and Somero 1973), allowing fish to exploit a variety of habitats and adapt to adverse conditions. Therefore, Yellowstone Lake cutthroat trout expressing hemoglobin with unique oxygen-binding properties might demonstrate increased resiliency to the extremes of physical activity described above.

Multiple Hemoglobin Components in Cutthroat and Rainbow Trout

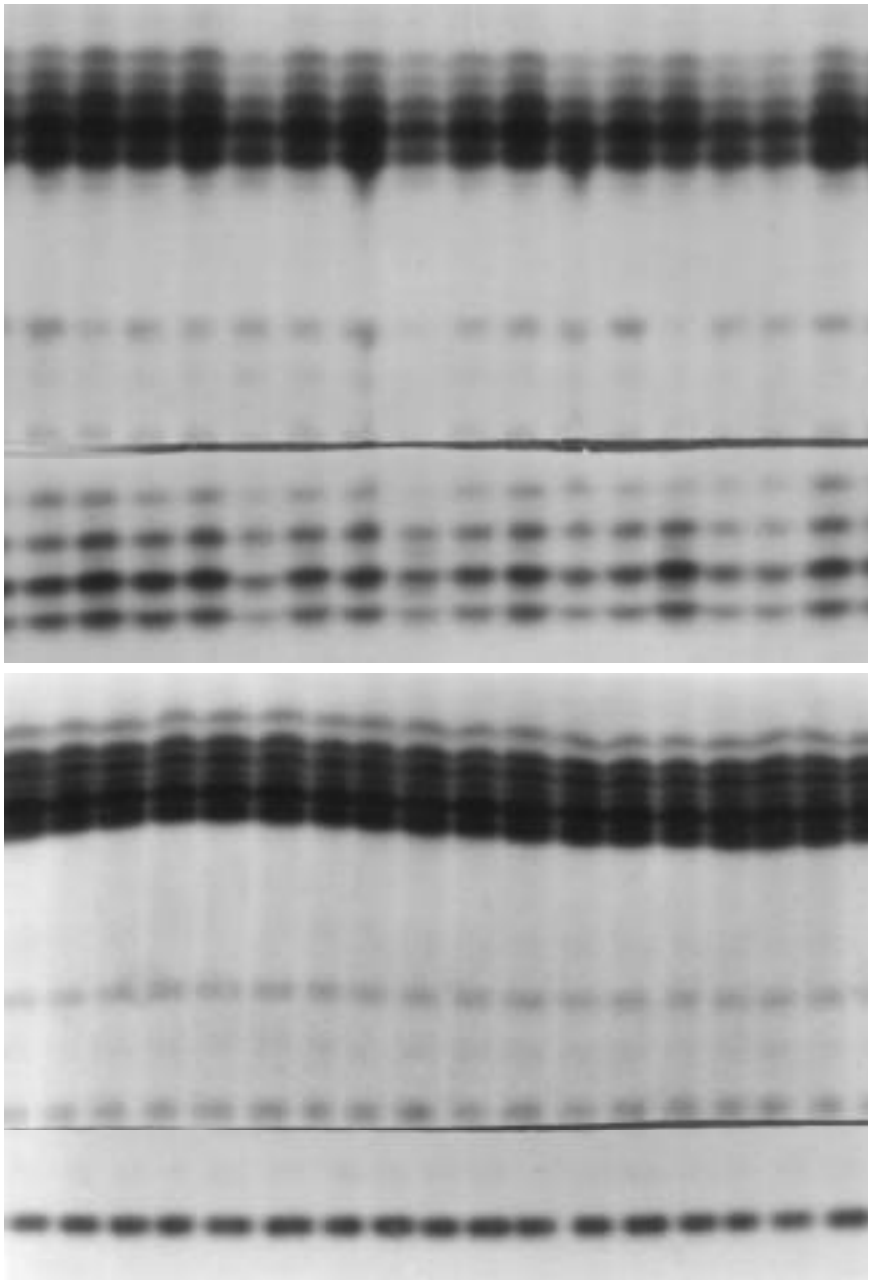
Multiple hemoglobin components in fish is a well-documented phenomenon, with cutthroat trout having twelve (Figure 1a) and rainbow trout having nine (Figure 1b) hemoglobin components (Braman et al.1977). A high-resolution starch gel electrophoresis method was developed to resolve eight negatively charged hemoglobin components from both species, all of which migrate coincidentally toward the positive electrode (anode). Rainbow trout have one and cutthroat trout have four positively charged hemoglobin components migrating toward the negative electrode (cathode). The single positively charged rainbow trout hemoglobin component migrates coincidentally with one of the four positively charged cutthroat trout hemoglobin components.

Hemoglobin Polymorphism in Yellowstone Lake Cutthroat Trout

Hemoglobin polymorphism due to allelic variation (Sick 1961; DeLigney 1969; Fyhn and Sullivan 1974; Fyhn and Sullivan 1975; Bonaventura et al. 1975; Perez and Rylander 1985; Giles and Rystephanuk 1989; Fyhn and Withler 1991) and ontogenetic variation (Wilkins 1968; Iuchi and Yamagami 1969; Giles and Vanstone 1976; Koch 1982; Wilkins 1985; Giles and Rystephanuk 1989) has been described in a variety of fish species, but not in cutthroat trout. Several cutthroat trout populations in the Intermountain West were examined for hemoglobin polymorphism by the starch gel electrophoresis method described above. All fish demonstrated the prototypical cutthroat trout phenotype with twelve hemoglobin components (Figure 1a). Yellowstone Lake cutthroat trout collected from the Peale Island area were also examined for hemoglobin polymorphism. Blood samples were collected on two occasions (September and October 1974) from a total of ninety-three fish. Variation in hemoglobin components migrating toward the cathode was observed in fish collected on both occasions (Braman et al. 1980; Figure 2). The polymorphism is complex in that there are concentration differences in hemoglobin components within a given sample in addition to variation in the number and concentration of hemoglobin components between samples. It is interesting to note that eight of the ninety-three fish sampled possessed the rainbow trout phenotype, with a single hemoglobin component migrating toward the cathode. These fish were not, by all apparent outward characteristics, cutthroat–rainbow (cuttbow) hybrids.

Additional Observations Made of Yellowstone Lake Cutthroat Trout

Fish sampled near Peale Island appeared to be adults ranging in size from 30 to 40 cm in length. Many of the fish were infested with unidentified ectoparasites on the body and, in particular, on the fins, where considerable damage was inflicted. A third sample of 50 cutthroat trout was collected one year later (1975)



Figures 1a and 1b. Starch gel electrophoresis of adult cutthroat trout (1a) and adult rain - bow trout (1b) hemoglobin components. Electrophoresis was performed as described in Braman et al. (1976). The anode (positive electrode) of the electrophoresis chamber is located at the top of the photo. The visible horizontal line running across the width of the gel in the photo represents the origin where hemoglobin samples were applied.

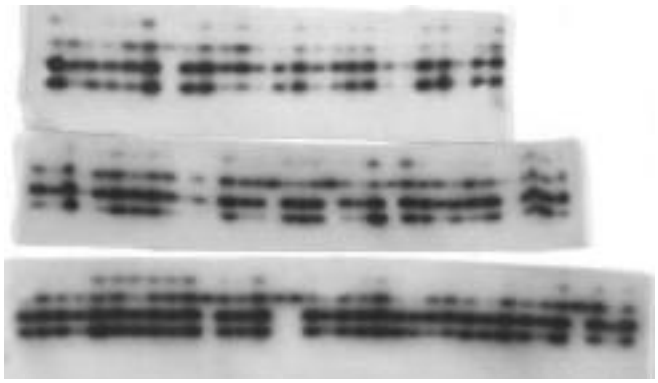


Figure 2. Three starch gel sections showing hemoglobin components migrating toward the cathode (negative electrode). Samples are from 93 adult Yellowstone Lake cutthroat trout. Electrophoresis was performed as described in Braman et al. (1980). Each section is oriented so that the origin is positioned at the top and the cathode is positioned at the bottom of each segment.

from a site approximately five miles north of Peale Island. These fish were also 30 to 40 cm in length, were not infested with ectoparasites, and did not demonstrate hemoglobin polymorphism. This second group of Yellowstone Lake cutthroat trout had the characteristic phenotype (i.e., having twelve hemoglobin components) shown in Figure 1a.

Plausible Explanations for the Observed Hemoglobin Polymorphism

Observed protein variation when using starch gel electrophoresis may result from artifacts generated during sample preparation and storage (Utter et al. 1974; Reinitz 1976). This is an unlikely explanation for hemoglobin polymorphism in Yellowstone Lake cutthroat trout because variation occurred exclusively in the hemoglobin components migrating toward the cathode. Hemoglobin components migrating toward the anode did not vary in number and concentration. If sample preparation and storage caused the polymorphic hemoglobin electrophoretic patterns, then all hemoglobin components from every population of fish would likely demonstrate variation, not just the hemoglobin components migrating toward the cathode, as in the Peale Island group of Yellowstone Lake cutthroat trout. In practice, identical electrophoretic patterns were obtained with freshly prepared and three-week-old hemoglobin samples. A hemoglobin sample stored longer than three weeks demonstrated degradation of all hemoglobin components, as evidenced by streaking of the entire electrophoretic pattern.

Another explanation for hemoglobin polymorphism in Yellowstone Lake cutthroat trout is ontogenetic variation (Wilkins 1968; Iuchi and Yamagami 1969; Giles and Vanstone 1976; Koch 1882; Wilkins 1985; Giles and Rystephanuk 1989). This hypothesis is unlikely because all fish examined appeared to be adults, 30 to 40 cm in length.

Hemoglobin polymorphism in Yellowstone Lake cutthroat trout could be

attributed to allelic variation and is complicated by the fact that rainbow trout genetic material was introduced into the Yellowstone Lake cutthroat trout gene pool as a result of stocking prior to 1915 (Jack L. Dean, personal communication). Allelic variation in cathodal hemoglobin components has been described for Arctic charr *(Salvelinus alpinus;* Giles and Rystephanuk 1989) and in anodal hemoglobin components for chinook salmon *(Oncorhynchus tshawytscha;* Fyhn and Withler 1991). Allelic variation resulting in polymorphic hemoglobin components of Yellowstone Lake cutthroat trout has not been confirmed. Breeding Yellowstone Lake cutthroat trout having known hemoglobin phenotypes, as well as performing crosses of Yellowstone Lake cutthroat trout with rainbow trout and scoring the phenotypes of the resulting offspring, will establish if the polymorphism is genetically based.

A further influence of rainbow trout genetic material on phenotypic expression of Yellowstone Lake cutthroat proteins is worth mentioning. The extent of introgression of anadromous rainbow trout *(Oncorhynchus mykiss irideus)* and coastal cutthroat trout *(O. clarki clarki)* was recently investigated by screening populations of these fish with amplified fragment length polymorphic (AFLP) and mitochondrial (mt) DNA markers (Young et al. 2001). Results of this work confirm that rainbow–cutthroat F_1 hybrids are produced from females of both species. Rainbow and cutthroat backcross hybrids were also detected, indicating that F_1 hybrids mate successfully with both rainbow and cutthroat parents. Hybrids were not found in all populations sampled and hybrid swarms were not evident. The data are consistent with the hypothesis that complete introgression of these two species is not possible due to an environment-dependent reduction in hybrid fitness. Screening Yellowstone Lake cutthroat trout with AFLP and mt DNA markers will aid in determining the extent and persistence of rainbow trout introgression due to stocking that occurred many years ago. AFLP markers are sensitive for identifying rainbow trout genetic material in cutthroat trout populations because, for the markers used by Young et al., rainbow trout did have cutthroat trout-diagnostic AFLP markers, while native cutthroat trout did not display any rainbow trout-diagnostic AFLP markers. Limiting the extent of introgression does not eliminate the possibility that Yellowstone Lake cutthroat trout harbor remnant rainbow trout hemoglobin alleles.

A second piece of circumstantial evidence obtained using a different experimental approach further reduces the importance of rainbow trout influence. Two-dimensional gel electrophoresis of serum proteins was used to distinguish native rainbow and cutthroat trout from cuttbow hybrids (Rourke and Wallace 1978). Results of these experiments show that serum protein profiles are different for native rainbow and cutthroat trout, but are equivalent for cuttbow and native cutthroat trout, suggesting that rainbow trout genetic material does not measurably alter the expression pattern of native cutthroat trout serum proteins.

Physiological stress represents another plausible explanation for the observed polymorphic hemoglobin patterns (Utter et al. 1974; Koch 1982). Circumstantial evidence in favor of this explanation is that Peale Island fish were infested with ectoparasites and had polymorphic hemoglobin components, while fish collect-

ed one year later five miles north of Peale Island were not infested with ectoparasites and did not have polymorphic hemoglobin components. However, a mechanism is lacking that links stress with variation in Yellowstone Lake cutthroat trout hemoglobin components migrating toward the cathode, and with the fact that several fish expressed the characteristic rainbow trout phenotype (i.e., having nine hemoglobin components).

Future Research

Cutthroat trout, rainbow trout, and other salmonids contain multiple hemoglobins that are divided into two groups. The anode group migrates toward the positive electrode during starch gel electrophoresis and contains hemoglobin components with relatively low isoelectric points. They are characterized by oxygen equilibria that are strongly dependent on pH, temperature, and ATP (adenosine triphosphate). The cathode group migrates toward the negative electrode during starch gel electrophoresis and contains hemoglobin components that are largely unaffected by pH, temperature, and ATP (Southard et al. 1986). Analogous anode and cathode groups of hemoglobin components are found in other teleost fishes, and it is hypothesized that the cathode group allows efficient uptake of oxygen at the gills as blood pH lowers during and following strenuous exertion (Powers and Edmundson 1972). Yellowstone Lake cutthroat trout demonstrate polymorphism in the cathode group of hemoglobin components. The physiological significance of this phenomenon deserves further investigation.

Yellowstone Lake cutthroat trout collected near Peale Island, many of which were infested with unidentified ectoparasites, demonstrated hemoglobin polymorphism. Fish sampled from a location approximately five miles north of Peale Island were not infested with ectoparasites and did not demonstrate hemoglobin polymorphism. It will be instructive to investigate whether hemoglobin polymorphism is a widespread occurrence in Yellowstone Lake cutthroat trout or if it is limited to fish confined to certain locations.

It is also important to establish whether hemoglobin polymorphism in the lake's cutthroat trout is due to allelic variation or is the result of stress.

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