



STRIPER 2000

Research Advances on Striped Bass and Its Hybrids

A Program Summary

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— Fred Wheaton

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INTRODUCTION

Aquaculture is the fastest growing segment of the agricultural industry. Several species have contributed to increasing production, among them, trout, catfish, oysters and tropical fish. Striped bass (*Morone saxatilis*) and its hybrids is another species that has been gaining in popularity among growers, largely because of its marketability, growth characteristics and adaptability to culture. Although striped bass culture has grown dramatically in this last decade, the industry faces a number of challenges if it is to sustain that growth: productivity must increase, costs must be optimized and marketing must develop innovative approaches. Meeting these challenges will depend on scientific advances that can lead to better control over broodstock reproduction, growth, nutrition and diseases.

Growers need more specific information on such issues as optimal environmental requirements, high performance strains and cost-efficient culture technology. To assess our current scientific understanding in these and other areas and to clarify how research can best assist the development of striped bass culture, the Maryland Agricultural Experiment Station organized Striper 2000 as a forum for bringing together scientists, members of the striped bass industry, extension specialists and agency representatives.

The program has been divided into major research areas that are contributing to the development of striped bass culture, namely, genetics, reproduction and growth, nutrition and feeding, production and technology, diseases, utilization, and policy issues. Discussion of these issues as they affect culture of striped bass and its hybrids is followed by an industry discussion of the challenges and opportunities that lay ahead.

Several organizations came together to sponsor the Striper 2000 Conference. The Maryland Agricultural Experiment Station has funded a number of projects over several years aimed at closing the life cycle of striped bass and its hybrids; most of this research will be summarized at this conference. Maryland Sea Grant has been funding striped bass research and is a co-sponsor of Striper 2000. Industry groups including the Striped Bass Growers Association and the Maryland Aquaculture Association also are conference participants and help focus the industry perspective. In addition, leading researchers and industry experts have been invited to round out this conference. This publication collects abstracts of all the papers presented at Striper 2000.

— Fred Wheaton

GROWTH OF THE STRIPED BASS AQUACULTURE INDUSTRY

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The culture of striped bass and striped bass hybrids achieved commercial viability in the late 1980s. This success resulted from the logical extension of culture methods that had been developed over the past hundred years in state and federal hatcheries at Weldon, Edenton, Moncks Corner, Richloam, and at several other locations. Some of the most significant accomplishments made at these facilities included the development of hormonal spawning methods, hybridization with white bass, and refinement of rearing techniques for the production of phase I fingerlings in ponds. Recent achievements by researchers in universities and in private companies have advanced these culture methods by attempting to develop better genetic stocks for domestic use, and are working on out-of-season spawning and tank rearing of fingerlings to provide a reliable supply of fingerlings on a year-round basis. This presentation will provide a brief review of these historical accomplishments and focus on the commercial developments over the past ten years. These developments include nursery operations, inventory management, nutrition and feeding, disease treatment, water quality control, waste water treatment, harvesting, packaging, and delivery. General information on production economics also will be presented.

Production statistics based on a survey of the leading 20 producers and information from state extension agents and aquaculture coordinators will be presented. Production volumes in 1997 from 95 hybrid striped bass registered producers was estimated to be 8.4 million pounds. Only 31 farms produce over 10,000 pounds each and five of the largest producers supplied nearly 6 million pounds, or 71% of the production. A majority of the product was grown in tanks (56%) and ponds (43%), with the balance from cages (1%). Production by major geographical region was estimated to be 47% in the west, 24% in the southeast, 15% in the mid-Atlantic, and 14% in the northeast. Sales of live product increased to over one million pounds (12%) of the total 1997 production. Over half (53%) of the production from the northeast was sold live, while 99% of the production from the southeast was sold fresh. Most of the live product was sold into the ethnic Asian markets in New York and Toronto.

The average FOB farm price for fresh product was estimated to be \$2.53 per pound in 1997, and \$3.06 per pound for live fish. Both prices were down 28% from original prices obtained ten years ago in 1986. Projections for 1998 indicate that production may increase to 12.9 million pounds and prices could fall below \$2.25 per pound. Some of the competition in 1997 came from commercially landed striped bass from the Chesapeake Bay and coastal fisheries of New England (4.7 million in 1996) and from the white bass commercial fishery in the Great Lakes (2.3 million in 1996). Based on the Atlantic States Fisheries Commission's Virtual Population Analysis, the total

OVERVIEW

allowable catch for striped bass is predicted to be 20 million pounds annually, with 15 million pounds allocated to the recreational fishery and 5 million pounds to the commercial fishery. Production of farm-raised hybrid striped bass for foreign markets is principally from four Kibbutz farms in Israel that produced approximately 600,000 pounds in 1997, which was sold in Western Europe, and about 6 million pounds from Taiwan and China, which was sold in Asia. The projected increase in production volumes and intensification of competition suggests a further decline in sales price in the future. To counter this trend, it is imperative that the industry expand marketing efforts to increase awareness of this product and to develop new markets, both domestic and foreign.

ADVANCES IN STRIPED BASS GENETICS

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Other than hybridization, little information exists regarding the qualitative, quantitative and manipulation genetics of striped bass. At Horn Point Laboratory we have been conducting seminal research examining heritability of certain commercially important traits in striped bass, evaluation of polyploid induction and polyploid performance under commercial conditions, sex-reversal methodologies involving both hormonal induction and gynogenesis through prevention of second polar body extrusion, and strain performance of the east coast populations of striped bass. In our heritability study efforts thus far, we examined the trait of airbladder inflation and determined that there was little exploitable genetic component for this trait and that it appears to be primarily environmentally mediated. Side by side trials with polyploid palmetto bass, sunshine bass, and striped bass in which triploids were compared to diploid siblings, only in the case of the striped bass did the triploid progeny perform (growth) as well as the diploids. Gynogens have been effectively produced using UV irradiated sperm from white perch as a marker and restoring diploidy by preventing extrusion of the second polar body during the second meiotic division through the use of hydrostatic pressure. An all-male population was effected by feeding a diet laced with 17 α -methyltestosterone during early larval-juvenile feeding 20-60 days post hatch. Lastly, in a replicated tank study multiple families of striped bass from the Gulf of Mexico and Florida Atlantic coast, South Carolina, Maryland, New York, and Canada were examined for a variety of commercially important traits (growth, survival, filet yields). While there was wide variation between the different geographical regions, the families from Maryland and Florida Gulf Coast populations had the overall best performance. The Canadian families were a close third with South Carolina and New York fish being the poorest performers.

DOMESTICATION AND SELECTIVE BREEDING OF STRIPED BASS

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For many plants and animals, successful selective breeding programs have been implemented with an enormous gain in production and product quality. However, in aquaculture, we have only just started to utilize this strategy for important finfish species. Most traditional genetic improvement programs are initiated through efforts to first domesticate and then establish selective breeding programs with well defined goals and/or heritable traits of importance. Recent studies have shown that selection, to increase growth, feed conversion ration, and survival, in rainbow trout for example, accelerates the rate of domestication. Variation in growth rate within and among striped bass families, originating from three geographical areas of Chesapeake Bay, produced by the Crane Aquaculture Facility, has recently been evaluated. Effects of stock, family, gender, and their interactions were evaluated using a mixed model ANOVA and best linear unbiased predictions of family merit were established. Significant differences in growth performance between distinct stocks indicate a potentially important source of variation to be exploited. Additional comparisons within and among seven, second filial generation (domestic) families of Chesapeake origin, indicated significant family and family x gender effects and further suggested that a combined within and between family breeding strategy could lead to rapid genetic progress. In a specific example, variation in weight within the fastest growing family compared had a mean weight of eighteen months of 454 grams with a range of 374–774 grams. Many individuals within the family exhibited performance well above the family mean. Imposing a selection intensity of 90% on this family would generate a subset of individuals with a mean weight of the 670 grams and would yield a potential selection differential of 176 grams for the succeeding generation.

INVESTIGATION INTO THE PROPERTIES OF STRIPED BASS SPERM AS A RESULT OF ACTIVATION

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The ability to perform selective breeding or compensate for seasonal variability while breeding striped bass in captivity could be greatly enhanced through the development of improved cryopreservation protocols for striped bass sperm. Many of the steps within the cryopreservation protocol, however, appear to come threateningly close to the physical and biological limits of these cells. Cryopreservation of a tissue requires the use of a cryoprotectant to prevent ice damage from occurring during freezing and thawing of the sample. Post-cryopreservation, the cryoprotectant must be removed from the system to avoid toxicity damage. One method to do this is to dilute the sample until the cryoprotectant concentrations cease to be toxic. However, during dilution, there is a possibility that the osmolality will drop to the point where activation occurs. This activation could strongly impact the success of fertilization by using cryopreserved spermatozoa. This study examined both the mitochondrial functionality and the membrane intactness of the cells subjected to varying osmolalities below the isotonic osmolality of the “neat” milt (approximately 300 mmol/kg). Striped bass sperm cells are only active at most 1 to 2 minutes and it is generally accepted that, once activated, sperm cells cannot become active again. Upon diluting the milt with deionized water (1:2, milt:water) to an osmolality of less than 110 mmol/kg for up to 5 hours, striped bass sperm cells do not exhibit significant numbers of lysed cells. The majority of sperm cells did not stain with propidium iodide but did stain with SYTO-16, indicating membrane intactness. However, these cells also did not show signs of motility nor was there evidence of mitochondrial activity, as indicated by staining with rhodamine 123. However, cells that remained in the 2:1 dilution with deionized water for 17 minutes or less and then were returned to isotonic for at least 2 hours, activated upon the subsequent addition of deionized water. The repetition of activation was only investigated through one cycle, but the reactivated samples appeared to have nearly the same motility as the control sample. One possible mechanism to explain this re-activation is that intact spermatozoa, once returned to an isotonic environment, are able to rebuild the electrical transmembrane potentials of the mitochondrial membranes. With active and functioning mitochondria, the cells are able to re-activate. This cycle or re-activation would likely cease as the stored energy of the cells is depleted.

PERFORMANCE COMPARISON OF GEOGRAPHICALLY DISTINCT STRAINS OF WHITE BASS TO PRODUCE SUNSHINE BASS

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White bass (*Morone chrysops*) broodstock were obtained from three locations encompassing the native range of this species: Arkansas River, Arkansas; Lake Erie, near Cleveland, Ohio; and Lake Poinsett, South Dakota. In spring 1996, white bass eggs of each strain were fertilized with fresh semen from the same strain or with extended striped bass (*M. saxatilis*) semen pooled from 9 males collected from the Arkansas River. Each strain of white bass and sunshine bass was stocked at 500,000/ha as 4-d post hatch larvae into quadruplicated earthen ponds recently filled and fertilized. The fish were offered fry meal (~50% crude protein) 21-d post stocking. Phase I fingerlings were harvested 35-d post stocking. Sunshine bass mean survival rates (12.5%) were significantly higher than white bass (2.6%). No differences in survival existed within the white bass or sunshine bass groups. Phase II sunshine bass were subsequently stocked in triplicated ponds at 25,000/ha. The fish were fed to satiation twice daily with a 40% crude protein diet, and harvested 100-d poststocking. Lake Erie sunshine bass mean weight (90.2 g) was significantly greater than Arkansas fish (58.4 g), but not South Dakota fish (69.0 g). Phase III sunshine bass were stocked in triplicated ponds at 5,000/ha, and fed in like manner as in Phase II. In fall 1997, ponds were harvested, and all fish reached marketable size with Lake Erie sunshine bass averaging 647 g, followed by Arkansas fish at 638 g, and South Dakota fish at 566 g. These weights were not significantly different. These results demonstrate the feasibility of raising sunshine bass to market size in earthen ponds in the midwest. Lake Erie white bass might offer some advantage as a source of broodfish relative to the strains compared and under the conditions in which they were raised in this study.

GENETIC ANALYSIS OF STRIPED BASS

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Striped bass exhibit less genetic variation than most other species. However, we have identified both single copy nuclear and microsatellite markers that can be used for the genetic analysis of striped bass. In some cases, high levels of genetic diversity can be seen at single genetic loci. These markers have been used to demonstrate that genetically distinct populations of striped bass are present in most river systems studied and that small populations tend to exhibit greater levels of genetic divergence than larger populations. In addition, we have shown that population substructure can occur within a single drainage system. In the Santee-Cooper river system, we have obtained evidence for three genetically distinct subpopulations despite the absence of physical barriers to mixing. We have also measured genetic variation among year classes within a single population and used this information to estimate the number of parents contributing to each year class. With regard to aquaculture, these genetic markers can be used to identify the progeny of specific crosses so that the performance of co-cultured families can be assessed or to develop a genetic map for striped bass.

MANIPULATION OF SPAWNING IN STRIPED BASS: FROM HORMONES TO GENES

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Intensive and cost-efficient farming of striped bass and its hybrids must rely on a predictable and continuous supply of seeds. However, as is the case with many other commercially important farmed fish, captive-reared striped bass ovaries do not develop beyond vitellogenesis. Consequently, final oocyte maturation (FOM), ovulation and spawning do not occur. By comparing the ovarian and hormonal status of females in confinement to females captured on the spawning grounds, we have demonstrated that the surge of the maturational gonadotropin (GtH II), which triggers FOM, ovulation, and spawning, does not occur in captive females, which remain in an “ineligible” state. Multiple injections of super-active analogs of the hypothalamic gonadotropin-releasing hormone (GnRHa) induce a short GtH II surge and early FOM, but not ovulation and spawning. However, sustained-administration of the GnRHa via polymer-based delivery systems, stimulates a prolonged secretion of GtH II followed by final ovarian development and spawning. The sustained-release GnRHa delivery system is presently used for commercial production of hybrid striped bass, among other species.

The failure of captive females to secrete GtH II indicates a deficiency of GnRH synthesis or release. We have recently found that the brains of striped bass and other perciform fish contain three native forms of GnRH, of which the sbGnRH form is a novel GnRH. Combining endocrine and molecular approaches, we have demonstrated that the novel sbGnRH is the most physiologically-relevant form for FOM, ovulation, and spawning. We have cloned and characterized the genes coding the three GnRHs from striped bass. Analyzing expression levels of the three GnRH genes and the pituitary levels of the three peptides showed differences between captive and wild fish, suggesting an effect of confinement on the GnRH system. We are presently studying the regulation of GnRH gene expression in striped bass, in view of developing novel approaches, based on the manipulation of GnRH function at the level of its genes, for the control of spawning in farmed fish.

ISOLATION AND CHARACTERIZATION OF THE cDNA ENCODING THE OVARIAN GONADOTROPIN RECEPTOR IN STRIPED BASS (*MORONE SAXATILIS*)

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Many cultured species of teleosts (including the striped bass) will undergo appropriate gametogenesis in captivity but fail to spontaneously ovulate. In general, this problem can be tempered with injections of hCG to induce spawning, although with variable success. In order to enhance spawning success, oocyte diameter is used as a measure of developmental progress; however, this process of visual examination is highly subjective. The imprecise nature of this test often results in attempts to induce spawning in brood stock containing oocytes that are insufficiently developed. The working hypothesis of this research project is that the follicular expression of the receptor for the maturational gonadotropin (GtH II) may be a more precise measure of maturational competence of fully developed oocytes. Blood-borne GtH II activates its receptor expressed on the follicular granulosa cells to stimulate the production of the maturation inducing steroid which, in turn, induces the oocyte to mature and ovulate. Therefore, an appropriate level of ovarian expression of the GtH II receptor (GtH-R II) is required for successful induction of final oocyte maturation. In order to develop an accurate measure of GtH-R II expression, we set out to isolate and characterize the cDNA encoding the striped bass GtH-R II. A high-quality cDNA library was constructed from poly(A)-mRNA isolated from oocytes that were in the early phase of final oocyte maturation. Previously, a portion of a presumptive GtH-R was isolated by polymerase chain reaction utilizing degenerate oligonucleotide primers. This GtH-R amplicon was used as a probe to screen 420,000 plaques of which 52 plaques were identified as positive. Fifteen of these plaques were randomly selected to be purified, the inserts were subcloned into plasmids, and the resulting constructs were subjected to restriction digest analysis. Nine of the 15 isolates contained identical 2.9 kb inserts and 5 of the remaining isolates were truncated forms of the 2.9 kb clones. Alignment of the predicted amino acid sequence of the presumptive striped bass GtH-R II to other known forms of luteinizing hormone-receptors (LH-R), follicle stimulating hormone-receptor (FSH-R), and thyroid stimulating hormone-receptor (TSH-R) will be discussed. The utility of this clone for the evaluation of GtH-R II expression and its correlation to successfully induced spawning events will be introduced.

MANIPULATION OF THE SOMATOTROPIC AXIS IN STRIPED BASS: POTENTIAL TO ALTER GROWTH AND PRODUCTION EFFICIENCY

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Two experiments were conducted to explore the potential to manipulate the somatotrophic axis in striped bass and thereby alter production efficiency. The objective of Experiment I was to test the hypothesis that bovine growth hormone (bGH) and bovine placental lactogen (bPL) increase growth in striped bass in a dose dependent manner. The first experiment was designed to test the effects of exogenously administered growth hormone on striped bass growth efficiency and body composition. Variables tested were length gain, weight gain, feed conversion, blood growth, hormone concentration, and body composition. Every two weeks for a total of 14 weeks, fish were injected with a dose of 0, 1, or 10 ug/gm bodyweight (BW) bGH or bPL. At this time, fish were also weighed and measured for length. Fish for proximate analysis and blood samples were obtained at the beginning, middle, and end of the 14 week period. At the ug/gm BW dose, bPL ($P < 0.09$) and bGH ($P < 0.11$) increased DW. Tendencies toward increased length gain were also seen in these treated groups ($P < 0.19$ & $P < 0.13$, respectively). Proximate analysis indicated that bGH increased protein content (10 ug/gm; $P < .10$) and decreased fat (1 and 10 ug/gm; $P < .10$) at 14 weeks. From these results, we conclude that bGH and bPL are effective in increasing body growth in striped bass, and offer a method to increase the efficiency of striped bass production in Maryland.

Despite the positive results obtained in the experiment described above, one limitation of bGH for commercial application is that it must be injected; this would not be acceptable to producers. Rather, a compound that stimulates fish to secrete more endogenous GH, and is orally active, would have tremendous utility. Such a compound that is available for use in fish is arginine. Arginine increases GH in mammals through a hypothalamic mechanism. However, a similar effect in striped bass (or any aquatic species) has not been described to our knowledge. Thus, Experiment 2 was designed to determine if augmentation of dietary arginine increases endogenous GH secretion and growth in striped bass. A dose response study was conducted with arginine supplemented directly into the diet of striped bass for 10 weeks. Diets were identical except for increasing levels of arginine (1.6, 3.2, & 6.4%). Glutamic acid was used to maintain isonitrogenous diets in the low

arginine diets. Relative to the fish consuming the 1.6% arginine diet, 6.4% arginine increased ($P < .03$) BW; the 3.2% diet had intermediate effect on growth. Currently, a radioimmunoassay for striped bass GH is being validated to determine if arginine influences the level of endogenously produced GH. Regardless of the mechanism, however, our results suggest that supplying higher levels of arginine in the diet of striped bass can increase growth.

EFFECT OF SALINITY AND TEMPERATURE ON RELATIVE GROWTH PERFORMANCE IN ESTUARINE AND FRESHWATER STRAINS OF STRIPED BASS

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The use of salinity to ease stress and improve growth performance of juvenile striped bass has become a “rule of thumb” of aquaculture practitioners. We examined the effect of salinity-temperature combinations for two strains of striped bass: a freshwater “strain” (South Carolina) and an estuarine “strain” (Chesapeake Bay). Three salinity levels (0, 7, and 15 ppt) and three temperature levels (20, 24, and 28° C) were examined employing a Latin square design with two replicates. Juveniles used for the first 15-d experiment, for which analysis has been completed, ranged between 40 and 60 mm, SL. Daily specific growth rates ranged from 2.2 to 15.6 percent per day with highest growth rates occurring at the highest temperature level and at the intermediate salinity level. Highest growth efficiency (range 14.1 to 43.7 percent) occurred at the intermediate salinity, but temperature had no significant effect. Feeding rate (range 15 to 40 percent body weight per day) was positively related to temperature; salinity had no significant influence. Strain did not significantly affect growth or feeding rates, or growth efficiency. Results indicate that higher growth rates at higher temperatures are achieved through increased feeding rather than increased growth efficiency. Improvement in growth rates at 7 ppt were due to higher growth efficiency rather than higher feeding rates at that salinity level. Therefore, low levels of salinity (i.e., 7 ppt) should improve efficiency of juvenile grow-out by increasing feed conversion efficiency. Because there was no effect of strain in the first experiment, these results should have general application to both freshwater and estuarine populations of striped bass.

INDEPENDENT REGULATORY EFFECTS OF PHOTOPERIOD AND WATER TEMPERATURE ON STRIPED BASS (*MORONE SAXATILIS*) REPRODUCTION

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Full development of hybrid striped bass farming will require domestication of broodstock and complete control of the timing of their reproductive cycles so that fingerling production can be accomplished continuously throughout the year. We previously verified that the schedule of maturation in these temperate basses is set by annual cycles of daylength (photoperiod) and water temperature. In the present study, we further investigated photothermal control of striped bass maturation by exposing adult fish to various annual cycles of daylength and water temperature and sampling them throughout their gametogenic cycle to evaluate a series of maturational parameters. Females exposed to a natural photothermal cycle (Control group) or a natural thermal cycle with constant long daylength (Temperature group) had similar profiles of ovarian growth and maturation, including blood plasma levels of sex steroid hormones and the egg yolk precursor, vitellogenin (Vg). Several females from each of these treatment groups were induced to spawn successfully. In contrast, females exposed to constant thermal conditions with or without a natural annual cycle of daylength (Photoperiod and Static groups, respectively) failed to complete ovarian growth and never reached a stage of maturity suitable for induced spawning. Maturation of these females was characterized by diminished circulating levels of gonadal steroid hormones and Vg, resulting in insufficient Vg incorporation into developing oocytes and decreased oocyte growth. In males, plasma androgen levels and the incidence of spermiation showed a similar dependence on cycling water temperature. All of the male striped bass spermiated at some time during the study, and a relatively high percentage of those held under constant thermal conditions (Photoperiod and Static groups) were always spermiating. The results of this study indicate that the annual cycle of water temperature plays a prominent role in the initiation, maintenance, and termination of the striped bass reproductive cycle. Development of domestic striped bass broodstocks and broodstock husbandry systems will likely require complete control of water temperature during the critical periods of reproductive maturation.

BALANCING AMINO ACID REQUIREMENTS AND CARBOHYDRATE TOLERANCE IN DIETS FORMULATED FOR STRIPED BASS, *MORONE SAXATILIS*

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Continued and future development of the striped bass aquaculture industry will require the development of lower cost, more efficiently utilized diets which meet the species' specific nutrient requirements. Substituting lower cost plant feedstuff for higher cost animal feedstuffs could potentially reduce feed costs by as much as 50%. Common problems associated with feeding plant feedstuffs to fish include: 1) dietary limitations of essential amino acids (EAA); 2) poor utilization of EAA; and 3) low tolerance of high dietary carbohydrate. In order to determine the EAA requirements of striped bass, fillets were analyzed for amino acid composition to ascertain A/E ratios [(EAA/total EAA) x 1000]. The use of fillet A/E ratios allows for the simultaneous estimation of EAA requirements. In two experiments, diets with graded levels of EAA were fed to juvenile striped bass, such that dietary A/E ratios were maintained similar to fillet A/E ratios. Statistical analysis of weight gain, feed conversion, and nitrogen balance indicated significant differences ($P < 0.05$) among treatments. Non-linear regression analysis of the response criteria pooled from both experiments yielded the following estimates of dietary EAA requirements when digestible energy equaled 3200 kcal/100 g diet (g/100 g dry diet): arginine, 1.4; histidine, 0.6; isoleucine, 0.9; leucine, 1.9; lysine, 2.2; methionine+cystine, 1.0; phenylalanine+tyrosine, 1.7; threonine, 1.1; tryptophan, 0.3; and valine, 1.0. In a third experiment, low-temperature herring fish meal, soybean meal, corn gluten meal, and peanut meal were bio-assayed for amino acid availability. Corn gluten meal had a significantly ($P < 0.05$) lower availability coefficient for lysine, and peanut meal had significantly lower availability coefficients for histidine, isoleucine, leucine and lysine. There were no significant differences among treatments for availability of arginine, threonine, valine, and nonessential amino acids with the exception of cystine. Statistically there were no differences between soybean meal and herring meal for any nutrient tested. Two experiments were performed to determine dietary carbohydrate tolerance. Diets contained graded levels of carbohydrate as glucose or corn starch and were fed for a period of one or two months, respectively. Parameters measured included glucose tolerance, weight gain, feed conversion ratio, and hepatosomatic index (HSI). Glucose tolerance was determined following an oral glucose challenge. Glucose tolerance tests indicated a prolonged hyperglycemia with a greater tolerance for dietary carbohydrate levels as high as 12.5% but less than 25%, irrespective of carbohydrate complexity. Significant differences ($P < 0.05$) were detected between 15 and 20% carbohydrate for both weight gain and feed conversion ratios. HSI data indi-

cated a significant increase ($P < 0.05$) in liver size for all diets containing carbohydrate versus the control. Determination of the quantitative EAA requirements, availability of amino acids in practical plant feedstuffs, and the realization that striped bass are able to utilize both simple and complex carbohydrates at levels of 15–20% of the diet allows for the development of a low-cost species specific diet for the striped bass. Several diets have been formulated utilizing these data, and tested in both laboratory and practical conditions.

ASPECTS OF ESSENTIAL FATTY ACID FUNCTION IN GROWTH AND REPRODUCTION OF STRIPED BASS (*MORONE SAXATILIS*)

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Striped bass (*Morone saxatilis*) and its hybrids require n-3 highly unsaturated fatty acids (HUFAs) in their diet for growth and reproduction. Our laboratory has explored the effects of dietary levels of an essential n-3 fatty acid, docosahexanoic acid (DHA) on the rates of intestinal assimilation in striped bass, white bass and their hybrids and examined the timing and selectivity of deposition of DHA into ovaries of female striped bass.

Striped bass and white bass were fed one of two isocaloric diets that differed only in levels of HUFAs. Fish fed the low HUFA diet grew at a slower rate and exhibited lower rates of intestinal proline uptake rates *in vitro* relative to fish fed the high HUFA diet. Hybrid striped bass were fed isocaloric diets that differed in the ratio of DHA to eicosapentanoic acid (EPA). Three dietary treatments exhibited different growth rates, but not different intestinal uptake rates for proline, suggesting that dietary lipids were affecting growth rates by physiological processes other than intestinal absorption rates.

Analysis of lipid class and fatty acid profiles of male and female striped bass showed that all lipid classes in the plasma varied seasonally and phospholipid levels were also lower in females than males. The fatty acid compositions of each of the lipid classes in the plasma exhibited little variation with sampling date or sex. The findings that wax esters were not detectable in the plasma and the presence of lipolytic activity against phospholipids and triglycerides in ovaries, suggests that wax esters in the eggs of striped bass are synthesized within the ovaries. Purified striped bass vitellogenin had a lipid content of 20.1%. Over 80% of this lipid was phospholipid, primarily phosphatidylcholine. DHA content of striped bass vitellogenin was low (13%) relative to plasma lipid contents, but comparable to levels in egg lipids. DHA contents of ova from captive fish were comparable to samples from wild fish, but were nearly 2-fold higher than levels in the diet. This suggests that striped bass can preferentially deposit DHA in developing oocytes relative to their dietary intake.

INFLUENCE OF AQUEOUS AND DIETARY CALCIUM LEVELS ON GROWTH, SURVIVAL, AND BLOOD CHEMISTRY VALUES OF PHASE I AND PHASE II STRIPED BASS, *MORONE SAXATILIS*

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Two studies were conducted to determine: 1) if after a combination of handling and transportation stresses the optimum (as determined by survival rates and blood osmolarity and electrolyte levels) level of aqueous calcium for the receiving water of phase II striped bass lies above the current recommended value of 80 - 100 mg/L and to determine if this response is dependent on the calcium content of the water the fish were acclimated to prior to the administration of the stresses; and 2) if aqueous calcium levels will have an effect on the survival, growth, feed conversion efficiency (FCE), and blood osmolarity and electrolyte (sodium, potassium, and calcium) levels of phase I striped bass. Research in the first study indicated that there was a significant positive impact on survival, blood calcium levels, and blood osmolarity when the fish were transported into water with calcium levels above 250 mg/L. Fish placed into receiving waters containing either 30 or 100 mg/L calcium experienced very high mortality rates and showed greatly depressed levels of blood osmolarity and calcium. This research would indicate that the previous striped bass guidelines for aqueous calcium may in fact be too low and that higher levels are required for optimum survival rates. Discussion will also be presented on the impact that dietary calcium had on reducing the impact of transportation stresses.

NUTRITION OF LARVAL STRIPED BASS AND ITS HYBRIDS

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Larval striped bass and hybrids are currently fed a diet of live food. Hybrids are normally reared in ponds which have been prepared so that abundant live zooplankton species are available for them to feed on. Stripers are often raised in hatcheries which rely on brine shrimp nauplii as live food. The study of larval nutrition is important for two reasons. First, the rearing of fish on nutritionally inadequate live diets can lead to physiologically inferior fish that may be less resistant to stress and disease. Second, live food such as brine shrimp can be very expensive and the costs of production might be reduced if formulated diets could be used to replace live food. Fish reared in ponds are unlikely to suffer from nutritional inadequacies, because the variety of species available to them represents the natural food to which the two parent species have adapted over evolutionary time. Fish reared in hatcheries are more likely to suffer from nutritional inadequacies (both because of a single-species diet like brine shrimp and because of the stressful conditions of intensive rearing) and to benefit from development of formulated diets.

Studies in the 1980s demonstrated that a great deal of nutritional variability exists even in live food like brine shrimp nauplii and that variability can affect striped bass larval growth and survival. Procedures and commercial products are now available to improve the nutritional quality of nauplii. Unfortunately, periodic shortages of the cysts from which nauplii hatch mean that price per pound can vary from \$15 to \$45 and increasing worldwide demand could easily drive the price higher. The need for a formulated diet replacement for brine shrimp has never been greater, but commercially available diets can only partially, not fully, replace brine shrimp.

Formulated diets for larval fish must be available in the water column, palatable, digestible, and nutritious. The ideal formulated diet would be neutrally buoyant in the water, of small enough size to be easily ingested by the fish, soft and “tasty,” able to be broken down by the fish’s limited larval digestive capabilities, and with a nutrient content like that of natural zooplankton and simultaneously resistant to leaching of essential nutrients while in the water. All of these requirements present problems, but they may be solvable by an interdisciplinary team of engineers, nutritionists, biologists and aquaculturists. Because classical nutrition studies with fish require formulated diets whose composition can be manipulated and because larval fish cannot currently thrive on such diets, we do not have adequate knowledge of any nutrient requirements for larval fish. We do have some knowledge of some nutrient requirements for larval striped bass.

The digestive tract of striped bass is relatively undeveloped until the stomach appears at the time of transition to the juvenile stage, at which point weaning to a formulated diet is possible. Understanding of the processing of diets by the rudimentary larval digestive tract will be necessary for the development of adequate formulated diets for larvae. Studies using techniques of histology and immunocytochemistry will aid our search for that understanding.

OVERVIEW OF NUTRITIONAL ADVANCEMENTS WITH HYBRID STRIPED BASS

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Aquacultural production of hybrid crosses between striped bass (*Morone saxatilis*) and white bass (*M. chrysops*) as food and sport fish has increased substantially over the past decade. Our laboratory has conducted a variety of nutritional investigations with sunshine bass (*Morone chrysops* ♀ x *M. saxatilis* ♂) over the past several years to support the development of more nutritious and cost-effective diets for these fish. One area of research has concerned the establishment of some of the most critical nutritional requirements of sunshine bass. Quantitative requirements of these fish for many diet constituents have been determined including crude protein and specific amino acids such as lysine, methionine and threonine, protein:energy ratio, essential fatty acids, and selected minerals including phosphorus and zinc. Additional studies have been conducted to investigate intermediary metabolism of energy-yielding nutrients. In particular, the metabolism for soluble carbohydrates has been characterized. Investigations of nutrient and energy availability from practical feedstuffs also have been conducted in this laboratory with sunshine bass. Integration of this information concerning nutrient requirements and metabolism with that of feedstuff quality has led to the development of diet formulations for more efficient and sustainable production of these fishes in aquaculture.

WATER QUALITY IN FOUR RECIRCULATING AQUACULTURAL UNITS USING DIFFERENT FILTRATION SYSTEMS

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Four recirculating aquacultural systems were constructed. Each contained a 2 m diameter fiber glass culture tank, a biofilter, and a solids removal filter. System one consisted of a tank, bead filter, and a rotating screen; system two consisted of a culture tank, a trickling filter and a settling basin. System three consisted of a culture tank, a trickling filter, and a rotating screen; system four consisted of a Culture tank, a bead filter and a settling basin. All four systems were loaded as evenly as possible with hybrid striped bass and the water quality was monitored in an attempt to determine how the systems design influenced the water quality under similar loads. The following water quality parameters were monitored: temperature; TAN, nitrite, and total organic Carbon (TOC) concentrations; pH, and alkalinity.

Results indicate that there was a significant difference in the concentrations of total ammonia nitrogen (TAN), nitrate nitrogen and TOC between systems having a trickling filter and a bead filter as the biofilter. Nitrite nitrogen was the only measured parameter showing a significance difference between the systems with the rotary screen and the settling basin. Overall water quality parameters for the four systems are compared and the differences are discussed.

RECIRCULATING AQUACULTURAL SYSTEMS AND THE CHALLENGES FOR THE CULTURE OF STRIPED BASS AND ITS HYBRIDS

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Intensive culture of striped bass and its hybrids has steadily gained in popularity over the last two decades and recirculating aquacultural systems can be one of several possible production methods. Recirculating aquacultural systems use filters to remove wastes and maintain water quality, including solids removal, carbon dioxide stripping, and ammonia removal. The potential advantages of recirculating aquacultural systems over less intensive methods (e.g., ponds or flow through systems) are smaller space and lower water requirements. However, these advantages come with a price, namely more technology requirements and greater costs for both physical plant and operating costs. This presentation will outline some of the challenges to recirculating aquaculture systems and explore some ways to overcome the burden associated with them.

One challenge for recirculating system owners and designers is the myriad of configurations and components available from which to choose. After the system is designed, the biofilters must be conditioned and readied for use. Operating a recirculating aquacultural system is similar in many respects to other fish culture operations, but there are some differences that must be learned and experienced before operations become routine. For example, routine tasks like changing feeding levels cannot be done in recirculating systems as they are in pond or flow through systems. Treating fish for disease or parasites requires that biofilters are protected from potentially harmful chemicals, which even at therapeutic levels can kill desired bacterial populations. Overcoming the challenges requires diligent record keeping, constant attention to routine details, and the ability to anticipate impacts to the fish or system resulting from necessary changes.

OZONE APPLICATION IN RECIRCULATING SYSTEMS REARING HYBRID STRIPED BASS

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Ozone application in recirculating aquacultural systems addresses several water treatment goals including dissolved waste removal, enhanced nitrification, and fine solids control. Therefore, ozone application can lead to reduced wastewater discharged, improved fish growth, and a reduced oxygen requirement. However, the cost of ozone application is substantial, requiring a rational analysis of its benefits before recommending it to commercial operators. At present, there exists no well defined scientific criterion for ozone application in recirculating systems.

The Maryland Agricultural Experiment Station has recently funded two projects that aim to quantify the benefits of ozone application in recirculating aquacultural systems rearing hybrid striped bass. Specific objectives of the project are: (1) comparing the physical and chemical properties of dissolved and particulate waste (water quality) in an ozonated system with those of a control system, (2) comparing the ozonated system and control as to fish growth performance, (3) comparing the ozonated and control systems with regard to biofilter and particulate filter performances, and (4) comparing the quantifiable operating costs per kg of fish production under the two treatments.

To quantify ozone demand, relationships between ozone consumption and change in selected water quality parameters will be developed using a bench-scale approach similar to that used in drinking water studies. This will help identify the best water quality parameter(s) that should be used for predicting ozone demand of a recirculating aquaculture system. Work on ozone demand quantification is currently in progress.

Experiments will be conducted to compare the performance of two recirculating aquacultural systems, identical in size and configuration except that one will use ozone application and the other will not. Water quality, fish growth, and major operating expenses will be monitored and recorded in both systems for statistical comparison. The location of ozone application within the recirculating system and the time of application will also be evaluated. Results, thus obtained, should be directly applicable to a commercial recirculating striped bass culture system irrespective of its size, intensity of production, feeding rate, and other system parameters.

HYBRID STRIPED BASS PRODUCTION TECHNOLOGY

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Commercial production of hybrid striped bass (HSB) as foodfish began in the mid 1980s. Fingerling production to support the industry is still dependent on pond production although a few farms are working on fingerling production in tanks. Foodfish production levels in the past decade have increased from 10,000 pounds in 1986, to 8.4 million pounds in 1997 based on a recent Striped Bass Growers Association survey. Production technology can be divided into three types, tanks, ponds, and cages. Tank, pond, and cage culture accounted for 56, 43, and 1 percent of foodfish HSB production in 1997.

Most tank culture systems use some form of water reuse. Production in these systems is intensive and growth is managed by controlling such variables as temperature, density, and feeding rate. Pure oxygen is usually used to maintain suitable dissolved oxygen levels and appropriate back-up systems must be in place to protect against power outages.

Pond culture of HSB is a two-year cycle. Most producers stock small fingerlings (about 1 gram) at 10,000 to 20,000 per acre during the first year and then transfer them to growout ponds at 3,000 - 4,000 fish per acre for the second year. The ratio of fingerling ponds to growout ponds on individual farms is around 1:3 or 1:4. All ponds are equipped with electric aerators and tractor powered backup aerators must be available to protect against plankton die-offs that result in low oxygen. Water quality management is the key to successful production in ponds.

Cage culture is used in a few situations where wild water or pond water resources are available that cannot be used in any other way. Fish are stocked at high densities and water quality and dissolved oxygen are maintained by natural water movement through the cages.

USE OF BACKCROSS HYBRID STRIPED BASS FOR AQUACULTURE

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During the past five years, a number of studies were conducted in South Carolina to characterize the potential use of backcrossed hybrid striped bass (female sunshine bass x male striped bass) for aquaculture. Hatchery production of backcrosses was highly variable among years and within hatchery trials each year. Female sunshine bass could be induced to ovulate using HCG and or GNRH implants, and could be strip spawned or tank spawned. Egg fertilization rates varied from 0 to about 65% (among trials, mean 19.4%) while hatch rates varied from 0 to about 45% (among trials, mean 13.7% using either spawning technique. Feeding trials with recently hatched fry indicated that live *Artemia nauplii* were a suitable first feed.

Backcrosses were tolerant of brackish water. Eggs incubated at 9 and 11 g/l salinity had a hatch rate of 35 and 30% respectively, as compared to 55% for those hatched in freshwater. In 96 hour studies, two day post-hatch juveniles exhibited 100% survival at 10 g/l salinity and 0% survival at 15 g/l. However, forty day post hatch juveniles exhibited 100% survival in salinities for four month at various salinities. Growth was similar at salinities 1, 10, and 20 g/l and lower at 30 g/l. Survival was highest (87.2%) at 0 g/l and similar at 10-30 g/l treatments (mean 59.9%). However, a bacterial infection occurred which more severely impacted fish reared above 0 g/l salinity.

Five pond nursery trials were conducted using typical striped bass culture techniques. First feeding fry were stocked at a density of 600,000/ha and reared for 1-2 months at 6-8 g/l salinity water. Mean survival was 56% (range 21-70%) and mean harvest size was 0.32 g. A one-year study compared performance of backcrosses to sunshine bass and striped bass. At harvest of phase I (9 months), no differences ($P < .05$) were detected in mean size, specific growth, or feed conversion. However, an *Amyloodinium* infection affected survival of the backcrosses (74%) more so than the sunshine bass (87%) or striped bass (91%). At conclusion of phase 2 (3.5 months), there were no differences in size, survival, feed conversion, or specific growth. Similarly, results of a demonstration trial with an instate farmer indicated that performance was similar to the sunshine bass typically reared at the facility. Also, a limited marketing test indicated that they were priced similar to other striped bass hybrids.

The above information indicates that the backcrossed hybrids can serve as an alternative culture candidate for hybrid bass farmers.

MOLECULAR CHARACTERIZATION OF AN AQUAREOVIRUS ISOLATED FROM STRIPED BASS

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During the past 15 years, a large number of viruses with characteristics of the family *Reoviridae* have been isolated from a wide variety of aquatic animals. Although the majority were isolated during the routine examination of apparently healthy fish, some were isolated from fish that were also infected with bacteria or from fish populations undergoing chronic or large mortalities. The molecular characteristics of an aquareovirus (SBR strain) isolated from a moribund striped bass were examined. The virions are icosahedral, 70–75 nm in diameter with a double-layered capsid and a genome of 11 segments of double-stranded RNA. They form plaque-like areas or syncytia as a characteristic cytopathic effect when grown in fish cell cultures. Although these viruses share some features with the Reovirus and Rotavirus genera, they have been classified as a new genus, Aquareovirus, based on their host range, optimal growth temperature, and lack of cross-reactions with other reoviruses by RNA-RNA hybridization and serological test methods. Using reciprocal RNA-RNA dot blot hybridizations under high, medium, and low stringency conditions, six different genogroups (A through F) have been defined from among the 50+ isolates made from sites around the world that we have examined. Genogroups A (19 members) and B (20 members) were the largest, and Northern blots between these groups showed that segment 10 (which codes for the major outer capsid protein) was the most variable gene. Examination of proteins in purified SBR virus and lysates of virus infected cells showed 12 proteins ranging in molecular weight from 130 to 15 kDa, 7 were structural and 5 were non-structural. Cell-free translation of total virion RNA in a rabbit reticulocyte system resulted in the synthesis of proteins that were both qualitatively and quantitatively very similar to those found in infected cells. Segments 1–10 of the SBR virus genome encoded one protein each while segment 11 encoded 2 proteins. The proteins were found to be virus specific by immunoprecipitations. The major outer capsid protein, VP7, was found to be glycosylated and its removal by trypsin markedly enhanced viral infectivity; however, the electrophoretic mobility of VP7 was not altered when untreated and deglycosylated proteins were compared indicating the presence of short oligosaccharide chains. The 3-D image reconstruction from cryoelectron micrographs of the SBR virus displays a marked similarity to the mammalian reovirus intermediate subviral particle suggesting a close evolutionary relationship. One notable structural distinction is that the aquareoviruses lack the hemagglutinin spikes seen in reoviruses.

RISK ANALYSIS FOR PATHOGEN INTRODUCTION INTO MARYLAND STRIPED BASS VIA LIVE FISH, FISH EGGS OR FISH PRODUCT IMPORTATION

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Aquaculture producers, animal health regulatory officials, veterinarians, fish health professionals and natural resource agencies continue to look for methods to evaluate existing knowledge to prioritize relative risks associated with potential pathogens so that risk management strategies can be targeted in the most cost-effective manner. A collection of methods known as risk analysis has emerged to support rational decision-making in the face of uncertainty. Risk Analysis is a blend of hazard identification, risk assessment, risk management and risk communication. Risk, as it relates to Maryland striped bass, is the measure of the probability of the introduction of pathogens through the importation of live fish, fish eggs or fish products. The hazard identification process seeks to establish which pathogens are of concern and how they may be introduced. Risk assessment is the process of estimating the probability or likelihood of pathogen introduction as well as the associated implications. The goal of risk management is to reduce both the likelihood and implications of the introduction of pathogens into Maryland striped bass. The involvement of all potentially affected parties in the overall process is the goal of risk communication. This is an important, but often over-looked, aspect of the risk analysis continuum.

This particular risk analysis may be described using five main objectives. First, the involvement of fish producers, game fishermen, fish management veterinarians, fish health professionals and regulatory officials, consumers, importers and exporters in *communications* concerning pathogens of striped bass and analysis of the risk of their introduction into Maryland. Second, to *assess the risk* for introduction of bacterial, viral, or parasitic pathogens into the Maryland striped bass population from imported live fish, fish eggs or fish products. Third, to *prioritize these risks* based on the likelihood of their occurrence and the magnitude of the impacts should introduction occur. Fourth, to *evaluate current risk management strategies* and propose alternative or additional options for the cost-effective reduction of the risk of pathogen introduction. The final step and ultimate goal is to establish an ongoing process of risk analysis for striped bass and generate information in multiple formats to support rational decision-making by regulatory officials, industry groups, aquaculture producers and other affected parties.

The above activities were eclipsed by the focus on *Pfiesteria*, which dominated the industry throughout the late summer and fall of 1997. Nevertheless, the *Pfiesteria* situation provided the opportunity to gain further insights into industry players and the diverse concerns of aquaculture

producers and consumers. The events provided an excellent living laboratory to examine the reactions of producers and consumers to the recognition of a new pathogen in Maryland fish.

Relationships were established with the Maryland Department of Agriculture and the Maryland Department of Natural Resources to further document the pathogens which have been identified in the Maryland striped bass population in aquaculture and in the wild. Further meetings are planned for this spring with extension personnel, at both the university and state government levels, in order to more thoroughly assess the current thoughts on possible emerging diseases in both the wild and aquacultured fish in Maryland.

DISEASES OF SERIOUS CONCERN TO MORONE AQUACULTURE

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As in other aquaculture endeavors, disease continues to pose a threat to the successful production of *Morone*. The ability of these fish to thrive in both freshwater and marine environments places them at risk of exposure to a wide range of pathogens. While many infectious and noninfectious diseases have been documented in *Morone*, this talk will focus on those problems which are the most serious current or potential threats to *Morone* aquaculture. Included are the protozoan parasites (*Amyloodinium*), worm parasites (“grubs”), bacterial infections (columnaris, aeromonads, mycobacteriosis, pasteurellosis, streptococcosis/enterococcosis), water molds and algal toxicoses (*Pfiesteria*). The continued success of *Morone* aquaculture will depend upon a constant improvement in disease management. In this arena, the most pressing needs are for registration of critically needed therapeutics, improved certification procedures to detect and eliminate/exclude pathogens from culture systems and effective vaccines for control of important pathogens. Also needed are the development of better methods for the detection and reduction of stress, which usually plays an important role in disease outbreaks.

PRODUCT DEVELOPMENT: POTENTIAL OF STRIPED BASS

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Currently, most aquacultured products are marketed unprocessed or minimally processed product, e.g. dressed fish. Although further processing offers the potential for adding value, the industry has been slow to innovate. Several factors are responsible for this, including high costs with insufficient margins and limited food technology expertise in most aquaculture organizations. Food safety regulatory requirements for processed products are another deterrent for some producers, especially for products considered to be high risk, including ready-to-eat and vacuum packaged items. However, opportunities do exist for value-added products and the early trend is likely to continue as it has in other food industries.

The Maryland Sea Grant Program is involved in several projects related to seafood product development and food regulatory compliance. The Rural Development Center (University of Maryland Eastern Shore) recently funded a study to determine the feasibility of freezing rockfish for year around distribution and to evaluate other value-added products for expanding the product lines of an existing seafood cooperative and other processors. Project investigators will: (1) assist members of the Smith Island Crabmeat Cooperative, processors in Somerset County, Maryland, and seafood distributors to optimize facilities use and labor during the off-season, (2) develop one or more products, initially with striped bass, which meets cost and regulatory requirements and the needs of customers; (3) determine product requirements related to packaging and processing, by conducting controlled shelflife studies; (4) conduct market surveys to determine the product's acceptability to commercial buyers and consumers; (5) develop a Sanitation Standard Operating Procedure and HACCP program applicable to the selected products; and (6) assess the profitability and sustainability of a seafood cooperative based on expanding the Smith Island Crabmeat Cooperative to include, or network with, other processors in Somerset county.

Maryland Sea Grant Extension is also actively involved in the Seafood HACCP Alliance, including development of models and resource materials for industry. Eight certification courses have been offered to date which meet federal and state training requirements. Alternative monitoring strategies under HACCP were studied in crab processing facilities in 1997. Results suggest that various manual and electronic methods for documenting critical control points are feasible when procedures are consistently implemented.

Processors of aquacultured seafood should consider several important issues prior to introducing a new aquaculture produce. Conduct a thorough market analysis. Meet with customers and determine their interest in one or more products under consideration. Be prepared to answer questions related to availability, packaging and quality, such as expected shelf-life. Price will be an impor-

tant concern but should be quoted only after thorough study. Each restaurant and retail chain has specifications which may be unique to them. A bake-and-serve item is of no interest to those restaurants which equip their kitchens with fryers only. A highly convenient product (fully formulated, IQF, heat-and-serve) may fit nicely with space and time constraints in a family-style restaurant but prove far less suitable for white tablecloth markets. Plate coverage and portion control are often important issues. Do some homework and seek their input. Be aware that some products may require hefty slotting fees to gain display space at retail. Conduct storage studies to determine the most cost-effective processing and packaging options for inventory management.

The new U.S. Food and Drug Administration regulations based on Hazard Analysis Critical Control Point (HACCP) must be fully considered. Understand all of the species and process related hazards associated with the product. The regulations require an analysis of these hazards, monitoring and recordkeeping where potential problems exist. Also, key sanitation requirements must be met and documented. Most firms should seek assistance when assessing hazards and appropriate control strategies.