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Recommendations for Conservation of Formosa Landlocked Salmon

The participants at the International Symposium on Formosa Landlocked Salmon and Masu Salmon recommend the following guidelines:

Dr. John R. Gold

(Regents Professor, Texas A&M University, USA)

The conservationists who want to preserve a "pure" Taiwan salmon appear to be of the view that what's left represent a "pure" population. Given Dr. Gwo's data that there is hardly any genetic variation in present-day Taiwan salmon (to be sure in sequences presumed to be selectively neutral), here is the question: Were "pure" Taiwan salmon prior to human occupation genetically depauperate? ("Depauperate" is a fancy word for genetically impoverished.) It is my guess that if one catalogued average levels of microsatellite variation in all other extant species of salmon, one would not find any species with such low levels of genetic variation. If so, one could then make the argument that present-day Taiwan salmon likely are not "pure" at all but have become something they were not intended to be (i.e., genetically depauperate) before humans came along and altered the habitat and climate. The main point would be that present-day Taiwan salmon are not "pure" at all but have become something very different from what "pure" Taiwan salmon were before humans came along. To argue otherwise would be to hypothesize that Taiwan salmon, eventually over the course of time, would have become genetically impoverished and ultimately have gone extinct, even in the absence of humans. This could well be the case; however, if so, it would appear without question that humans have accelerated significantly the decline.

My suggestion was that one might consider bringing in some new alleles into Taiwan salmon by crossing with its closest phylogenetic relative (which I assume would be one of the subspecies of *Oncorhynchus masou* in Japan). The suggestion was based on the observation that there is very little genetic variation in present-day Taiwan salmon. I would have to search the literature more thoroughly, but my "sense" is that two di-allelic microsatellite loci out of 13 assayed is among the lowest reported for a gonochoristic fish. As I mentioned at the roundtable discussion, one of the points noted in the restoration plan was to introduce Taiwan salmon into other streams/creeks, in part so that genetic exchange could occur. Introducing Taiwan salmon into other parts of the drainage is, of course, a very good plan. However, if there is little to no genetic variation, genetic mixing of fish from different streams/creeks won't generate very many new genetic combinations.

As to crossing Taiwan salmon with one of the subspecies of *O. masou*, my recommendation would be to use female Taiwan salmon and male masu salmon. The reason for this is to avoid any issues related to maternal effects. As I listened to the very excellent presentations by Professors Arai and Abe, I had the sense that maternal effects may have played a role in some of the interspecies crosses where either success was very low or the cross worked in one direction but not the other. In addition, maternal effects themselves are, in fact, a polygenic trait that is acted upon by natural selection. The point here is that if there are

maternal effects in Taiwan salmon (and I would be very surprised if there were not), it would be important to attempt to insure that the maternal-effect genes are those of Taiwan salmon. Following generation of F1 hybrids, the next series of crosses would be backcrosses of F1 males to female Taiwan salmon. Again, the point of using female Taiwan salmon is not to "lose" any maternal effect genes (or at least to minimize their loss).

The research approach I would suggest is to carry out the above and to assay a number of genetic markers to determine the percentage of new alleles coming in from masu salmon into Taiwan salmon. My preference would be microsatellites and I would think a suitable number would be at least 50 (if not more). I prefer microsatellites to AFLPs and to SNPs as microsatellites are codominant (an advantage over AFLPs) and often have well more than two alleles (an advantage over SNPs).

Once one had the microsatellite primers in hand, one would assay both Taiwan salmon and the males of masu salmon that would be used in the cross. The F1 hybrids, of course, should have one allele from Taiwan salmon and one allele from the masu salmon used in the cross. The interesting question is the percentage of Taiwan salmon (or masu salmon) alleles in the F1 backcross progeny. This will be the initial "test" of the success of introducing "new" alleles into Taiwan salmon. Subsequent crosses could be continuous backcrossing with female Taiwan salmon.

A last (but very important) point is that I don't necessarily recommend that one start introducing the backcrossed fish into the "wild" (at least not for a period of time). Rather, I recommend that the crosses be made and that the introgression of masu alleles into Taiwan salmon be followed experimentally. It also might be important at the same time to investigate any number of biological parameters (morphology, physiology, etcetera) in the backcross progeny and make comparisons with "pure" Taiwan salmon. The point here is that introducing alleles from masu salmon is likely the only way in "real time" to introduce new alleles into Taiwan salmon. Mutation would work but only over a millennium (and I would be surprised if Taiwan salmon survived that long given their present genetically impoverished state). However, the process and any potential biological impacts need to be studied before any action is to be taken. If nothing else, having the information *a priori* on this alternative would be imminently preferable to having to carry out the alternative in the absence of any data. That is, suppose in 5-10 years the survival of Taiwan salmon drops precipitously because of environmental change (e.g., global warming) and the inability of Taiwan salmon to adapt (because they have little to no genetic diversity). One might then considering crossing in "new" alleles from masu salmon as a last desperate measure. It would be beneficial at that time if there already were scientific data on the cross and its ramifications.

Dr. Koji Maekawa

(Professor Emeritus, Hokkaido University, Japan)

Formosa masu salmon in Taiwan is a very important endemic subspecies of masu salmon, because it represents the most southern limit of the masu salmon distribution, and was probably distributed during a glacial age more than 100,000 years ago. The status or existence of this fish may also be symbolic in the conservation of nature in Taiwan.

We suggest here how this subspecies should be preserved. First, according to many studies on the maintenance of populations of masu salmon (e.g., Maekawa 2004), Formosa masu salmon habitats (all river and creaks for potential distribution) and riverine forests have to be conserved. The latter may provide salmon habitat and foods (eg. Nakano and colleagues, see reference in Maekawa 2004). During the excursion at the symposium I found that creeks and riverine forests look suitable for Formosa salmon and also observed many spawning events during the stay in the national park, suggesting the continued existence of a wild Formosa salmon population. However, one of the most important problems for preservation of this salmon will be impassable dams (erosion control dams), because of fragmentation of habitats (leading to decrease of genetic diversity) and leading to decreases in spawning grounds. I suggest that these dams should be removed or improved as soon as possible.

Secondly, rearing of rainbow trout in the hatchery must be stopped in future because of the possibility of introducing disease as Dr. Onozato and other participants have suggested. Thirdly, may I remind you of some problems with releasing fish reared in a hatchery? Such releases may lead sometimes to increase salmon population, but this should be recognized to have some risk.

In summary, after all, I would like to emphasize that to increase wild population and habitats will be primarily more important to preserve Formosa salmon population than releasing a fish or others and to hope that further studies will be conducted, especially, about population ecology and others.

Dr. Francis Juanes

(Professor, University of Massachusetts, USA)

I have three recommendations. First, it is critical to devise strategies to increase genetic diversity of Formosa salmon, but the mechanisms used to do that are controversial and should be carefully debated. Second, the stocking strategies and goals need to be clarified. Stocking with Formosa salmon alone will not increase genetic variability, but could increase habitat range and fish numbers. All participants warned of the great risk of keeping rainbow trout in the hatchery alongside salmon because of the dangers of escape and disease transmission. Third, the effects of agricultural activities need to be carefully examined as they are likely having a detrimental effect on Formosa salmon as indicated through increased observations of malformations. Similarly, habitat connectivity is critical for salmon populations, and dams fragment and modify habitat. Are there future plans for more dams to be destroyed, and if not, can fish passage facilities be constructed? Ultimately, the park managers need to decide what preservation means, is it increase in fish numbers, habitat range or genetic variability? Formosa landlocked salmon probably require all three to prevent extinction.

Dr. Satoshi Awata

(National Research Institute of Fisheries Science, Japan)

I have some comments about the conservation of Formosa 'dam-locked' salmon. Throughout the symposium, I noticed that the researchers from Taiwan and Japan have accumulated many background data regarding phylogeny, migration patterns, and population

persistence. I think that the most important point for the future is to bring a conservation plan into practice. For example, such a plan could include rehabilitation and dam destruction. Releasing salmons to the habitat where the Formosa salmon have disappeared from is advisable. Also you should continue to destroy dams. However, you should be circumspect in destroying dams because it is possible that dam destruction also brings negative impacts on fish population. Hence, it is important to consider which dams should be destroyed first, and second how many dams should be destroyed (I think there is no need to destroy all dams). I do not know enough about the progress of taxonomical and ecological studies in Shei-Pa National Park. However, there may be several new species and biologically and ecologically important species in the area. Hence, I encourage Taiwanese researchers to study other animals as well as the salmon such as birds, reptiles and fishes. If many new species and biologically important species (also endangered species) are found, many more people will pay attention to the importance of Shei-Pa National Park. And also the government will increase funds for conservation and preservation of the Formosa salmon and the National Park. However, you should be cautious about how many researchers are allowed to study in the area. Having too many researchers could lead to habitat destruction. I hope these comments are useful for you and future Formosa 'dam-locked' salmon.