The Dry Method of Otolith Mass Marking

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Keywords: Salmon mass marking, otolith, dry method of otolith marking, hatchery, incubator, salmon embryos.

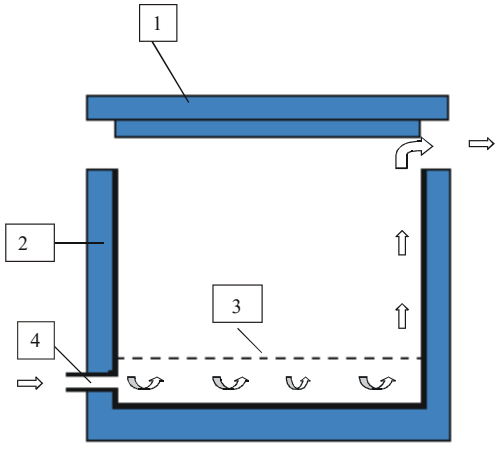
Marking has been traditionally used in fish hatcheries to identify fishes of artificial origin. There are many methods of marking such as fin amputation, opercula punching, branding, application of substances to dye the tissues of the organism, external and implanted radiotelemetry and magnetic tags, marking with radioactive isotopes, etc. All these marking methods have their advantages and flaws, but few are appropriate for mass marking of small fishes such as chum and pink salmon fry raised at the hatcheries.

Mass marking methods based on marking otoliths have been intensively developed in recent years. The countries primarily using these marking technologies are the United States, Canada, Japan and Russia. The most widely used is the method of thermal marking of otoliths developed by American scientists (Volk et al. 1990, 1999; Munk and Geiger 1998).

In Russia, we use the dry method for marking salmon otoliths in addition to thermal marking. The idea arose several years ago while studying the reasons for the development of pseudo-rings (additional rings) on embryonic salmonid otoliths. It was noticed that such pseudo-rings appear on the otoliths of embryos lying inside the packed clusters of eggs where the water flow is minimum or null. We hypothesized that there was a relation between the water flow intensity and formation of the otolith rings, and proposed to use the artificial cessation of water flow in incubators to induce recognizable rings on the otoliths of salmon embryos. The principle of this method, as well as that of the thermal marking, is based on the fact that environmental changes cause adequate physiological reactions in fishes to change the thickness of the daily calcium and protein increments in otoliths. Should the influence of this factor be periodical in nature, its periodicity manifests itself as a sequence of dark and light rings of certain width. In our opinion, in the process of dry marking the abrupt changes of ambient conditions put the organism under stress resulting in physiological changes. We feel that the same thing happens when thermal marking is used, where changes in increment characteristics are the result of physiological reactions to changes in ambient parameters.

The dry method of otolith marking is based on periodical changes of the water regime for incubation of eggs. The eggs are dried in incubators according to pre-determined schedules, usually at 24 hours intervals. During one marking cycle, when one dark and one light ring are formed, the eggs are kept dry for 24 hours (without water, in a humid atmosphere), and washed with water during the next 24 hours (with the incubator working in a normal operating mode). To retain a humid atmosphere and prevent abrupt changes in temperatures, the incubators should be covered with a plastic film or any heat-insulating material. When using Atkins and NOPAD incubators, circulation of water should be provided around the bottom and the side panels. The eggs should be thoroughly stirred prior to water drainage to prevent the egg clusters from packing and improve ventilation. The period of embryonic development, during which it may be possible to use the dry marking method, begins from the embryo's eye pigmentation (eyed-egg) stage and lasts until hatching. The length of this "marking window" depends on thermal conditions of the incubation process and may vary at different hatcheries and regions. At the hatcheries of the Magadan Region, this period usually lasts from 20 to 35 days.

Fig. 1. Isothermal incubator working in a normal operating mode. 1 – cover, 2 – styrofoam, 3 – mesh tray with eggs, 4 – water inlet.



The first tests of the dry marking method were conducted in 1996 on chum salmon embryos, and showed that periodic drying of eggs stimulates the formation of otolith rings with the same periodicity. However, in these experiments during interruption of the water flow, the temperature of the incubated eggs gradually equilibrated with the ambient air temperature, and the otolith rings could form not only due to drying of eggs, but also due to the effect caused by the temperature difference in accordance with the mechanisms of the thermal marking.

We conducted another series of experiments using temperature-controlled incubators (Fig. 1) that allowed us to

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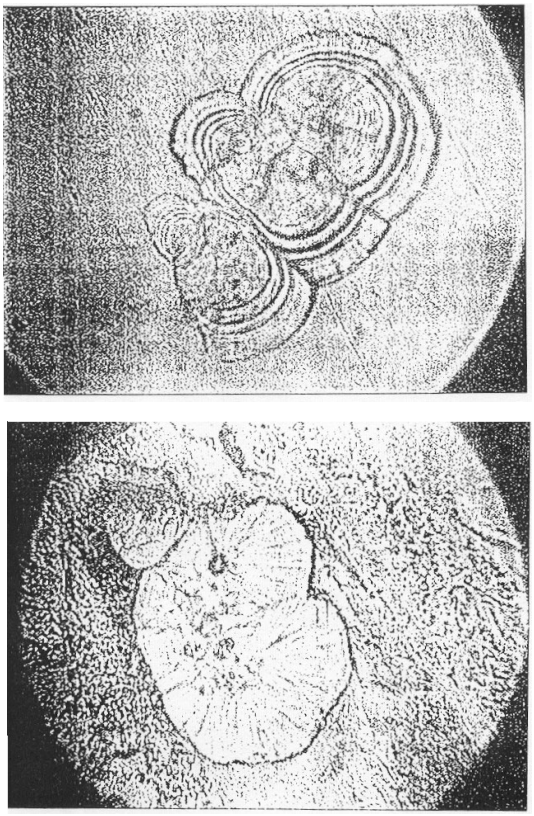
keep the temperature unchanged when drying the eggs. Over the six days experimental period, the water temperature fluctuated only 0.6°C. Three drying cycles of 24 hours followed by 24 hours of normal water flow resulted in the formation of three rings on the otoliths of pink salmon embryos (Fig. 2). The results indicated that the dry method of salmon marking caused the formation of an otolith mark that does not depend on temperature changes, but occurs due to some other factors related to the egg's drying.

During the mass marking of coho and chum salmon from brood year 2000 at the Yana Hatchery, the temperature also remained relatively constant. The water temperature in the incubator water-supply system ranged from 2.9 to 3.1°C during the 13 days of marking. The air temperature at the hatchery varied from 2.8 to 3.0°C. Distinct marks were found on the otoliths upon completion of the marking process (Fig. 3). One hundred embryonic coho salmon otoliths were inspected to evaluate the quality of the marks and similar marks were formed in all otoliths. American NOPAD incubators were used at the Yana Hatchery for dry marking. Japanese Atkins incubators were used to conduct the other experiments. During marking, the layer of eggs in incubators varied from 2–3 cm up to 25–30 cm (from 1 to 12 inches). The marking was conducted with alternating dry and wet intervals of either 24 hours or 12 hours. In the year 2000, the hatcheries of the Russian Far East released almost 4.5 million salmon marked by the dry method.

Fig. 2. Dry mark on the otolith of a pink salmon embryo (above) and control otolith of a pink salmon embryo (below) in the Kulkuty River, 1998.



The dry method of otolith mass marking is based on the ability of the salmon eggs to normally develop in a humid atmosphere. We discovered that the drying of eggs for12 or 24 hours does not lead to an increase in mortality rate. The quantities of dead eggs during the marking process and at other stages of ontogenesis (larva incubation and fry raising) remained the same in both experimental and control samples. In some of our experiments, we allowed the eggs to be incubated in a humid atmosphere for 440 hours (18 days). Figure 4 shows the mark consisting of 12 rings. Mortality rate of marked eggs remained the same as the eggs hatching under normal conditions. We also found no difference between drying and wet eggs with regard to either hydro fraction content or in a water flow resistance test applied to the fry.

People working at the hatcheries know that fish farmers often use the drying of eggs in practice. For instance, the eggs are dried for a short period of time for sorting. We can also give examples of more continuous incubation of eggs in a humid atmosphere. In the Russian Far East, such incubation has been used at the Ushki Hatchery in Kamchatka since 1934. The eggs of sockeye, chum, coho and chinook salmon were sometimes incubated in a humid atmosphere for 50–60 days. The released fry demonstrated good vitality (Rassokhina 1998).

Fig. 3. Dry mark on the otolith of a coho salmon embryo in Yana Hatchery, 2001.

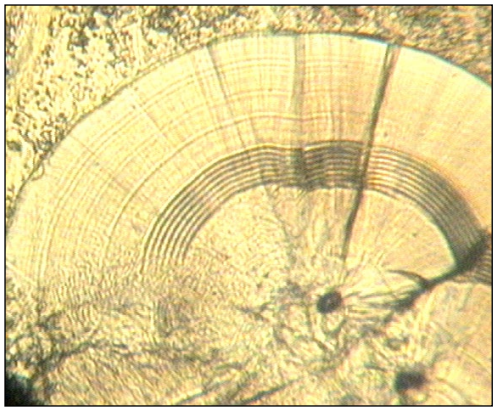
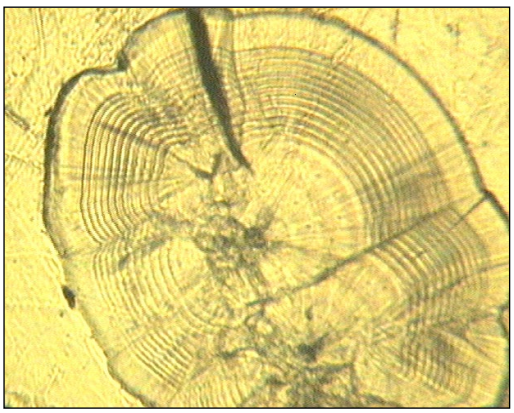


Fig. 4. Dry mark on the otolith of a chum salmon embryo in Ola Hatchery, 1998.



The drying of eggs was officially accepted in fish farming for salmon from the genus *Oncorhynchus* (Smirnov 1958) and *Salmo salar* (Yandovskaya et al. 1996). At the present time, drying of the salmon eggs at the "eyed" stage is being used for the transportation of eggs and for incubation in a humid atmosphere (Mikhailenko and Sokhnov 1997; Tiaptirganov et al. 1997). It should be noted that during either marking or transportation, the eggs should be kept in a humid atmosphere and the upper layer of eggs should not be allowed to dry.

The flaw of the dry method of marking is that it cannot be used for marking of the salmon larvae and fry. In general, the dry marking method is simple, convenient and requires no special (including electrical) equipment. The marking can be conducted separately in each incubator within the optimum time period. The dry marking method can be applied at any hatchery and can also be used for salmon farming in field conditions.

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