
Selective Breeding of Specific Pathogen-Free (SPF) Shrimp for High Health and Increased Growth

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Abstract

World shrimp farming depends on seed that is either gathered from the wild or produced in hatcheries by wild-caught spawners. This approach is inherently unreliable. In contrast, great success has been achieved through selective breeding in meat production technologies such as poultry, cattle and swine. It is likely that shrimp farming's future will include selective breeding and domestication. Shrimp diseases have had a devastating effect on shrimp farming in both the United States and around the world. In other meat production industries, modern growers rely on certified virus-free stock to avoid virus-caused diseases. Continued expansion of the global shrimp industry will require reliable supplies of virus-free shrimp.

Based on these assumptions, the U.S. Marine Shrimp Farming Program initiated a project in 1989 to develop reliable supplies of specific pathogen-free (SPF) *P. vannamei* for the U.S. industry. The project includes a selective breeding program to improve the quality of the SPF stocks. This paper describes our approach and initial results in developing a selective breeding program for improving the SPF stock production performance.

Introduction

World shrimp farmers produced over 633,000 MT of penaeid shrimp in 1990 (Rosenberry, 1991). Worth more than \$2.5 billion, farmed shrimp supplied over 25% of the world's demand. Nearly all of this production was derived from seed either gathered from the wild or produced from wild-caught spawners. Because of the inherent instability of this approach and the tre-

mendous success of breeding programs in other meat production technologies, shrimp farming's future will likely include selective breeding and domestication. Shrimp farmers will use improved stocks, bred for optimal performance in culture.

In the last several years, shrimp diseases have had a devastating effect on U.S. and world shrimp farming. Diseases increase risk, deterring invest-

manuscript is a synthesis of the recommendations provided by the advisory group. It also describes progress to date toward establishing a selective breeding program for SPF *P. vannamei*.

Goals of the SPF Breeding Program

The goal of the breeding program is to develop a faster-growing cultured shrimp (*P. vannamei*) through selective breeding.

The specific objectives of the SPF breeding program are:

- To maintain the SPF status of stocks;
- To avoid inbreeding; and
- To improve shrimp growth and survival to market size (20 g).

Establishing the Founder SPF Stock

Building on the SPF concept developed for livestock industries, the SPF shrimp program was initiated in 1989 (Wyban et al., 1992). Since *P. vannamei* is the principal shrimp species cultured in the United States and the rest of the Western Hemisphere (Rosenberry, 1991), it was chosen as the target species for the program.

The definition of what constitutes an SPF shrimp population follows the guidelines developed by the International Council for the Exploration of the Sea for working with exotic species (ICES, 1988). Only disease-causing microbes that can be reliably diagnosed

Table 1. A working list of excludable pathogens of *Penaeus vannamei*.

Group	Pathogen
Virus	Infectious hypodermal and hematopoietic necrosis virus (IHHNV)
Virus	<i>Baculovirus penaei</i> type-A baculovirus (BP)
Virus	Hepatopancreatic parvo-like virus (HPV)
Protozoan	Microsporidians
Protozoan	Gregarines
Protozoan	Haplosporidians
Metazoan parasites	Nematodes and cestodes

and physically excluded from a facility are considered. Diagnosable microbes that cause economically significant disease in *P. vannamei* and that can be excluded from a facility (specific pathogens) are listed in Table 1.

Development of a historical record through ongoing screening is necessary to insure SPF status. Throughout this manuscript, broodstock shrimp that have passed through this rigorous process are referred to as "SPF broodstock." When SPF broodstock are transferred to a commercial facility, they and the nauplii and postlarvae derived from those broodstock are referred to as "high health" shrimp, because their SPF status is no longer certain, and a new historical record for that facility must be established.

In June 1989, postlarval *P. vannamei* were imported from a hatchery in Mexico. Following preliminary SPF diagnosis by histology, these shrimp were shipped to Hawaii and maintained in

quarantine. Bioassays confirmed that these shrimp were IHHN-free and repeated histopathology examinations indicated they were also free of the other excludable pathogens. They were then shipped to OI's SPF shrimp quarantine facility (OI-Keahuolu) on the island of Hawaii. Postlarvae produced from these "tentative" SPF broodstock were diagnosed as SPF, thus confirming the stock's full SPF status. This founding group of SPF *P. vannamei* is referred to as Kona Population 1.

Addition of New Genetic Material

The original population of SPF shrimp probably represents a narrow genetic sampling of the species. To avoid detrimental founder effects, the breeding program was advised to start with the widest possible sampling of the species (Shultz, 1986; Gall, 1990). However, rigorous screening of new stock additions must be employed to protect the valuable SPF status of the first population.

Numerous attempts to acquire additional samples of SPF postlarvae from the wild to expand the gene pool of the SPF stock have been unsuccessful. IHHN virus is now widespread in wild stocks of *P. vannamei* throughout its range (Lightner et al., 1990; Pantoja-Morales and Lightner, 1991; Lotz et al., 1991). A new approach to developing SPF populations involving nondestructive individual broodstock screening using gene probe diagnostic procedures is currently being tested (Lightner, this volume).

A genetic diversity analysis comparing *P. vannamei* within its natural range to Kona Population 1 was recommended (Lannan, 1980) and has been initiated. Researchers from Tufts University and Worcester Polytechnical Institute are using molecular techniques (both nuclear and mitochondrial DNA) for this purpose. Similar techniques have been used in other marine invertebrates (Brown and Paynter, 1991). In addition to providing measures of diversity within the SPF population(s), the molecular techniques used for diversity analysis may also yield "marker genes" that can be used in the breeding program, if they can be correlated with growth.

A comparison of genetic variation in a farmed population of *P. vannamei* with three natural populations across the species range using allozyme techniques found very low levels of variation and heterozygosity in all four stocks, and very low levels of differentiation between the wild populations (Sunden and Davis, 1991). The authors concluded that allele frequencies among populations throughout the species range have little variation. However, traits of economic (breeding) importance may be under different selective pressures across the range, and differentiation between populations or locales could be significant.

Breeding Program Design

In maintaining captive broodstocks, there are two goals that impose conflicting requirements for management. The

first goal requires procedures that avoid detrimental inbreeding of the captive broodstock. The second goal requires genetic manipulation to improve production performance. To preclude conflict, the SPF breeding program consists of two levels: multiple discrete populations and multiple maternal lineage families within each population (Fig. 1). A similar two-level broodstock management program is used in Norway to breed Atlantic Salmon (Refstie, 1990).

Previous selective breeding efforts for reproductive quality indicated that *P. vannamei* responds to selection, and reproductive quality can be improved by selective breeding (Wyban et al., submitted). Realized heritability estimated for several quantitative variables including nauplii/spawn, spawning frequency and hatch rate were compared in two selected families against nonselected controls. Two different full-sib families from the two outstanding reproductive females with the highest hatch rates, mating/spawning frequency, and lifetime nauplii production were reared to adult size. Two independent experiments were conducted comparing the selected families against nonselected controls. Selected females far out-produced the nonselected controls in both experiments, indicating that *P. vannamei* will respond to selection for a quantitative character.

Because much of the fundamental knowledge about inheritance of economic traits in shrimp is unknown, a number of foundation experiments were recommended. Best opportunities

to improve shrimp performance by selective breeding will be verified by systematic, scientific methods to optimize the effectiveness of the breeding program to improve economically important traits for shrimp culture.

The objectives of these foundation experiments are:

- To determine male and female contributions to the quantitative traits, growth and survival;
- To estimate variance components (additive and nonadditive genetic variation, and nongenetic variation) and phenotypic, genetic and environmental correlations.

If there is additive genetic variance for growth rate, individual selection will be applied. Responses from each generation will add to previous gains — as steps in a stairway. If there is heterosis for growth, it will be utilized to add to the effects of individual selection. While crossbreeding must be repeated each generation, it has the advantage that one's competitors cannot duplicate the specific crosses.

A "sub-line" system will be used in the breeding program. A population (resulting from one importation) will be subdivided into multiple, genetically isolated, maternal lineage families. The system is based on the theory that because of inbreeding, genetic variance (V) within a family will go to zero as the inbreeding coefficient (F) goes to 1. As the variance between families in-

SPF Shrimp Breeding Scheme

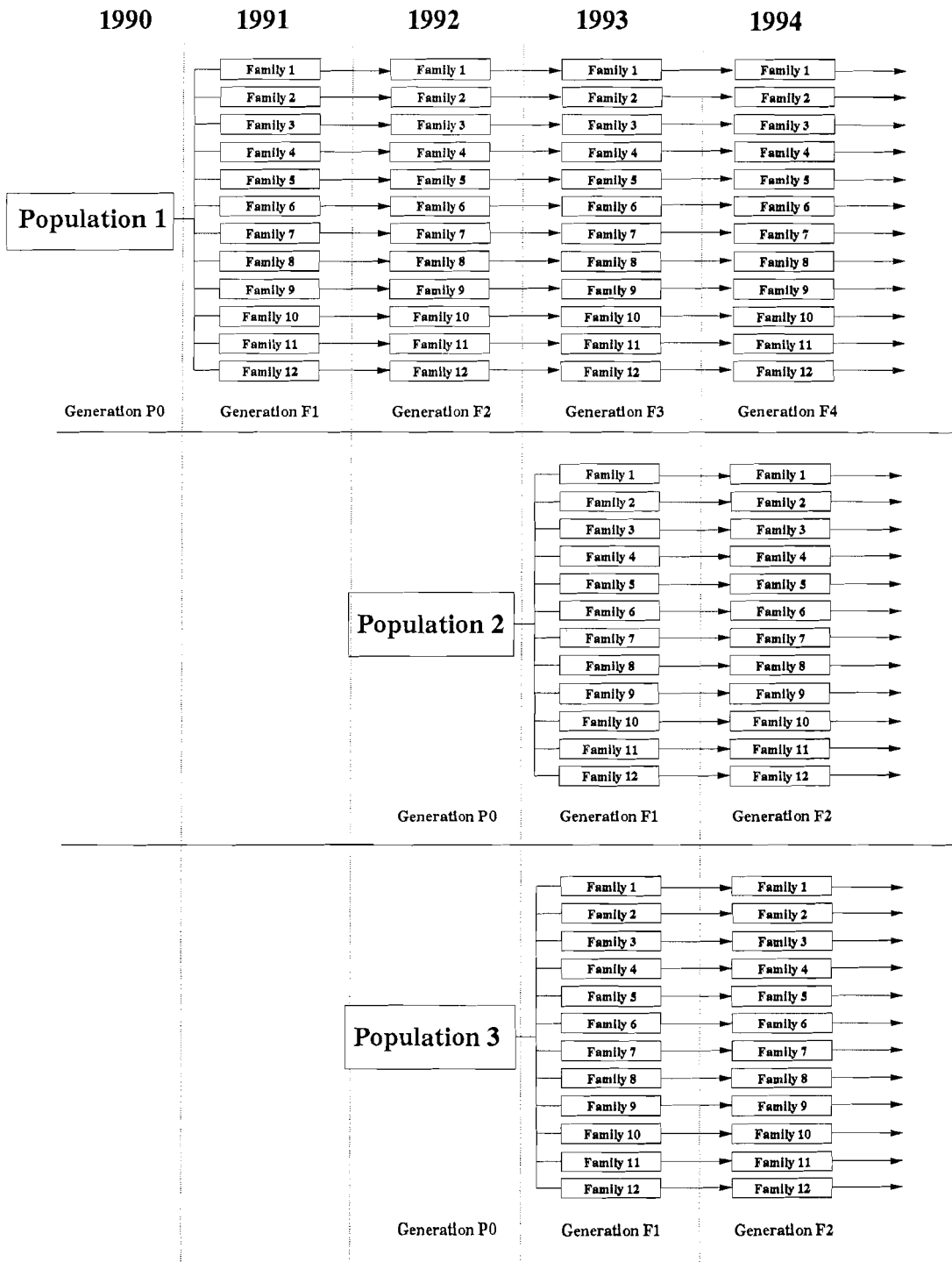


Figure 1. SPF shrimp breeding scheme. Genetically discrete populations are subdivided and maintained in 12 individual families.

creases, the total variance of the population increases to $2V$. In reality, V and F never reach these extremes and selection plays an important modifying role.

SPF Broodstock Pedigrees

Current procedures permit accurate identification of the maternal parent of a cross, but the paternal parent is not determined. Therefore, pedigrees based on maternal families will be maintained for each SPF population. To ensure continuity of the pedigrees, the progeny resulting from the mating of each maternal line will be reared in separate tanks up to market size, when they are large enough to be individually tagged with bird bands. The families will then be combined and mass reared in broodstock ponds.

Mating Scheme

In each generation, two selected females from each maternal line family will be mated with two males chosen at random from the broodstock, and no male will be mated to more than one female. The following mating plan will ensure that the effective population size is maximized:

1. Stock four females from each of 12 families, and four males from each of 12 families into maturation tanks.
2. Collect all females mated during an active one-week period (to eliminate multiple matings by a single male); spawn at least one female from each family and stock individual larval-rearing systems.
3. For families that have not successfully reproduced, artificial insemination will be used with randomly chosen males to produce progeny from the remaining lines.
4. Nauplii from each family will be reared in separate 20-L buckets to PL10 stage. Each family will then be moved outdoors and reared separately in 5,000-L tanks up to 15 to 20 g, when individuals will be tagged with bird band eye tags.
5. At tagging size, the largest 20% of males and 20% of females in each family will be selected and tagged; smaller animals will be discarded.

Selection for Growth Rate

Improved growth rate to market size is the number one breeding priority for production cost reduction (Gjedrem, 1985). Sensitivity analysis of breakeven price to input costs and performance in intensive shrimp culture found that survival and growth rate had the greatest effects of all factors studied (Wyban et al., 1988). Thus, improvement of these two factors are the principal targets for the selective breeding program. The phenotypic correlation between weight at market age (average 20 g population) and adult weight (when selected for breeding) will be determined early in the program. If the correlation is high, shrimp will not

have to be weighed at market age, or both weights can be used for selection. If the correlation is low, selection will be at market age. Whole shrimp weight will be used as the criterion for selection.

Selection for disease resistance was not recommended by the advisory group. Knowledge of shrimp diseases to allow effective exposures or to adequately measure shrimp response is insufficient at this time. This technical problem has been described as it relates to testing efficacy in drugs for shrimp (Bell, this volume).

If genotype-environment interaction is important, separate stocks for different environments must be developed. If there is no interaction, ranking of strains or crosses for breeding value is the same in different environments, and the selection scheme can concentrate on developing the best strain for all environments. In Salmonid fishes and tilapia, genotype-environment interaction is negligible (Gjedrem, 1985). Early in the program, experiments will be conducted to determine whether genotype-environment interaction is important.

Facilities

Successful implementation of the SPF breeding program requires a network of facilities. To introduce new populations, a quarantine is required where untested "tentative" SPF stock can be maintained and tested. When adults, the "tentative" stocks are moved to a

quarantine reproduction facility and their resultant offspring are tested. If the offspring are SPF, then these new stocks can be introduced to the SPF nucleus breeding center.

Nucleus Breeding Centers

The nucleus breeding center (NBC) is where the SPF breeding program is implemented. It was unanimously recommended by the breeding advisory group that two SPF facilities at separate, isolated locations be established. Two or more facilities are required to reduce risk of losing broodstocks because of contamination with infectious agents. All of the selective breeding activities are conducted at the NBCs, which are operated under strict quarantine procedures. Each facility should be capable of producing and maintaining at least 48 families in four populations. Postlarvae from the best strains produced by the selection program will be distributed from the NBCs to broodstock multiplication centers (Fig. 2).

Multiplication Centers

The nucleus breeding centers are not designed to produce enough broodstock to satisfy industry demand. Regional multiplication centers to produce SPF broodstock for commercial distribution must be developed. This approach follows the model developed in Norway for breeding Atlantic Salmon (Refstie, 1990). These centers will obtain improved SPF postlarvae from the NBCs to be cultured to broodstock for commercial distribution. The multipli-

cation centers must have full quarantine capabilities in order to maintain high-health status and exclude pathogens throughout the one-year broodstock culture cycle. The resulting high-health broodstock will then be transferred to commercial hatcheries for seed production.

Progress Toward Goals

As of April 1992, Kona Population 1 has been subdivided into 12 separate full-sib families and two of these families reached the F2 generation. Performance data collected for each family are listed in Table 2.

Each family was spawned from a single female mated with a random male, and larvae were reared in separate 20-L buckets. At PL10, shrimp in each family were stocked into separate outdoor tanks (5,000 L). Nurseries were harvested at PL58-PL65. Three hundred

Table 2. Growth performance data recorded for each family in SPF breeding program.

Stage	Performance Data
Z2	Survival (%)
PL10	Survival (%)
PL30	Mean individual weight (g)
PL45	Mean individual weight (g)
PL45	Coefficient of variation (CV) in size (%)
PL45	Survival (%)
PL45	FCR
PL60	Mean weight (g)
PL60	CV (%)
PL60	Survival (%)
PL60	FCR
PL60 to 20 weeks	Biweekly individual weight (g)
20 weeks	Mean weight (g)
20 weeks	CV (%)
20 weeks	Survival (%)
20 weeks	FCR

fifty random shrimp from each family were restocked into growout, and bi-weekly growth rates were monitored during growout until harvest after 20

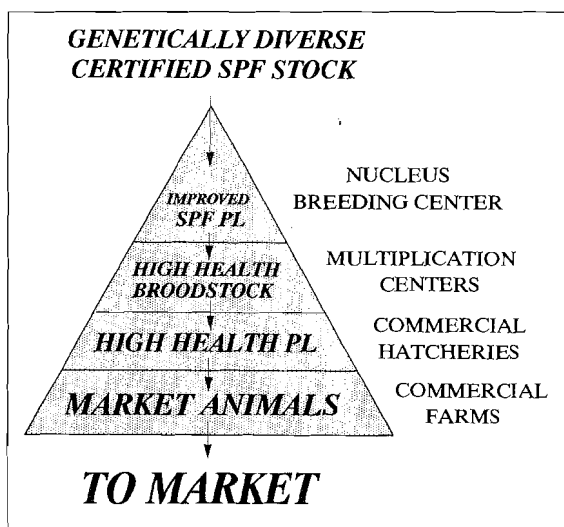


Figure 2. Production pyramid for high health, genetically improved shrimp. Shrimp only move down the pyramid. Shrimp products are listed inside the pyramid; facilities are listed to the right.

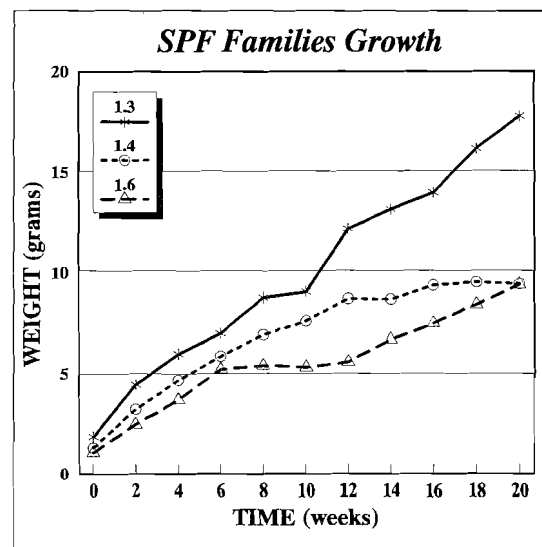


Figure 3. Growth of three SPF shrimp families of identical age cultured under identical conditions.

weeks. Figure 3 illustrates growth of three families spawned on the same day (same age) and grown under identical conditions. The difference in growth among the three families indicates there is significant diversity for growth among the families in Population 1.

As of April 1992, seven families that reached selection size (20 weeks in growout) have been harvested. Their relative production performance is plotted in Figure 4. Families were ranked by mean size at 20 weeks and coefficient of variation (CV), food conversion ratio (FCR), and survival were also plotted using the same family ranking. These seven families were not all reared

simultaneously (as in Fig. 3), so environmental differences (temperature) could have contributed some of the variation in growth. Nonetheless, the substantial variability in growth among these families suggests there may be sufficient genetic variation to improve *P. vannamei* growth rate by selective breeding. In addition, the significant negative correlations of CV ($p < .01$) and FCR ($p < .05$) with growth suggest that the best family in terms of growth is also the best in other production criteria.

The breeding of SPF shrimp for high health and improved growth is only beginning. Based on these preliminary results and knowledge of the success enjoyed by breeders of other meat animals, significant opportunities to improve shrimp performance and advance the industry are waiting.

Conclusions

- A single population of SPF *P. vannamei* has been established in Hawaii and is called Kona Population 1.
- A breeding program designed to protect their SPF status, avoid unnecessary inbreeding, and improve growth and survival through selective breeding has been established.
- Kona Population 1 has been subdivided into 12 full-sib families.

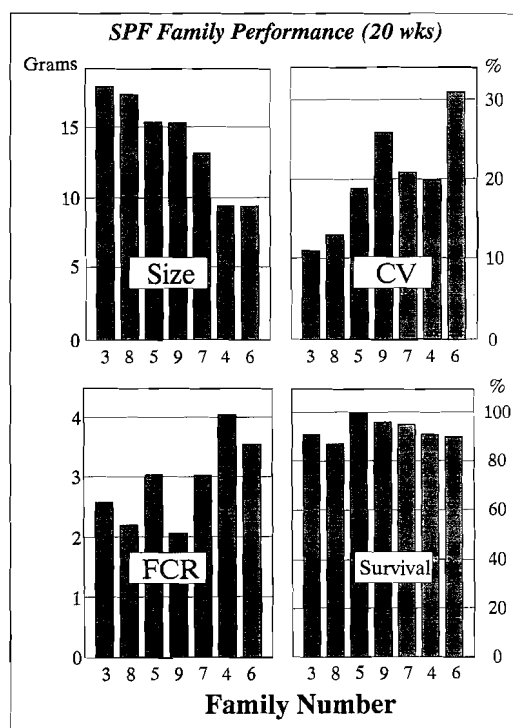


Figure 4. Growth performance of seven SPF families ranked by size at 20 weeks in growout. Using the same ranking, coefficient of variation (CV) in individual size, feed conversion ratio (FCR) and survival are also listed.

- Significant variation in growth among families was observed.
- A strong correlation between growth to market size and size uniformity and feed conversion efficiency was observed.

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