Is Small-hold Tropical Aquaculture in a Genetic Plunge Towards Extinction?

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Abstract

Tropical shrimp aquaculture is in a disease-induced crisis of lost production. The response to this crisis currently focuses on microbiology and pathology, quarantine, and transboundary transfer of shrimp. The crisis also involves an interaction between shrimp genetics and various human interests including protection of intellectual property. Breeders of high-quality strains generally employ (and are encouraged to employ) some form of "breeder lock" that generates inbreeding when broodstocks are "copied". Smaller hatcheries sell these copied, inbred shrimp to farmers, who thereby increase the likelihood of losing their crops to disease. The joint behavior of breeders, hatcheries and farmers causes inbreeding to accumulate in tropical regions.

The depressive effect of inbreeding on disease resistance is exceptionally strong in shrimp, as shown in a re-analysis of published field and experimental data. Inbreeding increases the severity and frequency of disease through a variety of mechanisms. We have relatively few, marker-based estimates of accumulated inbreeding in any non-pedigreed shrimp aquaculture system. Simulation shows, however, that locked post larvae (PLs) can be distinguished from copies in broodstocks and farm ponds, given appropriate analysis of genetic markers.

Culture of stocks certified to be free of specified pathogens (specific pathogen free or SPF stocks) is strongly recommended and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate, beneficial and necessary. But insofar as they increase the commercial value of proprietary genetic strains, such regulations may also increase the likelihood of copying, and thus inbreeding at farm level and ever-increasing susceptibility to disease and climate stress (Doyle, 2014a).

The intellectual property value of disease-resistant strains will be extremely high and intellectual property rights are fundamental to science-based economic innovation. Breeders will, and must, continue to protect their genetic improvement programs with genetic locks, especially in regions where judicial sanctions are ineffective. The regulatory objective should be to encourage biosecurity and genetic progress while discouraging copying and consequent inbreeding.

The current consensus that inbreeding is unimportant may therefore be out of date. Inbreeding may be amplifying the severity of diseases (including the major current threats: white spot syndrome virus or WSSV, infectious hypodermal and hematopoietic necrosis virus or IHHNV and early mortality syndrome or EMS (acute hepatopancreatic necrosis disease or AHPND). Continuing to ignore the interaction between inbreeding and disease may become a fatal error for tropical shrimp aquaculture.

Keywords: tropical shrimp aquaculture, inbreeding, disease resistance, biosecurity, genetic progress

Introduction

Shrimp production in Asian farm ponds rose continuously from 1992 until 2010, when 2.5 million metric tons were harvested and 45 million people employed (FAO, 2013b). In 2011, a sudden increase in losses from disease caused production to fall, and in 2012, it fell again (Anderson and Valderrama, 2013). In 2012, disease was ranked as the greatest challenge in a global survey of the aquaculture industry (Anderson and Valderrama, 2013) and as much as 40% of tropical shrimp production was thought to be lost to disease (Stentiford *et al.*, 2012).

Social and economic fall-out from this crisis is described in an FAO newsletter (Reantaso, 2012) as, *devastating impacts including direct production losses, therefore loss of food availability; direct and indirect impacts on income and livelihoods/ employment; increased operating costs; restrictions on trade; impacts on biodiversity; loss of market share or investment; loss of consumer confidence, and in some cases, collapse of the sector*.

To date, discussion of the shrimp diseases has focused on the microbiology of aquaculture pathogens and the regulations needed to limit their spread locally and across national boundaries (FAO, 2008; Reantaso, 2012; Jones, 2012; Lightner, 2012). In this essay, I take a wider perspective, that of a geneticist and evolutionary biologist. I argue that the ultimate cause of the crisis is an agro-economic system that locks shrimp breeders, hatcheries and farmers into behavior that induces critical levels of inbreeding at farm level. The inbreeding manifests itself as increased susceptibility to disease and frequency of epidemics over vast areas of Asia, Central and South America, Africa and the Middle East. This global disease crisis may therefore continue to get worse until its roots in human behavior are addressed.

Agro-economic system that leads to inbreeding in farm shrimp populations

Interactions between breeders, hatcheries and farmers

The (aquacultural) agro-economic system of tropical shrimp farming comprises a transfer of genetic material, in the form of adult spawners, juvenile shrimp and post-larvae (PLs), through a network of interconnected transactions between breeders, hatcheries and farmers (Doyle, 2014b). These relationships must be described here in some detail because, while they are central to the proposed mechanism that links agro-economics, genetics and an agricultural disease crisis, they may be unfamiliar to many readers. Figure 1 shows the relationships in a diagram that describes the essential aspect of relationships in many parts of the world.

At the top of the schematic diagram in Figure 1 is a breeder, either private or public sector, who maintains a broodstock with due attention to genetic improvement and minimization of inbreeding ("family breeding program" (Gjedrem *et al.*, 2012)). The breeder provides broodstock animals as juvenile or adult spawners to a hatchery, which produces the young animals (nauplii or post-larvae) sold to farmers for grow-out.

Spawners sent by breeders to hatcheries generally represent only a fraction of the total allelic diversity in the breeder's own broodstock (Gjedrem *et al.*, 2012; Rye, 2012). Often, the subset supplied to

a hatchery comprises only two full-sib families of spawners, each containing thousands of brothers and sisters. The intention of the breeder is that the hatchery will produce post-larvae (PLs) by mating these animals according to instructions that specify which spawners to mate together to produce high-quality offspring for sale.

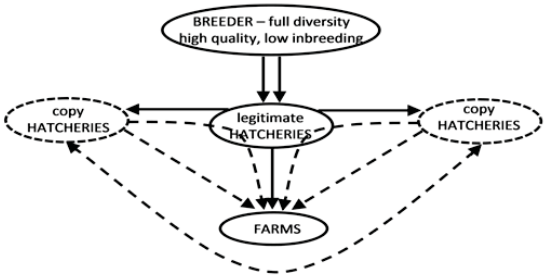

Figure 1. Gene flow through the lock-copy interactions among breeders, hatcheries and farms.

PLs flow onwards from hatcheries to farmers along two channels, only one of which is optimized by mating instructions from the breeders. Called here "legitimate" and shown as solid arrows in Figure 1, PLs in this distribution channel are intended to have maximal uniformity and minimal inbreeding. The flow of genetic material along the solid arrows in Figure 1 is similar to that recommended as good practice by Ponzoni *et al.,* (2012) in their Figure 3.

The "copy" distribution channel shown as dashed arrows in Figure 1 carries PLs or spawners that are diverted from the legitimate channel – either by the hatchery itself or by farmers – and grown to maturity as broodstock in "copy hatcheries". The offspring of these copy spawners will be inbred to varying degrees depending on the genetic composition of the legitimate channel at the point where diversion takes place. Moreover, hatcheries tend to spawn as few shrimp as possible due to the fecundity of shrimp, and often use a highly skewed sex ratio because this enables them to maintain fewer brood shrimp (FAO, 2008). The inbreeding level in the copy channel is expected to range from 0.125 to 0.25 among PLs in the first generation in copy farms and as high as 0.375 in the second (Figure 2) and Doyle (2014b).

The "breeder lock" that generates inbreeding

The primary objective of the legitimate channel (solid arrows) in Figure 1 is to provide highly uniform, non-inbred seed. Aan important secondary objective is to protect the breeder's intellectual property because breeding programs are expensive and breeders protect their investment in various ways, both contractual (Ogden and Weigel, 2007) and biological. The most widely used biological defense against copiers is the "breeder lock", a mating scheme that produces highly inbred offspring in the copy channel (Doyle, 2014a, 2014b). There are many possible types of breeder lock (Sellars and Preston, 2008; Janhunen *et al.*, 2012) but the simplest is probably the one illustrated in Figure 2, which has been reproduced with some changes from Doyle *et al.* (2006). Batches of many millions of seed animals are frequently descended from just two pairs of grandparents, or four full-sib families of grandparents, as shown in Figure 2. PLs from hatcheries that propagate seed according to instructions provided by the breeder normally give good results. However, seed produced by copiers are, as the breeders intend them to be, inbred and give poor growth and survival (Doyle *et al.*, 2006; Sellars and Preston, 2008; Gjedrem *et al.*, 2012; Janhunen *et al.*, 2012; Ponzoni *et al.*, 2012).

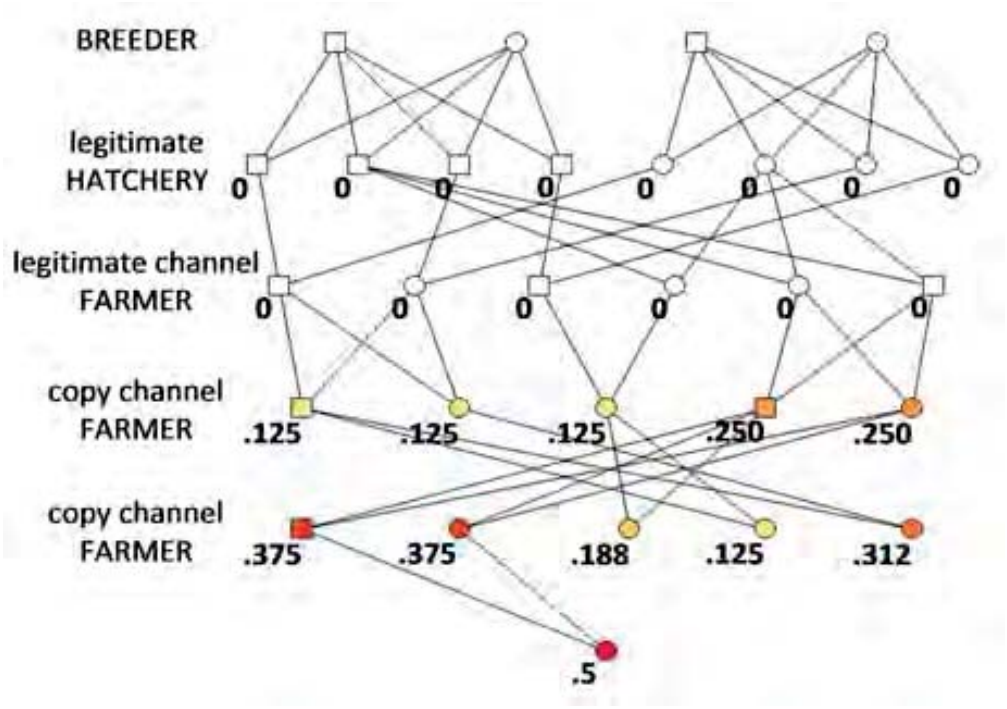

Figure 2. Increase of inbreeding in copied PLs. At the top of the diagram are two pairs of grandparents in the source broodstock. Kinship between these pairs (source kinship) is unspecified but breeders strive to keep it low. The numbers show inbreeding (F), additional to that from source kinship, at successive generations along lines of descent to hatcheries and then to farmers. F=0.125, equivalent to offspring of double first cousins; F=0.25, full sibs; F=0.5, self-fertilization. The diagram is merely a schematic: actual levels obtained by locking a real broodstock will depend on other operational factors such as the number of offspring in each family, sex ratios etc.

The breeder lock in one form or another is widely used, defended and recommended for protecting the intellectual property of breeders (Doyle *et al.*, 2006; Ponzoni *et al.*, 2012).

Copy hatcheries disseminate inbred shrimp

In the world of tropical aquaculture, an improved strain is generally copied shortly after it appears. Due to the high reproductive capacity of fish and crustaceans, unauthorized reproduction and use of improved stocks tend to be widespread for many species (Rye, 2012). Other hatcheries propagate the strain and sell later generations to farmers. They also mix inbred, copied animals with animals in the legitimate distribution channel and sell the mix to unsuspecting farmers, as illustrated in Figure 1. These activities have been grouped with poor broodstock management as "malpractice" (Ponzoni *et al.*, 2012). When hatcheries copy from breeders or other hatcheries, there is an immediate, large decrease in genotypic diversity and a large increase in inbreeding. Copying hatcheries receive only a fraction of the genetic diversity possessed by breeders even when there is no lock. This is ultimately due to the high and variable fecundity of shrimp, which allows very few females to produce enough offspring to stock a farm or provide the next generation of breeders (FAO, 2008). Cumulative loss of genetic diversity over time and during transfers is well documented in shrimp (Benzie, 2009) and other aquacultural species (Doyle *et al.*, 2001).

Although hatcheries in the copy channel may try to circumvent the locks by mating males and females from different hatcheries in the legitimate channel (Fig 1), this tactic is mostly futile. There are generally very few breeder companies – often only one – supplying the legitimate channel with non-inbred stock in any one aquacultural region (Ponzoni *et al.*, 2010; Ponzoni *et al.*, 2012). The breeder usually tries to supply all its client hatcheries with spawners from the same limited group of broodstock families. Again, this helps protect intellectual property as well as supply PLs from top-quality broodstock families.

Estimates of the global extent of copying

Most production of penaeids now depends on domesticated stocks (Stentiford *et al.*, 2012). As *Penaeus vannamei* is an exotic species in Asia, Africa and the Middle East, it is entirely dependent on domesticated broodstocks in those regions.

Rye (Rye, 2012; Gjedrem *et al.*, 2012) estimates that production from uncontrolled breeding programs constitutes more than 90% of worldwide hatchery production from all species. There is as yet no individual estimate for any shrimp species, but people directly involved in the tropical shrimp industry believe copying to be substantial. The following "guesstimates" have been offered as personal communications with permission to cite the source by name: Thailand, conservatively, 50% copied (Mr. Robins McIntosh); Ecuador > 90% copied, Honduras ≈ 50% copied, Mexico > 90% copied, Nicaragua ≈ 50% copied, Panama < 10% copied, Venezuela > 90% copied (Mr. José B. Martinez, Panama.) These estimates are in general agreement with consensus estimates developed during a recent international workshop on the possible connection between inbreeding and shrimp disease (NACA, 2014).

The designation "copied" in the preceding paragraph by no means implies that all copying involves a breeder lock following the highly non-random gene flow in Figure 1. It includes any broodstock that was initiated with restricted genetic diversity and propagated thereafter without a pedigreed family structure to limit inbreeding. A study of genetic erosion in wild and cultivated populations of *Penaeus monodon* here in the Philippines (Xu *et al.*, 2001) provides an exceptionally clear demonstration of this process. Preliminary though it is, the information given above is all we currently have on the extent of copying in shrimp broodstocks. It may be taken as informative to an order of magnitude – that is to say, when properly estimated, production from copied broodstock is likely to be closer to 70% than 7% of the total.

Estimating degree of inbreeding

The obvious way to estimate inbreeding is through surveys of microsatellite diversity in farms receiving PLs through the legitimate and copy channels. The difficulty of doing this might surprise those unfamiliar with the practice of shrimp aquaculture. In idealized, large populations where mating is random the relationship between observed heterozygosity and various definitions of inbreeding is predictable from simple combinatorial rules (Halliburton, 2004). The structure of aquacultural populations is too complex for this approach.

In terrestrial agricultural populations that are divided into sub-groups (breeds, farms, herds etc.), estimates of inbreeding derived from the ratio of observed to expected heterozygosity are often an artifact of unrecognized heterogeneity within samples (Hedrick, 2012; Hedrick, 2013) and works cited therein. Even within a single batch, individuals could on average be either highly inbred or highly outbred, relative to random-mating expectations based on neutral marker data from the same batch, if the batch is part of a breeder locking protocol, kinship-minimizing protocol or some other mating scheme other than haphazard.

Another technical problem is that field estimates from microsatellite markers are usually close to zero, and often negative. Furthermore, low as they are, such estimates are biased upwards by null alleles and unrecognized population substructure. These technical caveats provide another reason for non-specialists to conclude that the impact of inbreeding is likely to be small (Doyle, 2014b).

Although there are notable exceptions (Bierne *et al.*, 2000), most microsatellite estimates actually provide no direct information about inbreeding in aquacultural broodstocks. There are two related difficulties with these estimates. Firstly, the indicators of inbreeding most often reported are deviation from Hardy-Weinberg equilibrium and/or the fixation index, Fis. Secondly, Analysis of Molecular Variance (AMOVA) and simpler procedures for estimating H-W and Fis are usually based on allele frequencies in the same set of samples for which the estimate is made.

The fixation index, Fis, is an indicator of non-randomness in the mating system and thus indicates a potential cause of inbreeding but does not directly measure inbreeding (Templeton and Read, 1994; Waples, 2015). Deliberate non-random mating is rare in aquaculture except in the lock-copy situation shown in Figure 1. Instead, inbreeding in aquaculture broodstocks nearly always accumulates owing to bottlenecks, small population sizes, unequal fecundity and other random processes, rather than deliberate consanguineous mating. "In finite populations, some individuals mate with biological relatives just by chance and inbreeding in the pedigree sense is the result" (Templeton and Read, 1994). The resulting inbreeding will not produce a significantly positive Fis so long as mating is random. Even the offspring of a population of full-sib brothers and sisters, Fped = 0.25, shows neither H-W deviation nor positive Fis if the siblings mated at random.

The second difficulty with Fis and related indicators of inbreeding/non-random mating arises when allele frequencies are calculated from the samples for which estimates are to be made. "Fis is defined with respect to the populations that are included in the sample, either through population-specific estimates or through the average of those estimates" (Holsinger and Weir, 2009). The practical and conceptual difficulty arising from this is beautifully explained by Wang (2014), whose paper should be studied by anyone seeking further information. "Frequently genotype or allele frequency data are unavailable from an ancestral population, and allele frequencies used in calculating relatedness have to be estimated from the current sample in which relatedness between individuals is being calculated. This practice effectively uses the current

population (sample) as reference, and an estimator conforming to the correlation concept of relatedness should give an average estimate of zero. This is true regardless of the actual relatedness among individuals in the sample, as shown by simulation (Doyle, 2014b) and analytical results in Wang's 2014 study."

This may surprise non-specialists who believe that inbreeding is likely to be unimportant when in fact we have hardly any direct evidence concerning inbreeding in hatcheries that lack pedigree data.

The obvious solution to both difficulties is to use maximum likelihood estimators such as Wang's trioML rather than Fis, and base inbreeding estimates on reference allele frequencies from an earlier generation, as in the trioML(B) estimator of Figure 2. An appropriate reference is the generation that would be called the founder of the pedigree had pedigree records been kept. A survey of *P. vannamei* broodstocks in Mexico (Perez-Enriquez *et al.*, 2009) is a good example of this approach. For their study of *P. stylirostris* in New Caledonia, Bierne *et al.,* (2000) used published data from wild populations of other penaeid species – a daring move.

It is also possible to estimate inbreeding – inaccurately, for many reasons – from estimates of gene diversity or heterozygosity in the reference generation and the harmonic mean of the effective population number over the intervening generations if these quantities happen to be known, e.g. in Coombs *et al.,* (2009). If data from two or more generations can be obtained, a variety of inbreeding estimators are available e.g. in Hoehn *et al.,* (2012), Waples (2015) and references therein. The reference should, ideally, include only animals that actually contribute to subsequent broodstock generations, and not all the animals in the reference population, many of which may not have reproduced (Lacy, 1995).

At levels of population structure higher than the batch, e.g. farm, hatchery and geographical region, it is actually more useful to ignore observed heterozygosity (as an uninterpretable artifact) and instead pay attention only to the biogeographically and temporal structure of allele number (Jost, 2008; Gregorius, 2010). As generations follow one another there will be a monotonic, inverse relationship between allele number and accumulated inbreeding in an aquacultural region. The correlation will be mainly due to the passage of time and the ineluctable, irreversible loss of genetic material caused by too-small broodstocks, artificial and natural selection, variable reproductive success, serial transfers of subsets of broodstock from hatchery to hatchery, irrational or careless breeding and deliberate breeder locks. The correlation will grow stronger as time passes, and declining allele diversity will reflect the cumulative erosion of the regional genetic environment for aquaculture.

Microsatellites are not the only markers available. The technology of high-throughput sequencing is developing rapidly and it may soon be possible use high-density genomic data to routinely estimate the inbreeding of individuals with an accuracy close to that obtainable with pedigree data (Li *et al.*, 2011). With a sufficient number of genome-wide markers, the relationship of any pair of individuals can be inferred by estimating their kinship coefficient, independent of sample composition or hidden population structure (Manichaikul *et al.*, 2010).

The most straight-forward – and immediately available – way to estimate inbreeding depression (as distinct from pedigree inbreeding) would probably be to measure it directly, by hybridizing samples of hatchery spawners with an unrelated tester strain or strains. Inbreeding depression would then be evaluated by comparing fitness traits of inbreds and outbreds in standardized stress tests, disease challenges etc.

Inbreeding increases susceptibility to disease and other stresses

Inbreeding depression is the decrease in growth and other traits, most importantly reproductive success and survival that is seen in inbred relative to outbred animals and populations (Lynch and Walsh, 1998). Inbreeding depression is especially severe in environments where survival is low, even in outbred populations, owing to poor nutrition, extreme temperatures, the presence of pathogens or a myriad of other possible stressors alone or in combination (Frankham *et al.*, 2002; Liao and Reed, 2009; Bijlsma and Loeschcke, 2012; Cheptou and Donohue, 2011; Cheptou and Donohue, 2013; Enders and Nunney, 2012; Reed *et al.*, 2012).

Remarkably, data from many plant and animal taxa and many different kinds of natural and artificial stressors can be fitted to a common regression line of inbreeding depression against stress (Fox and Reed, 2010). Stress was defined in Fox and Reed's (2010) meta-analysis as the proportional decrease in survival of outbred individuals in a stressful environment compared to a benign environment. Shrimp, like other animals, are affected more strongly by stress when inbred. Inbred and outbred experimental populations of the mysid shrimp *Americamysis bahia* differed greatly in their survival under low salinity stress, and genetic load was much higher in stressful environments for several fitness indices (Markert *et al.*, 2010). The authors note that this is actually an underestimate of the amplification of genetic load by stress because many of their inbred lines did not survive long enough to be tested.

The fit of *P. vannamei* and other shrimp species to the meta-analysis regression of Fox *et al.,* (Fox and Reed, 2010) cannot simply be assumed. Several aquacultural species, like *P. vannamei* and oysters, have exceptionally high fecundities and "huge" inbreeding loads (Bierne *et al.*, 2000). Inbreeding depression in oysters, which have fecundities on the order of 106 offspring per spawn, has been studied in considerable detail (Bierne *et al.*, 1998; Launey and Hedgecock, 2001; Plough, 2012) and found to be high. The shrimp *P. vannamei* has a fecundity on the order of 10^5 offspring per spawn.

Inbreeding increases mortality from viral disease in P. vannamei

Two viruses, white spot syndrome virus (WSSV) and Taura syndrome virus (TSV), bore most of the responsibility for the global economic loss from disease in shrimp as of 2011 (Stentiford *et al.*, 2012). A new disease, Early Mortality Syndrome disease, EMS/AHPND, has recently become the most serious disease problem facing tropical shrimp aquaculture. EMS appears to be a septicaemic vibriosis involving at least two *Vibrio* species infected by a bacteriophage (Lightner *et al.*, 2013; FAO, 2013a).

Penaeus vannamei is by far the dominant shrimp species in aquaculture (FAO, 2013b; Anderson and Valderrama,

2013). Mortality induced by exposure to both of these viruses has been examined at various levels of inbreeding in a population of *P. vannamei* at the Oceanic Institute, in Hawaii (Moss *et al.*, 2008; Moss *et al.*, 2007). Re-analysis of the Oceanic Institute data reveals that the interaction between inbreeding load in *P. vannamei* and disease stress is significantly stronger than the regression meta-analysis of Fox *et al.,* (Fox and Reed, 2010). High as it is, *P vannamei* inbreeding loads under disease stress are comparable to loads in oysters (Bierne *et al.*, 1998; Launey and Hedgecock, 2001; Plough, 2012).

An important and possibly unique field study shows the effect of inbreeding mortality from disease in the farmed shrimp *Penaeus stylirostris* in New Caledonia (Bierne *et al.*, 2000; Goyard *et al.*, 2008). Both components of yield, mortality and growth, were depressed by inbreeding that accumulated slowly over many generations (not rapidly, as in the lock-copy system described here). Inbreeding depression was especially evident in years when the environment was poor and overall yields low. This work is particularly relevant at this time because the disease affecting *P. stylirostris* was a septicaemia caused by a species of *Vibrio*, the bacterium which has recently been implicated (Lightner *et al.*, 2013; FAO, 2013a) in the current EMS (or AHPND) disease crisis.

Inbred and outbred experimental populations of the mysid shrimp *Americamysis bahia* differed greatly in their survival under low salinity stress, and genetic load was much higher in stressful environments for several fitness indices (Markert *et al.*, 2010). The authors note that this is actually an underestimate of the amplification of genetic load by stress because many of their inbred lines did not survive long enough to be tested.

There appears to be no published experimental data relating inbreeding to disease or any other stress in aquacultural shrimp species, other than the data from Moss *et al.,* (Moss *et al.*, 2008; Moss *et al.*, 2007) and the *P. stylirostris* study in New Caledonia (Bierne *et al.*, 2000; Goyard *et al.*, 2008). Shrimp are routinely challenged for a variety of diseases and other stresses in breeding programs. Usable data must therefore exist unexamined, or at any rate unpublished, in the files of many family breeding program that keep pedigree and mortality records.

Monoculture and the incidence of epidemics

Epidemiological theory (Lively, 2010; Keesing *et al.*, 2006; Keesing *et al.*, 2010; King and Lively, 2012) and observation suggest that the incidence of epidemics is inversely proportional to the genotypic diversity of the host population, a relationship called the *dilution effect or monoculture effect* (reviewed by Ostfeld and Keesing 2012). Increases in the prevalence of infection in the wild are associated with genetic bottlenecking and inbreeding induced by founder effects and/or mating systems (King and Lively, 2012). Small, genotypically uniform populations of endangered species are especially prone to epidemics, as are populations at the edge of a recent range expansion (instances cited in King and Lively, 2012).

The breeder lock illustrated in Figure 2 leads to low levels of genotypic diversity within farms and farming regions. The legitimate and copy distribution channels both contribute to a restriction in genotypic diversity but it is particularly true in the

copy distribution channel. Hatcheries that copy from other hatcheries start with founder populations that will, in many cases, have been deliberately locked. In such cases the low genotypic diversity results both from random founder effects (small sample of available allele diversity) and mating system (founders deliberately related by descent, e.g. double first cousins).

It appears from modelling exercises that small increases in genotypic diversity can cause dramatic reductions in the likelihood of an epidemic outbreak (Lively, 2010). The effect is distinct from increased disease susceptibility through inbreeding depression, although in practice inbreeding is usually associated with low genetic diversity, as explained above. In Lively`s theoretical model (Lively, 2010) the incidence of epidemics is inversely proportional to genotypic diversity in the host population, and epidemics are less severe and die out more quickly in genetically diverse populations. Under the assumptions of some models, small increases in allelic diversity dramatically reduces pathogen load even in very large host populations (King and Lively, 2012). The effect is expected to be greater when the pathogen is not host – specific (Ostfeld and Keesing, 2012). It is therefore worth noting that more than 93 species of arthropods are reported to be hosts of WSSV (Moss *et al.*, 2012), one of the worst shrimp disease viruses.

Verifiable information might persuade farmers to avoid the copy channel

Intellectual property rights are fundamental to science-based economic innovation. Breeders will continue to protect their genetic improvement programs with breeder locks that generate inbreeding when copied, especially in regions where judicial sanctions are ineffective. Farmers are well aware of inbreeding depression, as previously mentioned, and may have a good notion of how broodstocks are managed by copy hatcheries in their local areas. However, farmers often cannot be sure the seed animals they purchase are not inbred even when they buy from supposedly legitimate hatcheries. Their puzzling reluctance to pay more for genetically superior aquaculture stock (Ponzoni *et al.*, 2009; Gjedrem *et al.*, 2012) could be due in part to lack of credible information. This possibility has already been noted (Ponzoni *et al.*, 2012).

If verifiable information by legitimate breeders and hatcheries are available, for example through a national broodstock information exchange network (Doyle, 2015), farmers could more easily choose to avoid the copy distribution channel shown in Figure 1. “Certificates of authenticity” have been offered by some breeders, but this strategy fails when the certificates too are copied. Certificates offered to date have been missing the essential element of verifiability. Verification is technically easy in principle. A certificate from the breeder attesting that a particular batch is 100% heterozygous for two particular alleles at a particular locus (both specified in the certificate) would be sufficient to verify that the batch is a first-generation hybrid, i.e. minimally inbred.

Could breeders be persuaded to provide verifiable genetic information to farmers? Some individual breeders might see a marketing advantage in doing so. As a group, breeders as well as farmers might come to realize the pernicious, cumulative effect of the collective behavior of breeders, hatcheries and farmers on the

whole aquaculture sector and therefore on themselves. In disease-ridden environments even the most modern, isolated and bio-secure breeding facilities are at risk.

Unintended consequences of disease-control policies that ignore genetic side effects

International organizations concerned with aquaculture, including the Food and Agriculture Organization of the United Nations (FAO), are responding vigorously to the disease crisis by developing regulations and codes of practice for transferring aquacultural stocks between and within regions, and also by promoting standard and guidelines for disease detection, identification and surveillance (FAO, 2008; Reantaso, 2012). The culture of stocks that are certified to be free of specified pathogens (SPF stocks) is strongly recommended, and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate and beneficial from a strictly microbiological perspective.

It appears that those concerned with disease control are not yet thinking of the genetic consequences of their policies and recommendations. Neither the word "inbreeding", nor the phrases "genetic erosion" or "host diversity" (pertaining to monoculture effect) appear in any of the discussions of aquaculture diseases and disease-related regulations that I found published in 2012 or in the first half of 2013, totalling more than 300 pages (Murray, 2013; Stentiford *et al.*, 2012; Lightner, 2012; Moss *et al.*, 2012; FAO, 2013b; Chamberlain, 2013; Flegel, 2012; Jones, 2012; Kibenge *et al.*, 2012; Reantaso, 2012; NACA, 2013). Although farmers themselves often blame inbreeding for poor yields from their ponds their concerns have been deprecated and dismissed (FAO, 2008, p. 13).

In this review I suggest that disease crises in tropical shrimp aquaculture may be growing more severe and more frequent owing to an agro-economic system that generates genetic erosion at farm level. Genetic erosion results from a pattern of human behavior in which breeders protect intellectual property through breeder locks (expressed only when broodstock is "copied"), copying hatcheries sell inbred offspring, and farmers stock their ponds with seed animals they are unable to evaluate. The resulting inbreeding and low genotypic diversity increase susceptibility to disease, which leads to more infected individuals and farms and thus, by standard epidemiological reasoning, increases the frequency and severity of epidemics.

The hypothesis is not that inbreeding triggers shrimp diseases – which have myriad environmental and other immediate causes – but that inbreeding increases the prevalence and severity of disease, and that inbreeding is accumulating on regional scales. We may be making a dangerous mistake in treating the torrent of shrimp diseases of the past decade as isolated events with independent, microbiological causes, describable with some (unknown but invariant) distribution of risk. The distribution of risk may be shifting towards higher values in a farming system experiencing genetic erosion.

Culture of stocks certified to be free of specified pathogens (SPF stocks) is strongly recommended and only SPF stocks can now be legally imported into most jurisdictions. These recommendations are appropriate, beneficial and necessary.

But insofar as they increase the value of proprietary, high-quality SPF strains, such regulations may also increase the use of breeder locks and the likelihood of copying, and thus inbreeding at farm level (Doyle, 2014a, 2014b). Intellectual property rights are fundamental to science-based economic innovation. Breeders will, and should, continue to protect their genetic improvement programs with breeder locks that generate inbreeding when copied, especially in regions where judicial sanctions are ineffective. The intellectual property value of disease-resistant strains will be extremely high.

The current consensus that inbreeding is unimportant may therefore be out of date. Inbreeding may be amplifying the severity of diseases, including the major current threats: WSSV, IHHNV and EMS (or AHPND). The regulatory objective should be to encourage biosecurity and genetic progress while at the same time discouraging copying and consequent inbreeding.

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