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Study on the Intestinal Microflora of Salmonids

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Abstract: A study on the actual condition and changes of normal bacterial flora in the intestines of salmonids was carried out to determine the actual status of bacterial flora in the digestive tract, particularly in the intestines, in the course of their life cycle, i. e. migration from fresh water to the sea and back to the original river.

From the results of this study, it would be concluded as follows.

The intestinal microflora of healthy salmonids are mainly composed of the genus Aeromonas and the family Enterobacteriaceae of a so called terrestrial type, if they are living in fresh water. Contrarily, the flora are mainly composed of the genus Vibrio of marine or halophilic type when they are living in sea water. If fish move to the sea or upstream a river in their life cycle, the flora in their intestines would undergo changes in order to adequaely adapt the fish themselves for their living environments.

Studies on the intestinal microflora of fish have long been done from the viewpoint of food hygiene such as spoilage of fish (Stewart 1932; Gibbons 1934; Dyer 1947; Kaneko 1971a, b and c) and contaminated fish due to the enteric bacteria of human or animal origin (Geldrich and Clarke 1966) and also from that nutrition and fish diseases (Prévot *et al.* 1957; Kaiser 1961; Okutani *et al.* 1964; Kashiwada and Tejima 1966a and b; Tejima and Kashiwada 1967, 1969; Okutani and Kitada 1968a and b; Yakov 1968; Kashiwada *et al.* 1970). And the presence of a lot of bacteria in a digestive tract especially in the intestines has been recognized by a great number of researchers (Liston 1956; Shewan 1961; Mattheis 1966a, b, c and d; Ozaki 1972).

Bacterial flora on the body surface and gills are unlike the specific bacteria to be found in the intestinal flora of fish in general as many papers reported before (Liston 1954, 1957; Spencer 1961; Aiso *et al.* 1968; Okuzumi and Horie 1969; Chung and Kou 1973; Trust 1974). However for many years, there has been suport for the view that fish do not have any "specific bacterial flora normally existing in the intestines (Obst 1919; Margolis 1953; Sorimachi and Egusa 1971). As for a normal intestinal bacterial flora, however, there are many problems unsolved, such as, what normal flora is, how dominant species appear, where they come from and how they affect fish (Rae 1965; Seki 1972; Sera and Kimata 1972; Sera and Ishida 1972a and b; Sera *et al.* 1972; Ozaki 1972).

It is most important to identify the normal intestinal bacterial flora of fish not only in the studies of fish diseases but also in the interpretation of their physiology. In other words, unless the normal intestinal microflora of fish has been identified, no correct judgement could be made on the direct or indirect relationship between the organism isolated from fish used as test samples and their effects upon the diseases or the physiology of fish.

From the standpoint mentioned above, the authors have carried out a study on the actual conditions and the changes of normal bacterial flora in the intestines of fish by selecting salmonids as the test samples to determine the actual status of bacterial flora in the digestive tract, particularly in the intestines, in the

course of their life cycle, i. e. migration from fresh water to the sea and back to the original river.

Materials and Methods

1. Materials

As shown in Table 1, five groups were selected as tested materials in consideration of the life of salmonids. They were subjected to examination to determine the microbial flora and counts of viable microorganisms in the intestines. And simultaneously, experiments were carried out for the comparative microbial evaluation of their environmental water, diets and zoo-plankton which form their food. The details are described as follows:

(1) Fish reared in fresh water and sea water (groups 1–1, 1–2)

As the fish rearing in fresh water, a total of 27 masu salmon (*Oncorhynchus masou*) and king salmon (*O. tschawytscha*) (body length; 13.5–25.5 cm, body weight; 29.5–215.0 g), were reared in Mori Branch of Hokkaido Fish Hatchery (or simply called Mori Hatchery), were selected for test materials. As the fish rearing in sea water, 5 king salmon (length; 35–44 cm, weight; 785–1,300 g), were transferred the previous year from Mori Hatchery into the Oshoro Bay Culture Station of Hokkaido Fish Hatchery (also referred to as

TABLE 1. Materials

group	sampling place	sampling date	species of fish	No. of fish exam.	B. L.*6 (cm) average	B. W.*7 (g) average	other samples
1.–Fish reared in fresh and sea water							
1–1.	Mori H.*1	June '71 ~ July '72	masu salmon	22	19.6	97.5	supplying water
			king salmon	5	16.3	46.0	pond water
1–2.	Oshoro Bay S.*2	Oct. '71	king salmon	5	38.4	997.0	sea water
2.–Fish in artificially transplanting period from fresh water into sea water and reared in fresh water without being fed							
2–1.	Marine C.C.*3	Oct. ~ Nov. '72	masu salmon	24	20.3	107.3	sea water
2–2.		Nov. '72		5	21.0	106.2	fresh water
2–3.		June ~ July '72		30	17.5	59.0	diet
2–4.	Nanae-cho*4	June '73		33	15.0	41.7	
3.–Mature fish cultured in fresh water							
3–1.	Mori H.	Aug., Oct. '72 Aug. '74	masu salmon	17	25.5	218.6	
4.–Fish living in open sea							
4–1.	Bering Sea	June '74	sockeye salmon	12	57.4	2436	sea water
			pink salmon	6	47.0	1218	zoo-plankton
			chum salmon	12	55.7	2045	
			king salmon	3	54.0	1890	
4–2.	Coast of Hokkaido	Sep. '73	7 species (without salmonids)	11	36.5	500	zoo-plankton
4–3.	East Bering Sea	July '74	6 species (without salmonids)	14	39.0	661	
5.–Anadoromous fish							
5–1.	Oshima H*5	Sep. '72	pink salmon	9	55.2	—	
5–2.		Nov. '72, '73	chum salmon	20	72.2	4142	

cf. *1: Mori Branch of Hokkaido Fish Hatchery, *2: Oshoro Bay Culture Station of Hokkaido Fish Hatchery, *3: Hokkaido Marine Cultivation Center, *4: private fish farm in Nanae-cho, *5: Oshima Branch of Hokkaido Salmon Hatchery, *6: body length, *7: body weight

Oshoro Bay Station) which were selected as test materials. The water in which test fish live was also collected for the test, respectively from two places.

(2) Fish in artificial transplanting period from fresh water into sea water and fish reared in fresh water without feeding (groups 2-1, 2-2, 2-3, 2-4)

Ninety two masu salmon after being developed silvering cultured in Mori Hatchery (length; 12.5–23.5 cm, weight; 25.0–134.0 g) were transferred to the Hokkaido Marine Cultivation Center and a private fish farm in Nanae-cho, a village near Hakodate, and were divided into 4 groups. Three groups were transferred from fresh water into sea water in the manner illustrated in Fig. 2 and 1 group which was not fed, was transferred to fresh water.

(3) Mature fish cultured in fresh water

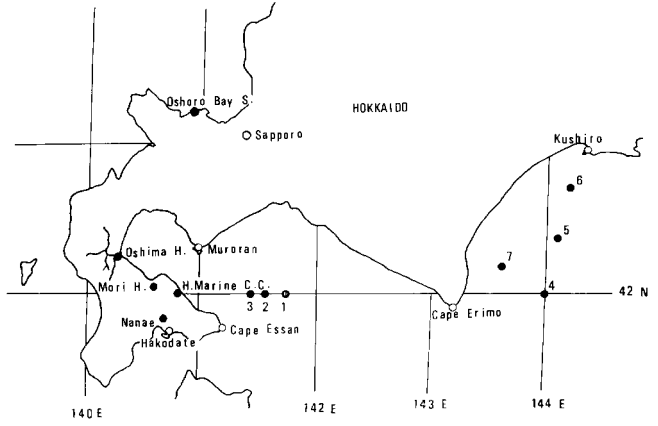

FIGURE 1. Location of the sampling stations.

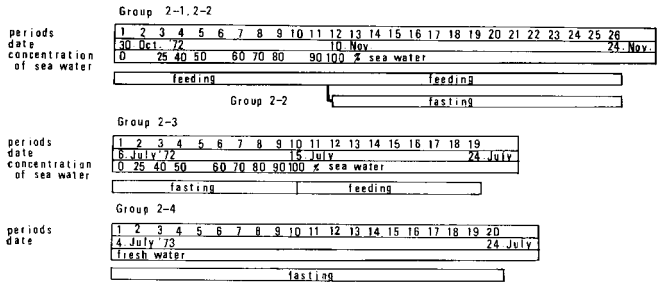

FIGURE 2. Rearing conditions at the each stages in artificially transplanting period from fresh water into sea water and fish reared in fresh water without being fed.

(group 3)

Seventeen of masu salmon which reached their mature stage in Mori Hatchery (length; 19.5–29.5 cm. weight; 96–445 g) were provided as test sample

(4) Fish living in open sea (groups 4–1, 4–2, 4–3)

Thirty two salmonid of 4 species (*O. nerka, O. keta, O. gorbuscha,* and *O. tschawyscha*: length; 43.8–62.8 cm, weight; 1,100–3,600 g) were caught in the Bering Sea (Fig. 3, Stations A–G). Sea water and several kinds of zoo-plankton (Calanus, Parathemisto, Euphausia and Tissanoessa) feeding organisms for fish, were also given for experiment.

(5) Anadromous fish (groups 5–1, 5–2)

Nine adult pink salmon (*O. gorbuscha*: length; 48–52 cm) caught at rivermouth for collecting their eggs by the Oshima Branch of Hokkaido Salmon Hatchery were reared about 3 months in fresh water without feeding, and 20 anadromous chum salmon (*O. keta*) caught for same purpose (length; 59–78 cm, weight; 2,400–5,300 g) were employed as test samples.

TABLE 2. Composition of the media used

	FWA*1 (g)	SWA*2 (g)
Poly pepton (Daigo)	5.0	5.0
Beef extract (Kyokuto)	2.5	2.5
Yeast extract (Daigo)	2.5	2.5
K_2HPO_4	0.2	0.2
$MgSO_4$	0.05	—
Glucose	1.0	1.0
NaCl	5.0	—
Agar (Kyokuto)	15.0	15.0
Artificial sea water*3	—	750 ml
Distilled water	1000 ml	250 ml
Adjusted to pH.	7.5	7.8

cf. *1; fresh water agar, *2; sea water agar, *3; Herbst's artificial sea water, composition; (g/100 ml: NaCl 3.0, KCl 0.07, $MgSO_4$ 0.26, $MgCl_2$ 0.5, $CaSO_4$ 0.1)

The location where those test samples were collected is listed in Figs. 1 and 3.

The materials of goups 1, 2, 3 and 5 were carried to our laboratory kept in ice box, while the materials of group 4 were immediately subjected to the experiments in the labo-

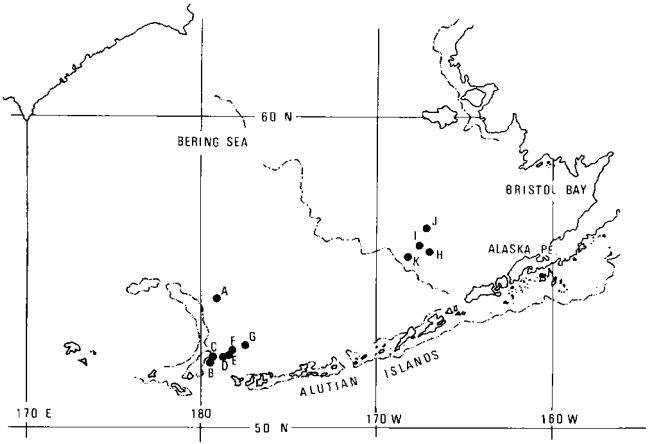

FIGURE 3. Location of the sampling stations in the Bering Sea

ratory on the ship.

2. Basal media and culture condition

The fresh water medium and sea water medium shown in Table 2 were employed and culture was carried out for 5 days under aerobic conditions at 25°C. The above mentioned media and culture conditions were determined by the preliminary test using the same materials.

3. Method for determination of number of viable microorganisms and method for classification of isolates

At the early stage of this study (group 1), the intestinal contents were aseptically taken from the anus after a disinfecation of the fish surface. And thereafter, the intestinal contens or mucus substances were taken aseptically from the intestinal tract by way of removing the intestinal tract from the end of the pylorus to 1 cm before the anus by abdominal operation. The materials were diluted with saline or artificial sea water in accordance with the 10 fold dilution method. The number of colonies was determined using a surface smear plate method with 0.2 ml of diluted sample in acordance with the regular procedure. The number of viable microorganisms were indicated by the number of viable cells per 1 g weight of every sample in the case of intestinal contents, diet and zoo-plankton, and by the number per 1 ml in the case of water sample.

The isolation of strains was made by fishing up every colony within a designated area (20–30 colonies) of the plate of suitable dilutions of individual test samples various characters of those isolates were examined in accordance with the regular procedure and a classification on the genus level was carried out according to the method proposed by Shewan *et al.* (1960), as illustrated in Fig. 4.

4. Bacterial type classification on the basis of requirement of inorganic salts for growth

Isolates from intestinal contents were subjected to a study of bacterial types on the basis of requirement for inorganic salts in growing process according to the method proposed by Hidaka (1964, 1965) and Hidaka and Sakai (1968).

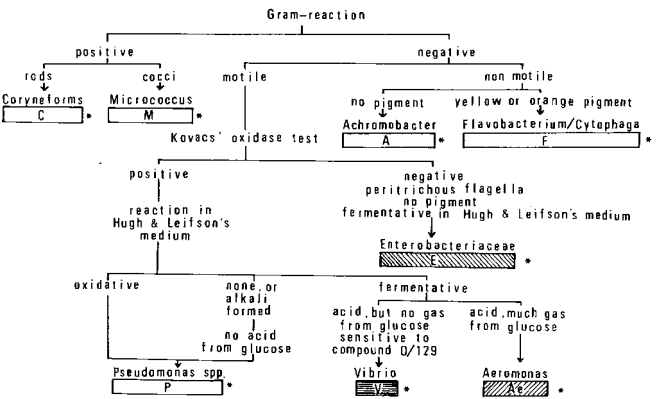

FIGURE 4. An outline of the sequence of tests used in the screening of isolates. (by Shewan, J. M., G. Hobbs, and W. Hodgkiss 1960)

cf. *: These abbreviations were used in following Fig. 5 and Fig. 7~Fig. 12

5. Susceptibility to low pH and bile

Isolates from the intestinal contents, water samples, and diets of the tested fish in the individual groups of 1–1, 1–2 and 2–1 were subjected to a study on their susceptibility to low pH and bile in accordance with the method proposed by Sera and Kimata (1972), Sera and Ishida (1972a and b) and Sera *et al.* (1972).

Results

1. Intestinal microflora and number of viable microorganisms

(1) Fish reared in fresh water and sea water (groups 1–1, 1–2)

The number of viable microorganisms of water samples from both rearing environments was constantly 10^2/ml all the year round. However, the number of microorganisms varied in the case of the intestines of the reared fish ranging between 10^3–10^7/g which is higher than in environmental water. As shown in Fig. 5 and 12, the genus Aeromonas and family Enterobacteriaceae represented the majority microflora in the intestines of fish reared in fresh water, while the genus Vibrio represented the majority in the fish reared in sea water. All of those cases were a striking contrast to the dominance of the genera Achromobacter, Flavobacterium/Cytophaga, and Pseudomonas in both environmental water.

(2) Fish in artificially transplanting period from fresh water into sea water and fish reared in fresh water without being fed (groups 2–1, 2–2, 2–3, 2–4)

Number of viable microorganisms;

In the group 2–1 shown in Fig. 2 which was transferred with feeding, the number of intestinal viable microorganisms was nearly at a constant level even during the period of transferring to sea water. However, in the group 2–2 reared in sea water without feeding for 2 weeks after the establishment of transfer into sea water, the number of intestinal viable microorganisms was found to be reduced. In the group 2–3, fish transferred into sea

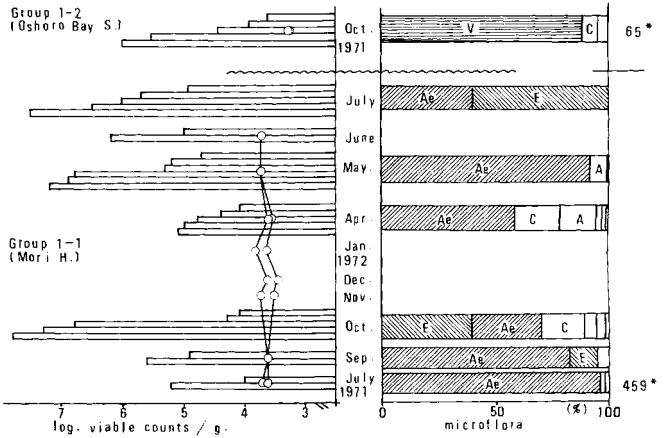

FIGURE 5. Intestinal microflora and number of viable microorganisms of masu salmon (*O. masou*) and king salmon (*O. tschawytscha*) reared in fresh water and sea water.
cf. ○—○: viable counts /ml in water tested, *: number of the isolates employed

water without feeding, the number of viable microorganisms in the intestines tended to decrease as the concentration of sea water increased, as shown in Fig. 6. However, following a subsequent feeding, the number tended to increase.

On the other hand, in group 2–4, fish reared in fresh water without feeding, the number of intestinal viable microrganisms decreased as time elapsed, but the decrease was not so great as in group 2–3.

The number of viable microorganisms in the environmental water samples during the experiment were 10^2–10^3/ml, while the number in the diets was 10^5/g.

Intestinal microflora;

As shown in Fig. 7, as the concentration of sea water increased in the rearing water of the group 2–1, the proportion of genus Pseudomonas became greater than that of the genus

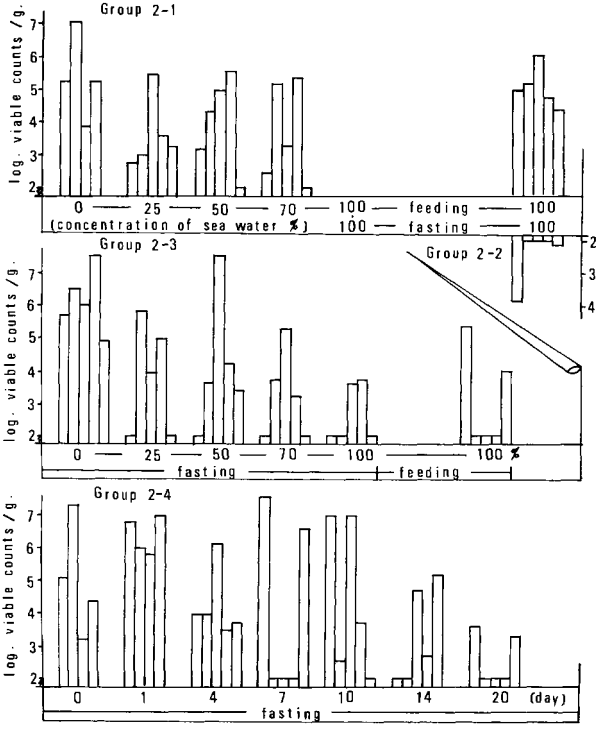

FIGURE 6. Number of viable microorganisms in the intestines of masu salmon (*O. masou*) in artificially transplanting period from fresh water into sea water and reared in fresh water without being fed.

Aeromonas and the family Enterobacteriaceae, of which are the main intestinal flora of the starting period. Then the genus Vibrio became greater with the progress of stage, thus taking place of the genus Pseudomonas and finally became the main flora in the late stage. In the group 2-2, of which feeding was stopped after transfer into 100% sea water, the genus Vibrio still represented the majority of the flora. In the group 2-3, a similar tendency was noted at the initial stage of the flora like with group 2-1, as the concentration of sea water increased, the flora became more complicated and took place in lieu of those of the initial stage, the genera Pseudomonas, Micrococcus, Coryneforms, the genus Achromobacter, and yeast. But, eventually, the yeast and genus Pseudomonas became the largest proportion of the flora. However, after subsequent feeding, the flora became mainly composed the genus Micrococus and Coryneforms thus being similar to the flora in diets. In the group 2-4, the proportion of the genus Aeromonas tended to decrease

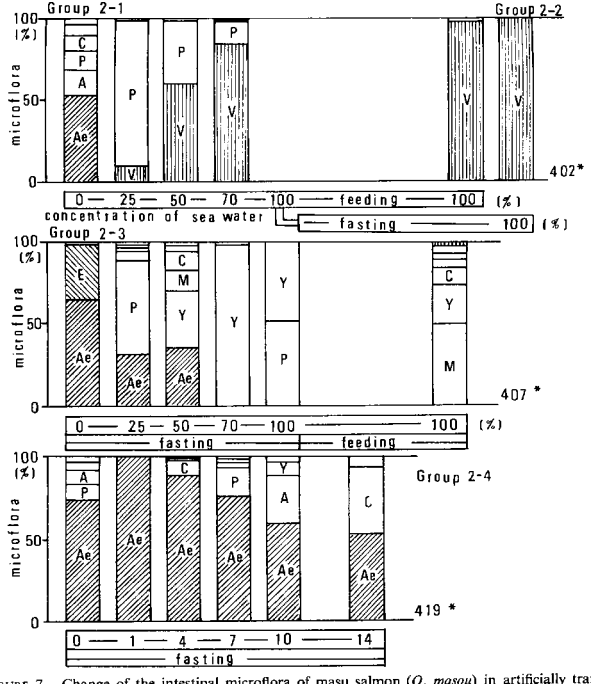

FIGURE 7. Change of the intestinal microflora of masu salmon (*O. masou*) in artificially transplanting period from fresh water into sea water and reared in fresh water without being fed.
cf. *: number of the isolates employed

gradually, but no significant changes were noted until the 10th day of rearing without feeding. On the 14th day, Coryneforms predominated in some samples.

(3) Mature fish cultured in fresh water (group 3)

A very small amount of contents, if not all, was found in the digestive tracts of the tested fish, a fact which indicated that they were in a fasting condition because of the eating habits of the salmon at this stage. The number of intestinal viable microorganisms, shown in Fig. 8, appear to be extremely few, and in half of the tested fish of 10^2–10^5/g and the largest proportion of the flora was yeast and Coryneforms, while the genus Aeromonas showed 20–40%.

(4) Fish living in open sea (groups 4–1, 4–2, 4–3)

The number of viable microorganisms in the intestines of those fish showed, as shown in Fig. 9, present a relatively high variation of 10^2–10^7/g, among the fish, but higher than the number in environmental sea water (average about 10/ml). The highest number in the pink salmon amounted to 10^7/g, to be followed by the sockeye salmon 10^6–10^7/g, the chum salmon 10^5–10^7/g, and the king salmon 10^2–10^5/g. This trend tended to be in proportion to the volume of contents in the intestines.

The number of viable microorganisms in several kinds of zoo-planktons which represented the main sources of food for these fish amounted to 10^2–10^5/g.

The microflora in the intestines of those fish generally comprised the genus Vibrio mainly, something like 69%. The proportion of the genus Vibrio was decreasingly less in the order of pink salmon, sockeye salmon, chum salmon and king salmon, indicating a correlation between the percentage of the genus Vibrio and the number of viable microorganisms in the intestines. About 40% of the strains isolated from those test samples were luminous bacteria. The microflora of both sea water and zooplankton were the genera Pseudomonas and Achromobacter at the rate of 98% in this particular station in the sea.

The results about several marine fish other

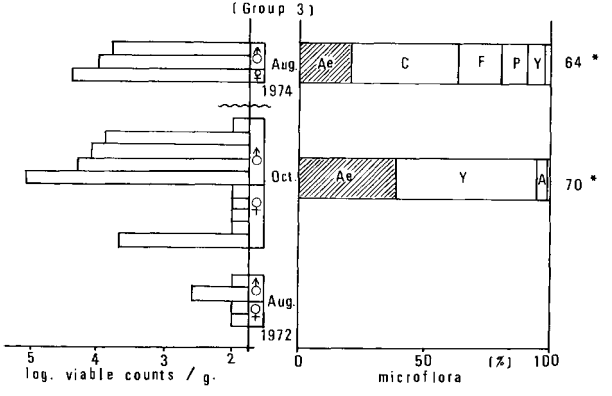

FIGURE 8. Intestinal microflora and number of viable microorganisms of mature masu salmon (*O. masou*) cultured in fresh water.
cf. *: number of the isolates employed

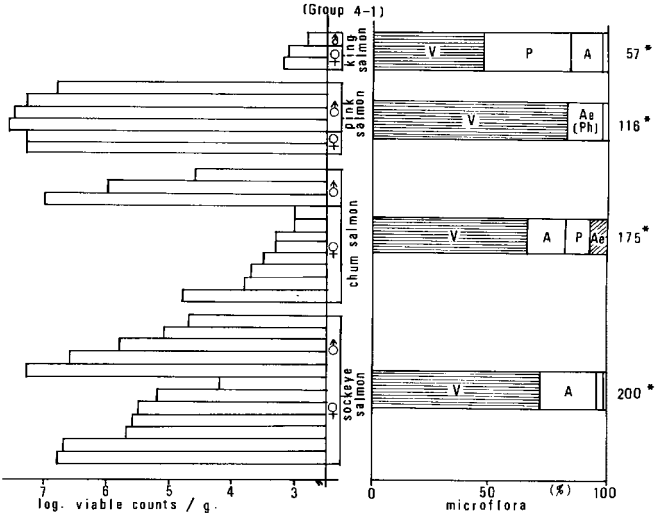

FIGURE 9. Intestinal microflora and number of viable microorganisms of salmon living in open sea.
cf. *: number of the isolates employed

than salmonids, which were observed for control, are shown in Fig. 10. In 12 fish from 7 species caught in the coastal sea of Hokkaido Island, the number of intestinal viable microorganisms varied 10^2–10^7/g, regardless of species, size or sex of the fish. Of these flora, the genus Vibrio showed 61%, while the genus Aeromonas with photogenesity by our classification showed 21% (genus Photobacterium by Bergey's Manual 8th ed. Buchanan and Gibbons 1974). On the other hand, with 14 fish from 6 species caught in the eastern Bering Sea, the intestinal viable microorganisms were 10^2–10^7/g and the flora was mainly the genus Vibrio (61%) and 40% of all isolates were photogenic.

As shown in Fig. 12 the microflora in zooplanktons collected in the coastal sea of Hokkaido Island, the genera Pseudomonas, Vibrio and Achromobacter were found to be predominant. Especially the genus Vibrio showed very high percentage.

(5) Anadromous fish (groups 5–1, 5–2)

As shown in Fig. 11, 10^2–10^7/g of intestinal viable microorganisms were detected in both pink salmon under rearing after their upstream run and chum salmon during their upstream run. Hardly any influence of fasting had been noted during the upstream run. In pink salmon, the genera Vibrio and Aeromonas represented the majority of intestinal microflora, which the genera Vibrio, Aeromonas and Pseudomonas were dominant in chum salmon.

2. Classification by type of representative isolates on the basis of requirement of inorganic salts for growth

Table 3 indicates the results of isolates from

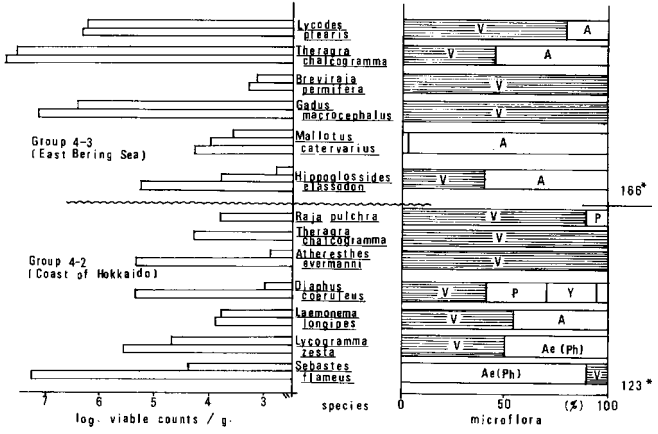

FIGURE 10. Intestinal microflora and number of viable microorganisms of several marine fish living in open sea.
cf. *: number of the isolates employed

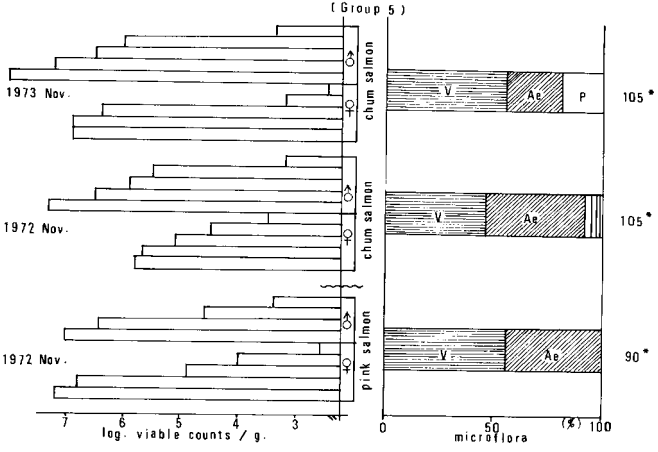

FIGURE 11. Intestinal microflora and number of viable microorganisms of anadromous pink salmon (*O. gorbuscha*) and chum salmon (*O. keta*).
cf. *: number of the isolates employed

TABLE 3. Typing of isolates by inorganic salt requirement for growth

Genus	Number of strains isolated from intestinal contents of fresh-water salmon			transplanting periods salmon			sea-water salmon		anadromous salmon	
	T*1	H*2	M*3	T	H	M	T	H, M	T	H, M
Aeromonas	28	0	0	30	0	0	0	24	45	9
Enterobacteriaceae	27	0	0	—	—	—	—	—	1	0
Pseudomonas	—	—	—	12	13	0	0	16	25	26
Vibrio	—	—	—	0	30	0	0	128	0	107
Achromobacter	10	0	0	—	—	—	0	36	1	0

cf. *1: Terrestrial type, *2: Halophilic type, *3: Marine type

TABLE 4. Susceptibility of isolates to bile and low pH

Genus	Number of strains isolated from intestinal contents S. b.*1 (≧0.5)	S. h.*2	environmental waters S. b. (≧0.5)	S. h.	diets S. b. (≧0.5)	S. h.
Aeromonas	18/26*	23/26*	6/ 9*	9/ 9*	—	—
Enterobacteriaceae	27/27	27/27	21/23	22/23	—	—
Pseudomonas	1/26	14/26	16/30	26/30	—	—
Vibrio	16/50	30/50	3/ 3	0/ 3	—	—
Micrococcus	—	—	0/14	2/14	0/12*	2/12
Coryneforms	—	—	5/12	2/12	0/ 2	1/ 2
Achromobacter	—	—	9/19	7/19	—	—
Flavobacterium/ Cytophaga	—	—	1/16	3/16	—	—

cf. * : number of the strains employed

*1: S. b. value = Turbidity of basal culture containing bile (2%) / Turbidity of bile free basal culture

*2: S. h. value = Turbidity of basal culture at pH. 5.5 / Turbidity of basal culture at pH. 7.5

fish intestines, environmental water, and diets of tested fish, into the types of terrestrial, halophilic, and marine from the requirement of inorganic salts. Of the isolates from intestinal contents of tested fish, all of the genus Aeromonas of fresh water fish origin were of terrestrial type, while all the genus Vibrio of sea water fish origin were either of halophilic or marine types. All of the genus Pseudomonas derived from the fish during their transfer period into sea water and from the anadromous fish were nearly 50% of the terrestrial and 50% of the halophilic types.

3. Susceptibility to low pH and bile of represetative isolates

The results of observation of susceptibility to low pH (5.5) and bile (2%) of isolates from intestinal contents, environmental water and diets of fish of groups 1 and 2–1 are shown in Table 4. The genus Aeromonas, the family Enterobacteriaceae and the genus Vibrio were found to be resistant to low pH and bile, whereas most Coryneforms, the genera Micrococcus, Achromobacter and Flavobacterium/Cytophaga were found to have sensitivity to them.

Discussion

The above mentioned results are summarized in Fig. 12. The tested fish living in fresh water were only those cultured in Mori

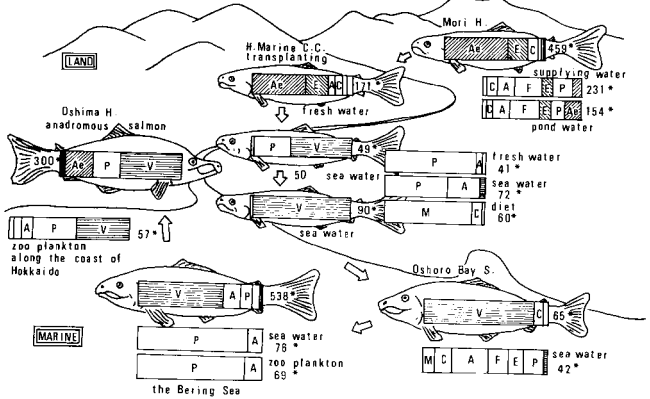

FIGURE 12. Change of the intestinal microflora in the course of salmonid life cycle.
cf. *: number of the isolates employed

Hatchery, so that no generalized conclusion can be drawn, but unlike the microflora occurring in the culturing water, i. e. the genera Achromobacter, Flavobacterium/Cytophaga, Pseudomonas, Aeromonas or the family Enterobacteriaceae, the flora in the intestines was predominantly of the genus Aeromonas and the family Enterobacteriaceae, and could be assumed to be indicative of the main intestinal microflora of the fish grown in fresh water.

The above results of this study resemble those of Mattheis (1966a, b, c, and d) who had reported that the predominant microflora in the intestines of fresh water fish were the genus Aeromonas, the family Enterobacteriaceae and the genus Pseudomonas, and also the results agreed with the report of Trust (1974) indicating that the genera Aeromonas, Enterobacter and Acinetobacter as the main constituent of fish intestinal microflora.

From the comparison between the microflora of the water to pond and water in pond a proportional increase of the genus Aeromonas could be noted in those of the latter. This fact may be attributable to the effect of microflora in the intestines of the reared fish.

In the fish reared in sea water, the genus Vibrio could be detected at the high percentage in the intestines, unlike the microflora of environmental sea water such as the genera Achromobacter, Pseudomonas, Flavobacterium/Cytophaga, the family Enterobacteriaceae or Coryneforms. This finding suggested the presence of microflora was also specific for the intestines of marine fish. This result agrees with the reports of Liston (1954, 1957), Okuzumi and Horie (1969), Kaneko (1971a, b, and c), Sera and Kimata (1972), Sera and Ishida (1972a and b), and Sera *et al.* (1972), which indicated the predominance of genus Vibrio in the microflora of the intestines of marine fish, and the reports of Colwell (1962), Shrivastava and Floodgate (1966), and Newman *et al.* (1972), which indicated a higher frequency of the genus Vibrio in the microflora of fish intestines than in the flora of fish living environments.

In the tested fish transferred artificially to sea water, the effects of microflora in the sea

water on intestinal microflora of non fed group were noted, which could be attributed to fasting. Whereas, in the group with feeding, the genus Aeromonas of the terrestrial type which was predominant in the intestinal microflora at the initial stage was gradually replaced with the genus Pseudomonas of terrestrial or halophilic type as the concentration of sea water in the rearing water increased. These were further replaced with the genus Vibrio that came to be predominant in the flora. This result may coincide with the difference of intestinal microflora of fish reared in sea water from those of fish reared in fresh water like our finding above.

The above tested fish were cultured with different diets in three different places, but no effects of diet upon the intestinal microflora could be seen. This finding may support the report of Sera and Kimata (1972) reporting that the intestinal microflora of the fish are not affected by feeding.

Most of the isolates from fish intestines were fast to low pH and bile and it is supposed that intestinal microorganisms would be selected by the fish itself, not by feeding or environmental conditions. Fish intestinal microflora seen after feeding following the transfer to sea water without feeding was similar to microflora of their diets and has no resistance to low pH. This finding may be claimed to be a specific phenomenon due to the weakening of tested fish transferred into sea water without feeding.

On the other hand, in the group without feeding in fresh water, the number of viable microorganisms decreased gradually with the day of non feeding and also the proportion of the genus Aeromonas gradually decreased with the passing day. However, the residence of the genus Aeromonas in intestines was found about 10–14 days after. This trend was noted also in the other tested groups. These results are a little different from the reports of Obst (1919), Hunter (1920), and Sorimachi and Egusa (1971), claiming that no bacteria could be detected from the intestines of fasting fish. Hence, the above mentioned finding would suggest that, unless the tested fish are weakened, the number of viable microorganisms in the intestines would be reduced following culture without feeding, but their flora would retain the groups of the initially existing microorganisms for a considerable long period.

In the mature fish cultured in fresh water, a notable decrease in the intestinal viable microorganisms was noted, and no microorganisms were found in half of the tested fish. In addition, yeast and Coryneforms showed the highest proportion of the flora in intestines. These results may be attributable to the effect of fasting, in view of the nature of fish of those kinds to terminate eating as they reach maturity.

As described above, the intestinal microflora of the fish reared in sea water were predominantly of the genus Vibrio in our experiment. The genus Vibrio would be inevitably detected from those fish, because Shimizu and Aiso (1962) and Shimizu *et al.* (1971) reported that generally the genus Vibrio was the main bacterial flora of sea water and plankton at the season of relatively high temperature along the coast of Japan. However it may be justifiably accepted that the genus Vibrio and its related organisms would be the main ones of the microflora in the intestines of marine fish, in view of the fact that it showed a percentage as high as 61–69% out of the intestinal microflora through the observation on the tested salmonids and several other marine fish which were collected in the open sea.

A correlation could be seen between the number of viable microorganisms in the intestines of those fish and microflora in their intestines. This trend can suggest the possibility of correlation of microflora with food chain, but hardly any genus Vibrio could be found in the sea water or feeding organisms. Hence, there was no ostensible relationship found as suggested above. It may be assumed that the digestive tract of fish might have abili-

ty to select microorganisms, and the genus Vibrio taken into the intestines from sea water or diet might have selectively propagated in the intestinal tracts.

In the anadromous fish during their running upstream, the number of viable microorganisms in their intestines could be detected in the range of 10^2–10^7/g, which was unlike the reports of Hunter (1920) who claimed that the digestive tracts of salmon running upstream for egg laying were almost aseptic. Particularly, in tested pink salmon, large number of viable microorganisms could be noted in intestines despite their prior rearing for about 3 months in fresh water. Hence, the above mentioned finding would support the report of Mattheis (1966d) claiming that, although the number of viable microorganisms, in the intestines of salmonids are reduced following fasting, they can still be detected even after fasting for about 4.5 months. The intestinal microflora of those tested fish were mainly the genera Vibrio and Aeromonas, both of the halophilic and terrestrial type. This finding is great interest because it is a sharp contrast with the finding in the tested fish which were artificially transplanted to sea water. The fact is that the genus Vibrio can be detected in high percentage even after prolonged rearing without feeding. This might suggest that there would be some difference between the genus Aeromonas which can be frequently noted in the intestinal microflora of fresh water fish and the genus Vibrio in marine fish, in terms of the nature of normal intestinal flora. This difference should be cleared by further study.

From our findings above, it would be concluded that the intestinal microflora of healthy salmonids are mainly composed of the genus Aeromonas and family Enterobacteriaceae of a so called terrestrial type, if they are living in fresh water, on the other hand, the flora are mainly composed of genus Vibrio of marine or halophilic type when living in sea water. If fish move to the sea or upstream a river in their life cycle, the flora in their intestines would undergo changes in order to adequately adapt the fish themselves for their living environments.

According to the results of the experiments of our coworker (Ugajin 1974), it was found that the genus Aeromonas, the family Enterobacteriaceae, and genus Vibrio, the main constituent of intestinal microflora of salmonids, were extremely uniform on the level of their species, and that flora were composed of very limited species, such as *Aeromonas punctata or hydrophila, Hafnia alvei*, and *Vibrio fischeri*. Also from these finding, it can be assumed that fish have ability to select their microorganisms in their digestive tract.

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