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| Year 2023 | **Summary of Thesis** |
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(Title)
Study on vertebral bone strength and bone quality in land-locked and sea run forms of Oncorhynchus nerka

**[Background]** Bone morphology and size are primarily determined by age and the mechanical environment of the bone, and astronauts who have been in microgravity environment for long periods of time experience a decrease in bone mass. Microgravity environments also occur underwater, where gravity and buoyancy are balanced. As fish swim underwater while receiving external forces from all directions, their bones develop even under the microgravity environment. Furthermore, as fish propel themselves by moving their tails, the external forces they receive differ depending on the position of their spine, which is thought to affect bone development. We have previously evaluated the bone strength and bone quality of the vertebral bones in four-year-old (4Y) land-locked Oncorhynchus nerka (kokanee salmon) and sea run Oncorhynchus nerka (sockeye salmon), and examined the effects of living environment on bone. In this study, we characterized and compared vertebral bone strength and bone quality between 4Y kokanee salmon (4Y Kokanee) and one- to four-year-old sockeye salmon (1Y, 2Y, 3Y, and 4Y Sockeye) to clarify the effects of vertebrae position and age. We also evaluated the vertebral bone strength and bone quality in 2Y and 3Y Sockeye, which were deformed fish with abnormal vertebral morphology, and examined the effects of deformation on bone. **[Materials and Methods]** Vertebrae removed from 4Y Kokanee (n=10) provided by Shikototsuko Fishermans Cooperative, and 1Y (n=10), 2Y (n=11, deformed n=5), 3Y (n=8, deformed n=4) and 4Y (n=10) Sockeye provided by the National Institute for Fisheries Research and Education, the “Japan Fisheries Research and Education Agency”. The 4Y Kokanee and Sockeye were grown in a natural environment after hatching, and the 1Y to 3Y Sockeye were raised in freshwater tanks. Vertebrae were characterized using compression tests, infrared imaging and micro-Raman spectroscopy.

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**[Results] 1. Vertebral bone position-dependent changes in bone strength and bone quality.** The size of the vertebrae of 4Y Kokanee varied depending on their position, with the 39th vertebra behind the dorsal fin showing significantly higher values for both diameter and length compared to the cephalic vertebrae. In terms of the mechanical properties of the vertebrae in the 4Y Kokanee, the ultimate load and energy absorption were significantly higher in the vertebra just below (26th) and behind (39th) the dorsal fin compared to the cephalic vertebra (7th). On the other hand, there were no differences in the material constants, such as ultimate stress and toughness according to the position. Bone quality in both 4Y Kokanee and Sockeye revealed that the degree of mineralization in the vertebrae behind the dorsal fin of was significantly higher than that of the vertebrae directly below the dorsal fin. **2. Changes in bone strength and bone quality with increasing age**. The average diameter and length of sockeye salmon vertebrae increased with age, and those of the 4Y Sockeye were significantly larger than those of the 1Y Sockeye. The values of ultimate load and energy absorption also increased with age, and the 4Y Sockeye showed significantly higher values than did the 1Y and 2Y Sockeye. On the other hand, ultimate stress and toughness of 3Y Sockeye reared in freshwater tanks were significantly larger than those of 1Y and 4Y Sockeye (ocean→river), and the elastic modulus decreased with increasing age. Mineral maturity and carbonate-to-phosphate ratio were significantly larger in the 3Y and 4Y Sockeye than in the 1Y Sockeye in FTIR images. Raman spectra showed that the crystallinity increased with increasing age. **3. Bone strength and quality in bone deformity.** No significant differences were observed between deformed bones and normal bones in terms of bone strength and bone quality in the 2Y and 3Y Sockeye reared in freshwater tanks. **[Discussion]** The vertebrae of salmon that grow in a natural environment increase in size and degree of mineralization at the rear of the dorsal fin, where force is applied during swimming, resulting in higher ultimate load and energy absorption values. The reason why the ultimate stress and toughness of the 3Y Sockeye were greater than that of the 4Y sockeye grown in a natural environment is that they were constantly provided with high-quality food, in addition to being exposed to external forces due to the limited environment. On the other hand, although the deformed bones observed in salmon reared in freshwater differed in shape from normal bones, there were no major differences in their mineral composition or mechanical properties. The maturation of apatite crystals observed with age influenced the improvement of vertebral strength. **[Conclusion]** The shape, size, and quality of their vertebrae in salmonids change due to external forces applied to them during swimming.

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