



Chemical Marking of Hatchery Fish: Techniques and Applications

Alizarin red S solution

Alizarin red S solution can be made up from scratch using alizarin red S powder. We have tested 0.1% solutions of alizarin red S on golden perch and found that fish took a long time to recover and significant negative effects on growth and mortality (see Crook *et al.* 2009). We also tested 0.05% solutions and found no effects on growth and mortality when the fish were exposed to the salt solution for 5 minutes and to the alizarin red S solution for 10 minutes. For normal hatchery operations, we would recommend the use of 0.05% solutions to minimise effects on fish whilst still producing relatively high quality marks. Alizarin red S solutions can be re-used several times, however, mark intensity will decline as the solution becomes diluted and the alizarin red S is used up. We do not recommend using a solution for any more than 5 applications. Alizarin red S solutions should always be aerated using oxygen to ensure suitable conditions for fish respiration during the marking procedure. Small amounts of an anti-foaming agent (e.g. Dow Corning® 2-3101 Antifoam Emulsion) can be added to reduce foaming if it becomes a problem.

Ingredients

Hatchery water (same as fish holding water)

Alizarin red S (1,2 dihydroxyanthraquinone sodium sulfonate)

1.0 M Sodium hydroxide

1.0 M Hydrochloric acid

Dow Corning® 2-3101 Antifoam Emulsion

(refer to specific MSDS's for safety information on the above chemicals).

To make a 0.05% solution, add 0.5 g of alizarin red S powder per 1 L of hatchery water and stir well. The alizarin red S will cause a large decrease in the pH of the solution (from about 7 to 3) and is relatively insoluble at the lowered pH. If pH does not decrease when the alizarin red S powder is added to the water, contact your supplier and do not use the solution.

Continue to stir well and adjust the pH of the solution back to that of the original hatchery water by gradually adding 1.0 M sodium hydroxide (NaOH) with a pipette to dissolve the alizarin red S powder. Do this very carefully and regularly check the pH of the solution with a pH meter to ensure you don't overshoot (pH may take a while to respond to addition of NaOH, so take your time). If you overshoot and the solution becomes alkaline, adjust back by adding small amounts hydrochloric acid.

After use, alizarin red S solution must not be disposed of by pouring into drain or otherwise disposed of into sewers or waterways (refer to your local environmental regulations prior to disposing of any chemicals used for marking fish).

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Calcein solution

Calcein solution can be made up from scratch using calcein powder or purchased as a pre-made liquid concentrate. The calcein powder is available in Australia from suppliers of chemical products (we purchase ours from Sigma-Aldrich). The pre-made concentrate, sold as “SE-Mark”, is only available from Western Chemicals Inc. in the USA. We have tested 1% and 0.5% solutions made from calcein powder on golden perch, Murray cod, trout cod and Australian bass and found no effects on growth or mortality (Crook *et al.* 2009; unpubl. data). We also found that a 1% solution provides only marginally more intense marks in golden perch than the 0.5% solution. Thus, for normal hatchery operations, we would recommend the use of 0.5% solutions to minimise costs whilst still producing excellent marks. Calcein solutions can be re-used several times, however, mark intensity will decline as the solution becomes diluted and the calcein is used up. We do not recommend using a solution for any more than 5 applications. Calcein solutions should always be aerated using oxygen to ensure suitable conditions for fish respiration during the marking procedure. Small amounts of an anti-foaming agent (e.g. Dow Corning® 2-3101 Antifoam Emulsion) can be added to reduce foaming if it becomes a problem.

Ingredients

Hatchery water (same as fish holding water)

Calcein powder (2,4-bis-[N,N’-{dicarbo methyl}-aminomethyl] fluorescein)

1.0 M Sodium hydroxide

1.0 M Hydrochloric acid

Dow Corning® 2-3101 Antifoam Emulsion

(refer to specific MSDS's for safety information on the above chemicals).

To make a 0.5% solution, add 5 g of calcein powder per 1 L of hatchery water and stir well. The calcein will cause a large decrease in the pH of the solution (from about 7 to 3) and is relatively insoluble at the lowered pH. If pH does not decrease when the calcein powder is added to the water, contact your supplier and do not use the solution.

Continue to stir well and adjust the pH of the solution back to that of the original hatchery water by gradually adding 1.0 M sodium hydroxide (NaOH) with a pipette to dissolve the calcein powder. Do this very carefully and regularly check the pH of the solution with a pH meter to ensure you don't overshoot (pH may take a while to respond to addition of NaOH, so take your time). If you overshoot and the solution becomes alkaline, adjust back carefully by adding small amounts of hydrochloric acid.

After use, calcein solution must not be disposed of by pouring into drain or otherwise disposed of into sewers or waterways (refer to your local environmental regulations prior to disposing of any chemicals used for marking fish).

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Osmotic induction marking

ALWAYS wear gloves, protective eye glasses and a lab coat when marking fish with fluorescent dyes. Avoid direct contact with dye solutions and always handle chemicals according to the MSDS. Clean up any spills immediately.

To mark the fish, very carefully pour all solutions into suitable containers. ALWAYS conduct a test run on a small number of individuals (e.g. 30) before attempting to mark a large number of fish to ensure that solutions are working correctly and that fish are able to cope with marking.

Ensure that solutions are well aerated to maintain suitable conditions for fish respiration during marking. Also, ensure that the temperatures of the marking solutions are within about 1°C of the holding water to avoid stress associated with temperature change. Use an electronic timer with an alarm to ensure that fish are held in the solutions for the correct amount of time.

Generally, the aim of osmotic induction marking is to mark as many fish as possible with each application. This requires holding fish in much higher densities than would be considered as part of normal hatchery practice. We have successfully marked up to 8,000 fingerlings in a single application using 10 L of calcein solution. The very high densities of fish and submersion in salt and dye solutions cause a short-term stress on the fish - the key to successful osmotic induction marking is to minimise any additional stressors.

Once fish have been concentrated and placed in a holding sieve (see next page), lower the sieve into the salt solution for 5 minutes (or 3 minutes if concerned about excessive stress). The fish will immediately lose equilibrium and will float to the surface because of the high salinity. This is perfectly normal. If re-using the solution, quickly dip the sieve into fresh hatchery water after the salt immersion and allow excess liquid to drain off. Then place sieve into the calcein or alizarin red S solution for 10 minutes. If necessary, cover with a lid to prevent splashing of dye. Foaming may occur in both the salt and dye solutions, particularly if the solutions are re-used several times. Small amounts of an anti-foaming agent (e.g. Dow Corning® 2-3101 Antifoam Emulsion) can be added to reduce foaming if it becomes a problem. That's it.

After marking, drain and rinse off excess dye solution and place the fish back into their original hatchery water. Allow at least 24 hours for recovery prior to shipment for stocking. It is to be expected that some fish will show signs of stress immediately after marking and they may take an hour or two to fully recover. We have not observed increased rates of mortality in our trials with golden perch, trout cod, Murray cod and Australian bass. However, all hatchery operations are different, so undertake enough trials to ensure that you are comfortable with the techniques for your own specific circumstances.

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Salt solution

For osmotic induction marking, a very strong salt solution is required to provide the osmotic stress necessary to induce rapid uptake of the fluorescent marker. The salinity necessary to achieve this is 5%, or approx. 1.5 times the salinity of seawater. We have found that golden perch, trout cod, Murray cod and Australian bass are able to cope very well with being held in 5% salt solution for 5 minutes and longer. Fish will show some signs of stress while in the salt solution - if this becomes a concern, the immersion time can be reduced from 5 minutes to 3 minutes without greatly affecting mark uptake. We do not recommend reducing the strength of the salt solution for osmotic induction marking, as this may significantly reduce mark quality. The salt solution should be made up using the same water that the fish are being held in and we recommend the use of a commercially available natural salt (we use "Lake Charm" salt) rather than sodium chloride or refined salt. The salt solution should always be aerated using oxygen to ensure suitable conditions for fish respiration during the marking procedure. Small amounts of an anti-foaming agent (e.g. Dow Corning® 2-3101 Antifoam Emulsion) can be added to reduce foaming if it becomes a problem.

Ingredients

Hatchery water (same as fish holding water)

Natural salt

Dow Corning® 2-3101 Antifoam Emulsion (for safety information, refer to MSDS).

To make up the solution, simply add 50 g of salt per litre of hatchery water and stir well until fully dissolved. This is a lot of salt to dissolve, so it will take several minutes before the salt fully dissolves. There should be no need to adjust the pH of the salt solution.

In most situations, salt solution can be disposed of by diluting in freshwater and pouring down drain (refer to your local environmental regulations prior to disposing of any chemicals used for marking fish. For further information on use and disposal of chemicals see Regulatory Issues).

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