Anderson Chen

Molecular and Genetic Approaches to the Conservation Efforts of *Thunnus* Group

It has become increasingly obvious that stringent government regulation can no longer adequately address the severe issue of overfishing. As global fish consumerism has shifted to a farm and ranch-based aquaculture model, the ability for supply to keep up with the voracious demands of the international market has ameliorated the burdens of wild stock populations. More noticeably, however, is that certain valuable fish species still trail behind this change in farmed production, leading to an accelerated rate of loss that worries researchers. Numerous studies and experimental research has been completed in an attempt to lay the foundation for Bluefin tuna conservation, with population genetic and larval genomic expression data as tools for the potential establishment of sustainable methods for tuna cultivation. The problem remains that as of right now, passive research and aggregation of conclusions drawn from vast amounts of genetic data yields no practical way of active conservation. Thus, current forays into tuna aquaculture is immensely valuable, and a more appropriate direction of research, for accelerated development of sustainable consumption of one of the most valuable food resources on the planet.

Though low in quantity, tuna farms have existed for decades, with varying success in their respective countries. However, sustainability remains an issue; traditional tuna farms have always operated on a capture-based method. This entails the whole of the farming activity to depend on juveniles caught in the wild and reared in captivity. Completion of a full life cycle on a commercial scale is a massive endeavor that requires controlled conditions through long periods of time and investment of resources. It wasn't until recently that researchers were able to replicate the natural reproductive cycle of certain tuna species. Given the high mortality rates of juvenile tuna, transporting the fish from towing cages presents an obstacle, as "transfer action is a crucial activity as specimens may suffer severe stress that may lead to death." (1) Though only recorded at a mere 2%, high mortality rates within the aquaculture sites nevertheless remain an issue due to adverse environmental conditions. Various crucial sustainment data is also generally neglected, as recommended isolation from anthropogenic interference for the fish has led to "growth, food intake and feed conversion rates [that] have never been estimated accurately by farmers." (1) Overfeeding during the "fattening" stage of farming has also been a misguided common practice; calculated feed conversion ratios have generous estimates at best as well. Capture-based aquaculture relies on retrieving juvenile "seeds" from wild stock populations. In other words, overlap with the fishing sector is unavoidable and despite using fewer specimens than haphazard trawl fishing, is not sustainable in the long run; both factors into the depletion of spawning stock biomass. As such, we must now turn to molecular-based rearing techniques that allow for the completion of tuna life cycles, allowing for the production of a positive feedback system in which juveniles hatched and raised in captivity go on to contribute to farming stock.

The first issue at hand is the feed conversion ratio. Apex predators such as Bluefin tuna must achieve a diet that balances economic factors and sufficient protein output. Given that shifts in diets such as overfeeding and incorrect distribution of nutrients can lead to larval mortality, feed composition is an integral aspect of aquaculture, similar to its terrestrial counterparts. There are a couple ways to tackle this that have gained traction within the community: emulation of natural prey selectivity and experimentation with bait preparation that may potentially increase growth. As mentioned before, the high rate of digestive function and antioxidant development in gene

expression of Bluefin tuna larvae restricts the availability of traditional feed. (2) The former involved the selection of two prominent types of natural food source, seabreams and bonitos. Cultivated strains of both were achieved in large tanks, and timed and controlled feeding of Pacific Bluefin tuna were done along with random feeding to generate preference data. Size and species preference observed indicated foraging behavior, and may help in establishing appropriate baits for raised tuna. Smaller tuna seemed to prefer the bonito preys that are smaller in size, whereas larger ones aimed for the larger seabream food source. Alternative feeding of different food sources may be appropriate for different life stages of farmed tuna, by actively "select[ing] the small-sized prey, a strategy that can maximize energy intake per unit of time when encounter rates, handling times and capture success between prey sizes differ." (3) Experimentation with different bait compositions poses a more complicated task. A realistic approach would be to analyze genetic expression of larval digestive systems through different long-term experimentations. First, on a macro scale, observed mortality and growth can be determined. Then, with the larvae that have observed accelerated development, genetic screening can be done in which RNA extraction and cDNA establishment can help isolate expression genes that respond well to the respective bait type. This style of double screening can speed up the process, with the con being the long life cycles of larval development that would require large sample sizes to be efficient. The emphasis and focus on feed is not without reason, as the primary stage of larval mortality occurs in the fattening stage. Solving the feed efficiency problem also alleviates other areas of concern such as environmental impacts of wonton bait collection and avoidance of food-based marine diseases, not to mention the economic savings involved.

In an era where genetics has now played a large role in providing large quantities of food with desirable traits, the transgenic movement has now spread to the fishing industry. While commonly consumed species of fish are now being raised for traits that confer size and nutritional advantages, larger fish of higher value still struggle to be farmed. However, the genetic process can still be applied for potentially facilitated tuna rearing. Spermatogonial transplant may be achieved with a recipient species that are similar enough genetically and physiologically to species such as the Bluefin tuna. In one such experiment, the Eastern little tuna was used for its short generation cycle, small size, and similarities with its Pacific counterpart. Post-transplantation, gonadotropin-releasing hormone was used to induce spawning in 9 to 14 ELT broodstocks. What followed was a surge in fertilization and hatching rates that accompanied egg collection numbers of 50,000 to 170,000 per day. DNA genotyping revealed through parentage assignment that " 3 of the 5 implanted females produced viable offspring, and one of these females participated in up to nine consecutive spawning events." (4) A second trial was completed with sample size 12 (6 females) that were kept together before the trial and then injected with hormone pellets. 11 days post-transplant, the mean fertilization (61%) and hatching (42%) remained sufficiently high to demonstrate the effectiveness of GnRHa-induced spawning, offering a relatively simple technique that may lead to development of "a surrogate broodstock technology" for Pacific Bluefin tuna. (4)

Transgenic fish, backed by its ubiquity for much of the consumed seafood in nations today, thus represents a strong contender for the primary method of establishing sustainable tuna farms. Enhanced growth rate and feed efficiency may be difficult to achieve given the carnivorous diet of tuna, but other traits may be desirable. Shorter generation times, resilient broodstock, low-mortality larva, and resistance to water turbidity, diseases, and other adverse conditions are all genetic attributes that may benefit from transgene development. Like the aforementioned Little

Eastern tuna, genetically similar and less-threatened tuna species may be used to identify and screen for profitable traits. These fish can realistically have larger sample sizes and achieve results at a faster rate. Hormone-injections aside, pronuclear injections in which linear DNA is inserted into an egg impregnated by a sperm is also possible given modern technology, despite it being an infrequent event that depends strongly on its introductory location. Once Atlantic or Pacific species of *Thuunus* can be induced to spawn and confer traits of short but stable generation cycles, the process naturally facilitates itself. Isolation of RNA expression and the physical gene involved, as well as the intricate process of transgene transplant and safe hormone induction, are all highly complicated, time-consuming, and expensive processes that have disproportionately low success rates; yet if achieved, may offer a chance for truly sustainable yields of these fish.

Perhaps the most important aspect of tuna aquaculture to consider is the successful completion of a life cycle. Selective breeding using such methods as hormone-induced spawning and pronuclear injections are only viable if the resulting larva can fully develop into reproductive adults. Successful husbandry, of course, relies on genetic data and life history of tuna species, reacting appropriately and observing the cause of high mortality rates at every stage. According to an aquaculture research paper on the completion of the *Thunnus orientalis* life cycle, "major obstacles that remain in the establishment of PBT hatchery production technology originate from their biological traits." (5) Firstly, spawning in captivity is still relatively unpredictable, as knowing the temporal range of spawns does not translate to a precise, controlled factor. The key factors of spawning are still unknown, hence the reliance on hormone-induced spawning as a possible means to generate sufficient stock. As mentioned before, high mortality rates given any combination of factors (environment, feed, nutrition) may contribute to stock collapse. Improved techniques so far through the years have only brought larval and juvenile rearing to a measly 4%. (5) The molecular intricacies of the PBT's volatile digestive system also present complications in terms of cannibalism, as the need for a high protein diet has ensured the adaptation to fish consumption, especially given the ease of access to a constant food source in the form of cohorts. Hypersensitivity to physical stimuli coupled with high-speed swimming within cages and tanks all contribute to the constant deaths of captive tuna. The long-term experiment of Sawada and co. thus began with wild-caught broodstock PBT, as the natural wild stock (sample size 281) had a hatching rate of more than 80%. After sufficient eggs have been collected, artificial larval rearing begins as feeding schedule ranged from DHA-enriched rotifers to fish larva and minced meat. 0.07% of the artificial stock survived to the next stage. This F1 PBT broodstock was left alone to spawn, with observed unstable spawning. An F2 generation rearing began as more eggs were collected. This F2 generation saw an astounding 97.1% hatching rate out of 1.63 million eggs, but mortality rates through larval development nonetheless remained high due to a culmination of "trauma by collision (33.4% mortality)", "iridoviral infection (56.5% mortality)", and "collision with nets (after growth to adulthood)." (5) Despite soaring mortality rates, successful hatching, and most importantly growth to adulthood, nevertheless was possible through tightly controlled conditions. The experiment served to point out the glaring problems that exist in traditional aquaculture methods today, citing strict regulation of rearing conditions in order to avoid mortality rates and allow for facilitated selective breeding.

In present day, researchers have come a long way in monitoring the population trends and composition of major tuna species. Large amounts of genetic data and population genetic structure have pointed to glaring problems in sustainable fishing and laid the foundations for establishing sustainable methods of tuna consumption. However, we must now turn to

experimental studies that focus on molecular approaches to realistic tuna rearing. Traditional tuna aquacultures have long been insufficient in terms of sustainability and consistency. Production output is restricted by high mortality rates, and wild stock populations remain burdened by the constant need for wild-caught juveniles. For the sake of conservation efforts and to emulate the success of fish farms of prominent species, two key factors must be accounted for. First, the fertilization and hatching success rates must be consistent, and mortality rates from adverse conditions must be marginal at best. Completion of tuna life cycle is the fundamental key to all other genetic work such as selective breeding and hormone-induced spawning. This includes highly regulated environmental control such as light, temperature, and density levels, as well as molecular-based nutrient feed ratios of certain types of bait foods. Feed ratios remain an integral process in sustainability, so optimal bait composition depends on natural prey selectivity and genetic expression of digestive system functions. The second factor revolves around the implementation of an efficient transgene system that would allow for selection of traits to propagate production output, including disease resistance and shorter generation cycles. Pronuclear injections, followed by hormone-induced spawning and consistent completion of sexual maturity to adulthood would hopefully incite a positive feedback system in which the same genetic sequence can be screened and selected for within a large-scale farm operation. With appropriate backing by genetic data, molecular and experimental approaches to active sustainable farming become possible. We have begun to move towards adapting large predatory, marine fish to our familiar farming system, hopefully with more tact in regards to environmental impact and efficiency.

Works Cited

1. Ottolenghi, F. 2008. Capture-based aquaculture of bluefin tuna. In A. Lovatelli and P.F. Holthus (eds). Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No. 508. Rome, FAO. pp. 169–182.
2. Mazurais, David, Denis Covès, Nikos Papandroulakis, Aurelio Ortega, Elisabeth Desbruyeres, Christine Huelvan, Marie Madeleine Le Gall, Fernando De La Gandara, and Chantal Louise Cahu. "Gene Expression Pattern of Digestive and Antioxidant Enzymes during the Larval Development of Reared Atlantic Bluefin Tuna (ABFT), Thunnus Thynnus L." *Aquaculture Research Aquac Res* (2014).
3. Reglero, P., E. Blanco, A. Ortega, O. Fiksen, F. De La Gandara, M. Seoka, F. J. Viguri, and A. Folkvord. "Prey Selectivity in Piscivorous Bluefin Tuna Larvae Reared in the Laboratory." Journal of Plankton Research 37.1 (2014): 2-5. Web.
4. Yazawa, Ryosuke, Yutaka Takeuchi, Kotaro Amezawa, Kenta Sato, Gaku Iwata, Naoki Kabeya, and Goro Yoshizaki. "GnRHa-induced Spawning of the Eastern Little Tuna (Euthynnus Affinis) in a 70-m3 Land-based Tank." Aquaculture 442 (2015): 58-68. Web.
5. Sawada, Yoshifumi, Tokihiko Okada, Shigeru Miyashita, Osamu Murata, and Hidemi Kumai. "Completion of the Pacific Bluefin Tuna Thunnus Orientalis (Temminck Et Schlegel) Life Cycle." Aquaculture Research Aquac Research 36.5 (2005): 413-21. Web.