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# Reproductive Management of Freshwater Ornamental Fish

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**Reproductive management of freshwater ornamental fish is a discipline of increasing importance. Vigorous growth in the aquarium hobby in the United States, continued foreign competition for livestock sales, governmental concerns over medications, conservation of endangered species, and the use of ornamentals as laboratory animals are just a few factors escalating the need for veterinary expertise. This article is an introduction to ornamental fish reproduction and covers both generalities and specifics. Veterinarians must have a basic understanding of the wide range of fish reproductive techniques and management in order to guide their training and provide service to individuals in these matters.**

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Ornamental fish are the No. 1 choice of pet in the United States, with retail sales for pet fish and fish products estimated at 1.34 billion dollars in 1994 alone, an increase of 49% from 1993.<sup>1</sup> Approximately \$463 million of this income went toward live fish, and \$88.4 million went toward over-the-counter (OTC) fish medications.<sup>1</sup>

Many factors have escalated the need for veterinary proficiency in all aspects of the industry. The growing popularity of fish as low-maintenance pets, advances in the science and technology of aquarium keeping, and trends toward governmental regulation of OTC medications are a few major factors. Conservation concerns increase as habitat destruction and other causes throughout the world drive more species toward extinction. The hobby and industry have become major players in fish species conservation. Many producers, more advanced hobbyists, and biologists, through brute trial and error or academic research, are developing various repro-

ductive techniques and paving the way for propagation of species in decline.<sup>2,3</sup> Many fish exist only because of the hobby.<sup>4,5</sup> Zoos and public aquaria are becoming more active in fish conservation. Also, fish have become the mice of the 1980s and 1990s in research, with more work being conducted on all aspects of reproduction and development. Clearly, reproductive management of ornamental fish has become an important discipline.

## Origins of the Species

Where do all these fish come from? About 95% of the ornamental fish produced in the United States originate from Florida, with most production facilities centered around Tampa (Hillsborough and Polk Counties) and Miami for both airport accessibility and historical reasons. Fish also enter the United States from South America, the Far East, and Africa. Miami, Los Angeles, and New York are the major ports of entry. The Far East exports large numbers of farmed and wild-caught fish and is a major competitor to Florida's ornamental operations.

The industry in Florida has grown considerably since its humble beginnings in the early 1930s and continues to thrive. In 1993, approximately 187 producers of ornamental tropical fish netted 46.7 million dollars.<sup>6</sup> This is substantial considering the sizable markup on the wholesale and retail end, where a fish that a Florida producer sells for 15 cents may go for \$2.89 in a northern retail store.<sup>7</sup>

Numerous facilities outside of Florida round out total overall production in the United States. In addition to a few large- and medium-sized hatchery operations outside of Florida, there are many smaller basement or fish room setups run by advanced hobbyists for whom breeding may or may not be a primary source of income and who sell either to wholesalers, to hobbyists through specialized trade journals or clubs, or directly to retailers. Intermediate hobbyists also

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frequently begin to experiment with fish breeding as a logical next step in the hobby.

There are hundreds of varieties of ornamental fish available at the average pet store or through specialty mail-order catalogs or clubs. The reproductive strategies of these fish are varied: Some are complex, and many are not fully understood. Several very good references detail species-specific requirements and are highly recommended for initial background information.<sup>8-16</sup>

However, certain families are more commonly bred and have more standardized protocols for reproductive management. In Florida, these include the following families: Characidae, or tetras; Cyprinidae, which includes barbs, danios, freshwater sharks, koi, and goldfish; Belontiidae, also known as the anabantids or labyrinth fish, which includes the gouramis, fighting fish, and related species; Poeciliidae, which includes the live-bearing guppies, swordtails, platies, and mollies; Melanotaenidae, better known as the rainbowfish; Cichlidae, the cichlids, which includes angelfish and discus; various species of catfish, including the common plecostomus, *Hypostomus plecostomus*, and some related members of the Loricariidae; as well as members of the *Corydoras* catfish family, Callichthyidae. Although the Cyprinodontidae, also known as the killifish, are not as commonly farm raised, many hobbyists obtain and breed these species through the American Killifish Association. Other specialized hobbyist groups, such as the American Cichlid Association, the North American Catfish Society, Ltd, the American Live-Bearer Association, and the Rainbowfish Study Group, promote breeding of their particular species of interest and exchange of livestock—breeders, eggs, fry, or juveniles—among their respective membership. These organizations are excellent resources for current natural breeding techniques. Also, numerous local aquarium societies often have very knowledgeable advanced fish keepers who are good sources of information and potentially good clients.

### **Fish Reproduction and the General Practitioner**

Aquariums and ornamental fish were, for the longest time, considered veterinary black boxes. Fortunately, both nonveterinary and veterinary fish health specialists have made considerable

inroads into ornamental fish medicine, although an ocean of baseline information remains uncharted. Most breeders already have a good understanding of the basics of fish keeping. Consequently, the veterinarian must be well grounded in the basics of fish medicine and reproduction. Several very good fish health references are available,<sup>10,17-22</sup> but nothing compares with hands-on learning. Interested veterinarians should attend one of the many fish health short courses now being offered each year. The American Veterinary Medical Association and International Association for Aquatic Animal Medicine are excellent resources for course information and contacts.

Much of this article is devoted to basic reproductive techniques. An understanding of some of the generalities and some of the specifics is a prerequisite to proper veterinary care.

### **Key Steps to Successful Reproduction**

Reproduction of any species requires successful completion of a number of key steps: broodstock selection; broodstock conditioning; spawning; hatching; and larvae/fry survival/ growth. Most problems can be traced to mismanagement of one or more of these steps.

#### **Broodstock Selection**

Broodstock must be strong and vigorous, be in good health and color, and have excellent conformation with no deformities. Attention to detail during selection ensures higher-quality offspring. Conditioned and sexually mature fish often differ in size, coloration, finnage, body shape, and behavior from immature or unconditioned specimens. Older and younger breeders are less reproductively sound. Older breeders may show decreased productivity either because of age or because of acquisition of age—related diseases. Younger breeders often are less fertile and in certain species may show greater cannibalism and less parental care.<sup>10</sup> Greater success in compatibility and hormonal synchrony and assurance of the presence of both males and females are possible through the purchase of 6 to 12 juveniles of the same age of differing sizes because males will often grow more quickly than females.<sup>10</sup> Excessively colorful juveniles of certain species, especially cichlids, may have been exposed to testosterone to enhance coloration. These should not be chosen

for breeding purposes. Inbreeding depression, caused by an increase in the proportion of homozygous traits in a population, usually does not play a major role in early filial generations, and some species of *Xiphophorus* have been inbred 57 generations without major deterioration. However, it should always be considered a potential problem.<sup>10</sup>

### Broodstock Conditioning

Conditioning is achieved through optimal water quality, ample and appropriate nutrition and habitat, and reduction or elimination of parasite/pathogen loads. Many production-oriented operations separate most species by sex during the conditioning period to optimize nutrient use toward gamete formation and away from behavioral distractions or untimely spawnings. Most broodstock, in particular omnivores and carnivores, require high-quality protein foods that contain essential fatty acids, carbohydrates, vitamins, and minerals, with adequate levels of digestible energy, three to six times a day. Appropriate habitat is important for all species of fish, but special consideration must be given to more behaviorally advanced or aggressive species. Cichlids fit both categories, and, in addition to water-quality related densities, behaviorally oriented stocking densities as well as male-to-female ratios are critical to successful survival of broodstock, conditioning, and reproduction.

New broodstock should be quarantined for several weeks before entry into the general population and examined periodically for parasites and other pathogens. External and internal parasites reduce reproductive capacity. Wild-caught and pond-raised broodstock are more likely to have significant parasite loads than tank-raised fish, although the latter can be just as loaded if water quality and other factors have not been kept at a premium. In large production operations, a sample of broodfish should be examined for parasites before conditioning, especially if these fish are new breeders. Any fish showing signs of illness or weakness should be chosen. If broodstock is valuable, population is small, or in hobbyist situations, if sacrifice is not an option, survival procedures for routine parasite checks can be used. There are excellent references for parasite identification and treatment; however, species-specific susceptibilities and sensitivities to treatment exist.<sup>10,17-22</sup>

Internal parasites are often a problem in

angelfish, discus, other cichlids, kissing gourami, and occasionally in goldfish, with presence of high numbers of the intestinal flagellates *Spironucleus/Hexamita*, being closely linked to reduced fertility, reduced hatchability, fry stunting, and decreased fry survival.<sup>23</sup> Other gastrointestinal parasites such as nematodes and tapeworms also should be eliminated. Fresh fecals are a good alternative to sacrifice and necropsy for detection of internal parasitism, but these are not 100% accurate and must be fresh.

### Spawning

For commonly bred fish, this is a relatively simple task. However, spawning is one of the most difficult hurdles to overcome when "cracking" a new species, that is, a species that has never been bred before, or, in the fish farming community, a species that has not been bred commercially. Ease of conditioning and spawning and numbers produced per spawn are some of the critical factors used to determine the commercial viability of a species. For many species, factors that trigger spawning are well known. Some of these include photoperiod, water temperature, water quality (pH, hardness, total dissolved solids [TDS], dissolved oxygen [DO], salinity, alkalinity), flooding, water current, tide and cycles of the moon, weather cycles (atmospheric pressure, rainfall), spawning substrate, (eg, the presence of aquatic plants such as Java moss, sticks, gravel, ledges, or spawning caves), or the presence of other species.<sup>24</sup>

Induction of spawning by hormonal injections is used for species that are too difficult to trigger naturally or are more efficiently triggered manually. Some of these include Rainbow Sharks, Red-Tail Black Sharks, Iridescent Sharks, koi, goldfish, and Tinfoil Barbs, although many other species have been spawned this way but not reported in the literature.

A cascade of events leads to release of mature gametes from ovaries and testes. Either proper environmental stimuli or hormones acting at the level of the hypothalamus, pituitary, or gonads will effect successful release. Frequently used products include carp pituitary extracts (2.5 to 12 mg/kg); purified gonadotropins, such as human chorionic gonadotropin (60 to 2,300 IU/kg); luteinizing hormone-releasing hormone analogues (5 to 100 µg/kg), alone or in combination with dopamine blockers; or steroids used to stimulate gametes directly. These

hormones are used alone or in combination, and calculated dosages are often split between two injections, 6 to 24 hours apart, each a percentage of the total.<sup>25</sup>

Usually, conditioned broodstock are outwardly more robust, with females being full in the abdominal area. Eggs from mature females are sampled by insertion of the properly sized catheter, either flexible or rigid polyethylene or vinyl tubing, with a rounded end to prevent damage, into the reproductive tract with gentle aspiration by syringe. Immature eggs are smaller and clearer than mature eggs. As eggs approach maturity, the nucleus (germinal vesicle) migrates from the center of the egg toward the animal pole, which contains the micropyle through which the sperm enters. Microscopically there should be a relatively high percentage of large, mature eggs that have a granular but not degenerating cytoplasm, with eccentric nuclei. In males, application of gentle pressure near the vent will usually allow assessment of the presence and quality of sperm.<sup>26</sup> When broodfish are ready for induction, one injection or two injections at a predetermined interval are administered, depending on the hormone used and the species. Because of species-specific egg stabilities after release from the ovary (maximum period between ovulation and deterioration of egg quality for Common Carp is 50 to 80 minutes and for Red-Tail Black Sharks is 15 to 30 minutes), females should be checked for ovulation periodically, every 30 to 45 minutes, by gentle pressure around the vent area, starting a few hours before the female should be ready. She has ovulated when pressure around the vent area produces a stream of eggs. Once eggs have been ovulated, the females and males can either be tank spawned, manually stripped, or, in species that are not easily stripped, their eggs and sperm can be surgically removed. In tank spawning, females are placed with males, and female egg release stimulates male sperm release. During manual stripping procedures, the female is anesthetized with tricaine methane sulfonate or quinaldine, carefully dried around the vent area, to prevent anesthetic and/or water from contaminating and/or activating the eggs, and then held over a bowl and massaged over the abdomen toward the vent to gently force the eggs into the bowl. Once she is spent, the male is prepared in the same way. His milt is allowed to flow over the eggs and is then mixed gently with them. Water is

then mixed in to activate the egg and permit fertilization.<sup>27</sup> After fertilization, the eggs are either placed into modified MacDonald hatching jars, which are designed to keep eggs in continuous gentle movement via flow of oxygen-rich water through them, and allowed to hatch out, or the eggs are first treated, if adhesive, to prevent adhesion during the incubation period. The water source must be clean and at the correct temperature. Ultraviolet sterilizers will help eliminate potential bacterial pathogens but may not reduce fungal spores.

Once a protocol has been established, the veterinarian should verify that proper procedures are followed. Procedures that ensure aseptic technique and stability of the product are very important and often overlooked.

### Hatching

Excellent water quality is always a must. For many species, except those whose young absolutely require parental care, breeders are removed from the spawning area, or eggs are removed and allowed to incubate in a separate container that, depending on species and water quality, may require antibacterial or antifungal chemicals. Many different chemicals are used by breeders at all levels with varying degrees of success. These include Mar-Oxy (Mardel Laboratories, Inc, Glendale Heights, IL), Marvel Med (Eules, TX), acriflavine, methylene blue, Congo red, iodophors, or other dyes. Salt or formalin dips also have been advocated for some. Dosages vary widely, and species-specific sensitivities do exist. Aeration is important but must be moderated to avoid damage to the developing embryos. If possible, eggs that are white or otherwise obviously infected with fungus or bacteria should be carefully removed, without disturbing the rest of the spawn, to avoid spread of the infection.

### Larvae and Fry Survival and Growth

Good water quality, appropriate habitat, and proper nutrition are critical to survival of larval and fry stages. Fry are very sensitive to water quality perturbations. Their small size makes them equally susceptible to physical disturbances, such as excess light, current, or turbulence.

### Nutrition

Most basic requirements have been extrapolated from comprehensive studies conducted on

food fish (eg. Channel Catfish, salmonids, carp). Detailed information for all life stages of all species is lacking, making conditioning of certain species for reproduction difficult and experimental. The following factors further complicate matters: in ponds and in planted aquaria, natural foods are an important source, especially in nonintensive culture; feeding alters water quality; and losses in nutritional value will occur if prepared food is not consumed immediately.<sup>28</sup>

Omnivorous and carnivorous broodstock condition with commercial fish meals and flakes, beef heart, squid, brine shrimp, and a variety of other live or frozen foods including *Daphnia*, bloodworms, krill, mosquito larvae, white/grindal worms, glassworms, and wingless fruit flies. Because wild-caught foods can be vectors for fish parasites and other pathogens, live foods are best obtained from an artificial culture. Herbivorous broodstock need a more vegetarian diet. Spirulina, a high-protein algal food containing many natural color-enhancing carotenoids, is often included in the diet of breeders as well as fry. *Tubifex* worms are an undesirable food because of their potential to introduce pathogenic bacteria and parasites.

Protein levels are important for optimal growth. There are 10 essential amino acids for most fish: arginine, lysine, histidine, leucine, isoleucine, valine, methionine, threonine, tryptophan, and phenylalanine. Although absolute requirements for species will differ, essential fatty acids include  $\omega$ -3 and  $\omega$ -6 fatty acids: linoleic and linolenic acid, both higher in plant oils, and eicosapentaenoic acid and docosahexaenoic acid, both higher in marine oils. Additional lipid requirements include phospholipids, lecithin, and cholesterol.<sup>29</sup> These fatty acids allow for more flexibility and permeability of membrane phospholipids and are also important for egg hatchability.<sup>28</sup> In addition to these and other functions, proteins and lipids are considered better energy sources than carbohydrates. Approximately 10% lipid (depending on species and life stage) will optimize protein utilization, but excess lipid will reduce intake of other important nutrients. Many fish have a limited capacity to digest raw carbohydrates, although they are always present in commercial diets.<sup>28,29</sup>

Vitamins required for growth and reproduction are similar to those required by other animals and include the fat-soluble vitamins A,

D, E, and K and the water-soluble vitamins C, B<sub>12</sub>, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, biotin, inositol, choline, and folic acid. Specific requirements depend on species, stocking density, culture conditions (eg, extensive v intensive, pond, raceway, or tank), temperature, presence of toxicants, stress, age, and reproductive status.<sup>28</sup>

Vitamin deficiencies have been recognized in several species. A few of these deficiencies follow: Vitamin C deficiency results in lordosis and scoliosis in Channel Catfish and salmonids and results in deformed opercula in salmonids. Vitamin C is important for immune system function, with increased resistance to bacterial infections observed in fish that are fed vitamin C-rich diets. Vitamin E deficiency causes muscular dystrophy and other problems in Channel Catfish, carp, and salmonids. Thiamin deficiency causes dark skin in Channel Catfish and neurological signs in Channel Catfish, carp, and salmonids. Pantothenic acid deficiency causes clubbed and exudative gills in Channel Catfish and salmonids and anemia in Channel Catfish, salmonids, and carp.<sup>28</sup>

Minerals in fish have the additional function of osmoregulation, which is the maintenance of osmotic balance between body fluids in the fish and the water surrounding the fish. Minerals such as calcium are commonly absorbed across the gills, or in marine fish, which tend to drink more water, through the digestive tract. Phosphorus is usually not as concentrated in natural waters and must be supplemented in the diet.<sup>28</sup> Other minerals may or may not be easily absorbed through the water.

Juvenile fish require as much as 5% to 9% body weight daily and benefit from more frequent feedings than older fish for optimal growth and development. Early life stages require higher protein and lipid levels than later stages. For example, Channel Catfish of 3-g size require twice as much protein as 50-g fish, and four times as much protein as 250-g fish.<sup>28</sup> *Tilapia* require 50% to 60% protein as fry and 30% to 40% as adults.<sup>28</sup> Earliest life stages of common carp need 40% protein and 9% lipid, with older stages requiring 36% protein and 7% lipid, and 30% protein and 5% lipid needed for maintenance.<sup>29</sup> However, digestible energy must be proportionate to protein levels or inefficient consumption, especially of prepared feeds, will occur.

Live foods, such as brine shrimp (*Artemia*), *Daphnia*, rotifers, and infusoria are frequently first foods for fry. *Artemia*, available frozen, live, or encysted, are fed commonly to all life stages. Adult shrimp are approximately 60% protein and 10% lipid dry weight (DW). Brine shrimp eggs or cysts are available to be hatched out into nauplii (earliest life stages) for feeding smaller fish and fry. Nauplii are 42% protein and 20% lipid DW, and unlike the adults, are deficient in histidine, methionine, phenylalanine, and threonine. However, nauplii are ideal for the fry of many species because of their size: 400 to 500  $\mu\text{m}$  length x 250  $\mu\text{m}$  width versus 8,000 or more  $\mu\text{m}$  adult length.<sup>30</sup> Care must be used to remove all hatched and unhatched cyst shells from the culture before feeding. These are indigestible and can cause intestinal obstructions in fry.<sup>30</sup>

*Daphnia*, also known as water fleas, are cultured by hobbyists and professionals for food. *Daphnia* average 50% protein DW and 20% to 27% fat in adults versus 4% to 6% in juveniles. Newly hatched *daphnia* of the *Moina* genera are 430  $\mu\text{m}$ , whereas adults average 700 to 1,500  $\mu\text{m}$ .<sup>30</sup>

Rotifers, common in natural ponds and specially prepared grow-out ponds, can also be cultured in-house by breeders. They are an excellent first food for many fry because of their high nutritional value and small size (123 to 292  $\mu\text{m}$  in length x 200  $\mu\text{m}$  width).<sup>30</sup> In artificial culture, rotifers must be fed cultured microalgae in order to provide essential fatty acids required by the fry. Rotifers average 70% to 75% protein and 17% to 22% fat, DW.<sup>30</sup>

Many breeders have developed their own home recipes, which may or may not be 100% adequate. These diets should be analyzed for content, especially if results are unfavorable.

Appropriate food storage is important. Moisture encourages fungal contamination, resulting in mycotoxin production. Also, poor storage, either in high-humidity or high-temperature areas, results in more rapid deterioration of both macronutrients and micronutrients.

Coloration, an important trait of ornamental fish, may be muted in fish fed 100% processed foods without appropriate levels of color-enhancing elements. Intensive production, especially in indoor closed recirculating systems where processed feeds are the major source of nutrition, can result in reduction in color intensities.<sup>31</sup>

Most natural color enhancers include carotenoids, sources of red, orange, and yellow colors in fish. Carotenoid bound to protein can produce blue and green colorations.<sup>21</sup> Important sources of carotenoids are crustaceans (krill, shrimp, crabs, and their by-products) that contain astaxanthin, which is considered an excellent source of pigment in koi<sup>21</sup>; spirulina, an algae that provides beta-carotene and zeaxanthin; and other plants, such as marigold, squash flowers, and paprika. Some commercially produced pigment products such as BioRed (Athens, GA), which contains capxanthin, lutein, zeaxanthin, and cryptoxanthin, may hold even more promise.<sup>21,31</sup>

Many companies have formulated all-purpose ornamental fish foods, processed into flakes, pellets, or wafers, for the lucrative retail market. Some also sell processed foods for fry. A few companies are working with individual producers to create more species and stage-specific diets.

The bottom line is that although some basic research has been completed for ornamental fish, nutritionally complete conditioning and specific growth formulations are not yet readily available for all species. Appropriate nutrition is best met through an intelligent, broad, and varied approach, including both processed and frozen or cultured live foods in the diet, taking species-specific requirements into account. Baseline information can be taken from species' natural diets.

### Basic Reproductive Strategies

The veterinarian who understands the basic biology and reproductive strategies of commonly bred fish species will not only have a better frame of reference from which to pose questions and solve problems but will also instill much greater confidence in the client. Loisel presents a very useful scheme, distilling the hundreds of commonly bred species into several categories based on similarity of reproductive patterns. The following categories, with a few simplifications, are based on his scholarship<sup>10,11</sup>: (1) live-bearers; (2) egg layers with no parental care—[a] egg layers, type 1 egg scatterers, [b] egg layers, type 2 egg scatterers without diapause, and [c] annual fishes, type 2, with diapause; and (3) egg layers with parental care—[a] labyrinth fish (bubblenest

builders), [b] cichlids (monogamous and polygamous).

### Live-Bearers

Dawes gives a useful definition for freshwater live-bearers<sup>9</sup>:

*A live-bearer is a freshwater aquarium fish that employs internal fertilization of eggs followed by either their deposition after a shorter or longer period of time or their retention within the body of the female until the moment of birth.*

Live-bearers use many substrategies. The most popular members of this group, the guppies, mollies, platies, and swordtails, belong to the family Poeciliidae and have a specialized copulatory structure, the gonopodium, which is a modification of the third, fourth, and fifth anal fin rays found in males. By contrast, females have a more normal anal fin. After the male's anal fin has metamorphosed into this structure, linear growth ceases.<sup>10</sup> Thus, later maturing males tend to grow larger than earlier maturing ones. Older female breeders may be more prone to egg binding.<sup>9</sup> During mating, relatively intimate contact allows sperm packets, known as spermatozeugmata, to be introduced into the female genital aperture by means of this gonopodium. Similar structures exist in *Anableps anableps*, the four-eyed fish. In the Half-Beak Fish, the structure is called the andropodium.<sup>9</sup>

In some species, the spermatozeugmata burst and immediately fertilize all mature eggs within the female's follicles. In other species, the spermatozeugmata is imbedded in the ovarian cavity wall and can be used continuously by the female to fertilize different batches of eggs.<sup>9</sup> This becomes a problem if selective breeding is attempted in already fertilized females. Most common live-bearing females will become much fuller when pregnant and will develop a gravid spot, a dark area over the vent. Hybridization occurs readily between many species within the same genus and accounts for many of the domesticated color, fin, and size variations.

With regard to embryonic nutrition, a range of adaptations exists from those whose embryos derive complete nutrition from the yolk (lecithotrophy) to those whose embryos receive significant contributions from the mother (specialized matrotrophy). In general, lecithotrophic species (such as guppies) have offspring that weigh significantly less than their egg, whereas special-

ized matrotrophs have offspring that weigh significantly more.<sup>9</sup>

Guppies, platies, swordtails, and variatus have a gestation period of about 4 to 6 weeks, with fry averaging 50 to 100. Mollies require a bit more time, from 6 to 10 weeks, depending on the species. Toward the end of this period, stresses should be avoided. Occasionally, premature egg release occurs. If this happens late in gestation, fry may survive.<sup>9</sup>

On live-bearer farms in Florida, most species are pond spawned, with sorting, grading, and culling occurring periodically. In an aquarium, production is maximized and potential cannibalism is reduced by removing other adult fish, using a breeding trap or cage that holds the female while permitting the fry to escape into the rest of the tank or by otherwise permitting cover for the fry through the use of floating plants or mops made of nontoxic yarn.

Fry can be raised on standard powdered foods, *Artemia nauplii*, and microworms, among others.<sup>10</sup> Sexes should be separated as soon as possible to avoid unwanted pregnancies.

### Egg Layers With No Parental Care

**Type 1 egg scatterers.** The majority of "bread and butter" ornamental fish, the fish most commonly raised and bred for the industry, are type 1 egg scatterers. These include many members of the Cyprinidae: the barbs, danios, goldfish, koi, and rasboras; the Characidae, tetras; the Kissing Gourami; the *Corydoras* Catfish; and others. Many of these species are sexed according to body shape, females being much more easily separated by abdominal fullness when conditioned. All species in this group produce offspring that hatch into a yolk sac stage before becoming free swimming.

Stimuli to spawn for many of these species is water quality related, with such parameters as pH, hardness, alkalinity, temperature, or TDS playing important roles. Each species should be properly researched for exact requirements. Spawning substrates should be provided. Some fish prefer plants or plant-like materials like Java moss, Spanish moss, or mops made of nontoxic yarn on which to spawn. Others prefer a hard substrate, whereas still others release nonadhesive eggs.

Some species prefer to be pair spawned, whereas others spawn best as a group. Regard-

less, spawning tanks should be as clean as possible and properly sized and equipped to reduce cannibalism. After spawning, broodstock are usually removed, and eggs are allowed to incubate and hatch. Water quality must be optimal. Breeders often use some type of disinfectant such as methylene blue or acriflavine to prevent bacteria or fungus from contaminating the eggs. Unfertilized eggs or bad eggs will turn opaque or white and often become covered with *Saprolegnia*. This can be very dangerous because the fungus spreads rapidly and can easily destroy a spawn, unless kept in check.

In 12 to 48 hours, the eggs hatch and yolk sac fry emerge. These young continue to feed off their yolk sacs for about 4 to 7 days until they become free swimming. At this time they should be properly fed. Good water quality must be maintained.<sup>10</sup>

**Type 2 egg scatterers.** Type 2 egg scatterers include many species of killifish (Cyprinodontidae) and rainbowfish (Melanotaenidae and related families). Most species of killies and rainbowfish exhibit sexual dichromatism, with males usually being much more colorful. Males also frequently have more elaborate finnage. Fry are advanced and fully mobile soon after hatching and do not undergo a diapause (period of arrested development) as do annual killifish.

Selection of killifish broodstock is critical because killies in general are shorter lived. Older and larger adults are less productive. Many prefer to spawn in plants or mops. When conditioned, trios of one male and two females are introduced into tanks where several mops have been placed, floating and/or weighted on the bottom, depending on the killies' preferences. If efficiency is not critical, a heavily planted tank provides spawning areas and fry shelters. Killifish breeders often use mops made from nontoxic synthetic yarns that have been boiled or bleached for sterility. Fish appear to prefer darker or green mops.<sup>10</sup> After the fish have spawned, the eggs need several hours to harden before being carefully pried off with forceps and placed in 2 mL of water per zygote. Egg production will become steady for some time and will continue if breeders are fed during spawning periods. Eggs spawned more than 3 days apart should be separated. Egg condition should be monitored and bad eggs should be removed. Spent females and/or males are removed and reconditioned,

while new females or trios are placed together and allowed to spawn.<sup>10</sup>

Rainbowfish; natives primarily of Australia and New Guinea, breed easily in ponds in Florida, but production is better controlled indoors. Rainbows condition best if given some live foods in their diet because their primary food source in the wild is insects and their larvae, primarily ants and small crustaceans.<sup>8</sup> Rainbows may be pair spawned or group spawned with two to three females per male. Spawning mops or Java moss should be provided as substrate. Females will lay 2 to 30 eggs every day for a few weeks. Eggs should be removed or they may be eaten. Hatching may take anywhere from a week for *Melanotaenia spp* to 14 to 24 days for other species.<sup>8</sup>

Fry may be fed after 12 hours. Powdered commercial fry foods as well as appropriately sized live foods (infusoria, microworms, or *Artemia nauplii*) can be fed.<sup>8,10</sup> Shell grit added to the rearing tank increases survivability and growth.<sup>8</sup>

**Annual fishes—type 2 egg scatterers with diapause.** This group of egg scatterers is comprised of those species of killifish that have evolved to live and breed in temporary bodies of water, eg, during wet seasons in various parts of the world. Consequently, breeding these fish in captivity requires that fertilized eggs experience something akin to the drying up of the pool, followed by another "wet season," in order for proper development to occur. A period of diapause ("resting phase") during embryonic development is common to all these fish, although the length of time required to complete this period varies among species. This group matures rapidly—some within several weeks after hatching—has short life spans, and is sexually dimorphic. Potential breeders should be young fish, and trios of one male and two females are recommended for breeding. Keeping annuals at temperatures below 77 °F will prolong their natural and reproductive life. These fish prefer soft, neutral to slightly acid water.<sup>10</sup>

Sexes should be conditioned separately to prevent males from fighting and to avoid undesired spawnings. Spawning tanks should have good water quality and should be furnished with hiding areas for spent females. In addition, a container with preconditioned sterilized peat is necessary as a spawning substrate. Depending on the species, breeding pairs will either bury them-

selves completely within the peat or bury only their eggs.<sup>10</sup>

After several days of spawning, the peat is removed, and water gently squeezed out of it. The eggs are fairly hard and can take gentle manipulation. When a sufficient amount of water has been removed, so that the peat is still damp but not dripping, the peat is placed in a tight container, such as a plastic bag within a plastic bag, and stored for 6 weeks to 9 months, depending on species, temperature, and moisture content of the peat.<sup>10</sup> The peat moisture content should be checked periodically to avoid desiccation.

After the prescribed time for diapause, the peat and eggs are placed in a shallow container of conditioned water. Eggs hatch between 1 to 6 hours later. After this period, the peat should be prepared as before for storage for a few more weeks. Multiple rewettings are required to obtain all the eggs because of varying periods of diapause. Consistently low numbers of fry from the same clump of peat after repeated attempts to stimulate hatching may be caused by a poor spawn (few eggs), death of embryos (as evidenced by numerous fungused eggs), or inability of fry to hatch properly.<sup>10</sup> If numerous eyed eggs are seen, and time in storage has been sufficient to permit proper development for that species, then the eggs may benefit from slight increases in the carbon dioxide content of the water.

Fry can be fed *Artemia* nauplii. To avoid cannibalism, sorting by size is recommended. Sexes should be separated as soon as possible to prevent male aggression over females.

### Egg Layers With Parental Care

**Labyrinth fish (bubblenest builders).** Labyrinth fish, commonly referred to as anabantids, have an accessory respiratory apparatus called the labyrinth organ comprised of heavily vascularized lamellae and located in the head region above the gills. This organ allows them to use atmospheric oxygen, that is, breath air, under low dissolved oxygen conditions.<sup>13</sup>

Labyrinth fish actually comprise four major families: (1) Anabantidae; (2) Osphronemidae; (3) Helostomidae, a monogeneric family that includes the Kissing Gourami, which can be considered a type 1 egg scatterer<sup>10</sup>; and (4) Belontiidae, which includes gouramis of the *Trichogaster* and *Colisa* genera, Paradise Fish, the Siamese

Fighting Fish, *Betta splendens*, and kin.<sup>13</sup> Males and females are more easily distinguished by color during nonbreeding periods. Males tend to have longer and fuller dorsal, anal and pelvic fins, and much brighter coloration.<sup>13</sup>

Labyrinth fish differ in reproductive strategies. Some (Blue Gourami) produce floating eggs (containing an oil droplet of lower density than water) that are cared for in bubblenests. Others produce sinking eggs, which are cared for in bubblenests (*Betta splendens*) or mouth brooded (Chocolate Gourami, other species of *Betta*), ie, incubated within the parent's mouth. The particular strategy will determine the number of eggs in a spawn.<sup>13</sup> Bubblenests, built by the male before spawning, are organized masses of mucus-coated bubbles that allow him to care for brood in a confined area by keeping eggs together near the surface. Sinking eggs that are not able to stay near the surface usually do not hatch, and if they do, the fry will not survive.<sup>13</sup>

Labyrinth fish come from slow-moving or standing waters, often highly organic, cohabited by numerous aquatic insects and their larvae as well as worms and small crustaceans.<sup>13</sup> Consequently, live or frozen foods of insect or crustacean origin such as glassworms, frozen brine shrimp, or bloodworms are useful for conditioning, although diets can be supplemented with flakes and other processed foods. A few labyrinth fish may require more plant life such as spinach, peas, or plant flakes in their diet in order to condition properly.<sup>10,13</sup> Conditioning takes from 2 to 3 weeks.

Most of the common labyrinth fish spawn in a range of pH and hardness conditions as long as extremes are not encountered; however, many prefer warmer temperatures (78 to 82° F).<sup>13</sup> They are usually spawned in pairs, but either a large enough tank or enough cover (floating plants, mops, or submerged flower pots) must be provided to avoid aggression by the male against the female. A male may kill the female if she is not yet ready to spawn, or after spawning, while he protects the nest. After spawning, females should be removed if tank space is limited. Breeders often remove broodstock after spawning because parental care of floating egg layers is not as crucial in captivity as in the wild, and cannibalism may occur after fry become mobile.

Fry should be fed infusoria, then micro-worms, or newly hatched *Artemia*. Although *Arte-*

*mia* nauplii may be too large, some of the more aggressive fry will be able to consume the shrimp by eating it one piece at a time. Of critical importance is the period during which the labyrinth organ becomes fully functional. During this interval, which occurs after several weeks, fry are sensitive to cool atmospheric air; therefore attempts to keep a pocket of warm air above the tank, eg, by means of a tight cover, are crucial. When most of the fry can be seen gulping air at the surface regularly, this critical period is over.

Male Siamese Fighting Fish (*Betta splendens*) are very aggressive toward each other and should be separated and kept individually at about 3 to 4 months of age.

**Cichlids.** The Cichlidae is the most diverse and complex group of ornamentals in the industry, both physically and behaviorally. Because many species are quite spectacularly marked, cichlids have been equated with marine fish with regards to coloration, beauty, and complexity. However, complexity does not come without a price. In addition to considerations applicable to all types of fish such as water quality, stocking densities, nutrition, and the like, the cichlid breeder must also consider mating patterns when designing a management scheme for reproduction.

Cichlids can be divided into two major categories, monogamous and polygamous species. Monogamous species, such as angelfish and discus, are monogamous for one reproductive cycle and do not necessarily form a lifelong bond.<sup>11</sup> Polygamous cichlids have multiple spawning partners, either one or both sexes, during one reproductive period.<sup>11</sup>

Polygamy can be further subdivided into harem polygyny or open polygamy.<sup>11</sup> Haremically polygynous species, such as many of the Dwarf Cichlids (eg, the *Apistogramma* spp), tend to have one dominant male whose territory envelopes numerous smaller female territories. The male spawns with each female, is chased away, and goes to another female. Meanwhile, the female who has just spawned begins defending a much larger area in preparation for her fry.<sup>11</sup> Openly polygamous species exhibit one of three major patterns<sup>11</sup>: (1) general aterritorial polygamy, meaning that breeders spawn wherever the female releases eggs (eg, *Tropheus* sp); (2) persistent display site polygamy, where a single male defends one display and spawning site (eg, the Haplochromines such as *Pseudotropheus* sp and

*Labeotropheus* sp from Lake Malawi in Africa, known commonly as mbuna, or "rockfish," because of their close association with rocky territories); or (3) lek or arena-type polygamy, which is characterized by the temporary male occupation of small tightly clustered territories, which are often directly adjacent to other male territories (as in other non—mbuna Malawi Haplochromines as well as many of their counterparts, such as those from Lake Victoria).<sup>11</sup>

Frequently, non-cichlid fish are kept with cichlids to act as dither fish or target fish. Dither fish are used to calm skittish cichlids such as discus, angelfish, or Dwarf Cichlids by acting as "white noise." They assure the cichlids that all is well. Dither fish should be nonaggressive and large enough not to be a meal. Danios, some live-bearers, and rainbowfish are often used as dithers. Target fish are fish used as targets of aggression by biparentally custodially species. They should be species hardy enough to handle aggression.<sup>11</sup>

Cichlids are also diverse in their spawning methods. Angelfish and discus are substrate spawners, requiring a clean site on which to lay their eggs. Breeders often use slate pieces or plastic plants as substrates. The haremically polygynous *Apistogrammas* are cave spawners, preferring to spawn in inverted clay flower pots or pieces of polyvinyl chloride in captivity. On the other hand, the *Lamprologus* sp from Lake Tanganyika absolutely require the correct size shell in which to lay their eggs. As mentioned previously, many of the openly polygamous species are mouthbrooders, with the female picking up her eggs immediately after she lays them and then stimulating the male to fertilize the eggs within her buccal cavity. This is accomplished in some species by the female's nipping directly at the male's vent, and in others by the female nipping at egg dummies, or pseudo-ocelli, which are brightly colored spots on the anal fin of the male that resemble eggs. After fertilization, the female retreats to a sheltered area to incubate her eggs.<sup>11</sup>

There is some correlation between mating system type and care of the offspring, with exceptions. In general, monogamous species have biparental care of offspring. In discus, fry will procure nourishment for the first week or two by feeding on secretions from specialized skin glands on their parents, although breeders

have been able to raise fry successfully on live foods alone. On the other hand, most polygamous species are maternal mouthbrooders, with the female being solely responsible for incubating and protecting eggs and fry in her buccal cavity.<sup>11</sup>

So what does all of this mean to the general practitioner? Especially when dealing with cichlids, it is important to determine whether the species is being housed and managed correctly. An injured fish may be treated, but unless the cause of the injury is remedied, more losses will follow. For monogamous species, problems often arise, resulting in aggression or injury if the pair is not at the same stage of the reproductive cycle.<sup>11</sup> For polygamous species, male-to-female ratios and aquarium or tank size appropriate for the size of the fish and their general territories are the most important keys to successful breeding. Many problems arise because tank space is too small to support multiple territories. A group of one male and three or four females may require only a 30-gallon tank for water-quality reasons but may require a 75-gallon tank for behavioral reasons. If too few females are present for a given male and those females present are not reproductively ready, they may be injured or killed. If there are too many males, the less dominant males may fall victim to the dominant male. Stress from aggression is not always obvious. After spawning, especially among haremically polygynous species, the female's territory expands greatly, so unless the tank has enough space or hiding areas to enable the male to leave the female's territory, he will be injured.<sup>11</sup>

### Water Quality

When working within the context of a breeding operation, a deeper appreciation of some of the intricacies of water chemistry and biology are mandatory. DO, ammonia, nitrite, pH, temperature, chlorine, hardness, alkalinity, calcium, iron, TDS and/or conductivity, toxins, and cleanliness become far more important for conditioning, spawning, hatching, and larval/fry growth and survival than they are during simple maintenance.

Fish generally have a more narrow range of parameters under which they will condition successfully compared with those for maintenance.

Temperature is one of the more important parameters in this respect, although any water quality stresses such as high ammonia and nitrite levels will take energy and nutrients away from gamete production. Goldfish and koi condition under cooler temperatures (61 to 66 °F)<sup>12</sup> than most tropical fish (upper 70s to 80s).

The sublethal effects of toxins also must be considered when evaluating reproductive efficiency. These include heavy metals and pesticides, among others. Zebra Danios exposed to 5 ppm zinc for 9 days during gamete formation will show reduced spawn.<sup>32</sup> The same holds true for the flagfish (*Jordanella floridae*) and the guppy in the ppb (parts per billion- $\mu\text{g/L}$ ) range. Guppies exposed to 0.36 to 17  $\mu\text{g/L}$  of zinc had clutch size reduced by 50%.<sup>32</sup>

Many of the "blackwater fish" from South America, such as members of the tetra family, spawn under conditions of more acidic pH, low hardness, and low TDS. For example, while the Neon Tetra, *Paracheirodon innesi*, can acclimate to a hardness of several hundred ppm  $\text{CaCO}_3$  and a pH of 8.0, it requires a hardness of 17 to 30 ppm or less and a pH of 5.0 to 6.0 in order to breed successfully.<sup>14</sup> The same holds true for temperature, DO, and other parameters. Some fish prefer to breed at much lower or higher temperatures or lower or higher DOs than those at which they can be successfully maintained. Goldfish and White Clouds (a relative of the Zebra Danio) breed at 68 to 72 °F.<sup>12,14</sup> By contrast, most tropical fish prefer temperatures in the high 70s to low 80s. Discus prefer warmer temperatures in which to live and breed (82 to 88 °F).<sup>11</sup>

Water quality is crucial to hatching, fry survival, and growth. The yolk sac or larval stage is the most sensitive stage in the life cycle, followed by the embryonic stage before gastrulation.<sup>32</sup> Poor nitrogen cycle maintenance (ammonia or nitrite levels), improper temperature, inadequate oxygenation, high organic loads, inadequate calcium levels, or toxic chemicals such as heavy metals or pesticides will reduce the percentage of hatch or prevent hatching altogether. Poor water quality will often lead to bacterial or fungal contamination of the developing eggs.

### Disinfection

Disinfection of tanks or vats, nets, siphon hoses, spawning substrates, and other equip-

**Table 1.** Broodstock General Stress and Illness Rule-Outs

Water quality	Examine ammonia, nitrite, temp, pH, DO, CO <sub>2</sub> , hardness, TDS, toxins (heavy metals, insecticides, etc) supersaturation
Nutrition	Evaluate diet
Habitat/substrate/lighting	Evaluate with species preferences in mind
Aggression/social stress	Evaluate for inappropriate sex ratios, too-dense population, size discrepancies, inappropriate cohabitants (other species)
Parasites-external, internal	Evaluate for predisposing stresses, history, signs, fin, gill, skin biopsy, fresh fecal or necropsy sample
Bacteria	Evaluate for predisposing stresses, history, signs, perform blood culture; or sacrifice and culture kidney, liver, spleen, brain
Selection	Evaluate for genetic/acquired disease; check history or necropsy for evidence of exogenous hormone-induced infertility (eg, testosterone administration)
Other	Biopsy or necropsy for viral, fungal disease, sample for clinical pathology (not too many normals presently known), or other

ment that may contact multiple tanks or systems will help greatly reduce the spread of pathogens. Initial cleaning should include scrubbing of surfaces to eliminate organic residues, which will reduce the efficacy of most disinfectants.

Many disinfectants exist, but those most commonly used for fish facilities include chlorine/bleach, quaternary ammonium compounds such as benzalkonium chloride, iodophors, and formalin, with the former two being preferred. Chlorine, at 200 to 1,000 ppm, is perhaps the most inexpensive, readily accessible, and potent but is

very rough on nets and is also very toxic to fish. All residues must be eliminated by adequate rinsing and/or neutralization procedures. Sodium thiosulfate, available in solution or as a crystal, readily neutralizes chlorine if used at approximately two to three times the concentration of chlorine, in ppm. Always double check, if in doubt, for presence of residues with a chlorine test kit. Quaternary ammonium compounds, such as benzalkonium chloride, are frequently used as a net dip and are most effective against bacteria. Effective concentrations vary depend-

**Table 2.** Broodstock Conditioning Failure and Spawning Failure Rule-Outs

Conditioning Failure Rule-Outs	
General health, stress, or illness problems.	See Table 1.
Nutrition	Evaluate for species, cf natural diet, review micronutrient/macronutrient components and quality, and examine feeding schedule/quantity
Age/maturity	Evaluate size and age for maturity
Seasonality/annual cycle(s)	Review natural spawning conditions, eg, photoperiod, season, temperature
Spawning failure rule-outs	
Conditioning problem	(see above)
Fertilization hampered	Evaluate management techniques, verify presence of both sexes and proper ratio
Natural triggers not provided	Evaluate photoperiod, water-quality parameters (especially temperature, pH, hardness, DO, salinity/TDS, alkalinity, tannins), flooding, water current, tides, lunar or weather cycles (atmospheric pressure, rainfall), spawning substrate, other species
Available space/substrate inadequate for natural behaviors	Review rituals, habits, courtship displays, etc; cannibalism of spawn?
Induced spawning failure:	Not conditioned (see above); females/males not properly checked for condition status (quantity/nuclear migration of eggs; sperm production/motility); hormone-incorrect, degraded, or contaminated; excess stress during preovulation procedures; water quality parameters: temperature or other inappropriate; stripping of gametes done too early or too late after ovulation/spermiation; injection method incorrect

**Table 3.** Poor Egg Hatch Rule-Outs

Water quality	Evaluate all parameters, including temperature, pH, DO, CO <sub>2</sub> , ammonia, nitrite, hardness, TDS, alkalinity, toxins (heavy metals, pesticides, hydrogen sulfide)
Pathogens	Evaluate spawn for fungus, bacteria, parasites, virus; examine water source and quality for pathogens; evaluate disinfection of spawn/hatch-related materials (containers, substrates, nets); consider egg disinfectants and ultraviolet sterilization of hatching water; evaluate broodstock for subclinical pathogens, eg, endoparasites
Improper development or fertilization	Inadequate broodstock conditioning/diet; female infertile; male infertile or did not fertilize eggs

ing on the manufacturer, but many work well at 10 to 20 ppm, followed by a rinse in clean water. Iodophors do not work very well in the presence of dirt and/or organics, and formalin, although commonly used at lower dosage rates to treat parasitic diseases, can be harmful to humans (and fish) at concentrations required for disinfection. Ethanol, 70%, is necessary for disinfection of *Mycobacterium*-infected materials. Finally, air drying reduces some pathogens, but not all, and the time required is usually not feasible in larger operations.

### Problem Solving

When working up a case involving a client who has called with fish reproduction difficulties, an accurate and complete history is paramount. The practitioner who can obtain some

**Table 4.** Larvae/Fry Poor Survival Rate Rule-Outs

Examine for presence of yolk sac/quality of yolk sac (in fry that should have them); (look for differences among fry); mortality before yolk sac absorption will more likely be owing to factors other than starvation.	
Broodstock improperly conditioned	(See Table 2)
Water quality parameters inappropriate	Review water quality requirements
Physical conditions rough	Handling, current, or aeration excessive
Pathogens	Examine for bacterial, fungal, parasitic, or viral etiology
Nutrition	Evaluate for composition, size, type of food, amount, proper removal of unhatched cysts or hatched shells (if fed <i>Artemia</i> ), which can cause impactions; evaluate broodstock diet

background information on that particular species stands a better chance of asking the right questions. This knowledge will also help determine whether the client is familiar with techniques commonly used for that species.

A good history will include, in addition to clinical signs and questions related more directly to the problem itself<sup>33</sup> (eg, basic aquarium/vat/pond setup and management practices including disinfection/sanitation protocols), the following: broodstock origin, selection, and management; previous reproductive activity and results; fry rearing conditions; nutritional programs; and an understanding of the client's level of expertise, involvement, and expectations.

Depending on the accuracy and completeness of the history, the extent of the problem, and the level of production involved, a site visit may or may not be warranted. Citino provides excellent guidelines for site visits of production-oriented facilities.<sup>34</sup> If an obvious illness is involved and the fish can handle the stress, the client can bring the fish along with a separate water sample to the clinic for basic workup.

**Table 5.** Larvae/Fry Deformities/Stunting Rule-Outs

Genetics or conditioning of broodstock	Evaluate
Water quality parameters	Evaluate during hatch, growth, R/O toxins; Examine feeding regimen and R/O nitrogenous waste buildup
Nutrition	Examine for adequacy of quantity, size, bioavailability, and nutritional content; evaluate broodstock diet
Density	Consider reduction in population density

However, if the problem is reproductive failure and not an obvious illness in broodstock or juvenile fish, then problem solving becomes more involved. Tables 1 through 5 are a guide to the most common causes of reproductive failure in fish. It should be noted that most problems are management related and all of the key steps necessary for successful reproduction are interrelated. Tables 1 and 2 highlight common broodstock problems. Table 3 lists rule-outs for poor hatch. Tables 4 and 5 provide rule-outs for larvae and fry problems.

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