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Marking Fingerling Salmon with Trace-Elements and Non-Radioactive Isotopes

By

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The Bureau of Commercial Fisheries at its Seattle Laboratory** is conducting experiments to develop a permanent mark that can be applied to fingerling salmon quickly, easily and economically, and that can be employed to mark the entire production of a hatchery or specific spawning area. The evaluation of hatchery or spawning area contribution to a fishery has depended upon the recovery of fish marked by clipping one or more fins. While fin-clipping has produced some data for evaluation, the method is time-consuming, expensive, requires the handling of each fish, and usually results in only a portion of the output being marked.

The use of trace elements or non-radioactive stable isotopes would eliminate the disadvantages of fin-clipping and would enable biologists to mark entire productions for identification.

The specifications of a perfect mark might be defined as follows:

(1) Not injurious to the fish.
(2) Has no effect on usual behavior patterns.
(3) Has no harmful effect on metabolism.
(4) Can be applied without handling the fish.
(5) Easily and readily applied.
(6) Can be applied to large numbers quickly and easily.
(7) Readily distinguishable to observers.
(8) Marked and unmarked population differences indistinguishable by predators.
(9) Inexpensive to apply.

With the exception of not being externally visible and easily distinguishable to observers, trace elements and non-radioactive stable isotopes appear to be a perfect mark.

Experiments by biologists have indicated that certain mineral salts can be assimilated into the bone matrix of most vertebrates during juvenile development with no harmful effect. These salts crystallize and remain with the organism in detectable amounts throughout its life cycle with little ion exchange. During their early life history in a freshwater environment, Pacific salmon assimilate certain elements necessary for sustained health and vigor. The radioisotopes of these elements are being used in some countries for permanently marking juvenile fish, and it appears reasonable to believe that contiguous non-radioactive (or stable) isotopes and certain trace elements could be used as a permanent mark for salmon fingerlings.

The present study is still in the primary phases, being only some five months old. Experiments are in progress to determine practical elements and isotopes that can be used in a marking program.

The first part has been the cation toxicity studies. We have one good guide on this. Shaw and Grushkin (1957) clearly demonstrated that the cation toxicity to aquatic organisms is directly correlated to the insolubility of the sulfide of the cation. We have checked this with sockeye salmon, and thus far the correlation holds true. Toxicity studies are still being continued. This phase is important for the study of cation metabolism in ionic solutions, since it determines lethal and permissible concentrations. If certain cations are toxic at low concentrations in solution, we then place emphasis on a dietary introduction study.

The actual determination of the retention of a cation can best be done (in most cases) by using the radioactive isotope of that cation in controlled closed system laboratory studies. Accurate information as to the most successful method of introduction can best be obtained in this manner also.

Once a cation has been demonstrated to be a bone-seeking retentive element, the next phase involves a study of introducing the non-radioactive isotopes. They will be introduced to test lots of fish by addition to their diet or by ionic solution. In the latter method, the fish are placed in a solution of the trace element for 24 to 48 hours. Chinook salmon (*Oncorhynchus tshawytscha*), blueback salmon (*O. nerka*) and rainbow trout (*Salmo gairdneri*) are being subjected to the medium. The chinook and blueback salmon will be released at their normal migration period. It will be necessary to hold fish (such as the rainbow trout) after the last introduction, and sample periodically until vestigial detection has been reached.

The marked fish will be identified by different procedures. Samples of the osseous tissue will be taken and reduced to an ash. The trace elements will be identified by either colorimetric analysis or neutron activation of the ash. The method chosen will depend on its sensitivity for a particular trace element.

Utilizing colorimetric analysis as a technique, one trace element mark would suffice for this, since a

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destructive analysis of the bone must be made. Neutron-activation analysis can be done on a non-destructive basis, and many trace elements can be detected in one sample by regulating activation time and reactor flux.

Fortunately, the most promising trace elements are the lanthanide series, and they are detectable by neutron-activation to quantities as low as 5×10^{-13}gm.

Durbin et al. (1956) have demonstrated that the average biological halflife of the entire lanthanide series in bone is 2.5 years.

In the case of the stable isotopes, only those which can be detected by neutron activation will be employed. The availability of enriched stable isotopes which can be produced cheaply is currently a problem which may not be resolved until the demands from industry become greater.

In practice, the elements or isotopes used would not be naturally available in either the freshwater or marine environment, and would thus be a product of the original introductory site when detected in a fish. Samples can be taken at any time during the life history and the analysis of osseous tissues will reveal their true origin. There appear to be sufficient trace elements and stable isotopes to initiate marking programs through a regulatory agency such as is being done now in the fin-clipping program.

Summary

The assimilation of certain trace elements and non-radioactive (or stable) isotopes into the osseous tissues of fingerling salmon is the basis of research now in progress. Elements have been selected which are not naturally available in the fishes' environment. They are introduced to the fish in their diet and through the water in their holding tanks.

Toxicity of the substances is being studied. Samples of osseous tissue will be taken and reduced to an ash. The trace elements and stable isotopes will be identified by colorimetric analysis or neutron activation.

References

SHAW, W. H. R., and B. GRUSHKIN 1957. The toxicity o ions to aquatic organisms. *Arch. Biochem.*, **67** (2): 447—452.

DURBIN, P. W., et al. 1956. Metabolism of the lanthanons in the rat. *Proc. Soc. Exp. Biol.* N. Y., **91**: 78—85.