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Cover image: Spawning group of Scleromystax barbatus. Photo: M. Hardman





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AGM 2017 and constitutional amendments

The Catfish Study Group's AGM was held on the 15th January 2017; two main points of constitutional change were proposed and one was voted upon.

> Following discussion, the proposal to reintroduce the



introduce the President role was withdrawn by its proposer.

• The proposal to change CSG membership was voted in. This means free membership achieved via the CSG website by submission of an email address has been replaced by paid CSG journal subscription.

The CSG constitution (available on our website) has been changed accordingly and minutes of the AGM are available on request.

An email was sent to the mailing list subscribers, in other words, members of the CSG before the 15th of January vote confirming the change in membership status. The CSG mailing list will continued to be used periodically to update its subscribers with new issues of the Journal and event information. However, our primary method of communication with those interested in the CSG is via our <u>Facebook</u> group.

As a journal subscriber, from the 15th of January you are a member of the CSG and will continue to be until you do not renew your Journal subscription. We will ensure you are kept up-to-date with governance matters on an ongoing basis as they arise via the Journal and via the email address you supplied when paying for the Journal.

As ever, any questions, please get in touch.



Chairman's report

It's that exciting time of year again when the CSG demonstrates its truly international credentials with the biggest and best catfish convention on the planet. Well, you wouldn't expect me to say any less would you



say any less would you - I'll leave the Chairs of the many other fantastic events around the world to blow their own trumpets!

I am extremely proud of our committee and the effort they have put into holding the 38th annual convention, all voluntarily – sacrificing many hours of their precious time to manage speakers, promotions, finances, sponsors, awards, accommodation, IT and of course delegates. I am sure you will all show your appreciation of their efforts when you attend the event and realise the enormity of the task to host a convention of this size.

Coincidentally, 2017 brings main speakers from Brazil, USA, Denmark and Germany (as per 2016 – but different speakers!), plus three evening talks from UK, Germany and the Netherlands. The overall package with nine talks over three days demonstrates what a world-class event is on offer. Our sponsors have come-up-trumps again with donations of aquatic products for the massive prize draws and some mini auctions, during the event. All sales tanks are booked with the promise of some usually unobtainable species of catfish on offer.

Most of all, I'm looking forward to meeting my catfish friends at the event, and making some new ones. I expect that with all the planning things will run smoothly, but if there are any issues please have a chat with any of the committee members at the event, or the extremely helpful hotel team.

Looking back over recent CSG events, there is a write-up on the February Spring' auction, one of the most successful of recent years, plus the main outcome of the recent Annual General Meeting.

Back in the fish house, I've been spending a lot of time preparing fish for display at the Convention – including a few surprise inclusions, but have also set up a few tanks for hopefully breeding some recent acquisitions including Corydoras gracilis, C. eversi and the gold form of Aspidoras C125. So much to do and so little time!

Mark <u>chairman@catfishstudygroup.org</u>



Diary dates 2017				
Date	Event	Location		
TBA	Away day with Wiltshire Plecos	TBA		
9–11 June	Cory-vention (Ian Fuller)	Brittania Hotel, Wigan WN6 oSR		
9 July	Castleford AS catfish & loach show	Lock Lane Centre, Castleford WF10 2LW		
24 September	Open show and auction	Derwent Hall, Darwen BD3 oDQ		
27–29 October	L-number days	Hanover, Germany		
19 November	Autumn auction	Derwent Hall, Darwen BD3 oDQ		
10 December	Christmas meeting	Derwent Hall, Darwen BD3 oDQ		

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Breeding *Panaqolus albivermis* (L204), the flash pleco (Siluriformes: Loricariidae)

By Jacqueline Heijmen Bennett-Leaver



Fig. 1. Adult female Panaqolus albivermis. Photo: Jacqueline Heijmen Bennett-Leaver

Panaqolus albivermis is one of my favorite fish. I got my first fish in 2006, but they turned out to be all males, and among the 30 or so I bought (and subsequently re-sold) over the next two years, all but one of them was a female. For some reason, I found it very difficult to find a female. However, after finally ending up with a pair, I kept them for four years without them showing any interest in spawning. Due to equipment failure, I lost the male so I gave the female to a friend for her to try and breed them.

About two years later, a member of a forum showed me his group of *P. albivermis* and asked if I could sex them. I was amazed to see what looked like a group composed of two males and



Fig. 2. Group of *P. albivermis*; two males and four females. Photo: Jacqueline Heijmen Bennett-Leaver.

four females! I told him if he ever wanted to sell them I would be very interested in taking the entire group. A year later, he did and I brought them home (Fig. 2) in September 2013.

Sexing

The males are easy to recognize. They are covered in odontodes. That does make them a little less attractive than the females and makes them more dark grey than the high-contrast females (Figs.1 & 3). I noticed that females can also develop odontodes when they are not in breeding condition. This can make it difficult to spot them soon after being imported. If you're not sure, there are a few other things you can check.

One of the best sexual differences can be seen in their pectoral fins. In males, these are short and rounded. In females they are longer and pointed. Adult females that are in good condition, you can also look at the vent, which should have small orange papillae (Fig. 4). This is something I have noticed on different species including *Peckoltia* and *Spectracanthicus*. But not all have this feature.



Fig. 3. Adult male and female P. albivermis. Photos: Jacqueline Heijmen Bennett-Leaver.

Maintenance and care

I'm a very laid back hobbyist breeder. I don't do fancy stuff such as making my own water with reverse osmosis units or simulate dry and wet seasons. We are blessed with excellent soft water in our area so I keep it simple, using only tap water. Our pH is 7,2 , hardness 3° GH and a conductivity of around 300 microsiemens. I do believe in good maintenance, so regular water changes and giving good foods are top priorities. I change water in all my tanks two to three times a week. Once or twice a week I drain about 25% and then refill with cold water, and once a week I drain 80% and refill with warm water. In winter, I often skip the cold water refill and add warm water instead.



Fig. 4. Close-up of orange papillae behind the vent of adult female *P. albivermis.* Photo: Jacqueline Heijmen Bennett-Leaver.

My *Panaqolus albivermis* are housed in a breeding system consisting of seven 175L tanks, filtered by a large biological filter in a sump. I keep them in tanks without gravel or sand but provide some caves, slate and soft wood. The Dshaped caves I use (and which the male seems to prefer) are made from dark clay and about 25cm long, 5cm wide and 4-5cm tall. I have caves with a triangle profile, which I use a lot for other species, but they are not interested in them.

I don't use a circulation pump for extra flow.

Each tank has a sprinkler system above which gives a soft flow when the water comes into the tank. I can adjust it to make the intake a little stronger so more current is in the water. The filter adds all the needed oxygen to the water.

The lighting is medium on all tanks, I use LED strips. The fish don't need it, it is only there for my own convenience and because it looks nice. The light is on from about 14:00 till 19:00. After the lights go out, I try not to disturb the fish unless I really have to.

Just before the lights turn off I feed the fish. I feed them Repashy herbivore and carnivore formulas, nothing else. I have found that my group can eat a lot of food.

Spawning

As mentioned above, I don't specifically encourage my fish to breed. I simply try to provide them with everything they need. Observing them closely every day I started to notice some of the females were becoming fuller in the abdomen. Also, the two males had been fighting a lot with one suffering considerable damage to his fins and skin.

After every large water change they became noticeably more active, showing signs of prespawning behaviour. The dominant male set himself up in a cave (Fig. 5) and I found him in there almost every day after that. One of the females started to hang out near or alongside of the male's cave. In another spawning report of this species, the author mentioned these fish interacted via their long caudal-fin filaments, but in all the time I've spent watching my group I have not seen this behavior. In fact, the female always faced towards the front of the tank as the male is always head first in the cave, so it's more head-to-tail.

I did not notice any particular behaviour or activity before the female entered the male's cave to begin trapping. On several occasions, I have been working in the fish house and noticed the female alongside his cave, and when I was finished and looked again they were trapping.



Fig. 5. Male *P. albivermis* established in the spawning cave. Photo: Jacqueline Heijmen Bennett-Leaver.

Once underway, trapping is swift and spawning takes place within one or two days. The first time I didn't even notice them trapping and discovered the male incubating eggs on O2 January 2016. Unfortunately, this first batch of eggs was in poor condition, most likely due to my previous problems with endocrine disrupters caused by soluble plastics in my system (Bennett-Leaver, 2016), and the eggs died after a couple of days.

After several trappings without success and moving house in August, they spawned again on 27 October 2016. This time, the eggs looked good and I left them with the male and hoped for the best. During the first 24-48h, I collected a few loose eggs from outside the cave, but within three days these had unfortunately succumbed to fungus and the remaining eggs in the cave had disappeared. Another failed spawning.



Fig. 6. Artificial incubation of *P. albivermis* eggs. Photo: Jacqueline Heijmen Bennett-Leaver.

Although it's always the same male who has the eggs, both males trap females. Sometimes a female will trap with the sub-dominant male and then swap to the dominant male the day after. I also found other females will disturb a trapping at the moment the male gives the trapped female space to turn around in the cave. This can go on for weeks or even months before things go quiet again.

In trying to solve this problem, I once removed the sub-dominant male but this had the effect of stopping *all* spawning behavior. I returned him to the group. I have started to suspect that social factors within a spawning group are just as important as the right cave and water quality for a successful spawning.

They spawned for the third time on 14 December 2016. This time, I didn't take any chances and after 24 hours I collected the eggs and incubated them artificially. I use a very simple but effective set up; a 30L tank containing a heater and a small power filter with the eggs held in a plastic breeder box in of the flow (Fig. 6). I cover the tank and add an antifungus treatment to prevent any microbial attack.

Each day I performed small water changes, switched the breeder box for a clean one and gently cleaned the egg mass. The eggs developed day by day without problem and after only four days (120 hours), they began hatching! I had only lost a few eggs along the way and ended up with about 45 fry.

I transferred the fry from the incubator tank to the main breeding system, putting them in one of my fry boxes. I covered the bottom of the fry box with some gravel and added some shelter (Fig. 7). I have hatched and raised Panagolus sp. L397 before and learned that *Panagolus* can be very sensitive to the microbial film that grows on the glass and other surfaces. I hoped the gravel would keep the fry from the glass surface and improve their survival. It worked for most, but some fry developed a problem with the yolk sac after the fourth day. In these cases, the volk sac began filling with fluid and the fry eventually died (Fig. 7, inset). I have talked to many other breeders but no one really knows exactly why this happens. My opinion is that it has to do with bacteria and water conditions.



Fig. 7. Fry raising box and fry suffering yolk sac problem (inset). Photo: Jacqueline Heijmen Bennett-Leaver.

The rest of the fry developed quickly, dissolving their yolk sacks within 10 days (Figs. 8-9). Eleven days after hatching I started feeding Repashy herbivore formulas. I am delighted to report that the fry are doing very well and growing into small replicas of the adult fish with striking golden lines on a black body. You just got to love a nice Panaqolus albivermis!

References

Bennett-Leaver, J. H. 2016. Dealing with a silent assassin: endocrine disruptors in freshwater aquariums. *Journal of the Catfish Study Group* 17(2): 17-20.







Fig. 8. Development sequence of larvae in P. albivermis. Photos: Jacqueline Heijmen Bennett-Leaver.



Fig. 9. Development sequence of fry in P. albivermis. Photos: Jacqueline Heijmen Bennett-Leaver.

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Update on a spawning account of *Spatuloricaria puganensis* (Siluriformes: Loricariidae).

By Mark Walters



Fig. 1. Male *Spatuloricaria puganensis* brooding a developing egg plaque. Photo: Mark Walters.

I last wrote about my experiences with this giant whiptail in March 2016, following an unexpected spawning event which is believed to be a first for this species. By means of an update, the dozen youngsters which I raised continue to thrive in my tanks, happily co-habiting with their parents. My only comment on their development would be the relatively slow growth rate after 15 months, attaining a size of 80mm SL, although perfect miniatures of their parents with impressive 'whips' on the upper lobes of their caudal fins.

The two pairs of adults I originally purchased nearly two years ago have proven easy and relatively trouble-free to look after, although again I am surprised that the smaller adults have not yet reached that of the largest pair (over 250mm) which have been spawning. I would attribute the slow growth of both the youngsters and smaller adults to unknown limiting factors related to life in captivity. It could also be that they are a slow growing species, with associated longevity, although Michael Hardman tells me has not observed the same growth pattern in his fish from the same shipment, and that the low temperature I generally run in my fish-house (24°C) might explain the slow growth.

The young seem sensitive to routine tank maintenance or other sudden movements when they flee for cover. For this reason, I have not offered any of the young to fellow aquarists, fearing they could suffer in transit. In combination, these observations have deterred me from making any focused effort to spawn this species again – there are lots of loricariines I would like to keep tank space available for.

Spawning and raising *S. puganensis* has left me with several unanswered questions, which described a behaviour unlike that of other species in this genus, and I was obliged to incubate the eggs and raise the fry artificially. Specifically, my questions included:

- Is this species really a broadcast spawner?
- Does this species spawn seasonally in the aquarium?
- How do the parents interact during spawning?
- How long does spawning take to complete?

- Assuming many of the eggs in the first spawning were predated, how many eggs does a female produce in a typical spawn?
- Does the male exhibit any broodcare?
- What is the accurate incubation time for eggs at a given temperature?
- What is the behaviour of the male and fry during and after hatching, respectively?

I had an opportunity to answer some of these questions late in 2016. Up to this point, the fish had not shown any further signs of breeding behaviour. The male had reabsorbed his cheek odontodes a month after spawning and the females had not roed-up again. During October, I noticed increased interactions between the breeding pair and the first signs of the male developing his spawning gear. I was planning to move a few fish between tanks and decided to take the opportunity to give the giant whiptails the space they deserved.

I had a pair of Leporacanthicus sp. L240 which have spent the past few years in a 120gallon tank occasionally showing some interest in spawning. I'd decided a change was needed, so downsized them to a tank where I could perform more frequent water changes. This freed up their tank for the Spatuloricaria, providing a larger footprint for them to roam around in their search for food. I also took the opportunity for some aquascaping and in addition to the usual tree branches and other wood tangles, offered some hard substrate options. In this case, two house bricks and a roof slate. The logic was based on reports of two other Spatuloricaria species which had spawned on the underside of overhanging stones. I have spawned other loricariines (e.g., Sturisoma aureum) on fixed substrates so was keen to give my group of Spatuloricaria the option.



Fig. 2. Spawning pair of *S. puganensis* at the start of spawning, female to the left. Photo: Mark Walters.

After another month, the large male had developed a full flush of cheek odontodes and the largest female (his previous mate) looked to be swollen with eggs. A few days later, with no obvious triggers on my part, the two fish were displaying over the slate (Fig. 2). The male had darkened in colour and was observed shimmying excitedly in the presence of the female, who retained her usual light brown colouration.

A few days later, one morning I noticed eggs spilling over the slate with a few falling onto the sand substrate below the cave structure. After a few hours, the pair had improved their efforts and a single layer of eggs was gradually laid on the slate surface. This behaviour continued and 18 hours later, the male was actively guarding an impressive plaque of eggs (Fig. 3). Similar to other loricariids, the female played no further part in spawning or care of the eggs.



Fig. 3. Male $S.\ puganensis$ brooding a freshly spawned egg plaque. Photo: Mark Walters.

So, that was the first few questions answered; yes, *S. puganensis* is a fixed-substrate spawner and no, the first spawning event was not typical. The male continued to protect his eggs for 10 days, before I started to take more interest in when they might hatch. Eggs laid in the previous spawn began hatching after 10-12 days, although I suspected this was too soon as fry hatched with large yolk sacs and they seemed underdeveloped compared to other loricariines I have spawned.

So that I could give the fry the best chance of survival, I decided to move the brooding male and eggs to a rearing tank. The male's protective instinct meant I could lift the slate into a submerged tub, with him still performing his duties. I took the opportunity to photograph the eggs so that I could count them accurately – over 400! I moved the slate, eggs and male into a prepared 40 gallon tank elsewhere in the same system. The male seemed unperturbed and continued caring for the eggs (Fig. 4).



Fig. 4. Close up of eggs of *S. puganensis* after 10 days of development. Photo: Mark Walters.

In the rearing tank, it was easier to observe his behaviour and monitor egg development. I was fascinated by the way the male used his fringed lips (Fig. 1) to methodically clean the eggs. I assume he was removing any debris, checking for dead eggs and helping to ensure they were well oxygenated.

Eggs began hatching after 12 days and continued over the next 48 hours, with the last few taking over 14 days to emerge! The male was quite active during this period, moving over the eggs, perhaps making sure that the fry made it out of their eggs when ready to. From observing other Loricariinae with cheek or snout odontodes (e.g., Farlowella and Sturisoma), I wonder if they use the short bristles to help fry hatch by rupturing the egg membranes. Whether this is the case, or if the eggs need any intervention at all, it offers a suggestion for the development odontodes seasonal of in Spatuloricaria and other loricariines.

Watching the male also led me to suspect he might be consuming an egg or two, and I began to worry he might begin feeding on the larvae. I moved him back to the spawning tank and I was delighted to see hundreds of healthy fry in the rearing tank that seemed to have smaller yolk sacs compared to the first spawning event. This agrees with my thoughts about the earlier clutch hatching too soon.

My usual anxiety now set in about offering the right foods at the right time, although in a more natural rearing tank – to which I had added leaves and branches – provided plenty of surface area for aufwuchs to develop and serve as a first food. I also began adding small quantities of Spirulina powder and microworms after a few days before increasing the quantity and variety of foods over the coming days and weeks. The rearing tank also had the advantage of being on a circulatory system so water conditions remained stable than a small individual tank.

After four months, I now have a tank containing a few hundred juvenile *Spatuloricaria* actively feeding on *Artemia* nauplii, powdered tablet/flake and agar-gel foods (Fig. 5). I expect it will be another 12 months before they are ready to share with other fish keepers, but they make for an interesting display in their own right. Whether I will be able to continue raising future broods the adults produce, even only once a year is another matter!



Fig. 5. Juvenile *S puganensis* in the rearing aquarium after four weeks. Photo: Mark Walters.

Report on the 2017 Spring auction

By Mark Walters

The CSG has held its three auctions a year for as long as I have been a member. My first CSG auction was in 2005, when I filled my boots with tank-bred corys that have fed my catfish obsession to this day. Over the past 12 years, I've obtained most of my rare fish from auctions, many tank bred and some which have led to breeding successes of my own.

As a committee member wearing many hats, I now organise the event and perform auctioneering duties with welcome support from volunteer runners, book-keepers, raffle ticket sellers and hard-working canteen staff.



Peckoltia sp. L397. Photo: Terry Gargan

My enthusiasm for the auctions has been on a roller-coaster over recent years with a few events not generating the interest necessary to justify the effort. However, our auctions have built on the success of earlier events and now enjoy repeat booking from vendors and more and more buyers coming through the doors having learned about the quality, rarity and excellent bargains that can be had on the day.



Corydoras gracilis. Photo: Colin Eveson

The Spring 2017 auction was the largest I have been involved in. Brisk selling of fishes, equipment and literature from 20 lots lasted

four hours, with prices remaining fair for sellers and buyers throughout. As a result, we are delighted to report a record sales commission. This has eased the concerns of our Treasurer at this expensive time of the year when we pay for the travel and accommodation of our convention speakers.

Unfortunately, we don't have the resources to record a list of species sold, but I can recall over 40 corydoradines, 20+ plecos, a dozen or so loricariines, numerous woodcats, mochokids, doradids and lots of other fishes besides. Highlights for me were breeding groups of *Corydoras gracilis*, a breeding pair of *Ancistrus* L184 and a fin-perfect *Pseudacanthicus pitanga*!

One lot contained six boxes of aquatic books, which were battled over by enthusiastic bidders, including sets of the Baensch Atlas, rare *Corydoras* books, Aqualogs and some weighty cichlid atlases.



Synodontis lucipinnis. Photo: Danny Blundell

If you missed this one, we hold a smaller auction during the open show (September 24) and another regular auction in the Autumn (November 19). Sellers can reserve lots up to two months in advance right up to the day itself. Come along to our next auction and enjoy a friendly bidding war or a delicious hot pie from our award-winning canteen!

> LIVE EISH RUSH

How do corys fertilize their eggs? - An evaluation of thru-gut insemination and observation of a novel component of spawning behaviour (Callichthyidae: *Corydoras*).

By Michael Hardman



Fig.1. Pair of Corydoras sp. C126. Male below and female inverted showing eggs in pelvic-fin basket. Photo: Rita Dahl Aspevik

Corys are firm favourites among aquarists for many reasons. For me, their greatest appeal lies in their fascinating reproductive biology. Most of us will have witnessed their spawning when males chase females and repeatedly form an embrace (or *T-position*) during which eggs are released into a basket formed by the female's pelvic fins (Fig. 1). The embrace is held for a few seconds (Fig.2), the pair rest for several more, and then the female begins searching for a suitable surface to place the eggs on. Several minutes later, once the eggs are successfully placed and the pelvic fins are empty, the spawning cycle repeats perhaps 10-50 times over several hours.

Although variation exists in the number and size of eggs released, the intensity of spawning, and preferred site and pattern of egg placement, most corydoradines (i.e., *Aspidoras, Corydoras* and *Scleromystax*) spawn in this way (Breder & Rosen, 1966; Fuller, 2012; <u>Corydoras Spawning Reports</u>).

Spawning embraces and adhesive eggs are seen in many other catfish families. Some

loricariines (e.g., Loricariichthys) carry a raft of fertilized eggs on an expanded lower lip. Many ariids (e.g., Bagre) and a few claroteines (e.g., Phyllonemus, Lophiobagrus) are oral incubators. Some banjo catfishes (e.g., Aspredo) develop Platystacus, specialized structures on their bellies from which fertilized eggs are suspended and possibly nourished (Breder & Rosen, 1966).

The reproductive strategies and behaviours shown by catfishes are overwhelming and may be involved in their persistence and diversification through time. Examples can be found of other bony fishes that reproduce in similar ways to catfishes, but the transport and placement of adhesive eggs released into a pelvic-fin basket is unique to corydoradines and, possibly, some hypoptopomatines.

While eggs in fin baskets (Fig. 1) are something of a novelty, the observation is uncontroversial. However, one of the perennial questions concerning corydoradine reproduction concerns *when*, *where* and *how* the eggs are fertilized. In what is considered to be the first



Fig. 2. Spawning embrace of *Corydoras fowleri*; female to the left. Photo: Roland van Ouwerkerk.

captive spawning of a cory, Carbonnier (1881) noted their peculiar reproduction of *Callichthys fasciatus* (syn. *Corydoras paleatus*):

"At the moment of fecundation the female brings together her ventral fins, after the fashion of two open fans united by their edges, and thus forms a sort of cul-de-sac, at the bottom of which the aperture of the ovaries opens. The fecundating elements of the male are imprisoned in this sort of membranous sac; and when, a few moments afterwards, the eggs arrive in the same place, they will find themselves bathed in a liquid very rich in spermatozoids."



Fig. 3. Pierre Carbonnier (1828-1883); the first aquarist to report on cory spawning behaviour. Photo: Creative Commons.

Evidently, Carbonnier (Fig. 3) believed that eggs were fertilized by sperm that were already *inside* the basket when the eggs are released, but gave no explanation as to how the sperm got there.

In their 941-page classic Modes of Reproduction in Fishes, Breder and Rosen (1966) explained how from the late nineteenth century onwards, aquarists and ichthyologists have speculated on the mechanics of egg insemination in corydoradine catfishes. CSG meetings and social media threads suggest this debate continues today, and I spent some time in 2013 thinking about this issue in light of some papers published in 1995 and 2002, and making observations that might shed some light on this 140-year-old question.

There are basically three competing explanations for how the sperm reach the egg surface. Sperm are released by the male during the embrace and: 1. travel along the ventral contours of the spawning pair before entering the pelvic-fin basket, or; 2. are held in the mouth of the female until being placed onto a surface immediately prior to egg placement, or; 3. are swallowed by the female and passed through the gut and released into the pelvic-fin basket via the anus immediately prior to eggs being released. From here on, I will be referring to these mechanisms as *external* (1), oral (2), and thru-qut (3).

In most cases, authors have discussed this in terms of what they *think* is happening rather than by employing a robust experimental approach or a detailed study of environmental samples taken during spawning. Most aquarists tend to prefer either external or oral mechanisms and reject thru-gut without presenting any strong evidence either way. The first experimental approach to this question was provided by Masanori Kohda and colleagues at the Osaka City University in Japan (Kohda *et al.*, 1994, 2002).

Kohda *et al.* (1995, 2002) approached the problem by trying to answer three specific questions: 1. *who* fertilizes the eggs?; 2. *when* are the eggs fertilized?, and; 3. *how* are the eggs fertilized?

They answered the first question by allowing albino females to spawn with a mixture of albino

and wild-type (i.e., normally pigmented) males. Typically, albino fish are born from albino



Fig.4. The principle behind experiment 2 in Kohda *et al.* (1995). Normally pigmented (wild type) *C. aeneus* produce similar offspring. Only when both parents are albino will all the offspring be albino. Photos: Creative Commons.

parents. If either parent is pure wild-type, all the offspring will look wild-type but will be "carrying" the albino allele in their DNA. By tracking which male the albino female spawned with, then collecting the resulting eggs and hatching either albino (sired by the albino male) or pigmented (sired by the wild type), Kohda *et al.* (1995, 2002) showed that the male that forms the embrace is the male that fertilizes the eggs released during that encounter (Fig. 4).

In a second experiment, Kohda *et al.* (1995, 2002) collected eggs from the pelvic-fin basket at different times after their release and monitored their fertilization rate. They determined that 90% of eggs were fertilized within 60 seconds of release into the pelvic-fin basket.

In the third and most controversial experiment, Kohda *et al.* (1995, 2002) claimed to have released a small amount of methylene blue dye into the mouth of a spawning female during an embrace and to have witnessed the dye exiting the anus into the pelvic-fin basket several seconds later (Fig. 5).

According to these results, it would appear that corys rely on thru-gut insemination (TGI) of their eggs. It is important to note that, as far as we know, such a mechanism is unique within the animal kingdom and anything that rare should be scrutinized *very* carefully. But, as scientists, we must accept the published and peer-reviewed work of our colleagues and assume their observations are accurate and the conclusions



Fig.5. Modified figure from Kohda *et al.* (1995) illustrating experiment 3 in which methylene blue dye is used to demonstrate sperm drinking and thru-gut insemination.

are correct unless we can find compelling evidence to reject them.

How might we scrutinize the claim that corvs are, in fact, swallowing sperm and passing it through their guts in a matter of seconds before releasing it, intact, into the pelvic-fin basket prior to the release of mature eggs? Sperm are microscopic and it is very difficult to observe them with the naked eye, especially during a spawning embrace that might only last a few seconds and usually takes place in a secluded corner of the aquarium. Confirmation of viable sperm in the hindgut of a spawning female is probably a technical impossibility. In the absence of direct methods to detect the sperm, we are left to consider *what must also be true* if female corys really are passing sperm through their guts.

Complex animals like corys exist as beautifully integrated miracles of biological engineering. If they are doing something as bizarre as drinking sperm, there will likely be tell-tale adaptations elsewhere in the animal. For example, in the sperm microanatomy (i.e., so that it can survive the hostile environment of the stomach and gut) and male reproductive organs, the female gut and its associated structures, and possibly the egg surface itself. We might also expect there to be some behavioural anomalies seen during spawning that only make sense if it is true. Also, if TGI is correct, it also has some requirements that we can evaluate with respect to what we know about fish reproductive biology generally - to see if it holds water, so to speak.

Corydoradine eggs are interesting objects. Anyone that has tried to remove them from plant leaves or glass panes of the aquarium will know that they can be surprisingly sticky, but adhesive property is based this on а morphological rather than chemical adaptation that is not known in other fish groups. The surface of the average cory egg is covered with a carpet of small stumps, or *papillae*, arranged in a somewhat regular lines (Fig. 6, Huysentruyt & Adriaens, 2005). When pressed, the papillae squeeze out the water in the microchannels to create a small sucker when the pressure is removed wherever they come into contact with a smooth surface - this is what the female is looking for and why she so diligently cleans any debris from the site to ensure a good purchase. In and of itself, the egg surface ultrastructure is fascinating and helps us to understand how they are made so sticky, but I'm not sure it has much to say about TGI. While the spaces between the papillae provide a refuge for sperm searching for the *micropyle* (a small hole leading into the egg nucleus and the gateway to fertilization), they might also serve to trap them in a sort of maze.

What about the journey that must be made by the sperm from the male genital opening to the egg surface? How far does the sperm have to travel? According to Kohda *et al.* (1995, 2002), it only takes around 5-7 seconds for the dye to be swallowed and released at the anus, so how fast does the sperm have to move (or *be* moved) to make it through? Is this implied speed comparable to what we know about fish sperm generally?



Fig.6. Scanning electron micrographs of the egg surface (left) and micropylar region (right) in *Corydoras aeneus*. Reproduced with permission from Huysentruyt and Adriaens (2005).



Fig. 7. Dissection sequence of female *C. schultzei*. a. intact specimen. b. with body plates and skin partially removed. c. with pectoral girdle removed showing compact mass of viscera. d. with ovaries removed, the remaining mass contains the stomach, gut, liver and other minor organs. Note the tightly coiled intestine as it sits in its natural state. Photos: Michael Hardman.

To answer these questions, I first dissected the stomach and gut from a preserved female *Corydoras schultzei* (Fig. 7). For this individual, and others I've looked at over the years, the stomach and gut in corys is simple and when carefully teased out is approximately the same length as the fish without the tail, in this case ca. 50mm (or 50,000 μ m) (Fig. 8). If we assume the dye experiment showed that sperm takes ca. 6 seconds to make it through the gut (ca. 50,000 μ m), that's an approximate swimming speed of ca. 8,300 μ m (or 8.3mm) per second, and 40 times faster than the fastest swimming speeds of fish sperm that have been measured so far (i.e., 200 μ m/s in *Lepomis* and *Gadus*)! With this in mind, it's not likely the sperm are swimming through the gut.

What about the microanatomy of the sperm? Is it in anyway specialized to suggest an adaptation to TGI? Fortunately, Maria Spadella and colleagues at the Universidade Estadual de Campinas and Universidade Estadual Paulista have studied the sperm of *C. aeneus* and found it to be rather unspecialized and not showing any adaptations for high-speed swimming such as a large flagellum (Spadella *et al.*, 2007).

Morphology of the testes can also reflect reproductive strategy. Cory testes are unremarkable, but sperm density is low, suggesting that the fertilization rate is high and sperm competition is limited. The seminal vesicle, located at the end of the spermproduction line, is a gland that secretes the seminal fluid in which sperm are released during spawning. In corys, the glands are well developed and produce a thick, mucus-enriched fluid that is believed to provide protection and serves to contain the sperm and delay their activation after release by the male (Mazzoldi et al., 2007).

In summary, research into sperm and testicular morphology suggests that corys enjoy highly-efficient insemination and that sperm



Fig.8. Stomach (foregut) and intestine (midgut and hindgut) dissected and straightened to estimate length. Photo: Michael Hardman.



Fig.9. Medial (internal, left image) and lateral (external, right image) view of left half of the pectoral girdle in *C. schultzei*. Red arrows indicate approximate location and action of adductor muscles serving the pectoral-fin spine. Photos: Michael Hardman.

must be brought into close contact with the egg surface because they are not adapted for highspeed or long-distance swimming. The form and size of the seminal vesicle suggest sperm are packed together and protected in a thick fluid package. Together, these results *support* TGI.

So how is the sperm packet moved through the gut? If sperm are not swimming, there are two mechanisms by which they could be actively transported from the mouth to the anus: involuntary peristalsis (rhythmic contractions of muscle rings in the intestinal wall that slowly push food and feces through the gut) or a voluntary priming pump (controlled contraction of skeletal muscle to squeeze sperm through the gut like toothpaste from a tube). The blue dye experiment of Kohda et al. (1995, 2002) suggests the travel time is ca. 5-7 seconds, which favours the latter mechanism. Peristalsis is simply too slow; food and feces spend several hours in the gut under this mechanism. If TGI relies on a priming pump, we should be able to find the muscles involved with a careful

dissection of the body cavity.

The body cavity is a cone-shaped space sitting behind the bony wall created by the cleithrum and pectoral girdle and bounded by the rib cage, pelvic girdle and belly. The cavity contains a soft mass of organs, blood vessels, membranes and fat stores. The gut twists and turns through this mass to connect the muscular simple stomach to the anus.

There are few muscles on the inside of the body cavity. There are two pairs of muscles that operate the pectoral-fin spines; the adductor pectoralis superficialis and adductor pectoralis profundus (Fig. 9). These muscles insert on the base of the pectoral-fin spine and originate on surfaces of the the cleithrum and scapulocoracoid, respectively. Thev lie somewhat close to the muscular stomach (which may be under voluntary control in bony fish), and their contraction could help increase internal pressure at the front of the body cavity and provide the priming pump we're looking for. also a pair of muscle There is straps



Fig.10. Stills taken from video of a spawning embrace of *C. davidsandsi*. a.the male clasps the barbels of the female to his lower flank, note the pectoral fins are held away from the body of the female (ventilation of the gills temporarily ceases); b. the embrace continues and the female draws her pectoral fins tightly against her flanks; c. the male releases the female and her pectoral fins are again held away from the body; d. the female rests on the substrate with pectoral fins held away from the body and resumes ventilating the gills. Photos: Michael Hardman.

(the *infracarinales*) that extend between the pectoral and pelvic girdles on the inside of the belly skin, but the bony scutes of corys limit movement here and it seems unlikely they are involved.

In order for the pectoral-spine muscles to serve as an internal pump, they would need to contract during the spawning embrace and, as a consequence, we should see the pectoral fins being pulled towards the flanks. After reviewing some video footage I took of a spawning embrace involving C. davidsandsi, I noticed precisely this behaviour during the embrace and have since seen it in other spawning corys (Fig. 10). I hadn't noticed it before and, if it does not provide evidence of a priming pump mechanism, then its existence needs an explanation. Put another way, if not to increase internal pressure, why else would female corys retract their pectoral fins during a spawning embrace (Fig. 10b)?

These new observations tie the anatomy (pectoral-fin muscles) to а previously unreported aspect of the spawning behaviour (pectoral pump) and, in combination with sperm and gonad morphology, support TGI as the mechanism by which corys fertilize their eggs. However, the convoluted nature of the gut is surely a difficult conduit through which to selectively transport a fluid packet of sperm, and we might expect to see female corvs defecating and expelling any swallowed air in the early stages of spawning. Although TGI remains a contentious explanation, the evidence presented here seems to be consistent with it and I've so far seen nothing that convincingly rejects it.

One of the main stumbling blocks of TGI concerns the requirement that the female gut must be empty during spawning; a sperm packet would have a hard time being selectively transported through food and feces. This could be investigated by monitoring female feeding behaviour prior to and during spawning events, and confirming egg fertility/infertility of any embraces formed. This would be something that aquarists could provide valuable information on.

I will be posting the complete video of the spawning embrace in the <u>CSG facebook group</u> and invite your comments and alternative explanations there.

Acknowledgments

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