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The Egg and the Egg-Development in Killies

The following thoughts on the egg-problem within killies, in some way, is a hypothesis, built upon the research which Dr. W. Foersch made mostly on *Cynolebias bellottii*, but also on *Aphyosemion sjoestedti*, *Aph. coeruleum* and *Pterolebias longipinnis*. Foerschs' results are mixed up with some thought and results of my own.

The egg-laying-tooth-carps or killies or Cyprinodontidae mostly spawn rather big eggs being from 1.25 to 2.0 m in diameter. Some species spawn small eggs, and among the aquarium-kept species *Cynolebias (Cynopoecilus) ladigesi* (so-called "splendens") possibly have the smallest eggs, these being only 0.8-1.0 mm in diameter. Eggs of the two most common "red-tails" the *Nothobranchius guntheri* and *N. palmquisti* (?) also have small eggs, these being about 1.0 mm or more or less. The biggest eggs I found in *Aplocheilus lineatus*, the Golden Tail Rivulus and the Procatopus species, these eggs measured 1.8-1.9 mm. The volume of these big eggs then is 10-12 times the volume of eggs "ladigesi".

The egg-membrane often is very tough (at least in most species within the Rivuliinii or following Myers latest (?) division the Procatopodinae, the Rivulinae and possibly also the Oryziatinae and may be composed by several layers (Siegel's discovery, see DATZ 1958). This tough egg-membrane gives good protection to the embryo (larvae) during development, which, compared with most other species of aquarium fishes, take rather long time. Breeders should remember, that the egg-membrane only get its full strength some hours after spawning, and therefore should not be handled before that time. The single egg of *Pterolebias longipinnis* will stand a pressure of up to 500 grams (!!!). It seems as if the strength of the membrane decrease during the development of the embryo, but not when no development starts in the egg (resting eggs, "ruhende Eier" in German), even when such eggs have been without any development at all for two years, they seem to keep the strength rater unchanged.

The membrane is often provided with filaments, which no doubt primarily act as anchors, adhering the egg to the vegetation. Very often (in particular in species which normally spawn in fine plants) the filaments are concentrated in one or two poles of the surface of the egg and in the poles the filaments then often are much longer than the other filaments. Very long filaments are found in the Oryzias (perhaps also in Cubanichthys) where the females carry the eggs (up to 50) for some time or even until hatching. The filaments then form a "rope" anchored at the anus of the female.

In some of the bottom spawning species and also in some of the species, which sometimes spawn in plants, roots, etc. but sometimes at the surface of the bottom layer, the filaments could be reduced in their length and then also often much more evenly distributed over the whole surface of the egg. In *Aphyosemion "filamentosum"* (also called "arnoldi" or "gardneri"), I cannot find any filaments at all, and no peat adheres to these eggs, so the eggs are very easily washed out of any peat. In this species also the surface of the membrane have a particular pattern, close to the pattern on eggs of *Aph. sjoestedti* (which adhere much peat, but have no filaments?) and in eggs of *Aph. calabaricus*. On eggs of Cynolebias, and Pterolebias the filaments are very much reduced and only "buds"" are seen. In Nothobranchius the filaments are often so hard, that they stand out from the egg-surface just like "hairs" in particular in *Nothobranchius guntheri* and *N. palmquisti*. In *Nothobranchius melanospilus* (which have much larger eggs) the hairs are not so hard and rarely stand out from the surface. These eggs do not adhere to pieces of peat as do most other eggs of the bottom-spawning species.

Eggs of *Cynolebias (Cynopoecilus) melanotaenia* and *C. ladigesi* have a particular type of hairs. Here (maybe) several single hairs have formed a short "rope" which stands out very stiff from the surface of the egg and is ending in a sort of brush. These eggs also do not adhere to any particles of peat when they are washed out. Perhaps in these two genera the hairs have no adhering (to plants) properties, but serves in the way that they protect the egg against small displacements in the mud, when this dries up and also forms a zone of air around the egg, and thus prevents the egg from losing too much water and forms an isolating layer against temperature changes. In these genera the hairs are very evenly distributed over the surface of the egg. The surface of eggs of killies very often shows a certain pattern, perhaps related to the filaments, but I have not been able to trace a key to these patterns, if they form any key at all.

The egg of killies normally will develop into a hatchable fry within 1 to 4 weeks, depending on temperatures and the species of killie concerned. But in some species, the development of the embryo into a hatchable fry is delayed and does not result in the hatching at the normal due time. The fry stays inside the egg and lives there for days, weeks or even months. This particular state of egg-development we may call (Foersch) "Resting Fry". First of all, we find "Resting Fry" in the species, which are called "Annual Fishes," that is the Nothobranchius, the Cynolebias, the Pterolebias and Aphyosemion (only one species, perhaps more, but not all species in this genus). The eggs of these fishes may survive the drying up of the ponds and other small waters in which they live in nature. But also we find resting fry in many other species, which are not considered to be annual fishes: Aplocheilichthys, Oryzias (?) and perhaps other genera. Once I tried to explain some of the properties of the resting fry in this way as an osmotic phenomenon.

The newly spawned eggs contain mostly highly molecularous components such as fats, proteins, etc. Even in distilled water the osmotic pressure inside the egg cannot be more than slightly larger than the osmotic pressure outside the egg-membrane. As the membrane is permeable (see later) to oxygen molecules, water molecules etc., the water pressure may adjust itself between the inner and the outer liquid. As the embryo develops and feeds on the yolk it gives away waste-products and if at least some of these are not able to penetrate the membrane, the salinity inside the egg will increase and as will the osmotic pressure. If now the salinity of the water in which the eggs are kept is higher than normal to the species (compared with its natural waters) the inner pressure will not increase to that value and this will "burst" the membrane. Therefore the poor fry needs to make a higher osmotic pressure, and this it forms by "eating his own tissues" by degenerating and decomposing himself until the salinity reach the right value. But when eggs are kept in water of lower salinity than normal to the species, the fry will come out too early (*Aph. petersi*, Procatopus spec.). If eggs with "resting fry" are kept in water of certain salinity, they may be hatched only by putting the eggs into distilled water. Then they often will hatch in a few minutes. As an experiment I once hatched out many half developed fry of *Aplocheilus lineatus* by putting them into distilled water. Together with my younger brother, 9 years ago, I was breeding the *Aplocheilichthys macrophthalmus* ("lampeye") in our normal tap-water, which is alkaline (alkalinity about 5.0, that is about 14 German degrees of temporary hardness) and of a salinity of about 300 ppm measured (electrical) as sodium chloride. We had good and fertile eggs, but always they turned out to be "resting fry", which died inside eggs after a few weeks. Then we tried to hatch them by putting the eggs into distilled water, and always this gave good fry in a few minutes.

I do not believe in this simple theory any more. The problems indeed are not so simple. The hatching of "resting fry" by putting ripe eggs into distilled water (or any water with much lower conductivity than the water, where the eggs were stored) more seems to be a "dynamic phenomenon" than a "static one" and do not support the above mentioned theory, that will say (if the membrane is permeable to the simple salts in water also) that the water molecules (when putting eggs from water with higher salinity into water with lower salinity) penetrate the egg quicker than the various salts inside the egg can get out. This certainly will increase the inner pressure and this may burst the membrane. But... when I bred the *Aplocheilichthys macrophthalmus* in rainwater, there was no "resting fry" and eggs hatched in due time. Certainly they always did this. Here again the "theory of osmosis" could be used, but after all, the rainwater not only had much lower salinity (conductivity) but also was slightly acid, whereas the tap water was rather alkaline indeed. Therefore I think we should concentrate the study of the properties of the egg-membrane, that will say its permeability to oxygen (resting eggs) and various simple salts (resting fry) under various conditions and in particular in dependence of the pH of the water. Many years ago I read some paper prepared by Ruttner (German biologist or water chemist?). He wrote that the permeability of cell-membranes in aquatic vegetations to a very great degree was under influence of the pH of the water.

"Resting fry" also may be hatched by adding "dry food" to the water. The fry often will hatch long time before the water gets cloudy, that will say within the first 1-8 hours. We do not know what acts in this type of artificial hatching. Perhaps certain bacteria? Perhaps certain enzymes? However the method works very well indeed and has always proved to be superior to the "osmotic method". I found that "resting fry" of species which commonly are not considered as "annual fishes" always could be hatched using distilled water or pure rainwater (if they have been stored in water of higher salinity) whereas ripe eggs of the "annuals" very often would not hatch by this method, but came to good hatchings when I used the "dry food method". "They may have been very hungry, and came out as soon they saw the good dry food" as Jack (Scheidnass) wrote me once.

Foersch found, that "resting fry" of *Cynolebias bellottii* lived in eggs for up to a year. Also resting fry of other "real annuals" seems to be able to live inside eggs in fully developed state for that long time or even more. But fry of the "common killies" certainly will not be able to survive such a long resting period. If you keep eggs with "resting fry" in water, you may inspect the circulation of the bold-elements and by the speed of circulation you may judge the deepness of the dormant life of the embryo. Very often you will find the circulation to be so slow, that there seems to be no circulation at all. However, if you wait a little you will see the sudden results of the few heart-pulsations.

No doubt the state of "resting fry" gives the species a chance to survive in nature, when the natural waters dry up for some short time. You may force the eggs of most killies into the state of "resting fry" simply by drying up the eggs and keep them in moist air. Very many species have been shipped as live eggs in this state. Most eggs are tough enough to stand the drying out in perlon or in peat.

But the state of "resting fry" is not the only resting state in the development of eggs within the killies. If you breed the so-called "annual fishes" within killies. The Cynolebias, the Pterolebias and the Nothobranchius and after spawning wash out the eggs and place them only with little coarse peat or without any peat in small containers on shallow water, you will find that not all the eggs will show an embryo or trace of an embryo after 2-3 weeks.

These eggs could be called by the name of "resting eggs" (Foersch). To my opinion these eggs represent a very interesting object for simple "home-made" research. Under the above mentioned conditions, that is to say, a good supply on oxygen to the eggs (shallow water, no peat and at least only little and coarse peat) I never found resting eggs in any of my Aphyosemion (10 different species) except the "sjoestedti", a species which also in other respects differs from the other aquarium-kept species. But in the 4 "species" of Nothobranchius (palmquisti, guntheri, melanospilus and kuntae) there always were at least some "resting eggs" in batches of eggs which were kept in water (or dried more or less hard). The same happens in Cynolebias (belottii, nigripinnis and whitei) and "Cynopoecilus" (ladigesi and melanotaenia) and Pterolebias (longipinnis and peruensis). There were no "resting eggs" in the various crosses within Aphyosemion (except "sjoe" male to "cog" female) out of 50 eggs, only one egg did not catch fungus and this sole egg stayed transparent (with some trace of "embryo" (or better a formless mass of cells) for some months). In this genus, until now I have had 16 different types of crosses which showed development of an embryo in the eggs. But in the 6 (7) types of crosses with the Nothobranchius (I kept last year) only 3 (4) there were only "resting eggs" which did not develop any embryo ("traces of embryo" in some eggs) within the months they were under inspection. After some months the yolk in these eggs decomposed and fell into more and more oil drops. None of the eggs from crossings in this genus gave any hybrid, which grew up to maturity, but hatching was observed in 3 combinations. N. palmquisti male to N. guntheri female seems to be the most promising combination which no doubt would give you live-able fry. But the "tuberculosis" took mine when they were about the show color.

The Cynolebias crossings easily gave eggs, but may get fungus. Until now only 3 combinations gave eggs that stayed transparent after 3 weeks, but none have showed the smallest trace of development of an embryo.

The cross *Cynolebias (Cynopoecilus) ladigesi* male to *C. melanotaenia* female (250 eggs) gave resting eggs as well as eggs with normal development. Here is the data of this cross (8 different batches, each 10-15 eggs have now been mailed as experiments, so I do not have the exact data for all eggs).

- First cross with only one male to one (later two) females gave during one week only 16 eggs. Of these only one did not catch fungus. 12 days after spawning there was a small embryo in this egg. After 23 days there was black pigmentation. As the fry did not hatch within 55 days after spawning, the sole egg was dried up in peat.

- Next spawning had two small male "ladigesi" and 4 small females "melantoaenia". Spawning in fine mud with only little coarse peat (in order to prevent the eggs to clot) 21-23 C. Water type the usual one (300 ppm NaCl, pH about 6.8)

- 30 March. I lifted out 15 eggs with big (nearly hatchable) embryo. These eggs were dried in peat (hatched 11 April, 12 fry came out, all fine). 10 eggs were reserved for experiments (water filled ampulles, no air, sealed) in order to try to force them into the state of "resting eggs".

- On 03 April, 4 batches with total 60 eggs (mostly without any embryo) were shipped to "egg-friends".

- 04 April, eggs counted, there were still 169 (169, 60, 15, 10 = 254). 11 April the 169 eggs were sorted: 52 with big, pigmented embryo, 117 with no embryo or only a transparent embryo.

- 15 April another 60 eggs (4 batches of 15) were shipped to friends, in the few days (11-15 April) many more of the "transparent eggs" have got black pigmentation. Today there are (16 April) only 46 transparent eggs in the bowl, together with 20 with ore or less pigmentation. The fry that hatched on 11 April are just as fine and sound as the pure "melanotania" I hatched at the same time in order to have something to compare to. Of course, these two broods are not mixed, but have their own tanks.

But the state of "resting eggs" is not the same as found in the above mentioned species within Cynolebias, Pterolebias and Nothobranchius. Something points in the direction that newly spawned eggs of many other killies may be forced into the state of "resting eggs" by reducing of the oxygen concentration in the medium, in which the eggs are stored (water, peat, etc.). When I recall my first fumbling steps in the breeding of "annual fishes" (1955, Aphyosemion "filamentosum") the thing that was most interesting was the fact that eggs of this semi-annual fish did not develop, if they stayed in the fine mud I used in my spawning tanks. As these eggs so easily are washed out of the mud, because they do not adhere to peat, I again and again found transparent eggs in the mud in tanks where no "filamentosum" had been for months. I these eggs after washing were stored on shallow water without any peat, some of them got fungus, but all the rest developed a livable embryo within the coming two-three weeks. The embryos turned into the state of "resting fry" when they had finished their development and only after some months they hatched out as feeble fry, most of which were able to live. Rarely was I able to force them out by using distilled water, but dry food always was effective. In 1956 I washed out the bottom mud of a tank where no killies had been kept for at least 5 months. There were hundreds of transparent eggs; none showed any trace of embryo. After drying up of this mud, no fry hatched, so possibly these eggs were too old. Unfortunately I did not search for eggs in this mud after the first watering.

A breeder of killies, who once had the *Cynolebias (Cynopoecilus) ladigesi* in his tanks again and again, will find the small, so characteristic eggs of this species, when he washes out his spawnings of other species. The "ladigesi" is the "weed of the killie-breeder", but fortunately the fry is so small, that it is not able to eat fry of the species you want to raise. Again and again I hatch this species from peat, where I never thought any "ladigesi" have been for years. By this way I always have a stock of this species, even as many are fed to bigger killies in order to keep down the stock. Even from a sample of peat which had been dried, and certainly very hard, for an unbroken period of 450 days one fry of this species came out and was found.

In order to control the theory of the "oxygen-concentration" in connection with the state of "resting eggs" in killies others than annuals I now take from most spawnings some eggs which are stored in small ampulles, about 10-20 in each (the same ampulles which I used for shipping of eggs), without any air-bubble, cork on and parafined at the "cork-end". These interesting experiments are recent and should go on for some years before one may draw any conclusion from them, but until now only very few eggs in some of the batches (never all in one batch) have changed in a way that may be explained as the death of the egg. Here I have several of these ampulles. 10 eggs *C. ladigesi/melanotaenia* cross, only 3 weeks in ampulle, all fine, no trace to spot of an embryo; 4 eggs of *C. melanotaenia*, in ampulle for 11 weeks, all fine no trace of an embryo; 5 eggs same species, 9 weeks, fine and transparent.

From a sample of mud, spawned in Feb. 1957 at a local breeder of *Cynolebias nigripinnis* in March 1959 after drying of the mud for about three weeks hatched about 100 fry and washed out about 200-300 eggs, most of which were transparent. 100 of these eggs, with or without any embryo, were at once put into very fine (dirty) mud and stored in a glass totally filled with water and airtight closed. These should stand for at least (?) year before they once more are washed out and controlled. But I also sorted out some eggs at various states of development in order to control how they would behave in the small ampulles, where they could be controlled without opening of the glass. They have now been in their ampulles for 6 weeks: 4 eggs with big pigmented embryo; all dead and partly decomposed; 6 eggs with non-pigmented embryo seems to be all right; also 6 eggs at the same state and 10 eggs without any trace of an embryo.

The research on "resting eggs" may be of a certain interest to zoologists and biologists. The breeders of killies however would no doubt be very interested, if they were able to control this state of development. At present we are able to store some species up to one year and even more by keeping the eggs in dry peat (not completely dry indeed, but as moist as a good smoking-tobacco or a little drier). In this way we are able to reduce the number of species which we keep "in water" as fry or adults. During winter-time, when live food is scarce, we can "dry up" some of the species, which will not take dry food and in spring when the live food shoals again, we may raise new stocks from the dry peat. All my 4 "species" of Nothobranchius have been sleeping this winter as eggs in peat in airtight glasses. Now all 4 species are in the tanks again and (except *N. palmquisti*, which as usually gave a lot of males) in pairs. But... if we could force eggs of any species (indeed this is a very optimistic thought) into the state of "resting eggs" and keep them in this state for many months (or even for years) this would be real progress. However, we certainly do not know all the factors which govern the state of "resting eggs" and much research have to be done on this interesting subject. Perhaps we should try to find out the properties of the membrane of the eggs, because if some factors could reduce the "pores" of the membrane in a way that oxygen can not penetrate the membrane, we may be close to the solution of the "resting eggs" in the Cynolebias and other real annual fishes.

Foersch divided several batches of eggs from spawnings in equal portions and found that the percentage of "resting eggs" differed in the two parts of the same spawning. So the factors that control the percentage of resting eggs could not only be some factors of inheritance.

Among aquarists who work on killies, there is a common belief, that repeated drying and watering may start the development in the "resting eggs". Perhaps this helps, but not at all in all cases (remember the 5 year egg of *Cynolebias bellottii* at Dr. Foersch). Foersch also found that the temperature did not influence on the "percentage" of "resting eggs" in different batches, but that extreme low temperatures, that will say, temperatures close to the freezing point of water, turned more eggs into the dormant life of "resting eggs".

Foersch found that the batches of eggs gave the lowest "percentage" of resting eggs, when he dried up the peat as soon after spawning as possible. This was with *Cynolebias bellottii*. But in *Cynolebias melanotaenia* and in Nothobranchius palmquisti, I found the contrary to be the case. But as I dry up eggs much more than most other aquarists, my very sudden and "hard" drying up may have stopped the development in eggs of the several batches of these two species. In most of these breedings I divided the lot of eggs in two portions. One was stored on shallow water in order to keep an eye on the development. The dry peat was stored at the same temperature with lots of air in the airtight plastic-boxes. When the eggs in the water after some weeks showed a ripe fry, I watered the peat and found (in some cases) no development in eggs or at least by far not so many eggs with embryos as is in the eggs which were stored in water.