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“Rash” Skin Disease of Rainbow Trout

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“Rash”, a subchronic, debilitating and non-fatal inflammatory skin disease has been found in cultured rainbow trout (*Oncorhynchus mykiss*) in several areas of Japan over the past five years. This condition was observed from small fish (11 cm in body length) to mature-size animals, however, most of the affected fish were market size (in Japan, 20 cm/120 g). Morbidity sometimes reaches as high as 48% and causes a significant economic impact, since those fish lose commercial value. A self-limited clinical course and healing of the lesion could be observed after 6 to 8 weeks. “Rash” signs included the presence of bright red, non-raised, ulcerated or not, defined to petechial scattered lesions on the ventral and/or lateral surfaces of the fish. Histopathological features included a subchronic focal to non-focal, non-suppurative dermatitis with various degrees of ulceration and an extensive mononuclear inflammatory infiltration. “Rash” etiology still remains unknown.

Key words: Rash, skin disease, rainbow trout, etiology, histology

Several skin disorders affect wild and cultured rainbow trout (*Oncorhynchus mykiss*). Skin diseases include columnaris disease (*Flexibacter columnaris*), coldwater disease (*F. psychrophilus*), furunculosis (*Aeromonas salmonicida*), vibriosis (*Vibrio anguillarum*, *V. ordalii*), bacterial kidney disease and spawning rash (*Renibacterium salmoninarum*), ectoparasites (Platyhelminthes, Branchiura, Copepoda etc.) and fungal infections (*Saprolegnia* spp.). Other skin anomalies include ulcerative dermal necrosis (might be of photosensitizer origin) (Roberts, 1989) and strawberry disease. Potential etiology of strawberry disease includes a rickettsiae member*⁶, an adeno-like virus (Fleury *et al.*, 1985) or a local allergic reaction to endo- or exotoxins produced by

intestinal microflora (Olson *et al.*, 1985). Among the non-infectious skin diseases, sunburn can be cited (Roberts, 1989).

“Rash” skin disease of rainbow trout is another disorder that has been identified in at least 20 fish farms in Japan over the past five years. It is a serious economic problem to aquaculture producers because this disorder affects market size rainbow trout (about 20 cm/120 g) and its incidence reaches 48%. Affected fish are rejected for human consumption, compromising their value. The etiology of “Rash” is unknown.

The purpose of this report is to describe clinical signs, histopathological characteristics and distribution in Japan and to suggest potential etiology of “Rash”.

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*⁶ Oman, E. P. (1990): Distribution and possible cause of strawberry disease in salmonids. Master Thesis, University of Idaho, U.S.A.

Materials and Methods

Epidemiological studies

Ninety percent of the Japanese rainbow trout production is concentrated in Shizuoka, Yamanashi, Aichi, Nagano and Gifu Prefectures. Investigations concerning distribution, incidence and possible determinants of "Rash" skin disease were conducted through a survey among the prefectural fisheries experimental stations and the Fuji Trout Farmers Cooperative. The following parameters were measured: size and species of fish affected, prevalence, size and types of lesion, seasonality, water source and temperature, nutrition, other diseases, routine vaccination and treatment regime, mortality and morbidity, and husbandry parameters.

Clinical evaluations

A survey of bacterial flora of fish was performed at three hatcheries with a history of "Rash". Samples of the skin (lesioned and normal areas), spleen, kidney and blood were processed as smears, imprints and histopathological sections. Specimens were also inoculated onto Brain Heart Infusion Agar (BHIA) and Tryptic Soy Agar (TSA). Cultures were incubated at 20°C for 7 days and observed daily. Smears and imprints were stained with Gram, Giemsa and methylene blue stains and examined by light microscopy. Tissues for histology were fixed in 10% buffered formalin and examined histologically with Hematoxylin & Eosin, Brown and Breen Gram, Pinkerton-Rickettsia, and Giemsa staining procedures (Luna, 1968).

Homogenates from different types of lesions were inoculated onto cell culture monolayers. Briefly, "Rash" lesions were ground with a mortar and pestle containing sterile sand and diluted 1:10 in Eagle's Minimum Essential Medium (Nissui®). The homogenate was centrifuged for 5 min at 5,000 rpm at 4°C and filtered through a 0.45 µm membrane filter (Millipore®). The filtrate extract was then inoculated onto various cell lines cultured in 24-well microplates (at a density of approximately 2×10^5 cells/well), incubated and observed daily for signs of cytopathic effects. Each cell line was incubated at 15°C for 14 days. Forty different cell lines were tested, i.e. AF-29, AS-6, ASE, BB, BF-2, CCO, CHH-1, CHSE-214, EK-1, EO-2, EPC, EPG, FHM, FRF, GSE, HF-1, JSKG, KO-6, KRE, KRE-2,

MSE, PAS, PF, RF-1, RTE, RTE-2, RTH, RTG-2, RTT, SBK, SE, SEH, SET, SF-2, SHH, STE-137, WF-1, WF-2, WSF and YNK (Yoshimizu, 1991).

Results

Epidemiological study

"Rash" was identified by the presence of a bright red, non-raised, ulcerated or not, defined to petechial, scattered lesion on the ventral and/or lateral surfaces of the fish. It was a self-limited disease but sometimes could debilitate the general health of fish. When mortality occurred it appeared to be related to a secondary infection, such as saprolegniosis and/or *Ichthyophonus* disease. Other diseases that affect trout farms in areas where "Rash" had been identified included infectious hematopoietic necrosis (IHN), coldwater disease and furunculosis. In an attempt to control these diseases, treatments using antibiotics such as sulphamonomethoxine and oxytetracycline were utilized but appeared to have no effect on "Rash". In some farms, fish were vaccinated against *Vibrio anguillarum* and *V. ordalii* with a commercial vaccine and some farmers have pointed out a positive correlation between "Rash" incidence and anti-vibriosis vaccination, but it had not been confirmed.

"Rash" was observed only in rainbow trout. It was not identified in any other cultured salmonid species in Japan, such as amago salmon (*Oncorhynchus rhodurus*), or coho salmon (*O. kisutch*). "Rash" was observed on rainbow trout at Shizuoka, Yamanashi, Nagano and Aichi Prefectures. The highest morbidity recorded was 48%. In one survey "Rash" appeared to increase during the summer (Fig. 1). The disease incidence in fish kept in first use water was negligible, however the incidence increased when fish were held in water that had been used three or more times.

"Rash" were observed from small fish (11 cm in body length) to mature-size animals, but predominantly markert size rainbow trout were affected (in Japan, 20 cm/120 g). Signs of the disease were seldom observed in fish weighting more than 200 g. Additionally, nutrition effects and the reoccurrence of "Rash" in previously infected fish were not observed.

Clinical examinations

Attempts to identify the bacteria that had grown

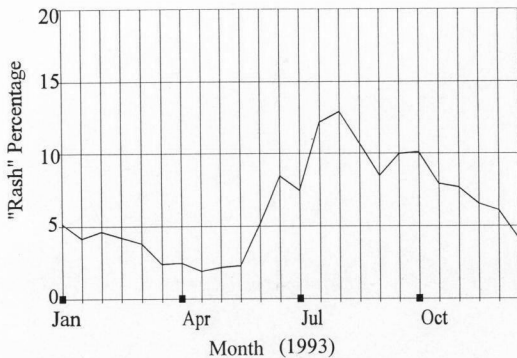


Fig. 1. "Rash" mean incidence in seven trout farms of Fujinomiya area in Shizuoka prefecture in 1993.

in cultures were not conclusive. When fixed, stained and observed by light microscopy, bacteria were most bacillus and coccus. This was similar to what was observed in stained smears. *Ichthyophonus hoferi* endospores were identified in smears from kidney of affected and unaffected fish. No cytopathic effect was observed in the inoculated cell cultures.

"Rash" seemed to debilitate the general health condition of the fish, allowing opportunistic infections to establish. Saprolegniosis was the most common. It generally was associated with ulcerated lesions and could lead to death, mainly when it had progressed to the gills. Filamentous bacteria were observed, affecting the dorsal and caudal fins, but they were also detected in "Rash" unaffected fish.

Histopathological study

Histological sections failed to show any bacteria or fungi. Pinkerton-Rickettsia stained sections did not indicate any appearance of rickettsiae. The unique clinical signs of "Rash" was distinguished by the presence of lesions in the skin and no involvement of any other organ. In general the gross appearance of "Rash" was characterized by the presence of a bright red, non-raised, ulcerated or not, defined to petechial, scattered lesion on the ventral and/or lateral surfaces of the fish. Three different types of lesions were observed: petechial, yellowish and ulcerated. The petechial type was distinguished by small red spots (about 1–2 mm in diameter) scattered mainly on the ventral surface (Fig. 2A), and it usually appeared in approximately 20% of the affected fish. The most common lesion was termed yellowish, with a prevalence of 75%. It was characterized by a non-raised, non-defined, reddish to yellowish lesion

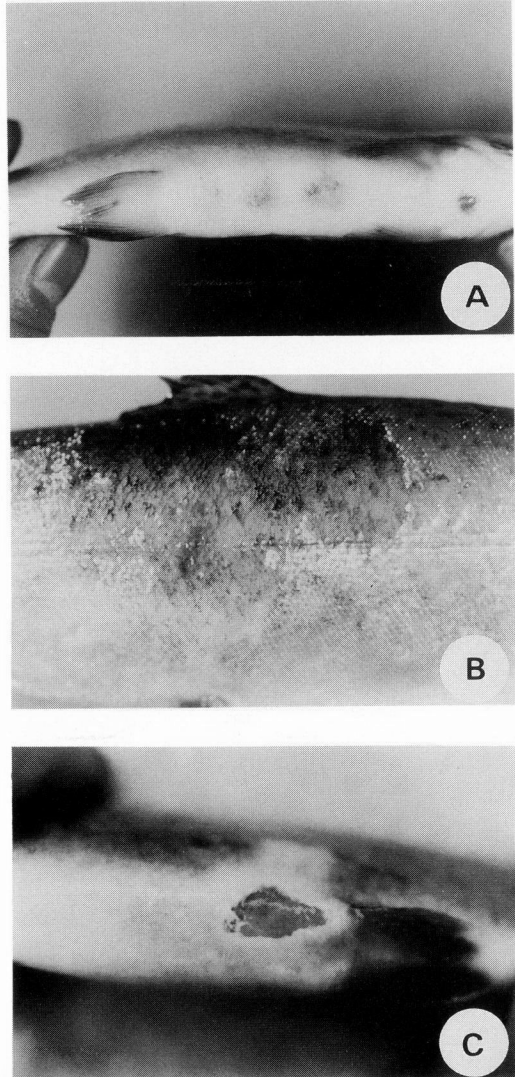


Fig. 2. (a) "Rash" petechial type; (b) yellowish type and (c) ulcerative type.

(diameter varies from 1 to 5 cm), occasionally with light ulceration. It generally was localized in the lateral part of the body, but could also be seen on ventral surface (Fig. 2B). The most severe type of lesion observed was the ulcerated. It was a bright red, non-raised, circular (about 1 cm in diameter) that exposed the muscular layer (Fig. 2C). This type of lesion was usually observed in 5% of the lesions. It differed from the yellowish type because the degree of ulceration was more severe and no yellowish halo was present around the lesion. Although the gross

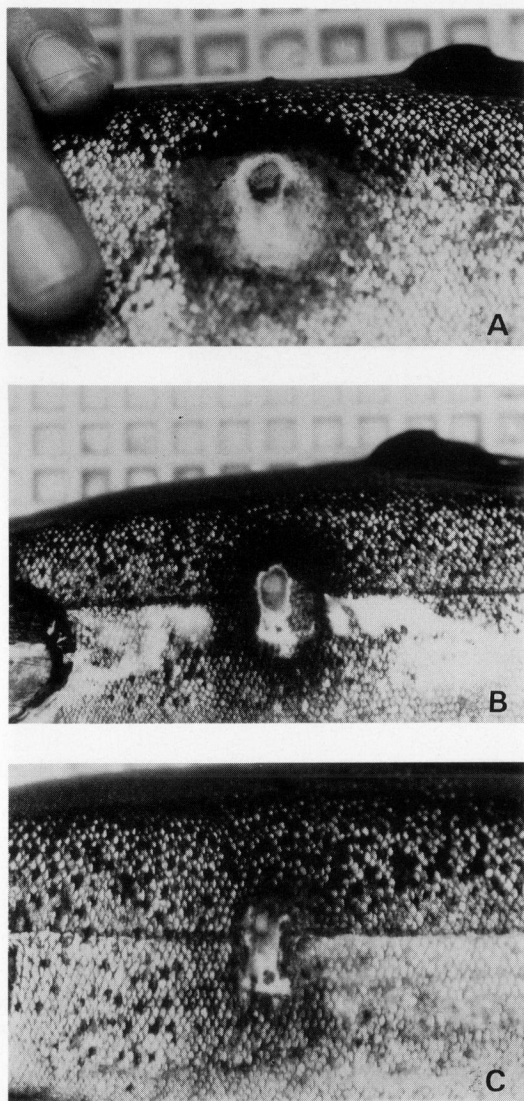


Fig. 3. "Rash" lesion (ulcerative type) monitoring showing its self-healing process. (A) lesion aspect at the beginning of the monitoring; (B) lesion aspect after 10 days and (C) lesion aspect after 25 days. Complete healing of the lesion was reached at 45 days after the monitoring started. Fish was kept in a five times used water raceway.

appearance of the lesions had differed, all of them were considered "Rash". This was based on the fact that all three types had a similar histopathological feature, each of them exhibited a self-limited clinical course under the same conditions, spontaneous heal-

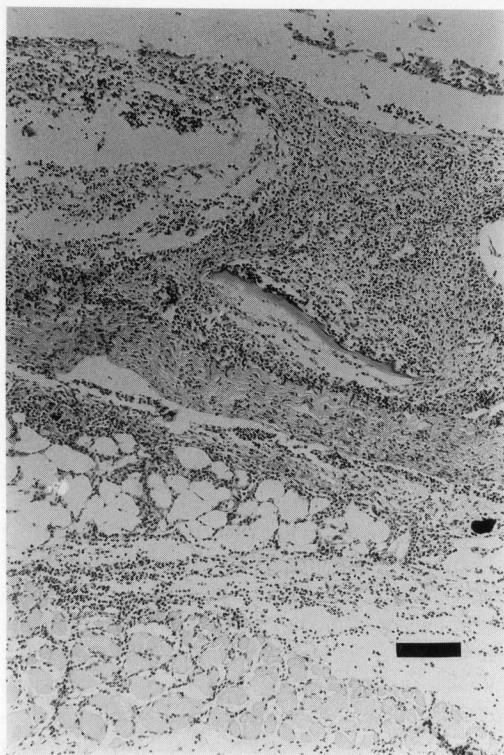


Fig. 4. "Rash" histopathological feature. An extensive inflammatory infiltrate can be observed from the epidermis to the muscle bundles (Bar: 100 μ m).

ing was observed after 4 to 8 weeks and no etiologic agent was identified (Fig. 3).

The histopathologic features of each lesion were characterized by subchronic, focal to non-focal, non-suppurative dermatitis with various degrees of ulceration and an extensive mononuclear inflammatory infiltration. The infiltrate appeared to be composed of lymphocytes, monocytes and melano-macrophages. Granulocytes were not seen, but might be present at early stages of the lesion. Thickening of the skin was observed and a extensive infiltrate was usually localized in the epidermis, dermis, hypodermis, reaching into the muscle bundles in the more severe cases (Fig. 4). Vascular changes included extensive dilation and congestion of blood vessels and capillaries with evidence of hemorrhage. In later stages of the lesion, healing was indicated by the presence of fibroblasts and deposition of collagen.

Discussion

The epidemiological study just provided a few indications regarding "Rash" characteristics. The increasing "Rash" incidence during summer suggested a seasonality of this disease. This fact could be correlated with the amount of handling that fish were subjected during this period. Also, in summer, fish density increased and data from Nagano Prefectural Fisheries Experimental Station indicated that the occurrence of "Rash" was higher in fish kept at a density greater than 35 kg/m³ (personal communication)*⁷. The epizootiology of "Rash" could also be related to increasing water temperature. This varied according to the water source. Temperature of ground water was approximately 14.5°C throughout the year and in surface water it varied from 5° to 22°C. This variation in water temperature might interfere with the physical-chemical and microbiological characteristics of the water which could influence disease occurrence.

In general, a used water raceway has a poor environmental conditions for fish; its physical-chemical properties are altered (elevated rate of ammonia, decreased pH, increased turbidity, decreased dissolved oxygen, etc.), and there is an increased microbial load. These elements could contribute to fish stress, and lead diseases or other syndromes. The cause of this increased occurrence in reused water should be investigated in future studies.

A size-dependence factor should be considered in "Rash" pathogenesis, however, whether a state of immunity is developed against the disease is still unknown.

Due to the fact that no bacteria, fungi, rickettsiae or viruses had been detected, the microbiological studies require a more precise evaluation, including a comprehensive investigation of the microflora present in the fish, as well as in the environment. An ultrastructural survey is another tool that would aid in determining an infectious etiology and provide in-depth characterization of the histopathological features. Transmission experiments should also be

attempted to determine the mechanism(s) "Rash" manifests, which might also provide additional information about the etiology.

"Rash" seems not to be restricted to Japan, because a disease resembling "Rash" skin disease has caused economic problems in some farms in the United States of America, where incidence has reached approximately 50% in 100–200 g fish (data not shown).

The terminology "Rash" has a very wide meaning, since it refers to any eruption of the skin in spots or patches. A more accurate name is needed, but additional information concerning the etiology, pathogenesis, prevention and cure are required before this could be established.

Acknowledgments

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