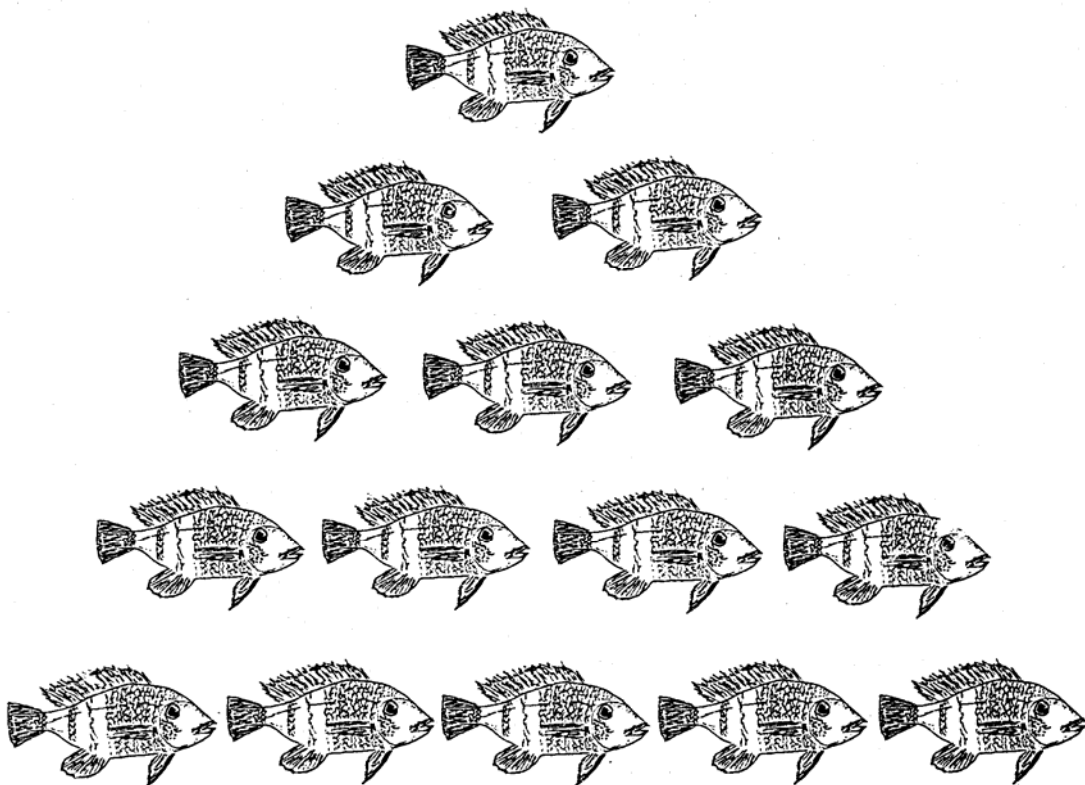


ISBN 1-895655-02-1

*Aquatic  
Conservation  
Network*

# Captive Breeding Guidelines

Edited by  
Robert V. Huntley and Roger W. Langton



## **Captive Breeding Guidelines**

**Published by:**

Aquatic Conservation Network, Inc.  
Canadian Registered Charity #0953471-52

This electronic version of **Captive Breeding Guidelines** is published by the North American Native Fishes Association (NANFA). Founded in 1972, NANFA is a not-for-profit, tax-exempt corporation dedicated to the appreciation, study and conservation of North America's indigenous fish fauna. Visit [www.nanfa.org](http://www.nanfa.org) for more information.

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Published in October, 1994  
ISBN 1-895655-02-1

**Cover Illustration:** Tim McCarthy

# Captive Breeding Guidelines

## FOREWORD

This document was developed by volunteer **Conservation Aquarists**. From initial drafts, to peer review, to final editing, this document exemplifies the role volunteers can play in the conservation movement. Included among the contributors are amateur aquarists as well as professionals who earn their living in scientific institutions, zoos and public aquariums. All of these people have an underlying belief there is a vital role for the non-scientist to play in the conservation of aquatic life. It is with this belief that these people have volunteered their time to this project; their contributions are gratefully acknowledged:

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# Captive Breeding Guidelines

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## **Captive Breeding Guidelines**

### ***Aquatic Conservation Network***

#### ***Conservation and Captive Breeding Goals***

1. *Support habitat protection as the first line of defense against the extinction of freshwater fishes.*
2. *When appropriate, establish viable captive breeding populations of endangered freshwater fishes in support of the preservation of biodiversity.*
3. *Offer holding and captive breeding services in support of conservation efforts by zoos and public aquariums.*
4. *Establish captive breeding guidelines to help maintain genetic diversity using the best scientific knowledge available.*
5. *Cooperate with scientific researchers when their findings are likely to yield information vital to the conservation of freshwater fishes.*
6. *Cooperate with appropriate government agencies responsible for regulating the possession and transfer of protected freshwater species.*
7. *Educate amateur aquarists regarding the need for standards that promote conservation in all aspects of the aquarium trade.*
8. *Foster public awareness of aquatic conservation issues and promote fundraising to assist in situ and ex situ conservation efforts.*

## Captive Breeding Guidelines

### GENERAL INTRODUCTION

As the months and years go by it will become increasingly apparent that many of the world's fish species are threatened with extinction. The continuing growth of human population and the consequent pressures placed upon limited resources will increase to unprecedented levels. Deforestation and other environmental disruptions will continue to degrade and destroy the habitats of many of the world's living creatures. Zoos and public aquariums are already operating under emergency conditions to save threatened animals. Due to these extraordinary circumstances, there is a role for the amateur to play in captive breeding programs. The full extent of that role is yet to be determined and will depend upon the commitments all parties have to the projects instituted. Ultimately, the success or failure of the **Aquatic Conservation Network's** efforts will depend upon whether or not cultural norms that support long-range conservation evolve quickly enough. In the meantime, we will need the support of each other to compensate for the weaknesses of human nature that so often make conservation efforts no more than a gesture, a wind tunnel of words that produces nothing.

There are about 24,000 fish species that have been described by scientists. It has been estimated by many conservation biologists (Ehrlich 1990, Wilson 1992, Andrews 1993) that from 25-50% of these species are in danger of becoming extinct by the year 2050. Since most of the captive breeding efforts are currently focused on saving endangered mammals, birds and reptiles, the amateur aquarist will find opportunities to take the lead in preserving for posterity fishes that can be successfully bred in captivity. Conservation resources are very limited and fish are likely to be neglected, with many species going extinct, unnoticed. A likely outcome of working to save the hobby will be that more and more fish that are extinct in nature will be found only in the tanks of aquarists. Serious aquarists of the future will need to learn a great deal about genetics and apply this information to their breeding programs. Many will come to realize that working with the guidance and support of professionals may be the most effective way to preserve their fish for future generations. Conversely, conservation professionals may learn that the amateur has an important contribution to make in saving parts of the earth's biodiversity. The extinction crisis is simply too overwhelming to ignore any potential resources. It is hoped that the current and future **conservation aquarists** will earn respect by becoming an important part of the practical process that acts to ensure the survival of endangered species.

This **Aquatic Conservation Network** document, *Captive Breeding Guidelines*, has been written for those who wish to participate in ACN captive breeding programs. The aim is to give guidance to all participants and help them decide what role they are willing to play in this important endeavor. Initially the ACN will begin its work with the **Madagascar Project** and, after experience is gained, move on to other areas. The number of species brought under ACN guidelines will depend upon the amount of commitment shown by amateurs and professionals relative to the announced programs. This effort represents an act of faith that there are enough volunteers who care about the preservation of threatened fish populations to make the various projects a success.

Every local, national and international aquarium society is urged to initiate a program to encourage conservation. No doubt only the most dedicated will manage to do the job and you are encouraged to be among them. The purpose of this publication is to assist conservation aquarists who are committed to achieving these goals.

Thanks go to Dr. Peter Burgess, Rob Huntley, Roger Langton, Mark Rosenqvist and Dr. Phillip Spokenberg whose efforts made this work possible.





# Captive Breeding Guidelines

## SECTION 1

### IN DEFENSE OF CAPTIVE BREEDING OF ENDANGERED FISH

by **Roger Langton**

*The extraordinary effort needed to care for and breed fish for the purposes of conservation requires that those who make the effort be philosophically committed to the task. Without a positive perspective and the resulting motivation, participants in Aquatic Conservation Network programs are likely to give their captive breeding efforts a very low priority. When this is the case, they will probably drop out of the program after a short period of time. This is not to say there are not many aspects of life that justifiably have a higher priority, but commitment to the captive breeding programs must have a high enough priority to ensure long-term participation.*

*There are many people who are skeptical about the value of keeping threatened animals alive through captive breeding and they will challenge the wisdom of these efforts. No doubt, ACN members have already given many of these issues considerable thought. In order to aid ACN participants to think more about their conservation efforts, Section One will be devoted to answering some of the most often heard objections to programs designed to keep endangered fish alive in aquariums. Perhaps the following will be useful in helping them answer those who will criticize and be skeptical about their efforts.*

*This section first appeared as an article "In Defense of Captive Breeding of Endangered Fish" in the March, 1993 issue of Aquatic Survival, the quarterly bulletin of the Aquatic Conservation Network.*



The debate over the value of captive breeding as a means of preserving species from extinction will probably go on for decades to come. Conservation-minded people are divided as to the value of this approach to the long term maintenance of biodiversity. The purpose of this article is to discuss the positive aspects of captive breeding by commenting on the points most frequently made by those who oppose or seriously question such efforts. Eight opposing statements will be examined in an effort to make the case that captive breeding efforts can and will make a valuable contribution to conservation.

**1. Humans are part of nature and the extinctions caused by their dominance and superiority are natural.**

It is obvious that this attitude works against conservation efforts. It reflects an anthropocentric perspective that places humans at the center of existence and assumes their superiority and the inherent inferiority of all other living things. This attitude is shared by many people, many of whom have not examined the assumptions by which they live their lives. This common attitude demonstrates a profound ignorance about how ecosystems work by failing to recognize the essential services that plants and animals provide in the maintenance of a viable environment. As a matter of fact, if these free services were to suddenly stop, *Homo sapiens* would become extinct in a very short time (Ehrlich, 1988; Wilson, 1992).

Another component of this position is the belief that humans cannot destroy the environment even if they wanted to and that nature will always be able to rapidly repair any damage that might be done. Along with this is an optimistic belief that technology will be able to solve any environmental problems that might arise. Many go so far as to say that those who wish to restrain human activity for the sake of the environment are perpetuating a hoax which represents the last vestiges of socialism or communism. In effect, environmentalists are seen as enemies of capitalism. Clearly, people who hold these attitudes do not offer much encouragement to those who wish to restrain or modify some human activities in order to protect the

## **Captive Breeding Guidelines**

viability of the planet. The reality is that we are presently in one of the great extinction spasms of geological history. E. O. Wilson estimates that currently 27,000 species of plants and animals go extinct every year. In the rainforest, due to human activity, this represents an extinction rate from 1,000 to 10,000 times faster than the normal rate over geological time (Wilson, 1992). Clearly, education is an important component of any conservation effort.

People with this view often fail to see that what sets humans apart from the rest of nature is their ability to understand the consequences of their behavior. Implied within this is the capacity to restrain behavior for the common good. It is this unique potential to be ethical that gives hope that a new value system will develop which gives reverence for the miracle each species represents. Those who support measures that ensure a high degree of biodiversity have faith that respect for all life and the consequent preservation of nature will eventually become the norm. This hope is based upon the increased realization that it is in the best interests of human survival to do so. At that point the extraordinary efforts of those involved in saving species from extinction will be more fully appreciated.

### **2. What good is the captive breeding of a species when its natural habitat is destroyed and there is no possibility of reintroduction?**

One of the expectations of people involved in captive breeding is that some fish species will eventually be reintroduced into their natural habitat. It is true that many habitats will not be able to support aquatic life for many decades or perhaps forever. The conservation aquarist chooses to maintain the fish even if the future of its habitat looks bleak. The hope is that habitat reconstruction will become an important area of research in the next few decades and that eventually many species will be placed back into nature even if the habitat has to be engineered by humans. If the fish no longer exist, this will not be an option.

In addition, the educational value of displaying fish that are threatened or extinct in nature should not be underestimated. It is hoped that such displays will help inform people of the value of biodiversity and may help bring about the conservation ethic mentioned earlier.

### **3. Long term captive breeding will eventually fail due to the inevitability of inbreeding and the subsequent loss of genetic diversity.**

Perhaps the greatest challenge presented to persons breeding fish for conservation is to maintain genetic diversity in their captive specimens. Fortunately, conservation biologists have done considerable research in the last two decades relating to genetic management. Their findings will give guidance to those involved in long term captive breeding programs. Still, most of this research has been done on mammals that produce small broods over extended period of time; this research may or may not apply to many fish species. More research will be necessary to refine breeding procedures to achieve this goal and these processes may differ for individual species. It is likely that some species will prove to be more resistant to inbreeding than others. Some fish species living in small habitats have survived hundreds of years of inbreeding and are, for all practical purposes, genetically identical e. g. desert pupfishes. Aquarists have maintained viable populations for decades despite a high degree of inbreeding. Experience among amateurs suggests that it is possible to eliminate lethal genes through selective breeding practices. But, to be fair, aquarists have also learned that continuous inbreeding can lead to deformities, disease, and the loss of a breeding group.

Despite all of these potential difficulties, the serious conservation aquarist will make every effort to begin a breeding program with as many unrelated founders as are available and add unrelated specimens to the program when available. Theoretically, it is possible to maintain a high percentage of the genetic variability present in the founders through careful breeding management.

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A high level of genetic diversity will be especially desirable, if not essential, when a species is to be reintroduced into a natural environment. The point of concern is well taken. Only time and experience will determine if these obstacles can be overcome. Not to try would be the greatest mistake. The opportunity to save a species seldom comes more than once.

### **4. Animals bred in captivity lose their survival instincts and become easy prey when reintroduced into their natural habitat.**

It is certainly true that captive bred fish learn some "unnatural" habits while living in aquariums. It is not unusual for fish to rush to the front of the tank in anticipation of being fed and to seemingly recognize the people who feed them. This behavior does not mean that the fish has lost the proper response to predators. The instincts are there, but have been compromised in the safe aquarium environment. If you drop a small fish into a tank containing large cichlids, there is often a wild chase with the small fish seeking and sometimes finding a safe hiding place. The panic shown in the behavior of the potential victim says a great deal about the existence of flight instincts in the face of danger, even in species that have been in captive environments for many generations. The question is, will the fish learn in time to revert to a more natural response? As biologists become more adept at the science of reintroduction, ways may be found to revive these survival mechanisms before fish are put back into nature.

### **5. Captive bred animals no longer participate in the process of evolution and, therefore, lose their importance to the natural world.**

Conservation aquarists have respect for the wisdom of nature in creating a wide variety of species and wish to see the process of evolution continue; this is an important motivational factor in their efforts. It is true that the interaction that occurs in a natural habitat is temporarily lost or greatly compromised in captive populations. It is also true that the adaptations that do occur are in response to an artificial environment and may not be useful in the wild. But the bottom line is clear, if animals do not exist, the possibility of future participation in the evolutionary process is lost forever. (Even in this context the potential for meaningful research exists, although this is a secondary consideration except in cases where research aids conservation efforts.)

### **6. The cost of keeping a species indefinitely in captivity is very high. Scarce funds should be used to protect habitat rather than maintain a species that is, for all practical purposes, extinct.**

If the potential for captive breeding is used as an excuse for not protecting habitat, then the concept is being misused. Habitat protection is always the first priority. As a general rule, it is only when the habitat is in grave and imminent peril that captive breeding comes into play. The bulldozer and chain saw are very capable of turning a pristine environment into a wasteland within a matter of days or weeks. Still, these decisions are often difficult. Conservation aquarists want to be able to obtain enough founders when captive breeding is indicated but they don't want to endanger a habitat further if there is any possibility of it being saved. This is where knowledge and good judgment come into play.

Other conservationists advocate that captive breeding programs should begin long before a species is on the brink of extinction. Indeed, when one considers the number of countries that are currently experiencing political instability, many of which have critical fish habitats under their jurisdiction, it would seem prudent to have captive populations outside of those areas. This approach suggests that the wise thing to do is establish these populations in anticipation that habitat destruction is likely within a few years. Such action is especially appropriate in those areas where conservation efforts are highly problematical. By the time everyone agrees that a habitat is ruined, it may be too late to acquire a viable population for captive breeding. The rainforest species of Sierra Leone and Cameroon would be good examples of fish that will probably be in

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trouble within this decade and plans could be made now to save them. It is a tough wire to walk.

If human population doubles during the next century, as it surely will, it is likely that from 30 to 60% of all species on earth will disappear by the year 2050 (Wilson, 1992).

**7. Saving fish for the hobby is wasting resources that could be used for more important human needs.**

Certainly, much of the motivation for amateur aquarists to participate in captive breeding programs will be to save the fish for the enjoyment of the hobby. Given the extent of the challenge to save even a few species, all persons of good will should be invited to participate. If aquarists are willing to follow the rules insuring the integrity of the breeding program, they should be encouraged to take part. The potential is enormous. It is likely that the hobbyist will become, before long, a conservation aquarist who understands the full significance of the work. Yes, human needs are important, but the survival of biodiversity on this planet must be our highest priority if we are to avoid the collapse of civilization and what Paul Ehrlich describes as "the equivalent of a nuclear winter" (Ehrlich, 1988).

**8. As a general rule, technological societies do not support the ethical principles necessary to sustain long term conservation efforts.**

The number of people focusing on conservation issues is growing but, for the most part, there are few fundamental changes that put significantly less stress on the environment. We tinker around but cannot face the hard choices necessary to make the difference. We are an intelligent species, but history may yet record that we have a fatal flaw- our capacity to use nature exceeds the capacity of nature to sustain our activities. Put another way, we may demonstrate that our capacity to restrain ourselves for the sake of posterity is weaker than our desire to satisfy every imaginable appetite in the present. If this latter characteristic proves to be true, then there is little of significance that separates us from the proverbial beast. Many know that we are heading for difficult times but, as in the case with the U. S. national debt, the necessary adjustments to solve the problems are difficult to contemplate and politically unpopular.

We all know what has to be done: reduce human population, walk more gently on the earth, love nature as our mother and father, move from material growth to personal growth, stop this unhealthy obsession with our species (ourselves), make room for all life and learn to love, or at least appreciate, all of nature as we love and appreciate ourselves. In the interest of our survival, change in this direction is an historical necessity. Is it too late? Maybe, but we have to try to change or we will be in danger of losing those qualities essential to our humanity.

# Captive Breeding Guidelines

## SECTION 2

### BREEDING FOR GENETIC DIVERSITY

by Roger Langton

*Section Two deals with the concept of breeding fish for genetic diversity. In many ways this will be the most challenging part of the program and the temptation to take short cuts will be ever present. The first thing any captive breeding program needs is a set of goals. Once the goals have been established, the degree of discipline needed in breeding the fish will become more evident.*

*If the goal is to breed fish for a relatively short period of time with no other purpose than enjoyment, it is not necessary to give much thought to the principles regarding the maintenance of genetic diversity. All that is needed is a healthy pair to start the process and to choose healthy breeding pairs from subsequent generations until the hobbyist decides to go on to some other species or until the population is no longer viable. If the goal is to keep a species "forever" in a captive state, the challenge will be to breed the healthiest and strongest specimens for several generations until the lethal genes are eliminated and, if successful, the captive population will, over time, be genetically identical. The fish will essentially be clones, and as long as they are not required to make severe environmental adaptations, the fish should remain in a healthy state indefinitely. On the other hand, if you intend to breed fish with the expectation of returning them to their natural habitat, the fish will need to maintain the adaptive mechanisms found within the natural population. The specific identity and source of origin of stock needs to be confirmed from the outset, preferably by reference to a recognised taxonomic specialist. Years of work could be wasted if the founders are misidentified or if their geographical origin or genetic integrity is in serious question. The breeder will need several unrelated pairs to begin the program and their breeding will have to be managed in a very disciplined way. Ideally, regardless of the goals, genetic diversity will be maintained because the future of many freshwater fishes is unknown and the option of reintroduction should remain viable.*

*Although it is not necessary to know a great deal about genetics to breed fish in the manner discussed here, it is useful to have a few fundamental principles in mind. The first part of Section Two gives a brief introduction to these principles and it is recommended that you read them carefully. It is important to emphasize that the examples given here are used only to illustrate one way of going about breeding for genetic diversity. Although the principles are the same, in many cases the process will need to be adapted to the breeding habits of a particular species. This section will start participants in the right direction but experience and new knowledge will move them toward new and improved methods. In future editions of Captive Breeding Guidelines, it is anticipated that this section will be expanded to include methods of breeding a variety of species that take into account their unique breeding patterns. The potential to learn and discover is almost unlimited.*



Much of the research regarding genetic diversity has been done during the last 20 years. The author has drawn heavily upon the contents of two journals, *Zoo Biology* and *Conservation Biology*. Both of these journals contain research useful in planning a program for maintaining genetic diversity among captive bred animals. It should be kept in mind that most of the research has been done on mammals with little specific information about fish. Still, the principles are basically the same. It is not the intent of this article to present a comprehensive explanation of the science of genetics but a few general principles should be kept in mind.

When a male and female fish come together to spawn, both individuals makes a genetic contribution to the offspring. Both animals make different contributions. In other words, males carry different genetic material than females and vice versa. Breeding seven males with three females will pass on a smaller gene pool than would result from spawning five males with five females. Sutcliffe (1992) has shown that three males and seven females (or vice versa) will result in an effective breeding population equivalent to 8.4 individuals, while five males and five females will represent an effective population of 10. **Effective population** refers to

## Captive Breeding Guidelines

the number of individuals that actually participate in breeding, not simply the total number in a particular population. This is an important consideration when beginning a breeding program designed for long-term maintenance.

Alleles is the term used to refer to the specific genetic variation that is exchanged from the genes. In other words, "each gene within each locus may exist in one of several variations- alleles" (Tudge, 1992). Some alleles are fixed and will always produce the same result while others are variable and there may be several possible outcomes. One allele might give your fish tolerance to a certain disease or the capacity to adapt to cooler than normal water temperatures. Thus you will find genetic variation among fish that otherwise look identical. The more offspring a pair produces the more likely it is that all the genetic variation available in the two fish will be passed on to the next generation.

Alleles can be lost over time. This natural phenomenon is referred to as genetic drift. An allele may become rare in a natural population, and if by chance none of the fish possessing the rare allele get to breed, the genetic information is lost and will probably never be regained. Since most natural populations are genetically variable, genetic drift is likely to be countered by other forces acting on those populations.

Although this is a natural process, the loss is accelerated when inbreeding occurs. Siblings can only pass on what was given to them by their parents. Inbreeding changes combinations of alleles (genotype) resulting in diminished genetic diversity. A point will be reached when the species is probably doomed to extinction. Infertility, deformity and susceptibility to disease are among the possible consequences. Thus, if a captive species is to remain viable, a large gene pool is desirable. The longer the species is to be maintained, the greater the need to breed the species in such a way as to conserve a large percentage of the genetic diversity. Theory suggests (Ralls & Ballou 1986; Tudge 1992) that a population of 500 individuals of equal sex ratio is needed for indefinite survival.

If your goal is to keep fish for a long period of time, you need founders that are unrelated. Founders are the original parents of your captive generations. The more founders, the more genetic diversity will be available for future generations.

Hybrids (offspring resulting from the crossing of two different species) are to be avoided because they do not represent the fish as it exists in nature and are seldom viable for very long. For conservationists, producing hybrids is a waste of time except for scientific research in determining relationships among species. Hybrids should not take precious time, space and effort that could otherwise be used for conservation efforts. Obviously hybrids should never be mixed with pure populations but the danger exists if they are in the fishroom. It is prudent that hybrids not be allowed to exist in the same area if there is any danger of contaminating a valuable gene pool.

In addition, breeding between subspecies should be avoided because they are often adapted to different environmental conditions. This is especially true for fish that will eventually be returned to their natural habitat. Breeding between subspecies might be done as a last resort to save a species from extinction, but not as a general rule. One need only look at the confusion found among discus breeders as to the origin of their fish. The well-known discus breeder Schmidt-Focke (1990) has recently warned aquarists to stop mixing these fish and to get back to breeding pure species and subspecies. Otherwise this magnificent fish may be lost to the hobby.

Does this mean that if only one pair of fish is available that it is useless to try and conserve them? No, you should try because you might get lucky. For example, all of the hamsters currently kept by thousands of children and grownups came from one pregnant female. Just take a look at the variability found among them. Some of the desert pupfish, such as *Cyprinodon diabolis*, are genetically identical due to hundreds of years of

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inbreeding (Turner, 1974) and yet persist as a viable population. Because of this variation in tolerance to inbreeding, efforts should be made even when the founding population is small. Still, as a general rule, it is best to breed fish in a manner that will preserve as much genetic diversity as possible.

Ideally, preserving genetic diversity in a captive population means that you start with a founding group that carries within it most of the gene pool found in nature. Unfortunately this is not always easy to achieve. Part of the difficulty lies in the fact that most aquarists do not keep records regarding breeding lines. Even if you obtain fish from different hobbyists who live in various geographical areas, it is not unusual to find that all fish came from the same parents. In some cases, one pair has produced all captive fish of a certain species. In practice it means that you start out with fish that are available. Still, every effort should be made to obtain fish that are genetically unrelated. This will increase the odds that the gene pool will be larger than it would be if inbreeding has occurred.

Theory suggests (Ralls & Ballou 1986) that you need from 6B12 unrelated founder fish of an equal sex ratio to have a good chance for success over an extended period of time. Using this relatively small number of founders will likely mean they are not representative genetically of wild populations and the effects of inbreeding will lead to a fairly rapid loss of genetic diversity within a few generations. In addition, it may be unrealistic to believe aquarists can create an artificial environment that will provide the same selection pressures encountered in nature. The end result may be a species genetically very different from those found in the wild. The number of founders used to begin the captive breeding program will depend upon the specific goals of the program (e. g. returning a fish back into its natural range versus maintaining the fish for the aquarium trade) as well as the availability of breeding stock.

If your goal is to maintain a viable population during your lifetime as an aquarist, then here is a suggested procedure that should produce good results for at least a 40 year period. This example starts with a founding population of eight individuals, four males and four females. If all goes well a core breeding population of 32 individuals (16 males and 16 females) will be the effective population that contributes to each subsequent generation. This model can be easily modified upwards or downwards depending upon the goal to be achieved. Perhaps an effective breeding group of 500 individuals is necessary for a population to be maintained indefinitely while fewer than eight founders would work well for aquarists with more modest goals. Working with a group of aquarists is advisable when the tank space each person can contribute is limited. Cooperating groups can exchange fish from time to time to expand the gene pool. Space, time, commitment and life's circumstances will eventually determine what is accomplished.

For the purpose of going through the proposed breeding process, *Fundulopanchax gardneri*, a well known killifish, will be used as the species being maintained. Some species will be much more difficult to maintain than others but many fish can be successfully maintained using this method. This model will be a combination of random and manipulated breeding procedures designed to ensure genetic diversity for a 40 year period.

The set up requires 20 tanks and several small containers for egg incubation and for housing fry during the first few days after hatching. *F. gardneri* will require eight 2 ½ - gallon (9.5 liter), four 20-gallon (75 liter) and eight 10-gallon (38 liter) tanks. For most purposes it is better to err on the side of larger rather than smaller tanks (See Figure 1).

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The first step is to set up four 2 ½ -gallon (9.5 liter) tanks for the founding breeders. Each tank will contain a spawning mop and a sponge filter. Tanks are labeled A, B, C and D. The breeding pairs from the eight founding individuals are chosen randomly. No effort should be made to make judgments about which are the most desirable specimens etc. It is assumed the fish are healthy and in prime condition. Obviously, all eight founding fish are used in the breeding program. Keeping in mind the data required to fill out the studbook (brood numbers etc.) will make the record keeping requirements easier.

Eggs are picked from the spawning mops and water incubated in petri dishes or other appropriate containers. Each container is labeled A, B, C, or D to keep the source of the eggs accurate. It is important to pay attention

when putting eggs in containers or eggs from more than one breeding line can accidentally get mixed. When the eggs hatch, in about 14 days, the fry are placed in appropriate containers until they are eating well and ready to be transferred to larger quarters. Again, the containers should be labeled accurately. It is desirable that at least 30 fish from each pair be produced. In the case of *F. gardneri*, this should not prove to be difficult.

When the fry are ready, they are put in the appropriate 20-gallon (75 liter) tank and raised to breeding size. The rearing tanks should be labeled to keep track of breeding lines. The fry produced by breeders labeled A should be placed and reared separately in a rearing tank labeled A. Fry from tank B go into rearing tank B and so on for all four breeding lines. These fish will be considered the F<sub>1</sub> generation relative to the founding individuals and will eventually become the parents of the F<sub>2</sub> generation and so on.

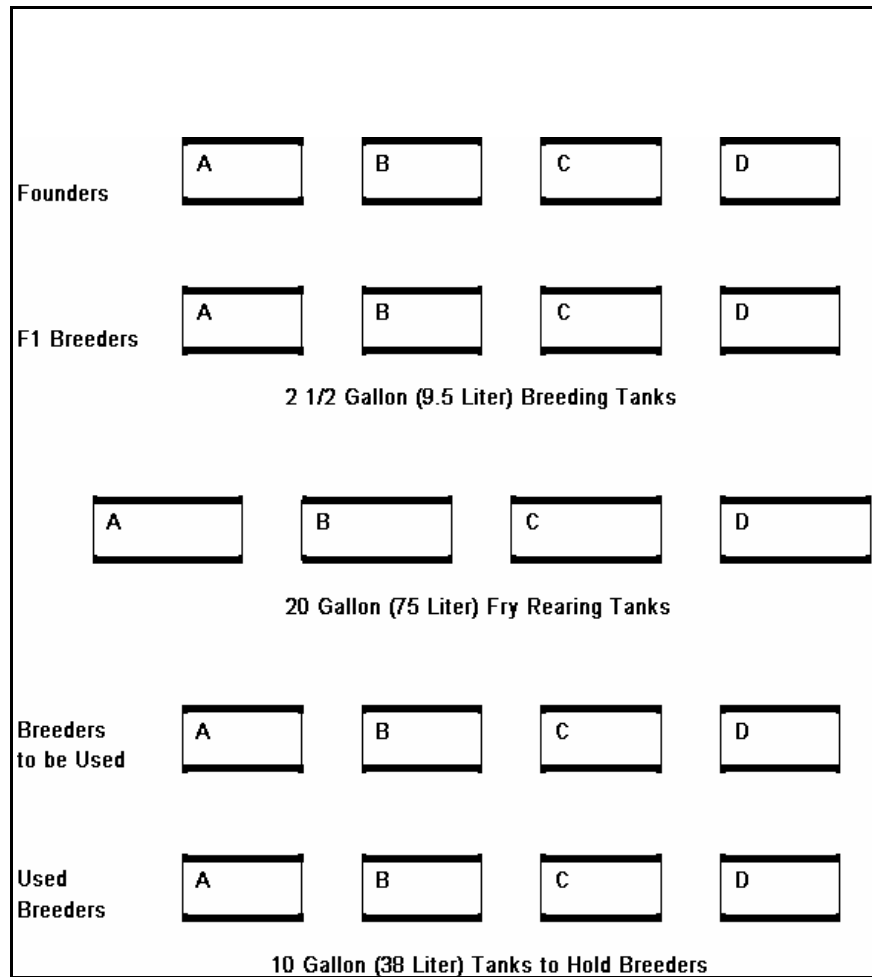


Figure 1 - Core Breeding Set Up



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Because genetic drift is likely to occur with each generation, it is important to have some patience and not breed the fish too early. The idea here is to produce no more than one new generation each year. If you breed fish at intervals of eight months, 60 generations will be produced in the 40 year period causing a greater loss of genetic diversity than would be the case if one generation is produced each year. Of course, some fish, such as South American annuals, will need to be bred more often if they are to reproduce during their prime. Other fish will be commonly bred every two years or even longer intervals. For *F. gardneri*, one year seems appropriate. In addition, it is likely that some genetic information will be lost as a result of adapting to the aquarium environment, especially after several decades of breeding.

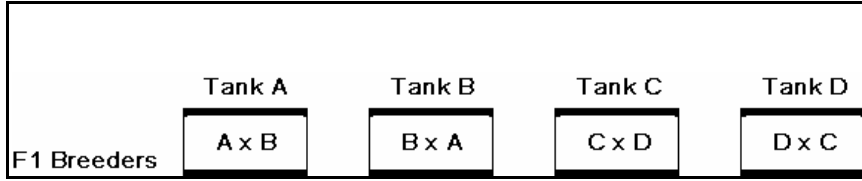
When the F<sub>2</sub> offspring are near full maturity, four males and four females should be chosen **randomly** from the same breeding line and placed in a 10-gallon (38 liter) tank. The only selection to be used is to avoid deformed or diseased fish. This should be done for each of the four breeding lines with each tank being carefully labeled A, B, C, or D. When all four 10-gallon (38 liter) tanks have four males and four females from the four founding pairs, you will have a core of 32 fish. It is this core of breeders, all of which will participate in producing the next generation, that will provide the genetic viability of your species. It is wise to keep the remaining fish in the 20-gallon (75 liter) tanks in the event that the fish chosen for breeding die or become diseased. Redundancy is an important aspect of this process and especially important if the fish is extinct in the wild. After the conservation requirements are met, the excess fish can be distributed to other aquarists.

At this point it is important to emphasize that fish used to produce the next generation be chosen randomly. Although selection may counter the effects of inbreeding to a certain extent, the question remains as to what you select for? Selection causes genetic change and there may be correlated responses that produce other, less desirable characteristics. Because of this risk, a random approach is recommended in this model.

After the core breeding stock has reached one year of age, the fish should be bred to produce the F<sub>2</sub> generation. Remember that all 32 fish will be used to pass on their genes to the next generation. This may seem difficult to accomplish but with a little planning it can be done within the framework of a diverse and active life. The founding breeders are now held in reserve in case of serious problems with the offspring (redundancy). Otherwise they can be used to produce fish and eggs for distribution; this latter activity should be kept separate from the core program. By the time you are ready to spawn the F<sub>2</sub> generation, the founding breeders may have died or be past their prime, i.e. *F. gardneri* example.

The next step is to set up four additional 2 ½ -gallon (9.5 liter) tanks to house the F<sub>1</sub> breeders. A suggested pattern would be as follows: A randomly chosen pair from the A and B lines (e.g. a male from tank A and a female from tank B or vice versa.) are set up in the new breeding tank which becomes the new tank labeled A. A pair from the B and A lines is set up in a tank labeled B. A pair from the C and D lines is set up in a tank labeled C and a pair from the D and C lines is set up in tank labeled D. It is important to keep track of which two lines are being bred in tanks A, B etc. These same patterns are repeated until each of the 32 core individuals has been used for breeding. Since breeding lines A X B and B X A (the same is true for C X D and D X C) use the same two sources to produce the new breeding lines, be sure to breed only four fish from each established line. Four fish will be used from line A and four from line B to produce the new line A. The same is true when producing the new line B. Four fish from line C and four from line D will produce the new line C. The same is true for the new line D (See Figure 2).

## Captive Breeding Guidelines



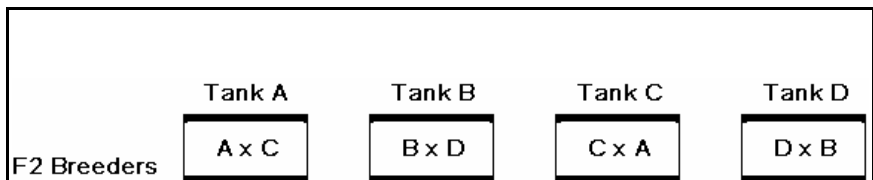
**Figure 2** – 2 ½ Gallon (9.5 Liter) Breeding Tanks with Breeding Combinations for F<sub>1</sub> Breeders.

It is not necessary to breed fish in all possible combinations. It can be done, but is more than most aquarists can handle and is not required for the 40 year model being presented. It is important to emphasize that males and females are to be chosen randomly for this second round and all subsequent rounds of core breeding. Once a given pair has produced four or five viable eggs, a second pair, using the same random procedure can be placed in the spawning tank. Keep this up until all fish in the 32 core program have spawned. This is where the final four tanks are used. After a given pair has spawned, say from strains A and B, it can be placed in the appropriate 10-gallon (38 liter) tank. A separate tank for each breeding line is needed to ensure the spawned fish's usefulness in the event of unforeseen problems. The unspawned fish will still be in the original 10-gallon (38 liter) tanks. This way you can keep track of which fish have spawned and which have not (See Figure 1).

It is now time to remove any remaining fish in the 20-gallon (75 liter) tanks to make room for the F<sub>2</sub> generation. It is important to clean the tanks thoroughly to ensure that no eggs or disease are present. After accurately labeling and preparing each tank, the offspring can be introduced and raised to maturity. Problems, of course, may arise relative to the number of fry that survive to adulthood, sex ratio etc. Much of your success will be due to consistent monitoring of water quality and general good maintenance practices. A dose of good luck wouldn't hurt either.

Producing the F<sub>3</sub> generation comes about by crossing A & B lines with C & D lines. The recommended pattern can be seen in Figure 3. After that you can repeat the same sequence used for the F<sub>1</sub> and F<sub>2</sub> generations with future generations. Following this pattern will ensure that some of the genetic information from all eight founders will be represented in each of the four breeding lines.

Many aquarists will not have the tank space to breed fish in the manner suggested here. In such cases it is desirable to cooperate with another aquarist to accomplish the task. Figure 4 gives an example of how this might be accomplished. It is advisable to make this arrangement between aquarists who communicate regularly to ensure that each participant is operating with the same assumptions. This will increase the chances that both aquarists will have fish ready when it is time to exchange specimens for the next breeding sequence.



**Figure 3** – 2 ½ Gallon (9.5 Liter) Breeding Tanks with Breeding Combinations for F<sub>2</sub> Breeders

## Captive Breeding Guidelines

The preceding example is given to illustrate in a concrete way the principles involved when breeding fish to maintain genetic diversity. It is important to emphasize that many fish will have to be housed and bred in a very different manner. Cichlids, for example, do not spawn in synthetic mops, but lay their eggs in clusters on rocks, flowerpots and other solid objects; others are mouthbrooders. In these cases the fry will be chosen randomly, rather than a few eggs incubated and hatched separately for introduction into the next generations' core population. The *F. gardneri* example is less complicated because the sexes are easily distinguished. For many cichlids this is not the case and fish will have to pair off before choices can be made. Obviously more work and patience will be required and the tank set up will be considerably different from the example given here. Needless to say, tetras, catfish

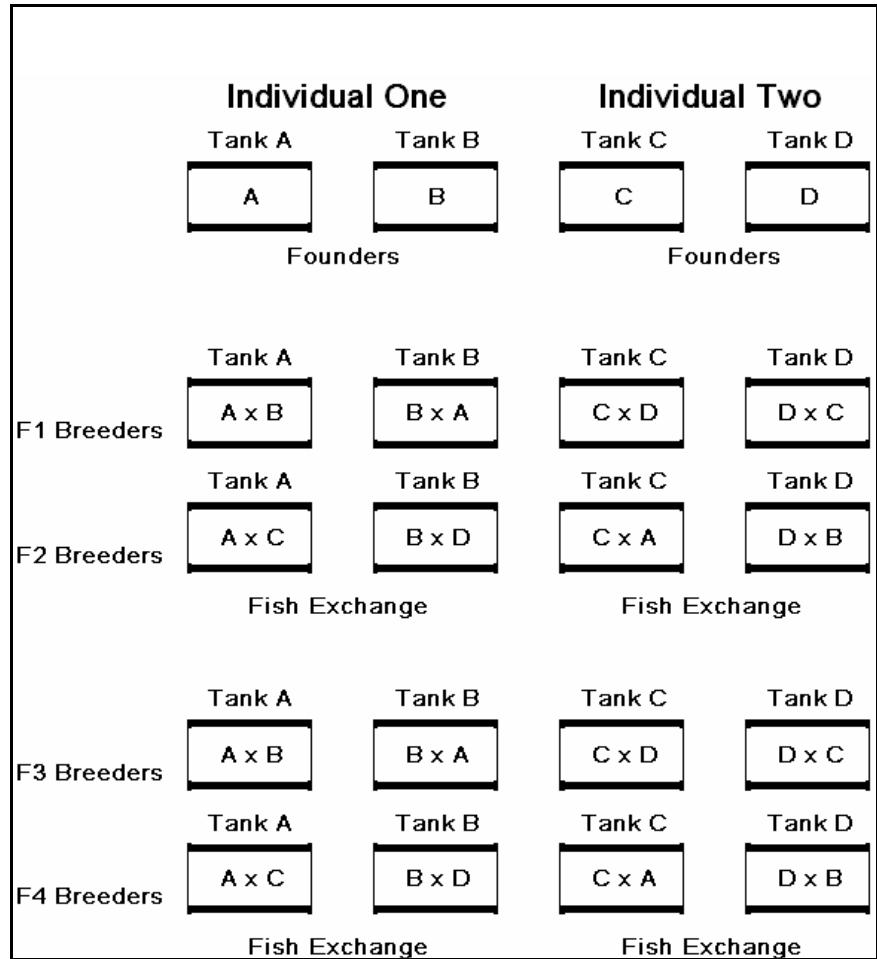


Figure 4 - Plan for Cooperating Individuals

and other groups of fish will require further refinements. It will be up to the **conservation aquarist** to make the innovations necessary to achieve the best results. When a successful approach is developed, it is hoped that those aquarists will write of their experiences and pass them on to others.

The importance of keeping accurate records should be clear at this point. A studbook form can be used to jot down the origins of each founding fish and keep aquarists on track regarding breeding procedures. (See Figure 5.) Then, if they want to know if someone's fish are closely related to theirs, they might have the answer. If aquarists are fortunate enough to find people who will cooperate with them by exchanging fish and information, the task will be more pleasant. Also it is critical to be able to prove that your fish are captive bred in the event they are subsequently listed as threatened or endangered. A strong record will help avoid possible legal difficulties. Along with this, the demonstration that aquarists are dedicated to the task of conserving nature, will likely open the door for more participation by amateurs in the future.

In the meantime, many fish that are currently available to aquarists will become extinct in nature. The decision whether or not to take responsibility for their survival will be up to them. To quote Edward O. Wilson, "...every scrap of biological diversity is priceless, to be learned and cherished, and never to be surrendered without a struggle."

## Captive Breeding Guidelines

### Studbook Form

Name of species: \_\_\_\_\_ Date \_\_\_\_\_

Original description reference: \_\_\_\_\_

Location information: \_\_\_\_\_

Date obtained and from whom: \_\_\_\_\_

Sex: \_\_\_\_\_ Age and condition: \_\_\_\_\_

Pair: \_\_\_\_\_ (Check if it is a pair from the same generation)

Generation from the wild: \_\_\_\_\_

Generation from the founders: \_\_\_\_\_

Original collector, date, and owner for each generation, if known:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Is this a founder? \_\_\_\_\_

Breeding line: \_\_\_\_\_

New introduction to established breeding program: \_\_\_\_\_

Notes: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Figure 5 - Studbook Form

# Captive Breeding Guidelines

## SECTION 3

### ENVIRONMENTAL CONTROL

by Mark Rosenqvist

*The environmental control section will try to cover most aspects of a fish's surroundings that might affect its health and ability to reproduce. These things can be physical, such as water chemistry or temperature, or they can be things which could induce physiological stress, such as size and shape of the tank, type and placement of furnishings in our tank, or how different species are mixed.*

*Keep in mind that there is a direct connection between a fish's environment and its health. Many health problems in fish are caused specifically by less than satisfactory environmental conditions. Even most pathogenic organisms such as bacteria and parasites can be controlled without chemicals or high tech filtration if environmental parameters are properly maintained.*

*There is also a less obvious connection between a fish's environment and its ability to take in and utilize nutrients. Temperature not only affects a fish's ability to feed but it also affects the rate of digestion. Even if all the factors in the fish's physical environment are good, psychological stresses, such as overcrowding or bullying, can cause it to stop feeding.*

*A successful captive breeding program will be greatly enhanced if high standards of husbandry are used. The ACN's commitment to conservation can only be fulfilled for the long term if each species is supplied the very best environment in which to live and breed. The purpose of this section is to remind and instruct conservation aquarists of some of the important factors that make this possible.*

*The section discusses only general principles. The aquarist will have to consult appropriate textbooks for specific information. In time, it is anticipated that customized husbandry protocols relating to particular ACN breeding programs will be developed and possibly take the form of ACN Taxon (or Species) Management Accounts. If an aquarist is breaking new ground, there is going to be some risk involved and the aquarist may be encouraged to cautiously experiment and investigate.*



When most people think of a fish's environment, they usually think of the physical aspects such as heat and water quality. These are the factors that most directly affect the functioning of a fish's mechanical and chemical systems. Because we are dealing with captive maintenance projects that will presumably last over a long period of time and for many generations of fish, it must be remembered that the genetic health of the fish populations must be protected by insuring the physical health of the individual fish.

#### **Temperature**

Temperature has many specific effects on fish. Metabolic activity, oxygen availability, and disease resistance are all dependent on temperature. Because fish are cold-blooded, or poikilothermic, their metabolism is directly linked to temperature. Digestion, excretion, respiration, and other chemical processes are slowest at colder temperatures and speed up as external temperature increases.

Fish tend to acclimate to temperature decreases better than the reverse. This is for two reasons. As temperatures increase, so does a fish's respiration. This is due to the increased need for oxygen as metabolism speeds up. This forced metabolic increase could be stressful. Unfortunately, as the temperature increases, dissolved oxygen in the water decreases. These two conditions combine to create serious problems for fish

## **Captive Breeding Guidelines**

trying to acclimate to warmer temperatures. The reverse is true of temperature decreases. The fish's metabolism slows down while even more dissolved oxygen becomes available. As long as the temperature stays above any particular species minimum requirement, a fish can handle relatively large temperature decreases. It has been said by many writers that when acclimating fish, a 2°F (approx. 1°C) change per day is safe. If you do not know the constitution of a particular species you are working with, this is probably a good idea. However, if you are familiar with the fish being acclimated, you can be a little less strict with your time table. Since unplanned temperature changes of any kind aren't good, it is important to provide the best system of temperature control possible. This is relatively easy to accomplish with modern aquarium heaters.

As a rule of thumb, 5 watts of power per gallon (approx. three liters) of water will comfortably raise the temperature in an aquarium 10°F (5.5°C) above the room temperature. If you need to raise the water temperature more than this, more power may be needed to avoid overworking the heater.

A good idea in any situation, but especially in larger aquariums, is the use of two smaller heaters instead of one larger one. If one of the heaters fails, the other will still maintain an acceptable minimum temperature. If one fails to turn off, it will not be strong enough to cook your fish. Two heaters will also give you better distribution of heat in your aquarium. Finally, with two heaters working together, each works less and lasts longer.

### **Light**

Light is a physical factor in the fish's environment that is sometimes misunderstood as to its importance. In the wild, light provides the energy needed for natural ecosystems to function. Algae and other plants are grown, providing the initial nutrients for most aquatic food chains. Heat is also provided, enabling cold blooded organisms to function. For fish and some invertebrates, sunlight provides some of the seasonal cues needed to stimulate spawning behavior.

Light of the proper intensity, spectrum, and duration is easily provided once the fish's needs are known. Incandescent bulbs are inexpensive and easy to get. They are, however, wasteful of power. Much of this wasted power is given off as heat. This can be a major problem, especially if you need to provide a lot of light and you are working with fish that are sensitive to elevated water temperatures. Incandescent lights also tend to be very restricted so far as light spectrum is concerned.

Metal halide bulbs are much more efficient than incandescent lights. They put out a much greater intensity and possess a wider spectrum of light. There are two major drawbacks to metal halide bulbs. First is the amount of heat they produce which is at least as great as incandescent, and it usually needs to be eliminated somehow. Second is their price. Although an equivalent bank of fluorescent lights would cost almost as much as any particular metal halide, the flexibility gained by using fluorescent lights is much greater. Metal halide bulbs are good for specific applications such as concentrating light in a reef tank.

Fluorescent bulbs combine energy efficiency, wide spectral range, and ease of installation and maintenance, which make them the best choice of lighting for most applications.

Although fairly high power, high intensity fluorescent lights are available, a better choice might be the use of multiple lower power tubes. There are several advantages to this. If one bulb goes, your total light intensity won't be affected too much. Multiple bulbs make it easier to simulate seasonal and even daily changes of light intensity. By careful distribution of the bulbs, changes of light direction can be used to simulate morning and evening light conditions.

## **Captive Breeding Guidelines**

### **Water Quality**

Water quality is one of the most important things to consider when caring for fish. The chemical makeup of water will affect the transfer of gases, nutrients, and wastes into and out of the fish's body. Knowledge of a fish's natural environment will give you important clues as to water hardness, pH, water flow and as well as other characteristics which may be critical to the well being of a particular species. A few of these factors will be discussed here.

### **Filtration**

There are many types of filtration and each has a specific function in any aquarium system. With any filtration, there are a few basic ideas that should be considered.

Keep it simple. The more involved and automated your system, the greater that chance of drastic consequences if something should go wrong. Within reason, the more that you personally have to do with your aquarium, the quicker you will develop the inner sense that will tell you when something is wrong, when water changes are called for, or when to leave things alone.

Just as not using enough of the right filtration can be bad, so can too much filtration. A fish's natural environment is constantly changing in many ways. Controlling the fish's artificial environment too tightly can leave them unprepared for sudden changes due to mistakes or equipment failure. Likewise, sterilizing your systems will leave your fish susceptible to inadvertent disease introductions because they have not been allowed to develop immunities.

Many types of filtration are meant to be separated from the aquarium itself. Biological filtration is the most important type, at least in freshwater aquaria. It is still usually done in the aquarium by an undergravel filter. For various reasons which will be discussed further on, biological filtration should be done outside of the aquarium if at all possible. Besides trying to make this form of filtration more efficient, doing all your filtration outside the aquarium will eliminate almost all direct interference with the daily lives of your fish.

### **Biological Filtration**

Biological filtration is often the main type of filtration in a system. The nitrifying bacteria living on the filter substrate take care of ammonia excreted by the fish, changing it first to nitrite, than to nitrate. Additionally, the filter bed is home to many other types of microscopic, and larger, organisms that break down organic particles such as uneaten food and feces.

If you are using an undergravel filter, use airlifts to move the water. If you must use powerheads, be sure to use the type that allows the outflow water to be aerated. This is important because the water coming from under the filter plate has been stripped of much of its oxygen by the activity of the filter organisms. Biological filter media must be cleaned occasionally, making systems which operate outside the tank advantageous. A major disadvantage of having your biological filtration in the tank itself is the disturbance caused during maintenance. Locating biological filtration outside of the tank eliminates this problem.

The wet/dry filter is a form of biological filtration that has been used by marine aquarists for several years as a way to increase efficiency and decrease disturbance in the tank. Wet/dry filters use many types of filter material including woven fiber mats, shaved PVC strips, and various types of molded plastic shaped. These things are all meant to provide plenty of surface area for the attachment of bacteria.

## **Captive Breeding Guidelines**

Because any wet/dry filter needs a sump in which to collect filtered water for return to the aquarium, they lend themselves to the simple addition of other types of filtration, such as carbon and water softeners. Heaters can also be placed in the sump which will keep them out of the aquarium where they might be damaged by larger fish, or cause damage to fish if they break.

Biological filters work by passing oxygenated water over bacteria covered filter substrate. If a lot of particulate material is drawn through its substrate, it can build up enough to impede water flow and inhibit the biological function of the filter. If at all possible, water passing through a biological filter should be prefiltered for particulate. Prefiltering will greatly reduce maintenance, and will help avoid the temporary chemistry imbalances brought on by damage caused during routine cleaning of the filter substrate. Wet/dry filters typically use some type of prefilter. Undergravel filters should preferably be reverse flow, with water being pre-filtered before going under the gravel.

### **Mechanical Filtration**

Mechanical filtration includes many different methods, all aimed at removing solids from water. These solids range from plant debris to microbes. Removing solids is easy in theory, but can require some finesse to get the most out of a particular system.

Regardless of what you are trying to filter out, efficient filtration depends upon the system design. The most common type of filter on the market is the magnetic drive overflow filter. These work by drawing water into the open filter box and allowing it to flow through the filter material and back into the tank. The advantage to these filters lies in their low price and ease of maintenance. The main disadvantage is the limited amount of filtration that they can do. Because the water passively flows through the filter material, only particles down to around 20 microns can be effectively filtered. Because water is not being forced through the filter material, it will take the easiest route, and not go through at all if heavily loaded with particulates.

Another type of filter that hangs on the back of the aquarium is the siphon type. In this design, water is siphoned into an open filter box, drawn down through filter materials, and then returned to the aquarium by a direct drive pump. This type of filter is less limited because water is actually pulled through the filter. This means that more of a detritus coating can build up on filter material before water flow stops. This allows smaller particles (5-10 microns) to be pulled out of the water. It also means that less changing of filter material is required. The main disadvantage of this type of filter is that its operation depends on the proper functioning of the inflow siphon. A drop in water level, siphon tube blockage, or air bubbles in the siphon tube, can all cause the siphon to stop, and with it, the filter. This means a fair amount of attention must be given to the system.

The next, and most efficient type of mechanical filtration system, is the pressurized filter. These filters come in many configurations, but the basic design uses a sealed container filled with filter material. The water is forced through the filter material by a pump on either the intake or outflow of the filter container. Because the container is sealed, water must go through the filter material. Much finer filtering can be achieved using this type of filter. Depending on the specific design and the type of filter material used, filtration down to 0.5 microns can easily be achieved. Pressurized filters tend to be more complex, and they are usually constructed with closer tolerances than other types. This means that maintenance can be somewhat more involved. Although these filters will run longer than most other filters before the flow stops, it is important to watch filter outflow. When clogged, water flow drops off rapidly.



## **Captive Breeding Guidelines**

### **Filter Media**

There are many types of filter media. Most are meant to be used for very specific purposes. Depending on what media is used, mechanical filters will remove particles from 50 microns down to 0.5 microns.

Woven or spun synthetic fibers are most commonly used for coarse filtration. This type of filter media is used first in-line in most filters to remove large particulates down to 20 to 50 microns. Using a coarse media first will keep your finer filter materials from clogging up as fast, allowing more time between maintenance. More tightly woven fibers, as well as foam and molded or pressed mineral materials will remove particulates at sizes between 5 and 20 microns. For most purposes, this is the finest filtration needed for a continuously running system. Except for bacteria, viruses, and the smallest protists, all particulates will be removed.

To really polish the water, and in the event of bacterial or protozoan outbreaks, various crushed and processed mineral materials (such as diatomaceous earth) can be used. These materials, and their associated filter equipment, are designed to remove particulates down to 0.5 microns. This will remove everything except viruses and the smallest bacteria. Because this type of filtration is so efficient, it becomes clogged quickly, and is not recommended for continuous use. It should not be used for more than a few hours at a time.

### **Water Changes**

It has often been said by advanced aquarists that the single most important thing you can do to keep your fish healthy is to make regular water changes. Clearly, fish need many human interventions to keep them healthy and stimulate them to breed, but water changes must rank near the top of any maintenance list. Changing 20% to 40% of the water volume once every week or two is appropriate in most circumstances. It is important that the water used has the same characteristics as found in the tank regarding temperature, pH, etc., before being introduced.

### **Psychological Environment**

A fish's physical environment can be absolutely perfect and it still won't breed. Besides water quality, temperature and light, there are psychological needs that must be met before fish will reproduce. Although some things are less obvious as problems, a few things stand out. Overcrowding is definitely a problem. If fish are crowded together they usually have trouble establishing natural behavior patterns.

In the wild, many species of fish coexist. This is because they have relatively unlimited space and can, for the most part, stay out of each others' way. In the aquarium, forcing different species to coexist in close quarters with each other may make conflicting behavior patterns worse and inhibit or stop breeding behavior.

Disturbances outside the tank are just as important as the ones inside. Putting the aquarium in a high traffic area where, for example, doors are being opened and shut all day, will keep the fish in a constant low level state of stress. This may easily prevent them from breeding.

Besides the above mentioned, there are other factors that may or may not apply to specific species but that might in general, be important to consider. The size and shape of the aquarium may be very important. Obviously, fish that are one foot (30 cm) long are not likely to spawn in a ten gallon (38 liter) tank (even if they could live there) but the opposite might also be true. Small schooling fish might need lots of room to move around to allow for the proper group behavior. Shape of the aquarium is just as important. That same school of fish might only need fifty gallons (190 liters) of water to breed in but if the tank is tall and skinny, they may not be able to move back and forth enough. What might be better is a shallow tank that has a large

## **Captive Breeding Guidelines**

surface area. When deciding on what size and shape of aquarium to use, don't restrict yourself to conventional aquariums. Wading pools, horse troughs and plastic dish tubs might all be better than a conventional aquarium depending on the species of fish being considered.

A very important issue for conservation breeders is how closely they should try to duplicate a fish's natural environment. Research is necessary to determine those factors which may influence the genetic makeup of a fish population. If there is any reasonable doubt as to the importance of a specific factor, be it food type, water chemistry, yearly light cycle etc., it must be reproduced, if possible. Breeders must remain flexible in order to allow changes in their setups as new information becomes available.

The proper balance between the use of barriers and open space is very important. Often, schooling fish just need enough room to swim naturally in a group. Open space is often needed by some territorial species because they need to have a clear field of view. Other territorial species need closely spaced barriers to give them an easily defendable territory of manageable size. Although some schooling fish need open space, they also need to have something to hide behind when startled or stressed. Barriers can be almost anything: PVC pipe caves, rocks, driftwood, plants and plastic tank dividers all have their specific uses. Bottom substrate may or may not be important to use. If possible, do your biological filtering outside the aquarium. This allows you to decide on the use of substrate solely by whether or not the fish really need it. Many open water schooling fish rarely get near the bottom while other fish such as fire eels and loaches are frequently buried under whatever they can find. Some fish are spooked by clear bottom glass. This can be remodeled by painting the outside bottom of the tank or even by allowing a layer of algae to grow.

If you do use a substrate, what type will depend on whether or not you are using an undergravel filter and, beyond that, what fish you are working with and what habitat you are reproducing. Gravel, sand, dead leaves, mud and almost anything else could be used as substrate. If you use an undergravel filter, gravel in the 3-5 mm size range is best since it allows the maximum amount of nitrifying bacterial growth without the danger of packing causing channeling through the filter bed.

Without an undergravel filter, the choice will come down to what is best for the specific fish being bred. An important thing to remember is that the higher the organic content of the substrate, the harder the filtration system will have to work to maintain stable water quality. Whenever possible, use non-organic substrate materials. Also remember that the smaller and finer the material is, the more likely that anaerobic conditions will occur causing the release of various toxic compounds.

## **Nutrition**

Proper nutrition, another very important area of concern, is essential to the success of any long term captive breeding program. Giving your fish the proper types and quantities of food will ensure that they are in the best physical condition for breeding. Not only will the fish be in the proper condition, but proper nutrition will also ensure that your adults will produce healthy eggs and young.

Frequently, feeding behavior, and structures associated with feeding, are used to help determine the taxonomy of a particular species. This makes it extremely important to know the feeding habits of your fish. Over many generations, radical changes in diet could reduce a captive-bred population's ability to survive in a restored natural environment, if that is a goal of the program. Because of this, it is important to feed a diet that is identical or similar to what the fish gets in the wild. Fresh foods such as live food organisms or fresh plant material, presented in a way that is consistent with their natural habitat, should always be the first diet until it is certain that specific dietary factors are not an evolutionary influence.

There can be no hard and fast rules about the quantity of food to give your fish. It will depend on many things

## Captive Breeding Guidelines

including species, age, current physical condition and type of food. This is also true of how often you should feed them. There is a wide variety of foods available to the fish breeder. With many species, the best way to ensure the best nutrition possible is to feed a large variety of foods. Although some fish will only accept a narrow range of foods, most will take all kinds of things if they are introduced to them slowly and allowed to get used to them.

**Dry prepared foods** are an excellent place to start if the fish will eat them. The larger companies have done a great deal of research to be certain that the basic nutritional content of their foods is at the proper levels. They have also tried as much as possible to get their foods to your fish while they are still fresh. The variety of dry foods is incredible. Flakes, pellets, sticks and granules are all available. The food companies have gone to great lengths to put their foods into formats that will be acceptable to the greatest variety of fish. Assuming your fish will eat dry food, you might need to choose the food with the shape, size, density and nutrient make-up that most closely resembles what your fish eat in the wild. Obviously, dry food doesn't physically resemble much of anything that fish naturally eat but it would be a mistake to feed vegetable based flake foods to carnivorous bottom feeders such as catfish. Knowledge of your fishes food preferences in nature will aid you in making the right choices.

**Frozen foods** are a more natural source of nutrients than flake food. A wide variety of food organisms are available in this form. A problem with frozen foods is that the freezing process ruptures cell membranes and body coverings; this allows vital nutrients from the body fluids to leak out into the surrounding water and lower the nutritional value of the food. Because of this fluid leakage, frozen foods must be rinsed before feeding or they might quickly foul the aquarium water.

**Preserved foods** have the advantage that the various food organisms are kept whole and storage is very easy. Unfortunately, selection is still somewhat limited, and although rinsing removes most preservatives, some may remain and be ingested by the fish. The long term effects of these preservatives on fish are not well known.

**Freeze dried foods** are processed in a way that removes only their water content. All nutrients are left in place. Like frozen foods, a variety of freeze dried foods are available. A concern with these foods is that they break up very easily, leaving dust and pieces too small to use at the bottom of the container.

If at all possible, some type of fresh food should always be given to any fish you are trying to breed. **Live foods** are probably the best way to give your fish a balanced diet. Live foods contain nutritional components that might not be possible to duplicate in a dry diet. Even though various prepared foods start out live, nutrients are usually lost during processing. Feeding live foods leaves their nutritional value intact. Fresh foods can also encourage natural feeding behaviors, a potential factor in stimulating successful spawning behavior. It is often best to culture live food organisms yourself. If this is not possible, try to stick with cultured foods purchased from a reputable source. This will eliminate, as much as possible, the possibility of introducing disease organisms through the fish's food. There are many types of live food on the market today. Many are available at the local pet stores. Artemia, various worms and insect larvae as well as feeder fish can usually be found in your area. Many other types of food organisms can be obtained through mail order ads in aquarium magazines. Most organisms purchased through the mail come as starter cultures. Rotifers, wingless fruit flies, microworms and vinegar eels can all be obtained in this way.

The importance of maintaining the proper environmental conditions for your fishes cannot be emphasized enough. Given all of the other problems connected with long-term conservation efforts, providing adequate conditions for most fishes is relatively easy.

## Captive Breeding Guidelines

### SECTION 4

#### FISH HEALTH AND HYGIENE

by Peter Burgess

*The maintenance of fish in good health is obviously fundamental to a successful breeding program. Broodstock which are in top condition will be more likely to breed and produce larger numbers of healthy offspring. Unfortunately, fish, just as with humans, are prone to a vast range of health problems, such as tumors, organ failure, genetic defects, dietary disorders, and infections due to viruses, bacteria, fungi, protozoa, and worms.*

*The control of infectious diseases is especially important in breeding programs as stock will continually be transferred between aquarists. Also, wild-caught founder stock may have poor levels of resistance to certain diseases, and may themselves be carrying infections, some possibly of unknown pathogenicity.*

*By adhering to the following guidelines, the aquarist should be able to avoid many of the pitfalls which can lead to disease outbreaks.*



#### Quarantine Regimes

Quarantine is extremely important when acquiring stocks of fish, regardless of their source. The purpose of quarantine is to monitor the fish in isolation for any signs of disease which can be treated before the fish are allowed to come into contact with the existing stock. Even if you acquire seemingly healthy fish which have been kept under ideal aquarium conditions, you should still quarantine them. The stress of transporting them and introducing them to unfamiliar surroundings may cause the fish to develop infections. Quarantine means keeping new fish completely separate from your existing stock. The quarantine tank should therefore have its own "dedicated" set of immersible equipment (heater, filter, nets, etc.) and decor (plants, substrate, rocks, etc.), these items never being used in the stock tanks. It is very important to ensure that water from the quarantine tank cannot accidentally enter the stock systems, so be careful when siphoning water and when netting quarantined fish as their splashing inevitably leads to air-borne water droplets. It is worth remembering that a single drop of water can harbor millions of deadly pathogens. For this reason it is recommended that the quarantine tank(s) be kept physically apart from the stock tanks, ideally in a separate room.

The time of the quarantine period is important. For wild caught founder stock (which are more likely to be harboring a variety of infections), six weeks quarantine is the recommended minimum. For captive bred stock, four weeks should suffice. Avoid acquiring fish which have had a recent history of infection - try to wait a few weeks, if possible. It must be remembered that quarantine can only reduce the chances of spreading disease, it is not a foolproof method of disease prevention. For example, some chronic infections and internal parasites may not become apparent during the quarantine period. A fish which has been quarantined cannot, therefore, be considered to be completely disease-free.

It is likely that some aquarists will maintain their breeder stocks in close proximity to their pet fish, such as in fish houses. In these situations, the aquarist should have set(s) of immersible equipment (heaters, thermometers, nets, etc.) which are used solely for the breeders tanks in order to avoid cross-infections.

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### Health Monitoring

You should regularly check all of your fish for signs of ill health. In particular, keep a close watch on fish which have recently spawned or are guarding fry or have been bullied by their mating partners or tank-mates; these individuals may be highly stressed and consequently be more susceptible to infection. Fry may also be at risk until their immune systems have fully developed.

The best time to check your fish is when you feed them. Many species become very active as soon as food is dropped into the tank. An unusually sluggish response to food may be the first warning signs of a health problem.

Signs of ill-health in fishes include:

- unusual swimming behavior (not to be confused with spawning behavior);
- increased or labored respiration (= fast gill movements);
- repeated rubbing or "flashing" against hard surfaces;
- protruding scales (often more noticeable when viewed from above);
- clamped fins;
- bulging eyes;
- cloudy eyes;
- thin, emaciated appearance;\*\*\*\*
- erosion at the fin extremities;
- long, stringy faeces;
- blotches, spots, lumps, ulcers, or cotton-like growths on the body or fins.

Any of these symptoms should give cause for concern and prompt immediate investigation.

### Record Keeping

Try to get into the habit of maintaining records of fish health problems. Outbreaks of disease in breeder stocks should be documented with respect to symptoms, mortality levels, diagnosis (if known) and effective treatment(s). Fish species vary in their susceptibility to certain diseases, conversely fish pathogens exhibit varying degrees of host specificity. Disease information can prove very useful for the long-term management of the fish species in question.

### Disease Outbreaks

Sooner or later, you are bound to experience a health problem with your fishes - it happens to the most experienced aquarists. The important thing is to be able to quickly identify the problem and initiate a suitable remedy. A detailed account of the various diseases which can affect fish is beyond the scope of this document, however the following guidelines may prove useful.

We must consider that many species of fish do not live very long, some tetras and killifishes being "annual" in the wild, dying within 12 months. Aquarists who are maintaining large breeding programs involving hundreds of fish will likely experience mortalities on a frequent, perhaps daily, basis. This is simply because a proportion of the stock have reached old age. It is when a notable increase in mortalities occurs or includes young fish or associated clinical symptoms that the aquarist should be concerned.

In the case of a mysterious outbreak of fish mortalities or symptoms of ill-health always first consider whether these may reflect a water quality problem. Check the water temperature, pH, nitrite, and ammonia

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levels - are they all within acceptable limits? Is the aerator and/or filter working correctly? Is there an excessive build up of organic matter in the tank which could cause oxygen depletion? Is there a possibility of metal poisoning (e.g. from iron or copper) or contamination with detergents, soaps or perfumes (introduced via the hands)? Have you been using paints, glues, solvents, or insecticides in the vicinity of your fish? - these substances are potentially harmful to aquatic life. Is it possible that any dead or damaged fish may be the result of bullying or attempted predation by their tank mates?

If the water quality has deteriorated badly, it is usually necessary to move all the fish (not just the sick ones) to another tank containing water at optimal conditions. Increase the aeration as many adverse water conditions (e.g. ammonia poisoning) can cause gill damage thereby reducing the fish's ability to respire efficiently.

If you still have not discovered the cause, you may have an infectious disease problem on your hands. In any case, bear in mind that poor water quality can weaken the fish and make them prone to infections.

If you suspect an infectious disease, it is important to diagnose the causative agent in order to select an effective cure. For the commoner types of disease, such as whitespot, you may be able to make your own diagnosis, perhaps with the aid of a fish health book. You should avoid the temptation to add a "cure-all" remedy or antibiotics if you are not sure of the cause - many chemical treatments are themselves stressful to fish and may only make matters worse. If in any doubt, seek expert help as soon as is possible. It is important not to delay as some disease pathogens can cause high fish mortalities within as little as one or two days.

### **Nutritional Disease Problems**

It is worth bearing in mind that poor nutrition can give rise to disease. The correct storage of dried foods, such as pellets and flakes, is very important. Dried foods which have been held for extended periods under very warm or damp conditions may have lost some of their vitamin activity. This can result in vitamin deficiency diseases which often manifest as spine deformities, cloudy eyes, and skin hemorrhaging. Food which has become damp should be discarded as it may be moldy (i.e. contain fungal growth) and possibly be contaminated with fungal toxins (= mycotoxins). Food containing mycotoxins can be lethal to fish. In order to avoid these storage problems it is best to buy small quantities of food which will last you no more than 6 months, rather than purchasing vast amounts which may take years to use up. Don't store the food container on the top of a tank next to the lights, as it will get hot and deteriorate.

### **Live Foods as Agents of Disease**

The feeding of live foods which have been collected from the wild presents a serious risk of introducing disease. For example, daphnia, copepods, and tubifex worms can harbor the intermediate stages of helminth (= "worm") and protozoal parasites and the water in which they are collected can contain pathogenic bacteria and viruses. The fish louse *Argulus* and fish leech may also be accidentally introduced into the aquarium along with live foods. It is therefore wise to feed only those live foods which are cultivated under artificial conditions (refer to the Nutrition section). Tubifex, bloodworm, daphnia, and many other natural foods are commercially available in pre-sterilized, frozen packs and these are perfectly safe to feed.

### **Genetic Causes of Disease**

This is highly relevant to captive breeding programs where there may be an increased possibility of inbreeding effects or other genetic causes of disease. The following clinical symptoms can arise through gene mutations:

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- Body deformities such as shortened body. Observed in guppies.
- Skeletal deformities. Bent or twisted backbone. Note that this symptom can also arise from vitamin deficiency.
- "Saddleback". This can be caused by a dominant gene which is lethal in the homozygous condition. Fish exhibiting saddleback should be destroyed in order to help eliminate the gene from the stock.
- Neoplasia (e.g. melanomas - black growths on the body, and melanosarcomas). These may reflect a genetic disorder. Reported in *Xiphophorous* hybrids.
- Stress. The aquarist should be aware that many genes influence the fish's tolerance to environment conditions such as water temperature. An increasing intolerance by successive generations to minor fluctuations in environmental conditions (such as during water changes or following movement to other tanks) should be noted.

Any of the above symptoms should be reported to the ACN as they may reflect a genetic disorder which could have serious implications for the breeding program.

## Vertical Disease Transmission

Certain infectious diseases can be transferred from parents to progeny (= vertical transmission) which could seriously affect a breeding program. Vertical transmission has been recorded for a few viral and bacterial infections, including fish tuberculosis (= fish TB). Outbreaks of fish TB should therefore reported to the ACN.

## Useful Items of Fish Health Equipment

- quarantine tank(s) and "dedicated" equipment;
- basic remedies for curing protozoal, bacterial and fungal infections;
- sterilizing solution for disinfecting nets (such as bleaches or iodophores, available from your aquarium supplier);
- pipettes (for adding treatments to water);
- a good quality magnifying lens;
- reading lamp or similar light-source to aid close-up observations of sick fish and for fish dissections;
- dissection board (plastic tray will suffice), scissors, scalpel and forceps;
- 70% or stronger alcohol or methylated spirits to clean dissecting instruments and swab down dissecting trays;
- fish health textbook;
- list of names and addresses of specialist veterinary surgeons or fish health experts, and fish pathology laboratories.

The purchase of a compound microscope will greatly assist disease diagnosis. It will also be useful for checking egg development. (It will additionally prove an excellent educational instrument for the whole family).

## Submission of Specimens for Disease Diagnosis

If you are unable to diagnose the cause of disease then you will need to consult a veterinary surgeon or fish health scientist. If you consult a vet, make sure that s/he has specialist experience and training in fish health (the general veterinary training is usually poor with regards to fish health). Your national veterinary association should be able to give you the names and addresses of specialized vets practicing in your area. In exceptional cases, you may need the facilities of a fish health laboratory which can undertake bacteriology and histology. Again, your veterinary association or local vet should be able to provide a list of suitable

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establishments.

In most cases, it is far easier to diagnose an infectious disease on a live fish. The live fish may exhibit tell-tale clinical symptoms which will not be apparent from a dead specimen. Also, remember that once a fish has died, certain skin and external parasites (e.g. the whitespot parasite, *Ichthyophthirius*) will tend to leave their host and therefore may not be detected at autopsy. If you have several fish in various stages of infection, bring along specimens of each as this may assist diagnosis. Your vet may also request that you bring a sample of aquarium water. Also, pass on any information which you think may be relevant.

It may become necessary to submit the fish to a specialized laboratory for bacteriological testing. This should enable the identification of the bacterium causing the disease as well as assessing which antibiotics will be effective in eradicating it. Unfortunately, it is not recommended to take bacteriological samples from dead fish due to the rapid post-mortem invasion of the tissues by a whole variety of bacteria which will make diagnosis very difficult. Bacteriology is equally impossible with preserved fish as the fixative will kill the bacteria. Live, clinically infected fish should be taken to the diagnostic laboratory where skin swabs will be taken. Regrettably, it may be necessary to swab internal organs in which case the fish will have to be freshly killed. The sacrifice of a single fish must be considered necessary as this may improve the chances of saving the lives of the other infected fish.

In some cases, a microscopical examination of the fish's tissues and internal organs will need to be undertaken, i.e. histology. Histological investigations will only be meaningful if they are undertaken on tissues which have not decomposed. Fish which have been dead for some hours are not likely to provide good histological samples. Fish and tissues for histology should be fixed as soon as possible after death with the aid of a formalin preservative. (Never put live fish in formalin - it is extremely cruel to kill them this way). Formalin is an aqueous solution of formaldehyde gas. Standard formalin is 40% formaldehyde in water. You will need to dilute the solution to 10% formalin (= 4% formaldehyde) using tap water or preferably distilled water. Be careful, formalin can cause extreme irritation to your eyes and respiratory tract. Make up formalin solutions in a well ventilated room or outside and keep it well away from your aquaria, pets and children! Dead fish can be placed in a jar of formalin, large specimens (over 10 cm length) should have their belly slit from anus to gill cover in order to achieve good fixation of the internal organs. For sending fish through the post, fix them for 1-2 days and then wrap them in cloth (e.g. cheesecloth) which has been soaked in 10% formalin. Place the wrapped fish in at least two layers of plastic bag which should be adequately sealed. Package in a strong container containing relevant information about the fish, such as clinical symptoms, etc. If you are requested to submit specific tissues, cut the relevant tissue into small blocks (no greater than 1 cm cubes) and fix them in 40 times their volume of formalin solution for at least 24 hours prior to submission to the lab. The blocks can then be transferred to a smaller volume of fixative for postage. Remember, formalin fumes are very unpleasant and any leakages during shipment in the post could result in unpleasant and expensive consequences! You should check with your national mail or postal company (or with the fish lab) whether it is acceptable to send formalin-fixed material by mail.

The submission of fish or tissues for bacteriology and histology can prove expensive. It may be wise, therefore, to seek advice as to whether these investigations are really necessary. Finally, never send sick fish through the post - it is cruel.

### **Materials for Fixation and Autopsy:**

- glass containers with firmly fitting lids, large enough to hold preserved fish;
- small glass bottles with screw caps for holding tissue sections. These containers must be able to resist breaking in shipment;
- graduated measuring cylinder for diluting formalin;



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- water-proof gloves (e.g. wash-up gloves) for handling preserved fish and formalin-soaked cloth (never immerse bare hands in formalin);
- formalin fixative (= 10% formalin solution = 4% formaldehyde);
- scissors or scalpel for cutting tissue blocks and for slitting the body cavities of large specimens;
- muslin or cheesecloth for wrapping specimens;
- strong plastic bags and elastic rubber bands (to prevent escape of formalin fumes during shipment);
- strong packaging.

## Humane Killing of Fish

When a fish is very sick, perhaps as a result of ill health or simply through old-age, it is much kinder to destroy it rather than allow it to suffer in agony. There is overwhelming scientific evidence that fish experience pain and stress, something we should all be aware of during our fish keeping practices. The humane way to kill a small fish is to wrap the lower body firmly in a dry cloth and strike the exposed head firmly and swiftly against a hard object (wall or floor) - this should be done with force - it may sound barbaric but it is an effective and rapid method. For large fish, death by anesthetic overdose is recommended (recommended anesthetics are benzocaine and MS222). If you are not familiar with applying fish anesthetics then it is important that you seek help from an aquarist or veterinary surgeon who is experienced in their use. Following either method, death should be confirmed by destroying the brain with the aid of a scalpel or strong scissors.

In the case of a sick fish which is very rare or founder stock, the ACN may wish to be informed of the need to kill the fish and may require the dead specimen for taxonomic or other purposes. Never flush a live or dead fish in the public water system as this can result in transmitting disease to wild fish populations.

**Further Reading:** The aquarist should have access to an illustrated textbook on fish diseases. This will greatly assist in disease identification and selection of suitable treatments. Suggested books include:

- Andrews, Chris; Adrian Exell and Neville Carrington. *Interpet Manual of Fish Health*.
- Post, George. *TFH Textbook of Fish Health*.
- Untergasser, Dieter. 1989. *Handbook of Fish Diseases*. TFH Publications.

# Captive Breeding Guidelines

## SECTION 5

### ACN CAPTIVE BREEDING PROGRAMS

by Rob Huntley

*With so many fish threatened with extinction, it is unrealistic to think that the members of the ACN can preserve the genetic diversity of each and every endangered species. The choice is: "Do we preserve a few species in an optimal fashion?" or "Do we preserve as many species as possible within the limits of available resources?". Certainly, questions of immediacy of need, prospects for reintroduction, availability of founder stock, probability of breeding program success, etc., enter into the equation. In many cases we are faced with inadequate information, and for most of those cases we are left making an intuitive decision. Further, many of these fish are available in the hobby and the opportunity to develop an orderly means of preserving them is staring us in the face, regardless of the species' relative conservation status. The ACN is examining three approaches to captive propagation of candidate species:*

- 1) **Species Survival Approach** - strict genetic tracking and database management (studbooks);
- 2) **Species Registry Approach** - fish registry (database) and mechanisms for periodic and systematic exchanges of stocks but not necessarily with strict genetic tracking of program fish;
- 3) **Exploratory Approach** - establishing husbandry methods and life history research on species which are not necessarily endangered but will make a viable contribution to species survival and science.

*Initially, a few species may be chosen to gain experience using the **Species Survival Approach** in order to establish methodologies and to determine the complexities and costs of such techniques. The Lake Victoria Species Survival Plan (American Zoo and Aquarium Association) is a model which the ACN could choose to adopt. However, in the foreseeable future, most species will be controlled under the **Species Registry Approach** in an effort to secure as many species as possible from complete and irreversible extinction. In addition, a number of species may be considered under the **Exploratory Approach** whereby the vast experience that hobbyists have in fish breeding can serve as an expertise pool to assist academic/scientific institutions.*

*None of these three approaches are necessarily limited to endangered fish species. In fact, in some cases endangered species may be difficult to acquire because of protection laws or access. The ACN can work at establishing protocols for species that are vulnerable due to isolation, limited range, habitat destruction, etc., but which are not yet officially listed as endangered. Candidate species will be selected so that work on that species can be targeted before quantities of founders become limiting.*

*This section outlines ways in which aquarists can become involved in conservation, both directly (maintaining fish) and indirectly (in support of the work of others who are maintaining fish).*



### Participation in ACN Captive Breeding Programs

The ACN directs aquarists into conservation breeding initiatives which are designed under the supervision and guidance of scientific and legal authorities. Aquarists participating in ACN initiatives are required to follow ACN guidelines, and directives from the ACN Species Coordinators for the species with which they are involved.

Screening is important to ensure the appropriate selection of private individuals for participation in a captive breeding project. However the rules are not intended to be so strict so as to severely limit the number of people who qualify. A balance has been sought between a) the technical and theoretical requirements and; b)

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the probability of aquarists being willing to conform. Consideration has also been given to what are perceived to be the minimum requirements for scientific endorsement of the ACN's work.

### **Selection Criteria**

The ACN aims to develop professionally endorsed programs in species maintenance and conservation breeding; foster appropriate participation by private individuals; establish codes of appropriate behavior; and make every effort to ensure that participating members operate in compliance with national and other regulatory processes and legal restrictions.

Four main areas are identified in terms of requirements of program participants:

- abilities and experience;
- seriousness and dedication to the cause;
- willingness to comply with the rules; and
- availability of the minimum equipment and other resources in support of their involvement.

Essentially, the type of participant needed is one who shares the conservation philosophy and subscribes to the ACN Conservation and Captive Breeding Goals listed at the beginning of this document. People with personal or corporate agendas aimed at the exploitation of endangered species need not apply. This is by no means intended to exclude corporate participation. Participants in ACN conservation projects do so simply and clearly because they wish to contribute to saving endangered species from extinction. The ACN is looking for people who have a genuine interest in conserving nature and place this as their highest priority in all captive breeding efforts. Thus, participants devote personal and/or corporate time and resources to the cause with no expectation of compensation for their commitment.

Prospective participants will first be asked to complete a questionnaire which is intended to identify how they are positioned with respect to the four main selection criteria listed above. A list of the questions included in the questionnaire is found in Appendix A.

Secondly, persons who successfully pass through the questionnaire stage will be assigned to a "Tier Level" as outlined below. Except for special circumstances, which will be examined on a case by case basis, all new entrants to the program will be assigned to the lowest level, Tier Level 3.

### **Tier System**

A three tier breeding program is being implemented by the ACN.

Tier 1 includes top level breeders with proven experience and reliability who may be considered for the rarest and/or most difficult to raise fish in the program. Tier 1 breeders follow prescribed guidelines in maintaining a breeding population of ACN program species. Tier 1 breeders can be assigned to work with species designated under any of the three approaches outlined previously (Species Survival, Species Registry, Exploratory).

Tier 2 is the intermediate level where participants are eligible to maintain fishes which are currently listed as threatened either in the IUCN Red Data List of Threatened Animals, national red lists or identified as being of particular concern by the ACN or other organizations. This eligibility naturally depends on the availability of the species to the ACN as well as species specific considerations. Tier 2 breeders are assigned to priority species in the "Species Registry Approach".

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Tier 3 is the entry level for newcomers to the program who are assigned moderately threatened species. In order to gain experience, hobbyists in the tier 3 would be challenged under established criteria to reproduce environmentally threatened fish species that are still relatively common in the hobby trade (thanks to fish farms, etc.) or else species genetically similar or with comparable behavior, maintenance and breeding requirements to species at serious risk. Included in these would be species which, if current trends continue, are expected to be included as a "red listed" species within the next ten years. Stock would often be available through commercial channels.

In due course, newcomers to the program will be given the opportunity to demonstrate their abilities and commitment with not-so-rare species or species related to endangered species and having similar husbandry and/or reproductive requirements. In this way, participants will show that they understand the requirements for maintaining and potentially breeding endangered species before being considered for high risk fishes with limited availability.

In the near future, specific criteria will be developed for determining who qualifies for advancement to the next tier. The criteria could include such things as simply having breeding success, demonstrating organization and recordkeeping abilities, and factors such as success at mimicking nature in the aquarium environment and the advancement of husbandry methods. A committee to evaluate applications for advancement will also be instituted.

### **Basic Rules of Compliance for Participation by Private Individuals in ACN Conservation Breeding Programs** (adapted from Loiselle (1992))

Captive populations are all that stands between some species and total extinction. As a result, captive populations are critical, and their caretakers are participating in a critically important endeavor. This endeavor is so important that uniform rules must govern it. These rules are not designed to be merely annoyances with no purpose, but instead are meant to put the conservation and maintenance of captive populations on a sound scientific and carefully documented footing. These rules will indeed help the private conservation breeders to gain the recognition they need for their efforts, and will be essential in gaining acceptance in the general conservation movement for these critical, if small scale, private efforts. With regards to records management (next section), the stakes are simply too high to not have uniform documentation of breeding programs.

The following outlines the expectations held by the ACN of all program participants:

1. Founder stocks of species represented in captive breeding programs will be provided to private individuals on the basis of long-term breeding loans.
2. As conditions of such loans, individual participants agree to the following conditions:
  - a. to dispose of founder stock animals by sale or gift only with the permission of the ACN and the institution that supplied them;
  - b. to return founder stock animals when requested to do so by the ACN or the institution that supplied them;
  - c. to promptly report the death of any founder stock animals to the ACN and the institution that supplied them;
  - d. to exchange founder stock with other participants in the program only with the permission of the ACN Species Coordinator;

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- e. to make available gratis to the ACN or to the institution that supplied founder stock animals, quantities of progeny fish which conform to the number and genetic variation requirements and any other species specific requirements as set forth by the Species Coordinator. These quantities will be subject to, among other things, the degree of involvement of other participants as well as the conservation status of the species concerned. Participants should look to the Species Coordinator to spell out the specific needs for a given species.
  - f. to offer first refusal of purchase to the ACN, at a previously negotiated, mutually agreeable price, any fish which are deemed by the Species Coordinator to be surplus to program needs. Second refusal of purchase will be offered to the institution that supplied founder stock.
  - g. to report twice yearly to the ACN Species Coordinator on the status of species under the aquarist's management.
3. Where a breeder is accepted into the program and already has stocks of the species in question, or where a breeder wishes to introduce new individuals of the species which have been personally obtained from a source outside the program, the participant agrees to the following conditions:
- a. to introduce new individuals of the species in question to the ACN breeding program only with the permission of the ACN Species Coordinator. Such introductions to the program must not contravene statutory controls concerning the collection, possession or transportation of the species. If the species is collected from the wild, this must be done so only with the permission of wildlife agencies of the country in question and in accordance with generally agreed goals in conservation (either *in situ* or *ex situ*);
  - b. to make an "in kind" donation of these animals to the ACN. When this donation is from persons in countries where the ACN has registered charity status (currently only in Canada but with application presently being made in the United States of America) the ACN will consult with the appropriate agency to determine whether the donor can be provided with and official receipt for income tax purposes. If this is found to be in accordance with taxation requirements, the ACN will subsequently establish appropriate procedures;
  - c. when excessive costs have been incurred to acquire the animals, the ACN "may" elect to reimburse a portion of this cost at a previously negotiated, mutually agreeable amount. As in point "b", a tax receipt may be issued for the donated portion of the cost where appropriate. Alternatively, the ACN may decide that the animals should be accepted into the breeding program but ownership will continue to reside with the person introducing the animals. ACN policy on this matter will be clarified in the near future;
  - d. donated or purchased animals become property of the ACN, making them subject to all of the conditions of loans, as outlined in point 2;
  - e. regardless of whether the animals were donated to the ACN, purchased from the breeder by the ACN, or still owned by the breeder, offspring derived from these newly introduced ACN program animals are subject to the terms outlined in 2(e) and 2(f).
4. Individual participants are free to dispose of any fry refused by the ACN and the institution that supplied founder stock in any manner they deem fit (note the exception in the next paragraph) with the understanding that such individuals are no longer considered to be part of the captive breeding

## **Captive Breeding Guidelines**

program and can be brought back into it only with the express approval of the ACN Species Coordinator. Such disposal of fish must not contravene existing local or international legislation concerning the trade or wild release of living specimens and/or procedures for the proper disposal of dead specimens under existing disease or fish and wildlife regulations.

There are concerns about participants selling surplus fishes for profit which, among other things might draw criticism towards the work of the ACN and jeopardize the charitable status of the organization's activities. This is an issue that needs to be examined more closely and will be one focus of a proposed ACN Ethics Review Committee (see section below on this proposed committee). In the interim, the production of fish which are surplus to program needs will be discouraged and selling or trading of surplus fishes will only be permitted on a restricted basis and only with the consent of the Species Coordinator.

5. Individuals no longer wishing to participate in the program agree to return to the ACN or the institution that supplied them with founder stock, sufficient numbers and genetic variation from their stocks to ensure that the program can continue as prescribed. The requirements for stock returns will be determined by the Species Coordinator and will depend, among other things, on the status of the individual's breeding program, the number of other participants supporting the program having comparable stocks, and the security of the breeding program for that species as a whole.
6. That arrangements be made, in the event of the death of the participant or his/her sudden inability to continue in the program, for the rapid and coordinated transfer of program fish to another program member or other arrangement registered with and found satisfactory by the Species Coordinator. It is of vital importance that these arrangements ensure that studbook numbers and the specimens being transferred remain together. These arrangements must be stated in writing and kept on file with the Species Coordinator and updated as and when required. For Tier 1 breeders, both the "next of kin" and "executor of the estate" must be notified of these arrangements, be informed of the urgency in fulfilling these arrangements, and be given appropriate ACN contact names and telephone numbers (Species Coordinator and ACN Head Office).

### **Proposed ACN Ethics Review Committee**

It is difficult to conceive that ACN protocol for captive breeding of endangered species can be thoroughly enforced for an international network of conservation breeders. The costs of doing so are simply too high. In time, some mechanisms will undoubtedly be adopted to minimize identified problem areas (such as the sale of surplus fishes which was identified earlier). There is a requirement for an "ACN Code of Ethics" to be developed. Thus, the establishment of an ACN Ethics Review Committee is seen as a priority undertaking. The degree of control exercised will be continually reviewed by this Committee to recommend policy and evaluate violations. Regulatory mechanisms will be implemented by the ACN Board of Directors as and when necessary and feasible. But overall, faith in each other and our abilities is what will largely determine the success of our efforts.

### **Honesty and Integrity**

Breeders who are accepted into the program have certified that they will comply with the rules and procedures outlined in this document. However, this means that to a large degree the onus is on the individual to be accurate and honest in his or her work. Where personal morals have inspired an individual to work for the integrity of nature, it is self defeating for them to disregard their own personal integrity in the process. Further, the whole purpose of the exercise will be jeopardized along with the work of many others. Thus, it seems reasonable to presume that screened participants will do their utmost to observe the rules.

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The rule of thumb for an individual conservation breeder has to be: "If there is ever room for doubt, don't guess. If you think there has been a mistake, start over." It is essential to be cautious in our work as errors can cause irreversible damage. Honest mistakes are a common element of human existence. But let's minimize them ... and certainly, let's not compound them.

One of the most common mistakes is to "contaminate" the gene pool. Since the ACN's major concern in its conservation programs is to preserve the gene pool, contamination due to the production of hybrids or offspring that are crosses between subspecies would require that the fish be withdrawn from the program. For example, there are at least two color morphs of *Pachypanchax omalonotus*, yellow and blue. If the status of these color variations is not clear, it would be essential not to mix them. In other words, such breeding could turn out to be between sibling species or distinct subspecies. In both cases the gene pool needs to be kept separate for conservation purposes until it is definitely known that both color forms are produced by both color morphs in nature, in which case mixing would not be a problem. As it turns out, in the case of *Pachypanchax omalonotus*, breeding pairs will produce both color varieties and are the same species. The Species Coordinators, being advised by the most knowledgeable people available, will be able to make such determinations and advise the participants. When knowledge is not available, it is advisable to err on the side on caution. Two fish that appear to be identical may or may not be the same species or identified population. We have to be sure before we enter them into the breeding program. A mistake will require that the entire captive breeding population being kept by that individual will have to be removed from the conservation effort. In some cases, this could be fatal to the survival of the species. Thus, a participant making an honest mistake must report the occurrence as soon as possible with no fear of retribution.

### **Record Keeping and Data Management**

The Species Coordinator and Studbook Keeper are the two key persons involved with the management of the captive breeding populations at the species level. These persons do not necessarily have to be fish keepers. In fact, the positions might be best suited to individuals who are retired, handicapped, or where other aspects of their day-to-day lives or accommodations would interfere with direct participation as a conservation breeder.

There may even be scope for ACN members to participate as Species Coordinators or Studbook Keepers for non-ACN programs should such opportunities arise at a future date (e.g. the Lake Victoria Species Survival Plan of the AZA).

### **Species Coordinators**

The primary person responsible for coordinating the captive breeding program and breeder network for a given species will be the "Species Coordinator". He/She will be required to develop and/or disseminate information concerning effective methods of husbandry and propagation of the species, and manage the breeding population according to principles which strive to safeguard long term genetic viability.

It is essential that Species Coordinators have a well entrenched understanding of fish husbandry and reproduction, particular knowledge of the species they are charged with "coordinating", be organized and able to keep good records, be good correspondents, have no inhibitions about making appropriate contacts to resolve problems; and have flexibility to deal with urgent issues as and when they arise.

### **Studbook Keepers**

Studbook Keepers must also demonstrate that they are organized in keeping records and be good correspondents. They should also have good knowledge of the husbandry and reproduction of the species to provide advice to the Species Coordinator as required.

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Species Coordinators and Studbook Keepers may also participate as conservation breeders if this does not jeopardize their primary ACN responsibilities .

### Studbooks<sup>1</sup>

A studbook will be developed for each program species to adequately track populations. Such tracking is essential in rare species, as it allows for effective breeding management, population documentation, and population maintenance. Without studbook data it is impossible to track the individual components of a population, and some components can easily be lost due to chance errors of undirected programs. The studbook will be held by the "Studbook Keeper" and will be periodically updated and a copy submitted to ACN headquarters for updating the central ACN Studbook Reference Database. Studbook Keepers will have the primary responsibility for maintaining all historical records (the studbook) of captive breeding of the species in the ACN program. The Studbook Keeper will work closely with the Species Coordinator and could conceivably maintain the studbooks for more than one program under different Species Coordinators. All of these records will be transferred to his/her successor(s). The ACN Studbook Reference Database serves as a backup system and a readily available source of information for administrative needs.

The studbook will be fairly rudimentary in the case of the "Species Registry Approach", but will adhere to the basic principles of studbook maintenance so as to permit upgrading to a genetic tracking system required in the "Species Survival Approach" at a future date. The AAZPA guidelines for studbooks, Species Survival Plans and the responsibilities of Species Coordinators and Studbook Keepers will be followed to the extent possible.

The studbook will serve as a focus for the activities and communication of the breeders of the species. It will also serve very usefully as a constant census instrument to keep track of the numbers of breeding animals of the species, which is essential information in a conservation program.

All fish in the species propagation program need to be in the studbook. This documentation obviously needs to start somewhere, and it is best started with the origin of breeding fish currently being used. The information desired on all broods which produced breeding fish is as follows:

- wild caught or not
- if wild caught
  - location
  - time
  - collector
- if not wild caught
  - source of male parent
  - source of female parent
  - breeder's name
  - date of spawn, hatch or emergence
  - any comments applicable of individual or brood.
  - breeding status of brood (young, old, active)

Studbooks should be arranged around broods of fish. Fish are propagated by the mating of males of a single brood with females of a single brood. This works best if the male brood is different from the female. In species with monogamous mating systems the choice of a mate will obviously be met simply by the fish's insistence that this be so. This being the case, it is important that monogamous choices be made from among

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<sup>1</sup>Assistance in writing this section came from Phillip Sponenberg



## Captive Breeding Guidelines

genetically unrelated partners. It is equally important, though, in colony or polygamous mating species to limit the breeding population to males from one source brood and females from one source brood. This is the only way to effectively track the genetic material through the generations, which is the ultimate goal of any conservation effort which conforms to the "Species Survival Approach" outlined previously. This constraint on parents of broods will track the genetic material better than if groups of males and females of different broods are used as breeders together, since it then becomes impossible in many cases to assign parentage to the resulting fish.

Each brood needs to be assigned a studbook number. Included in the studbook entry for each brood are the brood numbers of the male parent(s), female parent(s), date of spawn, hatch or emergence (whichever is appropriate for the given species), breeder, and any comments relevant to the brood. The brood number is assigned sequentially on the basis of date, so close coordination of breeders is needed. Breeders will assign temporary brood numbers based on species abbreviation (predetermined), the breeder's ACN breeder registration code, the date (yy-mm-dd), and the breeder's own sequentially determined brood number. These broods will subsequently be reassigned sequential studbook codes by the Studbook Keeper.

Information exchanges between the breeders of a species and the Species Coordinator will take place quarterly for some species and semi-annually for others with copies of studbook update information being sent also to the Studbook Keeper.

Records to be kept on the aquaria include the brood number of males, brood number of females, and the species. The participants in the breeder network need to make the commitment to providing timely feedback to quarterly or semi-annual questionnaires that are sent out by the Species Coordinator. Failure to respond to more than one questionnaire will dictate removal from the program.

By tracking the brood parentage in this manner it becomes possible to assure that all lineages are participating in breeding the succeeding generations. Breeding programs need to be established for each species, and need to be tailored for each individual situation. These programs need to assure that conservation genetics principles are applied, and will entail orderly exchange of fish among collections to assure even genetic representation in succeeding generations. (See Section Three for an example of a short studbook form. Also, see Appendix B & C for Wildlife Conservation and Management Committee (AZA) Guidelines.)

It is important that individual breeders report specific information such as birth abnormalities, disease, etc. and that these circumstances be adequately logged by the Species Coordinator. There is also a requirement for quarantine protocols to be observed and recorded with respect to ACN stocks as well as private stocks, and ACN stocks and private stocks must always be kept separate.

### **Division of Skills**

Some aquarists have demonstrated excellent skills in conditioning, spawning and first-feeding of fish but have very limited facilities for raising large numbers of fry on to adults. Conversely, there are many other aquarists who are less skilled in breeding fish but nevertheless have the basic knowledge and sufficient tank space in order to "grow on" large numbers of fish. The division of skills and facilities is a common feature of many aquaculture practices and could be considered for certain ACN breeding programs. This system might be particularly relevant in the case of species which grow to a large size, the broodstock being held and spawned by amateur aquarists and the juvenile fish transferred to public aquaria or other large holding systems for growing on. The highly skilled fish breeder is one of the greatest assets of the amateur community, and should not be excluded from ACN breeding programs simply because they have limited aquarium facilities.

## **Captive Breeding Guidelines**

### **Breeder Network Communications**

As already outlined, the main communication between breeders of a particular species will take place through the Species Coordinator in the course of updating the Studbook. There is scope, in some circumstances, for communications among breeders associated with fishes from closely related species or common geographical areas. Hopefully, mechanisms will arise as required, such as special communication circulars within components of the ACN network. It is anticipated that channels of information flow will develop such that program updates and other relevant information will be reported as appropriate in the ACN quarterly bulletin *Aquatic Survival*.

Mechanisms will also be sought to relay to a wider audience the relevant information from ACN captive breeding programs to encourage new participants.

### **Aquarium Societies and Liaison with ACN Programs**

The ACN has recently developed an Affiliate Club Program to educate aquarists about the role they can play in conservation, not necessarily by participating a formal ACN breeding program, but also in their own societies and in their hobby on a day to day basis. As the program moves along, the ACN intends to develop support services to aid societies in forming their own species maintenance and conservation programs. Societies participating as ACN affiliates will sign an affiliation agreement. A sample of the agreement is found in Appendix D.

Aquarist organizations are invited to participate further in ACN programs by appointing an ACN representative whose purpose would be to help communicate and organize captive breeding efforts by their organization's members. Consideration will be given to establishing training sessions in conjunction with ACN conferences, both for ACN representatives to local societies, as well as for Species Coordinators and Studbook Keepers.

### **Motivational Factors**

The long term commitment that is asked of persons involved in conservation breeding deserves some kind of public recognition. Some aquarists may devote several years of their lives to one or two species of fish before passing on his or her successes to another breeder in the network. Very dedicated individuals will pursue this task for the rest of their lives, and many already have a longstanding record of saving endangered species from extinction. Some of these people have likely come and gone, and their accomplishments have gone relatively unnoticed. The record of the work these persons did should be unearthed and put on prominent display before this information is lost. These few predecessors should be appropriately recognized for their contribution to a field to which so many more of us are now devoting ourselves. Their accomplishments should be an inspiration to us all.

It is very difficult to conceive of ways to satisfactorily reward persons who dedicate so much of themselves on a volunteer basis. Human nature often dictates "an hour's pay for an hour's work". Short of paying people a salary so that the work seems economically justified, any sort of award system appears to be mere tokenism. Nevertheless, an awards system, if well managed, can provide a great sense of satisfaction to deserving members. A coveted award can be more valuable to someone who's dedication to the cause transcends financial compensation. Thus, the ACN aims to provide awards to its deserving volunteers.

Although the precise format of an ACN Conservation Breeder Awards Program has yet to be finalised, several ideas are being considered. Clearly, the traditional mechanism of awarding breeding points simply for successfully breeding a species, and then accumulating these points by breeding many species, does not work

## Captive Breeding Guidelines

in conservation breeding - in fact it can have a deleterious effect. If recognition and awards are given to persons demonstrating high turnover of species and it is only necessary to produce youngsters (and not viable next generation adults), there is little guarantee for the long term captive maintenance of a species. In fact, dedicating oneself to one or two species, for long term conservation initiatives, means that one has to forego the opportunity of demonstrating superior skills as a breeder and obtaining the deserved recognition under these traditional programs. Further, rare species are often sought out by participants in the traditional breeder award programs, either because the point rating system awards higher points for rare species, or else the individual has bred so many species that there are only the rare ones left as options. In either case, we likely have an example of a high turnover breeder, and soon after the fish are acquired, they are discarded. Such specimens, for the sake of the conservation of the species, would have been much better incorporated into a program that disqualified them from traditional breeder awards programs, but contributed to a conservation oriented award based on different criteria. Any aquarium society could implement such an award system. The ACN will most definitely adopt this type of approach.

Some ideas being considered include:

1. Recognition as a Tier 1, 2 or 3 breeder by awarding a plaque, medal or certificate (possibly represented as gold, silver and bronze respectively);
2. Recognition for long term maintenance of a particular species or participation in a specific project by awarding 1 year, 2 year, 3 year, etc. pins or certificates;
3. Recognition of group efforts (e.g. aquarium societies);
4. Recognition for supporting activities such as research, writing, Species Coordinators, Studbook Keepers and other volunteer work;
5. Public announcements of milestone achievements of members through the bulletin *Aquatic Survival*, press releases, magazine articles and a "highlights" section of the annual report and directories (membership directory, breeder directory);

## Non-Breeder Roles

### Species Coordinators, Studbook Keepers and Database Management

The potential for non-breeders to assume the responsibilities of Species Coordinators and Studbook Keepers was discussed in the earlier section on "Record Keeping and Data Management". In addition to these roles, computer database management is clearly an important prospective area for volunteer support. Many zoos are already engaged in major programs for database development in livestock management (e.g. ARKS protocol), but these are not necessarily the most appropriate for fish breeding programs. It is, for example, not always possible to identify both parents of a brood. The CERCI program for 'colony management' currently under development may help to resolve this problem.

### Holding and Shipment Facilities

Although not fully considered yet at the time of writing, it seems clear that the ACN will require holding facilities. Thus, there is conceivably a role for volunteers to donate a portion of their facility to this function, and for yet others to play a volunteer role as part of the work force dedicated to supporting a relatively large

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holding facility (or conceivably a fully funded breeding facility with additional holding capability). This discussion is somewhat hypothetical at this point, but is presented here as an example of the types of contributions that may one day be required of ACN members.

The reasons behind the requirement for holding facilities are several and include:

- holding fish in transit between the wild and the breeders (and potentially vice versa in restocking programs) particularly where shipments have to be sorted and divided;
- holding fish in quarantine;
- holding fish from participants who have died or quickly exit the program for some other reason;
- moderating fish transfers between breeders;
- holding fish while a breeder's systems are cleaned or he/she relocates;
- to facilitate regular stock exchanges where one has to discharge stock before accepting a delivery;

Depending upon the need, the ACN may have to acquire its own facilities, enter into contract with fish farms or transhippers, develop cooperative arrangements with public aquaria, research institutions, universities, etc. or with an amateur aquarium society willing to undertake this responsibility. In the short term, but less manageable on a large scale, is the use of an aquarist's home to hold small shipments or part of all of the stocks of a program breeder who needs space during an exchange of stocks with another breeder.

Whatever the circumstances, certain criteria will have to be developed and implemented for ACN registered holding facilities.

### ACN Research Programs

There are many unknowns in the field of aquatic conservation. Even where research has been done, the information is not always readily available to each and every aquarist who could use it. Thus, there is a strong need for persons to assimilate information from the literature, from raw research data, and from general and often unrecorded observations of aquarists, into a form that is meaningful to both the private aquarist and the scientist.

There are possibly two ways to look at the approach to be taken by an ACN Research Associate.

- 1) The person can follow their personal interests, indicate that a certain area is where they plan to make a contribution (so that redundancies might be identified in advance); and provide the results of that research in a form that can be disseminated to the individuals who would most benefit. In the case of general interest information, their results might be published as a separate report or an article in *Aquatic Survival* (the ACN's quarterly bulletin). Studies might include such topics as habitat loss, geographic studies, species specific studies, husbandry techniques, genetic principles, etc.
- 2) Alternatively, the person could respond to a specific research requirement identified by the ACN and which would directly contribute to an ongoing or proposed program of the organization (e.g ACN Madagascar Project). In this case, there may be specific guidelines to follow so as not to overlap the contribution of other volunteers. The results of this research would be made available as described in point 1) with the added opportunity for inclusion in special comprehensive reports compiled from the inputs of several contributors.

Naturally, there is the long term scope for ACN associating with research programs in other institutions, cooperating with certain graduate student programs, etc. Research here could focus on, say, laboratory oriented research and possibly coordinate inputs from private breeders in the breeder network. Studies might

## **Captive Breeding Guidelines**

include such things as inbreeding experiments, hybridization work, fecundity studies, etc. But such projects will have to be considered in light of the potential diversion of limited and dedicated resources, so that they do not jeopardize priority programs and conservation objectives of the organization.

The ACN does not have a formal research mechanism at the time of writing and many of the points put forward here are hypothetical. But volunteers with a flair for research and writing are invited to contribute at any time.

### **Administration Oriented Activities**

A long term goal of the organization is to operate a sufficiently large program base and to secure sufficient funding to operate a permanent secretariat. With permanent office staff to carry the large part of the administrative burden, the ACN will be able to give greater assurance that the work it proposes to do, will get done.

In the meantime, and also to a large degree after achieving full staffing, the ACN employs volunteers to fulfil various administrative needs. Such services are required for the breeding network to function effectively. Persons who would like to assist should contact the head office and make known their particular skills or interests.

### **Other Activities**

There may be other skills which members would be able to bring to the organization. For example, fish photography or video filming could be useful in a number of ways, such as recording juvenile and adult colouration (which is usually lost following specimen preservation), and documenting conspecific interactions including spawning behaviour - especially with regards to rare or "first time" spawnings. Photographic records of successful aquarium layouts would be useful and all of these resources could be deposited with the ACN in order to build up a library of material for production of popular articles, press releases, scientific papers and talks.

Other opportunities to assist undoubtedly exist.

## Captive Breeding Guidelines

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## Captive Breeding Guidelines

### APPENDIX A

#### QUESTIONNAIRE FOR PROSPECTIVE PARTICIPANTS IN ACN CONSERVATION BREEDING PROGRAMS

*The following is a list of the questions included in the questionnaire completed by prospective conservation breeders of the ACN. The exact questionnaire might differ slightly at the time of application as the procedures evolve. The primary purpose in asking prospective participants to complete this questionnaire is to allow the ACN and the Species Coordinators to assist them in becoming conservation aquarists. The questionnaire is extensive and may highlight some factors which the aquarist had not previously considered. A poor rating on this questionnaire does not necessarily disqualify a person from participating, but might instead serve to highlight areas for improvement through training or study. At the end of the questionnaire, applicants are asked to certify with their signature that they have responded truthfully to this questionnaire and that if accepted into the program, they agree to follow the ACN Code of Ethics:*

#### **A. ABILITIES AND EXPERIENCE**

1. Are you an amateur or professional aquarist and how long has been your involvement?
2. Describe your involvement as an aquarist.
3. List the scientific names (species) of fish with which you have had breeding success and where offspring were raised to reproductive age. Indicate whether the species were maintained over several generations. It would assist if you could categorize these by family.
4. List the scientific names (species) of fish with which you have had breeding success but where offspring were not raised to reproductive age. Indicate whether the species were maintained over several generations. It would assist if you could categorize these by family.
5. List other species (scientific names) that you have successfully maintained in captivity for more than two years (but without attempts and/or success at breeding). It would assist if you could categorize these by family.
6. Describe any academic qualifications which contribute to your fish keeping abilities.
7. Describe any work experiences which contribute to your fish keeping abilities.
8. Describe any personal research or other undertakings which contribute to your fish keeping abilities.
9. Please rate your knowledge (Poor, Fair, Good, Above Average, Excellent) with respect to each of the following separate items: Fish Breeding, Genetics, Water Quality, Fish Nutrition, Fish Health, Fish Behavior, Fish Ecology, Fish Taxonomy, Fish Evolution, etc.

#### **B. SERIOUSNESS AND DEDICATION TO THE CAUSE**

1. What prompted you to join the Aquatic Conservation Network?
2. Why do you wish to take part in a captive breeding program for endangered fish?
3. Please describe your specific conservation interests, if any (in terms of fish families, species,

## **Captive Breeding Guidelines**

geographic areas, etc.). If you have no specific interests but are willing to consider any ACN program species, please say so.

4. How many hours per week will you be able to devote to conservation fish keeping activities (including husbandry, record keeping, correspondence, etc.)?
5. Are you able to inspect your fish every day and feed them as required? If not, is an experienced aquarist available to do this for you when you are absent?
6. Do you have a "positive" support system? Are your family members and close friends supportive of your philosophical commitment to endangered species conservation and captive breeding, and your plans to devote significant time, money and other resources to this activity?

### **C. WILLINGNESS TO COMPLY WITH THE RULES**

1. Will you comply with the rules and procedures outlined in the section titled "Basic Rules of Compliance for Participation by Private Individuals in ACN Conservation Breeding Programs" of the ACN document *Captive Breeding Guidelines*?
2. Are you willing you notify your next of kin, and at least one other dependable local aquarist, of the procedures that should be carried out in the event of your death or sudden inability to continue in the program, for the rapid and coordinated transfer of program fish to a successor, and to register these procedures with a designated official of the Aquatic Conservation Network?
3. If it becomes necessary (particularly with respect to highly endangered species), are you willing to indicate in your will, or in a letter to accompany your will, the procedures that should be carried out by the executor of your estate for the urgent and adequate maintenance and systematic transfer of your endangered stocks to a successor? Do you also agree to file copies of these procedures with a designated official of the Aquatic Conservation Network? (If required, the ACN may provide a standard format letter, which may be modified if necessary, to minimize solicitor's fees)

### **D. EQUIPMENT AND OTHER RESOURCES**

1. In terms of water volume, what capacity are you presently equipped with to devote to conservation fish keeping ("presently equipped" means fully serviced in terms of lighting, heating, filtration, etc.)? (Please specify liters, U.S. gallons or Imperial gallons).
2. Please provide a general breakdown of your available equipment in terms of type of unit (aquarium, pond, trough, etc.), size (volume), shape, and number of units. Are any of these units linked via flow-through systems? Such systems may have potential disease transmission problems. If you answered "Yes", please explain the systems in question (provide a diagram if it simplifies the explanation).
3. Would you consider additional investment and/or vacating existing equipment of present stocks in lieu of conservation fish keeping? If "Yes", by what proportion (%) would you be able or willing to expand your conservation breeding activities (making full allowance for additional time and resources required)? What factor(s), if any, limit this additional involvement from being even greater? (e.g. time, money, space, etc.). Over what time frame would you consider such expansion? Please explain, making clear also any concerns you may have.
4. Please supply the information about the pretreatment of your source(s) of water in terms of chlorine,



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chloramine and other pretreatments by yourself or by municipal sources. Is there scope for the introduction of fish disease through water supplies? Is there potential for the introduction of biologically harmful pollutants? Potential predators?

5. Please supply the following information about your source(s) of water: pH, pH 48 hours after drawing supply, Hardness (mg/l CaCO<sub>3</sub>), Ammonia, Nitrite, Nitrate, Dissolved Oxygen, Carbon Dioxide.
6. Describe water treatments (if any) utilized to make water from each source appropriate for your fish keeping. (e.g. pH adjustment, ion-exchange resins, etc.)
7. How do you dispose of water from your systems (e.g. municipal sewer system with filtration plant, storm sewer output to local water body, septic tank, other - specify):
8. Are there local regulations pertaining to properties of water effluents from household, industrial and aquaculture facilities for your type of disposal practice?
9. Do you conform to these regulations? And at what level of expansion of your operations would you be legally bound to consider post-use filtration and water treatment systems? Do you already use such systems? Are these because of a regulatory requirement or in consideration of the environment? Please comment.
10. Are there possibilities for escape of live fishes from your system to native water sources? If yes, please explain. A "Yes" answer may mean exclusion from the ACN captive breeding program. If "Yes", what would be required to stop this from occurring?
11. How do you dispose of dead fish?
12. Are you prepared to preserve specimens for taxonomic/genetic purposes?
13. Do you have access to live foods? What types? What are your sources (in terms of wild, cultured, purchased through pet stores, mail order or scientific supply houses)?
14. Are you aware of the disease risks associated with the feeding of certain live foods (especially tubifex, daphnia/copepods)?
15. Do you have access to literature on: fish health, water quality management, fish nutrition, fish breeding, genetics.
16. Please give the name, address, telephone and fax numbers of the nearest Fish Health Diagnostic Laboratory (or Veterinary Surgeon) that will provide services to aquarium fish keepers?
17. Are you familiar with the procedures for using their services?
18. Do you keep the necessary materials for making urgent submissions to their laboratory?
19. Are you willing to assume responsibility for the costs of these services if required (including packaging and shipping fees)?
20. Unless funding is obtained for specific ACN projects, you may be asked to contribute to the cost of

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the fish being assigned to you. Nevertheless, the fish would still be considered to be on loan to you as outlined in the section titled "Basic Rules of Compliance for Participation by Private Individuals in ACN Conservation Breeding Programs" of the ACN document *Captive Breeding Guidelines*. Within reasonable limits, and at a predetermined price, are you willing to consider contributing to the costs of purchasing these fish and/or the packaging and transportation costs?

21. What professional contacts or correspondents (fisheries scientists, public aquarium personnel, researchers for tropical fish industry, etc.) do you have that might be helpful to your work as a conservation breeder?
  
22. List Aquarium Organizations to which you are a member, and executive or committee positions you have held.

# Captive Breeding Guidelines

## APPENDIX B

### GUIDELINES FOR MINIMUM CONTENTS OF AZA WCMC APPROVED STUDBOOKS AND UPDATES

#### STUDBOOKS

All of the following information should be included in the studbook each time it is published so that the studbook is a complete document.

#### 1) INTRODUCTION

- a) Systematics
  - historical record/list of common and scientific names
- b) Status of Wild Populations
  - USFW
  - CITES
  - primary cause of decline in wild populations if applicable
- c) Distribution of Wild Populations
  - former range
  - current range
- d) Brief Description of Ecology of Wild Populations
  - social structure (solitary, pairs, groups etc.)
  - mating systems (polygyny, monogamy, polyandry)
  - activity cycle (nocturnal, diurnal, crepuscular)
  - diet type (carnivore, omnivore, herbivore, browser, etc.)
  - use of space (territory, home range, migration, etc.)

#### 2) GENERATION INFORMATION ABOUT STUDBOOK

- a) Geographic Scope of Studbook
  - Regional or International. State which countries are included (e.g., if North American regional are Caribbean islands included?)
- b) Time Scale
  - list date of first entry and the date through which the data are current
- c) Description of Data Fields
  - should include definitions of symbols used (e.g. F,M,U for sex, WILD, UNK, MULT, for Dam ID, etc.)
- d) Capture Locations of Founders, If Known
- e) Conventions Used in Studbook
  - what day/month is recorded for an event when actual date is not known?
  - for egg laying species what does the birth date represent (e.g., laying, pipping, hatching)
  - what is used as capture locations if specific site is not known (e.g., continent, region, country etc.)
- f) List of institutions by SPARKS mnemonic with addresses

#### 3) STUDBOOK

- a) Listing of Living and Historical Populations

#### 4) BIBLIOGRAPHY

## **Captive Breeding Guidelines**

- a) Relevant literature on the Studbook Species

### **UPDATES**

#### **1) INTRODUCTION**

- a) Species Current Common and Scientific Name
- b) Dates Covered by Update
- c) Dates of Most Recent Publication of Full Studbook
- d) Summary
  - total number of births, deaths, imports, exports, and transfers during time period covered in update

#### **2) UPDATE**

- a) Births
  - list by date or by institution
- b) Deaths
  - list by date or by institution
- c) Transfers
  - list by date or by institution
  - include imports and exports

#### **3) BIBLIOGRAPHY UPDATE**

Source: Willis/Mellen, April, 1992, *AZA (formerly AAZPA) Conservation Resource Guide*.

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## **Captive Breeding Guidelines**

### **APPENDIX C**

#### **RESPONSIBILITIES OF KEEPERS OF AZA WCMC APPROVED STUDBOOKS**

(Summarized from AZA (formerly AAZPA) Conservation Resource Guide)

##### **PRIMARY GOAL**

Maintain and distribute an accurate database on the species or taxon of interest so that the captive population can be effectively managed both demographically and genetically, and so that other conservation projects involving the taxon can benefit from this baseline biological data.

##### **RESPONSIBILITIES**

- 1) Publish and distribute the first edition of complete regional and international studbooks within 18 and 24 months of AZA WCMC or IUDZG approval, respectively.
- 2) Publish and distribute annual updates to the studbook.
- 3) Publish and distribute the complete studbook every three years.
- 4) Distribute disk copy of the studbook annually.
- 5) Serve on the relevant SSP and Regional Taxon Advisory Group.
- 6) Copy the AZA (formerly AAZPA) Director of Conservation Science, AZA WCMC Chair and AZA (formerly AAZPA) Studbook Advisor on all significant correspondence.
- 7) Conversion of existing studbooks to SPARKS format as soon as possible is strongly recommended.

##### **DISTRIBUTION OF THE STUDBOOK**

(Distribution list is not included here. It is formalized but updated as necessary)

excerpts from Shoemaker/AZA WCMC/C&S Office

April, 1992

## Captive Breeding Guidelines

### APPENDIX D

#### AQUATIC CONSERVATION NETWORK AFFILIATE CLUB PROGRAM

Affiliate clubs agree to foster the following goals and attitudes:

1. Recognize that conservation efforts are sustained by a sincere respect for the living creatures that share the planet with us, combined with a desire to help preserve them.
2. Encourage long-term breeding practices that maintain species for several generations. Organize breeder award programs that reward the aquarist who keeps and breeds a species for many years.
3. Make every effort to ensure that wild fish are obtained legally and that purchasing or collecting wild fish does not put increased pressure on endangered fish in the wild. The purchase of captive-bred fish is encouraged.
4. Keep only those fish that can be cared for properly, giving each fish the space necessary to grow to its maximum size while maintaining optimum health and life span.
5. Learn how to breed fish in a manner that helps maintain genetic diversity and encourage club members to put this knowledge into practice in their breeding programs.
6. Breed fish in such a way that they maintain the characteristics of the species as found in nature and avoid breeding for distortions in color, shape, fin size etc.
7. Learn as much as possible about the natural habitat of the fish that interest club members and alert others when the fish are threatened with extinction.
8. Sponsor at least one conservation oriented program a year and, when possible, provide financial support for *in-situ* or *ex-situ* conservation programs. Move in the direction of developing more Conservation Aquarists in the club.
9. **Never** dispose of fish or other aquatic animals by "dumping" them into waters that are not part of their natural habitat.
10. When the club, or a group within the club, feels it is ready to commit to the discipline of long-term conservation efforts, volunteer to join one of the ACN captive breeding programs.

The \_\_\_\_\_ (Name of Society) agrees to foster the goals and attitudes of the ACN Affiliate Club Program. I am an official representative of this society:

Name: \_\_\_\_\_ Title: \_\_\_\_\_

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

The annual fee for affiliation is \$10 (Canadian or U.S. currency) per year plus the annual \$25 ACN Membership Fee (Total = \$35). Send applications for the ACN Affiliate Club Program to the Aquatic Conservation Network, 540 Roosevelt Avenue, Ottawa, Ontario, Canada K2A 1Z8.

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### APPENDIX E

#### DEVELOPMENT OF CRITERIA FOR ESTABLISHING NEW CAPTIVE BREEDING PROGRAMS

*During the 1992 meeting of the Captive Breeding Specialist Group (CBSG) of the Species Survival Commission, World Conservation Union, much effort centered on drafting criteria that could be used to guide the development of new captive breeding initiatives. On the premise that many of the taxon specialist groups will be attempting to develop such criteria, it was suggested that a broadly based working group be established, charged with the development of general guidelines. With that in mind, the Primate Working Group, has drafted guidelines for review and further development by the CBSG. The text of that draft is printed here as food for thought in development of ACN captive breeding initiatives.*

#### DRAFT GUIDELINES FOR DEVELOPING NEW CAPTIVE BREEDING PROGRAMS

Captive breeding programs should occur in support of, not as a substitute for, wild populations. They represent one component of a holistic conservation effort that promotes species conservation through habitat preservation, education, training, research, and in some instances, intensive *in-situ* population management. As part of a comprehensive conservation strategy, it is important that initiation of new captive breeding programs be undertaken in a manner consistent with the following guidelines:

1. The captive breeding program should not divert resources designated for higher priority habitat conservation efforts or *in-situ* management. Recognizing that many resources available for captive breeding are not available for use *in-situ* conservation, captive programs should be developed in a manner that generates support for conservation and education efforts in the country of origin.
2. The decision to establish or not to establish a program should be based on the best available scientific information on the species and habitat status and on the ability to manage the species in captivity. Informed decisions should:
  - a. Be the result of consideration of the impact and effectiveness of various management options and should identify captive breeding as only one part of a comprehensive conservation strategy.
  - b. Reflect consideration of priorities identified in the Conservation Assessment and Management Plan and Regional Collections plans.
  - c. Make every effort to identify and incorporate founder stock currently in captivity but not in a managed population (pets, confiscated animals, etc.), before considering removal from the wild population.
  - d. Except in the cases of imminent local extinctions an evaluation (PHVA - Population and Habitat Viability Analysis; or PVA - Population Viability Analysis) of the impact of removing sufficient or additional founder stock from the wild populations.
3. The program should be developed in collaboration and partnership with regional and local resource managers, field biologists and captive breeding specialists. It should be reviewed by appropriate members of the SSC (including field biologists and captive managers) before initiation.
  - a. A management committee consisting whenever possible of wildlife authorities in the range country, field biologists and captive breeding specialists should be set up to oversee

## **Captive Breeding Guidelines**

management of each species, or group of species. The management committee should be responsible for insuring that a studbook is initiated and maintained, and that appropriate planning occurs in the contexts of a comprehensive conservation strategy.

- b. The management committee should ensure that the captive population is managed in accordance with defined demographic and genetic goals that will contribute to recovery and survival of the species.
  - c. Participating institutions should be identified on the basis of past records in husbandry, captive management and breeding of the particular or related taxon, and with due regard to past records of cooperation in coordinated managed breeding programs.
  - d. Participation in the program should be dependent on each party's signing and agreement of participation. Any party which violates this agreement should be excluded from future participation in the program.
4. Whenever possible, captive breeding efforts should be initiated in the country of origin.
  5. Ownership of animals in the captive breeding efforts should remain with the country or origin or with the management committee. Animals within the program should be managed without regard to commercial considerations.
  6. Programs should be initiated before the population has reached the stage where so few individuals exist as to make the success of the program unlikely. The International Union for the Conservation of Nature (IUCN) policy statement on captive breeding suggests that "Management to best reduce the risk of extinction requires the establishment of support captive breeding populations ... when the wild population is still in the thousands". Except in "rescue" situations, programs should not begin unless there is a high probability that a sufficient founder population (e.g. 20-30 individuals) can be acquired over a reasonable time period.
  7. Husbandry protocols should be developed as the first stage in initiating captive breeding programs whenever possible. Protocols should reflect experience with closely related taxa already in captivity that can serve as "models", or, in instances where the species' biology is poorly understood, may require research with a relatively small number of wild caught individuals. Whenever possible, preliminary research should occur in the country of origin. Following the inception of a program, the management committee should actively promote continuing research to increase knowledge of the biology of the species.

Source: CBSG News, Vol. 3 No. 3, 1992, P. 27. (CBSG News, 12101 Johnny Cake Ridge Road, Apple Valley, Minnesota 55124. Phone: (612) 431-9325. Fax: (612) 432-2757.



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## APPENDIX F

### THE IUCN POLICY STATEMENT ON CAPTIVE BREEDING

Prepared by the  
SSC Captive Breeding Specialist Group  
As approved by the  
22nd Meeting of the IUCN Council  
Gland, Switzerland  
September 4, 1987

#### SUMMARY

Habitat protection alone is not sufficient if the expressed goal of the World Conservation Strategy, the maintenance of biotic diversity, is to be achieved. Establishment of self-sustaining captive populations and other supportive intervention will be needed to avoid the loss of many species, especially those at high risk in greatly reduced, highly fragmented, and disturbed habitats. Captive breeding programmes need to be established before species are reduced to critically low numbers, and thereafter need to be co-ordinated internationally according to sound biological principles, with a view to the maintaining or re-establishment of viable populations in the wild.

#### PROBLEM STATEMENT

IUCN data indicate that about 3 percent of terrestrial Earth is gazetted for protection. Some of this and much of the other 97 per cent is becoming untenable for many species, and remaining populations are being greatly reduced and fragmented. From modern populations biology one can predict that many species will be lost under these conditions. On average more than one mammal, bird, or reptile species has been lost in each year this century. Since extinctions of most taxa outside these groups are not recorded, the loss rate for all species is much higher.

Certain groups of species are at particularly high risk, especially forms with restricted distribution, those of large body size, those of high economic value, those at the top of food chains, and those which occur only in climax habitats. Species in these categories are likely to be lost first, but a wide range of other forms are also at risk. Conservation over the long term will require management to reduce risk, including ex situ populations which could support and interact demographically and genetically with wild populations.

#### FEASIBILITY

Over 3,000 vertebrate species are being bred in zoos and other captive animal facilities. When a serious attempt is made, most species breed in captivity, and viable populations can be maintained over the long term. A wealth of experience is available in these institutions, including husbandry, veterinary medicine, reproductive biology, behavior, and genetics. They offer space for supporting populations of many threatened taxa, using resources not competitive with those for in situ conservation. Such captive stocks have in the past provided critical support for some wild populations (e.g. American bison, *Bison bison*), and have been the sole escape from extinction for others which have since been re-introduced to the wild (e.g. Arabian oryx, *Oryx leucoryx*).

#### RECOMMENDATION

IUCN urges that those national and international organizations and those individual institutions concerned

## Captive Breeding Guidelines

with maintaining wild animals in captivity commit themselves to a general policy of developing demographically self-sustaining captive populations of endangered species wherever necessary.

### SUGGESTED PROTOCOL

**WHAT:** The specific problems of the species concerned need to be considered, and appropriate aims for a captive breeding program made explicit.

**WHEN:** The vulnerability of small populations has been consistently underestimated. This has erroneously shifted the timing of establishment of captive populations to the last moment, when the crisis is enormous and when extinction is probable. Therefore, timely recognition of such situations is critical, and is dependent on information on wild population status, particularly that provided by the IUCN/Conservation Monitoring Centre. Management to best reduce the risk of extinction requires the establishment of supporting captive populations much earlier, preferably when the wild population is still in the thousands. Vertebrate taxa with a current census below one thousand individuals in the wild require close and swift cooperation between field conservationists and captive breeding specialists, to make their efforts complementary and minimize the likelihood of the extinction of these taxa.

**HOW:** Captive populations need to be founded and managed according to sound scientific principles for the primary purpose of securing the survival of species through stable, self-sustaining captive populations. Stable captive populations preserve the options of reintroduction and/or supplementation of wild populations.

A framework of international cooperation and coordination between captive breeding institutions holding species at risk must be based upon agreement to cooperatively manage such species for demographic security and genetic diversity. The IUCN/SSC Captive Breeding Specialist Group is an appropriate advisory body concerning captive breeding sciences and resources.

Captive programs involving species at risk should be conducted primarily for the benefit of the species and without commercial transactions. Acquisition of animals for such programs should not encourage commercial ventures or trade. Whenever possible, captive programs should be carried out in parallel with field studies and conservation efforts aimed at the species in its natural environment.

# Captive Breeding Guidelines

## APPENDIX G

### GLOSSARY OF ABBREVIATIONS AND TERMS

#### List of Abbreviations

AAZPA -	American Association of Zoological Parks and Aquariums (former name for the current AZA)
ACN -	Aquatic Conservation Network
ARKS -	Animal Records Keeping System
AZA -	American Zoo and Aquarium Association (formerly the AAZPA)
C&S -	Conservation and Science Office (component of the AZA)
CBSG -	Captive Breeding Specialist Group (a component of the IUCN)
CERCI -	(a computer program for "colony management")
CITES -	Convention on International Trade in Endangered Species of Wild Fauna and Flora
FFTAG -	Freshwater Fishes Tazon Advisory Group (a component of the AZA)
IUCN -	World Conservation Union (formerly the International Union for the Conservation of Nature and Natural Resources)
IUDZG -	International Union of Directors of Zoological Gardens
SPARKS -	Single Population Analysis and Record Keeping System
SSP -	Species Survival Plan (a component of the AZA)
VSSP -	Lake Victoria Species Survival Plan (a component of the AZA under the direction of the FFTAG)
WCMC -	Wildlife Conservation and Management Committee (a component of the AZA)
WCMC -	World Conservation Monitoring Centre (a component of the IUCN)

#### Glossary of Terms

biodiversity -	The variety of life on earth, or some part of it. It includes genetic or inherited diversity, taxonomic diversity such as species, genera or family diversity, and ecological or habitat diversity. Some also include ecological services or functions.
critical -	See "threatened".
endangered -	See "threatened".
<i>ex situ</i> -	Conserve through captive breeding.
extinct -	Species not definitely located in the wild during the past 50 years (criterion as used by the Convention on International Trade in Endangered Species of Wild Fauna and Flora).
fecundity -	The quality or power of reproduction.
founders -	The original specimens participating in a captive breeding program.
habitat -	The natural home of a plant or animal.
hybrid -	The offspring of two animals or plants of different species.
hybridization -	To produce by cross-breeding.
inbreeding -	Mating animals of the same bloodstock.
<i>in situ</i> -	Conserve in the original habitat.
monogamous -	Having a single mate.
polygamous -	Having more than one mate.
progeny -	Offspring.
propagation -	Multiplying or reproducing by generation.
red list -	1990 IUCN Red List of Threatened Animals (earlier editions were published and future revisions of the list are anticipated)- the term also applies to lists of endangered or threatened plants or animals produced on a national or regional basis.

## Captive Breeding Guidelines

### Species

- Survival Plan - Strategy of the American Zoo and Aquarium Association whereby animals are managed by populations and species rather than on an individual animal basis. The SSP originated in 1981 in response to a need to address the genetic and demographic problems associated with the maintenance of small captive populations over long periods of time. Each SSP program, under the direction of a species coordinator and elected propagation group, allows a number of institutions to manage individual animals collectively as one large population.
- studbook - A record of the history of a captive population, including genealogies of individuals and a listing of the various locations in which individuals have been held. Studbooks are used primarily for monitoring and managing captive populations. The data can be used to assess whether a population is stable, increasing or decreasing in numbers. It can also be used to make breeding decisions so that genetic variation can be retained and close inbreeding can be avoided.
- threatened - Mace & Lande proposed three categories of threat (plus "extinct"). These are:
- **critical** - 50% probability of extinction within 5 years or 2 generations, whichever is longer.
  - **endangered** - 20% probability of extinction within 20 years or 10 generations, whichever is longer.
  - **vulnerable** - 10% probability of extinction within 100 years.
- vulnerable - See "threatened"

### This glossary was derived from information drawn from various sources including:

- AAZPA. *Conservation Resource Guide*. AAZPA Conservation and Science Office.
- Canadian Centre for Biodiversity, Canadian Museum of Nature
- *Collins English Dictionary*
- IUCN. 1990. *Red Data Book*. IUCN (World Conservation Union)
- Mace, G.M. and R. Lande. 1991. Assessing Extinction threats: Toward a Reevaluation of IUCN Threatened Species Categories. *Conservation Biology*. Vol. 5, No. 2. June 1991, pg. 148-157.

ISBN 1-895655-02-1

*Aquatic Conservation Network*