Pigment Alteration as a Method of Permanantly
Marking Dungeness Crabs (<u>Cancer magister</u>) and
Other Crustaceans

A proposed study by:

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INTRODUCTION

The technique of freeze branding was developed by Dr. R. K. Farrell during the 1960°s as an alternative to fire branding domestic livestock.

Today, freeze branding is internationally used for cattle and horse ident—
ification. In addition to many species of haired animals successfully
freeze branded, non-haired animals such as whales, fish and snakes have
been marked using the same concepts developed for marking haired animals.

Freeze brands were maintained on rattlesnakes and gopher snakes through
numberous sheds of the skin. (unpublished data - Stroud) It was this
success with snakes that prompted the initial investigation into the possibility of freeze branding crustaceans with the hope that the depigmentation
caused by the process would be reproduced in each new exoskeleton.

The need for a method of marking crabs, shrimp and lobsters for fisheries research and management is well documented. The marking techniques current—
ly available are not wholely satisfactory. A good marking technique should produce an identifiable mark that will remain thoughout the life of the animal, will not interfear with the act of molting, will not cause a differential mortality in marked animals by increasing susceptability to predation or disease, is inexpensive and adaptable to field procedures, and is easily recovered or recognized in commercial or sport harvests.

Our first attempts were directed at placing freeze brands on crayfish (Pacifastacus trowbridgi). Areas of depigmentation were produced on the exoskeleton using a copper branding iron chilled to -700 with dry ice and ethanol mixture. Immediately after branding, the mark was visiable for approximately 5 minutes and then dissappeared. However, the mark reappear-

ed as a deffinate depigmented area in the newly formed exoskeleton when the crayfish molted. After a second molt, the depigmentation was still easily observable. (unpublished data- Stroud and Farrell) The experiment was not carried further due to the death of the crayfish from other causes.

In the spring of 1973, a group of small Dungeness crabs (<u>Cancer magister</u>) were branded and maintained at the Oregon Fish Commission Laboratory, Newport, Oregon. The figures 1 - 5 and 7 depict some of the results of freeze branding crabs with various methods. All of the marked animals have undergone at least one molt. The marks are not observable untill after molting. No difficulty of mortality due to the branding or molting was noted. Figure 6, 8, and 9 represent a group of crabs that were shipped to Pullman, Washington for branding with a lazer beam. The marks appeared approximately 3 mos.after the crab was lazed when the old shell was shed reveling the depigmented, scared new shell.

BASIC CONCEPT INVOLVED

Both freeze and lazer marking have as their common objective the selective disruption or distruction of the pigment producing cells, the melanocytes. Idealy, all surrounding cells would be unaffected by the process. With the freeze branding process, the microtubules that are responsible for the transport of the melanin granules thoughout the processess of the melanocytes are theorectally disrupted mechanically by the formation of intracellular ice crystals. With the lazer marking process, the melanocyte is destroyed by light energy of a specific wave length and intensity. The melanocyte is located among the endocuticle forming cells and is active in contributing pigment granules to the newly forming exoskeleton.

Therefore, theoretically it should be possible to permanantly depigment areas in the form of a symbol in the shellforming membrane. All subsequent exoskeletons produced by this area will be also theoretically depigmented.

PROFOSED FUTURE RESEARCH

We feel that our preliminary experiments have shown that it may be possible to cause depigmentation in a form of some symbol in the exoskeleton by a process of selective injury to the shell forming areas of the integument of the crab. This can be done by both freeze branding and lazer branding techniques.

However, there are many unanswered questions and refinements of the techniques that must be researched before these processes can be developed into a usuable tool for the management and study of the commercially important crustacean fisheries. The following questions are some examples and pertain to both freeze and lazer branding.

- 1. Is the mark permanant thoughout the life of the animal?
- 2. Does the marking process cause any increased mortality in marked animals by interfearing with the normal molting process or increasing susceptability to predation or disease.
- 3. What is the best location on the shell to brand and what is the best period in the development of the exoskeleton to mark.
- Wo What modification in the techniques will be need to sucessfully mark different life stages or different species?
- 5. Can the techniques be adapted to field conditions?
- 6. What is the cost envolved on a per individual basis?

To answer some of these questions, it is our intention to submit to the Oregon State University Sea Grant Program a request for funding of a project that will be carried out at the Marine Scienc Center on the Dungeness crab (<u>C. magister</u>). Information gained on the marking of Dungeness crab (<u>C. magister</u>) may be applicable to lobsters, kingcrabs and possibly even shrimp. The general approach will be to mark animals using various variations of the techniques and following the course of the marks with gross and histological observations. The use of a lazer beam will be available for some marking of crabs at the Marine Science Center if funds become available to move a lazer and the supporting equipment in the possession of Dr. R.K. Farrell in Pullman, Washington.

A letter of support, to be incooperated in the appendix of the grant proposal, would be appreciated and would increase the chances of successful funding. The letter should state that the project, if successful would be of benefit to your organization in the management and study of the crustacean fisheries in your area.

Figure 1-Freeze branded Crab-Exposure to cupper branding iron for 5 seconds at -70°C.

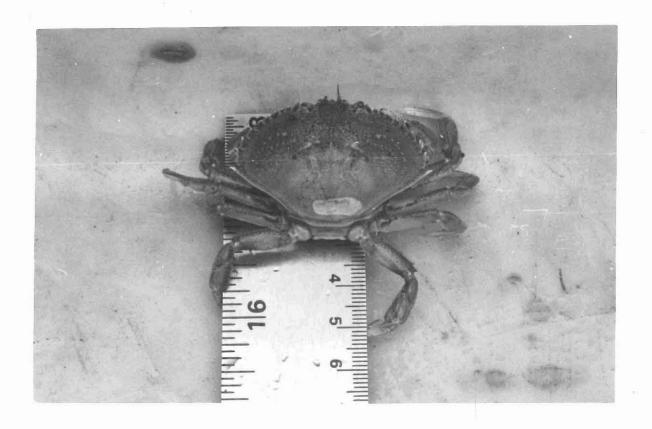




Figure 2-Close up of Brand above.

Figure 3-Close up of 2nd Crab branded in the same manner as Crab in fig.1.



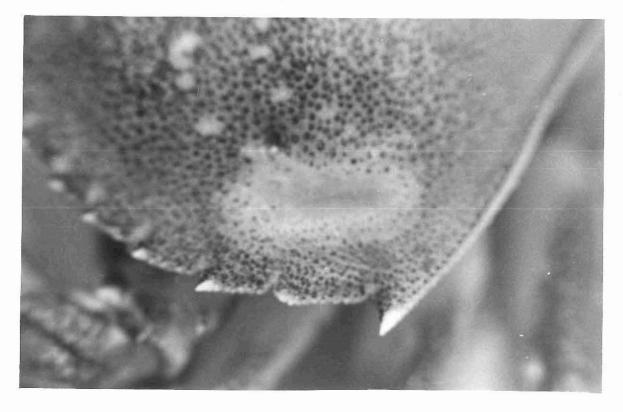


Figure 4-Close up of Freeze Brand-Exposure to cupper branding iron for 5 seconds at -21°C .

Figure 5-The results of over and under exposure to freeze tranding. Crab on left with shell defects from exposure to cupper iron for 15 and 20 seconds at -70° C. Crab on right exposured for 3 seconds to copper branding iron at -70° C.





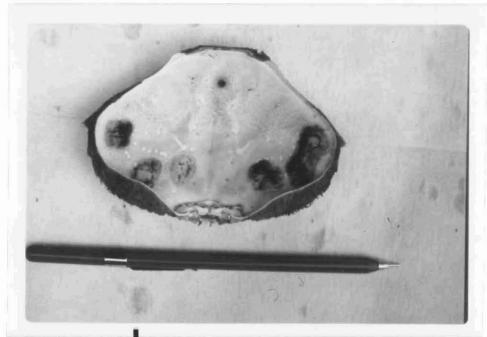
Figure 6-Lazer branded crab-marks are due to exposure to ruby lazer, wave length 6943 Å at 4.5 4.6 4.7 4.8 4.9 5.0 (left to right) kilovoltage



Figure 7-Freeze brand - Exposure to copper branding iron for 5 sec. at -21 C



Figure 8 Lazer branded crab exposed to ruby lazer, 6943 A at various kilovoltage levels



-Figure 9 Shed Shell from lazer branded crab in Figure 8