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HEMOGLOBIN STABILITY IN BULL SHARKS (1)

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ABSTRACT No significant differences in oxyhemoglobin affinity, or electrophoretic patterns of hemoglobin were found in 18 bull sharks collected in selected regions of Lake Nicaragua, the Rio San Juan, and the Caribbean Sea. The half-saturation of hemoglobin with oxygen (P_{50}) was 11 and 17 mm Hg at pH 7.4 and 6.8, respectively (25° C, 3% hemoglobin solution, potassium phosphate buffer, 0.3 ionic strength). Electrophoresis resolved the hemoglobin into a minor and a major band. Planimetry of densitometric recordings showed that the major band constituted 54% of the total hemoglobin; the minor band, 46%. On the basis of these hemoglobin studies, no subspeciation of bull sharks in Lake Nicaragua was identified, although marine bull sharks have free access to the lake and have been there, at least, since 1535; the synonymy of *Carcharhinus nicaraguensis* with *C. leucas* was confirmed.

Lake Nicaragua is the largest lake in Central America. It is approximately 100 miles long, 45 miles wide, and its deepest point is 250 feet. The lake is drained into the Caribbean Sea by the 110 mile-long Rio San Juan which contains eight major rapids. In 1535 de Oviedo recorded sharks present in Lake Nicaragua. Gill and Bransford (1877) described the shark in Lake Nicaragua as being a landlocked, distinct species, *Eulamia nicaraguensis*. Jordan (1887) renamed the lake shark *Carcharhinus nicaraguensis*. Beginning with Gill (1893), all of the succeeding reports, check-lists, and catalogues appearing until 1961 referred to the lake shark by one or the other of these two scientific names (Burke, in press). After a morphological study of four preserved lake sharks (Bigelow and Schroeder, '48), and one specimen and a jaw, *C. nicaraguensis* was placed in synonymy with *Carcharhinus leucas*, the marine bull shark (Bigelow and Schroeder, '61). The

synonymy received support from Thorson et al. ('66) whose measurements on bull sharks from Lake Nicaragua and the Rio San Juan compared favorably with similar data obtained from body measurements made on pelagic bull sharks (Schwartz, '60; Clark and von Schmidt, '65). In relation to synonymy, it was of interest to investigate hemoglobin, a stable genetic trait, in bull sharks taken from both fresh and salt water. If no variation occurs in bull shark hemoglobin (1) synonymy, based on morphometric data, is supported, and (2) non-variable hemoglobin would indicate no drift toward subspeciation and reproductive isolation (Sick, '61; Gorman and Dessauer, '65; Mourant, '70).

MATERIALS AND METHODS Eighteen mature male and female bull sharks were captured: eight in selected regions of the lake, four in the river, four in the river estuaries, and two in the sea. After immobilizing a bull shark by head blows, blood samples were removed from the heart (Burke, '62) using vacutainers, (1mg/ml EDTA) and 1½ inch, 20-gauge needles. The vacutainers, containing blood samples, were packaged in ice, and air-mailed to MCV where hemoglobin solutions were prepared (Dementi and Burke, '72) for oxyhemoglobin affinity curves (Burke, '66) and starch-gel electrophoresis (Smithies, '59). A portion of the hemoglobin solution used in determining an oxyhemoglobin affinity curve was electrophoresed using borate buffer at pH 8.7. At times it was possible to prepare hemoglobin solutions in Nicaragua, and ship them in containers with dry ice. Hemoglobin analyses were performed within 48-72 hr from the time of blood collection.

RESULTS The oxygen pressure at which 50% of the hemoglobin of a fresh water bull shark is saturated (P_{50}) is 11 mm Hg at pH 7.4; at pH 6.8, the P_{50} is 17 mm Hg; for a marine bull shark the respective values vary less than one mm Hg at half-saturation (fig. 1). Duplicate analyses at both pH 7.4 and pH 6.8 were made on bull sharks from the lake, river estuaries, and sea. There was

no significant difference between the P_{50} determined for the lake bull shark group and the P_{50} of each of the other groups. There was no sexual dimorphism.

Upon electrophoresis, hemoglobin from each bull shark was resolved into two bands identified by benzidine stain. A minor band migrated 16% faster than a major band. Planimetry of densitometer recordings (at 560 mμ) of the two bands revealed that the major band constituted 54% of the total hemoglobin and the minor one, 46%.

DISCUSSION Polymorphic hemoglobins are characteristic of fishes (Buhler and Shanks, '59; Chandrasekhar, '59; Manwell et al., '63; Burke, '65; Wilkins and Iles, '66; Riggs, '70). Hemoglobin is such a stable genetic character that it has been used to identify subspecific populations which are isolated, reproductively, within a species (Sick, '61; Gorman and Dessauer, '65; Mourant, '70). The constancy of the P_{50} and the stable hemoglobin pattern found in each bull shark indicates the homogeneity of bull shark hemoglobin and supports the synonymy of Carcharhinus nicaraguensis with C. leucas. Carcharhinus species do migrate into lakes and rivers from the ocean around the world (Burke, in press), but there is no direct evidence that C. leucas breeds in fresh water (Jensen, '72). Although both anadromous and catadromous migration of C. leucas occurs between Lake Nicaragua and the Caribbean Sea via the Rio San Juan (Thorson, '71), the non-variability of hemoglobin in C. leucas reported here indicates no drift toward subspeciation, and supports the vigorous contention of Herre ('55) that "sharks do not breed in freshwater, but ----return to the sea to breed".

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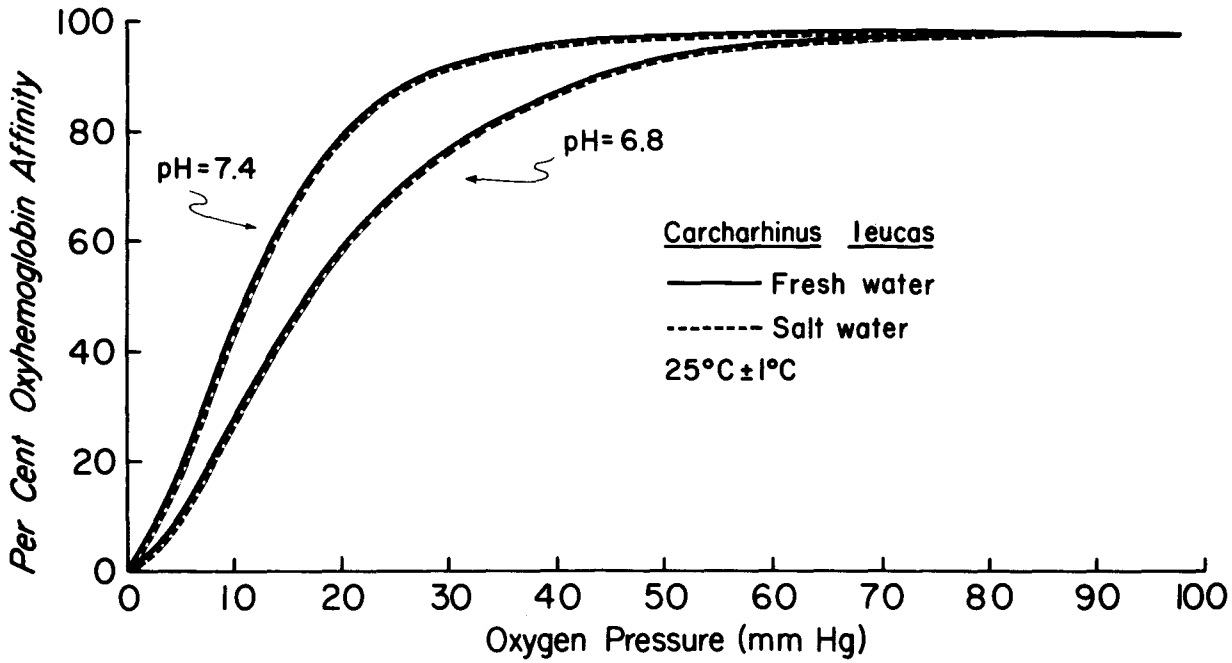
FIGURE LEGEND

1 Oxyhemoglobin affinity curves at 25 ± 1° C for Carcharhinus leucas from both fresh and salt water. At pH 7.4, P_{50} is 11 mm Hg; at pH 6.8 P_{50} is 17 mm Hg.


Per Cent Oxyhemoglobin Affinity
100
80
60
40
20
0
pH=7.4
pH=6.8
Carcharhinus leucas
Fresh water
Salt water
25°C±1°C
0
10
20
30
40
50
60
70
80
90
100
Oxygen Pressure (mm Hg)

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