Fish Mating Experiment in Space — What It Aimed at and How It Was Prepared

Kenichi Ijiri

Radioisotope Center, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

Abstract The code name 'MEDAKA' was given to the fish experiment in the IML-2 (the second International Microgravity Laboratory), a Space-shuttle mission (STS-65) carried out in July 1994. Medaka is the Japanese name for a small fresh-water fish, *Oryzias latipes*. This experiment titled 'Mating behavior of the fish Medaka and development of their eggs in space' aimed to present data for designing the future fish-culture in space. The Medaka experiment accomplished its objectives to the point of 100 %. The fish mated, laid eggs in space, and these eggs developed normally to hatching (coming out as a baby fish) under microgravity. Its success totally depended on selection of the four fish sent to space. This paper describes the aims of the IML-2 Medaka fish experiment and how it was prepared, together with a brief report on what were achieved in space.

Key Words: IML-2, space biology, mating behavior, space-originated fry, fish, Medaka

As one of the space biology experiments for the second International Microgravity Laboratory (IML-2/STS-65) carried out in the summer of 1994, four of a small Japanese killifish (Medaka) made a space travel of 15 days. These four Medaka fish performed their successful mating behavior in space for the first time among vertebrate animals. Moreover, the eggs laid developed normally till hatching fry (baby fish) in space. In this paper, I will report this Medaka fish experiment in space, with an emphasis on its aims and on how it was prepared.

1. Objectives

1.1 Objectives of IML-2 Medaka experiment

This particular space experiment has been planned in order to obtain fundamental data and to present some idea on designing the aquaculture (fish culture) style in space. The following special themes have been investigated using the fish Medaka in the Shuttle flight of 2-week duration.

1) Whether fish can carry out mating behavior and lay eggs or not under microgravity (Mating and spawning), 2) When eggs are laid through mating in space, whether all the processes from fertilization to hatching (coming out as a baby fish) take place normally or not under microgravity (Embryonic development), and 3) Letting the space-flown fish continue to mate and lay eggs on the ground after the Shuttle lands, whether any effects can be detected or not on their descendants (Effects on offsprings).

Beside these, in this mission, I have tried to solve one important problem for the multi-generation studies of this fish in Space Station Alpha. In the IML-2 experiment, inside the water-tank for the adult fish, a place for eggs they laid and fry not for the adult fish was set (two-generation aquarium). Thus, checking whether this separation mechanism works or not in space was also one of the objectives of the present experiment, the result of which may be useful for designing the new aquatic animal experiment unit, possibly used in JEM (Japanese Experiment Module) in the Space Station.

1.2 A long-range objective of this Medaka experiment -- Fundamental studies for fish culture

Received March 10, 1995

Accepted March 27, 1995

Address for correspondence: Dr. K. Ijiri
Radioisotope Center, University of Tokyo,
2-11-16 Yayoi, Bunkyo-ku, Tokyo 113, Japan

in space

Aquaculture (fish culture) in space will be important protein resources when human start to stay and live in space. Especially, Japan is good at making a variety of processed food from fish meat, and such techniques may be useful also in future space life. However, at present there is not any precise image yet of fish culture in space. Listed in Fig.1 are check points (steps) for realization of fish culture in space. Along the steps in Fig.1, IML-2 Medaka experiment has been planned. The data of space experiments like this one may tell us which of these steps are feasible or not under microgravity (micro-G). Depending on 'Yes' or 'No' obtained from the results of space experiments, procedures taken in the future fish-culture or even at its experimental stages on Space Station may become totally different ones.

If 'No' was answered for any step in Fig.1, an alternative procedure should be employed thereafter. The more steps are checked for 'Yes' or 'No', the procedures to take such as listed in Fig.1 become more clear. This is the reason we should approach this problem now, not waiting the construction of Space Station, checking as many steps as we can answer still in Space-shuttle experiments.

Let us briefly explain each step in Fig.1. As listed in Fig.1, 'Can fish mate and spawn eggs under micro-G?' is the first question to be asked. What Fig. 1 shows is that if the answer to this question was 'No', i.e., mating-behavior of fish was found to be impossible in micro-G, we should employ 'artificial insemination' as an alternative method. Either through natural way of mating between male and female, or through artificial insemination between sperm suspension and eggs taken out from female fish, (2)Eggs are fertilized normally? and (3)If fertilized, then fertilized eggs (i.e., embryos) develop normally? under micro-G are the next points to be checked. When these were answered in the negative, the fish culture in space has to use another procedure, i.e., transporting embryos or fry (baby fish) from the Earth. At present, the techniques for freezing fish embryos and thawing them alive have almost been established, and this transport procedure is possible. Freezing baby fish alive is difficult. If

necessary, they should be sent being kept in water from the Earth. In any case of sending embryos or fry from the Earth, a totally autonomous fish-culture system cannot be attained. It has to depend on the Earth for its constant supply of fish embryos or fry.

When 'No' is given to the question (4)Can baby fish be grown up to adult in micro-G?, I don't think it is any more worth carrying out studies for fish culture in microgravity. Rather, all the efforts are to be devoted to the research for fish culture under several 10th of G level, i.e., on Lunar base (about 1/6 of G) or at somewhere of 0.1 to 1.0 G levels artificially generated in a gigantic centrifuge, expecting a gravity-generating space colony be realized in future for human habitation. On the other hand, for the step answered 'Yes' both on the 1-G level (on the Earth) and micro-G level, it is reasonable to assume that the step can also be possible in any G level of 0.1 to 1.0.

Let us go back to the check points for micro-G in Fig.1. After (5)Germ cells develop normally? and (6)The 2nd-generation fish mate and spawn eggs? are answered 'Yes', breeding of the Medaka fish through many generations will be challenged. Meantime, incorporation of the fish as a component of CELSS (Controlled Ecological Life Support System) should also be tried. In multi-generation studies, if lucky, it might be expected that an emergence of mutant fish which is highly adaptive to micro-G. If such special gene(s) which exhibit powerful tolerance to micro-G are found in the Medaka fish, the transfer of such trait to any edible (and larger) fish can be attempted. Even now, such gene transfer method, i.e., production of a transgenic fish has already been a well-established technique.

Among the steps listed in Fig.1, space experiments to check the steps numbered (4) or more will take a long duration of months or more, and are to be carried out on Space Station. The steps the present IML-2 Medaka experiment may give definite answers to are (1)-(3). When 'Yes' or 'No' is given to (1)-(3), as explained above, the result may decide some procedures to be employed or not for the future fish-culture in space. Therefore, the importance of the IML-2 Medaka fish experiment is that it is aiming to

carry out all the checks we can do in Space shuttle (in 2-week mission), and with its results to consider and plan the further fish experiments on the Space Station.

In this IML-2 mission, for the adult fish

brought back to the Earth, development of the eggs they lay on each day after landing will also be observed, which may offer a part of information on germ-cell formation in space, i.e., the check point (5).

Fig.1 Check points for realization of fish culture in space. For details, see text.

[Why this small fish to space ?]

As mentioned above, fish culture in space is our ultimate goal. It is not, however, our idea at all to eat this fish Medaka in space. The fish is tiny and not edible (No Japanese eat this fish). The Medaka fish is a good experimental vertebrate species, with many different strains, easiness to breed, and short life-cycle (eggs develop to mature fish within 3 months). It is my belief that the space studies using this fish species for mating, spawning, development, adaptation to micro-G, possible emergence of mutant fish and so on, should be carried out first, and studies directed to large edible fish later. This could be the most efficient way toward the realization of fish culture in space.

Fig.2 Medaka fish, male (upper fish) and female (lower one). A cluster of eggs is visible on the belly of the female.

When conducting multi-generation studies using this Medaka fish in Space Station, there is a chance to obtain mutant fish which exhibit a better adaptation to micro-G. Once we got such mutant fish in Medaka (inherited to its descendants), transfer of DNA containing gene(s) responsible for such useful trait of Medaka fish into DNA of any edible fish (plaice, trout, cod, etc.) is a possible technique even at present time.

2. Details on what to be investigated

2.1 Medaka -- Its general features

Listed below are several characteristics of this small killifish, Medaka. Only those necessary for understanding this IML-2 Medaka experiment are listed.

Medaka (a small killifish)

Classification: fish(teleost), *Oryzias latipes*

Adult fish: 0.7 g in weight, 3 cm in length

Female and Male are different

in the shape of Dorsal fin and Anal fin

Conditions for mating and spawning:

Light 14-h light / 10-h dark

Temperature 23 - 28 °C

Spawning habit:

Once started spawning, fish spawn eggs everyday.

Within 2 hours after the light gets on, fish mate and spawn eggs.

Fig.3 Male and female of Medaka fish.

Eggs and Embryos:

Egg size 1.0 mm (diameter)

Number of eggs ca. 20 eggs /female/ day

Hatching ca. 10 days after fertilization
(at 24 °C)

2.2 Mating behavior of Medaka fish

To check whether this fish can carry out mating behavior in space or not is one of the objectives of this space experiment. It has been generally known that fish loop when exposed to micro-G. However, through many parabolic flights using airplane, we have found a particular strain of Medaka which do not loop at all under micro-G. Mating behavior can be expected only for such a fish strain tolerant to micro-G. This tolerance to micro-G is inheritable from parents to children, to grand-children and so on.

The strain of Medaka fish to be sent to space was thus decided. However, it was not the end. It was the beginning of the selection process toward the final four Medaka fish. Among the population of this strain, we paid much effort to select four fish which are at the best conditions immediately before placing them into the Medaka water-tank. Since this is one-shot experiment after full-time preparation of four years, selection was done employing many procedures, details of which are given in 3.3.

[Mating behavior]

A series of behaviors exhibited in mating and egg-laying are shown in Fig.4. From the video-images of Medaka fish taken in space, analysis of mating behavior will be performed using behavior patterns shown in Fig.4.

Fig.4 Mating behavior of Medaka fish (from T. Iwamatsu, 1993).

[20 seconds vs. 2 weeks]

It was a great success that we found fish strain(s) which are tolerant to micro-G. Such tolerance to micro-G is specific to the strain, thus genetically determined. The results from IML-2 experiment will extend studies to clarify the nature of genes involved in such tolerance. This is quite an interesting subject, since it deals with the connection between the information of DNA and the actual behavior of fish under micro-G.

However, such is a conclusion drawn from the repeated experiment of 20-second micro-G using jet-airplane (Figs. 5 and 6). All we have watched was the fish behavior in 20 seconds, i.e., the fish behavior corresponding to the first 20 seconds of 2 weeks (mission duration). Does the fish strain selected under 20-second micro-G show tolerance

also to a longer period of micro-G? This will be answered only in the actual space flight, and it is the time to test our working hypothesis on which our selection method was based. It is also of interest to see whether the difference exists in fish behavior between micro-G of 20 seconds and 2 weeks, and degree of adaptation to micro-G changes or not at different days in 2 weeks.

Fig.5 A jet-airplane used for parabolic flights.

Fig.6 Micro-G realized in a parabolic flight.

2.3 'Space food' for Medaka fish

In the past, it was common that fish were kept without food during the space flight, e.g., koi in SL-J (STS-47, 1992) and goldfish in IML-2. Since this IML-2 Medaka experiment expects fish to perform mating and laying eggs, food is indispensable to fish in space. 'Space food' for Medaka, especially for this mission was developed and produced by a Japanese company 'Kyorin-Foods' (a company providing 'Hikari' brand of

fish food in United States), together with our repeated tests on the fish kept in the water-tank actually used for space experiment.

Fig.7 Space food for Medaka fish.

As illustrated in Fig.8, the food-supplying apparatus has four divisions (separate rooms), into which this space food can be placed. Before the launch, food in each division is kept dry, being insulated from the water of the tank. An extra piece of food has been placed inside the tank for the time before the launch. When in space the astronaut drives food-cylinder one division-length to the left, division A goes to the place where an opening exists, there the exposed food absorbs water in. This special food when absorbs water swells and increases its volume to finally about 3 times large. Such swelling sends the food up from the opening, so that fish easily can eat it. Fish can also eat the rest of food, putting his head inside that division. A few days later, the astronaut pushes the cylinder again, bringing the division B to the opening. This procedure now restores division A. Thus, the remaining food in division A becomes insulated again from the water..

Fig.8 An illustration of the food-supplying apparatus used in Medaka water-tank.

2.4 Embryonic development in space

In this fish Medaka the egg envelope is transparent, and the developing embryonic body inside is easy to see (Fig.9). Moreover, some of the internal structure can be detected due to the transparency of body surface itself. To study how the fertilized eggs develop under micro-G is important since it also gives us informations of how on the Earth the gravity plays a role in the development. In this IML-2 Medaka experiment, three types of embryonic samples were studied for their development in space. The first one is, of course, the eggs laid in space. The other two types are the eggs fertilized already on the Earth.

Fig.9 Developmental stages for Medaka embryos. Stages are expressed in number.

a) Development of eggs laid in space

Fertilized eggs become baby fish (hatched fry) about 10 days after fertilization at 24 °C. Since IML-2 is a two-week mission, if fish mate on the first few days we may detect baby fish and observe their behavior in space. This will be an experiment, checking all the processes from fertilization (or even before the fertilization) till hatching under micro-G. However, if fish had not performed mating behavior and therefore no eggs had been laid in space, the experiment itself is not possible. Thinking such case might have occurred, two other experiments, b) and c) as shown below were designed.

b) Eggs fertilized on the ground: Its development and behavior of fry hatched in space

Two small mesh-boxes were set inside the water-tank. In these mesh-boxes, eggs fertilized already on the ground were placed. In one mesh-

box, eggs of HO5 strain were placed, and those of ccT strain in the other mesh-box.

In the laboratory (Room 185) at KSC (Kennedy Space Center), embryos already developed to the eye-pigmented stage (stage 26/27) were placed in the mesh-boxes and together with other samples, were loaded into the Spacelab of the Shuttle 30 hours before the launch. The stage of embryos was determined so as to see their hatching mainly around MET (mission elapsed time) 4 day to 6 day, so that their swimming behavior can be video-recorded in pre-scheduled time. During the mission, video pictures of the embryos and hatched fry were taped and also partially down-linked at real time.

Swimming patterns of hatched fry of the two Medaka strains (HO5, ccT) will be compared. There is a reason to use the fry of these strains. Adult fish of HO5 strain exhibit many loopings in 20 sec micro-G, however, as mentioned above, adult fish of ccT strain do not. The observation of swimming behavior of the fry of these strains, especially immediately after hatching in micro-G is of our interest. It will give us informations on the development of neural integration responsible for posture-control in fish.

Fig.10 Fish of HO5 strain are exhibiting looping behavior when being exposed to micro-G in a parabolic flight.

c) Eggs fertilized on the ground: Developmental capacity of ccT and other strains

The third experiment was for the comparison of developmental capacity between embryos of ccT and other strains. Embryos whose development is at stage 11 (0-day embryos) and

stage 17(1-day embryos) were put in the disc-shaped mesh-box (Fig.11) and placed at the rear portion of goldfish aquarium. Unfortunately, for these embryos no video pictures were taken in space, and their development and ability to hatch were checked 2 weeks later, i.e., immediately after landing of the Shuttle.

Fig.11 The disc-type mesh-box for the embryos prepared on the ground.

As mentioned earlier, selection of adult fish were based on their tolerance to micro-G or ability to swim normally under micro-G, and ccT strain was chosen. However, if embryos of ccT strain were found extremely sensitive or labile to micro-G, the strain might not be a good candidate for the future use in Space-Station experiments. To get such information is an objective of this experiment c).

2.5 Two-generation aquarium

Multi-generation studies on this fish species, i.e., carrying out all the processes from eggs to adult and repetition of such life-cycle many times in space, will be our next goal in Space Station. For its preparatory work, a simple structure of two-generation aquarium was designed, i.e., separating embryos and fry from adult fish. This is because when kept together adult fish eat the eggs they have laid, and even hatched fry may sometimes look delicious to the adult.

Thus, it is necessary to devise a mechanism for separating embryos and fry from the adult fish. A flow of water in the Medaka tank is a must in

this life-support system. Water oxygenated after passing an artificial lung and biologically filtered by bacteria at the rear portion of AAEU(Aquatic Animal Experiment Unit, provided by Japan), is constantly flowing into the Medaka tank, and the same volume of water is leaving there to be oxygenated and filtered again. We have come to use this water-flow in the tank also for such separation purpose.

Near the outlet of water tank, we set a separation plate of large meshes through which no adult fish can pass. Eggs at first attached to the belly of female fish later leave her body and float inside the tank, then carried by a water-flow pass through the mesh. Some eggs may be attached to the mesh-plate itself by the female. Thus, beneath the mesh-plate there is a kids' room, i.e., a place for keeping embryos safe from the adult. There a web of thread which has a very sticky nature to eggs has been set. Immediately before the outlet of the tank, a piece of sponge is also placed. This sponge was set for the place of hatched fry, together for the catching the dust. Its 3-dimensional mesh structure offers fry a comfortable place to stay.

Fig.12 Separated area for embryos and fry (a picture taken in space). The Crew is pointing the newly-laid eggs.

2.6 Effects on offsprings

When adult fish are recovered to the ground, their spawning will be checked everyday. Tracing of egg-laying and development of the eggs laid will be carried out everyday for a month or more. This will give us informations about the effects of micro-G on gametogenesis, i.e., sperm-

forming process in testes of males and egg-forming process in ovaries of females.

3. Ground-based studies and preparation

3.1 Strain of adult fish loaded in the Shuttle

Four adult Medaka fish (two fish for each sex) of ccT strain will be put in the water-tank installed in AAEU. From the parabolic-flight experiments, we have found this strain of fish swim normally under micro-G (say, tolerant to micro-G).

Fig.13 A fish strain which is tolerant to micro-G. All the fish are swimming normal though they are now under micro-G conditions.

So far, two Medaka strains have been found tolerant to micro-G in such airplane experiments. One is a strain named ccT and the other is HNI-II strain. However, through various tests, fish of the latter strain have been found quite nervous, and difficult to maintain in good conditions for mating and laying eggs in the small water-tank used in the IML-2 experiment. Beside its small volume, the existence of water-flow inside the tank was to give stresses to the fish. Since the other water-tanks (used for newts) of AAEU and Medaka tank share the water and the newts prefer temperature of 20-22 °C, water temperature is to be set near at the lowest value (23 or 24 °C) for Medaka fish to sustain mating. In repeated experiments, the ccT strain has shown a higher tolerancy to such environmental stresses than the other strain. Therefore, in this mission, we decided to send 4 adult fish of ccT strain to space. How among the fish of this strain the final 4 fish were chosen will be described below.

3.2 Selection procedures for space Medaka (in Japan)

1) About 2,000 Medaka fish (ccT strain) of 1 year old, have been prepared at our lab in University of Tokyo in the spring of 1994. This is the strain kept for a long time through many generations at the lab. A year before, i.e. from the spring to the summer of 1993, ten thousands of eggs of this strain were collected in our lab and they have been reared to 2,000 adult fish in outdoor conditions.

2) In April, from size and shape about 1,500 fish were chosen and transferred to indoor conditions, where light-cycle and temperature can be controlled.

3) Using a 'Rotating striped-drum apparatus', each fish was tested for their ability to follow the striped-drum movement, rotated at a certain high speed. This is a test, which is from the airplane studies known to be related to the ability of posture-control under micro-G. About 900 fish passed this test.

Fig.14 A test using a Rotating striped-drum apparatus.

4) Since on orbit fish cannot depend on gravity for their posture-control, we decided to provide an oriented lighting for helping their posture-control, i.e., expecting fish may always turn his back (dorsal side) to the light source. This reflex is called 'Dorsal-light reaction'. We tried to select fish showing a good reaction to light. Throughout the selection process, two methods of Dorsal-light reaction test (method I and method II) were employed (details will be described elsewhere).

For a test of each fish, the method II takes a longer time than the method I. Therefore, in Japan the Dorsal-light reaction test was done only using the method I.

Fig.15 Dorsal-light reaction test (method I). The lamps repeat lighting in turn.

5) Fish which passed the tests as described in 3) and 4) were then transferred to the room, of which light-dark time was gradually shifted to the light-dark time at KSC, Florida (day and night are almost opposite to that of Japan). After checking mating and egg-laying capacity, two females and two males were put in a mini-vessel. In total, 114 mini-vessels (i.e. 4 fish x 114 vessels = 456 fish) were set up. The mini-vessel is of the same volume as the Medaka water-tank used in space. At the beginning of June, 82 vessel-teams have remained.

Fig.16 Bottles for hand-carrying fish aboard airplane.

6) At the beginning of June, 300 fish (i.e., 75 vessel-teams of 4 fish) were transferred from Tokyo to KSC (Room 185 in the building called Hangar-L). They were transferred in two ways for a safety purpose. One by air cargo and the other carried by Principal Investigator (KI) in the airplane. Additionally, 100 fish of different strains were also sent by air cargo. These fish were for the purpose of egg-collection, and had not been selected as above.

3.3 Selection procedures for space Medaka (at KSC)

7) At KSC, the same set-ups of mini-vessels were realized.

8) For 300 fish (i.e., 75 vessel-teams), the Rotating striped-drum test was given to each fish. As a consequence, some teams were removed.

Fig.17 Dorsal-light reaction test (method II). The light always comes from the left side.

Fig.18 The final Medaka-astronauts selected.

9) Then, a new method of Dorsal-light reaction test (the method II) was employed for further selection of fish.

10) From the fish conditions observed by eyes and also from their spawning record over 2 months (of course, the recent record was given a highly weighted considerations), the final candidate of 4 fish (one vessel-team) was chosen.

4. In-flight report

4.1 A brief summary of events

Four fish (two males and two females) were put in the water-tank of AAEU. They were kept under temperature- and light-controlled conditions (24 °C, 14-h light and 10-h dark). With an interval of a few days, Payload Crew sent the new food to the accessible position in the food-supplying apparatus. Since the fish had been mating everyday before loaded to the Shuttle, if not suffered much damage in micro-G, the fish may mate and lay eggs also in space. Each day, mating behavior should be completed within 2 hours after the transition from dark period to light period. The Payload Crew checked spawning every day. On MET 2d, 4d and 7d, the video camera recorded their activity for two hours immediately after lighting, and this was an enough time to record the fish mating behavior.

Newly-laid eggs first form a cluster on the belly of female fish, then after an hour or so, they leave the body. Eggs detached from the female body travel along the water-flow to an area

Fig.19 The four Medaka fish in the water-tank, beside which a Japanese-shrine amulet was placed for their safe space-travel.

separated by a mesh plate. The crew carried out close-up video observations of the developing embryos and hatched fry. All the samples, i.e., adult fish, fry and embryos returned safely to the Earth.

Following are events in the IML-2 Medaka space-experiment listed with time-course order (details will be described elsewhere).

1) Adult fish (2 males and 2 females, of orange-colored type, ccT strain) were put in the Medaka water-tank. These four fish were named as 'Cosmo' and 'Genki' (meaning active) for males, 'Yume' (dream) and 'Miki' (future) for females. These names had been proposed to us by boys and girls of YAC (Young Astronaut Club) in Japan.

2) Water tank was loaded into the Spacelab, about 30 hours before the launch.

3) Space shuttle Columbia had a lift off just on a scheduled time. When examined at 9 hours after the launch, no eggs were detected. Then, about 24 hours after the launch, our Japanese Payload Specialist, Chiaki Mukai reported 3 newly-laid eggs by her visual inspection.

Fig.20 A fish eating the food in space.

4) In this fish Medaka, mating and laying eggs and fertilization of eggs all take place almost at the same time (Fig.4). Therefore, from her report we could tell that male and female fish did mate and laid eggs in space.

The spawning habit of this fish is, once they started mating they continue to mate and lay eggs once every day. So, as expected, on the next day

again they mated and laid eggs in space, and Chiaki Mukai reported number of eggs increased to the total of 10 in the tank.

5) The next day, i.e., between the 3rd day and 4th day of the mission (the end of MET 2 d), the Crew (Don Thomas) set up the video-camera and tried to record the mating behavior of the fish. And again, male and female fish mated.

Lucky enough, they did mate in the midst of video down-link time, so that I could see with my own eyes this dramatic scene, not waiting the arrival of recorded video-tapes after the landing of Shuttle. The scene is shown in Fig.21.

6) Crew reported the existence of many eggs in the tank and most of these eggs had well-developed body with black pigmented eyes.

Fig.21 Mating in space. Male and female together at the center of the tank. The first time reported in vertebrates.

Fig.22 Many eggs laid in space have developed normally, showing two black-pigmented eyes.

7) On MET 4 d, mating behavior was again video-recorded, and Payload Commander, Rick Hieb took a good video-picture of a female fish with eggs at her belly.

8) Most eggs seemed developing normally (Fig.22), and for those eggs laid on the early days of the mission baby fish (hatched fry) were expected. Usually at 24 °C, fertilized eggs will become hatched fry about 10 days later. However, due to the trouble (death) in newt tanks, water temperature has been lowered to 23 °C, which may have somewhat prolonged the time of hatching.

9) At the end of 12th day, a baby fish was detected by Chiaki Mukai when she carried out a routine visual-inspection. Video-recording is not scheduled on this day. However, she decided to take video pictures of the swimming baby. With the help of Payload Commander, Rick Hieb, she successfully video-recorded the picture of a baby fish swimming with its mother (for the picture, see K. Ijiri, 1993: *Biological Sciences in Space*, Vol.8 (No.4), p.231-233). On that evening, the picture was broadcast as a Crew's edition for NASA's mission high-light TV program.

Fig.23 A space-originated fry.

10) The Shuttle (orbiter) extended its stay in space with one day, and landed on KSC in the morning of July 23. After having watched its landing, we were waiting for a NASA's vehicle to bring the Medaka water-tank to KSC laboratory (Hangar-L). When we saw the four adult fish were lying down at the bottom of the tank, we all were shocked. The fish looked as dead (Fig.24).

However, checking their fins and gills were moving all right, we soon understood that they were only having a trouble from the gravity force on the Earth. Then one by one, fish began to swim or tried to swim. Though quite awkward they swam, a smile came back to our face.

Fig.24 Adult fish 6 hours after landing.

11) Another surprise. Eight space-hatched baby fish (see details in 4.3 a)) were detected in the area separated with a mesh-plate. An extra day of extended flight-duration may have brought about this increase in the number of fry hatched in space.

12) Adult fish returned from space had forgotten how to swim in the world of a gravity force. In space, for about 15 days they did not have to use air bladder (swim bladder) at all, and had forgotten how to use it properly. On the Earth, fish tried to swim upward, however, after a second or so they dropped again onto the bottom of the tank. They were also swimming without much use of tail-fin. This probably was because the fish had not used their tail-fin in space so frequently as they usually do on the Earth.

On the contrary, eight baby fish hatched in space from the eggs laid in space (i.e., truly space-originated fry) could swim normally, showing no difference in their behavior at all from the behavior of ground-kept fry.

13) It took fully 3 days for the adult fish to recall how to swim on the Earth. From the 4th day after landing, adult fish swam normally.

A week after landing (July 30), we came back to Japan with the fish. In our lab, we paired Genki

(male) and Yume (female) and put them together in an aquarium, and paired Cosmo (male) and Miki (female) together in another aquarium. Both pairs mated and laid eggs in that evening of the arrival to the lab (July 30) i.e. at the corresponding time of the morning in Florida.

14) For each pair, eggs have been collected everyday, and their development was traced till hatching. Data were obtained for the number of eggs laid, % of fertilized eggs, % of embryos survived till hatching fry, etc. Any effects of the two-week space flight on these offsprings could not be detected.

15) These fry, i.e., babies of the space-flown adult Medaka were reared for a few months in our lab, and about 1,300 grown-ups have been obtained. These children of the space-flown Medaka have been distributed up to now to 285 locations over Japan (March 1995). Usually 3 to 8 fish were given to each location. A half of these places were elementary schools, nursery and public museums, and the other half were individuals, most of them being school children. We have been receiving many reports from these people whenever the fish sent to them laid eggs and baby fish hatched out of the eggs.

16) In our lab at University of Tokyo, the four Medaka fish flown in space are still fine, and have now grand-grand children (March 1995).

Space-originated baby fish have also grown up and now have their children.

4.2 Mating behavior of Medaka fish

As mentioned above, fish mated and laid eggs in space. Thinking of the fact they mated from the very first day of the mission, for the Medaka fish it seemed not hard to live under micro-G. However, post-flight analyses on video-tapes have revealed details on their life in space, of which results may suggest that they also have some stresses due to being exposed to micro-G. The details on this subject will be reported in other occasions.

4.3 Embryonic development in space

a) Development of eggs laid in space

For the event 11) reported above, some details will be given here. About 6 hours after the landing of Shuttle, the Medaka water-tank was handed

to us in the building Hangar-L at KSC. When we opened the tank, beside the four adult fish, we actually detected 11 baby fish (fry). With no water-flow inside the tank at this time, the fry came out of their area separated from the adult. After the immediate removal of adult fish, we observed swimming behavior of the fry and they were swimming normal.

From the detailed observation of their development and behavior, out of 11 fry there existed a possibility that 3 fry might have hatched within 6 hours, i.e., after the landing. For the purpose of carrying out further studies on the fry hatched truly in space, only the other 8 fry were named 'space-originated fry'. Such space-originated fry were either put into a fixative solution to cut their body sections for histological observation under microscopy or transferred to a new aquarium for rearing them to adult to study effects on their offsprings.

Beside the 11 fry, we also detected 27 embryos well-developed, all being at stages 32 or 33, i.e., stages immediately before hatching. Among these embryos alive, 26 hatched within two days and the last one hatched on the third day after landing. Beside these embryos, 5 dead eggs were detected. They had been either not fertilized or dead at very early stages, i.e., before stage 16 when the body-axis formation (elongation of embryonic body after gastrula stage) takes place. Since such eggs seemed laid at early times of the mission, it was impossible then to decide whether such dead eggs had been fertilized or not.

Thus, it was concluded that at least 43 eggs were laid in space. It is possible that in space some eggs might have been eaten by the adult fish before the eggs detached from the female belly were transferred to inside the area separated from adult. In that case, eggs laid actually in space might be more than 43 in number.

b) Eggs fertilized on the ground: Its development and behavior of fry hatched in space

Eggs already fertilized (i.e., embryos) on the ground were put in two mesh-boxes inside the tank 30 hours before the launch and loaded into the Shuttle. In one mesh-box, 18 embryos of ccT strain (12 embryos at stage 26/27, 6 embryos at stage 11) were placed, and in the other box 12

embryos of HO5 strain (stage 26/27).

Visual inspection of these embryos with naked eyes was done almost everyday, and video-recording was carried out on different 5 days. Most embryos became hatched fry around MET 4d, and Payload Crew reported that they all were swimming normally without exhibiting any looping behavior. From the video-pictures, we also confirmed hatched fry of neither strain of ccT nor of HO5 were looping at all in space.

Parabolic-flight experiments (using airplane) have told us that in adult fish, ccT is the strain which does not exhibit any looping behavior in micro-G, and on the contrary, HO5 strain loops many times in micro-G. The present observation of swimming behavior of hatched fry in space showed that though adult fish were genetically determined to loop or not in micro-G, their babies did not loop in micro-G whichever type their genes were.

c) Eggs fertilized on the ground: Developmental capacity of ccT and other strains

The 0-day embryos (stage 11) and 1-day embryos (stage 17) of ccT strain and other strains were loaded into the Shuttle 30 hours before launch. At the time of launch their stages should have advanced probably to stage 19 and stage 24 respectively. This experiment is to see the embryos at stages 19 and 24 can develop till hatching in space, and to check differences in developmental capacity between ccT and other strains. Other strains showed a lower percentage of hatching than ccT strain in space. On the ground, checking the effects of various radiations and drugs on the embryos, we know embryos of ccT strain are fairly resistant to various kinds of stresses. A similar tendency was noted this time for being in space. The results suggest that for both adult and embryos, ccT is a suitable strain for using them again in Space-Station experiment.