Effects of a simulated oil spill and importance of fast oil destruction at the early development phase of trout *Salmo trutta* in refrigerator conditions

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This study examines how serious the damage caused by an oil spill really can be for the spawn of trout *Salmo trutta* in its early development phase and how important it is to act fast at oil destruction. The study had two hypotheses. The first hypothesis assumed that it is possible to nurse the spawn of trout in typical fridge conditions. The second hypothesis was that the number of eggs surviving after an oil spill is in straight proportion to speed and effectiveness of oil destruction.

A test was carried out with five samples, which all had 200 fish eggs. A control sample wasn´t exposed to oil. Three of the samples were exposed to an oil spill, lasting 2, 6 or 12 hours, by sinking them into oily water. After the exposure time spawn was taken away from the oily water and rinsed with clean water. After this they were moved to aquariums which located in the fridge, where the growth of spawn was to take place. The fifth sample was permanently left in the oily water. The growth and survival of the spawn was observed until the last fish egg from the sample of permanent oil exposure perished.

The results show that with the sufficient amount of carefulness spawn can be nursed in fridge conditions, but the mortality rate is so high that it wouldn't be productive just for fish farming. The second hypothesis is not actually true, because, according to results, the sample with 2-hour oil exposure had a higher mortality rate compared to the sample, which was in the oily water 6 hours. But still the control sample had most survivors and the sample with permanent oil exposure eggs perished at a significantly faster rate. This proves that an oil spill has serious consequences for trout's growth from spawn to fish and the mortality rate of the spawn.

Contents

Abstract

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Biography

Riina Kilpeläinen and Sanni Nieminen are both 17-year-old students who study in Mikkelin Yhteiskoulun lukio. In free time they both like to listen to music and watch some movies.

1. Introduction

1.1 Ontogeny

Trout can be divided into three main groups, brown trout, river trout and sea trout. This study examines the brown trout *Salmo trutta*, which lives inter alia in the waters of Vuoksi on Lake Saimaa.

The tone of brown trout is usually a bit brownish or yellowish. There are black and ruddy or russet brown spots on the sides of the brown trout. There are more frosted spots on its tail and fins. There is usually an orange stripe on its adipose fin (Hilden etc. 1984). The brown trout is about 20-30 cm long and even the biggest individuals weigh under one kilogram (Koli, 1990).

The brown trout doesn’t migrate, but it prospers in small quickly flowing waters. It demands a gravel bottom for spawning and some deeper waters where it can winter (Hilden etc. 1984). In order to survive the trout needs, like other salmon fishes too, clean, brisk and well-oxygenated water, this limits the spreading of trout to some extent in Finland (Koli, 1990).

In Finland the trout spawns from the end of September to November and in bigger rivers a bit later than that (Koli, 1990). If possible, the brown trout prefers to spawn in quite shall strongly flowing waters with a gravel bottom. The number of eggs is 1000-1800 per one kilogram of fish. The size of eggs depends on the age, size and a state of nutrition of the female trout (Halme & Orpana, 1936).

After spawning, most eggs are fertilized in few seconds. There are many small holes on the zona layer of the egg, through which water slowly changes in the egg. There is also one bigger hole on the zona layer through which the sperm can get into the egg. Many sperm cells usually get into the egg through this mickropyle but only one sperm cell can get into the nucleus of the ovum.

When the egg gets into the water, it starts to expand and the water permeates the egg and it takes about 20 minutes. In this phase the spawn is soft and glutinous but after the water has permeated the egg, it will become hard and smooth. The fertilization must happen at that time because when the water permeates the egg the mickropyle gets closed too. After the fertilization the spawn will get harder and harder for about 2 days, even though the swelling of the egg takes only about 20 minutes.

The ovum is surrounded by a thin membrane. If this membrane breaks, the whole egg will die. From three to seven days after the fertilization, the egg is at its most sensitive phase because the membrane has not become thick yet. In that period of time eggs should not be moved. Fig 1 (Halme & Orpana, 1936) shows the development phases of American steelhead trout from the fertilization to the eye spot phase. The brown trout has the same phases in its growth but it takes longer because of the lower spawning temperature.

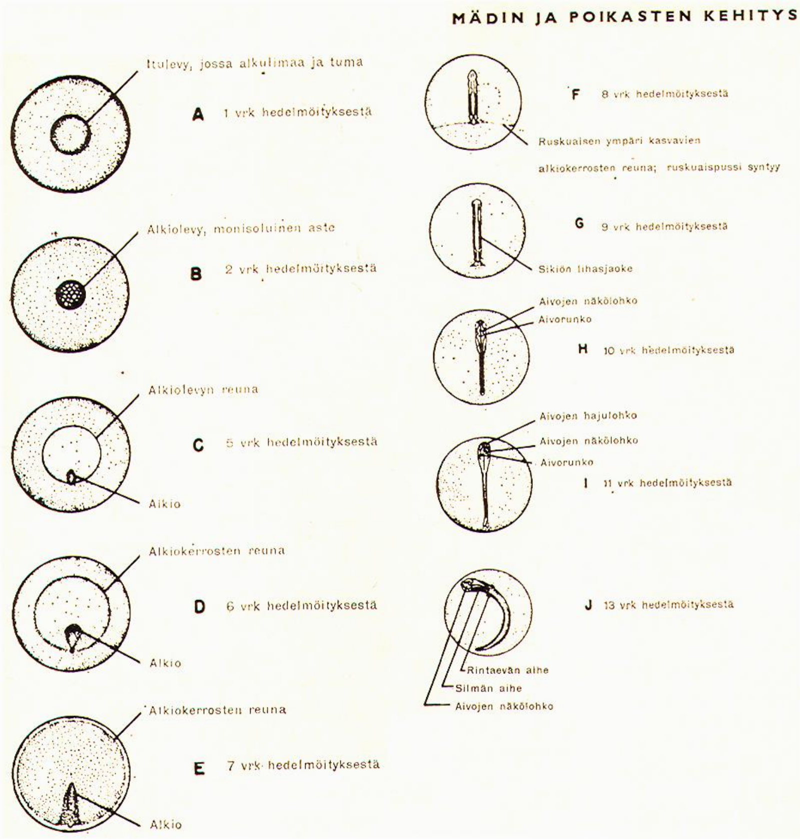

Fig 1. Development phases of American steelhead trout from fertilization to eye spot phase (Halme & Orpana, 1936).

When the spawn attains the eye spot phase (about 1-3 months after the fertilization) the membranes of the fertilized eggs have already become thick and the fertilised eggs can be separated from unfertilised eggs by so called shock therapy, where the eggs are moved rather roughly from one basin to another, which will cause the membranes of the unfertilised eggs to break. A week after reaching the eye spot phase, the spawn becomes more sensitive again and the moving of spawn should be avoided. When the spawn reaches the eye spot phase, it needs more oxygen than before. In terms of day degrees the results have been between 410 and 530 day degrees with the spawn of trout to the hatching (Halme & Orpana, 1936).

All fish are vertebrates and according to that they go under laws of animal experiments. These laws start to effect on a fry when it is not anymore on the phase of a foetus or an embryo. In the bio test of the examination, the spawn didn't attain the phase of development when animal experiment laws take place (EUR-lex & Finlex).

Different animal species have many very different ways to survive in nature. The survivorship curves of different species are often divided into three main groups: concave, convex and plain curves. (Fig 2: Ilkka Hanski etc. 1998). Species whose survival follow the concave model (I) do not get many offspring but almost all the newborn offspring survive, become adults and also have long lives. In the plain model (II) a few offspring are born and they die at a quite steady pace during the whole lifespan of the species. In the convex model (III) most newborn offspring die and only a small proportion of the population will survive and become adults. The survivorship curve of fish, also trout, follows the convex model. Even though there are lots of offspring, only a small proportion of all offspring attain adulthood (Ilkka Hanski, etc. 1998)

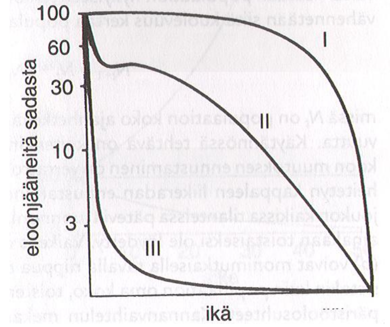

Fig 2. Three survivorship curves (Ilkka Hanski, etc. 1998)

1.2 Oil in the water

Light fuel oil mainly contains quite short alkane hydrocarbon-organic compounds, which evaporate and partly dissolve even in cold water. In heavy fuel oil the compositions of hydrocarbon-organic are usually longer and more complicated: they contain inter alia polycyclic aromatic hydrocarbons.

If oil spread in the water especially the planktons, bottom biota and the bird population would be at risk. For fish the long-term exposure to oil would cause damages in their tissue, scales and nervous system, which can develop into cancer tumours. Bottom biota would be at the risk if the oil slick will split into smaller slicks, lumps and drops, which will sink to the bottom (The Baltic Sea Portal).

If an oil spill took place on Lake Saimaa, its consequences would be very extensive. Damages caused by an oil accident are dependent on the size of the vessel and whether or not it is used for heavy or light fuel oil. The shipping routes run near the shores of Lake Saimaa, which is why the oil would reach land in very little time after an oil spill. In this case oil destruction would be very hard and the consequences far-reaching. It takes about ten years for an ecosystem to recover from an oil spill.

1.3 Oil destruction

Because of an increased amount of ship traffic the number of oil spills is also increasing around the world, even on Lake Saimaa, which is one of the trout’s natural habitats. Every year about 4000 ships operate on Lake Saimaa. The transportation of oil itself is forbidden on Lake Saimaa, but the ships that operate there can actually have 30 000-litre tanks. In addition, you do not have to report cargoes that weigh under1000 kg, so there is a great danger that some other hazardous substances are transported on Lake Saimaa. The most of the delivered cargo on Lake Saimaa is general cargo.

Oil spills on Lake Saimaa	
Year	Number
2001	13
2002	13
2003	7
2004	17
2005	19

Table 1. Oil spills on Lake Saimaa in the years 2001-2005

For example shipments of coal are transported regularly on Lake Saimaa and the sinking of a coal cargo would cause an oily surface on the water. One notable risk factor is also the roads and bridges that have been built near the shores, because a car accident on those roads would cause the oil to flow into the lake

The probability for an oil spill is not very high, but it is possible after all. Table 1 presents the number of oil spills on Lake Saimaa in the years 2001-2005. The probability for a spill on Lake Saimaa is about 0.16/1000 per voyage. (The Organization of the Fire and Rescue Department in the Water Area 2007).

Lake Saimaa is a very big lake (fig 3), so getting help to the location of the oil spill would take a variable amount of time depending on where the spill has happened. On Lake Saimaa the oil destruction belongs to the duties of the Fire and Rescue Department, which has the readiness to act in case of an oil spill. They will get to the scene of the accident in 20 minutes, and then the first oil barriers will be to put to their places and in about one hour the whole process of oil destruction is supposed to be in progress. The whole oil destruction takes averagely 2 hours. (Silmäri, oral announcement)

Fig 3. The location of Lake Saimaa in Finland

There are many towns on the shores of Lake Saimaa, and if there was an oil spill near these towns, the consequences would be hard to mend. For example the town of Savonlinna takes most of its drinking water from Lake Saimaa. There is a busy waterway nearby Savonlinna and the narrowness and strong current of which make it more prone to oil spills. Most of the few spills that have taken place on Lake Saimaa have happened there, but luckily they have not caused any big damages. If an oil spill happened on this waterway, it would ruin the shores of Savonlinna and it would prevent the town from taking drinking water from Lake Saimaa. Savonlinna has very limited groundwater resources, which would cause a shortage of water very soon.

There are not any regulations on the oil destruction equipment on the ships that operate on Lake Saimaa. Whereas big ports and towns like Varkaus, which storages oil, are supposed to have the readiness for oil destruction. But in any case the Fire and Rescue Department is the one that takes care of the oil destruction both in ports and further away on the lake. (Silmäri, oral announcement).

If an oil spill takes place, oil barriers are usually used and they prevent the spread of oil slicks, which makes it easier to remove the oil. If the oil has reached the shore, it has to be removed mechanically by shoveling or absorbing the oil. If the area exposed to oil is not very large, the oil can be removed from water with spill absorption materials (Koskinen, 2006).

1.4 The Finnish Game and Fisheries Research Institute and fish farming

The work of the Finnish Game and Fisheries Research Institute includes inter alia fish research and aquaculture in Finland. The areas of research are focused on the assessment of fish resources, the exploitation of fish stocks and the environment oriented fish research (The Finnish Game and Fisheries Research Institute).

Aquaculture is an important part of the work of the Finnish Game and Fisheries Research Institute especially at the department of Enonkoski at that is known as Saimaa Fisheries Research and Aquaculture.

The spawn that is needed in fish farming is taken from wild fish or from some fish hatcheries. If the spawn is taken from wild fish, it must be disinfected before starting the process of nursing the spawn. When the spawn and milt have been milked from the fish, they are mixed in the proportion of 1-3 ml of milt per 1 l of spawn. Then the spawn should be left alone for a while, before adding some water to the container and placing the spawn to the fish egg incubator. This period is very important for the fertilization of the spawn.

After fertilization there are many different methods of hatching the spawn, for example hatching in a funnel-shaped container or in a tub, depending on the fish species. Hatching in a tub is recommended for salmon fish, like trout.

1.5 Goals of the study

The first goal of the study was to find out if it is possible to hatch and nurse the spawn of trout in typical refrigerator conditions. This was examined by keeping the spawn in a fridge and by examining the survival rate of the spawn. The hypothesis was that with careful efforts it is possible to nurse the spawn of trout in typical refrigerator conditions.

In the study an attempt was made to show how important fast action at the oil destruction is in the early development phases of the spawn of trout. The damage caused by oil was examined by causing oil exposure to the spawn by using oil concentrates. This was carried out with the spawn that was nursed in refrigerator conditions. The mortality rate of the spawn and the length of the oil exposure were researched in the study. The hypothesis was that the length of the oil exposure is in straight proportion to the mortality rate: the longer the oil exposure, the more and the faster the spawn will perish.

2. Materials and methods

2.1 Spawn

The spawn which was used in this examination was milked from the trout population in the waters of Vuoksi and it was donated for the examination by Saimaa Fisheries Research and Aquaculture, which also helped with organizing of the examination. The spawn was fertilized and also transported from Enonkoski to Mikkeli in a same day. During the transportation the spawn was in a bag in which some oxygen was added for the transportation. The fertilization card of the spawn is shown in fig 4. After the transportation the spawn was put to strainer boxes which were placed in five different aquariums. The aquariums were put into the fridge where the temperature was +10°C. The temperature was lowered to +4 °C because it is closer to the temperature where wild trout usually spawn. The bio tests were carried out in the water where the temperature was +4 °C

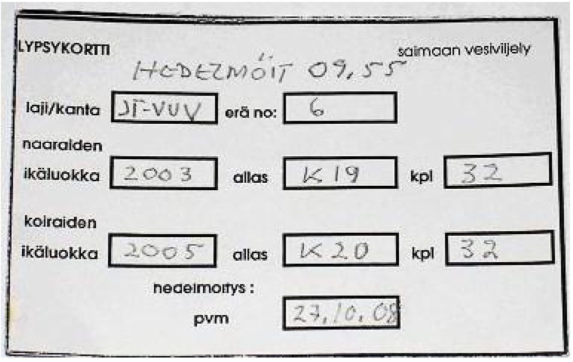
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Fig 4. The fertilization card of the spawn which was used in the study © Kirsi Idänpirtti

2.1 Materials

The spawn was nursed and observed in 10-liter (35x22x18) aquariums which were in the AEG ekoline refrigerator (Fig. 3). The fridge was bought from the recycling centre of Mikkeli. Altogether there were six aquariums: four of them were for the eggs which were exposed to oil, one aquarium was for the control group and one aquarium where the oil exposure took place. The aquariums were made of glass apart from one plastic aquarium where the oil spill was simulated. The aquariums came from Saimaa Fisheries Research and Aquaculture. The gasket of the glass aquariums was made of silicon. The amount of water was 6 litres per aquarium, but the aquarium where the oil spill was supposed to be simulated had only 2 litres of water in it.

In each aquarium there was a strainer box into which the spawn was evenly divided. Wooden tweezers and two plastic spoons were used for shifting the spawn and taking the death eggs out. Dophin KF-150 filter pumps and one EHEIM filter pump were used in the aquariums. The temperature in the fridge was measured by using an alcohol thermometer which was placed in the upper part of the fridge.

The raw water which was used in the study was groundwater from a drill well. The PH-value of water was about 6. The water was brought to the test side in 15-litre plastic canisters. A 1-litre measuring glass was used to divide the water into aquariums. The amount of oil was measured by using a pipette and the oil was added into the aquarium where the oil exposure was going to take place.

The oil that was used in this research was light fuel oil and it was taken from the ST1 gas station. Neste Oil delivers the fuel oil to ST1 stations. The content of sulphur in the oil was 0,1%. Two different fridges were used in this study: a Helkama –fridge for the spawn sample which was exposed to oil the whole time and an AEG ekoline fridge for all the other spawn samples. The AEG ekoline refrigerator is shown in fig 5.

Fig 5. The aquariums were placed in the fridge © Riina Kilpeläinen

2.3 Experiment

2.3.1 Bio test

The bio test was in progress 12 days in November 2008. The oil exposures of the spawn were ran through during two days and they were started 7 days after the fertilization. The fish eggs were in the strainer boxes during the whole experiment. There were in total 800 fish eggs in the strainer boxes (table 2).

Control	200 fish eggs	No oil exposure
1. bio test	200 fish eggs	2h oil exposure
2. bio test	200 fish eggs	6h oil exposure
3. bio test	200 fish eggs	12h oil exposure
4. bio test	200 fish eggs	Permanent oil exposure

Table 2. The fish eggs were divided into the aquariums for the experiment

The strainer box where the fish eggs were was sunk into the aquarium. Then the amount of oil was measured by using a pipette in the proportion of 1:1000 and the oil was added to the water in the aquarium. In other words, 2 litres of water and 2 millilitres of oil were used in this experiment. The instructions given by Lønning Voder, Falk-Petersen, Stene (1985) were followed when exposing the spawn to oil. The fish eggs were left in the oily water for 2, 6 or 12 hours. The oil wasn't changed during the bio test, but all oil spills were run through the same oily water.

After the oil exposure the fish eggs were immediately rinsed with 2 decilitres of groundwater. Then the fish eggs were put back into the same aquarium where they were before the oil exposure. One day after that the fish eggs were rinsed again with 5 decilitres of groundwater. These refrigerators were in different rooms so the strainer boxes containing the spawn had to be taken from one room to another during the different stages of the experiment.

2.3.2 Observation

After the oil exposure the spawn was observed every day and the number of dead eggs in each strainer box was written down. The aquariums were taken out of the refrigerator while counting the number of eggs and were taken back afterwards. The dead eggs were removed with wooden tweezers in order to prevent the formation of water mould.

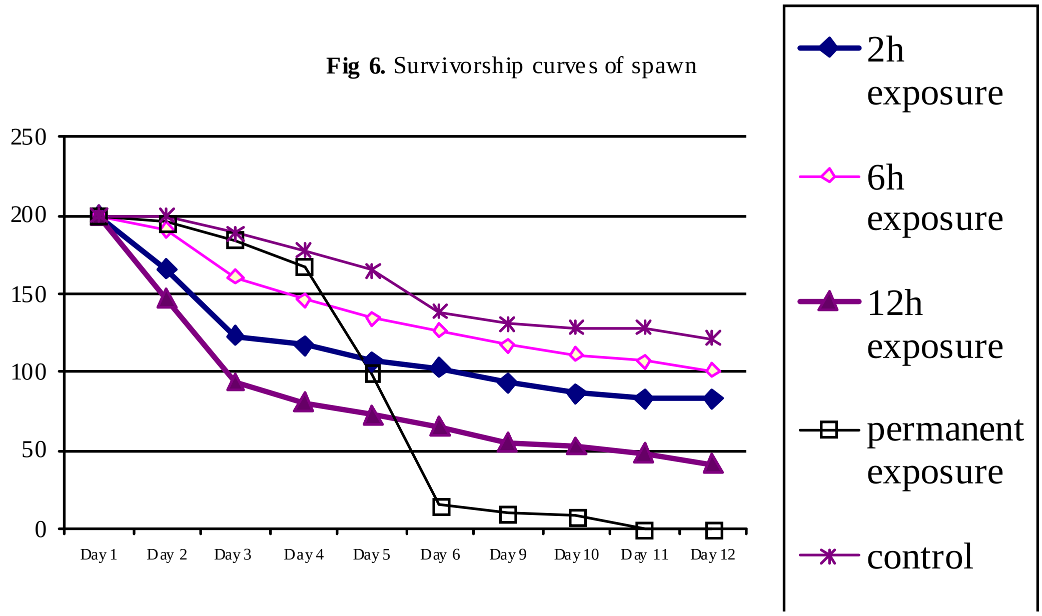
3. Results

3.1 Nursing the spawn in refrigerator

The spawn developed normally in refrigerator conditions and their different development phases were the very same as they are in fish hatcheries. The mortality rate of the spawn was relatively high due to the conditions in the refrigerator and the highly harmful effect of the oil. When the hatching of the fish eggs had taken place, only 5 eggs out of 800 eggs had survived.

3.2 The effects of oil at the early development of the spawn

Fig 6. Survivorship curves of spawn



The results of the second hypothesis are shown in curves where the results are organized according to the amount time being exposed to oil (fig 6). The figure begins with the first day when the fish eggs were exposed to oil and ends when the last fish egg from the sample with permanent oil exposure perished. This happened 12 days after starting the experiment and the bio test of this study was also completed at the same time.

The results show that the fish eggs in the sample which was permanently left in the oily water perished much faster than in the other samples where the oil was rinsed way after the exposure and notably faster than in the control sample, where the eggs were not exposed to oil at all. Mortality is particular fast in the sample with the permanent oil exposure between the days three and six.

The sample with the 12-hour oil exposure had the second highest mortality rate. However, in this sample the mortality rate was significantly lower than in the sample which was permanently left in the oily water. The sample with the 2-hour oil exposure had a higher mortality rate than the sample with the 6-hour oil exposure. When all the fish eggs exposure had totally perished over a half of the fish eggs in the control sample were still alive from the sample with the permanent oil (Appendix 1).

When the other samples were compared to the control sample, it was found out that the results differed significantly from the results of the control sample. The test was carried out by using the $\chi 2$ compatibility test.

2h test variable 172,759 (*** critical value 26,125)

6h	test variable 92,414 (*** critical value 26,125)
12h	test variable 454,146 (*** critical value 26,125)
Permanent exposure	test variable 483,501 (*** critical value 26,125)

4. Evaluation of the results

4.1 Nursing the spawn in refrigerator

The results show that fish eggs can be nursed successfully in quite primitive conditions in a refrigerator up to a certain point. But due to the low survival percentage of the fish eggs, trout farming in a fridge is not profitable, but for example when the development phases of the spawn are observed, the refrigerator conditions are suitable for the purpose. In the examination it is noteworthy that all the samples had as similar environments as possible, when the only variable was the oil spill and its duration. The purpose of the examination was to find out how important taking fast action in an oil accident was rather than how well spawn survives in a refrigerator.

The duration of the experiment is notable. If the fish eggs are supposed to be nursed until they hatch, the experiment will take several months. Rising the temperature for making the development faster is not recommended because the higher temperature may also increase the bacteria which are hazardous for the spawn. This is the reason why carrying out this experiment takes time and it is not possible to carry it out during one course at school. The experiment must be observed and all the dead fish eggs must be removed so that the water will stay clean during the whole experiment and you can also prevent the formation of water mould.

4.2 Effects of the oil exposure

The oil exposure took place about 7 days after the fertilisation. The most sensitive development phase of the spawn is about 3-7 days after the fertilisation. In other words, the fish eggs had just reached a better resistance after the membrane that surrounds the spawn egg had become thicker. This sensitive development phase (about 3 - 7 days) together with the timing of the oil exposure might have had an effect on the mortality rate. Those the fish eggs that were still at the more sensitive development phase were the ones which died first.

One reason for results could be that every bio test was carried out with same oil and the oil wasn’t changed between bio tests of different samples. The examination also suffers due to a lack of being repeated. This was caused by a time limit when the examination was supposed to run during a Finnish upper secondary school course (about 40 hours). Also a limited amount of work space and equipment was a reason why there were not several samples at the same time, or why the oil wasn't changed for each sample.

When the curves are examined, it could be clearly seen that the oil and the oil destruction have a direct impact on the survival of the spawn. The examination was carried out using light fuel oil which isn’t as hazardous as heavy fuel oil so it is clear that an accident with heavy fuel oil causes even greater damage. Although even in the examination using light fuel oil the damage is obvious in the results: The fish eggs in the control sample (with no oil exposure), die at a significantly slower pace than the fish eggs in the samples with the oil exposure. The sample with the 6-hour oil exposure had the second lowest mortality rate. The sample with the 2-hour oil exposure had thirdly lowest mortality. The reason for this is probably the fact that the fish eggs in the sample with the 2-hour oil exposure were in direct contact with the oily surface twice in a short period of time when they were sunk in the oily water and lifted from there. When the fish eggs were kept in the oily water for a longer period of time, for six hours, they had a longer pause between the direct contacts with the oil and they also had time to recover.

The survivorship curve of the sample with the permanent oil exposure falls significantly more radically than the curves of other samples. Mortality in this sample increases greatly on the third day. This shows that at the time the hazardous components of the oil start to affect the fish eggs and cause the higher mortality rate. The all other samples have survivorship curves the slopes of which become steeper at a very steady pace, whereas the survivor gentlest angle. The time of the oil exposure is not in straight proportion to the length of the oil exposure, but the survivorship curves are very symmetrical when compared to each other. When the samples which were exposed to oil are compared to the control sample, the values of the test variables show how the significance levels of the results are notable. The results are also logical when compared to the natural survivorship curve of fish.

According to the results the hypothesis about how the mortality rate is in straight proportion to the length of the oil exposure is not true. The time between the direct contacts with the oil also has a significant impact on the mortality rate. Also the different development phases of the spawn are noteworthy, because the damage caused by the oil also depends on the sensitivity of the spawn.

5. Summary

There were two hypotheses in this study and the first one was that it is possible to nurse the spawn of trout in ordinary refrigerator conditions. The second hypothesis assumed that the survival of the spawn is in straight proportion to the speed and effectiveness of oil destruction. A test was carried out with five samples, which all had 200 fish eggs. There was a control sample which was not exposed to oil at all. Three of the samples were exposed to an oil spill by sinking them into oily water. The oil exposure lasted 2, 6 or 12 hours. After the oil exposure the spawn was rinsed with clean water. The fifth sample was permanently left in the oily water. Then the fish eggs were placed into aquariums which were kept in two fridges and the spawn was to develop and grow there. The growth and survival of the spawn was observed until the last fish egg from the sample with the permanent oil exposure perished.

The results show that the mortality rate of the fish eggs was higher in those samples where the oil exposure lasted particularly long. Especially the fish eggs which were kept in the oily water the whole time perished very fast. The sample with the 2-hour oil exposure had a higher mortality rate

compared to the sample, which was kept in the oily water for 6 hours. The reason for this is probably the fact that the sample with the 2-hour oil exposure was in direct contact with the oil surface twice within a short amount of time when it was sunk in the oily water and when it was lifted from there.

The first hypothesis assumed that spawn can be nursed in refrigerator conditions. It is true that some fry could be nursed in fridge conditions in this study. Still you must take it into account that the mortality of fish eggs is much higher in a fridge than in fish hatcheries. In general it can be said that spawn could be nursed in quite primitive conditions if it is done carefully, but the mortality rate of the fish eggs is very high.

The second hypothesis is not true. The mortality of the spawn is not in straight proportion to the length of the oil exposure because the mortality rates of the samples with the 2-hour and 6-hour oil exposures differ from this supposition. However, the biggest number of fish eggs reached the hatching phase in the control sample and the fish eggs in the sample with the permanent oil exposure perished significantly faster. This shows that oil in the water has a big impact on trout's growth from spawn to fry and on the mortality rate of the fish eggs. That is why the speed of oil destruction is very important if an oil spill takes place. In other words, the extent of damage to the environment depends on how fast the oil destruction actually is.

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Oral announcements

- Arkko Pasi, Production manager of Saimaa Fisheries Research and Aquaculture in Enonkoski
- Silmäri Jyri, Fire Chief of Fire and Rescue Department of Southern Savo
- Toivola Vesa, Geologist of Southern Savo Regional Environment Centre

Appendixes

Appendix 1: The number of surviving eggs in the samples daily

2h

	alive pcs
10th November	166
11th November	123
12th November	117
13th November	107
14th November	103
17th November	93
18th November	87
19th November	83
20th November	80
21st November	75

6h

	alive pcs
10th November	191
11th November	161
12th November	146
13th November	134
14th November	127
17th November	117
18th November	111
19th November	107
20th November	105
21st November	102

12h

	alive pcs
10th November	147
11th November	94
12th November	81
13th November	73
14th November	65
17th November	55
18th November	53
19th November	48
20th November	42
21st November	39

permanent exposure

	alive pcs
10th November	
11th November	196
12th November	185
13th November	168
14th November	99
17th November	15
18th November	10
19th November	8
20th November	6
21stNovember	0

Control

	alive pcs
10th November	200
11th November	200
12th November	189
13th November	178
14th November	165
17th November	139
18th November	131
19th November	128
20th November	124
21st November	120