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GENETICS AND FISH HEALTH

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The planned selection of stocks to improve quality and production has been practiced in salmonid hatcheries for decades. However, emphasis in selection programs has been generally placed on phvsical characteristics such as size, coloration and growth rate, with little recognition of the long term genetic impact that such selection imposes on the stocks. Intensive selection for certain characteristics without a thorough appreciation of genetic principles can result in an eventual loss of fitness of the broodstock and the development of undesirable side effects, thus defeating the original objectives of the selection program.

Many technologies are available for brood stock development to improve the health of cultured stocks, but producers must be aware of the inherent advantages and disadvantages of each method. Such projects are, of necessity, long term with genetic gains only being made among successive generations. However, with careful planning and program control, the application of genetic principles in broodstock development can result in an improvement of fish health.

In selecting for disease resistance, one must be careful to differentiate between resistant and disease-free stocks. Resistant stocks do not develop clinical signs when infected but can be carriers of disease. Consequently, resistant fish can pose a disease risk if they are stocked in geographical areas where the disease is not already present.

BROODSTOCK MANIPULATION

Years of intensive breeding have developed lines of trout that are particularly adapted to fast growth in fish hatchery environments. Historically, selection criteria were aimed at a variety of traits, such as egg size and number, egg viability, fry survival, coloration and growth. Any quality lines that were

produced were then maintained by random mass selection; i.e. pooling the eggs of many selected females and fertilizing those eggs with sperm from many selected males. The resultant progeny would again be evaluated and the selection process repeated again.

Consistent with this approach, it seems logical to expect that disease resistance to be an end result of the natural selection process, and that this characteristic could be accelerated through a selective genetics program. However, refinement of existing methodologies to include the criterion of specific disease control requires sophisticated techniques of evaluation and control. Precautionary measures must be included to minimize inbreeding, and the program must continue to identify and select for fish stocks which display those traits which ensure efficient hatchery operations and/or a product well suited for the resource.

Procedures

Whereas the philosophy of "survival-of-the-fittest" is simple, the establishment of a selective breeding regime which supports a viable fisheries program is inherently difficult. The problem rests with the myriad of performance traits which characterize a successful program. Strain performance indices must be established which describe the most important characteristics for selection, depending on the final use of stocks produced. More importantly, the selection program must recognize that trait evaluations are comprehensive, and should therefore be based upon a long-term ranking effort. The following list describes some strain performance characteristics that are commonly used:

1. Qualities relevant to husbandry
   a. Quality of eggs - volume, number, size, percent hatch.
   b. Fry considerations - percent abnormal to swim-up, percent survival, disease resistance, tolerance to therapeutic treatments.
   c. Fingerling aspects - growth rate, disease resistance, food conversion efficiency, survivability, tolerance to therapeutic treatments.
   d. Adult qualities - growth rates, food conversion efficiency, survivability, disease resistance, crowding tolerance, quality of flesh, tolerance to warm temperatures, size uniformity, aesthetic appearance, longevity, broodstock potential, (early/late spawner; fecundity).
2. Qualities relevant to the resource
   a. Rehabilitation goals - stamina. cover-seeking behavior, survival, temperature tolerance, pH tolerance, natural reproduction potential, predator avoidance, ability to coexist with other species, longevity.
   b. "Put-and-take" goals - aesthetic appearance, (body form; low tin abrasion; markings and coloration), growth rate, fishing mortality (catchability), strong fighting tendencies, quality of flesh.

Implementation of a selective breeding program begins when individual values are assigned to each trait deemed important. These **values** can be changed at the discretion of those in charge of the breeding program to accommodate hatchery and fishery dynamics. As individual fish lots are evaluated, each is compared to the best performing lot and assigned a relative performance value. A comparative trait performance value can then be developed for each test

lot by multiplying each trait value by the corresponding relative values (Bedell and Gall 1968). The summarization of all trait performance values for each test lot would then constitute its strain performance index. This index value, expressed as a percent, represents the provisional strain value. It should be noted that strains with some very poor performance indices might possess outstanding singular trait performance values.

In a program where there is selection for disease resistance, it would be expected that survival after a challenge by the disease of importance would be assigned a high trait value. Selection could be accomplished by a challenge involving exposure to pre-determined densities of pathogens (Ehlinger 1964) to the test lot, or by rearing the fish in a water supply contaminated by the infectious agent. However, survival is only one of the desirable traits. In the case of diseases where vertical transmission occurs, this approach could lead to development of a carrier state. This is an undesirable situation, especially if disease carriers were to be introduced into new areas where the diseases did not otherwise occur.

Caution must be exercised since one can over-select for one trait at the expense of genetic variability. Spawning should also include at least 60 pairs (see Bedell and Gall 1968; Kincaid 1976a, 1976b; Ryman and Stahl 1980). Continued selection and cross breeding of those fish displaying the highest strain performance indices should result in greater resistance to the disease. When resistance has been established, future breedings should be planned to improve other selected traits without substantially reducing the performance level of any other important trait, and to continue to maintain maximum genetic variability withii brood stocks.

HATCHERY Management Implications

The principle of disease control through avoidance continues to be utilized by hatchery managers as a means of circumventing epizootics. The avoidance mechanisms generally used include a mixture of the following: control of the water supply (wells, UV treatment, etc.); control of the fish stocks (disease-free stock, surveillance, eradication); limiting the pathways to infection (segregate downstream culture activities from upstream activities); and disinfection of fish eggs and/or contaminated culture facilities. Such efforts are generally directed towards controlling the influx of any new diseases not normally associated with the station or region, and simultaneously, minimizing the effects of ubiquitous disease agents.

A difficulty arises in determining which of the disease avoidance mechanisms will pay the biggest dividends. Many program managers start by ensuring a clean hatchery site. They then obtain eggs or fish from hatchery stocks certified to be free of specific pathogens (Loftus 1975). This approach works well if other avoidance mechanisms continue. However, should the culture system become contaminated with a reportable infectious agent, there would be cause for alarm inasmuch as the disease-free stocks could possiblv lack genetic resistance to the disease. Moreover, new stocks may be unsuited to the existing environmental conditions (water quality, bacteria, etc.) associated with the fish culture station. Experience has demonstrated that the hatchery manager may experience some devastating mortalities if isolated disease-free stocks are transferred to different hatchery surroundings.

The dilemma of whether or not to use disease-free stocks is further hampered by the hatchery classification system. Because classification is based upon periodic inspections of hatcheries, it is possible fish health diagnosticians may miss an infection. This speculation is supported by recent authors (Mitchell and Hoffman 1981) who state that "attempts to isolate bacteria and viruses may be futile unless clinical signs of an infection are apparent." Furthermore, in their conclusion they relate that most diagnosticians do not certify fish to be disease free of specific diseases; they only examine the fish and report what they do or do not see. However, the agents of BKD, IPN, and IHN can be isolated by qualified inspectors in the absence of disease signs.

The bottom line of this discussion relates to those alternatives that examine the question: "Where can we go from here?" It has already been established (Dill 1973; Yamamoto and Kilistoff 1979) that disease control by avoidance has considerable potential benefit, as does the use of disease-free stock; but what about the use of selective stock manipulation to develop disease-free or disease-resistant brood lines? Snieszko, as early as 1953, reported that, for the control of viruses in cultured fish, "the long-range approach is the breeding of resistant strains of fish". Recent literature (Dill 1973; Wolf 1976; McIntyre and Amend 1978) also supports this philosophy: however, the use of avoidance and vaccines may be equally or more effective in the control of diseases.

Moreover, it has been suggested (Ehlinger 1964; Fujihara and Tramel1968; Wolf 1953; Winter et al. 1980) that the severity of common bacterial diseases can be reduced through controlled selection, wherein the host is challenged by the infectious agent. Therefore, if a specific disease becomes important enough to warrant the effort, a regimented breeding scheme could be established to select for those characteristics which reinforce survival after infection. Examples follow which demonstrate alternative approaches for the control of IPN. Modifications of this approach would be necessary for each disease, depending upon its etiology.

EXAMPLE 1

DEVELOPING IPN DISEASE-FREE BROODSTOCK

RATIONALE

Wolf et al. (1968), reported that the most effective method for controlling IPN epizootics lies in the capability to utilize virus-free broodstock, and to rear resultant fry and fingerlings in IPNV-free water. These brood fish could either be acquired through a tedious selection process or by direct transfer from a virus-free station. This technique suggests that one could recruit broodstock or eggs from an IPNV-free hatchery, and then successfully rear the progeny in virus-free water. Field experience, however, has demonstrated that this practice does not always accomodate the hatchery program. Recruited strains may not be suited for those environmental factors associated with a particular hatchery and severe mortalities may occur. From a production viewpoint, a more difficult approach might be to derive IPNV-free stock from those hatchery strains which already demonstrate desirable performance characteristics. If this is the chosen pathway it follows that the primary objective of such a program must be twofold: first, to

maintain the genetic variability of the strain which characterizes high quality performance; and second, to isolate those hatchery fish which are free of IPNV.

PROCEDURES

The development of IPNV-free broodstock will require a multi-faceted, regimented program. A standard protocol for selective breeding must also be included to maintain the genetic variability of the brood lines. Virological testing must determine which fish are carriers and presumptively IPNV-free fish must then be isolated and maintained in virus-free water. Spawning of single pairs (one male + one female) followed by egg/fry/fingerling isolation within separate but comparable rearing units (and virus-free water) will permit long-term evaluation to confirm that resultant progeny are indeed free of IPNV. Requirements for implementation of such a program are listed below (see Wolf et al. 1968):

1. Tag/mark adult fish and rear in virus-free water.
2. Screen prospective brood fish for IPNV using fecal examination.
3. Eliminate individuals carrying IPNV.
4. Screen sex products for IPNV.
5. Eliminate IPNV carriers.
6. Conduct pair matings and maintain each family lot as a separate entity in virus-free water. Be certain to select a minimum number of adults to supply at least 50 pairs of disease-free fish from each hatchery strain (Kincaid 1976a. 1976b; Ryman and Stahl 1980).
7. During egg, fry and fingerling stages, each individually spawned lot must be kept isolated from the other lots (within a virus-free water supply).
8. Each lot should be observed for IPN signs; suspect lots would be screened and eliminated if IPNV is verified.
9. If no signs develop, screening should be conducted 6-8 wk after the start of feeding; those lots confirmed to be virus-free could then by combined to form the initial virus-free complement from the original hatchery strain.

Precautionary CONSIDERATIONS

While these principles seem simple and straightforward, practical application could be hampered by the logistics associated with the number of strain replicates required (Falconer 1960; Hynes et al. 1981) and the required number of individual rearing units. Since the progeny are still susceptible to IPN infection, the isolation of virus-free stocks at fish culture stations may be impractical in those regions where IPNV is enzootic. Also there are many strains of IPNV, some of which are more virulent than others. Fish that appear resistant to some strains may be susceptible to the virulent strains. Furthermore, managers should be aware that resistant fish may carry subclinical infections and that their stocking into waters where IPNV is not endemic creates a definite risk of introducing the disease to wild, native stocks.

EXAMPLE 2

DEVELOPING IPN DISEASE-RESISTANT STOCK

RATIONALE

Resistance to viral diseases has been shown to be an heritable characteristic in a number of plants and animals. Correspondingly, comparisons of various strains of fish and IPNV has indicated that IPNV affects some fish more severely than others. Since this observation suggests IPN resistance, a breeding program could be initiated to measure differences in IPN resistance and to assign this performance characteristic a high trait value for use in selection. Continued selection might then develop lines of fish which would demonstrate a high degree of resistance to IPNV.

PROCEDURES

Initial contact with IPNV has been historically catastrophic with sudden and massive mortalities. However, at some fish culture stations which have encountered IPN disease for many years, a disease tolerance has developed to the point where hatchery managers feel they can "live with" the disease. By applying the principles of genetic selection, it seems possible that the natural selection process could be accelerated and improved upon.

Using the premise that IPNV disease normally kills the fastest growing fish, the main selection criterion should be the isolation of the largest surviving individuals since these fish may possess greater resistance to IPNV. While this process might minimize the effects of IPNV, it must be cautioned that the selection will be genetically very restrictive, so a large initial population of individuals must be utilized as future broodstock to maintain an adequate gene pool. A wise approach to this selection process would be to utilize the previous breeding program (Example 1) where performance characteristics are assigned trait values. This would permit the production manager to control the rate of selection by working out a methodology which conforms to the needs of the hatchery/fishery program. Taking this further, it may be possible to segregate non-carrier broodstock so that resultant eggs and fry would be both IPN resistant and free. A procedural outline follows:

1. Establish appropriate selection criteria which conform to management needs; assign a high trait value to those larger individuals that survive an IPN epizootic.
2. Continue to select for the resistance trait as long as other important traits are not appreciably impaired. Water supplies should harbor IPNV carriers.
3. A subgroup of fish which possess little natural resistance to IPNV should be maintained. These could then be used to challenge the developing resistant lines. Moribund fish from the susceptible sublot, which displays symptomatic IPN disease, could be ground and fed to those resistant families in order to intensify their chances of infection and to accelerate the selection process.

4. Maintain accurate and complete reference records for future comparison.
   a) Start with a population of 15,000 swim-up fry. After seven days, reduce the lots to 10,000 feeding fry (this will compensate for early differential mortality rates that occur between strains). Rearing units should be exact replicates with equal flows of virus-contaminated water.
   b) Record daily mortalities, water flow and temperatures, feed and feeding rates, etc.
   c) Summarize and graph the number of survivors at the end of each week.
   d) Summarize and graph the weekly accumulated mortalities, as a percent of those survivors at the beginning of each week.
5. When clinical signs of IPN develop, sample the fish and check for IPNV infection.
6. If no IPN signs develop, sample fish 6-8 wk after the start of feding and check for the presence of IPNV If these fish are negative, challenge them with IPNV-infected ground fish and recheck them after 2 wk.
7. The rate at which further IPNV resistance develops may eventually diminish to a very low level. At that point in time, and if further selection is deemed appropriate, more sophisticated testing using anti-IPN serum may prove meaningful. Taking this further, it may be possible to segregate non-carrier broodstock so that resultant eggs and fish would be both IPNV resistant and free (see Wolf 1953, 1976).

WILD BROODSTOCK - HATCHERY AND FISHERIES MANAGEMENT IMPLICATIONS

It has been postulated (Hynes et al. 1981) that captive hatchery fish stocks may undergo unintentional detrimental genetic changes as a result of selective breeding, and that subsequently, the planted species may not meet the expectations of a given fisheries management program. Consideration of this domestication effect is especially critical when management objectives include the rehabilitation of depleted recreational or commercial fish stocks. Fisheries managers must, therefore, consider the total needs of the fishery and communicate with aquaculturists so that an integrated approach can be established to maxmize the genetic integrity of managed stocks. To accomplish this end, progeny from wild broodstock should be reared within facilities that simulate the constraints associated with natural conditions.

The philosophy of matching the fish to the fishery is not new, and many authors (Allendorf and Phelps 1980; Barns 1972; Donaldson and Menasveta 1961; Flick and Webster 1962, 1964, 1976; Fraser 1981; Greene 1952; Horak 1972; Ihssen and Tai 1974; Krueger and Menze11979; Moyle 1969; Ryman and Stahl 1980; and Vincent 1960) have studied differences in performance among wild and domestic stocks. While fishery managers agree that wild stocks could contribute more to the fishery than captive hatchery stocks, the procurement and culture of wild broodstocks may only delay domestication. Because fish culture practices inadvertently result in genetic selection for the hatchery environment, the practice of utilizing truly wild fish stocks. as maintained within

the confines of traditional fish culture facilities, may only be a concept. Nevertheless, stock improvement toward accomplishing this goal would be possible if certain criteria can be accommodated. These are as follows:

1. Methods for identifying genetic changes within fish stocks.
   a) Identify the genetic composition of the original stocks to be preserved.
   b) Determine the changes in the genetic composition of both wild and captive stocks.
2. Methods of identifying phenotypic changes within fish stocks.
   a) Establish methods to differentiate between genotypic and phenotypic changes.
   b) Develop methods to determine whether phenotypic changes are the result of environmental or genetic factors.
3. Establishment of fish culture methods which preserve the genetic integrity of the stocks.
   a) Locate, identify, and characterize available fish stocks. Careful consideration must be given to closely match the genetic make-up of the stock to be used to rehabilitate the fisheries program (Krueger et al. 1981).
   b) Efficient management of specific stocks will require an increased number of highly versatile fish culture stations. These should be located as close as possible to those water bodies that contain the target stock (Hynes et al. 1981).
   c) Random sampling of the founding parents is essential; the sampling must cover the complete range of sizes, ages, spawning times, and spawning sites (Bedell and Gall 1968).
   d) Random mating procedures using a minimum of 60 males and 60 females should be an established protocol (Bedell and Gall 1968; Kincaid 1976a, 1976b; Ryman and Stahl 1980).
   e. Techniques such as rotational line crossing should be established as a protocol to reduce genetic inbreeding (Kincaid 1977).
   f. Periodic infusions of genetic material from wild stocks may be considered as a means of maintaining genetic variability, although this practice carries with it inherent risks of introducing diseases (Hynes et al. 1981).

While fisheries management has been traditionally characterized by typological (no difference between stocks) thinking (Schreck 1979), it is now apparent that administrators are aware of the stock concept (Hynes et al. 1981; Loftus 1976) and are ready to integrate the necessary disciplines into their management schemes. To implement this philosophy, managers must take a more holistic approach whereby elements are comprehensively perceived as dependent variables to some management goal.

Administratively, this effort must start at the top and then permeate throughout the system. Program development should not overemphasize one concept to the point where ideas become so polarized that the original goals may actually be hindered (Kutkuhn 1979). Ideally, the program should be flexible, open-ended, and above all, practical.

CONCLUSION

In summary, today's fish producer has many options available for broodstock development to improve fish health. Depending on what he wants to do with his

fish (e.g. restoration program, put-and-take fishery, direct food market, etc. I, he must use discretion and implement those options that best suit his needs. For this reason, no specific recommendations have been provided; rather, a selection of alternative approaches has been offered. The final decision rests in the hands of the manager who must match available resources with those approaches that will most effectively achieve the desired goals.

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