Subspecies identification of the *Oncorhynchus masou* complex in the Japanese Islands based on the mitochondrial DNA sequence

°Yusuke Tanaka[1], Shiro Itoi[1], Naoyuki Suguro[2], Jun-Ichi Tsuboi[3], Tomoya Taguchi[4] and Haruo Sugita[1]
([1] Department of Marine Science and Resources, Nihon University, [2] Kanagawa Prefectural Fisheries Research Institute, [3] Yamanashi Prefectural Fisheries Technology Center, [4] Miyazaki Prefectural Fisheries Experimental Station)

Introduction: Of the seven known species in the pacific salmons, the cherry salmon (*Oncorhynchus masou*) is confined to Asian rivers and coastal waters. The four known extant subspecies of the *O. masou* complex are Masu salmon (Yamame, *O. masou masou*), Amago salmon (*O. masou ishikawae*), Biwa salmon (*O. masou* ‘Biwa’) and Formosa salmon (*O. masou formosanus*). Many studies using microsatellite DNA loci and mitochondrial control region sequences have failed to resolve the relationship among the four subspecies of the *O. masou* complex. Therefore in this study, we attempted to classify the four subspecies of the *O. masou* complex based on the partial mitochondrial gene sequences.

Materials and methods: The samples, which thought to be original population, were collected from the Fuji River, Sgami River and Sugumo River systems. In addition, cultured samples were obtained from Kanagawa and Miyazaki Prefectures. Sequences of the complete mitochondrial DNA (mtDNA) of *O. masou masou* were compared with those of *O. masou ishikawae, O. masou* ‘Biwa’ and *O. masou formosanus* to find the useful region(s) for subspecies classification on the *O. masou* complex. Total genomic DNA was extracted from the muscle or ray fin. Partial DNA fragments were amplified by polymerase chain reaction (PCR). The amplified DNA fragment was subjected to sequencing analysis followed by phylogenetic analysis.

Results and discussion: The comparison of the nucleotide sequence for the *O. masou masou* mtDNA with those of other *O. masou* subspecies showed that there is no difference in the sequences of most genes on mtDNA among the *O. masou* complex. Only one gene, NADH dehydrogenase subunit 4 (*ND4*), showed difference in the sequence among *O. masou* subspecies, suggesting that the *ND4* gene was useful for subspecies classification. Subsequently, the sequences of the *O. masou masou* samples collected from several river systems were determined. As the result, the haplotypes were different among river systems investigated in this study, suggesting that *O. masou masou* have genetic structure inherent to each river system.