State of California
The Resources Agency
DEPARTMENT OF FISH AND GAME

Use of the Anesthetic Metomidate Hydochloride as an Aid in the Open-water Capture of Young-Of-The-Year Rockfish

by
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and
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MARINE RESOURCES

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THE USE OF THE ANESTHETIC METOMIDATE HYDROCHLORIDE AS AN AID IN OPEN WATER CAPTURE OF YOUNG-OF-THE-YEAR ROCKFISH 1/

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ABSTRACT

Many species of young-of-the-year (YOY) rockfish, Sebastes sp. move inshore into shallow reef areas in central California each year. We monitored the dates of arrival, relative abundance, species composition, lengths, and growth rates of these arrivals to help assess recruitment variability and stock success.

We tested the usefulness of the anesthetic metomidate hydrochloride as a non-lethal method to collect YOY rockfish to assess growth, abundance, and species composition. Earlier experiments by other researchers illustrated the usefulness of metomidate to collect benthic-oriented adult rockfish. Our research tested the possibility of using metomidate hydrochloride to collect open-water and benthic-oriented YOY rockfish. The anesthetic was applied and the fish captured by divers. Several concentrations of metomidate were tested, premixed both in fresh and salt water.

The anesthetic was not very useful in collecting open water YOY rockfish as the fish rapidly left the area of application and avoided being anesthetized. Increasing the concentration of metomidate did not increase success of capture of open-water YOY rockfish. However, the anesthetic metomidate worked very well in capturing benthic-oriented YOY rockfishes.

1/ Marine Resources Administrative Report No. 92-1

2/ and 4/ Marine Resources Division, 2201 Garden Road, Monterey, California 93940

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ACKNOWLEDGEMENTS

We would like to thank Wildlife Pharmaceuticals of Ft. Collins, Colorado, for helping us become licensed investigators to test the anesthetic metomidate hydrochloride for eventual licensing by FDA and for supplying the chemical to us.

Fisheries technicians Nicole Crane, Peter Nelson, and Michelle Paddack assisted in capturing anesthetized fish, video camera operations, visual observations and fish measurements and identifications.

Paul Reilly and Diana Watters reviewed the manuscript and offered invaluable suggestions.

INTRODUCTION

The Central California Marine Sport Fish Project, funded by the Federal Aid in Sport Fish Restoration Act, has been investigating the distribution and abundance of young-of-the-year (YOY) rockfish, Sebastes spp. for several years. The rockfish species under investigation reside on nearshore reefs. They release their larvae during the winter and early spring. Larvae are then entrained by ocean currents for 2 to 3 months. Usually, juveniles begin to appear from early March to late July on the sea floor at the seaward edge of nearshore reefs. They occur in small schools of five to fifty fish usually occupying less than 1 to 3m of water.

For several years, we have used custom-designed spears to collect YOY fish for identification and measurement. This is rather labor intensive and results in death and mutilation of specimens. Additionally, we suspect that the size composition of speared fish may not be representative of those we see. Species composition also may not be accurate as some species of YOY rockfish are more difficult to collect than others. We have tried dip nets, but numbers of fish collected have been too few for adequate assessment of size and species composition.

One objective of the project has been to develop methods of assessing growth and abundance that do not result in the death and removal of the fish from the population under investigation.

We have had consideraable success in assessing YOY rockfish abundance using visual transects and attraction devices (artificial habitats), but we still must handle fish to positively identify and measure them. Other investigators have used video transects (Ebeling et al. 1980) and electroshocking techniques (Stewart and Cameron 1974, Phillips and Scoloro 1980). Although those methods offer some promise, to date we have not found them practical for rockfish species in central California. Researchers have had success in live capture of marine fish with Quinaldine (Muench 1958) and with Tricaine methanesulfonate (MS 222) (Schoettger and Julin 1967, Jenkins and Smith 1990). We chose not to use those chemicals due to reported health risks to the user.

Research in the Pacific Northwest demonstrated that metomidate hydrochloride (metomidate) had a potential for use in open water collection in a marine environment (Matthews and Reavis 1990). They showed the drug to be effective in collecting adult rockfish in rocky marine habitat. Metomidate is not licensed by the U. S. Food and Drug Administration (USDA). Therefore, our first objective was to become investigational users as required by federal law to test the effectiveness of metomidate on open-water YOY rockfish. Our application is included in this report as Appendix A. Based upon our research needs, work by Matthews and Reavis (1990), and reduced user health risks associated with metomidate further studies testing efficacy for open water collection of marine species are warranted.

METHODS

Application for investigational user qualification with FDA and with the drug metomidate itself was obtained from Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado. We used a total of 80 gm of the chemical. To satisfy FDA requirements for investigational users, we developed a data sheet to record required information from each application of metomidate (Figure 1.). Information recorded included concentration of metomidate when different than the standard of 2 gm/l, subjective observations of fish behavior, and possible adverse effects on YOY fish from predatory species of invertebrates, fish, harbor seals and sea lions. We recorded species of fish exposed to metomidate, length of fish captured, concentration and volume of metomidate administered, distance of application from fish, time of response to each volume and concentration, time of recovery and any mortality (a fish was considered dead if no movement was observed one hour after application of the anesthetic).

The metomidate was weighed in 12 gm batches. One plastic screw cap bottle containing 12 gm of the powdered drug mixed with 6 l of fresh or salt water equaled a concentration of 2 gm/l. For a concentration of 4 gm/l we added 2 bottles of 12 grams each to the 6 l of water. All mixing was done on board R/V Melanops, a 25-foot cabin-enclosed Boston Whaler. Rubber gloves were worn when adding the powder to the water and all persons were very careful not to inhale the powder or dust. No face mask was

Metomidate Hydrochloride
INADA: _____

DATA COLLECTION SHEET
FREE WATER COLLECTION STUDY

Species: _____ Location: _____

Water Temp: _____ Date: _____

Habitat (coral, rock, etc.) _____

Stock Solution of Metomidate: _____ gm per _____ ml water.

Device used to deliver stock solution to fish: _____

Record of Use:

	ml Stock Solution Used	Distance from Fish	Time to Sedate	Time to Recover
FISH				
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

Record any Mortalities

Comments:

Investigator Signature _____
Date _____

Figure 1. Data sheet used for each application of metomidate.

required for use.

Usually, four divers participated in the collection. One applied the metomidate, one recorded fish reaction, one collected all fish immobilized and the fourth person either helped to collect fish, videotaped the procedure or simply observed and took notes.

The underwater application container was an accordion shaped plastic collapsible bottle of 7 l capacity with an open and close demand valve, manufactured under the brand name Olicamp. By squeezing the bottle between one's arm and side with the valve open, metomidate was released through a 3 foot section of 1/2 " surgical tubing attached to the valve stem. A one foot section of rigid 1" PVC pipe was attached to the terminal end of the surgical tubing to aid in application. Upon cessation of application the valve was closed so dilution with sea water would not occur in the tube or bottle.

The diver collecting fish used both a 12-in. diameter dip net and a one-person, folding lift-net measuring 2 ft by 3 ft on each of two sides.

We tested several hypotheses: (1) Reaction of fishes to metomidate premixed with freshwater was no different than metomidate premixed with sea water. (2) Reaction of open water fishes (those in schools more than one m above the substrate) showed no difference from exposure to two concentrations of metomidate (2 gms/l and 6 gms/l). (3) Reaction of benthic-oriented fishes to metomidate showed no difference from exposure to two concentrations of metomidate (2 gms/l and 6 gms/l). (4)

Open-water fishes react no differently than benthic-oriented fishes (fish within one m of substrate and solitary or in groups of less than 5) to the presence of metomidate. (5) Fish exposed to the anesthetic metomidate showed no permanent adverse effects following a recovery period not exceeding 1 hr.

For all fish captured total length was measured to the nearest millimeter. Fish were maintained and observed in floating containers at the water's surface until recovery was complete, or until 1 hr. had passed, whichever came first, and then released at the location of capture.

RESULTS AND DISCUSSION

Upon initial application of metomidate to open-water YOY rockfishes, a response was observed in the fish which suggested the metomidate premixed in fresh water was irritating to the fish. The observed responses included gaping of the mouth and opercula. This apparent irritation response by the fish was followed by the fish avoiding the area treated with the metomidate. In order to isolate possible factors eliciting these behavioral responses, we omitted the metomidate and filled two equal containers, one with fresh water and one with salt water and used yellow food coloring to provide us with a visual cue as to the area into which the solution had diffused. Upon application we observed no response by the fish to either the fresh or salt water solutions. A solution of metomidate and sea water was not tested initially because the supply of metomidate

was limited. We accepted the hypothesis that fish react equally and in the same manner to fresh or salt water. However, the fish did, in subsequent observations exhibit the same irritated response to both fresh and salt water when either contained metomidate.

Upon initial application of metomidate, open-water rockfish rapidly opened their operculas in a gaping manner for 1 to 2 seconds and then left the area of highest metomidate concentration. There was no noticeable difference in the avoidance reaction of open-water fishes to different (2 gm/l or 6 gm/l) concentrations of metomidate. We therefore accepted hypothesis number 2 that the response to different concentrations would be similar. The metomidate diluted rapidly in seawater and the combination of dilution and fish dispersal resulted in limited exposure of the fish to the concentrated solutions. Several of the fish were very near stupor and began to list as they left the test area, a sure sign of impending anesthesia. A total of 7 YOY rockfishes were captured and released in open-water habitat.

Benthic YOY fishes reacted similarly to 2gm/l and 6gm/l concentrations of metomidate. Upon initial application of metomidate, benthic-oriented rockfish rapidly opened their opercula, similarly to open-water fishes, but did not swim out of the concentrated metomidate. As a result, benthic-oriented YOY rockfish were much easier to anesthetize than open water species. Both the 6 gm/l and 2 gm/l concentrations were 100% effective. It appears that bottom-oriented fish do not leave the area when

they are being anesthetized. Both these statements support the third null hypothesis that benthic-oriented fishes would show no difference in response to different concentrations of premixed metomidate. This may be attributable to the fact that benthic rockfish orientate tactually and remain in water containing the more concentrated dilutions of the anesthetic. Open water fish, on the other hand, have no strong fidelity to their location and escape threats or unpleasant sensations by flight.

Open water YOY rockfish are able to avoid areas of seawater that have been treated with the anesthetic metomidate. Benthic-oriented YOY rockfish do not avoid the anesthetic metomidate but appear to remain close to the bottom for protection. We therefore reject the fourth hypothesis and accept the fact that open-water YOY rockfishes and benthic-oriented YOY rockfishes react differently to the application of metomidate.

Fishes took from 1-2 min to 1 hr. to recover from the application of the anesthetic. All fish except one apparently recovered fully based on observed behavior and were returned to the area of capture. The single fish which had not fully recovered after one hour received the strongest and longest application of metomidate. Upon returning it to the bottom, it was still slightly disoriented and moved sluggishly. It did seek protection in a small rocky crevice. Therefore we accept the fifth hypothesis that anesthetized fish and non-anesthetized fish behave and react the same when 1) the initial concentration of anesthesia applied was not excessive and 2) no more anesthetic was applied after the fish was anesthetized. Predation on YOY

rockfishes by large adult rockfishes and consequently on large adult rockfishes by harbor seals was a potential problem but was never observed.

CONCLUSIONS

We were unable to use metomidate to adequately sample young-of-the-year rockfish in open water. Open-water oriented YOY move out of the anesthetized area and only 7 were captured. The drug works well on benthic-oriented YOY which seek bottom habitat as a refuge. Once the YOYs orientate to the bottom they remain in the area containing the metomidate, become anesthetized and are easily captured.

Initial concentrations were increased to 3-fold (to 6gm/l) with no increase in effectiveness of the anesthetic on open water YOY rockfish. Three divers would be sufficient to capture fish when the anesthetic is effective. The method of application and the apparatus for applying the drug both worked successfully for benthic fish.

REFERENCES

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Appendix A

WILDLIFE PHARMACEUTICALS, INC.
PROTOCOL FOR FDA STUDY METOMIDATE HCL
INAD 4321

TITLE:

Efficacy of Metomidate Hydrochloride for Capture of Young-Of-Year rockfish (YOY), Sebastes spp., in Open Marine Habitat.

PURPOSE:

Demonstration of efficacy of metomidate hydrochloride solution for open water collection of free-ranging species.

INTRODUCTION:

Previous in-house trials using representatives of Salmonidae and Ictaluridae have demonstrated that metomidate hydrochloride is highly effective of producing sedation at 0.25 to 0.75 ppm and anesthesia at 6 to 10 ppm. Additionally, within the same studies the drug was shown to have an extremely high margin of safety. Additionally, research activity with cooperating researchers in the Pacific Northwest demonstrated that metomidate hydrochloride had a potential for use in open water collection in a marine environment. In this study, the drug was shown to be effective collecting rockfish within rocky marine habitat. It is based upon these studies and this information that further studies documenting and demonstrating an effective dose level for open water collection of marine species are warranted.

The Central California Sport Fish Survey, a project of the state of California funded by the federal Sport Fish Restoration Act, has for several years been investigating the distribution, abundance, and transport of young-of-the-year (YOY) rockfish, Sebastes spp. The rockfish species under investigation reside on nearshore reefs. They release their larvae during the winter and early spring. The larvae are then carried about by the ocean currents for two to three months. The juvenile fish then begin to appear on the sea floor at the seaward edge of the nearshore reefs. They occur in small schools of five to fifty fish usually occupying less than one cubic meter of water.

One of the objectives of the project has been to develop methods that do not require the death of the fish under investigation. We have had considerable success in assessing the abundance with visual transects and artificial habitats, but we still must

kill fish to positively identify them and to measure them. We have investigated the use of video and electroshocking, and although those methods still offer some promise for some situations, to date we have not found them practical for our routine investigations. Several years ago other researchers had success with Quinaldine and MS 222. We have chosen to not use those chemicals due to reported health risks to the user.

We are currently spearing the fish we need for identification and measurement. This is rather labor intensive, and results in death and mutilation of the specimen. Additionally, we suspect that the size and species composition of speared fish is not at all representative of those we see. We have tried to use dip nets, and although we can catch a few fish, they are too few for adequate assessment of size and species composition. We hope to have greater success with these dip nets if we can sedate the fish.

METHODS:

Metomidate hydrochloride will be mixed with water to produce a concentration of 2 gms/Liter. This diluted solution will be placed in a collapsible plastic squeeze bottle. A SCUBA diver will take this bottle to the sea floor, and scout for juvenile rockfish. Upon locating such a school of fish s/he will squirt 50 mL of the solution on the upcurrent side of the school, delivering the solution as close to the school as s/he can without dispersing the school. Additional solution may be applied if necessary to sufficiently sedate the fish for capture with a dip net.

The time and volume of solution required will be recorded, and each school collected will be stored separately. Another team of divers will spear fish at the same time and location. The number of fish collected per diver hour, and the size and species composition of fish collected with the two methods will be compared. A sample of the fish will be placed in sea water aboard the research vessel where their recovery rate will be observed for an hour and recorded. Some fish may be transported to a laboratory for observation for a week or more.

IDENTIFICATION OF TEST AND CONTROL ARTICLES:

Name: Metomidate hydrochloride

Chemical Name: (±)-methyl 1-(1-phenylethyl)-1H-imidazole-5-carboxylate monohydrochloride

Chemical Abstract Number: 5377-20-8

Code Number: 007997

SPONSOR:

Wildlife Pharmaceuticals, Inc.
1401 Duff Drive, Suite 600
Fort Collins, CO 80524

TESTING FACILITY:

State of California
Department of Fish and Game
2201 Garden Road
Monterey, CA 93940

PROPOSED DATES:

Upon approval of application to November, 1991 and then commencing again in April, 1992 to November, 1992.

JUSTIFICATION FOR TEST SYSTEM:

The research program of the California Department of Fish and Game provides opportunity and need for collection of juvenile rockfish. The safe and efficient collection of these valuable test animals is a critical part of the overall research program.

TEST SYSTEM IDENTIFICATION:

Number:	100-200
Body Weight Range (initial):	0.5 - 20 grams
Sex:	Unknown
Source of Supply:	Free-ranging in the areas of Monterey, California.
Species:	Sebastes spp. and Ophiodon elongatus
Strain:	N/A

Substrain: N/A

Age (initial): Young-of-year

PROCEDURE FOR IDENTIFICATION OF TEST SYSTEM:

Individual fish will be freeze branded or injected with latex paint.

EXPERIMENTAL DESIGN:

The fish used in this study will be captured under free-ranging conditions and brought under the management of the California Department of Fish and Game for additional studies. Weights and lengths will be taken by the investigator after collection.

METHOD OF ADMINISTRATION FOR TEST ANIMALS:

Metomidate hydrochloride is a 2 gm/Liter solution mixed with fresh water will be delivered by squirting 50 mL of the solution within 10 cm of the face of the fish.

EVALUATION OF THE EFFECTS:

Level One	No effect
Level Two	Fish is visibly effected, but not sedated sufficiently to capture.
Level Three	Fish is sedated to the point that fish can be captured with some effort by the investigator
Level Four	Complete anesthesia

Once the fish is placed in drug free water the time to return to normal behavior or presence of mortality will be noted.

DATA TO BE RECORDED:

Species

Length

Number of millimeters or metomidate solution delivered (time of application)

Distance from fishes

Time of response to various levels

Time to recovery

Presence of mortality

INVENTORY OF INVESTIGATIONAL DRUG:

- A sample of drug inventory sheet is attached.

TYPE OF CONTROL:

Each fish will serve as its own control as response to this drug is dramatic and self-evident. If facilities and personnel are available we will compare survival of those fish captured with metomidate and those fish captured solely by lift net.

METHODS FOR CONTROL OF BIAS:

None

DESCRIPTION AND/OR IDENTIFICATION OF DIET:

Non-applicable

ROUTE OF ADMINISTRATION OF TEST CONTROL ARTICLE AND REASON:

Route of administration will be inhalation of the drug over the gills.

TEST CONTROL ARTICLE:

Metomidate hydrochloride, 2% solution

OBSERVATION AND DATA COLLECTION:

Fish will be observed until effect or no effect is achieved according to the interpretation of the investigator.

RECORDS TO BE MAINTAINED:

1. Protocol
2. Protocol Amendments
3. Records of Receipt
4. Daily logs of Activities and Occurrences Associated with this study
5. Any Raw Data Sheets

DATE OF SPONSOR APPROVAL OF PROTOCOL:

SIGNATURE OF STUDY DIRECTOR:

STATISTICAL METHODS TO BE USED:

- None. As effect or no effect of test article is self-evident.

CONDUCT OF STUDY:

James L. Houk

TESTING FACILITIES OPERATION:

Research Laboratory

DISPOSITION OF SPECIMENS:

No specimen material will be collected during these trials

FINAL REPORT:

James L. Houk, Dr. William R. Lance

GOOD LABORATORY PRACTICE REGULATIONS:

The study will be conducted in compliance with Food and Drug Administration's "Good Laboratory Practices Regulations for Nonclinical Laboratory Studies" (21CFR, Part 58), and monitored for such compliance by the study, Quality Assurance Officer.

PERSONNEL:

James E. Hardwick
James L. Houk
David A. VenTresca

SIGNATURES:

James E. Hardwick

James L. Houk

David A. VenTresca

CHANGES IN OR REVISIONS OF APPROVED PROTOCOL AND REASONS:

SIGNATURE OF STUDY DIRECTOR:

DATE:

01 July 1991