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Chemical Tagging of Shells of Commercial Stock of Hatchery Clams

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11 Report Summary

Commercial clam hatcheries require a method of labeling or "tagging" clams so that they can be identified as hatchery reared. This is a requirement of the Queensland Department of Primary Industry, aimed at the prevention of the sale of clams collected illegally from the Great Barrier Reef Marine Park. Methods used for tagging to date have been labor intensive, therefore expensive, and suitable only for sales of small numbers of animals.

This study developed a cheap and relatively easy method of labelling commercially reared clams using a dye which stained the organic matrix of the shells. This dye, Erythrosine B, colours the organic matrix of the shell periphery a bright pink which remains in a band as new shell is laid down. The new shell is not discoloured. The dye meets all the criteria which the project identified as desirable for a chemical tag. i.e. It is:

1. Non-toxic to humans.
2. Non-toxic to the clam.
3. Cheap and requires minimal labour for application.
4. Artificial and its presence indicative of human intervention.
5. Easily identifiable.
6. Stains the shell of the clam at the time of exposure but does not detract aesthetically from the appearance of the animal.

Furthermore, the dye is currently used by the meat processing industry for the labelling of animal carcases and is approved for use in food for human consumption. This means that the use of the tagging process may be commenced immediately.

The clams require immersion in a dilute concentration of the dye for four days at a time at intervals of seven days apart. The time interval between each dye episode is to achieve a band of dyed shell sufficiently wide to be readily observed.

111 Background.

To enable large scale development of a commercial clam industry capable of supplying the growing demands of the aquarium trade and the exotic foods market, a clam labelling method is required. This is to satisfy the requirements of the Department of Primary Industry (DPI) which specifies that all clams sold must be identifiable as having originated in a commercial hatchery. This is to prevent the illegal harvest of clams from the Great Barrier Reef. Various methods of labelling or tagging have been employed until now, such as the use of indelible markers. These methods are all unsuitable for large numbers of animals as they are labour intensive and thus expensive, narrowing the profit margins on production so that the industry is not viable. The use of fish tags was suggested by DPI, however, it was calculated by the commercial hatchery at Fitzroy Island that the cost of tagging a single individual of the clam *Tridocna crocea* was greater than the anticipated profit margin. An alternative method of tagging which is acceptable to DPI is the chemical marking of the shell organic matrix of a growth band in juvenile clams. This project aimed at identifying such a chemical which met the requirements of both DPI and the commercial hatcheries.

IV Objectives.

The aim of the project was to test the affinity of various chemicals for the organic matrix of the clam *Tridacna crocea* and to identify one which was suitable to use for the labeling of clams in commercial hatcheries. A number of criteria was selected by which a suitable chemical could be identified:

1. Non-toxic to humans when ingested.
2. Non-toxic to clams.
3. Cheap to purchase.
4. Required minimal effort to apply to clams and did not necessitate the involvement of expensive equipment.
5. Artificial so that its presence in the animal can only be the result of human intervention.
6. Easy identification within the shell preferably without requiring the use of specialist equipment to detect its presence.
7. Capability of marking the shell in a manner which does not detract from it aesthetically.

Once a substance was identified as suitable for use as a "chemical tag" it was necessary to establish application concentration and the period of exposure required to mark the clams effectively.

V Introductory Technical Information.

The shells of all bivalves consist of calcium carbonate crystals inclosed in an organic matrix. The calcium carbonate crystals are usually either in the form of aragonite, as in the inner shell layer of Giant Clams of the Superfamily *Tridacnacea* or in the inner nacreous layers of pearl oysters of the Superfamily *Pteriacea* or *Ostreacea*, the edible oysters.

Calcium carbonate crystals e.g. the aragonite crystals of the *Tridacnacea* can be labelled by radioactive Ca"45 or by adding tetracycline to the water in which the animals are immersed. These procedures while useful experimentally are unsuitable for the present application. The former procedure was dismissed as Ca"45 is a gamma emittor and the latter involves viewing flourescence under U.V light for detection of the resultant altered band in the animal shell.

It is also probable that the metal ion content of the aragonite crystals can be altered by alteration in the concetration of these in the surrounding medium, especially the alteration of the concentration of divalent metal ions whose hydrated ionic radii is not greatly different from that of Calcium e.g. magnesium. However these manipulations are inappropriate to the solution of this problem as the use of sufficiently small alterations as to not deleteriously effect the animal would result in difficulty in detection in the resultant alterations to the metal ion content of the aragonite.

The alternative was to attempt to tag the organic matrix which sheaths the calcium carbonate crystals of the shell. It was important to find a method of dying the inner shell layer as it is laid down at the periphery as the outer shell layer near the umbo is commonly abraded in older bivalves. To determine the susceptibility to staining the peripheral inner shell layer of the *Tridacnacea* this part of the shell was lightly decalcified in 2% acetic acid rinsed to neutrality and stained with a variety of staining techniques. In the result the organic matrix in question stained purple with Mallorys trichrome, royal blue with Azocarmine, blue with Martuis yellow - brilliant crystal scarlet - analine blue (MSB), turquoise with Alcian Blue - M.S.B,and dark blue with Steedmans trichrome.

Since all of these stains are highly toxic none were useable in this context but as a result of the affinity of the organic matrix in question for these stains it appeared highly likely that a non-toxic protein stain would be useful.

For this reason the stains used by a meatworks to label different clases of meat for human consumption were an obvious place to start. Four different coloured stains, purple, brown, magenta and pinkish red were supplied by a local meatworks, and their affinity for the organic matrix of the inner shell layer of *Tridacna crocea* tested. In each case the organic matrix of the inner shell layer stained the same pinkish-red as was obtained by the use of the pinkish-red stain on it's own.

The commercial suppliers of these stains declined to say what were the ingredients of the purple brown and magenta stains but advised that the pinkish-red stain was Erythrosine B and that this stain was the basis for all four stains supplied.

This stain, Erythrosine B, was found to selectively stain the organic matrix of the inner shell layer and therefore further work to determine conditions required to produce useful results was confined to this substance.

VI Methods.

Individuals of *Tridacna crocea* (each approximately 4cm in length) were exposed to a range of dilutions of the dye Erythrosine B for varying lengths of time. The original dye solution used in the earlier trials was a commercially prepared liquid used by the meat processing industry. The Erythrosine concentration in this aqueous solution was 1.7g in 100ml. In addition, the solution contained small quantities of glycerine, alcohol and sugar. As these additions gave the dye properties which were not useful in the staining of clam shells, a stock solution was prepared using only Erythrosine B powder and distilled water (1.7g/100ml).

Clams used in the experiment were kept in a glass tank with an aeration system and continual water exchange. The tank was placed under aquarium lights (Osram "Daylight") to stimulate photosynthetic activity in the clam's symbiotic algae. While clams were exposed to the dye they were kept in smaller tanks (also aerated) within this large tank, so that the water temperature could be kept constant. The water in these smaller tanks was changed daily, and dye added.

Fifteen clams were immersed in Erythrosine solutions ranging from a concentration of 1ml of stock strength in 3 litres to 7ml in 3 litres, increased by 1ml/1 in each successive trial. The period of immersion of the clams in each trial varied from 1-5 days. The clams were then removed from these solutions and placed in clear water. Fifteen clams were left undyed as controls against which the extent of colour change and general features of health could be assessed.

Each day the colour of the shells of the dye immersed clams was recorded. The colour of the clams which had previously been removed from the dye tank was also noted to establish whether leaching of the dye had occured.

VII Results.

Erythrosine B was demonstrated to have stained the organic matrix of the shell of the clam so that it remained coloured after 3 months of exposure to clear water. At the concentrations used in this experiment (1-7ml stock solution in 3 litres), no adverse effects were noted to the health of the clam. After initial immersion in the dye solution most clams opened within one minute and no clam remained closed for longer than 15 minutes. Iridiophore brightness decreased over the duration of the period of immersion in the Erythrosine, possibly as a result of the different wavelengths of light reaching the cells. Higher concentrations of dye caused a more marked dimming of the iridophores. After placement in clear water, iridophore coloure returned to that of the control individuals.

The optimal dye concentration for the dying of the clam shells was 4ml/litre. Concentrations greater than this did not produce a more vivid colour over an equivalent time. The inner shell matrix dyed more strongly than the outer shell, and no evidence of the dye could be seen in the animal's soft tissues.

The immersion time for the most intense dye absorption by the shell organic matrix was 4 days. After this time the shells were dyed darkly and further immersion did not markedly deepen the colour.

VIII Discussion.

The dye, Erythrosine B was demonstrated to effectively stain the organic matrix of the shells of the clam *Tridacna crocea*. This substance met all the criteria originally specified for a substance suitable for the chemical labelling of hatchery clams. Erythrosine B is non-toxic to humans and is currently approved for use within the food industry. It is therefore suitable for clams destined for human consumption. The dye had no observed harmful effects on the metabolism of any of the sanimals used in the experiment, even at concentrations almost double those found to be optimal for the staining of the shells. As the dye is concentrated in the shell layer closest to the surface at the time of exposure, it affects only the shell of the clam at the stage when it is dyed. Later deposits of shell are unstained so that the appearance of the animal at later stages of development is the same as that of an individual which has not been treated. This is desirable for clams to be used for ornamental purposes in aquariums and for those used as food.

The cost of the labelling procudure using Erythrosine B is reasonable both in terms of financial outlay and terms of the labour and equipment involved. The recommended concentration of the dye is 68mg/litre. The approximate cost of the purchase of the powdered form of the dye is $20 for 25g. As it is anticipated that the labelling of the clams will occur when the animals are small, they can be placed in appropriately small tanks for treatment, necessitating the use of relatively low quantities of dye. The dyeing procedured requires no special equipment.

Erythrosine B is an artificial substance so its presence in the shell of a clam indicates unequivocally that at a stage in its development is was exposed to human intervention. The substance is obvious to the naked eye, appearing as a bright pink band in the naturally white shell substance. Its detection therefore does not require specially trained personnel or equipment.

The repeated exposure of the clams to the dye at intervals over 6 weeks ensures that new shell is stained as the clam grows creating a pink band of sufficient width to be easily seen. Over the duration of the study the colour remained very ovbvious. However, further assessment of the process will be required as the clams grow to ensure that the dye remains visible in large individuals. As the dye is water soluble some leaching may occur over extended periods. Studies addressing this will be undertaken at the commercial clam hatchery at Fitzroy Island where the use of the dye is currently being introduced to a test batch of animals.

If it is demonstrated that Erythrosine B does not leach out of the shell over an extended period the use of this chemical will provide a method of labeling clams to that the hatchery industry can meet the requirements of the Department of Primary Industry. Clams taken illegally from the Great Barrier Reef will be easily identifiable by an absence of dyed shell. The hatchery industry will have a method of marking their animals cheaply, removing the prohibitive costs involved in current labelling methods so that it may increase clam production and sales. The use of this technique may have further uses within the mariculture industry for the labelling of other types of shellfish.

1X Implications and Recommendations.

It is unclear at this stage what are the implications for other bivalve species. The dye is still to be trialled at the clam hatchery, and it is intended to expand that trial to test its use on pearl oysters. If as with the clams it specifically only stains the inner shell layer then only nacreous layer of the pearl oysters will stain.

This may curtail its use for the Pteriacea. A subsequent report will be submitted after the hatchery trial and the effect of the dye on pearl oysters will be reported then.

X Intellectual Property.

This project was undertaken to solve a specific industry problem. The matter of intellectual property does not arise as the point of this project was to generate knowledge for the industry.

XI Does not apply.

Covering Letter

1. As far as can be ascertained at the moment the objectives of this project have been fully achieveed. The fate of the dye in the shell layer over the long term will be known after hatchery trials as will any other problems encountered under these conditions. This information will be conveyed in a supplementary report.

2. Total Funds contributed.

a.	by F.R.D.C.	$7945.00
b.	by J.C.U. Townsville	$ 640.00
c.	by Reefarm Pty. Ltd.	$ 640.00
		$9225.00

While this project is continuing the costs of its completion are minimal and will be borne by J.C.U. and Reefarm Pty. Ltd.

3. If the efficacy of the method is confirmed by trials at the hatchery then it will be possible to legislate for the requirement that all hatchery clams be similarly tagged. Whether or not the method is applicable to other bivalves depends on further tests.

4 If the efficacy of the method under hatchery conditions is confirmed then this knowledge should be disseminated to all bodies which control relevant state fisheries.

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