Journal of the American Association for Laboratory Animal Science
Vol 63, No 5
September 2024
Pages 472–479

Comprehensive Husbandry Protocol for *Corydoras* Catfish and Many Other Amazonian Species

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A variety of fish species have proven instrumental in the investigation of evolution, behavior, ecology, and physiology, among many other fields. Many model systems (e.g., zebrafish, guppies, and three-spined sticklebacks) have been maintained by institutions and have had protocols written with respect to their husbandry. Here we present the protocols we have developed to maintain and breed a variety of *Corydoras* catfish species, which are native to the tropical Americas. *Corydoras* species are excellent systems for investigating behavior, ecology, and other topics, and our husbandry protocols would be suitable for nearly every species in the genus. In addition, these protocols are appropriate for a variety of softwater Amazonian species, and we present options for a variety of housing and husbandry conditions. On the whole, we suggest that, in a scientific laboratory setting, the use of remineralized reverse osmosis water is most appropriate and that in context, a single measure, total dissolved solids, can be used to monitor the water chemistry for water introduced to fish enclosures.

DOI: 10.30802/AALAS-JAALAS-24-039

Part I: Introduction

***Corydoras*: fascinating and understudied.** *Corydoras* is a genus of neotropical fish belonging to the armored catfish family Callichthyidae. As the most species-rich genus of catfish,[29] *Corydoras* diversity is so overwhelming that a system of "C-numbers" is in use to describe new species. Best known for their prevalence in the aquarium trade, *Corydoras* are of particular commercial interest in the Amazon River basin and various *Corydoras* species are among the most frequently exported aquarium fishes in the region.[26,37] They occupy essentially every freshwater aquatic niche in the neotropics and exhibit tremendous diversity of morphology and ecological strategies. Still, the adaptive radiation of this genus remains incompletely characterized and understudied.[31]

Much of what we know about *Corydoras* life histories comes from accounts of captive individuals, both in the hobby aquarium and the laboratory. *Corydoras* species are known for their longevity, often living 10 to 15 y.[21] Although these data from captive *Corydoras* cannot be extrapolated directly onto wild populations, *Corydoras* species are not seasonal fish and may live for many years in the small bodies of water they inhabit. The aquarium hobby has been instrumental in shedding light on this fascinating genus.[21,35]

Despite the millions of *Corydoras* lives playing out in homes of hobbyists around the world, the body of scientific literature on *Corydoras* behavior remains limited. One of the most obvious features of *Corydoras* behavior is their sociality: many *Corydoras* species form large aggregations[21,29] that contain males, females, and juveniles.[38] *Corydoras* are omnivorous social feeders and use their sensitive barbels to forage through substrate for invertebrates and detritus.[38] They are benthic fish that prefer slow-moving, shallow water less than 2 m in depth.[21] In the aquarium, *Corydoras* are particularly favored for their gentle demeanor and charming aggregative behaviors.[11,21]

The limited laboratory studies available reveal the immense potential of this genus for addressing questions related to behavior, evolution, and ecology. Their most notable social interactions are tactile nudges, which facilitate group cohesion and coordination.[33,34] This unique and understudied mode of communication underlies the essential sociality of the *Corydoras* genus. In addition, nudging develops in larval fish alongside morphologic development,[36] suggesting its importance in adult fish. This nudging behavior positions *Corydoras* catfish as a strong model system for investigating the role of individual influences and interindividual communication on group coordination, as many existing model systems for coordination do not exhibit clear individual to individual social interactions.[31]

Corydoras catfish are also a useful study system for questions related to morphology and predator defense. The defensive anatomy of this genus provides additional context for their longevity. *Corydoras* do not have scales and instead are covered by bony plates that interlock and protect the fish.[39,40] This is a deterrent to both predators and certain parasites.[21] *Corydoras* catfish also have dorsal, pectoral, and anal spines that can lock into place and increase the effective size of these fish, making them less accessible to predators.[24,39] These spines also produce a venom that is injected into potential predators.[39,43] This venom remains a source of great interest and ongoing research effort[15,42] but does not seem to be acutely dangerous to humans.[35] *Corydoras* catfish also exhibit a modified digestive lining that enables them to gulp and extract oxygen from

Submitted: 01 Apr 2024. Revision requested: 06 May 2024. Accepted: 22 Jun 2024.
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This article contains supplemental materials online.

atmospheric air; this allows them to survive in hypoxic water conditions, which are common in Amazonian waterways.[25]

Because of these features, *Corydoras* species tend to be hardy fish that enjoy relatively low predation pressure compared with other similarly sized fishes. The resilience of *Corydoras* is such that other catfish mimic the appearance of sympatric *Corydoras* species to reduce risk of predation.[2] Multiple instances of this occur in the loricariid catfish genus *Otolincus*, which is even known to form mixed-species shoals with *Corydoras* catfish in the wild.[39] *Corydoras* species have also evolved to mimic other species for the ostensible purposes of interspecific shoaling. Their existence underscores the behavioral and anatomic features of *Corydoras* that particularly adapt it to its social and physical environments.

The last uniquely fascinating feature of *Corydoras* biology that we wish to note is the highly unusual copulatory mechanism found in many species, i.e., sperm drinking, in which females drink their mates' seminal fluid during bouts of copulation.[20] This behavior ensures that all eggs laid in a given clutch are full siblings. The reproductive physiology presents many unanswered questions. *Corydoras* is a system ripe for discovery, with the potential to add essential insights to behavior, behavioral ecology, community ecology, physiology, and evolution.

Water conditions in the Amazon. Amazonian water conditions are characterized by extremely low hardness (both general hardness [GH] and carbonate hardness [KH]) and total dissolved solids (TDS). This causes the pH of Amazonian waterways to be markedly acidic. Our Peruvian population of CW097 *aeneus*-lineage fish was caught in a stream with a TDS of 17 ppm and a pH of 5.5, with a hardness of essentially zero, which is well below the limit for soft water: ~200 ppm TDS (safewater.org) or 50 to 60 ppm KH. Water conditions in certain localities of the Amazon basin can exhibit substantial seasonal variation,[39] and *Corydoras* species vary in their tolerance to water chemistry variation. In addition, some species of *Corydoras* and other Neotropical freshwater fish can survive in a variety of conditions[38] but will only breed in water conditions similar to those from their natural habitats.[8] One of the major challenges of maintaining sensitive species and breeding softwater Amazonian species more generally is replicating these distinctive natural water conditions over long timescales.

Water parameters are not stable with such low TDS in a laboratory setting. Nitrifying bacteria are inactivated at pH below 6, which stops biologic filtration.[41] Water conditions in captivity must be maintained to accommodate nitrifying bacteria while also maintaining ideal conditions for the fish. Below, we present the protocols we have tested and settled upon for the successful maintenance and rearing of *Corydoras* species.

Part II: Husbandry Protocols

We propose that the protocols we have developed are generally applicable to the *Corydoras* genus, as well as other sympatric species. These protocols have been in use since 2015 in 2 facilities. Rather than try to replicate the huge variation in seasonality and habitats of Amazonian waterways, we have settled on a general range of conditions within the chemical, physical, and logistical constraints of laboratory environments that have allowed us to produce thriving populations of even sensitive species. These are the conditions that provide a very effective compromise between stability and approximating natural conditions.

Fish sourcing. We have used these protocols on multiple species of *Corydoras* catfish from a variety of sources. In our first facility at the University of Cambridge, we maintained a large colony of captive bred *Corydoras aeneus*, and at our current *Corydoras* facility at the University of Maryland, we have wild-caught populations of 4 *Corydoras* species from 3 different sources, which are described in Appendix S1.

Housing. For long-term housing, we opt for standard glass aquariums, which allow easy monitoring of population health. Another advantage of using glass aquariums is that commonly available aquarium equipment (heaters, power filters, etc.) is generally designed for glass aquariums. Glass aquariums can be expensive, however, and we have found that aquariums measuring 76.2 cm × 30.5 cm × 45.7 cm (marketed as "29-gallon aquariums") strike a reasonable balance between cost-effectiveness and spaciousness (Figure 1). This allows for most adult *Corydoras* to be housed in sufficiently large group sizes (15 to 25). The 50.8 cm × 25.4 cm × 30.5-cm ("10-gallon") aquariums are an acceptable option for housing smaller species or for temporarily housing fry. For newborn fry, we isolate eggs in 19-L clear plastic aquaria with a single miniature sponge filter and very sparse decor for ease of cleanup (Figure 2). We continue to raise them in such a setup until they achieve at least a 1-cm standard length.

Plastic storage tubs are a more economical housing option. Our laboratory has used tubs ranging from 53 to 568 L for groups of varying sizes with great success. Scaling housing requirements up or down is a simple matter, allowing for adequate equipment and enrichment even in large shoals up to 100 *Corydoras*. For example, our 568-L pond uses largely the same materials as our other enclosures, sponge filters, PVC pipes, and sand; we just used more of each item to accommodate additional fish (Figure 3). Provided that the volume of the tank is large enough to give the stock enough horizontal space to live comfortably and provide enough water volume to keep nitrogenous wastes reasonably diluted, scaling up or down operations for rearing *Corydoras* is a straightforward endeavor.

We take care to provide our fish with sufficient enrichment so fish display natural behaviors in a captive environment. A crucial addition for *Corydoras* is the usage of a fine-grained sand substrate. Pool filter sand works well, as it is inert, widely available, and cost effective. Sand should be laid down in a thin layer approximately 0.5 to 1 cm in height at the bottom of the aquarium so *Corydoras* can engage in natural foraging behaviors but thin enough so fish can reach the bottom of the enclosure and avoid the accumulation of anaerobic bacteria, which produce poisonous gasses. Fine-grained sand is also purportedly

Figure 1. A social housing tank for adult *Corydoras aeneus*. This setup includes an over the back filter, 2 sponge filters, and enrichment items. There is a thin layer of sand on the bottom of the tank, less than 1-cm thick.

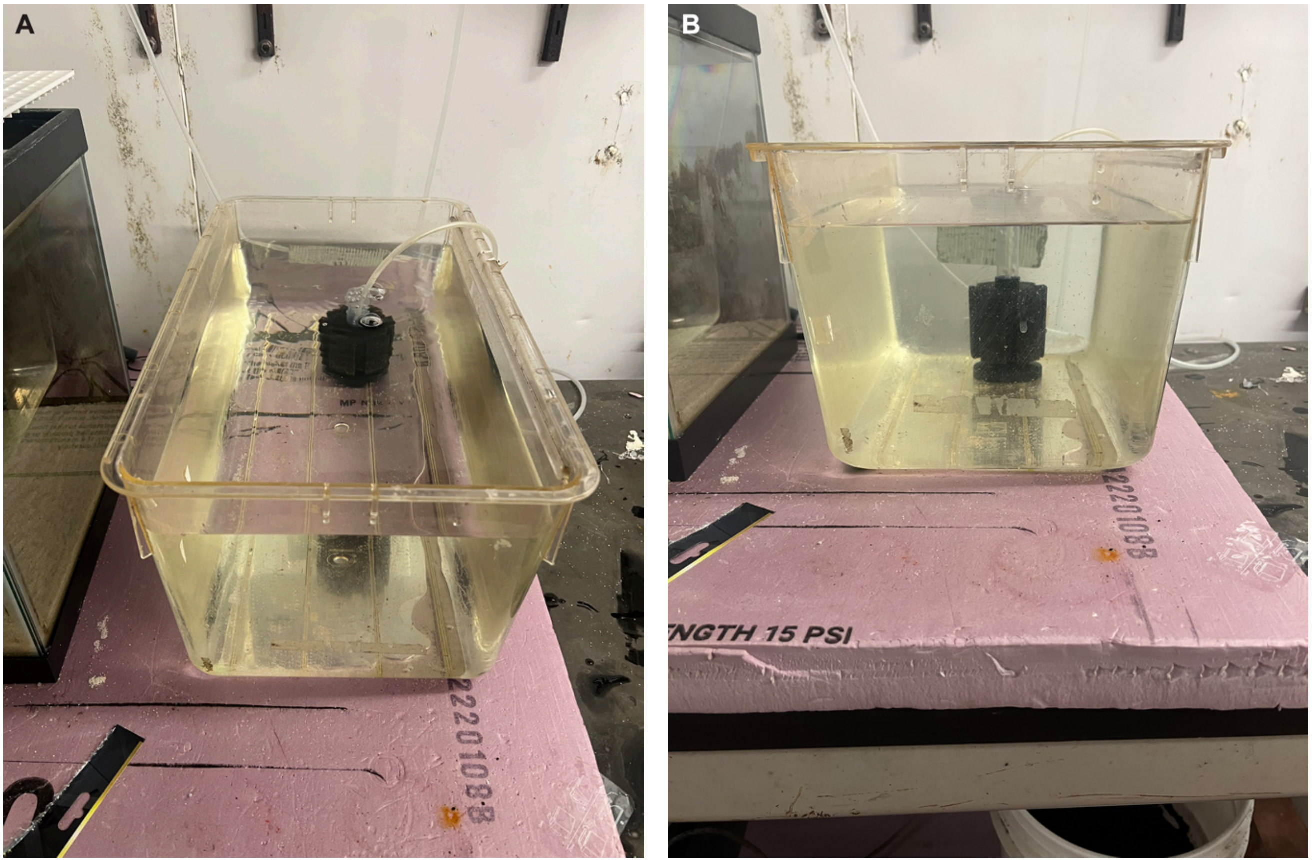

Figure 2. (A and B) A larval social housing tank, including (A) top view and (B) side view. The tank is bare bottomed for ease of cleaning and includes a cycled sponge filter, which contains microorganisms that fry (1 to 2 d posthatch) will feed on before they can be fed commercial feeds.

gentler on the barbels of catfish. PVC pipes and plastic plants also provide enrichment in long-term housing.

Lighting parameters. *Corydoras* catfish species have been shown to have very poor vision and as such are much less sensitive to light than many other species.[34] Other than our captive-bred lineages, our fish were primarily caught near the equator (Appendix S1), which means that in the wild they would have experienced a 12:12 light-dark cycle for the whole year. Therefore, we maintain our fish at a 12:12 light-dark cycle in the lab. *Corydoras* catfish do not have specialized lighting requirements, and in our facility at the University of Maryland, we use Lithonia Lighting brand fluorescent gas tube lighting (Luminaire model) that are suitable for highly humid conditions.

Figure 3. A pond or large social housing tank in a plastic bin. This set-up includes a large chamber filter, several sponge filters, enrichment items (e.g., PVC pipe and small pots), and a green Pothos plant. There is a thin layer of sand covering the bottom of the tank (less than 1 cm).

Our lights switch from light to dark abruptly, without any dimming. We have never observed any adverse effects from using this method. We have noticed via 24-hour video observation that fish activity temporarily reduces, then increases again, following the onset of the dark period.[14]

Water chemistry parameters. There are several important parameters to maintain water chemistry in a laboratory environment. Chlorine and chloramines are toxic and should be removed via at least 2 methods, such as reverse osmosis (RO) filtration and an aquarium dechlorinator. pH, GH, and KH are important parameters, however it is possible to manage these parameters simultaneously with the use of a proper remineralizer. In a separate receptacle, RO water should be mixed with remineralizer to create a consistent formulation. This formulation replaces water removed during water changes, which maximizes day-to-day stability. An airstone is kept bubbling in this receptacle at all times to facilitate dissolution of remineralizer and oxygenation of the water.

Our laboratory has been using Tropic Marin Re-mineral Tropic (Tropic Marin, Hünenberg, Switzerland) to remineralize RO water to a TDS of 95 to 105 ppm, with great success; typical parameter readings for this TDS range stay within the ranges of pH 6.5 to 7.2, GH 71.2 to 89 ppm, and K 35.6 to 53.4 ppm, which are highly suitable for soft-water Amazonian fish. We recommend a range of 95 to 105 ppm for TDS using our remineralizer, a measurement that results from a combination of the GH and KH measurements. For a quick water chemistry reference, see Appendix S3.

It thus becomes possible to quantify all 3 parameters using a single value (TDS). Each remineralizer product will affect these parameters in different ways, but once an appropriate product is identified, the caretaker can streamline the process of preparing water to meet the requirements of their fish using this simplified metric. With further experimentation, it is also possible to formulate a remineralizer from scratch by mixing the constituent mineral and salt components together in suitable amounts; we encourage others to experiment with this option.

Temperature. *Corydoras* are generally tropical fish that prefer a temperature range of 74 to 80 °F (23 to 27 °C).[21] It is often most practical to heat a whole room of aquariums to this temperature as compared with buying individual heaters for each tank. Different species have slightly different preferences, but all species we have kept (Appendix S1) have thrived under this temperature range.

Nitrogenous waste/biologic filtration. Successful captive rearing of all fish, including *Corydoras*, relies heavily on managing the nitrogen cycle in the context of biologic filtration. Management of the nitrogenous wastes produced naturally by fish is key to ensuring fish welfare. An appropriate microbiotic environment must be cultivated so waste ammonia is appropriately converted into nitrites, which are then converted into nitrates (a less toxic nitrogenous compound). In a mature system with efficient biologic filtration, there should be no detectable ammonia or nitrite, as these will immediately be converted into nitrates. Nitrates are removed with routine water changes or via other strategies. Heavily planted aquaria operate on the principle of the passive removal of nitrates, as plants take up nitrogenous compounds.[3] The golden pothos (*Epipremnum aureum*), a species that is not truly aquatic, has been propagated and used in our laboratory to mitigate the accumulation of nitrates without any harmful effects to the *Corydoras*. Rooted clippings are suspended in such a way that the roots are submerged in the water column while the leaves are above the water line.

High levels of dissolved oxygen (DO) are required for bacteria to process nitrogenous waste.[17] Electrically powered filters are suitable for maintaining DO levels. Biologic filtration capability will slowly grow with an increased bioload (e.g., adding stock to an aquatic system) and wane gradually if bioload is lowered. The doubling time of these bacteria is notably long, so the biologic filtration capability of an aquatic system will not reflect changes in bioload for a period of time. Therefore, care must be taken when adding more stock to an aquarium. It is good practice to perform regular water chemistry tests to make sure that there are no detectable amounts of ammonia or nitrite as the nitrifying bacteria multiply.

Before adding any stock to a new system, bacteria must be cultivated to support the bioload of prospective stock (a process termed "cycling"). This can be done in a variety of ways, but our laboratory has had most success by seeding with bacteria from an existing system with a mature biofilter. Perhaps the best way to seed a new aquatic system is to take filter media or a mature sponge filter from a healthy, mature aquarium: if an aquarium has any sign of potential disease, it should not be used to seed biofilters for new enclosures, as this can introduce pathogens; instead, follow quarantine procedures on the potentially afflicted tank and seed filters in a tank where all fish are obviously healthy. We routinely use this method to shorten cycling time down to a mere few days. However, regular testing of all relevant nitrogenous wastes, ammonia, nitrite, and nitrate, is still recommended.

We have safely kept the various *Corydoras* species in our laboratory with nitrate concentrations of 40 ppm or lower. It is crucial to devise an appropriate water change schedule that will maintain nitrate at appropriate levels. The rate of nitrate accumulation is dependent on the volume of water and stocking density in the system. For the most densely stocked aquariums in our laboratory, a 40% to 50% water change once a week keeps nitrates below 40 ppm. Less densely stocked aquatic systems safely follow the same schedule. For a more detailed discussion of this topic, see Appendix S2.

Feeding. The nutritional requirements of *Corydoras* species can be met with a variety of commercially available aquarium products and a regular feeding schedule. For basic maintenance of adult specimens, a once-daily feeding is sufficient for the health and well-being of these fish. *Corydoras* are primarily bottom-feeding fish, so they require a food that readily sinks. A granulated or pelleted sinking food, such as TetraColor XL Tropical Granules (and their UK-branded equivalent, Tetra Prima granules Tetra, Blacksburg, VA), works well as a staple diet, owing to the small size of the granules, its tendency to soften over time in water, and its ability to be pulverized easily for feeding smaller fish.

Although *Corydoras* can theoretically be fed a diet consisting entirely of these granules or pellets, enriching their diet with occasional feedings of other types of foods diversifies the feeding experience and nutrition. Frozen bloodworms and frozen brine shrimp are excellent options for larger species (i.e., CW097). For fry, subadult specimens, or smaller species, such as *Corydoras pygmaeus*, frozen baby brine shrimp, or live cultured microworms (*Panagrellus redivivus*) work well. The frozen food should be thawed first in a separate container before feeding. Microworms will crawl up the sides of the container and can be harvested by wiping them off the sides and swishing them directly into the aquarium. Our lab also uses a gel food, Repashy Community Plus (Repashy Specialty Pet Products, Oceanside, CA), as the soft, slow-dissolving nature of this food releases particles that even fry can easily consume. A table on recommended foods for various life stages and/or species is included in Appendix S3.

Determining a suitable amount of food to give takes some amount of trial and error, but some general guidelines to consider include that fish should consume all food quickly enough that no food remains to potentially foul the water and, at the same time, that enough food should be added so that every fish in the aquatic system has a chance to eat. *Corydoras* take longer to eat than mid- and surface-feeding fish, as they seem to locate food primarily through olfaction.[7] However, take care to remove all uneaten food that persists within a few hours after feeding as any unremoved speeds the growth of microorganisms.[4] Fish that are severely underfed exhibit sunken-in bellies, as do fish that are infected with internal parasites.[5] It is important to feed fish properly to rule out anorexia as a cause of a sunken appearance.

Health monitoring. Fish health is observable through apparent physical condition and behavior. Healthy *Corydoras* catfish have shiny armor, clear and alert eyes, long, tapered barbels, and intact fins (Figure 4). Healthy individuals move in social groups and frequently immerse their barbels in the sand substrate of their aquarium (sometimes so vigorously that their eyes are temporarily buried!). Fish are active, although they do rest on the substrate occasionally, and often move in stop-and-start patterns. We have never observed these fish being aggressive with conspecifics or other species,[12] which is very beneficial in aquarium settings.

Figure 4. A wild-caught *Corydoras* catfish (*Corydoras elegans*, in this case) from the Madre de Dios region of the Peruvian Amazon rainforest. RJR caught this fish in June 2011, and it participated in behavioral tests and was released at the same site its group was caught in; it is quite possible that this specimen is still alive. Notice this fish's shiny (almost iridescent, which is common) armor plates, clear, bright eyes, and intact, long barbels. This population does not exhibit elaborate markings, although other populations of *Corydoras elegans* have elaborate markings. This variation in markings and coloration makes these traits inconclusive for classifying species.

Even very well kept populations can occasionally result in small numbers of individuals presenting with poor health. The following are signs of illness in *Corydoras* species:

1) Loss of appetite;
2) Loss of balance and/or erratic swimming;
3) Red sores;
4) White fuzz-like growth on sores or pustule-like growths;
5) Swelling or cloudiness of the eyes;
6) Irregularities in fins (as though fins are ragged); and
7) Loss of color and skinny, wasted appearance.

Corydoras catfish do not have typical scales, as they are armored (which is typical for all members of the armored catfish family Callichthyidae, the family in which *Corydoras* catfish are placed), but their health indications are similar as scaled fish. Sick fish should be immediately quarantined in a hospital tank. Separate nets should be designated for use in the hospital tank.

Combinations of these symptoms indicate different illnesses. For example, signs 1 and 7 in combination indicate internal parasites, which are common in fish populations and easily treated with flubendazole (one or 2 doses). Any combination of signs 3, 4, 5, and 6 could indicate a bacterial infection, which is commonly treated with antibiotics such as nitrofurazone or minocycline. All treatments must be safe for scaleless fish, which sometimes require lower dosages and longer treatment times.

Over the nearly decade RJR has managed a facility of these fish, a very small proportion of individuals have presented with symptoms of illness, but to ensure fish welfare, we include this information.

Breeding strategy. Using the protocols we have established above, all of the species of *Corydoras* that we have maintained in our lab have spontaneously spawned, producing sometimes prodigious numbers of eggs. This has allowed our laboratory to deliberately raise young fish for developmental experiments.

Our experience from the lab is consistent with personal observations that increased feeding (twice per day) and increased water changes (twice per week) are associated with spawning.[35] Other than these, we have not needed to undertake specific actions to induce breeding, which happens sporadically throughout the year in our mixed-sex social housing tanks. We did not deliberately put together specific sex ratios, but due to the unique sperm drinking behavior of *Corydoras* species,[20] sperm must be produced with fluid that allows the sperm to survive the female's digestive tract.[10] For this reason, sperm seem to be particularly costly to produce, and it is thought that having a higher ratio of males to females (2:1 males to females has been suggested) is ideal for spawning[12]; commercial breeding of albino *Corydoras aeneus* utilizes this ratio.[23] Multiple other factors are thought to be associated with breeding in these fish. In the wild, these fish spawn during the rainy season,[21] and spawns seem to be correlated with rainy weather and storm fronts.[32] Aquarists have also reported increased spawning after water changes with pure RO water, which simulates heavy rainfall.[35]

Further study is necessary to elucidate the effect of these conditions and measures, as well as their mechanism of inducing spawning. This would be particularly informative for studies that investigate the unique reproductive physiology of these fishes, which presents many open questions of great interest.

Special considerations for larval husbandry. Various considerations and comments regarding the husbandry of *Corydoras* fry have already been provided in each of the sections of this guide, but several specific points bear mention. Larger species such as our CW097 and *elegans*-lineage fish do best when the eggs are isolated in a separate rearing container for at least the first few months of their lives. Using a clear plastic tub with a cycled, mature sponge filter serves as a suitable environment for isolated eggs (Figure 2). The sponge filter should not be cleaned before placing it in the larval tank, as growth on these filters allows the fry to graze on microorganisms even when not actively being fed.

After the fry hatch, they should not be fed for 1 to 2 d as they absorb their yolk sacs (Figure 5). After this, a rotating selection of pulverized TetraColor XL Tropical Granules, thawed frozen baby brine shrimp, live microworms, and Repashy Community Plus may be fed to the fry twice daily (Figure 5). Live foods such as microworms can survive for several hours and offer a continuous food source. Uneaten food should be removed daily, and 50% water changes should be performed every other day. This strategy works well for the first one or 2 mo of life for larger species; leaving the eggs in the tank with adult stock has seldom yielded successful larvae, likely due to the eggs and newly hatched fry being eaten by the adults. We have found from our previous developmental work[36] that our *Corydoras* species' developmental timelines are overall similar to those described in *Corydoras paleatus*.[37] We present our designations for life stages in Appendix S3.

A variable but nontrivial (approximately 20% to 50%) proportion of the eggs are unfertilized or do not develop normally, and if left alone will begin exhibiting a thick fungal growth once they have spoiled or died. These eggs should be removed for the sake of cleanliness, because the fungal growth will spread to healthy eggs. Unfertilized eggs can be identified and removed even before the onset of fungal growth, as they will take on an opaque, milky white coloration that/contrasts quite clearly with the translucent fertilized eggs, usually within 24 h. It is advisable to remove as many unfertilized eggs as can be confidently identified before fungal growth threatens the development of healthy eggs.

Smaller species such as *Corydoras pygmaeus* and *Corydoras hastatus* present many more difficulties when rearing fry; thus, we usually opt to leave the eggs in the tanks with the adult stock. These species appear less likely to consume their own eggs and fry, so with each spawning event one may expect to see a small handful of fry survive to adulthood without any

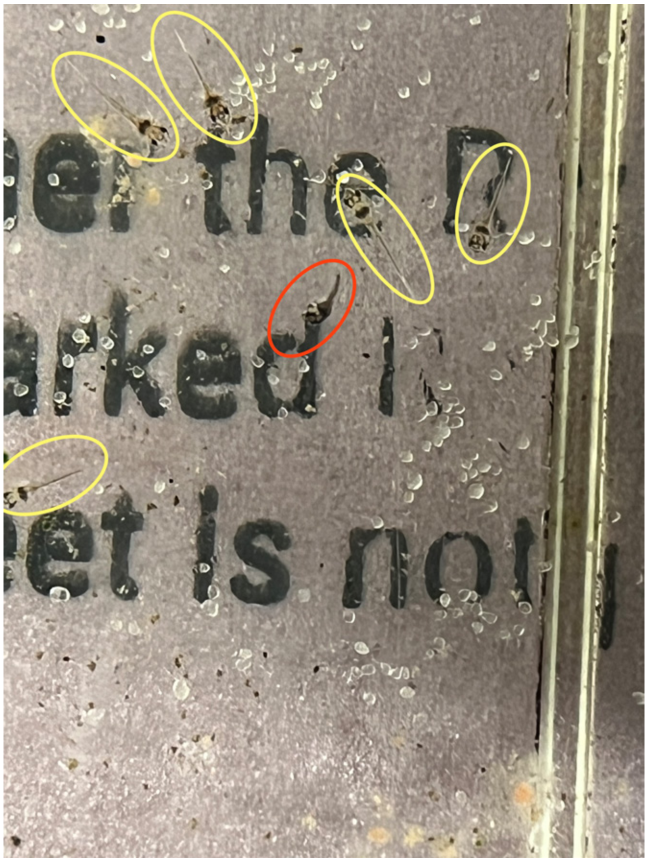

Figure 5. These are 5-d-old larvae, which have now absorbed their yolk sac and may be fed commercial feed, as described in Appendix S3. Yellow outlines indicate healthy, developmentally normal larvae. The red outline indicates a deformed larvae with a characteristic spinal curvature that occurs in a low percentage of larvae. All larvae appear "curved" before hatching, as they are constrained within the egg casing, but larvae that do not "unfurl" and have a straight spine shortly after hatching exhibit this deformity.

special intervention. However, with this method ultimate adult yields per batch of eggs are never as high as those of isolated fry. Should anyone wish to assess developmental aspects of *C. pygmaeus* and *C. hastatus* biology, we note here that hobbyist communities have developed strategies for raising large numbers of these smaller species[12] and would be a good place to start in developing a laboratory methodology.

Part III: Physiology Underlying Our Husbandry Protocol

Over the last 9 y of rearing *Corydoras* species for behavioral ecology investigations, we have learned that the management of *Corydoras* can be streamlined through the use of a remineralized RO strategy that focuses on a single measure, TDS, for water formulation. When deciding which parameters to most closely monitor and prioritize in an aquaculture system, one must find a balance between the natural conditions fish come from and practical husbandry considerations of an aquarium system. TDS, pH, KH, and GH are important to maintain within reasonable parameters for a successful tank ecosystem. We have found that by closely monitoring TDS of the incoming water during water changes, we have achieved stable levels of pH, GH, and KH that do not fluctuate outside the tolerance of *Corydoras*. We test all water parameters in established tanks (including ammonia, nitrites, nitrates, and pH) once a week in our facility and have never experienced large deviations in pH or unhealthy readings of nitrogenous compounds.

We developed this management system because of its practicality. The wild caught *Corydoras aenues* from which one of our laboratory populations descend were caught in the southern Peruvian Amazon (Madre de Dios region) in water that had a TDS of 17. Replicating this markedly low concentration of dissolved solids in aquaculture would prove difficult due to the biologic processes that characterize a relatively closed aquarium system (as compared with natural streams). When captive fish excrete ammonia, the nitrogen cycle converts it into nitrates, producing hydrogen ions as a byproduct. This lowers the pH of the water and causes rapid and unpredictable acidification of water in tanks. Prevention of this acidification at such a low TDS requires frequent water changes and proves impractical in a laboratory setting.

In addition, maintaining our fish on RO water remineralized to ~100 ppm TDS has led to stable pH that is not only well tolerated by fish, but enabled them to thrive. Since the pH tolerance of *Corydoras* is so wide,[21] if TDS is adjusted and kept within an appropriate range, then it becomes less necessary to intensively monitor pH. Using the remineralizer we have relied on, Tropic Marin Re-Mineral Tropic, we suggest that TDS can be measured directly with the use of RO water, without routine measurement of pH of water being prepared. With other remineralizers, or with bespoke remineralizers prepared for this purpose, care should be taken to assess the pH of prepared water to make sure the formula results in a pH within the tolerated range of the target species. By focusing on just TDS as our main water suitability parameter, we also reduce the labor involved in water treatment.

Our focus on TDS is informed by the fundamental osmoregulatory challenge freshwater fish face. From a physiologic standpoint, dissolved solids in water are critical for osmoregulation for freshwater teleosts, as the concentration of different dissolved solids affects osmotic gradients between the environment and the body. Freshwater fish take up large amounts of water via their gills, typically up to 50% of their body weight per hour.[13] This tasks the renal system with excreting excess water while reabsorbing important ions, that is potassium and magnesium, from the filtrate back into the body. In the nephron of a freshwater fish, typically all the ions that are filtered at the glomerulus are reabsorbed in the distal tubule, collecting duct, and urinary bladder.[13] This ensures that body tissues remain hyperosmotic relative to the environment and ion levels necessary for homeostasis are maintained. Thus, it follows that the TDS of water strongly affects the renal system of the freshwater teleost.

Many animals have a renal threshold, which is the concentration of a solute in filtrate (such as magnesium or phosphorus ions) when not all of the solute can be reabsorbed. It has been demonstrated that tilapia have a renal threshold for glucose,[22] and it stands to reason that, as freshwater fish are adapted to retain ions, they would have a high renal threshold to maximize ion uptake. Therefore, one can speculate that when freshwater fish are exposed to a TDS above their normal range, the ions are filtered at the glomerulus in higher levels, proportional to the high concentration of dissolved solutes that have been absorbed into the body. This contrasts to saltwater fish, which have adaptations to remove excess ions from their hypertonic environments.[44] If the concentration of these solutes in the filtrate exceeds the renal threshold, then the excess ions would be excreted with the urine; however, given that the renal system of freshwater fish is adapted to retain ions, the renal threshold would be relatively high, resulting in detrimental health effects if TDS is high and high solute retention leads to disruption in osmoregulation. We noticed strong negative health effects when we attempted to maintain *Corydoras aeneus* on local tap water in Cambridge, UK, where the tap water has a high TDS (approximately 350 to 400 ppm). These negative effects were resolved when we developed the husbandry protocols delineated here.

When pH is maintained within a tolerable range, there are no adverse effects to fish physiologic function, and our protocol ensures stable pH well within the range necessary for optimal *Corydoras* physiology. We do note, however, that overly acidic or alkaline water can become an issue for all fish species outside their tolerances. Water with a pH that is too acidic lowered the oxygen carrying capacity of hemoglobin in fishes,[16] and it has been theorized that acidic water negatively affects olfactory perception.[18] Basic pH values can also be a concern because, at pH values over 7, ammonia exists in its deprotonated, and more toxic, form; at acidic pH values, ammonia tends to exists as the conjugate acid ammonium (NH_4^+), and so high pH values not only tend to be outside the naturally occurring pHs in the Amazon, but also led to higher ammonia toxicity.[28] For these reasons, choosing a remineralizer that adequately buffers pH is critical to the success of this protocol.

In short, TDS is incredibly useful from a husbandry perspective, provided an appropriate remineralizer (commercial or custom made) is used. TDS can be used as a single parameter in preparing water instead of each parameter (pH, KH, GH, etc.) being considered individually. We do note that these parameters (pH, KH, GH, etc.) do need to be monitored in existing tanks, however incoming water chemistry may be evaluated solely using TDS. Because pH is naturally prone to fluctuation at the low conductivity (i.e., low TDS) that naturally occurs in the Amazon, Amazonian species generally tolerate a range of pH values. Therefore, in a laboratory setting, TDS stands in as a reliable indicator for pH levels under the protocol we prescribe, reducing the need for intensive pH monitoring.

Conclusion

The protocol we have developed has proven effective for several *Corydoras* species in different physical laboratories. As *Corydoras* is a geographically distinct genus that occurs only in the Neotropics (albeit with a broad range that essentially encompasses the tropical latitudes of North and South America), the general similarity in habitat water chemistry parameters suggests that this protocol should be effective for the vast majority of *Corydoras* species. We have used it to achieve a large degree of success in maintaining and rearing species from multiple *Corydoras* lineages, and we are confident that this is an appropriate husbandry regimen for *Corydoras* species with limited exceptions.

While we have developed the above protocol to serve the needs of our laboratory *Corydoras* populations, the conditions this protocol aims to replicate would serve a variety of softwater Amazonian species that may be of interest to various biologic disciplines. The *Corydoras* species we have in our lab naturally cooccur with a great variety of species that have proven useful to a variety of biologic disciplines, including various cichlids such as *Apistogramma* and *Cichlasoma* species, a variety of catfish clades including loricariids and *Scoloplax*, killifish from the Rivulidae family, trahira species from the Erythrinidae family, and a tremendous diversity of characins.[30] *Apistogramma* neotropical cichlids have proven useful for studying genetics, social hierarchy, physiology, and behavioral ecology,[6,9,19] as have *Cichlasoma* species.[1,27] The protocol we have suggested would be appropriate for most species from the range of our *Corydoras* population, including the tetra species *Hyphessobrycon elachys*, which lives in a mixed-species shoal with its mimic, *Corydoras hastatus*, in our current facility. Because of the way this protocol standardizes water chemistry that is naturally subject to high variation, we propose this protocol as a starting point for all Amazonian species and believe it will mitigate the inherent challenges of maintaining, breeding, and rearing these fish under laboratory conditions.

Supplementary Materials

Appendix S1. Genetic history, sourcing, and population notes on our *Corydoras* colonies.

Appendix S2. Nitrogenous waste/biological filtration.

Appendix S3. Tables for diet by life stage and water parameters.

Acknowledgments

We thank Mats Peterson for his advice in the early stages of development for this protocol. We also thank Dr. Eric Thomas for his advice while we were at the University of Maryland. We also acknowledge Dr. Beth Gillie and Dr. Thomas Roe for their support at the University of Cambridge.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The work done for this paper at the University of Maryland was funded by RJR's President's Postdoctoral Fellowship. The work done for this paper at the University of Cambridge was funded by the Hershel Smith Scholarship.

Author Contributions

Austin Chiang spearheaded the overall organization, wrote sections related to water chemistry, contributed to empirical methodological development of housing conditions at UMD, and provided commentary on the manuscript. Sullivan S S Haine wrote sections related to physiology, contributed to empirical methodological development of housing conditions at UMD, provided commentary on the manuscript and performed extensive revisions. Rebecca Goldring provided commentary on the manuscript, provided guidance on empirical methodological development of housing conditions at UMD and larval rearing, enriched all sections with additional insights, and provided feedback on the manuscript. Arne Jungwirth contributed to the empirical methodological development of housing conditions at Cambridge, guidance at UMD, and provided commentary on the manuscript. Munir Siddiqui contributed to the empirical methodological development of housing conditions at UMD, and provided commentary on the manuscript. Gerald Wilkinson provided support for empirical methodological development at UMD and commentary on the manuscript. Andrea Manica contributed support and guidance about empirical methodological development of housing conditions at Cambridge, and provided commentary on the manuscript. Riva J Riley developed the empirical protocols for housing conditions, larval husbandry, and other aspects of husbandry at Cambridge and UMD. This paper is based on the protocols developed for behavioral research. She contributed support and guidance for the writing of all aspects of this paper, wrote the introductory and housing conditions section, and extensively edited.

IACUC and ARRIVE Protocols

The protocols delineated above were approved by the appropriate committees associated with ethics in animal research at both the University of Cambridge and the University of Maryland. At the University of Cambridge, these protocols were approved via a nonregulated use of animals in scientific procedures application (consistent with UK's animal welfare legislation ASPA) through the University of Cambridge. They were approved through the University of Cambridge's Ethical Review Process and approved and presented by the Named Veterinary Surgeon and the Named Animal Care and Welfare Officer (NACWO) for the Zoology department. At the

University of Maryland (UMD), these protocols were approved as part of our Standard Operating Protocol (SOP) by the UMD IACUC (protocol no.: 2123596-1). These protocols were developed in accordance with the ARRIVE protocols.

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