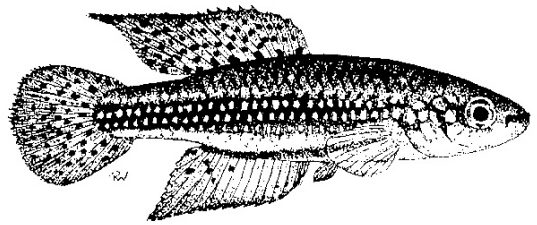

Cynopoecilus melanotaenia (Porto Alegre, Rio Grande do Sul, southern Brazil)
Drawing by R. H. Wildekamp

Based on these data we suggest the following conditions to the culture of *C. melanotaenia* in the laboratory: Temperature around 20°C, pH = 6.2 – 6.8 and hardness = 20 mg/L^{-1} of $CACO_3$, no aeration is needed. Arenzon et. al. (in press) showed that embryos of *C. melanotaenia* kept at 25°C may present morphological abnormalities and suggested a constant temperature of 20°C or a variable temperature of 16-25°C.

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Journal of the American Killifish Assoc. 35(5) 137-145 (2002)

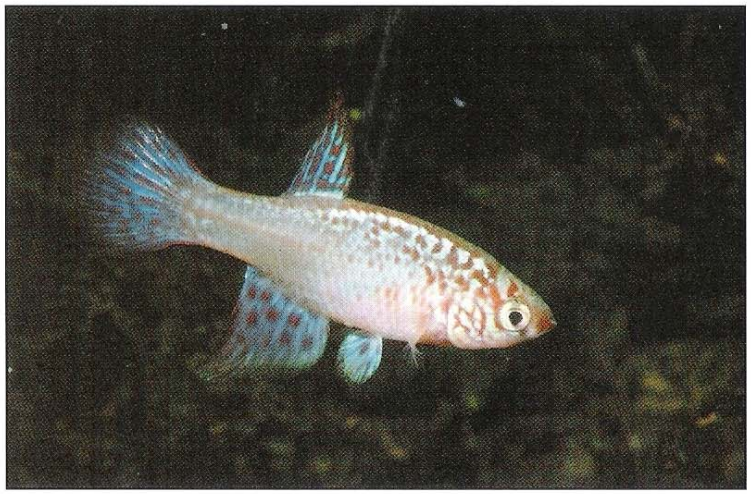
Water Incubation of South American Annual Killifish Eggs

Dan Katz

There is nothing more rewarding nor exciting within the aquarium hobby than wetting a bag of damp peat moss and seeing fry appear above this peat moss, almost miraculously, a few hours later. On the other hand, it is quite frustrating to go through the same work and the same "rituals" and to wet some peat moss and then to find no fry a few hours later. How many times has each of us annual fish hobbyists experienced this frustration? Too many times for me. Yet, I have frequently believed that I have done everything "right" only to have no hatch at all. Why didn't I get a hatch? Were there eggs in the peat in the first place? Did I hatch too early? Did I hatch too late? I never knew the answer to those questions. Eggs incubating in peat are a mystery. After 25 years of occasional successes, separated by frequent failures I have spent a good deal of time searching for ways to improve my yield of fry. While I have found a number of ways to obtain improved hatches employing variations on the "standard" method of spawning these fish and incubating their eggs in damp peat, none of these techniques has given me anywhere near the consistent high yields of fry as the water incubation process that I will describe in this article.

Please remember though that nature has designed the eggs of the South American annual fish to produce a relatively few fry from many eggs (a low yield) over a very wide range of environmental conditions. Whether the temperature is higher or lower than normal, whether the rains come prematurely early or on time or even miss a season, there are always some small fraction of the huge number of eggs in the substrate which are viable and ready to hatch to begin another generation. What we are trying to do in our fishrooms, on the other hand, is to find a fixed set of conditions to obtain a high yield of fry from the few eggs we can collect and that is not easy to do. We are trying to "fool Mother Nature".

Let me begin about 25 years ago, in the mid- 1970's when a fellow in England sent me a pair of a new *Nothobranchius* called "*Nothobranchius* from Olago". I asked him what the incubation time was for this fish, but he did not know. I was leery of spawning this new fish over peat and then not



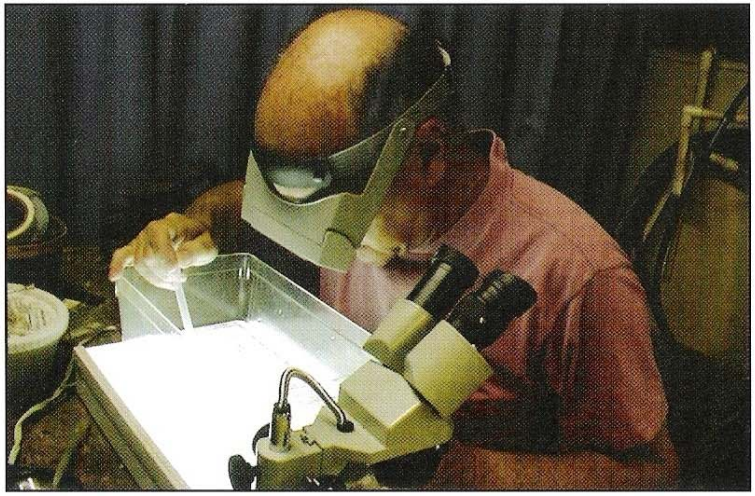
Maratecoara lacortei, male

Simpsonichthys adornatus, male
Photographs this page by John Brill

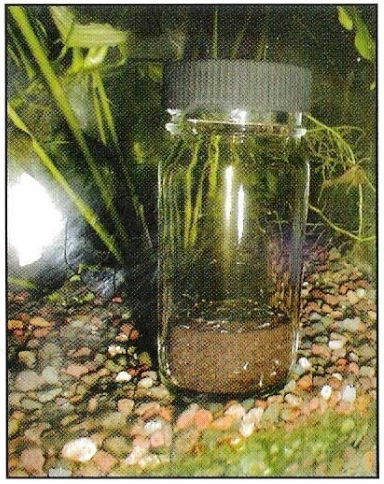
knowing how long to incubate the eggs, so I tried something different. I allowed the fish to spawn over a container of green sand in their tank and I collected the eggs from the green sand. Instead of putting these eggs in damp peat, I then placed a damp piece of paper towel on the bottom of a plastic container and placed the freshly collected eggs on top of the damp paper toweling. The container was sealed and I waited while the eggs developed into completed embryos.

A few months later, when the eggs looked "eyed-up" (fully developed) to me, I removed a few and put them in vial of water to hatch. I breathed into the vial to put some carbon dioxide into it, sealed it and then put it into my pocket and walked around with it to try to "force" the eggs to hatch. The eggs did hatch within a few hours. However, every time I did this, all of the resulting fry were bellysliders. This was frustrating and I talked about it to a few fellows at the next LIKA meeting. Barry Abrahams, who is the best breeder of annual fishes that I know, suggested that he had never seen any annual killie hatch and swim properly unless there was some peatmoss present during the hatching. As much as I respected Barry's opinion, I refused to believe it. How could that be true? But, I ran out of ideas to try and I was running out of *N.* sp. Olago eggs, so I tried Barry's suggestion in desperation. The next day I put a few of the embryonated eggs in my vial of water, added a pinch of peat moss, breathed in the vial, sealed it and put it in my pocket for a while. Within a few hours the eggs hatched and, much to my amazement, the fry swam. No bellysliders. I still refused to believe it was the peat moss that did it so I tried to think of other variables that might have been changed by the addition of the peat to the water in the vial. I hatched some eggs in water in the dark--bellysliders. I hatched some eggs in water with reduced pH--bellysliders. I hatched in some water with some salt added-- bellysliders. I repeated the test with added peatmoss--swimming fry. I then put peat in water for a few hours, filtered it through a fine net and added that peat extract to the eggs in the vial—also swimming fry. I don't understand it, but I believe it now; ***peat extract present during hatching can prevent or reduce the incidence of bellysliders in annual fish***. Ever since then, I have always added a fresh Jiffy 7® peat pellet to the water when I hatch my annual fish eggs.

I reported on these tests at the next LIKA meeting. We had a speaker at that meeting, Dr. Jules Markovsky, who was doing experimental work with *N. guentheri.* Jules told us that he never even dried his *N. guentheri* eggs. He simply water incubated them, force hatched them by just packing them close together in the tip of a narrow vial when they were developed and got no bellysliders. I later tried water incubating several species of Nothos and I found that, while Dr. Markofsky was certainly correct about water incubating *N. guentheri*, the other *Nothobranchius* species I tested, including *N. rachovii*, required peat extract in the hatch water in order for the fry to swim



The author checking eggs of annual killifish



Incubating eggs

properly. Without the peat extract, most, if not all, of the fry were bellysliders. I duly reported these results at a future LIKA meeting. Some time later, another LIKA member, Jerry Shapiro, used the combination of my peat extract data along with Jules Markovsky's water incubation results as the basis for a process he developed for water incubating all of his Nothos. Jerry reported on that process in JAKA (1,2). Twenty-five years later, Jerry is still raising Nothos and he hasn't dried an egg in all that time!

Now it would certainly be wonderful if we could use such a water incubating process for our South American annuals. But, before we can even try it, we need some eggs. Unfortunately, unlike Nothos, most of the South American annuals will not cooperate and lay many eggs in green sand. They much prefer a deeper substrate like peat moss. So we need a method for separating large numbers of killie eggs from peat moss. Twenty five years later and Barry Abrahams to the rescue again. There is a relatively straight forward method for separating eggs from peatmoss which other hobbyists have been using and Barry demonstrated if for me. The method is based on the differences in the settling velocities between killie eggs and peat moss. If you were to mix peatmoss and killie eggs in water and allow that mixture to begin to settle, the killie eggs would settle to the bottom of the container a little faster than would the peat moss. So, if you allow that mixture to begin to settle for a few seconds and then pour off the top half, or so, of the water you will be pouring away some of the peat but not the eggs, which have already settled to the bottom of the container. You can then add more water to the original container, mix it, and repeat the pouring off of the top portion after allowing a little settling time. If you keep doing this, what you are left with in the original container is all or most of the eggs in a small quantity of the original peat. My next step is to pour a little of this "egg concentrate" into a clear-bottom plastic container, add a little more water and then put that container onto a light box. If you don't have a light box, just shine a flashlight up through the bottom of the clear plastic container. If your eyes are good, you can see the eggs by looking down into the container. My eyes are getting a little older so I employ a head-mounted magnifier to assist me (see the photograph.) I use an eye dropper or plastic pipette to remove the eggs from the concentrate into another container. This process is repeated until I have removed as many eggs as I can find from the concentrate and placed them in a container of water. I have tested my technique and I find that I can locate and remove at least 90% of the eggs from a container of peat each time. It doesn't matter if a few eggs are missed, because the peat is then placed back in the tank with the spawning fish. Any missed eggs can be collected the next time that peat is examined.

Once you try this egg separation, you will be amazed at how productive certain species are, especially the smaller *Simpsonichthys* species.

Gnatholebias hoignei, male
Photograph by Tony Terceira

Simpsonichthys sp. BA-2-02
Photograph by Andre Carletto

It is not unusual for me to find several hundred eggs when going through the peat from a one week spawn of *S. alternatus, S. picturatus* or *S. magnificus*, for example.

Once I have the eggs in water, I put them in a small, closed plastic container- the type used to for storing leftover food. These containers hold anywhere from 1 cup of water to a pint or two. Water depth is between 0.5 and 1 inch. For most species of *Simpsonichthys*, I try to store the egg containers at between 75 and 80°F. Higher temperatures will speed development and may be useful for incubating eggs of slow developing species such as *S. magnificus* but be careful, because the higher temperatures can be fatal to the eggs of other species, such as *S. boitonei*. Note that I have not yet tried this process on any *Austrolebias* species. But, when I do, I will certainly use much lower incubation temperatures. I usually examine these eggs in water at least once or twice each week to remove any dead eggs. I also change water in the containers if there are many dead eggs or if there is a foul odor. Despite the fact that we have been warned for many years that light will adversely affect killie eggs, I have never taken pains to keep my eggs from the light. In fact, the eggs are stored in translucent containers, not very far away from a fluorescent light fixture. I have never detected any negative effect of light on my developing eggs.

For most species, some development can be seen in the eggs after a few weeks in water. Complete development can take anywhere from 1 month to more than 1 year, depending on the species and the incubation temperature. Two to three months is probably typical for the complete development of most *Simpsonichthys* species eggs at temperatures in the upper 70's °F. Eggs of the *Gnatholebias* species were fully incubated in about 6 or 7 months at those temperatures. It takes a little practice to know when the eggs are fully developed and ready to hatch. I use a strong magnifying glass or a low power microscope and look at the eye of the embryo. If you can see the golden ring around the eye, the embryo should be ready to hatch. Once I see that ring has formed, I usually wait at least 1 week before hatching them, just to make sure. For most species, once the eggs are eyed-up, you can even delay hatching for a month or two without any negative effects if you like. Occasionally, one or more of the eggs will hatch spontaneously in the incubating water-these are invariably belly sliders. Even if only a portion of the eggs are fully developed, I can hatch those eggs and leave the rest to complete their development in the water. This latter idea can be crucial to the successful propagation of a difficult species such as *S. marginatus* in which the incubation time for the eggs collected in a single batch is very variable.

I force hatch the fully developed eggs in peat extract. I usually keep a 2 gallon container with water and about 10 or 15 Jiffy 7® peat pellets in my fishroom. When I need peat in which to spawn some fish, I just use that peat.

The peat is never boiled. I just remove and discard the nylon netting from the pellets and put the pellets in a gallon or two of hot water and by the next day the peat is on the bottom and ready to use in a spawning container. The tea-colored water above this wet peat is the peat extract. I fill a small glass vial (any size container will do) about 90% full with this extract. I then use a pipette to place all of the fully developed eggs in that vial of peat extract and I add a pinch or two of the peat on top of the eggs. This pinch or two of peat is probably unnecessary, but I have not yet tried to leave it out. It's a part of my "ritual". I then put the cap on the vial loosely and place the vial on the bottom of a 55 gallon tank. The bottom of the vial is about 15 inches below the surface of the aquarium water. Many years ago, Bob Morenski (3) showed that the pressure at the bottom of a deep aquarium is a significant aid to force hatching eggs. Since reading his article, I have taken hatch-resistant eggs and placed them in containers in deep water and I have had several very positive results. This pressure hatching idea is so good that I now hatch all of my eggs, even those incubated in peat, in containers under deep water. Just remember that the top of the container has to be loose to allow the pressure of the deep water to be transmitted into the hatch container. This is very important. I usually see fry begin to hatch and appear on top of the peat within an hour or two. A photograph of the fry hatching in a vial is shown. I almost always remove the hatching container from the deep water after 3 or 4 hours, total. I am always afraid that the newly hatched fry will die due to lack of oxygen in the filled container so I take the container out of the 55 gallon tank and remove the lid to allow air to get to the water surface. A few hours later, when the fry are swimming, I pour the contents of the vial, or other hatching container, into a larger container with similar water.

Table 1 shows the results of 13 recent water incubation tests using 11 different species, including both short and long incubation time fishes. As you can see, there is some variation in the yields, but most yields seem to center near about 60% swimming fry from eyed-up eggs. The remainder of the eyed-up eggs either did not hatch or resulted in dead fry and bellysliders. Note that I have not reported the yield of eyed-up eggs from total eggs collected. Those yields are extremely variable. Usually, most of the eggs which are collected remain viable and wind up as eyed-up eggs. However, for some unexplained reason, an occasional batch of collected eggs just dies and funguses after a few days. Just be careful to check the freshly collected eggs in the incubating water 2 or 3 times in the first couple of weeks to ensure that any dead eggs are removed. Even if a few dead eggs left in with the good eggs do not result in a loss of the good eggs, they can cause a problem. Low oxygen (or high carbon dioxide-I'm not sure which) content in the incubation water will slow down the egg development. In fact, I have done tests in which I have compared the rate of development of side by side batches of eggs with an air

Results of Water Incubating Eggs

SPECIES	INCUBATION TIME	EYED UP EGGS	SWIMMING FRY	YIELD
	(in months)			
S. adornatus	1	65	44	68%
S. adornatus	?	35	24	69%
S. antenori	5.5	26	19	73%
S. boitonei	2	11	7	64%
S. marginatus	1.5 - 4	22	12	55%
S. ocellatus	2	8	8	100%
S. picturatus	1.75	92	55	60%
S. picturatus	3	7	6	86%
S. sp. BA-2-02	1	84	53	63%
S. trilineatus	2	59	20	34%
M. lacortei	4.5	17	12	71%
Gnath. hoignei	6.5	53	33	62%
Gnath. zonatus	4	11	5	45%

line bubbling beneath the water in one of the batches. The difference in the rate of development is startling; aeration can definitely speed the rate of development of water incubated eggs.

Now all of this may seem like it's a lot of work- and it is. But, it produces excellent results almost *every time*. The standard method of peat incubation is certainly less work, but the results of this water incubation process are so consistent and so good that it is definitely worth the extra effort. You don't have to do it too often. After all, how many fry do you need?

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