Resting Eggs

In a previous letter you will find some thoughts on the oxygen problem in the question of Resting Eggs. In order to find out if eggs might be kept for a long time under non-oxygen conditions I collected eggs of several species and stored them in completely water-filled ampoules (the normal 2 ccm ones) and closed them with cork and parafin. All eggs of Aphyosemion (calliurum, coeruleum, calabaricus, cognatum etc.) died (turned yellowish and turbid) after one or more weeks. When opening the ampoules the water gave away a strong smell of H2S. But in some ampoules containing eggs from Cynolebias nigripinnis, C. bellotti, C. melanotaenia, the eggs did not change and still were transparent and apparently alive.

In June my stock of ampoules was used and one evening I opened two ampoules in order to use these for mailing of eggs. These ampoules contained:

1) 5 eggs Cynopoecilus melanotaenia closed for 115 days.
2) 11 eggs cross C. ladigesi/melanotaenia closed for 75 days.

Water in ampoules was perfectly clear. A certain smell of H2S was traced. Parafin lock was unbroken. Now the 10 eggs C. ladigesi/melanotaenia all contain a hatchable embryo (one egg developed an embryo when in ampoule and this egg had a dead embryo when I opened the ampoule). The five eggs from Cyn. melanotaenia still are transparent and possibly alive.

This small experiment might be of great importance in the future mailing of eggs from "real annuals" as lots of eggs might be stored in very small containers and without any air. Also possibly during wintertime such eggs might be mailed by ordinary mail and then the risk of freezing the eggs will be much smaller when mailing eggs overseas.

Possibly also the experiments might guide to a solution of the problem of Resting Eggs in Killies. I certainly know that much more work must be done on this problem before a solution comes into reach. Now I am more sure that the oxygen concentration plays an important role in the development of this phase in eggs.

Resting Embryo

Unlike the case in the 1958 breeding of Nothobranchius melanospilus the (about 50 only) eggs that now
are stored on low water in cups do not develop normally giving a hatchable fry after about 4 weeks. In most eggs the development of the embryo stops at the phase "resting embryo" and stays in this phase for rather long time. This is also the case in *Nothobranchius kuhntae* (Griem's stock, possibly "orthonotus"). Here are some data.

**Nothobranchius kuhntae**:  
- two males and one female, very young. Spawning until 20 Apr. 59, peat washed out, found 36 eggs. 15 closed up in a 2 ccm ampoule, airtight. 21 on low water for examination. Egg measured: yolk = about 1.025 mm, egg about 1.20-1.25 mm. Egg oval. Yolk not quite regularly shaped. Eggs do not adhere to peat, but catch each other, forming balls. Breeding pair died a few days later. One egg in water got fungus. 8 more eggs were found when pair died.  
- 04 May 59: 27 eggs on low water: all eggs have small transparent embryo.  
- 30 May 59: 27 eggs on low water: two eggs have big embryo, near hatching point, 25 eggs unchanged since 04 May 59.  
- 17 June 59: 10 eggs (most with transparent embryo) mailed to Peter T.  
- 06 July 59: one fry hatched (Belly slider), 3 eggs have hatchable embryo, all other eggs seem to develop now.  
- 28 May 59: "15-egg-ampoule" opened. 12 eggs left, transparent, the 3 more eggs are turbid, dead. Strong smell of H2S, water turbid. All eggs inspected, no trace of an embryo.  
- 30 May 59: one more egg died.  
- 11 June 59: one egg has a small and transparent embryo, 10 are without any trace of an embryo.  
- 06 July 59: 4 eggs have embryo with black eyes, blood circulation. 3 have small transparent embryo. 4 eggs have no embryo. "Griem's stock".

**Nothobranchius melanospilus**:  
- (Henry Hansen's stock): two males and one female, very young. Spawning until 13 May 59: peat washed. Found 62 eggs. All placed on low water for examination.  
- 30 May 59: 58 eggs left. Eggs vary greatly in size, 17 eggs are dead. 41 eggs are sound: 3 have no trace of an embryo, 31 have small transparent embryo, 7 have pigmented embryo, growing.  
- 11 June 59: 6 eggs have big hatchable embryo, 32 eggs have small, transparent embryo, one egg has no embryo (yolk ball deformed).  
- 20 June 59: 13 ripe eggs dried up.  
- 06 June 59: 21 eggs have hatchable or nearly hatchable embryo, 2 eggs have small transparent embryo.

**Pterolebias longipinnis**: Development of Eggs.  
- Spawning 2 young males and one young female on fine mud until 10 May 59: peat washed out. Found 84 eggs. 2 of these had big, pigmented embryo (possibly eggs which had been in the very upper layer of the mud).  
- 23 May 59: 11 eggs are ripe, dried up in little peat. 30 May 59: 6 eggs have no embryo or very
small embryo (eggs adhere some small particles of peat and are difficult to inspect). 54 eggs have pigmented embryo, not at the same state of development.

- 15 June 59: water on small sample of peat, 11 sound fry hatched after 24 hours (two hours after watering I saw at least two fry swimming normally). 36 eggs in water are ripe (15 have been mailed on 17 June 59, most of these had only a very small embryo) and were dried up. Indeed the development in "longipinnis" is much quicker than in "peruensis". Also eggs of "longipinnis" have another pattern on membrane (fine dots). "peruensis" has small dots and large dots, not far from the pattern in "whitei". You will always be able to distinguish eggs of the two species of Pterolebias by the pattern on egg membrane, although eggs have equal size. *Pterolebias longipinnis* is Foersch's stock. *Pterolebias peruensis* is J. Scheidness' stock.

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**Cynolebias bellotti** Guevara's stock.

- 5 males and 13 females, all young, spawning until 02 May 59: 617 eggs washed out. Many of these eggs were mailed, so the results are not very exact indeed. Resting eggs mostly were mailed in order to be sure that they arrived alive.
- 30 May 59: all eggs stay on shallow water. 293 had an embryo with black pigmentation, 192 had no trace of embryo. Eggs are easily inspected, they do not adhere much peat.
- 09 June 59: eggs in plastic box begin to hatch by themselves. As eggs, also fry have different size. Biggest are 6.0 mm, smallest about 4.5 mm. 7 fry have total. 12 June 59: 6 more fry hatch. 13 June 59: 6 more fry hatched.
- 14 June 59: 106 fry hatched by themselves.
- 17 June 59: 23 more fry,
- 18 June 59: 33 more fry, these fry have big yolk balls. The 23 fry that hatched on 18 June 59 were placed in a small cup with only a few millimeters of water in order to see if they were able to be normal swimming fry. On 19 June, 17 of these 23 fry were normal swimming fry!!! As I had enough specimens of this species, I placed all belly sliders and normal fry together in a two liter glass. Now 07 July 59 only 3 fry are left, they measure 1.5-2 cm, all others were eaten up or died. They did not get much food, as food was scarce. It is interesting to see that the hatching of a few fry certainly ignites the hatching of most ripe eggs present in water. Also I did not believe that "bellotti" hatched in water were able to be normal fry.