

stretching caused stiffness increase in half of the samples tested. The stiffened samples showed weight loss. The stimulation by KASW caused stiffening and weight loss in all samples. The stiffer the dermis the larger the weight loss became. This result strongly suggested that water exudation was associated with stiffening.

TENSILIN DOES NOT INDUCE THE STIFFEST STATE OF HOLOTHURIAN DERMIS

○Masaki Tamori¹, Akira Yamada², Kazuhiro Oiwai², Tatsuo Motokawa¹

¹Department of Biological Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Meguro-ku, Tokyo 152-8551, Japan, ²Kansai Advanced Research Center, National Institute of Information and Communications Technology, Kobe, Hyogo 651-2492, Japan

The dermis of sea cucumbers is a catch connective tissue that changes its stiffness in response to stimulation. Tensilin, a protein isolated from sea cucumbers, is known to stiffen the dermis of holothurian body walls. We quantitatively examined the stiffening effect of tensilin by dynamic mechanical tests. Tensilin was isolated from the dermis of the sea cucumber *Holothuria leucospilota*. The sinusoidal tensile strain, whose maximal value in a cycle was 30%, was applied with the frequency at 0.3 Hz. The maximal stress divided by the maximal strain in a cycle was defined as stiffness. Tensilin stiffened the dermis in a soft state induced by immersing the dermis in Ca²⁺-free artificial sea water: the increased value of stiffness in tensilin was comparable to the stiffness of the dermis rested in artificial sea water with normal Ca²⁺ concentration (nASW) without stimulation. The dermis rested in nASW did not become stiffer when tensilin was applied. The dermis in nASW, however, showed marked stiffening response when stimulated by artificial sea water containing high concentration of K⁺. Thus our result showed that tensilin did not induce the stiffest state of the holothurian dermis.

MOLECULAR BIOLOGICAL STUDY ON OLFACTORY SYSTEM IMPRINTING-RELATED GENES IN LACUSTRINE SOCKEYE SALMON *ONCORHYNCHUS NERKA*

○Hiroshi Hino¹, Toshiharu Iwai², Masakane Yamashita², Hiroshi Ueda^{1,3}

¹Laboratory of Aquatic Ecosystem Conservation, Graduate School of Fisheries Sciences, Hokkaido University, Sapporo, Hokkaido 060-0809, Japan, ²Laboratory of Molecular and Cellular Interactions, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan, ³Laboratory of Aquatic Ecosystem Conservation, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Hokkaido 060-0809, Japan

During downstream migration, salmon are considered to imprint specific odors of their natal river, and return to their home river by following the imprinted odor memories. The olfactory hypothesis is suggesting that their olfactory neuron system plays an important role in homing migration. We attempted to identify olfactory imprinting-related genes in the olfactory system of lacustrine sockeye salmon using a subtractive hybridization method. A subtractive hybridization was performed in two directions. In the forward subtraction experiment, tester used the olfactory bulb of 1-year-old fish in the smolt term, and in reverse subtraction, the tester consisted of 3-year-old fish in the feeding migration term. After the forward subtractive cDNA library was constructed, differential screening was performed. We obtained a partial clone that showed a positive reaction in the forward subtraction probe only (Sockeye salmon Olfactory system Imprinting related Gene, SOIG). The SOIG was 1700 bp in length and only expressed in the olfactory epithelium, and not in other tissues. In the olfactory epithelium, the expression of SOIG mRNA was observed in the olfactory receptor cells and basal cells.

EFFECTS OF AMINO ACIDS ON SALMON HOME STREAM SELECTIVITY

○Yuzo Yamamoto¹, Takayuki Shouji², Hiroshi Ueda^{1,3}

¹Division of Environmental Science Creation, Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido 060-0809, Japan, ²Department of Fisheries, School of Marine Science and Technology, Tokai University, Shizuoka 424-8610, Japan, ³Laboratory of Aquatic Ecosystem Conservation, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Hokkaido 060-0809, Japan

Salmon are well known for their dramatic and accurate homing ability guided by the imprinted olfactory memory of their natal stream. However, these odor substances of natal stream and the mechanisms of olfactory imprinting remain to be clarified. According to recent studies, amino acids dissolved in the home stream water might be home stream substance for salmon. We carried out behavior experiments and electrophysiological experiments to test whether artificial amino acids mixtures have attractive effects on salmon upstream selectivity, and to examine the change in amino acid composition of the home stream on salmon olfactory nerve response. Homing chum salmon (*Oncorhynchus keta*) and homing lacustrine sockeye salmon (*O. nerka*) showed significant selectivity for artificial amino acids mixtures of their home stream water. Chum salmon could identify difference in artificial stream waters with and without L-Glu in the cross-adaptation experiment, but difference in amino acids composition did not reflect in the behavior experiments. These results strongly support the hypothesis that amino acids dissolved in stream waters are home stream substances for salmon.

A MALE-ATTRACTING PHEROMONE IN MASU SALMON

○Hidenobu Yambe¹, Shoji Kitamura², Michiya Kamio³, Shigeki Matsunaga⁴, Nobuhiro Fusetani^{4,5}, Fumio Yamazaki⁵

¹Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Sesoko, Motobu, Okinawa 905-0227, Japan, ²National Research Institute of Fisheries Science, Freshwater Research Division, Nikko Station, Chugushi, Nikko, Tochigi prefecture 321-1661, Japan, ³Department of Biology, Georgia State University, Atlanta, GA 30302-4010, USA, ⁴Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, ⁵Faculty of Fisheries, Hokkaido University, Minato-cho, Hakodate 041-8611, Japan

In the last 20 years, sex steroids and prostaglandins have been reported as sex pheromones in several teleosts. These pheromones are called hormonal pheromones because they or their precursors act as hormones in vertebrates. It has been speculated that such pheromones and the analogues are shared among many other species. However, it is hard to apply a concept of hormonal pheromones towards many other species that have their own species-specific manner in the reproduction. In this study, we identified a novel sex pheromone by using behavioral tests, liquid chromatography and spectroscopic analysis. In the spawning season, female masu salmon release a male-attracting pheromone in the urine. This pheromone is a metabolite of tryptophan, L-kynurenine that induces sexual excitement and locomotive behavior in mature males. The pheromone in this species is a compound other than hormonal pheromones.

PARTIAL BIVOLTINE IN EACH BROOD OF ARTEMIA DORMANT CYSTS

○Ziro Nambu, Shin Tanaka, Fumiko Nambu

Biology, Department of Medical Technology, School of Health Sciences, University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka 807-8555, Japan

Reproductive mode of the brine shrimp, *Artemia franciscana*, is composed of ovoviviparity (release of free-swimming nauplii from brood chamber) and oviparity (spawning encysted dormant gastrula embryos). Subitaneous cysts (hatch without delay) have been reported in rare case at a lower percentage. Here we reported that *Artemia* dormant cysts in each brood were partial bivoltine. They were composed of two types; one would hatch under suitable circumstances in one to three months after spawning, and the other would be dormant even under the appropriate environment for hatching and after passing the winter they would hatch in the spring. This was found for the first time by using the cysts that have never been dehydrated or desiccated.

TRANSFECTION OF BLOOD CELLS OF AN ASCIDIAN *ASCIDIA SYDNEIENSIS* SAMEA

○Tatsuya Ueki, Hiroyuki Kawano, Hitoshi Michibata

Department of Biological Science, Graduate School of Science, Hiroshima University, Hiroshima 739-8526, Japan

Ascidians are known to accumulate vanadium ions at extremely high concentration in the vacuole of vanadocytes (vanadium containing blood cells). To examine functions of genes responsible for vanadium accumulation such as vanadium binding proteins, metal transporters, or anion transporters, it is necessary to develop a transfection method for ascidian blood cells. Here we report transient transfection of ascidian blood cells with foreign DNAs by lipofection and polyamine methods. In a medium that contains DMEM/F12 and certain concentration of sodium chloride, we succeeded in introducing circular plasmid DNAs into blood cells of *Ascidia sydneiensis samea*. We used plasmid DNAs that contain a gene promoter for actin or Vanabin fused to green fluorescent protein, and observed green fluorescence in cytoplasm of blood cells. The efficiency of transfection was improved by reducing sodium chloride concentration.

STUDY ON LIGHT-SENSITIVITY OF MOTILE IRIDOPHORES IN THE DERMIS OF NEON TETRA, *PARACHEIRODON INNESI*

○Akiko Kasai, Noriko Oshima

Biomolecular Science Major, Graduate School of Science, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan

Motile iridophores present in lateral stripes of neon tetra respond to light directly and light irradiation causes a shift in the spectral reflectance to longer wavelengths. Each iridophore contains two stacks of light-reflecting platelets. A change in light intensity induces the change in the inclination of platelets, resulting in an alteration in the distance between them. In our previous study, we confirmed the expression of rhodopsin mRNA in the skin of neon tetra. Using skin preparation from lateral stripe, we lately found that cAMP accumulation within iridophores by treatment with forskolin or theophylline inhibited the photoresponses of the cells, which suggests the involvement of Gi in the responses. RT-PCR showed the expression of Gi mRNA in the skin, but Gt gene expression was not detected. Since Gts are generally essential components in the phototransduction in visual cells of vertebrates, it is interesting to compare the phototransduction system between the iridophores and visual cells of neon tetra. Therefore, we also examined the expression of Gt genes in some tissues including the retina.