

OUTLINE OF INTENDED ACTION TO IDENTIFY SEX FROM TISSUES OF EVISCERATED SAILFISH

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THE PROBLEM

A large portion of the catch of sailfishes is taken by longline fishing. In this fishery, the catch is processed promptly by head removal, evisceration, and freezing. Sex of the carcass cannot be determined by any existing approach available to field personnel. Because sailfish are strikingly dimorphic in size with respect to sex, sex must be known in order to make sense of age-related mortality.

AN APPROACH TO RESOLVING THE PROBLEM

Female fishes that are maturing carry the protein vitellogenin in their bloodstream. This protein is produced in the liver by specific activation of genes by the hormone estradiol. This hormone is not produced by maturing males, hence vitellogenin is female specific. The protein represents a major constituent of egg yolk, where it is cleaved into two smaller proteins (lipovitellin and phosvitin)--hence a means of detecting vitellogenin or either of its cleavage products would permit identification of mature and maturing females. Immature females would not be distinguishable from males, but the fishery is not believed to capture more than a few immature females.

METHODS

The phosvitin/lipovitellin complex was precipitated from gently macerated ovaries by adding 10 volumes of cold distilled water. Substantially pure lipovitellin was isolated from the complex dissolved in a salt solution by adding ammonium sulfate to 67% saturation, where phosvitin remained in solution and lipovitellin precipitated. Lipovitellin was dissolved in 1.2% NaCl and injected into rabbits three times over a six-week period. Rabbit serum was collected two weeks after the final injection and represents the "reagent" that will be used to test for presence of the female-specific protein. Initial assays will be done using 96-well microtiter plates with individual wells coated with protein extracts from red muscle, white muscle, blood, ovary, or kidney from sailfish samples of known sex.

After the immune reagent is added, then followed by incubation and washing, a goat antibody specific for rabbit immunoglobulin (and linked with an enzyme) is added. Reaction of the enzyme results in a colored product indicating that rabbit immunoglobulin was present, and hence that it was bound to female-specific protein. No binding, and hence no color reaction, is expected from male tissues.

MATERIALS ON HAND FOR STUDY

Blood, kidney, muscle, and gonad samples are available from 11 sailfish (both males and females) that were collected in May 1989 from a sailfish derby in Florida. These materials will be sufficient for all preliminary work. Suitability of antisera, and of the method in general, should be known late in September, 1989.

INITIAL RESULTS

Antiserums against lipovitellin from sailfish were prepared in two rabbits. Both of these antisera proved to be highly reactive against the protein used for immunization when tested by the enzyme-linked immunosorbant assay (ELISA). The assay was done in flat-bottomed plastic microplates (96 wells per plate). Binding of rabbit immunoglobulin to the antigen (lipovitellin, immobilized on the microplate wells by an alkaline buffer) was measured by adding goat anti-rabbit immunoglobulin-G conjugated to the enzyme alkaline phosphatase. A color reaction produced by providing the substrate for which alkaline phosphatase is specific was measured quantitatively in a Dynatech plate reader. Absorbance at 410 nm is proportional to the antigen concentration at appropriate antiserum dilutions.

Five-fold serial dilutions of antiserums were reacted with red muscle extracts from male and female sailfish. At antibody dilution of 15,725 reaction with female sailfish extracts was moderate (optical density 0.13-0.14) while extracts from males reacted weakly or not at all (optical density 0.00-0.02). The antiserums further reacted with serum from female Atlantic salmon, and with the protein phosvitin purified from domestic chicken eggs.

DISCUSSION

Initial results suggest that eviscerated male and female sailfish (sexually mature) carcasses can be distinguished from extracts obtained from small pieces of red muscle. These results are based on small samples and require confirmation by further work. The observation that phosvitin from hen eggs also reacts suggests that the protein was trapped in the insoluble precipitate of sailfish lipovitellin formed in ammonium sulfate (or alternatively, the two proteins might be structurally similar). This "contaminant" is not a disadvantage because the protein is also restricted to females. Crossreactivity with distantly related species suggests that the reagents developed may be useful in identifying sex from filets or gonadless carcasses of other species of fishes that are not immediately related to Istiophoridae (i.e. Xiphiidae).