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Biochemical characteristics of three migrated populations of chum salmon, *Oncorhynchus keta* for the spawning in Korea

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Abstract

Chum salmon (*Oncorhynchus keta*) is a species distributing widely in the North Pacific Ocean from the East Sea (Sea of Japan) as an estimated south limit to Gulf of Alaska via Bering Sea as a upper limit. The extensive geographic distributions, long-distance migrations, anadromous and semelparous life histories, and strong homing behaviors of Pacific salmon are major determinants of the highly subdivided population structures of these fish. The study focuses on the evaluation of spawning potential of the three chum salmon populations by determining some of biochemical and serological indicators, including AST and R/D ratio.

Introduction

Chum salmon (*Oncorhynchus keta*) has the widest natural distribution of all the Pacific salmon species, ranging northward from Korea in the western Pacific Ocean, through the Chukchi Sea in the Arctic, and south to the northwest United States in the eastern Pacific Ocean. The extensive geographic distributions, long-distance migrations, anadromous and semelparous life histories, and strong homing behaviors of Pacific salmon are major determinants of the highly subdivided population structures of these fish (Groot and Margolis, 1991). The strong instinct of chum salmon makes them return to the natal river and streams with low straying rates for spawning from ocean feeding areas. This behavior will lead to reproductive and partial genetic isolation in geographically distinct regional populations (Yoon, 2007).

Four underlying factors were derived from the analyses: 1) a nutritional factor composed of total protein, carbohydrate, RNA/DNA ratios in muscle and cholesterol in blood, 2) a tissue damage factor composed of the enzymes alanine aminotransferase, aspartate aminotransferase and creatine kinase, 3) a lipid metabolism factor composed of triglycerides and 4) a stress factor composed of glucose.

This study is focused on the determination of biochemical characteristics of different local chum salmon populations and to evaluate the some factors, blood chemistry, R/D ratio, biochemical composition as condition indexes for local chum salmon population.

Materials and Methods

Sampling

Thirty gravid females from the three locations, Namdaechun, Myoungpachun and Osipchun, from 21 to 23 November at Gangwondo in 2006 respectively. After measurement of total length and weight, blood was collected from caudal aorta with a 3 ml heparinized syringe, put into the centrifuge tube (kept on ice) and immediately measured for the blood chemistry of each population.

Blood chemistry

Eight factors, aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotranferase

(ALT; EC 2.6.1.2), glucose, creatine kinase, hemoglobin, urea, cholesterol, triglyceride in plasma were analyzed using commercial kits (Reflotron, Beringher Mannheim,Germany). According to the high concentration of some enzymes the blood was diluted with the physical saline.

RNA/DNA measurement

The nucleic acid content was determined fluorometrically, as described by Clemmesen (1994) and Belchier *et al*. (2004), with some modifications. The tissues from the bottom of the dorsal fin were freeze-dried (–50ºC, 24 h), weighed and homogenized on ice with Tris-ethylenediaminetetraacetic acid (EDTA) (TE; 5 mM Tris-HCl, 0.5 mM EDTA, pH 7.5) buffer solution (400 μl) using a hand pestle (duration 15–30 s). After centrifugation (8 min, 6000 rpm), the nucleic acids were extracted and purified from the homogenate and the total content was determined fluorometrically using ethidium bromide (EB) dye. Then, the RNA was digested with RNase and the remaining DNA was determined using EB. Detailed instructions can be found in Kim *et al*. (2005).

Total carbohydrate measurement

Using a phenol sulphuric acid assay, total carbohydrate content in tissue was measured.

Protein measurement

For protein analysis, a Bio-Rad protein assay kit II (Bio-Rad, Hercules, CA, USA) was used. From samples homogenized in TE buffer for RNA and DNA analysis, 100 μl homogenate + 900 μl distilled water + 250 μl Coomassie Brilliant Blue (G-250) were mixed in a microcentrifuge tube and measured at 595 nm (UV-spectrophotometer, Genesis 5; Genesis Technology Group, Miami, FL, USA). A standard curve was prepared for a range of 1 to 32 μg/ml BSA (Bovine serum albumin, 0.5 ml of a 2 mg/ml solution in water containing 2 mM sodium azide).

Results

The morphological characteristics of the local populations of gravid chum salmons in the east seaside of Korea are illustrated in Fig. 2. The mean folk length of Myungpachen was 59.6 ± 5.1 cm, Namdaechun 59.5 ± 4.5 cm and Oshipchun was 59.4 ± 6.4 cm. Total weight each populations were 2.2 ± 0.7 kg, 2.1 ± 0.5 kg and 2.2 ± 0.7 kg, respectively (Fig. 1). The mean Folk length and total weight between populations were not found the statistical differences.

The mean RNA content in the muscle were highest in Namdaechun population as 2.2 ± 0.5㎍/㎎ (Fig. 2). The RNA contents of Myungpachun and Oshipchun were 1.3 ± 0.5㎍/㎎ and 0.8 ± 0.2㎍/㎎, respectively. R/D ratio of Namdaechun recorded as 3.52 ± 0.7, Myungpachun as 2.9 ± 0.7 and Ohipchun as 2.4 ± 0.8. DNA content of these local populations showed very similar level of content (Myungpachun; 0.5 ± 0.3㎍/㎎,

Namdaechun; 0.6 ± 0.1㎍/㎎, Ohipchun; 0.4 ± 0.1㎍/㎎). According to the statistical analyze of Anova-test and post hoc comparison (Turkey's honest significant test, STATISTIKA, Germany), RNA content and R/D ratio of Namdachun population were significantly different to the Myunpachun and Oshipchun.

The results of the blood biochemistry showed very different pattern depending upon the analyzing factors (Fig. 3).

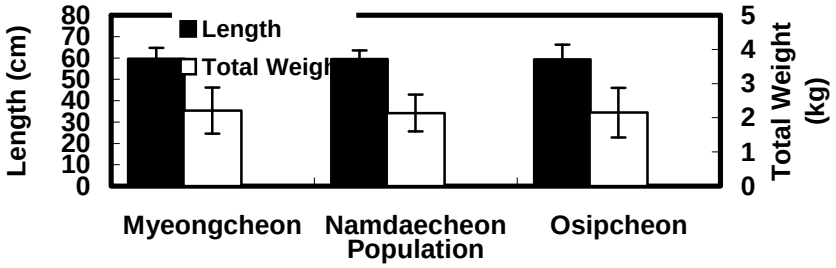

Fig. 1. Fork length and total weight of the three female *Oncorhynchus keta* populations migrating for spawning in the Korean waters.

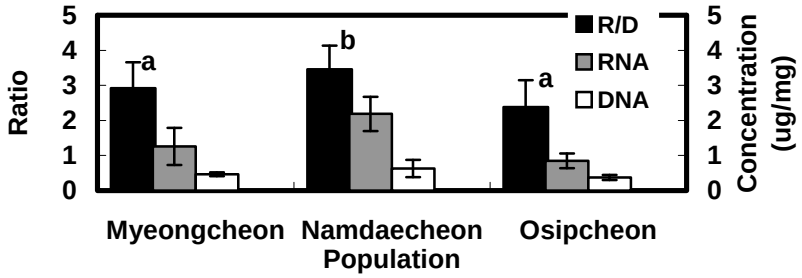

Fig. 2. RNA and DNA concentrations in dorsal muscle of chum salmons and its ratios as condition indices. Data are presented by means of ± S.D. (standard deviation, total n=90). Statistical analyze followed by ANOVA–test and Tuckey's honest significant test.

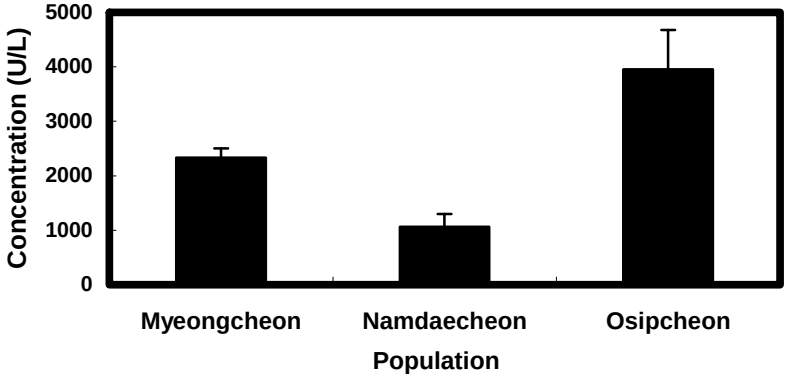

Fig. 3. Blood biochemical characteristics (AST) of gravid chum salmon females, *Oncorhynchus keta* migrated upwards to the three rivers in Korea. Error bars indicate standard error.

Discussion

In many researches about marine organisms, nucleic acids ratio was successfully used as a condition index (Anger and Hirche, 1990; Kim et al, 2006)

The use of the tissue R/D ratio as a condition index is derived from the role of nucleic acids (RNA and DNA) in protein synthesis and hence in growth. Variation in the number of cells in an organism due to cell division may be estimated from the DNA content, based on the assumption that the DNA content per nucleus is constant in somatic cells. Theoretically, the amount of RNA carrying genetic information from DNA to the sites of protein synthesis, the ribosomes, varies within the cell in proportion to the rate of protein synthesis (Brachet, 1961). In this study, the extracted tissue RNA and R/D ratio in the dorsal muscle of each population varied markedly, reflecting large differences in health.

ALT and AST are enzymes frequently used in the diagnosis of damage caused by pollutants and unsuitable environment in various tissues such as liver, muscle, and gills (De La Torre et al., 1999; 2000). AST is present both in the cytoplasm and in the mitochondria of the cells. After less severe cell damage the majority of the AST comes from the cytoplasm and only a small fraction from the mitochondria. Severe damage releases more mitochondrial enzyme. Elevated levels of transaminases can indicate myocardial infarction, liver disease, muscular dystrophy and organ damage. Increased serum activities of ALT are largely specific for liver parenchymal damage whereas AST is not a liver-specific enzyme. AST content in this study was very high in two populations (Myungpachun and Oshipchun). It assumed that the liver of gravid females were severely destroyed (Gordon, 1968)

Creatine synthesis begins in the kidney and is completed in the liver. It travels through the bloodstream to other tissues, particularly muscle where it reacts with ATP to form the high energy compound creatine phosphate, through creatine phosphokinase (CK) action (Marks et al., 1996).

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