NETWORK OF AQUACULTURE CENTRES IN ASIA-PACIFIC

Expert Consultation on Genetic Erosion Risk Analysis for Shrimp Diseases in Asia

PROCEEDINGS OF THE WORKSHOP

Maruay Garden Hotel, Bangkok, Thailand

13-14 November 2013

Prepared by the NACA Secretariat

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CONTEXT OF THE WORKSHOP

Shrimp aquaculture in tropical regions is facing a disease-induced catastrophe of lost production. It is estimated that more than 40% of tropical shrimp production is lost to disease annually. The devastating impacts of disease on lost incomes, livelihoods, increased operational costs, trade restrictions and loss of consumer confidence has been a subject of many consultations and policy dialogues. Discussions of disease crisis have to date been largely focused on identification of pathogens, guidelines and standards for disease detection and surveillance, regulations to limit trans-boundary movement of animals, and adoption of better management practices.

There is reason to believe that current broodstock management practices may induce genetic erosion that increases susceptibility to disease and vulnerability to epizootics:

1) Broodstock management as it is currently conducted in SE Asia, particularly by secondary and small-scale hatcheries, is likely to cause rapid accumulation of inbreeding and loss of genetic diversity ("genetic erosion") at farm level;
2) Inbreeding increases susceptibility to diseases and lowers the threshold for the outbreak of epidemics. This effect may be especially strong in shrimps;
3) Separately from its correlation with inbreeding, declining genetic diversity also increases the incidence of epizootics (the monoculture effect) and impedes the ability to adapt to stressful environments and changing climate;
4) These epidemiological effects of climate stress and inbreeding are likely to be multiplicative;
5) The possible role of genetic erosion in the incidence and prevalence of diseases and epizootics are not included in current discussions of the disease problem in tropical aquaculture.

The basic tenet for this Expert Consultation is that an important aggravating factor in the disease crisis is an agro-economic system that locks shrimp breeders, hatcheries and farmers into behaviour that induces high levels of inbreeding. If inbreeding does increase the severity and frequency of epidemics, this disease crisis will only get worse over vast areas of Asia, Central and South America, Africa and the Middle East until it is addressed.

This Expert Consultation was organized in conjunction with the annual meeting of the NACA Aquatic Animal Health Advisory Group (NACA-AG) to take advantage of the physical presence and expertise of a small group of world renowned Aquatic Animal Health experts from several national and international institutions. The list of participants and workshop agenda are presented in Annexes 1 and 2. This consultation is perhaps the first of its kind to bring together a balanced group of experts from diverse fields – epidemiology, microbiology, disease diagnostics & surveillance, aquaculture genetics, fish breeding, and evolutionary biology – to take a fresh, in-depth, and wider perspective on the possible interaction between genetic side-effects of broodstock management and the looming threat of aquatic animal diseases, in particular the contemporary shrimp disease crisis.

OBJECTIVE

The objective of the workshop is to evaluate the genetic erosion – disease connection in the light of the evidence currently available. The primary output is a preliminary appraisal plus recommendations for follow-up study.

DEFINITION OF GENETIC EROSION

The phrase “genetic erosion” was used informally during the workshop to include a variety of genetic phenomena including:

- inbreeding and inbreeding depression;
- loss of genetic (allele) diversity;
- loss of genotypic diversity (monoculture);
- loss of population diversity (replacement of many regional strains by a few, worldwide strains;
- loss of ability of domestic broodstocks to evolve further; and
- possible evolution of negative correlations between useful traits such as rapid growth and disease resistance.

All of these aspects of genetic erosion are likely to be occurring to some extent in aquaculture species, but discussion in the workshop mainly focussed on inbreeding and its effect on susceptibility to infectious disease and other stressors.

EXTENT OF BROODSTOCK COPYING: CURRENT KNOWLEDGE

A proportion of *Penaeus (Litopenaeus) vannamei* farm production comes from hatcheries that "copy" from other hatcheries by breeding animals collected from farm grow-out ponds. The resulting postlarvae (PLs) will be usually be inbred (often highly inbred), have low genetic diversity and are not specific pathogen free (SPF). It is also likely that some hatcheries are mixing legitimate, improved broodstock with farm-reared or 'copy' broodstock (http://www.enaca.org/modules/news/article.php?article_id=2001&title=artisinal-aquaculture-in-a-genetic-plunge-towards-extinction).

Workshop participants indicated that in Thailand, most copy hatcheries obtain breeders by purchasing animals from farmers who stock seed from legitimate hatcheries (first generation copies). Copy hatcheries only use first generation copies because they have found that serial copying results in slow growth. This places a limit on how much genetic erosion can take place. Copy breeders are chosen from farm ponds where growth is exceptionally good, although the animals in those ponds cannot be genetically superior to other ponds. Thai participants in the workshop estimated that 40% - 60% of farm production may come from copy hatcheries.

In India, the supply of PLs from legitimate hatcheries and legal imports falls far short of demand. Participants in the workshop estimated that the greater part of production must therefore come from copy hatcheries, possibly second- or third-generation copies.

In China, most the legitimate hatcheries use high-quality breeders imported from abroad. *P. vannamei* family breeding programs in China are mainly research-oriented at the present time. Copying does occur but the ratio of production from legitimate (imported) breeders and copied breeders could not be estimated by workshop participants, although it was thought to vary among regions.

***Consensus of the workshop*:**
Copying is widespread but varies greatly among countries and regions. More and better data are needed.

EXTENT OF BROODSTOCK COPYING AND GENETIC EROSION: MORE AND BETTER DATA

1) Copying may be inferred – quantitatively but very approximately – from the difference between total production by a country or region and PL production from imported or local high-quality broodstock. Although crude, such estimates should be useful as an indication of where better data should be collected.

2) Copies may be marketed as being from a breeding company's "improved line" when they actually are not, and may be inbred compared with improved lines as well as having lower genetic diversity. However, PLs from copy and legitimate hatcheries will have distinctly different genetic marker "signatures" in a batch of PLs, as was described during the workshop. These signatures are not technically difficult to observe by using microsatellites.

3) Genetic erosion at regional levels can be estimated by proper analysis of microsatellite or SNP survey data, including estimates of allele diversity, various types of F-statistics and related statistics and, because copying can be considered an extreme form of recent bottlenecking, multilocus linkage disequilibrium.

Note: A draft of a simple, numerical estimator of the extent of copying which uses currently available information (estimator 1) was prepared by Prof. Doyle immediately after the workshop. This estimator, provisionally called NACA Genetic Erosion Rapid Appraisal Protocol (NGERA), is presented in Annex 3.

***Consensus of the workshop*:**
Copying will result in genetic erosion but there is no quantitative estimate of the magnitude of loss caused by copying in any Asia-Pacific farm environment. There was agreement that the above estimation procedures are a promising basis for data collection and analysis.

PRELIMINARY DATA ON PERFORMANCE OF PLS FROM COPY HATCHERIES VS. LEGITIMATE HATCHERIES

One participant indicated that preliminary analysis of a farm survey shows that ponds stocked with PLs from backyard hatcheries grow more slowly but survive better than ponds stocked with PLs from legitimate hatcheries. This surprising finding was challenged by the other participants, who suggested many possible causes other than genetics. For instance, poor survival of nauplii in the copy hatcheries that pre-selects for survival at later stages. Animals that grow rapidly have a higher ingestion rate of pathogen-laden detritus and therefore a higher exposure to pathogens, etc.

***Consensus of the workshop*:**
This is a highly important observation if it can be replicated, but its genetic significance is unclear at present.

INTERACTION BETWEEN INBREEDING AND STRESS, INCLUDING STRESS FROM PATHOGENS

Evidence from a wide range of other taxa (e.g. *Drosophila*, mice, salmonids) consistently shows an inverse relationship between inbreeding and survival when exposed to pathogens, environmental stress, competition, temperature etc. A meta-analysis by Fox and Reed[1] suggests that inbreeding increases mortality from most or all causes in most or all organisms studied so far. Hybird *P. (L.) stylirostris* in New Caledonia[2] were shown to have much higher growth and biomass (2.5x) relative to inbred pure lines, when environmental conditions were poor. The difference was much less when environmental conditions were good.

There have been studies of inbreeding in shrimp but these have, for the most part, been carried out under benign conditions that do not provide evidence on the interaction between inbreeding and environmental stress, including stress from pathogens. Interpretation of a published viral challenge study in inbred shrimp showed that mortality increased with inbreeding, much faster than most other organisms in the Fox and Reed (2010) meta-analysis. Similar results have been found in conifers and oysters which, like shrimp, have very high fecundity. It is possible that inbreeding could increase rapidly but silently over a number of years when farming conditions are good, and only becoming evident when a serious stress is introduced, such as a novel pathogen, or a pathogen during poor weather.

***Consensus of the workshop*:**
Although direct data are scarce it is likely that shrimp, like other animals, suffer inbreeding depression (loss of viability and reproductive ability) under stressful conditions, including pathogen stress.

On **effect of inbreeding on susceptibility to disease**: As susceptibility (or vulnerability) to a pathogen increases for any reason, there is a reduction in the threshold for outbreak of an epizootic, according to conventional theory and experience in epidemiology. This was accepted as a truism by the participants.

DATA RELATING COPYING PREVALENCE AND EPIZOOTICS

Copying, including serial copying, is likely to be more prevalent in some areas than others. Areas with severe genetic erosion were referred to as "genetic slums" during the workshop. Whether the origin, frequency or severity of epizootics is related in time or space to the existence of genetic slums is not known, but could be ascertained from basic survey data using any of the methods outlined above.

[1] Fox, C.W. and D.H. Reed. 2010. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65 (1):246-258.
[2] Goyard, E., C. Goarant, D. Ansquer, P. Brun, S. de Decker, R. Dufour, C. Galinié, J.-M. Peignon, D. Pham, E. Vourey, Y. Harache, and J. Patrois. 2008. Cross breeding of different domesticated lines as a simple way for genetic improvement in small aquaculture industries: Heterosis and inbreeding effects on growth and survival rates of the Pacific blue shrimp *Penaeus* (*Litopenaeus*) *stylirostris*. Aquaculture 278 (1-4):43-50

OTHER DISEASE-RELATED RISKS FROM GENETIC EROSION

Monoculture. Loss of host (shrimp) genotypic diversity was mentioned as a risk factor from genetic erosion, as it is known that the likelihood of an outbreak of an epizootic increases as host genotypic diversity goes down[3].

Loss of adaptive capacity. Whether the genetic mechanisms that lead to reduced fitness of inbred organisms involve primarily, dominance, over-dominance, or epistasis was not discussed during the workshop. However, it was noted that genes that might be beneficial in specific situations (e.g. resistance for a specific, newly emerging disease) but are not recognized as such can be lost by genetic erosion and become unavailable for adaptive response to an epidemic; that is, erosion could prevent or delay recovery from an epizootic.

EFFECT OF GENETIC EROSION ON FREQUENCY OR SEVERITY OF EPIZOOTICS

Some participants accepted the hypothesis that motivated the workshop, namely, that significant genetic erosion is occurring in the farm environment and, through the effect of inbreeding under stressful conditions, may be increasing susceptibility to disease and the frequency and severity of epizootics. Other participants held that inbreeding depression must be irrelevant because contagious diseases have been breaking out and dying down since shrimp farming began, from many causes known and unknown, before much inbreeding can have taken place.

***Consensus of the workshop*:**
There was no consensus on whether accumulation of inbreeding at farm level affects the frequency and severity of epidemics, or is likely to do so in future.

POSSIBLE ALTERNATIVE REACTIONS TO THE COPYING OF HIGH-DIVERSITY BROODSTOCK

1) Do nothing about copying and wait until – as projected by some -- the environment becomes so contaminated by pathogens that all shrimp farming takes place in biosecure facilities. At that point depression of host disease resistance caused by inbreeding will have less relevance, as indicated in the draft NGERA protocol flowchart.

2) Reduce copying by requiring that all hatcheries have traceable broodstock and provide certificates of origin and genetic quality to farmers who purchase PLs.

3) Reduce copying by providing farmers with information about broodstock quality, whether or not regulations are in force. Farmers are generally aware of inbreeding depression and often ascribe their disease problems to it. They have difficulty basing their decisions on these concerns owing to lack of further information. Farmers cannot be sure the PLs they purchase are not inbred, even when they buy from supposedly legitimate hatcheries. If they were offered a verifiable "certificate of authenticity" by legitimate breeders and hatcheries, farmers might choose to avoid PLs from copy hatcheries even if they are cheaper.

[3] Lively, C.M. 2010. The effect of host genetic diversity on disease spread. American Naturalist 175 (6): 149-52.

Certificates should be verifiable if they are to be effective. It would be simple to verify such a certificate with a commercial kit designed for the purpose.

Even if legitimate breeders are unwilling to provide genetic information, it is not difficult, in principle, for farmers to ascertain whether a batch of PLs in a farm pond are first-generation hybrids offspring of parents that come from just two families of breeders, which is what legitimate hatcheries are normally selling. A kit based on a suite of 5 – 8 microsatellite markers and an accompanying, user-friendly laptop PC program could be designed to do that.

***Consensus of the workshop*:**
Doing nothing is not acceptable. A verifiable certification program could have the desired outcome and should be tried. Standard protocols for testing farm ponds for the genetic signatures of locking and copying should be developed. The current supply of PLs falls far short of demand in many areas, including China and India, and copying is likely to continue until this problem is solved.

IS GENETIC DIVERSITY NOW IN WORLD AQUACULTURE BROODSTOCKS SUSTAINABLE OVER THE LONG TERM?

In a word, yes. The focus of this workshop was not on broodstock genetic diversity *per se*, but on the consequences of sending very restricted sub-sets of the available diversity into grow-out environments where they are likely to be copied, become highly inbred in a single generation and susceptible to disease and other stressors.

Broodstocks in family breeding programs are, in many cases, being maintained so as to reduce the long term rate of diversity loss to a minimum. If low diversity eventually becomes a problem even in these high level broodstocks, solutions are available, including hybridization of stocks from widely different sources and/or gradual, controlled, introgression of new genetic material from wild populations.

Even though all broodstocks will probably have gone through one or more severe genetic bottlenecks, some of them deliberate, this need not necessarily put an end to broodstock evolution over the long term. It is known that genetic mechanisms exist that *increase* the additive genetic variance in populations that have a small number of founders (Barton and Turelli 2004[4]), sometimes (in theory) to a startling extent.

The rate of selection gain is steady or even increasing in *P. vannamei* broodstocks known to participants in the workshop.

***Consensus of the workshop*:**
There was only very brief discussion of this topic, and the consensus was that the world aggregate stock of genetic diversity in domesticated *P. vannamei* broodstocks is probably adequate. In other words, genetic erosion is a reversible feature of current farm management and not a necessary or permanent feature of the farm environment if all domesticated stocks are considered.

[4] Barton, N.H. and M. Turelli. 2004. Effects of genetic drift on variance components under a general model of epistasis. Evolution 58(10):2111-2132.

OVERALL CONCLUSION AND RECOMMENDATIONS

By and large, there was no clear evidence or consensus that shows the direct effect of the copy-hatchery system in terms of genetic erosion among succeeding generations of SPF *P. vannamei*. However, concerns are still there due to (1) the increasing prevalence and emergence of new diseases affecting the shrimp industry in the region, (2) widespread (but as yet un-quantified) copying and inbreeding, (3) the way inbreeding increases susceptibility to stress, (4) the way increased susceptibility to pathogens increases the likelihood of an epizootic.

The different stakeholders involved in the production system of shrimps also expressed their opinions about copy-hatcheries:

- big hatcheries don't like them for reason of IP protection and market share;
- microbiologists don't like them because they destroy the SPF status of shrimp stocks;
- some geneticists don't like them due to concerns of genetic erosion;
- farmers, however, like them because PLs produced from these SPF copies are cheaper compared to those produced from original SPF line; and,
- government is highly concerned about balancing the quantity against the quality of seed supply.

The genetic erosion question clearly needs more data before it can be assessed properly. Discussion results from this workshop brought the copy-hatchery system into sharp focus, for the first time, and had identified a number of potentially serious consequences. Discussion also brought out the value of a verifiable certification program for broodstock sources used for PL production, and standard procedures for assessing the lock-copy status of animals sampled from farm ponds.

With the continuous concern on many important shrimp diseases causing high economic losses among shrimp aquafarmers, further assessment is required on the direct or indirect effect of genetic erosion, especially on PLs supplied to farmers by copy-hatcheries. It is, therefore, recommended that a follow-up meeting on the copy-hatchery system and its various consequences should be undertaken. It is also necessary to find ways in improving the dissemination of PLs to minimize disease, genetic erosion and the interaction between the two, while ensuring the profitability of private sector broodstock development and farmer access to seed.

ANNEX 1

LIST OF PARTICIPANTS

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ANNEX 2

AGENDA

Day 1 (13 November, Wednesday)

13:30-17:00

(a) Opening Program: Messages from **Drs. Ambekar Eknath** and **Roger Doyle**

(b) Plenary Presentation by **Dr. Roger Doyle**, President, Genetic Computation Ltd. "Relationship between real-world broodstock management practices, inbreeding and severity of disease: the BHF – Nexus"

Group Photo

(c) General Discussions (moderated by **Dr. Ingo Ersnt**, Chairperson, NACA AAH-AG) Discussion to focus on terms and items needing clarification or additional support; Preliminary identification of areas of expertise required for follow-up analysis (epidemiology, ecology, genetics, microbiology and socio-economics); Identification of public and private sector donors to support the program

19:00

Workshop Dinner (hosted by)

Day 2 (14 November, Thursday)

(a) Summary of Conclusions from Day 1; Break into working groups to discuss in-depth various aspects of the problem (to be planned with WG leaders to be assigned)
(b) Working Group Meetings
(c) Short Presentation by the Working Groups
(d) Plenary Session: Statement of Consensus; Elaboration of elements for developing a Regional Program of Study and Plan of Action.

ANNEX 3

NACA Genetic Erosion Rapid Appraisal (NGERA) PROTOCOL

Evaluating the effect of genetic erosion on aquaculture production has three components, or tasks that must be accomplished:

1. The prevalence of genetic erosion in a defined geographical area (province, nation, region or global) should be estimated. **The protocol is concerned solely with this component.**

2. The rate or intensity of genetic erosion should be estimated.

3. The effect of genetic erosion on production by various mechanisms, including reduced survival and growth, increased susceptibility to existing pathogens and vulnerability to new ones, increased likelihood of epizootics should be estimated.

During our meeting we found a way to get a quick estimate of item (1), prevalence in a geographical region. Precise estimates will require survey work of various kinds, but a ”quick and dirty” estimate from the difference between broodstock importation and total production might be useful for identifying regions for more detailed study.

Therefore I’ve prepared a protocol for you to consider, provisionally named the NACA Genetic Erosion Rapid Appraisal, or NGERA protocol. NACA is placed at the front of the name because it was a joint creation from our workshop.

The NGERA protocol was written shortly after the workshop. Although it was inspired by the general discussion and depends on information provided by many people, there is no implication that any participant either disagrees or disagrees, as an individual, with the predictions of this simple model. Furthermore, the examples use simulated data to illustrate the effect of a range of input values. They are not intended to represent genetic erosion situation in actual countries.

The first figure below is the template of the protocol and the next two are what typical NGERAs for large production regions might look like.

The four numbers which are needed for a NGERA and suggested standards for getting them:

T. ***total production***, obtained from official government or international publications (metric tons)

A. ***estimated production from imported high quality breeders plus farms controlled by local high quality breeders***, obtained from official documents, corporate reports or information provided by responsible officers (metric tons).

B. **proportion of A** (production from all, known, high quality breeders including imports) that is not biosecure at farm level, estimated by responsible government officers (e.g. provincial fisheries officers). B is probably close to 1.0 in the current system.

C. **proportion of T-A** (farm production from breeders not known to be of high quality) that is not biosecure at farm level, estimated by responsible government officers. C is probably close to 1.0 in the current system.

The **output** of the NGERA calculator is expressed as the percentage of total production (T) that is estimated to have experienced genetic erosion and is therefore exposed to its consequences, whatever they are believed to be. The consequences will depend on how well the PLs are protected from the environment during grow-out.

For instance, “Region A”, shown on the next page, has the 200,000 MT annual production but only 30,000 MT can definitely be ascribed to PLs from (imported) breeders in family breeding programs that prevent genetic erosion. The calculator presumes that the missing production comes from copied breeders. There is no biosecurity at farm level so inbred PLs from copied breeders are exposed to disease and other environment stress during grow-out.

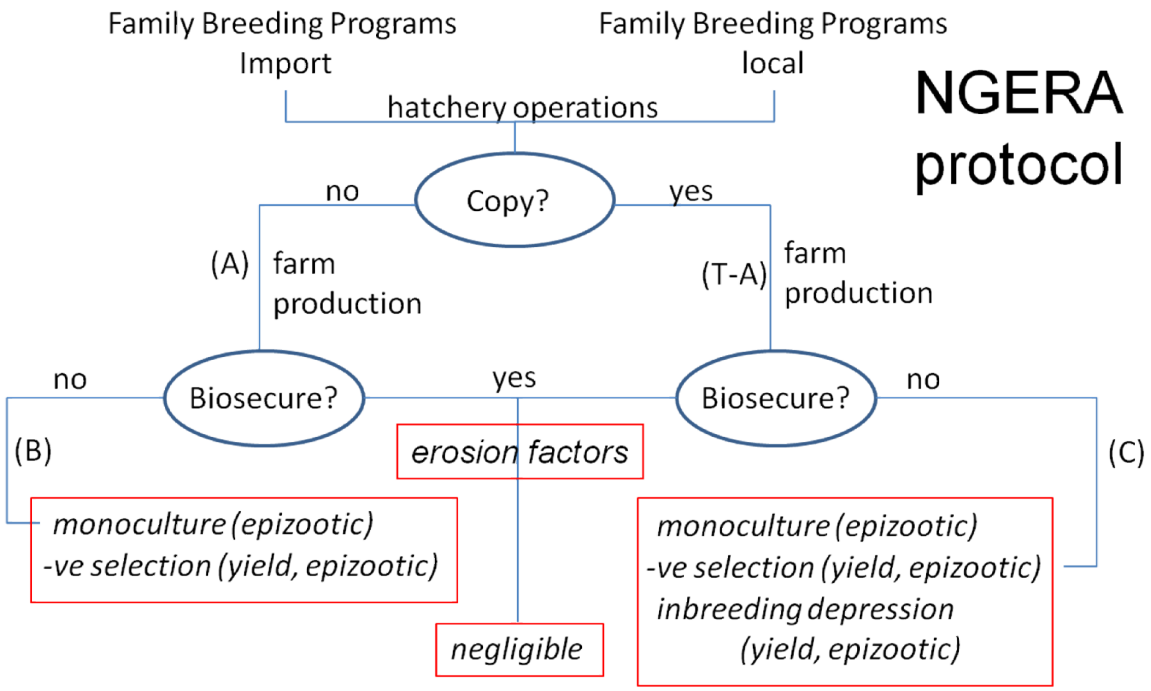
A bit of experimentation with the calculator shows that exposure to genetic erosion largely depends on two factors: the amount of production that comes from copying hatcheries and the exposure of PLs to the open environment during grow-out.

If PLs come from family breeding programs (not copy breeders) they are exposed to the potential consequences of monoculture and reverse selection only. They are not inbred. Consequences may include epidemic risk from monoculture and reverse selection (loss of fitness in open environments correlated with selection in biosecure breeding programs).

If PLs come from copy breeders they are exposed to the potential consequences of monoculture and reverse selection, plus they are inbred and may suffer depressed growth, survival and fecundity, plus increased susceptibility to existing epizootics and emerging epizootics..

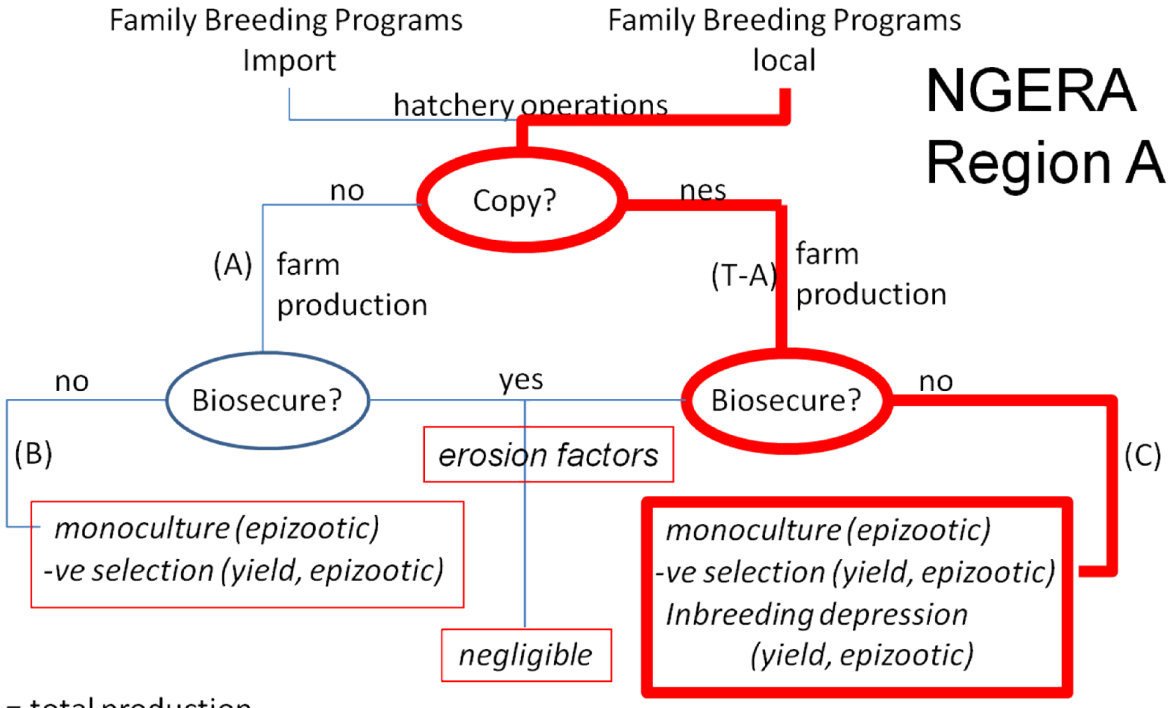
--- See flow charts on following pages ---

FLOW CHART template for all regions



T = total production
A = Estimated production from imported broodstock *plus* farms controlled by FBPs
B = proportion of A that is not biosecure
C = proportion of (T-A) that is not biosecure
A(B-C)/T + C = proportion of T exposed to monoculture & down selection
C(T-A)/T = proportion of T exposed to monoculture, down selection & inbreeding

FLOW CHART for region A



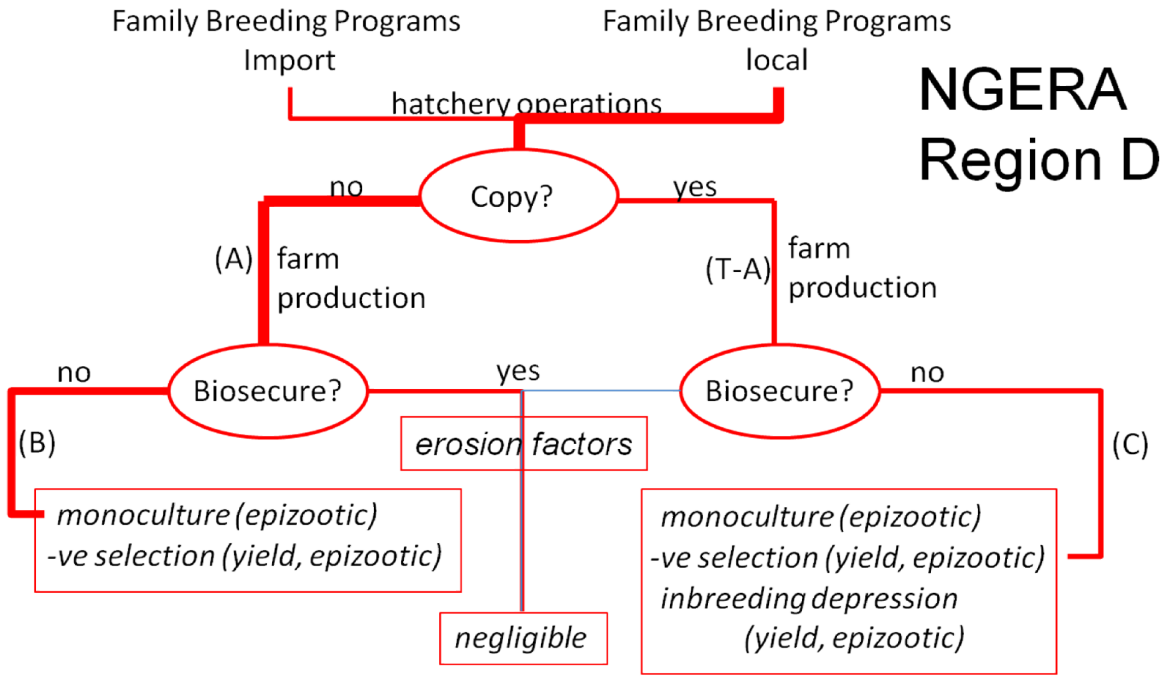
T = total production
A = Estimated production from imported broodstock *plus* farms controlled by FBPs
B = proportion of A that is not biosecure
C = proportion of (T-A) that is not biosecure
A(B-C)/T + C = proportion of T exposed to monoculture & down selection = 100%
C(T-A)/T = proportion of T exposed to monoculture, down selection & inbreeding = 85%

Calculator input and output

NGERA input:	T	A	B	C		GE exposure factors: monoculture + down sel (% total production)	GE exposure factors: monoculture + down sel + inbreeding (% total production)
Region A has high total production, very limited local biosecure family breeding facilities							
and imports a limited number of breeders. There is no biosecure farm growout.							
Region A	**200,000**	**30,000**	**1.00**	**1.00**		**100%**	**85%**

Full Excel file can be obtained from this link.

FLOW CHART for region D



T = total production
A = Estimated production from imported broodstock *plus* farms controlled by FBPs
B = proportion of A that is not biosecure
C = proportion of (T-A) that is not biosecure
A(B-C)/T + C = proportion of T exposed to monoculture & down selection = 94%
C(T-A)/T = proportion of T exposed to monoculture, down selection & inbreeding = 40%

Calculator input and output

NGERA input:	T	A	B	C		GE exposure factors: monoculture + down sel (% total production)	GE exposure factors: monoculture + down sel + inbreeding (% total production)
Region D has high total production, considerable local biosecure family breeding,							
moderate imports of SPF breeders and some biosecure farms							
Region D	**300,000**	**180,000**	**0.90**	**0.99**		**94%**	**40%**

Full Excel file can be obtained from this link.