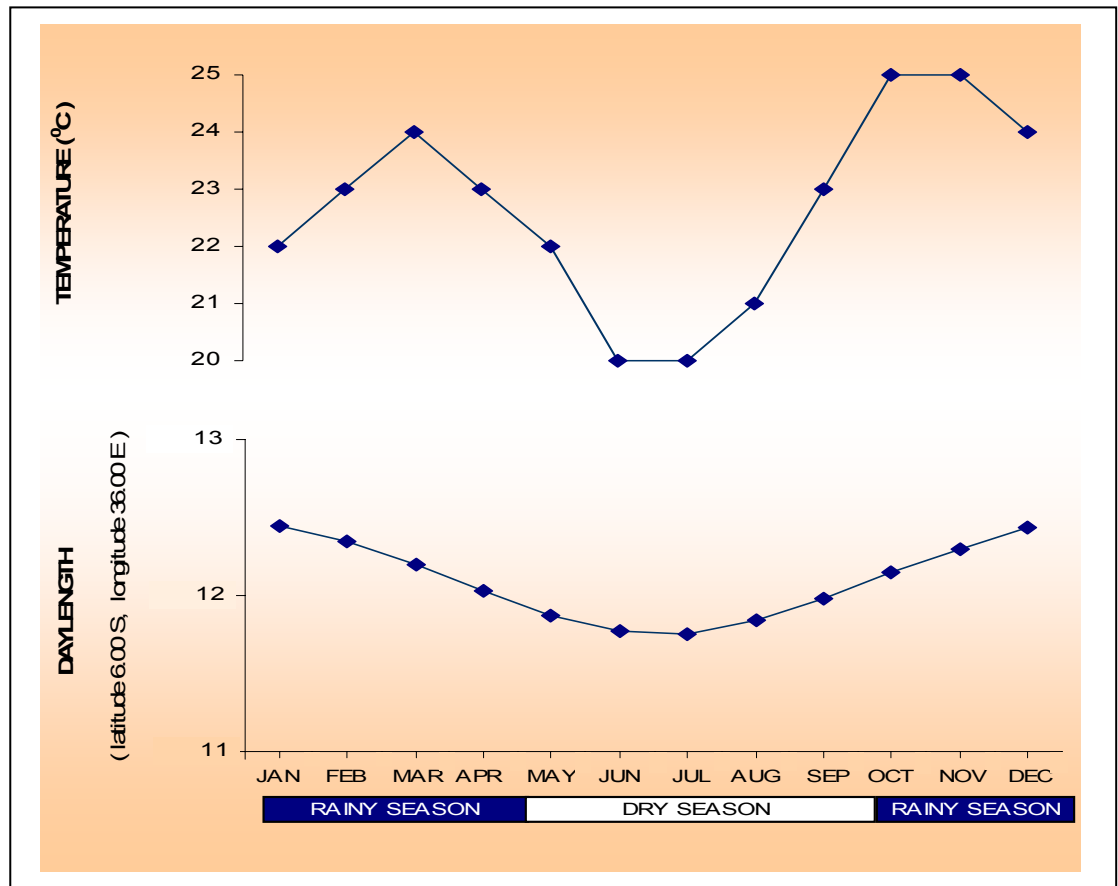


Figure 8. Rainfall pattern typical in the savannah region of Tanzania (Kasakela Station 942909, Source #3, Directorate of Meteorology, Tanzania)

Although the onset of the rainy season is variable, the beginning of the dry season occurs more predictably from year to year. If the pool fills with water in March, the active decomposition of organic matter and the stagnant nature of the pool would reduce the oxygen tension in the muddy bottom. The delayed-hatch embryos embedded in the substrate will be stimulated to hatch. Initially feeding on protozoans, the fry soon prey upon larger crustaceans. Sexual maturity is reached by the beginning of May. With adequate food, mostly insect larvae, a single female may lay as many as 20 eggs per day or approximately 2000 eggs within 3 months. The fertilized eggs develop to stage 20 at which point they undergo Diapause I. Further progression of development is inhibited by the reduced oxygen tension of the bottom mud and the presence of the adult-produced inhibitory substances. Furthermore, the decline of the amount of daylight below 12 h per day beginning in May is perceived by the maternal generation as a cue for the production of eggs that are pre-programmed to undergo Diapause II later on (Fig. 8)

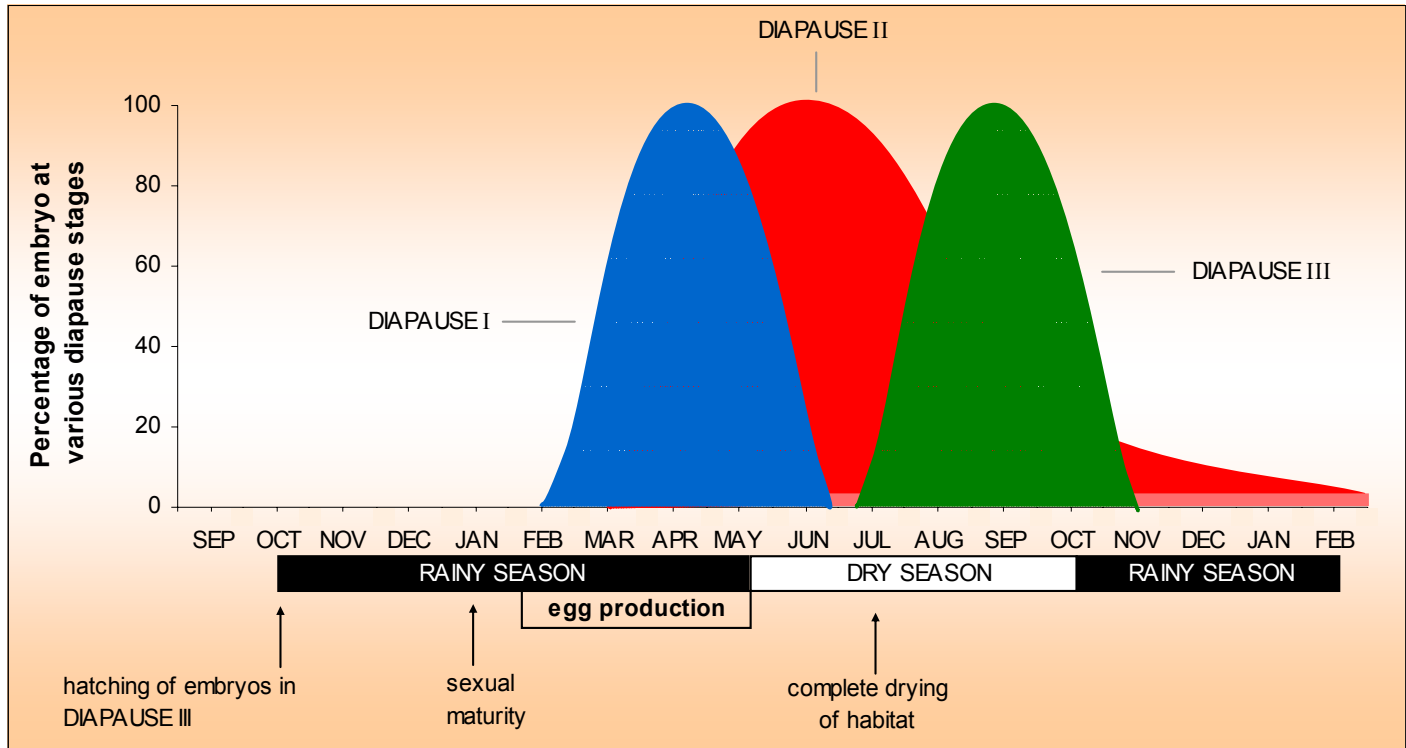


Figure 9. The pattern of recruitment of the embryonic population at various diapause stages at different periods of the year. This figure is a hypothetical pattern extrapolated from the biological studies in the laboratory and the conditions in the field.

All the adults are decimated by the drought occurring in July or August. The air temperature remains within the range conducive to survival despite the drought period (Fig. 8). The elimination of the inhibitory substances and the subsequent return of aerobic conditions to the substrate will act in concert to initiate development. The vast majority of the embryonic population then enters Diapause II. Small subpopulations may bypass this arrest period, continue development, and enter the delayed-hatch phase. The shift of the population into Diapause II is advantageous since it is the most resistant stage of development. Furthermore, partial desiccation prolongs the duration of Diapause II. Emergence from Diapause II occurs at variable schedules and, therefore, various subpopulations of delayed-hatch embryos are continuously produced throughout the dry season. If the rains do not arrive within 3 months, the delayed-hatch embryos are either forced to hatch when the yolk reserves are exhausted or die without hatching. If the initial showers are then followed by another dry spell, the subpopulations that hatch are similarly destroyed. However, the subpopulations that are sacrificed represent only a fraction of the population remaining in Diapause II. Recruitment into the delayed-hatch phase continues to replace the ones that are decimated.

The entire life cycle begins again when the depressions are completely filled with water. The embryonic population from the previous season remains in Diapause II and is inhibited from further development by the low oxygen tension and the adult-inhibitory factors. Continued recruitment into the delayed-hatch group ceases thus limiting the number of adults inhabiting the pool by the size of the initial hatch. This is of adaptive value since the isolated pond can support only a finite population of adults. This explains the absence of young fishes with sexually mature adults in their natural habitat. The embryonic population buried in the mud from the previous season is further augmented by another fresh generation of diapausing embryos spawned by the new adult population.

CONSIDERATIONS FOR FUTURE RESEARCH

Research on annual fishes have declined in the last two decades as basic biological studies are taken over by more biochemical and genomic studies in many other topics. Much remains unknown about how these unique fishes can survive in nature. Because they live in temporary pools of freshwater, habitat loss due to deforestation, agricultural pesticides and human encroachment are increasingly threatening the species with possible extinction. New species of annual fishes are being discovered and many more unknown species may have been lost to science and the aquarium trade already.

- Verification of the life cycle proposed in this paper by conducting field investigations on the stages of development found in natural habitats throughout the wet and dry seasons
- A comprehensive mapping of known locations of annual fish habitats
- Conservation of threatened species through transfer to secure locations and through maintaining the species in artificial conditions as repositories for future generations.
- Creating awareness of the value of the species for biomedical research.

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