Editorial

This combined issue of *Aquabyte* has a general review by yours truly, the Editor, on *ex-situ* conservation of germplasm, another article from Vietnam on integrated farming systems (our Vietnamese members are becoming frequent contributors to *Aquabyte*), and a summary report on the state of the NTAS and members' responses to the recent questionnaire. We would have liked to include more articles in this issue, but those at hand still require further editing and responses from authors. Please do send us more articles and please respond promptly when we send you edited manuscripts for attention. We have also included in this issue most of our regular features. More photos (original slides prefered) are requested for future Photosection use. In fact, we would welcome short "photo essays" like this issue's offering by Dr. Mark Prein. Please pick a theme from your work and illustrate it well with good photos and full explanatory legends. We will be publishing the NTAS members' directory soon. ***R.S.V. Pullin***

Ex-Situ Conservation of the Germplasm of Aquatic Organisms

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Background

Aquatic organisms (finfish, invertebrates, algae and other plants) seldom receive much attention in discussions and publications on *ex-situ* conservation of germplasm. Until recently, discussions on terrestrial and aquatic species were usually separate. For example, consultations on the management of global animal genetic resources (FAO 1990, 1992) were focused almost entirely on farmed livestock and poultry (buffalo, camelids, cattle, chicken, ducks and other poultry, goats, pigs and sheep): FAO had already published on conservation of genetic resources of fish (FAO/UNEP 1981). This separation is now lessening. In 1992, ICLARM and FAO both convened international meetings on aquatic genetic resources, with participation of terrestrial livestock and plant experts (FAO, in press; ICLARM 1992). Their findings should increase support for similar collaboration.

This paper is an attempt to summarize some of the major issues relating to *ex-situ* conservation of the germplasm of aquatic organisms and to give examples of some current activities and future possibilities. It supplements an earlier paper on *in-situ* conservation (Pullin 1990).

The main approaches to *ex-situ* germplasm conservation are captive breeding and cryopreservation. Storage of DNA may also become important but is not discussed in detail here. DNA can be obtained from a variety of tissue sources, including museum specimens and materials preserved in nature, although the theme of *Jurassic Park* - a renaissance of dinosaurs, engineered by entrepreneur geneticists from the DNA of blood cells preserved in the amber-trapped bodies of biting insects (Crichton 1990) - is unlikely to be imitated in aquaculture! The utility of stored fish DNA will depend largely upon parallel work on gene probes and gene libraries.

Breeding Histories and Aquatic Biodiversity

The centuries-long history of captive breeding of most terrestrial farm animals has few parallels in aquaculture. Only the common carp *(Cyprinus carpio)* and some hatchery strains of salmonids and catfishes, chiefly in Europe and North America, have lengthy, known breeding histories. Most aquaculture production derives from genetically uncharacterized broodstock. Even in Asia, most of the principal farmed fish species (cyprinids) were raised from wild collected 'riverine seed' until the 1960s, when hormone-induced spawning became available. The culture performance of many farmed aquatic organisms approximates to that of wildtypes, or may be worse because of indirect (negative) selection or inbreeding.

The high diversity of aquatic fauna and flora is also daunting for *ex-situ* conservation. Flint (1991) mentions the high biodiversity of some aquatic ecosystems (particularly coral reefs and other marine habitats - over 8,300 species are known from the West Pacific alone), points out the likelihood of new species being chosen for aquaculture and finds both *ex-situ* and *in-situ* conservation of aquatic species to be poorly documented and managed.

Some Current Activities

Against this demanding and changeable scenario, there is a growing awareness that *ex-situ* conservation of aquatic species needs more discussion and provision of more resources. The World Conservation Centre (IUCN) already coordinates some conservation activities for fish through its Species Survival Commission, including a Freshwater Fish Specialist Group. IUCN's Captive Breeding Specialist Group encourages some public aquaria to undertake captive breeding for nature conservation purposes and recognizes that captive breeding, cryopreservation of gametes and other tissues, and DNA collections can assist *ex-situ* conservation programs (IUCN 1991).

Recently, more international organizations have become involved in *ex-situ* conservation of aquatic species. In 1991, the International Union of Biological Sciences (IUBS) convened a workshop to develop a research agenda for biodiversity (Solbrig 1991). Aquatic species were prominently featured, though the agenda is largely devoted to research on biodiversity rather than *ex-situ* conservation *per se*.

In 1992, the International Fisheries Center (ICLARM) convened a workshop on International Concerns in the Use of Aquatic Germplasm (ICLARM 1992) and is now planning to coordinate work on *ex-situ* conservation of some farmed freshwater finfish (mainly carps and tilapias) through an International Network on Genetics in Aquaculture, to be established through UNDP support. ICLARM has also a large relational

database (FishBase) that contains records of finfish genetic diversity with details of the *in-situ* and *ex-situ* conservation status of species for which good information exists (Froese 1990; Pauly and Froese 1991; Agustin et al. 1992).

Most recently, in February 1993, the Smithsonian Institution and the United States Agency for International Development held a 'think-tank' on *ex-situ* conservation of biodiversity at which all living organisms, from bacteria to the so-called 'charismatic' species (pandas, rhinos, etc.) were on the agenda[1].

[1]Further details may be obtained from Dr. Leonard P. Hirsch, Smithsonian Institution, Office of International Relations, S. Dillon Ripley Center, Suite 3123, Washington, DC 20560, USA.

Captive Breeding

Captive breeding of aquatic organisms can be a useful aid to conservation but there are important caveats that apply to commercially farmed aquatic species and to species kept in aquaria or hatcheries for support to nature conservation efforts and for stocking sport and other fisheries.

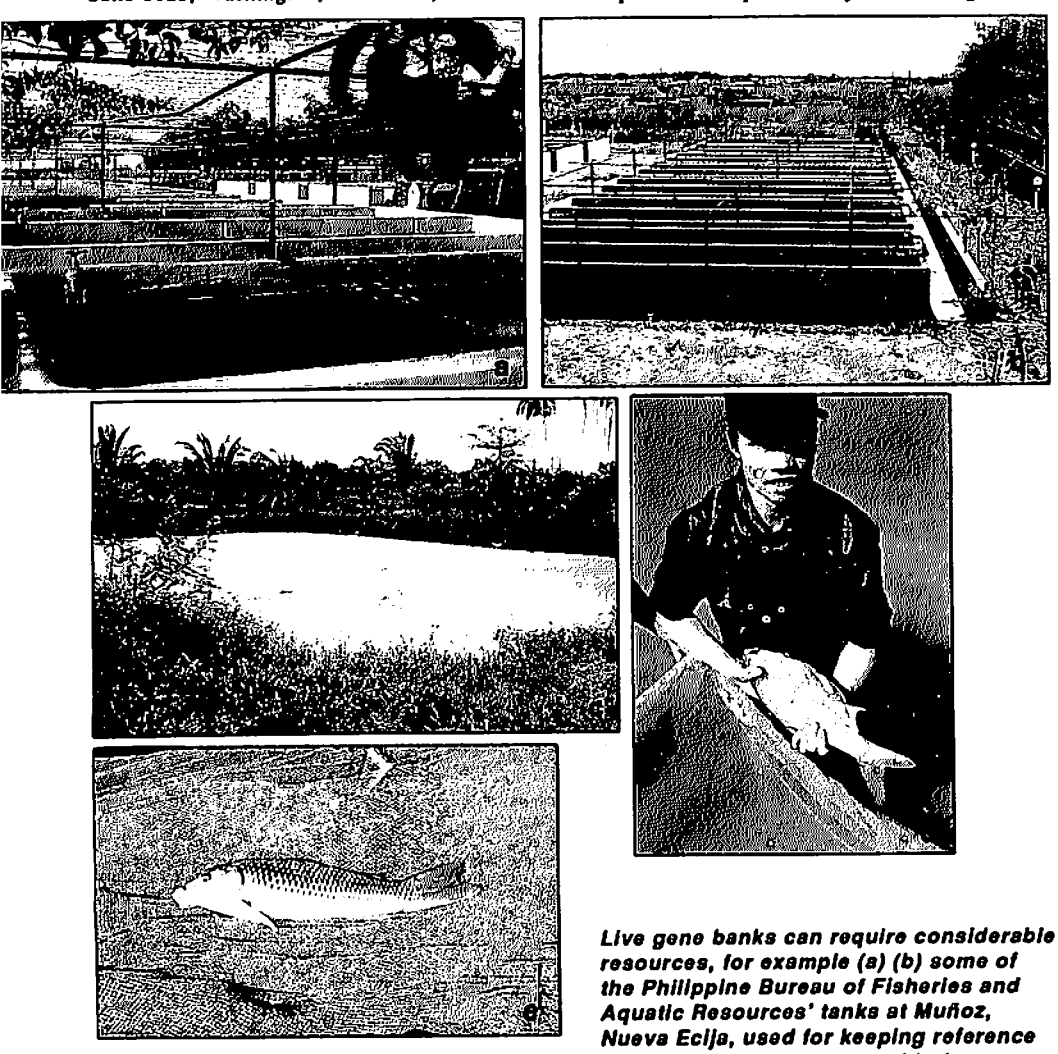
First, many species are reticent to mature and to spawn in captivity. It is often difficult to simulate in captivity the environmental conditions and cues/triggers that are necessary for successful reproduction; particularly for habitats such as rivers, coral reefs, open waters and the deep sea. Very occasionally, this difficulty can be turned to advantage. For example, the most prominent farmed species among the Chinese and Indian major carps mature in ponds but spawn only in running water after injections of exogenous gonadotropins or releasing hormones to stimulate their own pituitary glands. This means that captive broodstock of these species can be kept *together* in the same stagnant ponds. Separate facilities would be more expensive. These mixed populations can be separated and spawned artificially in special flowing water tanks when required. This does not apply to the common carp and some other cyprinids that spawn naturally in ponds and tanks, provided that required substrates (such as vegetation) are present, or to the tilapias, most of which spawn readily in tanks, ponds and cages and hybridize readily.

Second, many aquatic species have very high fecundity: often millions of eggs per spawning female. Hence, there is high mortality in captivity as in the wild, especially in early life history stages: fertilized eggs, larvae, postlarvae, fry, and fingerlings. This involves *natural* selection to the conditions of captivity, in addition to any artificial selection that may be applied.

Third, high fecundity and the high costs of keeping aquatic organisms encourage farmers to use small broodstock populations. This can lead to inbreeding and negative consequences for farmed or released stocks. For example, Eknath and Doyle (1985) found that Indian carp hatcheries had effective breeding numbers (N_e) ($N_e = 4 (♂) (♀)/(♂) + (♀)$) of only 3 to 30 and accumulated inbreeding of 2 to 17%[2].

Ryman and Laikre (1991) summarize the likely deleterious effects of captive supportive breeding in lowering the genetically effective population size of organisms in open systems. This is an important consideration for the stocking of hatchery-produced fish into open waters (so-called culture-based or enhanced fisheries, which are now enjoying a revival of interest) and to mass accidental escapes from fish farms. Day (1989) has summarized work on populations of the Sonoran topminnow *(Poeciliops occidentalis)* that were bred in captivity for restocking natural waters and became highly inbred.

Some texts, e.g., Jhingran and Pullin (1988) recommend a rule of thumb that a broodstock should comprise at least 50 breeding pairs. Tave (1986) and Smitherman and Tave (1987, 1988) treat N_e requirements more strictly: for example, "Requests to replicate reference



Live gene banks can require considerable resources, for example (a) (b) some of the Philippine Bureau of Fisheries and Aquatic Resources' tanks at Muñoz, Nueva Ecija, used for keeping reference collections of tilapia species, but soon to become a quarantine facility with the collections transferred to new custom-built ponds. Some institutions are making do with simple ponds, for example (c) a broodstock pond for native fish species at the Mekong Delta Fisheries Development Centre, part of the Research Institute for Aquaculture No. 2, Ho Chi Minh City, Vietnam. Valuable genetic resources are sometimes kept and bred with minimal resources and great dedication, for example (d) Vietnamese silver carp (Hypophthalmichthys harmandi) and (e) Vietnamese common carp (Cyprinus carpio) a variety known as 'white carp' at the Research Institute for Aquaculture No. 1, Hanoi, Vietnam. (Photos by the author)

[2]See Eknath, A. 1991. Simple broodstock management to control indirect selection and breeding: Indian carp example. Naga, ICLARM Q. 14(2):13-14.

populations ... should be filled by spawning 145-250 pairs in nets or tanks, which is an N_e of about 390-500".

In practice, most broodstock collections consist of fewer fish for logistical and cost reasons. For example, an important and well-established cyprinid live gene bank at Szarvas, Hungary maintains 50 individuals (25 ♂, 25 ♀) for 13 Hungarian and 12 other European and Asian common carp races, and three hybrid populations.

Cryopreservation

Erdahl (1982) summarized the relevance of cryopreservation to aquaculture and the present state-of-the-art remains similar: relatively easy cryopreservation of finfish semen but no success with their ova or embryos. Pursel and Johnson (1989) reviewed cryopreservation of animal germplasm and mentioned successes in cryopreservation of semen (at -196°C) from 25 species of finfish. This is a fairly simple technique that allows the establishment of haploid gene banks for finfish.

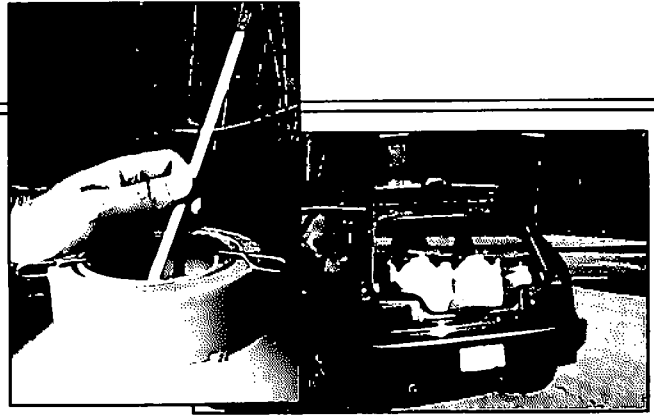
Recently, exciting advances have been made with organisms other than finfish. MTL Biotech Ltd., of Victoria B.C., Canada is a leading company in this field. It produces frozen marine algae (*Thalassiosira* and *Tetraselmis*) and eggs and early embryos of bivalve molluscs (e.g., *Mytilus* and *Crassostrea* spp.) which are all viable on thawing. These are used as feeds in fish hatcheries, but the technology is simple and could also be applied to gene banking. The same company anticipates future cryopreservation successes with crustacean embryos.

The Future

Ex-situ conservation can be expensive, but can also be a useful complement to *in-situ* efforts. It will definitely be an important part of future strategies for maintaining biodiversity of aquatic organisms, particularly for some of the species from inland waters and vulnerable marine habitats (e.g., coral reefs) that are most threatened by the impact of human interventions.

Decentralized, replicated live collections are possible in aquaria, educational and research establishments and aquaculture facilities, including private sector farms and hatcheries.

As field collection and freezing of finfish



Mobile cryopreservation 'hardware' - liquid nitrogen dewars in a car (right) and one 'unit' of cryopreserved Salmon sperm. The dewars contain enough units to fertilize two million ova. (Photos courtesy of IFGB)

semen become more commonplace, cryopreservation of spermatozoa will probably become increasingly important in conservation and in breeding programs. Cryopreservation of ova and embryos is much more difficult and, in the foreseeable future, will probably be possible only for a few invertebrate groups that have small eggs.

The overall problem is that the clear need to conserve aquatic biodiversity for use by future generations is not yet matched with clearly defined sets of present end-users who can and will pay for the required research development and for sustained activities. Aquaculture and enhanced fisheries are not yet widely developed in most countries. Their development will, hopefully, be accompanied by cost-effective means of conserving aquatic germplasm, *in-situ* and *ex-situ*.

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