WHITE SEABASS ENHANCEMENT PLAN

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California Department of Fish and Game
Marine Region

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White Seabass Enhancement Plan
Executive Summary

The White Seabass Enhancement Plan (WSEP) provides a framework for managing the Ocean Resources Enhancement and Hatchery Program (OREHP) in an environmentally sustainable manner. The WSEP presents detailed information on white seabass and the OREHP and establishes best management practices (BMPs) for hatchery and growout operations, fish health, genetics, and benthic monitoring. It also outlines methods on which to evaluate the OREHP and is designed to be flexible and adaptable to a wide range of future conditions. Minor changes can be made to the BMPs without the need to amend the WSEP by revising the other guidance documents for the OREHP. However, future research, environmental, biological, or economic changes of significance may create a need to amend the WSEP to ensure that the enhancement of white seabass is conducted in a responsible manner.

In 1983, the Legislature established the OREHP [Fish and Game Code (FGC) §6590 et. seq.] to conduct a program of basic and applied research into the artificial propagation, rearing, and stocking of important marine finfish species occurring in ocean waters off southern California. Initially, white seabass and California halibut were both chosen for use in the experimental stocking program; however, in 1990, research focused on white seabass because of the depressed condition of the stock at the time and its higher value to recreational and commercial fishermen.

Over the years, the Legislature has amended the intent language of the OREHP. Current legislation calls for a focus on determining if hatchery released fish can artificially enhance certain stocks of desirable species through increased production of fish and increased monitoring of fisheries to assess the hatchery contribution. The ultimate goal of the legislation is to enhance populations of marine finfish species important to California for their sport and commercial fishing value.

In 2006, the Legislature passed SB 201 (Simitian) Marine Finfish Aquaculture, which amended the statute related to marine aquaculture [FGC §15000 et. seq.]. The statute requires the preparation of an enhancement plan for any artificial propagation, rearing or stocking project for the purpose of recovery, restoration, or enhancement of native fish stocks carried out under either a scientific collecting permit, research permit, or the OREHP [FGC §15400(b)(10)(c)]. The plan shall provide for, among other things, monitoring and protecting of benthic habitat, the prevention of pollution, and the prevention of adverse impacts on wild fish stocks from disease, parasites, and genetic alterations. The legislation also designates the Fish and Game Commission (Commission) the authority to approve an enhancement plan.

To manage the State's commercial and recreational fisheries for white seabass, the Commission adopted a White Seabass Fishery Management Plan (WSFMP) in 2002. The WSFMP provides mainly for a fishery management program based on the concept of an Optimum Yield (estimated as a percentage of Maximum Sustainable Yield) with enforcement of take limits, including minimum size, daily bag, and seasonal restrictions.

Currently, the WSFMP does not include the OREHP as a management tool; however, if deemed successful, enhancement could be incorporated in the management of white seabass.

The WSEP currently includes twelve chapters and various appendices and supporting materials:

- **Chapter 1 – Background** outlines 11 components that are integral in developing, evaluating, and managing marine stock enhancement programs. It also lists the primary goal and objectives of the OREHP.
- **Chapter 2 – Biological Information for White Seabass** includes information on the biology and status of the stock.
- **Chapter 3 – History of the Fisheries** covers the historical white seabass catch of both the recreational and commercial fisheries.
- **Chapter 4 – History of Conservation and Enhancement Efforts** summarizes the white seabass regulations from 1931 to present and includes a history of the OREHP.
- **Chapter 5 – Hatchery Operations** describes the current operating procedures and BMPs for the white seabass hatchery.
- **Chapter 6 – Growout Facility Operations** describes the current operating procedures and BMPs for the white seabass growout facilities.
- **Chapter 7 – Fish Health Management** describes the prevention, identification, and treatment of many common white seabass pathogens, including non-infectious and infectious diseases. It also includes the BMPs for the Fish Health Management Program.
- **Chapter 8 – Regulatory Considerations** lists the permits and permissions required to operate the white seabass hatchery and growout facilities.
- **Chapter 9 – Environmental Considerations** describes the benthic monitoring program for the growout facilities, including a description of methods used and results of the initial testing for sulfide, reduced oxygen (redox) potential, total volatile solids, zinc, and copper. The BMPs for the growout facilities that identify interim threshold levels of sulfides are included as well.
- **Chapter 10 – Genetics** includes an overview of the three studies that apply to the genetics of and culturing/management practices for white seabass in southern California. In addition, the goals and objectives of the current genetics research plan are included.
- **Chapter 11 – Current Research and Future Needs** describes the juvenile and adult sampling programs that will be used to assess the proportion of hatchery-raised fish to the wild population.
- **Chapter 12 – Program Evaluation** outlines the methods that will be used to evaluate the OREHP. These methods include the creation of a Scientific Advisory Committee (SAC) and an Adaptive Management Plan (AMP); a stock assessment; an update of the bioeconomic model; and an analysis of the adult sampling, genetic management, and benthic monitoring programs. A plan for review and amendment of the WSEP is also included.

The primary goal of both the OREHP and the WSEP is to evaluate the economic and ecological feasibility of releasing hatchery-reared fish to restore depleted, endemic, marine fish populations to a higher, sustainable level. To achieve this goal, the following objectives must be realized: 1) develop and implement hatchery operation and growout methods that provide a supply of healthy and vigorous fish; 2) conduct the replenishment program in a manner that will avoid any significant environmental impacts resulting from operation of either the hatchery or pen rearing facilities; 3) maintain and assess a broodstock management plan that results in progeny being released that have genotypic diversity very similar to that of the wild population; 4) quantify contributions to the standing stock in definitive terms by tagging fish prior to release and assessing their survival in the field; 5) continue to develop, evaluate, and refine hatchery operations to maximize the potential for achieving the goal of the program and; 6) develop quantitative measures of success.

To work towards the goal of enhancement, the WSEP addresses each objective through BMPs and ongoing monitoring and evaluation. The BMPs have been developed to manage the program in a manner that will avoid any significant environmental impacts resulting from the operation of either the hatchery or growout facilities. These include, but are not limited to:

- Maintaining separate systems for each aspect of hatchery culture (broodstock, Juvenile 1 and 2 systems, raceway culture, experimental systems, and food production) (Objective 1);
- Maintaining water quality by sterilizing and filtering water at the hatchery and by maintaining clean nets and raceways in the field (Objective 1);
- Monitoring effects to the benthos from growout facility operations by visual inspection and sampling of sediment around growout facilities to analyze sediment free sulfides and redox potential (Objective 2).
- Assessing fish health daily at growout facilities (Objectives 1 and 2);
- Releasing only healthy fish that have been inspected by the Department of Fish and Game (Department) Fish Pathologist (Objectives 1 and 2);
- Rotating new broodstock (males and females) into the program following the procedures as described in the Comprehensive Hatchery Plan for Operation of the Leon Raymond Hubbard, Jr. Marine Fish Hatchery in Carlsbad, California (CHP) (Objective 3);
- Maximizing the genetic diversity of the parental contributions within the annual release total to the fullest extent practical by ensuring that cohorts of released fish are comprised of progeny from one to three female equivalents per run (Objective 3);
- Tagging all fish prior to transfer or release (Objective 4);
- Modifying the management of white seabass broodstock as new information becomes available (Objective 5).

The WSEP also includes a formal program evaluation, which will include the following components:

- Adult sampling program review and analysis
- White seabass stock assessment
- Bioeconomical model update/rewrite
- Juvenile release data review and analysis
- Genetic research plan and review
- Bethnic monitoring plan and review
- Results of ageing work
- Habitat assessment study at Santa Catalina Island

To assist the Department in managing the OREHP and evaluating the program, the Department will employ a Scientific Advisory Committee (SAC) made up of experts in white seabass biology, population biology, genetics, environmental quality, and fish pathology. The main purpose of the SAC is to have experts available to review proposed research aimed at evaluating the OREHP, review the AMP, and review the actual program evaluation when completed. The SAC will develop science-based criteria, based on the goals and objectives of the OREHP, to help evaluate the success of the program.

The Department also intends to develop an AMP, which will provide a mechanism to continuously evaluate the OREHP. The AMP would then be approved by the SAC and incorporated into the WSEP. The critical issues to be addressed by the AMP are: 1) maximizing the contribution potential of stocked fish through optimized culture and release strategies, 2) maintaining genetic diversity, 3) managing disease, and 4) minimizing impacts to the environment from the hatchery and growout facilities.

The WSEP lays out interim steps to ensure that the OREHP has every opportunity of successfully reaching its goals and objectives. If the OREHP proves successful, California recreational and commercial fishing may be more effectively managed by the inclusion of a significant new component (hatchery production) that eliminates natural fluctuations in recruitment that are typical of many fish populations in the wild. This could result in increased opportunities for recreational and commercial fishermen.

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List of Acronyms and Abbreviations

BMPs	Best Management Practices
BOD	Biological Oxygen Demand*
CCC	California Coastal Commission
CalCOFI	California Cooperative Oceanic Fisheries Investigations
CDP	Coastal Development Permit
CESA	California Endangered Species Act
CEQA	California Environmental Quality Act
CFIS	Commercial Fishery Information System
CHP	Comprehensive Hatchery Plan for Operation of the Leon Raymond Hubbard, Jr. Marine Fish Hatchery in Carlsbad, California
CNS	Central Nervous System*
CPFV	Commercial Passenger Fishing Vessel*
CRFS	California Recreational Fisheries Survey
CSF	Catalina Seabass Fund
CSUN	California State University, Northridge
CTR	California Toxics Rule*
CWT	Coded Wire Tag*
dph	Days Post Hatch
EEZ	Exclusive Economic Zone*
EFH	Essential Fish Habitat*
ELISA	Enzyme-linked Immunosorbent Assay*
ESA	Federal Endangered Species Act
FAT	Fluorescent Antibody Testing*
FCR	Food Conversion Rate*
GFC	Growout Facility Coordinator
GPM	Procedures Manual for Growout and Release of White Seabass (*Atractonscion nobilis*) as part of the Ocean Resources Enhancement and Hatchery Program (OREHP)
GSS	Gas Supersaturation*
HSWRI	Hubbs-SeaWorld Research Institute
LARWQCB	Los Angeles Regional Water Quality Control Board
LMMS	Larval Mass Mortality Syndrome*
LOP	Letter of Permission*
MCCS	Main Computer Control System
MLLW	Mean Lower Low Water
MLMA	Marine Life Management Act
MMPA	Marine Mammal Protection Act
MND	Mitigated Negative Declaration
MOA	Memorandum of Agreement
MRFSS	Marine Recreational Fisheries Statistical Survey
MSFCMA	Magnuson-Stevens Fishery Conservation and Management Act*
MS-222	Tricaine methanesulfonate*

NOAA	National Oceanic and Atmospheric Administration
NOAA Fisheries	NOAA's National Marine Fisheries Service
NPDES	National Pollution Discharge Elimination System Permit
OPP	Organophosphate Pesticides*
OREHP	Ocean Resources Enhancement and Hatchery Program
OREAP	Ocean Resources Enhancement Advisory Panel
OSP	Optimum Sustainable Population*
PBR	Potential Biological Removals*
PCR	Polymerase Chain Reaction*
PIT	Passive Integrated Transponder*
PSMFC	Pacific States Marine Fisheries Commission
RBC	Red Blood Cell
RecFIN	Recreational Fisheries Information Network
Redox	Reduced Oxygen
SAC	Scientific Advisory Committee
SCE	Southern California Edison
SDRWQCB	San Diego Regional Water Quality Control Board
SDSU	San Diego State University
SFA	Sustainable Fisheries Act
SFRA	Sport Fish Restoration Act
SLC	California State Lands Commission
SONGS	San Onofre Nuclear Generating Station
T&E	Threatened and Endangered Species
TDG	Total Dissolved Gas*
TEM	Transmission Electron Microscopy*
TGP	Total Gas Pressure*
TOC	Total Organic Carbon*
TVS	Total Volatile Solids*
UASC	United Anglers of Southern California
UCD	University of California, Davis
USACE	United States Army Corp of Engineers
USCG	United States Coast Guard
USDA	United States Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
VHS	Viral Hemorrhagic Septicemia*
VNN	Viral Nervous Necrosis*
VRG	Vantuna Research Group of Occidental College
VNNV	Viral Nervous Necrosis Virus*
WSEP	White Seabass Enhancement Plan
WSFMP	White Seabass Fishery Management Plan

*Defined in the Glossary of Terms and abbreviations

White Seabass Enhancement Plan
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Part 1 - The Ocean Resources Enhancement and Hatchery Program

Chapter 1. Background

1.1 Introduction and purpose of the enhancement plan

The passage of SB 201 (Simitian) Marine Finfish Aquaculture, in 2006, amended statute related to marine aquaculture [Fish and Game Code (FGC) §15000 et. seq.]. The statute requires the preparation of an enhancement plan for any artificial propagation, rearing or stocking project for the purpose of recovery, restoration, or enhancement of native fish stocks carried out under either a scientific collecting permit, research permit, or the Ocean Resources Enhancement and Hatchery Program (OREHP) [FGC §15400(b)(10)(c)]. The plan shall provide for, among other things, monitoring and protecting of benthic habitat, the prevention of pollution, and the prevention of adverse impacts on wild fish stocks from disease, parasites, and genetic alterations. The legislation also designated the Commission as the authority to approve an enhancement plan.

1.2 Components of a stock enhancement plan

Blankenship and Leber (1995) identified 10 components in developing, evaluating and managing marine fish stock enhancement programs. The components include the need to:

(1) Prioritize and select target species for enhancement;

(2) Develop a species management plan that identifies harvest opportunity, stock rebuilding goals, and genetic objectives;

(3) Define quantitative measures of success;

(4) Use genetic resource management to avoid deleterious genetic effects;

(5) Use disease and health management;

(6) Consider ecological, biological, and life history patterns when forming enhancement objectives and tactics;

(7) Identify released hatchery fish and assess stocking efforts;

(8) Use an empirical process for defining optimum release strategies;

(9) Identify economic and policy guidelines; and

(10) Use adaptive management.

Not stated in Blankenship and Leber (1995) but of concern to the OREHP is:

(11) Minimize the environmental effects of the hatchery and growout facilities.

The eleven items outlined above also cover the provisions of the Fish and Game Code relative to development of an enhancement plan. Specifically, component 11 covers monitoring and protecting the benthic habitat, and the prevention of pollution. Component 5 covers the prevention of adverse impacts on wild fish stocks from disease and parasites, while component 4 covers the prevention of genetic alterations. Table 1-1 outlines what has already been accomplished within the OREHP for each of these components and what remains to be done. Further discussion of each component can also be found in the following subsections.

Table 1-1. Timeline and progress to date for the OREHP.

Component	Subcomponent	Status	Location
Select target species for enhancement	Not applicable	Completed	Sections 1.2.1 and 4.2
Develop species management plan	Develop goals and objectives of the OREHP	Completed	Sections 1.2.2 and 1.4
	Identify and manage genetic structure of wild white seabass stock according to objectives of the OREHP	In progress - estimated completion date June 2014	Sections 1.2.2, 2.2, 6.3, and Chapter 10
	Estimate post-release survival	In progress – estimated completion date June 2014	Section 1.2.2 and Chapter 11
Define quantitative measures of success	Not applicable	Will be developed by June 2014	Section 1.2.3
Use genetic resource management	Determine geographical range of wild stock	Completed	Sections 1.2.4 and 2.2
	Determine effective broodstock population	Initial studies completed; further research in progress – estimated completion date June 2014	Chapter 10
	Develop genetic monitoring protocols	In progress – estimated completion date June 2014	Sections 10.3, 10.4, and 10.5
	Conduct genetic monitoring of broodstock and released progeny	In progress – estimated completion date June 2014	Sections 5.2.2, 5.3, 5.4, 6.3, 10.3, and 10.4
Use disease and health management	Develop protocols for routine sampling	Completed	Sections 1.2.5, 5.1, 6.5, 6.7.1, and Chapter 7
	Conduct research on novel pathogens to determine etiology and treatment	Ongoing/as needed	Chapter 7
	Develop protocols for treatment/euthanization	Completed – new pathogens to be added as needed	Chapter 7
Develop enhancement	Not applicable	In progress – estimated	Section 1.2.6 and

Table 1-1. Timeline and progress to date for the OREHP.

Component	Subcomponent	Status	Location
objectives and tactics		completion date June 2014	Chapters 2 and 3
Identify hatchery-raised fish and assess stocking efforts	Tag or mark all fish	Ongoing	Sections 1.2.7 and 5.6
	Develop juvenile sampling program	Completed	Sections 1.2.7 and 11.1
	Develop adult sampling program	Competed	Sections 1.2.7 and 11.2
Define optimum release strategies	Evaluate fish size at release	Completed	Section 1.2.8
	Evaluate release season	In progress – estimated completion date June 2014	Sections 1.2.8 and 11.1.3
	Evaluate release habitat	In progress – estimated completion date June 2014	Sections 2.2, 6.7, and 11.1
	Evaluate release magnitude	In progress – estimated completion date June 2014	Section 1.2.8 and 6.3
Identify economic and policy guidelines	Not applicable	Initial evaluation completed; update of evaluation estimated completion date June 2014	Section 1.2.9
Use adaptive management	Not applicable	In progress – estimated completion date June 2015	Section 1.2.10 and 12.2
Minimize environmental impacts	Identify best management practices at hatchery and growout facilities	Completed	Section 1.2.11 and Chapters 5 and 6
	Identify impacts to benthos and ways to minimize	In progress – estimated completion date June 2014	Chapter 9
	Identify permits and permissions	Completed	Chapter 8

1.2.1 Selecting target species

In the beginning, the Ocean Resources Enhancement Advisory Panel (OREAP) and the Department of Fish and Game (Department) selected two species, California halibut (*Paralichthys californicus*) and white seabass (*Atractoscion nobilis*), to begin developing culture methods. Original selection criteria included:

- Species indigenous to southern California
- Status as a diminished stock
- Economic value
- Both commercial and sport utilization
- Potential for success

During the first six years of the program, research focused on the capture, maintenance, spawning (both natural and artificial), and grow-out to release size for California halibut and white seabass. Additionally, work was undertaken to determine juvenile natural mortality, juvenile distribution in the wild, post-release survivability of hatchery reared

fish, and marking methods to identify hatchery reared fish in the wild. In 1990, the Department and the OREAP decided to focus the OREHP's limited funding on white seabass culture because California halibut commercial and recreational landings began to stabilize while white seabass landings continued to decline. In addition, white seabass was considered a more desirable species to both commercial and recreational fishers. For more information see Section 4.2.

1.2.2 Species management plan development

No formal species management plan, that identifies how the enhancement effort fits into the management of white seabass, was developed at the beginning of the program. However, the Comprehensive Hatchery Plan (CHP) for Operation of the Leon Raymond Hubbard, Jr. Marine Fish Hatchery in Carlsbad, California (Drawbridge and Okihiro 2007) and the Procedures Manual for Growout and Release of White Seabass (GPM) (*Atractoscion nobilis*) as part of the Ocean Resources Enhancement and Hatchery Program (OREHP) (Drawbridge and Okihiro 2007) cover most of the enhancement aspects of such a plan including goals and objectives of the OREHP, identification of genetic stocks to determine the population being enhanced, methods to maintain genetic diversity, and disease management. While the two documents do not estimate post-release survival, two research programs have been aimed at learning about post-release survival, the juvenile gill net sampling program (Section 11.1) and the adult head collection program (Section 11.2). While the adult head collection program is ongoing, the juvenile gill net sampling program operated from 1995 through 2008.

A separate document, the White Seabass Fishery Management Plan (WSFMP) (CDFG 2002), adopted by the Commission in 2002, covers the management of white seabass but does not include the OREHP as a management tool. The WSFMP was adopted pursuant to the Marine Life Management Act (MLMA) (AB 1241-Keeley; Fish and Game Code Section 7050 et. seq.), which required the development of a fishery management plan. The main goal of the MLMA is to ensure long-term resource conservation and sustainability. While the MLMA does not mention enhancement as a management tool, it does require the rebuilding of depressed stocks. Once the OREHP has been formally evaluated and if deemed successful, fishery managers can then consider incorporating enhancement into the management of white seabass.

1.2.3 Quantitative measures of success

To date, no quantifiable measures of success have been developed for the OREHP. Developing measures of success will be one of the tasks of the Scientific Advisory Committee (SAC) (Section 12.1). These measures of success should be based on the goals and objectives of the OREHP (Section 1.4) and should include criteria such as:

> *Hatchery releases will contribute at least X percent to the recreational and commercial landings annually.*

Monitoring will show less than Y percent change in the frequency of rare alleles after 5 years of hatchery releases.

Benthic monitoring will show less than Z percent change in key indicators attributable to growout pen operations between each round of benthic monitoring.

The measures of success should be specified by the SAC prior to the planned program evaluation by the Department.

1.2.4 Genetic resource management

The OREHP has made genetic resource management a priority since the early years of the program. Genetic resource management includes the genetic status of the stock to be enhanced, genetic goals of the enhancement program, and the approach for managing genetic impacts. Studies to examine the genetic structure of wild seabass were initiated in the mid-late 1980s and have ran parallel to the culture and assessment research (Bartley and Kent 1990).

One of the goals of the OREHP is to release cultured white seabass that have genetic diversity very similar to that of the wild population. The OREHP currently uses best management practices (BMPs) (Sections 5.2.3 and 5.3) to maximize the number of parents contributing to white seabass production. These BMPs will remain in place until a genetic management plan is developed and incorporated as part of the White Seabass Enhancement Plan (WSEP). The genetic management plan will be based on the results of genetic research currently being conducted by Hubbs-SeaWorld Research Institute (HSWRI) and should be completed and approved by the SAC within the next five years.

1.2.5 Fish health management

Maintaining fish health has always been a part of the OREHP. The goal is to ensure that no ill fish are released into the wild and that no novel disease is introduced into the wild white seabass population. To that end, the Department has committed a fulltime Fish Pathologist to the OREHP since the hatchery was built. HSWRI's resident veterinarian also participates in disease management for the OREHP. The current Fish Pathologist has greatly expanded our knowledge of pathogens affecting cultured and wild white seabass, enabling the OREHP to manage fish health effectively. Additionally, the OREHP routinely contracts with pathology researchers from the University of California, Davis (UCD). Chapter 7 details the BMPs for fish health management.

1.2.6 Enhancement objectives and tactics

An enhancement plan should contain all the available information regarding the ecological and biological mechanisms affecting the species to be enhanced. Information gaps should be filled by research projects designed to answer critical questions.

When the OREHP began, there was a lack of information regarding the early life stages of white seabass. By coordinating with local universities, several Master and PhD research projects were designed to expand our understanding of these early life stages. Dutton (1989), Donohoe (1990), and Kim (1987) investigated various aspects of white seabass larvae. Ragen (1990) estimated the pre-fishing biomass of white seabass and Franklin (1997) investigated the population structure of white seabass using DNA analysis. More recently, Smiley (2004) investigated the effects of gas supersaturation (GSS) on cultured white seabass.

The results of these studies and others have led to improved hatchery practices, provided information on the historical and current white seabass population, and helped define factors that can contribute to the success or failure of hatchery releases. Additionally, the research has helped to provide information that can be used during the program evaluation.

1.2.7 Identify hatchery-raised fish and assess stocking efforts

Since the OREHP's inception, all cultured white seabass have been marked. At first, fish were treated with oxytetracycline, a chemical marker used to mark time that is retained on the otolith and is visible under fluorescent light. As new technology developed, the OREHP began marking fish with coded wire tags (CWT) imbedded in the cheek muscle.

Since the mid-late1980s, the OREHP has contracted with researchers to develop juvenile and adult sampling programs to assess the proportion of hatchery-raised fish to the wild population. From 1988 to 2008, researchers at California State University, Northridge (CSUN); Occidental College; San Diego State University (SDSU); and HSWRI conducted a standardized gill net sampling survey designed to capture 1- to 4-year-old juvenile white seabass in shallow waters off southern California (Section 11.1). Initially, the survey focused on determining the distribution of young fish, but switched in 1996 to look at recruitment of 1-year-old fish and recovery of tagged fish. In the late 1990s, HSWRI researchers developed a sampling program to recover adult hatchery-raised white seabass from the commercial and recreational fisheries (Section 11.2). The program, which is ongoing, is aimed at scanning white seabass for the presence of a CWT. The results of both the juvenile and adult sampling programs will be used in evaluating the success of the OREHP.

1.2.8 Define optimum release strategies

Until the hatchery came online in late 1995, releases were very small and limited primarily to San Diego County. With the advent of the growout facilities, the hatchery releases have increased in size, frequency, and distribution throughout the Southern California Bight. The OREHP's current strategy is to release fish from the growout facilities during the spring, summer, and fall months because research has shown that white seabass have a higher survival rate during this time period than during other times in the year. Direct releases (fish released into the ocean without spending time at a

growout facility) will occur in the spring. At present, fish are released when they are 200 to 250 mm (8 to10 in.) total length (TL), based on the results of the bioeconomic model (Section 1.2.9), which suggests that this size yields the greatest return for the investment. Additionally, fish of this size are less vulnerable to disease when stressed than smaller fish. The OREHP also releases the majority of fish from the growout facilities, recognizing that these fish are more likely to survive than fish released directly in the ocean. Additional information on releases can be found in Section 6.7.

The Carlsbad hatchery was designed and constructed to support the production of more than 350,000 tagged juveniles per year. However, from 1996 to 2004, the OREHP was operating under a 125,000 fish annual release limit imposed by the California Coastal Commission (CCC) as a condition of the Coastal Development Permits (CDPs) for the growout facilities. This release limit was derived as a proportion of the breeding population that was housed at the hatchery in 1995. During that time, the hatchery increased the breeding population to 200 adult fish as specified in the original plan. Upon meeting the target broodstock population size and demonstrating the capacity to rear several hundred thousand juveniles, the OREHP requested and the CCC granted an increase to the release limit to 350,000 fish from 2004 until 2006. In 2007, the release limit dropped back to the earlier 125,000 fish because the breeding population decreased by 20 percent due to mortalities and the inability to rotate new broodstock into the hatchery.

In 2009, the Department and the OREAP submitted a request to the CCC proposing that the release limit be based on a proportion of the current breeding population housed at the hatchery (sliding scale release limit). The CCC agreed to this proposal, and the sliding scale release limit was implemented in 2010. Under this proposal, the annual release limit is calculated by dividing the current number of broodstock by 200 and multiplying that percentage by the production capability of 350,000. The current release limit is set at 287,000 fish. However, because the number of broad fish at the hatchery changes every few months due to mortalities or additions, the release limit is recalculated on January 10 and June 10 of each year.

1.2.9 Economic and policy objectives

The goals and objectives of the OREHP were developed early on (Section 1.4) and included determining if it was economically feasible. A bioeconomic model was developed by Botsford et al. (1988) to determine the feasibility of enhancement and guide research and planning. Based on 1988 fishing regulations and a natural mortality rate of 0.13, the cost per stocked fish was estimated to be $2.00. The bioeconomic model was developed before the hatchery was built and has not been updated to reflect hatchery operations or recent research on white seabass.

1.2.10 Adaptive management

Adaptive management provides a mechanism to adjust fish production and management via ongoing assessment of the different components of the enhancement

plan. For example, a critical component is the genetic management. As more is learned about the wild population, the contribution of broodstock to the production of progeny, and their recruitment to the adult population, the number of fish released annually can be adjusted upward or downward depending on their genetic diversity so that the genetic diversity of the wild population is not adversely impacted. The SAC will be instrumental in assessing the new information and whether changes in hatchery practices are needed to meet the goals and objectives of the enhancement plan.

1.2.11 Minimize environmental impacts

To ensure that impacts to the benthos are minimal and will remain minimal, the OREHP instituted a benthic monitoring program for all the growout facilities, with the exception of the land-based facility. BMPs for growout facilities (Sections 6.5, 9.1.5.2, 9.1.6.2, 9.1.7.2, 9.1.8.2, and 9.1.9.2) identify interim threshold levels of sulfides and other elements, along with steps to take if these thresholds are exceeded. By 2012, sufficient data should be collected that the SAC can use to evaluate these threshold levels, adjusting them as needed to protect the benthic environment around the growout facilities.

BMPs have been implemented at each facility that include monitoring feeding activity to minimize excess feed and associated fallout, cleaning raceways daily to prevent buildup of feces and feed, and cleaning the predator barriers and containment nets to keep water flowing through the facility.

The San Diego Regional Water Quality Control Board (SDRWQCB) does not require the hatchery to operate under a National Pollution Discharge Elimination System Permit (NPDES). However, the hatchery is required to monitor the intake and effluent flow volumes and pollutant levels and submit an annual monitoring report.

1.3 Background of the OREHP

The Department has managed the OREHP since 1983. The Legislature established the OREHP (FGC §6590 et. seq.) to conduct a program of basic and applied research into the artificial propagation, rearing and stocking of important marine finfish species occurring in ocean waters off southern California. Over the years, the Legislature has amended the intent language of the program with current legislation calling for a focus on determining if hatchery released fish can artificially enhance certain stocks of desirable species through increased hatchery production of fish and increased monitoring of fisheries to assess the hatchery contribution. The ultimate goal of the legislation is to enhance populations of marine finfish species important to California for their sport and commercial fishing value. White seabass have been chosen as the primary species on which to focus research.

The Department administers the OREHP, with the assistance of the 10-member Ocean Resources Enhancement Advisory Panel (OREAP). The Department's main contractor is HSWRI. HSWRI operates the marine fish hatchery that raises white seabass. As

part of their OREHP contractual obligations, HSWRI has developed the culture protocols required for the program, as well as the assessment techniques that will help evaluate the impact of the hatchery-reared fish on the recreational and commercial fisheries. A Department Fish Pathologist works in conjunction with HSWRI staff to investigate and manage disease issues within the OREHP. Researchers at SDSU and CSUN have also conducted research under contract with the Department to determine the relative amount of juvenile white seabass recruitment annually, for both wild and hatchery-raised fish.

In addition to these contractors, the OREHP receives considerable support (20,000 hours/year) from volunteers, primarily recreational angler groups, who own and operate the growout facilities in southern California. These growout facilities provide a cost-effective way to increase post-release survival by raising larger white seabass prior to release.

In addition to the OREHP-sponsored research and volunteer support, HSWRI and the Department have obtained research grants to support collaborative projects in fish health, physiology, systems design, post-release acoustic tracking, genetics, etc.

1.4 Goals of the OREHP

The primary goal of the OREHP is to evaluate the economic and ecological feasibility of releasing hatchery-reared fish to restore depleted, endemic, marine fish populations to a higher, sustainable level. Achievement of this enhancement goal will occur through completion of the following objectives:

(1) Develop and implement hatchery operation and growout methods that provide a supply of healthy and vigorous fish;

(2) Conduct the replenishment program in a manner that will avoid any significant environmental impacts resulting from operation of either the hatchery or pen rearing facilities;

(3) Maintain and assess a broodstock management plan that results in progeny being released that have genotypic diversity very similar to that of the wild population;

(4) Quantify contributions to the standing stock in definitive terms by tagging fish prior to release and assessing their survival in the field;

(5) Continue to develop, evaluate, and refine hatchery operations to maximize the potential for achieving the goal of the program;

(6) Develop quantitive measures of success.

Chapter 2. Biological Information for White Seabass

2.1 Description

Seven species of croakers (Family Sciaenidae) are native to the West Coast of the United States and off Baja California (Collins 1981). As a group, coakers exhibit strong estuarine ties during all or part of their lifecycle (Weinstein 1981). Most croakers emit sounds, which have been variously described as 'drumming', 'croaking', 'grunting', 'snoring', 'bellowing', 'purring', 'buzzing' and 'whistling' (Welsh and Breder 1923). These sounds are produced by vibrations of the swim bladder.

The white seabass, *Atractoscion nobilis*, is the largest croaker species in California waters (Thomas 1968). Adults are bluish to gray dorsally with dark speckling, and silver-to white-colored ventrally. Juveniles have several dark vertical bars. White seabass have been recorded to1.6 m (5.2 ft) total length and 42 kg (93 lbs); however, individuals larger than 27 kg (60 lbs) are rarely observed (Thomas 1968).

Fossil records of white seabass have been found in several southern California Pleistocene deposits and in a Pliocene site at San Diego. Some deposits are probably 10 to 12 million years old (Fitch and Lavenberg 1971).

2.2 Distribution, genetic stock structure, and migration

White seabass range over the continental shelf of the Eastern North Pacific Ocean from Juneau, Alaska, to Magdalena Bay, Baja California, Mexico. This species also inhabits the upper Gulf of California, Mexico, as a subpopulation that appears to be isolated from the coastal mainland megapopulation (or stock) (Thomas 1968).

California Cooperative Oceanic Fisheries Investigations (CalCOFI) zooplankton data collected between 1950 and 1978 indicate that white seabass larvae appear to settle out into coastal areas extending from Santa Rosa Island, California to Bahia Santa Maria, half way down the Baja California peninsula (Moser et al. 1983). Fifteen percent of documented occurrences were in California waters. Most of the larvae occurred from May to August and peaked in July. White seabass larvae were collected within San Francisco Bay (Richardson Bay) during a 1972 to 1973 study (Eldridge 1977). That timing of collections was correlated with upwelling in adjacent ocean waters.

In the past, it was assumed that white seabass in California waters consisted of non-resident fish that migrated into the Southern California Bight from Baja California, Mexico. However, white seabass off the coasts of California and Baja California, Mexico are currently considered to be part of the same breeding population, and the center of this population appears to be off central Baja California, Mexico (Moser et al. 1983, Vojkovich and Reed 1983, Franklin 1997).

Bartley and Kent (1990) attempted to describe the genetic structure of the white seabass population in the Southern California Bight. They also looked at the genetic diversity of hatchery fish. The results of the study showed that white seabass in the Southern California Bight region appear to be genetically similar.

Franklin (1997) examined white seabass DNA from fish collected between 1990 and 1995 in Californian and Mexican waters. He found that there were local spawning groups within the Southern California Bight that contribute to the genetic make-up of the population. Based on this research, Franklin (1997) concluded that the white seabass stock in the Eastern Pacific Ocean is composed of three components: northern, southern, and Sea of Cortez. The northern component of the white seabass populations ranges from Point Conception, California to central Baja California, Mexico (Franklin 1997).

Recruitment of young white seabass to coastal habitats in southern California is probably related to the strength and persistence of northward flowing warm water currents (Allen and Franklin 1992). However, the exact relationship is still unknown. Although previous white seabass tagging studies for migration have been unsuccessful (Maxwell 1977), hatchery-produced white seabass have been recaptured as far as 100 nautical miles from the point of release (Drawbridge et al. 2007). Catch data indicate that white seabass move northward with seasonally warming ocean temperatures (Skogsberg 1939, Radovich 1961, Karpov et al. 1995). For example, there were substantial commercial catches of white seabass near San Francisco Bay, Tomales Bay, and Monterey Bay during the early 1900s when ocean waters were warmer, followed by a long period in which landings from the central California coast were rare. Since 1999, commercial catches of white seabass have increased north of Point Conception (Table 2-1; CDFG, unpubl. data) possibly indicating a recent northward shift in the stock due to warmer waters brought up during El Niño/Southern Oscillation events.

Table 2-1. The commercial catch of white seabass (pounds) in the San Francisco Bay area, 1986 to 2008[1].

Year	Outside San Francisco Bay	Inside San Francisco Bay
1986	264	0
1987	0	0
1988	35	0
1989	69	0
1990	0	0
1991	0	0
1992	133	0
1993	184	0
1994	87	0
1995	175	0
1996	40	0
1997	1,531	19
1998	1,743	0

Table 2-1. The commercial catch of white seabass (pounds) in the San Francisco Bay area, 1986 to 2008[1].

Year	Outside San Francisco Bay	Inside San Francisco Bay
1999	1,324	0
2000	3,170	0
2001	5,492	20
2002	1,399	0
2003	3,986	253
2004	2,538	853
2005	5,214	0
2006	3,435	56
2007	8,493	29
2008	430	0

Note: 1. All data from CDFG's Commercial Fishery Information System (CFIS) landing data. Landings prior to 1986 are not available.

2.3 Age and growth

The age and growth of white seabass have been determined by reading scales and otoliths. Thomas (1968) used scales but found them difficult to read for individuals older than 13 years. A 711 mm (28 in.) white seabass (the minimum legal size) was determined to be 5 years old and weigh about 3 kg (7 lbs).

The white seabass length-weight relationship was described in Thomas (1968) by the equation:

$$W = 0.000015491 * L^{2.9216}$$

where length is in millimeters and weight is in grams. However, this may not be an accurate estimator for all lengths since only mature fish of both sexes were used in Thomas' (1968) calculations. Data from otoliths indicate that white seabass can grow very quickly, especially during the first 4 years (Table 2-2). A 1998 study by the Department, using sectioned otoliths from fish caught between 1991 and 1996, found that white seabass grow much faster than previously thought, indicating that larger individuals are considerably younger than previous estimates (CDFG 2002). The von Bertalanffy growth equation for juvenile and adult fishes of both sexes was calculated to be:

$$L_t = 1391\left[1 - e^{-0.0156(t+1.297)}\right]$$

Growth rates for males and females were not evaluated separately. The oldest fish aged was 27 years and measured 1,365 mm (54 in.) TL. These otolith data indicate that a 711 mm (28 in.) white seabass is approximately 3 years old. In contrast, the same fish would be 5 years old according to Thomas' (1968) scale data.

The age estimates based on otolith data were closer to those proposed by Clark (1930), who investigated white seabass gross gonadal development. She estimated fish less than 35 cm (13.7 in) were 1-year-old; fish between 35 and 65 cm (13.7 and 25.6 in.) were 2 years old; and fish larger than 75 cm (29.5 in) were 3 years old or older.

The discrepancies between Thomas' (1968) study and the more recent Department study may be partly due to the following: first, different ageing structures were used in each study; and second, the Department's study was conducted during a period of oceanic warming which may have influenced (increased) white seabass growth rates.

Table 2-2. Mean total length and weight at age for white seabass (taken from CDFG 2002).

Age class (years)	Mean length in mm (in.) using scales[1]	Mean length in mm (in.) using otoliths[2]	Weight in kg (lbs)
0	-	274 (10.8)	0.2 (0.5)
1	231 (9.1)	411 (16.2)	0.7 (1.5)
2	336 (13.2)	542 (21.3)	1.5 (3.3)
3	467 (18.4)	685 (27.0)	3.0 (6.6)
4	571 (22.5)	808 (31.8)	4.8 (10.7)
5	723 (28.5)	867 (34.1)	5.9 (13.1)
6	866 (34.1)	985 (38.8)	8.6 (19.0)
7	929 (36.6)	1,004 (39.5)	9.1 (20.1)
8	981 (38.6)	1,063 (41.8)	10.8 (23.8)
9	1,033 (40.7)	1,130 (44.5)	12.9 (28.4)
10	1,072 (42.2)	1,072 (42.5)	11.0 (24.4)
11	1,144 (45.0)	1,269 (50.0)	18.1 (39.9)
12	1,194 (47.0)	1,183 (46.6)	14.7 (32.5)
13	1,217 (47.9)	1,131 (44.5)	12.9 (28.5)
14	-	1,229 (48.4)	16.5 (36.3)
17	-	1,245 (49.0)	17.1 (37.7)
27	-	1,368 (53.7)	22.4 (49.3)

Note: 1. Data using scales from Thomas (1968).
2. Data using otoliths from CDFG unpublished data; small sample size for age classes 7 and older.

2.4 Reproduction, fecundity, and seasonality

The exact location of spawning areas have not been determined, but data indicate that peak spawning occurs in southern California from April through August (Skogsberg 1925). During this period, mature fish appear to congregate near shore, over rocky habitat, and near kelp beds (Thomas 1968).

Aalbers (2008) studied the spawning behavior and sound production of white seabass in a net pen off Santa Catalina Island and found that spawning occurred from March through July and peaked in May at a photoperiod of 14 hours. Most spawning occurred within the two hour period following sunset or from 19:00 to 20:00 hours Pacific Standard Time. White seabass spawned at every phase of the lunar cycle; but an increase in successive spawning events followed the new moon. Most spawning occurred in water temperatures from 15 to 18°C (59 to 64°F), and there was no

apparent correlation with tidal cycles. Seasonal and diel spawning periods were directly correlated with increases in the rate, intensity, and variety of white seabass sounds; this correlation may indicate that sounds function to enhance reproductive success (Aalbers 2008).

Aalbers and Drawbridge (2008) reported that gravid females are identifiable during courtship and spawning by shifts in behavior and the development of dark bars across the dorsal region. During numerous observed spawning events in a net pen off Santa Catalina Island, one to nine males were observed to tightly surround a gravid female and the resultant pack shuddered in unison as gametes were simultaneously broadcast into the water column. Five distinct types of sound were reportedly produced by white seabass: single and multiple pulse trains during courtship, drumrolls and thuds during spawning, and booms during yawning and burst swimming. During the actual release of gametes, a rapid succession of overlapping drumroll and thud sounds resulted in identifiable spawning chants lasting 7 to 55 seconds. Consistent physical, behavioral, and acoustical patterns during courtship and spawning indicated that white seabass utilize visual, tactile, and sonic cues to communicate their reproductive state.

A study of white seabass maturity in the late 1920s indicated that females begin maturing when they are near 607 mm (24 in.) TL, and males may reach sexual maturity at about 508 mm (20 in.) TL. All white seabass have probably spawned at least once by the time they reach 800 mm (31.5 in.) TL (Clark 1930).

White seabass have the largest eggs of the west coast sciaenids at approximately 1.24 mm. These eggs are buoyant and drift with the ocean currents. The dark-colored larvae appear to settle out in coastal areas (Moser et al. 1983). Fecundity has been estimated from ongoing artificial propagation of the species since 1984. Drawbridge (2003) reported that, in the hatchery setting, female seabass starting at 5 kg (11 lbs) released an average of 700,000 eggs per batch, increasing at a rate of approximately 100,000 eggs/kg as the females grew. The relationship between body size and fecundity was evident for fish up to 13 kg (29 lbs) but was not evaluated beyond that to see if it continued (Drawbridge 2003).

Although it has been reported that white seabass spawn more than once per season, the number of spawns per female and the spawning intervals for individual females are unknown. Drawbridge (2003) reported that an isolated female of 10 kg (22 lbs) released 1.2 and 1.4 million eggs during spawning events spaced 10 days apart.

2.5 Natural mortality

Thomas (1968) calculated a natural mortality rate of 0.303 for fish caught in commercial gill nets. These fish represented the majority of commercially-caught white seabass. Recently, natural mortality rates were determined for juvenile white seabass based on the OREHP data. Kent and Ford (1990) found that natural mortality rates range from 0.258 (1 and 2 year-old fish) to 0.117 (3 and 4 year-old fish). Likewise, MacCall et al. (1976) and Dayton and MacCall (1992) calculated natural mortality rates for white

seabass from the recreational and commercial fisheries that were significantly lower than Thomas' (1968) estimate (Table 2-3). In light of these values, it would seem that Thomas' estimate was an overestimate since natural mortality rates usually decline and level off as fish age.

Table 2-3. Estimates of white seabass natural mortality (M) (taken from CDFG 2002).

Source	M
Thomas (1968)	0.303
MacCall et al. (1976)	0.13
Kent and Ford (1990)	0.258 (1 to 2 yr old); 0.117 (3 to 4 yr old)
Dayton and MacCall (1992)	0.08

Chapter 3. History of the Fisheries

3.1 Introduction

During the past century, white seabass have been one of the most important commercial and recreational fisheries in California. The resource has been shared by recreational and commercial fishermen since the late 1890s. Historically, recreational fisherman have mainly caught white seabass using hook-and-line gear, while the commercial fishery has been comprised of fishermen who use set and drift gill nets or hook-and-line gear. Both recreational and commercial landings fluctuated during much of the 20th century; however, since the 1950s, the general trend has been one of decline. This decreasing trend in both commercial and recreational landings was an important factor in the decision to use white seabass in the OREHP as discussed in Section 4.2.

3.2 Recreational fishery

Recreational fishing for white seabass began around the turn of the century. Because of their size and elusive nature, white seabass are popular with anglers. The Avalon Tuna Club's weight records from the early 1900s include white seabass catch (Dayton and MacCall 1992) while historical records show that CPFV anglers, fishing in California waters, landed an average of 33,400 fish annually from 1947 to 1959 (Figure 3-1). The catch steadily declined to an average of 10,400 fish in the 1960s, 3,400 fish in the 1970s, and 1,200 fish in the 1980s. In the 1990s, the white seabass catch began to increase with an average of 3,000 fish. From 2000 through 2008, an annual average of 8,200 fish were caught, most likely a result of stronger recruitment of young white seabass in 1997 and 1998. Additional seabass are caught by divers and anglers aboard private boats, but accurate catches by these users are difficult to estimate.

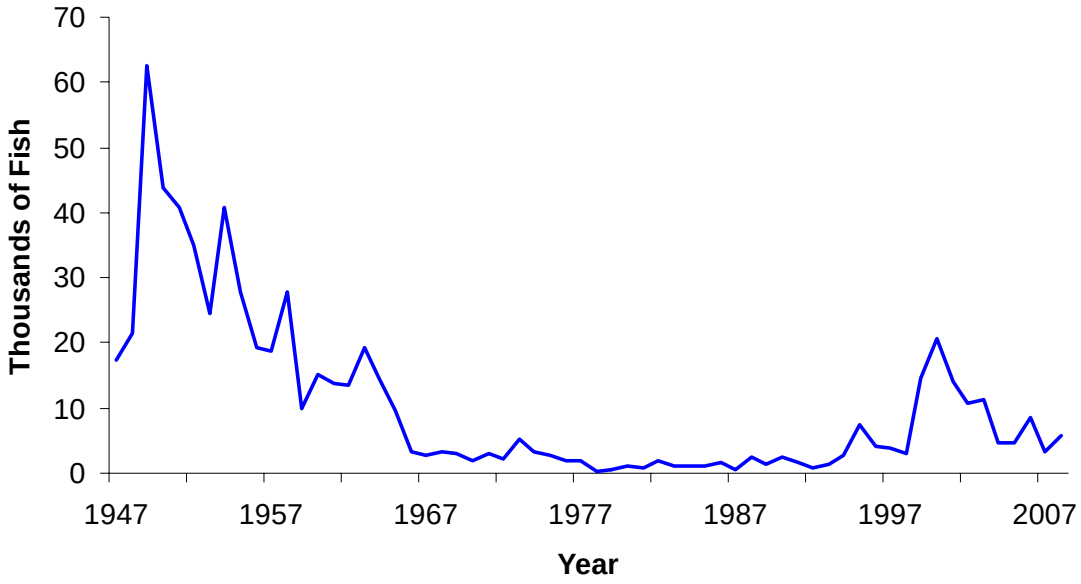

Figure 3-1. Recreational catch of white seabass in California, 1947 through 2008.

Notes:

1. Fish caught in U.S. waters only (does not include fish caught in Mexico and landed in California).
2. Recreational catch as reported by CPFV logbooks.

3.3 Commercial fishery

Commercial white seabass landings have fluctuated dramatically over the years. Landings were moderate during the late 1800s but grew impressively from 1889 to 1915. By 1904, over 950,000 pounds were landed annually. A peak in commercial white seabass landings came in 1959, when warm water increased white seabass availability and over three million pounds were landed (Figure 3-2). After the 1958-59 El Niño, landings sharply decreased in the 1960s and continued to decline during the 1970s and 1980s. Since 1999, however, landings have begun to increase, exceeding over 650,000 pounds in 2008 (Figure 3-2).

Today, catches of white seabass are concentrated along the coast from Point Conception to San Diego and around the Channel Islands. Catches from central and northern California were substantial during the late 1800s and early 1900s; however, the center of the fishery shifted to southern California by 1916 (CDFG 2002). Although the frequency of white seabass caught north of Point Conception has increased, these landings still represent less than 20 percent of the total California catch. An exception occurred in 2001, when 36 percent of commercial white seabass landings occurred north of Point Conception.

Historically, commercial catches were made using gill nets, hook-and-line, and round haul nets such as lamparas and purse seines. Purse seining was curtailed in the late 1920s because decreasing catches made it uneconomical. Since the take of white

seabass by round haul nets was prohibited in the early 1940s, gill nets have been the major commercial fishing gear. Set gill net fishing for white seabass within state waters was prohibited beginning in 1994. Today, drift gill netting is the primary fishing method used. Some commercial hook-and-line fishing takes place during the early spring in southern California when large white seabass are available. Further changes in take of white seabass due to gear regulations are discussed in Section 4.1.

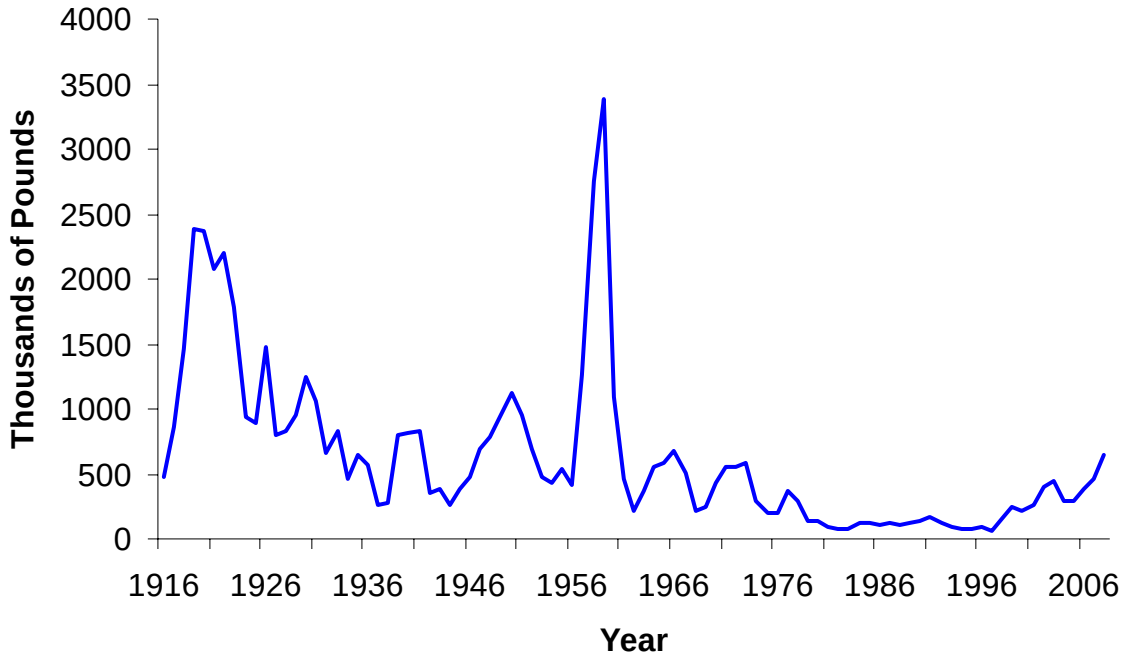

Figure 3-2. Commercial catch of white seabass in California, 1916 through 2008.

Notes:

1. Fish caught in U.S. waters only (does not include fish caught in Mexico and landed in California).
2. 1916 – 1935 commercial California catches from Heimann and Carlisle Jr. (1970).
3. 1936 – 1964 commercial California catches from Collyer (1949) and Thomas (1968).
4. 1965 – 2008 commercial landings from CDFG CFIS data.

Chapter 4. History of Conservation and Enhancement Efforts

4.1 Regulatory history

Declining white seabass landings in the late 1920s and during most of the 1930s led to a series of regulations designed to stabilize the catch (Young 1973). The first of these regulations was instituted in 1931, aimed primarily at the commercial fishery (Table 4-1). The first regulations enacted were a commercial fishing closure during May and June and a commercial minimum size limit of 711 mm (28 in.). The main purposes of these restrictions were to protect seabass during spawning, and to provide for spawning opportunities, at least twice, before the fish were caught (Skogsberg 1939). The use of purse seine and other roundhaul nets to take white seabass in waters off California was prohibited in 1940; however, their use in Mexican waters was still allowed and fishermen could transit through California waters with purse seine-caught fish under a Department-issued permit. In addition to this commercial gear change, a minimum gill net mesh size of 89 mm (3.5 in.) was also established in 1941. The gill net mesh size was increased to 152 mm (6 in.) in 1988. Four years later, California State Proposition 132 banned the use of gill and trammel nets in state waters along the mainland shore south of Point Arguello, Santa Barbara County, and 1 mile offshore or within 70 fm (128 m) around the Channel Islands. In 2002, the Commission banned the use of gill nets within 70 fm (128 m) from Point Reyes, Marin County to Point Arguello.

Table 4-1. Summary of White Seabass Regulations from 1931 to the Present (modified from Vojkovich and Reed 1983 and CDFG 2002).

Date (License required)	Season length	Size limit	Bag limit	Gear and area restrictions	Special conditions
1931-33 Commercial: license required	July 1-April 30	Commercial: Minimum size 28 in; no more than 5 fish less than 28 in	None	No nets within 4-mi radius of San Juan Pt., Orange Co.; bait nets only in Santa Monica Bay.	5 fish any size with hook & line, but may not be sold
1933-35 (same)	Hook & line all year	Same	May 1-Jun 30 (5 per day - hook & line)	Same	After Oct. 25, 1933, no fish may be sold from May 1-June 30
1935-37 (same)	No net fishing May 1-Aug 31	Same	May 1-Aug 31 500 lbs/person; 2500 lbs/boat	No nets in any Orange Co. waters (later rescinded)	Same
1937-39 Sportfish: license required	Same	Sportfish and Commercial: Minimum size 28 in; no more than 5 fish less than 28 in	Sportfish: 15/day for anyone on sportfish boat	Same	Sport-caught fish may not be sold
1939-41 (same)	Year round net fishing allowed	Same	Same	No purse seines. Gill net mesh size minimum 3 ½ in	Same
1941-49 (same)	Same	Same	Same	Same	Same
1949-53 (same)	Same	Same	Sportfish: 10/day/sport boat	Same	Same

Table 4-1. Summary of White Seabass Regulations from 1931 to the Present (modified from Vojkovich and Reed 1983 and CDFG 2002).

Date (License required)	Season length	Size limit	Bag limit	Gear and area restrictions	Special conditions
1953-57 (same)	Same	Same	Commercial: 1000 lbs/person/day; 5000 lbs/boat/day.	Same	Same
1957-71 (same)	Same	Sportfish: 2 fish less than 28 in	Sportfish: 10/day/sport boat	Same	Same
1971-73 (same)	Same	Sportfish and Commercial: No fish less than 28 in	Same	Same	Same
1973-78 (same)	Same	Sportfish and Commercial: One fish less than 28 in	Same	Same	Same
1978-80 (same)	Same	Sportfish and Commercial: No fish less than 28 in	Same	Same	Same
1980-82 (same)	Season closed Mar 15-Jun 15	Same	Sportfish: 3/day/person	Same	Logs required Permits required
1982-84 (same)	Same	Same	Same	Area closures for nets with mesh less than 6 in	Permits no longer required
1984-94 (same)	Same	Same	Sportfish: 1 white seabass/day/person during closed season	Same	Same
1994-00 (same)	Same	Same	Same	No Gill or trammel nets allowed 0-3 mi from shore along the mainland, or within 1 mi or waters less than 70 fm deep at the offshore islands from Point Arguello, Santa Barbara Co. to the United States - Mexico Border, and in waters less than 35 fm deep from Point Fermin, Los Angeles Co. to the south jetty Newport Harbor, Orange Co.	Same
2000-02 (same)	Same	Same	Commercial: 1 seabass/day/boat during closed season with gill net	Same	Same
2002-present (same)	Same	Same	Same	No gill or trammel nets allowed in waters less than 70 fm deep from Point Reyes, Marin Co, to Point Arguello, Santa Barbara Co.	Same

4.2 History of the OREHP

The OREHP began in 1983 as a result of legislation (Assembly Bill 1414) authored by California Assemblyman Larry Stirling. The legislation was adopted to fund research and development into the artificial propagation of marine finfish species whose populations had become depleted, with the intent of enhancing those populations.

To fund the program, the legislation required the purchase of an Ocean Enhancement Stamp by all recreational anglers and commercial passenger fishing vessels fishing south of Point Arguello, Santa Barbara County. Commercial fishermen are also required to purchase an Ocean Enhancement Stamp if they fish for white seabass south of Point Arguello. Since the late 1980s, the OREHP funding has been augmented by federal Sport Fish Restoration Act (SFRA) money.

Assembly Bill 1414 (Stirling) also created the OREAP consisting of academic and management agency scientists, representatives of both commercial and recreational fishing groups, and the aquaculture industry. The OREAP provides assistance to the Director of the Department in establishing policy and direction for the OREHP. Additionally, the annual budget for the OREHP is determined jointly by the OREAP and the Department.

In 1983, the OREAP identified white seabass and California halibut (*Paralichthys californicus)* as the most appropriate species for use in an experimental stocking program. Original selection criteria included:

- Species indigenous to southern California
- Status as a diminished stock
- Economic value
- Both commercial and sport utilization
- Potential for success

During the first six years of the program, research focused on the capture, maintenance, spawning (both natural and artificial), and grow-out to release size for white seabass and California halibut. Additionally, work was undertaken to determine juvenile natural mortality, juvenile distribution in the wild, post-release survivability of hatchery reared fish, and marking methods to identify hatchery reared fish in the wild. Finally, a cost/benefit model was developed to evaluate the economic feasibility of the OREHP.

Beginning in 1990, the OREHP research focused on white seabass with only limited effort on California halibut. The reduction in research on halibut was necessary because of limited funding and increased expenses associated with producing 100,000 white seabass annually for release. Raising and releasing a large number of juvenile white seabass was undertaken to gain experience with new hatchery protocols associated with increased production and provide juveniles for release and recapture studies. In addition, the recapture field work provided data on juvenile distribution and natural mortality.

To facilitate the rearing of increased numbers of white seabass, the OREHP accepted an offer by United Anglers of Southern California (UASC) to equip and run a growout facility at Channel Islands Harbor, Oxnard, California. This facility first accepted fish in 1992. Since then, an additional 12 volunteer growout facilities have come online at various sites from Santa Barbara to Mission Bay, San Diego. These facilities are operated by UASC chapters, nonprofit organizations, and HSWRI.

Concurrent with the passage of the OREHP legislation in 1992 that removed the OREHP's sunset provisions, the CCC authorized the use of $1.2 million in mitigation funds to be paid by Southern California Edison (SCE) for environmental effects of the San Onofre Nuclear Generating Station (SONGS). The mitigation funds were to be used by the OREHP for capital construction of a marine fish hatchery and enhanced recovery of fish in the field. A 1993 Memorandum of Agreement (MOA) between the CCC, Department, OREAP, and SCE covered financing, construction, and operation of the proposed hatchery. Construction began in July 1994, and the hatchery was dedicated on October 13, 1995.

Soon after initial completion of the hatchery, it became apparent that funding for construction was not adequate to totally build-out the facility, nor was Ocean Enhancement Stamp revenue sufficient to cover the costs of operating a larger facility. Additionally, field sampling to recover tagged fish was proving to be more costly than anticipated. Acting on a recommendation developed by the CCC staff in conjunction with the Department, the CCC authorized an additional $3.6 million in SONGS mitigation. The 1997 MOA between the CCC, Department, and OREAP stated that the funds were to be used to reduce the debt incurred during initial construction of the hatchery, to provide funding for equipment to build-out the hatchery, and to supplement operating funds over the next eight years.

Additional mitigation funding for the OREHP became available in 2003 as the result of a settlement between the Department and British Petroleum for the American Trader oil spill off Huntington Beach in 1991. Over $585,000 was given to the Department as mitigation for fish killed as a result of the spill. These funds were used by the OREHP to augment existing funding for hatchery operations, including release of juvenile fish into the ocean.

HSWRI owns and operates the hatchery but leases the land from NRG Cabrillo Power I LLC. When the hatchery was built, San Diego Gas & Electric was the landowner. In 1999, NRG Cabrillo Power Inc. purchased the land and continued with the hatchery's lease.

<u>The OREHP Milestones</u>

- October 1986 – the first experimental release of more than 2,000 juvenile white seabass took place at HSWRI's research facility in Mission Bay, San Diego, California
- March 1992 – first legal-sized oxytetracycline-marked hatchery-raised white seabass recapture
- October 1995 – the marine fish hatchery became operational
- June 1999 – first legal-sized coded wire-tagged hatchery-raised white seabass recapture
- 2001 – the first year more than 100,000 white seabass were released in southern California waters
- October 2004 – the 1,000,000th white seabass was released
- June 2007 – oldest (13 years) adult fish recovery

Part 2 - Best Management Practices

The CHP (Drawbridge and Okihiro 2007) covers all aspects of hatchery operations including plant management, broodstock care, egg production (spawning), nursery phase, and raceway culture. The GPM (Drawbridge and Okihiro 2007) covers the growout and subsequent release of juvenile white seabass. The BMPs are taken largely from these two documents.

Chapter 5. Hatchery Operations

5.1 Plant management and biosecurity measures

Biosecurity is an all encompassing concept whose primary goal is to prevent infectious disease agents from gaining entrance into the hatchery. Failing that, a secondary goal is to detect infectious diseases at the growout facilities and minimize spread. Components of biosecurity include: proper system layout and compartmentalization, water treatment and sterilization, equipment and system disinfection, and quarantine. Proper biosecurity remains one of the most important factors limiting hatchery production of healthy fish, and is critical in the prevention of disease spread to wild stocks. Biosecurity is dependent on: 1) equipment and systems within the hatchery; 2) protocols and procedures used by hatchery personnel; 3) proper training of hatchery personnel; and 4) the proper mind set.

The hatchery has seven separate systems: larval food production, broodstock, egg incubation, juvenile 1 (J1), juvenile 2 (J2), raceway culture, and experimental. These systems are compartmentalized with each system operating on a separate water system to reduce the chance of infection and the spread of disease. Except for raceway culture, each system is a recirculating water system. As the water is recirculated it passes through a series of filters (bead, floating media, and/or sand), foam fractionators, and UV sterilizers. Filtration is different for each system and based on the needs of the different life stages. All “make-up” water (replaces water lost during the recirculation process) is sterilized using an ozone system. Make-up water destined for egg incubation, the J1 system, and the J2 system goes through a sand filter prior to ozone treatment. Sea water for the raceways comes directly from Agua Hedionda Lagoon and is sand-filtered but not sterilized.

Water temperature and water turnover rate are controlled by the Main Computer Control System (MCCS). Filter backwashing is also controlled by the MCCS. The MCCS will automatically page hatchery personnel should a change in air or water flow occur.

To help prevent the spread of disease, each of the seven systems has equipment (i.e., nets, brooms, scrubbers) dedicated to that system. Iodine foot baths are placed at the entrance of each system to minimize transfer of contaminants, while physical barriers prevent foot bath avoidance. New gloves are applied as hatchery personnel move between any two systems.

Hatchery personnel are also well trained to detect stress and the early signs of a disease outbreak. Healthy fish have good color, intact skin and fins, and are not thin. In contrast, sick fish are often darkly pigmented or have a mottled appearance (spotty pigmentation associated with diffuse protozoal infestations). Healthy fish will also school up and orient themselves with the prevailing current. They are typically active, respond rapidly to external stimuli (e.g. food, pool vibrations), and have a strong net avoidance behavior. Sick fish, however, exhibit a range of abnormal behaviors, depending on the pathogen and severity of infection. Non-specific abnormal behaviors include anorexia, lethargy, and isolation. Specific behaviors include "flashing" (attempting to rub gills or skin against hard surfaces) associated with external parasites, and whirling or spinning associated with central nervous system (CNS) disease. Accurate descriptions of abnormal behavior or physical condition can often help the pathologist identify etiologic agents, even before necropsies are performed, or help narrow the search for the causative agent.

The SDRWQCB does not require the hatchery to operate under a NPDES permit. However, HSWRI is required to monitor the intake and affluent flow volumes and pollutant levels at the hatchery. Hatchery staff is also required to maintain self-monitoring reports and submit annual reports to the SDRWQCB.

BMPs for plant management and biosecurity

- Evaluate traffic patterns and maintain each system separately in accordance with the CHP to prevent the spread of disease
- Disinfect equipment and systems in accordance with the CHP
- Label disinfection stations with color-coded signage for chemicals
- Maintain regular maintenance schedule for sterilization stations
- Maintain water quality in each system in accordance with the CHP
- Maintain quarantine protocols in accordance with the CHP
- Maintain proper training of hatchery personnel
- Conduct monitoring of the intake and effluent flow volumes and pollutant levels as required by the SDRWQCB Monitoring and Reporting Program No. 2001-237
- Maintain self-monitoring reports and submit an annual reports to the SDRWQCB

5.2 Broodstock care

5.2.1 Broodstock care and feeding

Broodstock are maintained in four separate pools that are temperature and photoperiod-controlled by the MCCS. The temperature and photoperiod controls provide for year-round spawning by induction of spawning pool by pool so spawning duty is rotated among the fish by pool. Fifty broodstock are maintained in each of the four pools for a total of 200 broodstock.

Broodstock are fed a diet of frozen sardines five times per week at a ratio of 0.5 to 1.0 percent of body weight per day. The diet is enhanced with vitamin supplements injected into the sardines three days per week. All food handling is conducted in accordance with United States Department of Agriculture (USDA) standards for research facilities holding live vertebrate organisms.

The broodstock diet has changed over time as the nutritional requirements of white seabass have been examined. As a result, egg quality has increased dramatically compared to early years of OREHP operation. Research into broodstock nutritional requirements is ongoing.

5.2.2 Broodstock collection and holding

Each year, a surplus number of broodstock are collected by cooperative collecting trips conducted by HSWRI staff; these broodstock are maintained at HSWRI's net pen at Santa Catalina Harbor or at additional growout facilities if needed. On these trips, HSWRI staff and volunteers use hook-and-line to target fish approximately 610 mm (24 in.) TL. To help ensure that the genetic diversity of hatchery-released progeny will be similar to wild populations, broodstock are collected only from the northern component of the white seabass population range from Point Conception, California to central Baja California, Mexico. This surplus group does not contribute gametes to the enhancement effort but is available to replace broodstock at the hatchery that die or are removed from the system.

After capture, broodstock are weighed, measured, sexed, genotyped, and implanted with a Passive Integrated Transponder (PIT) tag for identification. This information is maintained in the broodstock tracking system, a Microsoft Access database that also includes information regarding fish transfers, disease treatments, and deaths. Broodstock are also scanned for a CWT. If a fish scans positive for a CWT (i.e., the fish was hatchery-raised), it cannot be used as a brood fish and is euathanized to ensure the genetic diversity of the broodstock.

Broodstock are transported to the mainland on the return leg following the delivery of juveniles to the growout facility whenever possible. Delivery of juveniles typically occurs twice a year. On the mainland, broodstock are held at either Sea World, San Diego, California or in one of the quarantine pools at the hatchery.

Because all new broodstock are assumed to be caring lethal pathogen, they must be quarantined before entering the hatchery system to prevent potential disease outbreaks. Broodstock holding facilities at Sea World and Santa Catalina Island offer some opportunities for initial quarantine, but a secondary quarantine is initiated at the hatchery to control for secondary infections that may be caused by handling stress. When the fish arrive at the hatchery, they are placed in quarantine pools that are plumbed for recirculation and supplied with ozonated water from the main hatchery building. The fish are isolated for 45 days and are observed daily for disease. Should new fish break out with disease, necropsy and appropriate diagnostics are performed to

determine etiologic agents. Euthanasia of all new arrivals is an option if some new arrivals break with a novel disease, or if the disease is known to be lethal and highly contagious.

A fifth breeding pool has been assembled and will be used initially to move existing brood fish into so the original systems can be upgraded. When all the pools have been upgraded, the fifth pool will serve as a quarantine and reserve pool that can hold new stock until they are needed in the rotation schedule. Temperature control in this system will allow the fish to acclimate to the temperature of the target pool (13 to 18°C; 55 to 64°F), which may vary considerably from ambient water (12-25°C; 54 to 77°F).

5.2.3 Broodstock rotation

The original broodstock management plan, developed by Bartley et al. (1995) recommended a 1:1 sex ratio in each of the four broodstock pools, replacing five percent of the stock each year (10 fish), and rotating five males between the four broodstock pools annually to increase genetic diversity. During the past ten years, new stock was added inconsistently, primarily to replace fish that have died or were euthanized for health reasons. The replacement of fish during this time period averaged approximately seven percent annually. Male fish were not rotated among pools because white seabass are very skittish and netting fish can result in other fish jumping out of the tank or injuring themselves on the side of the pool.

After reviewing the spawning characteristics of white seabass at the hatchery, a revised broodstock management plan was recently developed to ensure that future program genetic goals are met. The revised plan adjusts the sex ratios (male: female) in each of the broodstock pools to 40:60 to account for unequal reproductive contribution of the sexes. It also replaces brood fish at a rate of 25 per year to ensure genetic mixing and to better account for the effect of generation time on effective population size. The need to rotate five males between each of the four breeding pools has been mitigated by replacing more fish per year than the original plan. Modification of the revised broodstock management plan will occur, if needed, as new information becomes available.

BMPs for broodstock care

- Maintain a population of up to 200 white seabass broodstock distributed between the four breeding pools with a 40:60 sex ratio
- Maintain a surplus broodstock population offsite of the hatchery or in quarantine pools at the hatchery
- Maintain sanitary conditions in all food preparation areas according to USDA standards and those set forth in the CHP
- Obtain white seabass broodstock as needed, while maintaining appropriate permits and/or permissions and collecting all pertinent information for each fish
- Scan new broodstock for a CWT to insure that no recaptured hatchery progeny become broodstock

- Weigh, measure, sex, genotype, and implant new broodstock with a PIT tag for identification
- Hold new broodstock under quarantine at the hatchery for a minimum of 45 days
- Place incoming broodstock in the fifth broodstock pool to acclimate the fish to conditions in the main broodstock pool
- Rotate new stock (males and females) into the program on a regular basis without impacting the health of the fish or the general success of egg production

5.3 Egg production

HSWRI is currently maximizing the genetic diversity of the parental contributions within the annual release total to the fullest extent practical. The current operational protocol for the hatchery is to utilize one to three female equivilants (one female equivilant equals ~2 million eggs) per run for a total of 28 to 32 spawns per year. Eggs are obtained using the approach outlined below.

5.3.1 Spawning

Spawning is induced by increasing the temperature from 14 to 18°C (57 to 64°F) and photoperiod from 10 to 14 hour days to simulate spring/summer conditions. No hormone or other manipulation is needed to induce spawning. The temperature and photoperiod are maintained for three to four months and then slowly decreased to 14°C (57°F) and 10-hour days, respectively, to simulate winter or non-spawning conditions. The four broodstock pools temperature and light regimes are staggered so that one pool is in spawning mode throughout the year.

Based on hatchery observations, white seabass generally spawn in the early evening with one or more females and typically numerous males participating in each spawning event. While the exact period between individual female spawn events is not formally documented, it is believed to be 10 to 14 days based on hatchery observations.

5.3.2 Egg collection

Eggs are collected the morning following a spawn using a fine mesh net (<800 µm). The eggs are concentrated in a container and transferred to a clear graduated cylinder where the volume of eggs is determined. A conversion ratio of 585 eggs per ml is used to determine the number of eggs. Viable, undamaged eggs are buoyant and therefore concentrated at the top of the cylinder. Nonviable eggs settle to the bottom. While both viable and nonviable eggs are enumerated, only viable eggs are stocked for production.

BMPs for egg production

- Maintain broodstock pool conditions according to the CHP so that one pool is in spawning mode year round
- Collect eggs daily in accordance with the CHP, maintaining sanitary conditions, keeping only viable eggs for production and destroying nonviable eggs

5.4 Nursery phase

5.4.1 Incubation

The incubation system has a total of twelve tanks, holding 234,000 eggs each. Temperature is set at 18°C (64°F) to match the broodstock pool temperature, and eggs are disinfected in a 100 ppm formalin bath for one hour prior to placing them in incubation tanks. Eggs are collected from high quality spawns as characterized by the initial viability. Eggs are stocked at a consistent density of 150/L in each incubator. If a second spawn occurs within five to seven days of the first spawn, several incubators are drained and restocked with new eggs such that an equal number of incubators contain eggs from each available spawn. The precise partitioning of eggs among incubators is dictated by spawn volume. Spawning events that include eggs from multiple females are utilized more broadly (i.e. in more incubators) as needed.

White seabass eggs hatch at 48 hours and begin feeding at five days post hatch (dph). At this stage, white seabass larvae are fed only live, newly-hatched, and nutrient-enriched *Artemia* nauplii (*Artemia franciscanus*). The *Artemia* have to be enriched because they lose much of their nutritional value within an hour after hatching. The *Artemia* are rinsed in fresh water prior to feeding to reduce bacterial loading. Larvae are fed seven times a day and each feeding consists of a single batch of *Artemia*.

5.4.2 Juvenile 1 (J1) system

The J1 system consists of six 7,000 L (1,850 gallon) pools. The pools are stocked at 40 to 60 larvae/L, and the temperature is maintained at 23°C (73°F). Late larval white seabass are transferred from the incubation system to the J1 system via gravity feed at 12 dph.

At this time, larvae are introduced to dry pelleted feeds to wean them off of live feed (*Artemia*). The dry pelleted food is a custom-prepared feed containing 50 percent protein. The pellets are crumbled into small pieces (0.25 to 2.0 mm) by the manufacturer. These fish are fed at a rate of five percent of body weight per day. Around 18 dph, the amount of live feed is reduced until no live food is offered at approximately 25 dph.

5.4.3 Juvenile 2 (J2) system

Once the white seabass larvae have been weaned onto dry pellets (around 35 dph), they can be transferred to the J2 system which consists of four 7,000 L (1,850 gallon) circular pools and two 19,000 L (5,020 gallon) oval pools. As with the J1 system, temperature is maintained at 23°C (73°F) by the MCCS. Pools are manually siphoned once or twice a day to maintain high water quality.

Fish in the J2 system are fed at a rate of three to five percent of body weight per day. The fish are fed a commercially available pelleted diet using belt feeders. This feed

contains 50 percent protein, 14 percent fat, and has Vitamin C incorporated into it. The size of the pellets used in the J2 system is typically 3.0 or 4.0 mm, depending on the fish size.

Transfer of fish from the J2 system is dictated by the ambient water temperature of the receiving body of water, usually the raceways. The temperature in the J2 pools is gradually decreased to ambient temperatures. Differences in the temperature of the J2 system and the receiving body of water can stress the fish, thereby reducing the immune response. This is especially problematic because this transfer results in the fish's first exposure to disease as it leaves the sterile, filtered water of the hatchery system. During warm-water months (>18°C; 64°F), fish can be transferred at a smaller size (20 g, 80 to 90 dph) than during colder months where fish are held until they are larger (40 g, 120 dph).

BMPs for the nursery phase

- Maintain high quality water standards in accordance with the CHP
- Provide nutritious, high quality live and dry feed for larvae in accordance with the CHP

5.5 Raceway culture

The raceway system consists of eight 25 m^3 concrete raceways in a separate area enclosed by shade cloth-draped chain link fence away from the main hatchery. This system is flow-through (375 Lpm) with water coming from Agua Hedionda Lagoon through the raceways and then back into the lagoon. The water is not ozone or UV-sterilized but does pass through a sand filter and a low-head oxygenator to maintain proper dissolved oxygen levels (≥ 4.0 mg/L). Fish can be stocked in the raceways at a maximum density of 20 kg/m^3. Raceways are vacuumed manually once a day to remove detritus.

Fish are fed the same commercial diet as with the J2 system. However, fish in the raceways are fed by hand four times each day at a rate of two to three percent body weight per day.

Juvenile white seabass are susceptible to GSS disease caused by high levels of total dissolved gas (TDG) in the water (Smiley 2004). Ambient waters in Agua Hedionda Lagoon can have TDG levels as high as 120 percent. Degassers are used to remove some of the gases from lagoon waters before entering the raceway system. More information on GSS disease can be found in Section 7.2.1.

Currently, the raceways are out of operation due to disease issues. Because the raceway system is not on a recirculating water system, and thus has limited filtration, fish contained in it are more susceptible to certain types of disease. Future use of the raceways may rely on installation of a new recirculation and filtration systems for those facilities.

BMPs for raceway culture

- Stock raceways at no more than maximum density
- Vacuum raceways once a day
- Provide high quality, nutritious feed in accordance with the CHP

5.6 Fish tagging

Prior to transfer to a growout facility or direct release, all fish are tagged with a CWT, a sequentially-numbered, small (1.1 mm long by 0.25 mm diameter), magnetized, stainless steel wire tag. Each fish is tagged in the left cheek muscle below the posterior edge of the left eye. The beginning and end numbers are recorded for each batch of fish tagged. A batch of fish varies in number and is directly proportional to the size of the growout facility that will receive the fish. Thus, a batch of fish represents a proportion of one production run raised at the hatchery or from one growout facility. In this manner, tag returns can be attributed to a specific production run and release, allowing for more accurate estimates of growth, mortality and identifying patterns of movement. In previous years, binary codes were used to identify batches of fish in the same manner. This information is maintained by HSWRI in a central database.

The minimum size for tagging fish is approximately 2.0 g or 100 mm, based on the size of the target tissue (cheek muscle). Fish are tagged using a 5-person tagging station built by HSWRI. The tagging station consists of two major components. The upper component is a holding tank that is filled with ozonated water that is recirculated continuously. The lower unit has MS-222 laden water and is designed to deliver anesthetized fish to the taggers. Fish then pass through a quality control device that effectively separates tagged fish from untagged fish. Tagged fish are deposited into a five gallon bucket and then transferred into one of the J2 system tanks. This procedure ensures that 100 percent of the fish are tagged initially. Tag retention is measured again by subsampling fish one to two weeks after tagging and again just prior to release.

BMPs for fish tagging

- Tag all fish prior to leaving the hatchery for the growout facilities
- Subsample fish for tag retention before transport to the growout facility and before release
- Use sequentially-numbered tags so that tag returns can be attributed to individual releases
- Maintain tagging data in a central database

5.7 Fish transport

Fish are transferred to growout facilities and to remote release sites using different tanks, vehicles, and vessels. The configuration of the transport depends on the number of fish being transported and the conditions at the facility or release site. The most

commonly used transport tanks, however, are 1,500 L (400 gallon) and constructed of marine-grade aluminum; the size and shape of the tanks allow them to be easily loaded onto a pickup truck, flatbed truck, or boat. The tanks are designed with independent aeration systems, and as a back-up to the aerators, each tank is equipped with a 1.5 cubic meter cylinder of pure oxygen. Like the aerators, the cylinder and its associated components (i.e. regulator, flow meter, and diffusers) are attached to the tank, not the vehicle. The tanks are not recirculating and have no filtration system.

Fish are starved for 24 hours prior to transfer, and the tanks are stocked at a maximum density of 40 kg/m^3. Water from the holding facility is used in the tanks to transport the fish. Fritz Guard is added to the water to protect the ectodermal mucous layer, to maintain an appropriate electrolyte balance, and reduce the stress caused by transport. Oxygen supplied from a tank is used to maintain constant oxygenation. If water temperature at the receiving site is significantly different (>2.0°C; 4°F) than that in the tanks, water is pumped into the tanks to reduce the difference. Fish are then flushed from the tank using a flexible hose.

BMPs for transporting fish

- Maintain separate aeration systems for each tank
- Provide good water quality conditions for transport
- Maximum stocking density of 40 kg/m^3
- Acclimate fish to receiving body of water's temperature, if difference is greater than 2°C (4°F)

Chapter 6. Growout Facility Operations

6.1 General description

The first growout facility came online in 1992, and the OREHP now has 13 growout facilities capable of growing out almost 82,000 kg (1.1 million 200-mm fish) annually (Table 6-1). The facilities employ a traditional method of finfish culture, whereby either a net or fiberglass raceway is used to enclose the fish being cultured. Raceways have to be vacuumed daily and end screens periodically cleaned to maintain water quality within the system. Net pens do not have to be vacuumed; however, both the containment net and predator barrier have to be routinely cleaned to maintain water flow through the facility and to maintain facility stability.

The facility (net pen or raceway) is usually attached to a dock, although some are moored in open water. The net or raceway is supported by a frame that is buoyed by pontoons. This frame also provides support for walkways (1 m wide) that encircle the containment net and provides a sturdy platform to service the fish at the facility. In some cases, two or four nets are suspended from the frame. All water-based systems should be configured so that the raceway or containment net does not touch the bottom, even during minus tides. One facility is land-based and uses above-ground pools to enclose the fish. The volume of the growout facility varies at each location, ranging from 17.6 m^3 at the Huntington Harbor facility to 1,691.5 m^3 at HSWRI's Catalina Harbor facility.

Table 6-1. The OREHP growout facility growing volume.

Growout facility	Facility type	Total growing volume (m^3)	Maximum annual production (kg)
Quivera Basin, Mission Bay	1 net pen	31.6	951
San Diego Bay: Grape Steet	2 net pens	176.0	5,280
San Diego Bay: Southwest Yacht Club	1 fiberglass raceway	19.6	430
Agua Hedionda Lagoon	2 net pens	788.6	23,485
Dana Point Harbor	2 net pens	33.2	1000
Newport Harbor	4 fiberglass raceways	70.4	1,520
Huntington Harbor	1 fiberglass raceway	17.6	435
Catalina Harbor – Catalina Seabass Fund	4 net pens	258.8	7,765
Catalina Harbor – HSWRI	4 net pens	1,691.5	33,644
King Harbor	2 pools	45.5	683
Marina del Rey	2 fiberglass raceways	35.2	870
Channel Islands Harbor	3 net pens	172.8	5,185
Santa Barbara	1 net pen	93.7	1,410

The growout facilities are owned and operated by groups of volunteers associated with angler groups and nonprofit organizations. Two exceptions are the growout facilities at Agua Hedionda Lagoon and the larger growout facility at Catalina Harbor, which are owned and operated by HSWRI. Each growout facility has a growout facility operator

that manages the volunteer staff and communicates with HSWRI's Growout Facility Coordinator (GFC) and the Department's OREHP Coordinator. The volunteers are responsible for facility maintenance and care and feeding of the fish in accordance with the GPM. They are not liable for the loss of fish; however, the Site Selection Committee can review a facility's performance and/or facility design and require that the facility be redesigned to prevent fish escape or decommission a facility if the standards of the GPM are consistently not met. Fish food is provided by the OREHP and pathology support is provided by a Department Fish Pathologist.

Volunteers have to secure a site for their facility within the program area (Point Arguello, San Luis Obispo County, to the U.S.-Mexico border). The volunteer organization is responsible for all costs involved with obtaining a site and building a facility. Additionally, the organization must have liability insurance. The organization must submit a design for the net pen or raceway system and provide a list of volunteers to the OREHP's Site Selection Committee which will evaluate the location to ensure that it is suitable for white seabass culture. The Site Selection Committee consists of the GFC, a HSWRI staff member, the OREHP Coordinator, the Department's Fish Pathologist, and two growout facility operators. The Committee evaluates each facility, looking at fish health and operational considerations. Fish health considerations include, but are not limited to, degree of tidal flushing at the site, water depth at minus tides, water temperature, whether the location is close to bait receivers, fish cleaning stations or other sources of biological contamination, as well as proximity to fueling docks, sewage outfalls or thermal outfalls. Operational considerations, include but are not limited to, exposure to wind and currents, stability of the proposed facility, use of net pen or raceway, proximity to a dock for fish transport and electricity, security, and expandability.

The volunteer organization is responsible for obtaining all permits and permissions to operate the facility. The Department, as administrator of the OREHP, is a co-applicant on the CDP and State Lands Lease. Before beginning the permitting process, the organization should be authorized by the OREHP Site Selection Committee and the Department. Table 6-2 list the permits and permission required to operate a growout facility for the OREHP. See Chapter 8 for more information on required permits.

Table 6-2. Permits or permissions required to operate an OREHP growout facility.

Regulatory Authority	**Permit or Permission**
Department of Fish and Game	Permission to participate in the OREHP
Other State agencies:	
California Coastal Commission	Coastal Development Permit (CDP)
State Lands Commission	State Lands Lease is required if the tidelands have not been granted to a local authority
State Water Quality Control Board	401 Certification – in the past, this has been waived because the U.S. Army Corps of Engineers has not issued 404 permits
Regional Water Quality Control Board	Large facility (> 45 mt fish/year) – National Pollution Discharge Elimination System (NPDES) Permit Small facility (< 45 mt fish/year)– NPDES permit or

Table 6-2. Permits or permissions required to operate an OREHP growout facility.	
Regulatory Authority	**Permit or Permission**
	NPDES permit waiver (may contain monitoring requirements)
Federal agencies:	
U.S. Army Corps of Engineers	Large facility – 404 permit Small facility – letter of permission
U.S. Coast Guard	Private Aids to Navigation Permit
U.S. Fish and Wildlife Service	Section 7 Endangered Species Act Consultation (SFRA funding requirement as well)
NOAA Fisheries Service	Letter of permission indicating that no species of concern will be impacted
Local agencies:	
City, County, Port Authority	Requirements vary by location
Marina owner (private property)	Lease agreement/letter of permission. Needs to include lease agreement between landowner and marina owner if the marina owner does not own the property.

Growout facilities usually receive two batches of juvenile white seabass for growout annually. The first batch is transported in spring, coinciding with the increase of ambient water temperatures. These fish are held at the facility for a period of four to six months prior to their release. Daily fish culture and facility maintenance is performed by volunteers at the facility according to the GPM. After the first batch of fish is released the facility is typically fallowed for one to three months. During the fallow period, repairs and routine maintenance are performed as necessary. Usually, a second batch of fish for culture is transported to the facility in late fall before ambient seawater temperatures decline and the winter storm season begins. This batch will be held over winter at the facility until the following spring. Some growout facilities are located in areas with high storm runoff that can create a low salinity environment or areas with potentially severe weather. These facilities may lie fallow for the entire winter season.

6.1.1 Net pens

Fish containment nets are made from knotless nylon netting to minimize abrasions to the fish. Different mesh sizes are used for the containment nets corresponding to the size of the fish being held. A mesh size of 2.5 cm (1.0 in.) stretch is used to accommodate small 100 mm (4 in.) fish at stocking and a larger mesh size of 6.2 cm (2.4 in.) stretch may be used for larger, 200 mm (8 in.) fish. The predator nets, which are hung separately from, and outside of, the containment nets, are constructed of 15.0 to 20.0 cm (5.9 to 7.9 in.) stretch mesh netting, made of heavy gauge nylon or polypropylene. Colorful polypropylene netting is preferred because it is more visible underwater.

Both fish containment nets and predator nets are suspended from the handrails of each net pen and they are sufficiently weighted on the bottom to keep them taught, even in high currents. Taught nets are important to maintain a consistent rearing volume and to prevent predators from becoming entangled in the nets. Attachment rings are conveniently located along the perimeter of each net and in the center. The handrails

extend around each net pen on either side of the walkways and are elevated approximately 1.0 m (3 ft) above the water line. The containment net is suspended on the inside handrail and the predator net is hung from the outside handrail. This configuration effectively eliminates the risk of fish jumping out or predators jumping in. Each predator net encompasses a single containment net so that each net pen can function independently from the others if there is ever a desire to move one or more of them to another location. The other benefit to this design is the low profile of the system, approximately 1.0 m (3 ft) off the waterline, which reduces wind shear and visual impacts. Bird-netting is stretched across the top of each net pen to prevent birds from injuring or preying upon fish from above.

To ensure good water flow through the system both the predator net and the containment net must be cleaned periodically to remove biofouling organisms. Cleaning can be conducted in situ by utilitizing divers, hired by the growout facility operator, and a net scrubber, owned by HSWRI. Alternatively, nets can be removed from the water and replaced with new nets. Nets that are no longer useful need to be properly disposed of in an upland waste facility. In previous years, the use of antifoulants (copper sulfate-based) helped reduce the amount of fouling, but that practice has been discontinued.

6.1.2 Submerged raceways

Raceways are constructed of smooth fiberglass to minimize abrasions to the fish. At either end of each raceway is a removable, metal or plastic screen that allows for water exchange through the raceway while preventing fish escape. Different mesh sizes are used for the end screens corresponding to the size of the fish being held. Mesh sizes range from 1.3 cm (0.5 in.) to accommodate small, 100 mm (4 in.) fish at stocking and a larger mesh size of 2.5 cm (1.0 in.) may be used for larger, 200 mm (8 in.) fish.

Water levels within the raceway system are maintained at a minimum of 30.0 cm (11.8 in.) below the lip of the raceway to prevent fish from jumping out of the raceway. Screens constructed of shade cloth or other fine mesh materials are placed on top of the raceway to provide protection from avian predators as well as shade from the sun. The solid raceway structure provides a strong barrier that prevents harassment from predators below the water line. Above the water line, the outer perimeter of the facility is encompassed by a chain link fence to prevent intrusion from predators and to secure the facility from other trespassers.

Raceways have end screens that can become fouled and need to be cleaned or replaced to ensure good water flow through the system. Additionally, excess food and feces can accumulate on the bottom of the raceways. To maintain good water quality conditions, raceways should be vacuumed daily.

6.1.3 Land-based pools

The land-based facilities, which are adjacent to harbors, use vinyl above-ground swimming pools to house the fish. A pump system is used to provide water flow from

the harbor. Aerators are also used to increase the oxygen-carrying capacity of the water in the pools. A back-up generator is employed automatically when the power to the life support systems fails. The pools are housed within a tarped Quonset-hut type enclosure which prevents birds from entering and provides shade for the fish.

As with raceways, land-based pools can accumulate excess food and feces and should be vacuumed daily to maintain good water quality in the system.

BMPs for growout gacility operations

- Provide a secure environment for raising juvenile fish by maintaining the containment system in good working order
- Maintain adequate freeboard of the containment systems to prevent fish escape
- Provide appropriate barriers to predators both above and below the water
- Provide shade from the sun when systems are shallow
- Maintain good water quality conditions by removing biofouling as needed, and regularly vacuuming raceways and land-based pools
- For land-based systems, aerate water and provide a back-up generator to guard against power failures
- Maintain good communication among the growout facility operator, GFC, and the OREHP Coordinator

6.2 Stocking density

Fish are maintained at the facilities in modest densities of 12.0 to18.0 kg/m^3 to minimize the effects of crowding on fish health and water quality. For modeling purposes a time-at-release density of 15.0 kg/m^3 is used. The average size at release is 200 to 250 mm (8 to 10 in.) TL; this equates to approximately 200 fish/m^3.

BMPs for stocking density

- Stock fish into growout facilities based on a density at time-of-release of 12.0 to 18.0 kg/m^3 to minimize the effects of crowding on fish health and water quality

6.3 Annual release limit

With inception of the proposal for the Carlsbad hatchery, the OREHP planned on releasing approximately 350,000 juvenile white seabass annually into the ocean waters of southern California. All experimental protocols and economic evaluations were based on this production capability, and the hatchery was designed to produce that many juveniles annually. The broodstock management plan found within the CHP was based on analysis of the wild population's genetic variability and the projected number of broodstock required to minimize impacts to that population (Bartley et al. 1995). That

analysis estimated that 148 founders would be required; the OREHP took an even more conservative approach and committed to holding 200 brood fish in a 1:1 sex ratio.

When the hatchery was dedicated in 1995, the OREHP only had 70 brood fish. To calculate an annual release limit based on the number of brood fish at the hatchery, the Joint Panel, a now defunct advisory panel required by the MOA, divided the number of brood fish available in 1995 (70 individuals) by 200, and multiplied that percentage by the production capability of 350,000 to achieve an allowable production number of approximately 125,000 released juveniles (rounded up from 122,500).

This annual release limit was not approached until 2001, when 100,000 fish were released after culture techniques were refined sufficiently for large scale production. Further culture improvements, including the installation of an ozone sterilizer in 2004, greatly improved the survival of juvenile white seabass. In 2004, the OREHP petitioned the CCC to increase the release limit to 350,000 fish. The CCC granted this request, and the release limit was set at 350,000 fish from 2004 to 2006. In 2007, the release limit dropped back down to 125,000 fish because the number of brood fish at the hatchery decreased from 200 to 172.

In 2009, the Department and the OREAP submitted a request to the CCC proposing to increase the release limit to 287,000 juvenile white seabass per year (sliding scale release limit). This increase was based on the current breeding population housed at the hatchery as a proportion of the target broodstock size of 200. The CCC agreed to this proposal, and the sliding scale release limit was implemented in 2010. Under this proposal, the annual release is calculated by dividing the current number of broodstock by 200 and multiplying that percentage by the production capability of 350,000 to achieve an allowable production number of 287,000 released juvenile. Because the number of brood fish at the hatchery changes every few months due to mortalities or additions, the release limit is recalculated on January 10 and June 10 of each year.

Recent genetics research (Coykendall 2005) indicates that the effective population size of the broodstock may be smaller than Bartley's (1995) modeling predicted. As a result, additional research is being conducted to determine the effective population size at the hatchery. See Chapter 10 for more information on genetic considerations.

BMPs for the annual release limit

- Maintain a 350,000 fish release limit (calendar year) as long as there are 200 broodstock at the hatchery

6.4 Fish feed

Fish at the facilities are fed the same feed as in the J2 and raceway systems. Zinc is incorporated into the feed in a proteinated form so that it is biologically available and less likely to build up underneath the growout facility. Pellet size ranges from 2.5 to 6.0 mm depending on the size of the fish. Fish are fed at a daily ration of approximately one to three percent estimated average body weight (calculated monthly by measuring

20 fish and using a length-weight conversion) per day depending on water temperature. All the facilities have automatic feeders to distribute food. Food usage is recorded daily for each pen and is ultimately stored in a central database maintained by HSWRI for the Department.

Supplemental feeding is also done by hand each day in order to observe the feeding response of the fish as an indicator of fish health and appropriateness of current feeding levels. This observation is a valuable tool in the management of the feed distributed to the fish. If feeding rates diminish due to decreased water temperature, the change can be observed immediately, and a correlating reduction in the total amount of feed distributed daily through the automatic feeds can be made, preventing waste feed that can be deposited on the bottom of the raceway or beneath the net pen. Conversely, if an increase in fish appetite is observed, daily feeding rates can be increased accordingly, thus preventing weakened fish due to malnourishment.

BMPs for feeding fish

- Feed fish multiple times each day
- Hand feed fish daily to assess their health and feeding response
- Calculate daily ration at least once a month or as feeding response changes
- Provide a high quality fish feed based on white seabass nutritional needs

6.5 Monitoring

The growout facility operator ensures that volunteers are recording the amount of food put in the feeders and hand fed to the fish along with the number of dead fish removed from the facility each day in the daily log. At the end of each month the growout facility operator mails or faxes the daily log to HSWRI where the information is input into the central database. A growout facility operator may be asked to monitor various water conditions (i.e., temperature, salinity, ammonia) and will be provided with equipment to do so. All data collected should be written in the notes section of the daily log.

Monitoring includes the daily physical inspection of the facility, along with a general assessment of the overall condition of the fish and the number of mortalities. Under proper conditions, daily fish mortalities should not exceed a fraction of a percent (several individuals), although higher mortalities are not unusual right after transporting the fish to the growout facility. If a growout facility experiences higher mortalities for more than a few days, the growout facility operator should contact the GFC to arrange a fish health inspection with the Department's Fish Pathologist.

If someone notices that a rip in the containment net or a break in a raceway end screen has resulted in fish escaping the growout facility, the growout facility operator shall notify the GFC and the OREHP Coordinator immediately. The facility operator should estimate the percent of fish loss and be able to provide how and when the fish escaped. The rip or break should be repaired immediately to prevent further escape.

The GFC should visit the facility every three to five weeks to subsample the fish in a non-lethal way, taking length and weight measurements to assess growth. Based on these assessments, the containment net or raceway end screen can be changed for a larger mesh size, and the size of the pelleted feed can be increased.

HSWRI staff will also collect bottom samples for benthic monitoring from the growout facilities based on a three-year cycle. Sampling will occur between the period of one month prior to release to two months following release of fish from the facility. If a growout facility is empty and cannot be sampled, then it will be sampled the next time fish are grown out there. Benthic sampling will follow the protocol outlined in the Benthic Monitoring section of the GPM. HSWRI staff will analyze the samples in the field for free sulfides and redox potential. Subsamples will be saved for later analysis at the lab. Additional information regarding benthic monitoring can be found in Chapter 9.

BMPs for monitoring

- Assess fish health daily
- Remove and count fish mortalities daily
- If mortalities increase or fish health looks poor, contact the GFC to schedule a visit from the Department's Fish Pathologist
- Record data in the daily log
- At the end of each month, submit the daily logs to the GFC
- Conduct regular inspections of the physical components of the growout facility; make necessary repairs as soon as possible
- Notify the GFC and the OREHP Coordinator of any accidental releases within 24 hours
- The GFC should assess growth at the growout facility every three to five weeks
- Adjust feed size, containment net, or raceway end screen mesh size when appropriate
- Collect and analyze bottom samples for benthic monitoring according to protocols outlined in the GPM

6.6 Marine mammal interactions

Interactions with marine mammals can be avoided by proper siting, care, and maintenance of the growout facility. NOAA's National Marine Fisheries Service (NOAA Fisheries Service) has published a guideline of safe deterrence methods of marine mammals (NOAA 2008). They include the following:

- Passive deterrence measures – fencing, closely spaced posts, nets, or other types of physical barriers provided the potential for marine mammal entanglement is not increased.

- Active deterrence measures - mechanical or electrical noisemakers, water spray from a hose, sprinklers, blunt objects to prod animals, or crowder boards to herd animals.

Deterrence measures should not separate a female from her offspring; break the skin of an animal; result in dislocation of or fracture of bones, limbs, or other appendages; be directed at the head or eyes of an animal; or be used on seals and sea lions hauled out on unimproved property. Currently, the only deterrence measures approved by the OREHP are the chain link fencing that surrounds some facilities and barrier nets used below the water.

Any injury or mortality of a marine mammal must be reported within 48 hours of occurrence. NOAA Fisheries Service has defined a marine mammal injury as a wound or other physical harm. Signs of injury include, but are not limited to, visible blood flow, loss of or damage to an appendage or jaw, inability to use one or more appendages, asymmetry in the shape of body or body position, noticeable swelling or hemorrhage, laceration, puncture or rupture of eyeball, listless appearance or inability to defend itself, inability to swim or dive upon release from fishing gear, or signs of equilibrium imbalance. The Marine Mammal Authorization Program Mortality/Injury Reporting Form (OMB 0648-0292) should be filled out and faxed to the following individuals:

NOAA Fisheries Service -- fax: (301) 713-4060
Growout Facility Coordinator (GFC) -- fax: (760) 434-9502
OREHP Coordinator -- fax: (562) 342-7139

BMPs for marine mammal interactions

- Maintain proper siting, care, and maintenance of growout facility to avoid interactions with marine mammals
- Notify NOAA Fisheries Service, the GFC, and the OREHP Coordinator of any interactions with marine mammals within 48 hours

6.7 Fish releases

6.7.1 Final inspection and clearance

The growout facility operator should contact the GFC when the fish reach 200 to 250 mm to schedule a final inspection. The GFC will come to the growout facility along with the Department's Fish Pathologist to perform the final inspection, which includes a health check, length and weight measurements of a subsample of fish, and a final tag retention assessment. The fish cannot be released until they have been cleared by the Department's Fish Pathologist.

6.7.2 Coordinating the release

Once the fish are cleared for release, the growout facility operator and GFC will set a release date. The growout facility operator will schedule volunteers to assist with the release. All facilities will need volunteers to count the fish as they are released. At land-based facilities, additional volunteers are needed to crowd the fish in the pool, net them, and walk them to the ocean or a transport vehicle. At water-based facilities,

volunteers may be needed to help crowd the fish or pull up on the containment net or raceway end screen.

6.7.3 Releasing fish

On the day of the release, the GFC will demonstrate proper handling techniques which include using gloves and netting only a few fish at a time to minimize stress. Fish are to be released at the growout site, or nearest body of water for land-based facilities, and not transported to another site without permission from the GFC and the OREHP Coordinator. Additionally, fish should all be released within the same time period (1 to 2 days) to avoid biasing the post-release assessment of survival.

The OREHP's juvenile recruitment surveys and HSWRI's acoustic tracking studies (See Sections 11.1 and 11.3) have shown that juvenile white seabass inhabit shallow waters of embayments and the open coast. Thus, while off-site releases are not uncommon, the majority of white seabass are released at the growout site. The growout facility operator shall obtain permission of the GFC and the OREHP Coordinator before releasing fish at any site other than the growout facility.

If the media are invited to the release event, the GFC and the OREHP Coordinator should be contacted to provide accurate historical context for the event.

6.7.4 Remote releases

Juvenile white seabass can be transported from the hatchery or Catalina Harbor and released along the mainland coast. Juvenile white seabass are to be released in appropriate habitat (embayments or along the mainland coast in shallow water) within the Southern California Bight. There are no limitations on how many fish can be released at one site, except at Catalina Island. General practice is not to release more than 10,000 fish at one location. There are no limitations on distance from the growout site; however, longer distances, and thus longer transport times, can be more stressful to the fish. HSWRI is the only member of the OREHP allowed to conduct remote releases without advance permission of the Department.

6.7.5 Release limit at Catalina Island

The topography around Catalina Island is such that juvenile seabass released at the island are concentrated along a very narrow shelf surrounding the island. While the two growout facilities at Catalina Island are capable of growing out over 500,000 juvenile white seabass in a single production run, the OREHP has voluntarily limited releases at Catalina Island to 30,000 fish annually to minimize the potential for inter or intra-specific competitive interactions. The 30,000 fish release limit is not currently based on any scientific studies but rather as a "best guess" of what is appropriate. Directed studies (e.g. acoustic tracking) should be conducted to assess the availability of suitable habitat for juvenile white seabass at Catalina Island, assess the dispersion rate of white

seabass released at Catalina Island, and adjust the Catalina Island white seabass annual release limit accordingly.

6.7.6 Direct releases

Sometimes fish are held at the Carlsbad hatchery until they reach release size (200 to 250 mm; 8 to 10 in.). Once cleared for release by the Department's Fish Pathologist, these fish can be released from the raceways into Agua Hedionda Lagoon. Fish can also be transported to remote locations for release (i.e., Mission Bay, Oceanside) to more evenly distribute the fish along the mainland. HSWRI is the only member of the OREHP allowed to conduct remote releases without advance permission by the Department.

BMPs for fish releases

- Fish cannot be released until cleared for release by the GFC and the Department's Fish Pathologist
- The growout facility operator is responsible for requesting a final inspection from the GFC and for setting up the release event, including scheduling volunteers to help with release activities
- Fish are to be released at the growout facility site unless permission is granted by the Department in advance to release the fish remotely
- Proper fish handling techniques will be used during the release event
- The annual release limit for Catalina Island is 30,000 fish per calendar year
- Excess fish grown out at Catalina Island shall be transported to the mainland coast and released
- There are no limitations on remote releases (number of fish or distance); however, fish are to be released in the appropriate shallow water habitat

Chapter 7. Fish Health Management

7.1 Fish health management program

The fish health management program for the OREHP is under the supervision of a Department Fish Pathologist, with assistance from a HSWRI veterinary fish health specialist. This program includes prevention, identification, and treatment of many common white seabass pathogens, including non-infectious and infectious diseases. The goal of the program is to ensure that no sick fish are released into the wild and that no novel diseases or physical deformities are introduced to the wild white seabass population. This goal is achieved by the following protocol:

- Only healthy, asymptomatic fish can be transferred to the growout facilities or released into the wild

- Healthy, asymptomatic fish that have been exposed to a lethal, highly contagious pathogen known to occur in wild white seabass can be transferred to the growout facilities or released into the wild

- Healthy, asymptomatic fish that have been exposed to a lethal, highly contagious pathogen that is not known to occur in wild white seabass must be euthanized to prevent the introduction of new disease

To ensure only healthy, asymptomatic fish are released the program requires at least two health inspections by a Department Fish Pathologist or Department-approved Fish Pathologist: 1) before fish are transferred from the hatchery to the growout facility; and 2) prior to release into the wild. In addition to these routine inspections, a fish health inspection should be requested when hatchery staff or growout facility volunteers notice an increase in mortality or a change in fish behavior that lasts more than three days and is not associated with transport mortality.

All fish health inspections involve visual inspection and necropsy of three to ten fish per tank, net pen, or raceway. The inspection includes wet mount exams for parasites on gill and skin, and a thorough external and internal screen for gross abnormalities, parasites, and lesions. New and/or unusual pathogens or lesions are documented with line drawings and/or photography, which are subsequently used for identification and classification. If necessary, tissues are fixed in 10 percent formalin or Karnovsky's fixative, and followed with histopathology or electron microscopy. Unusual or new metatozan parasites are fixed in ethanol and sent to outside parasitologists for identification. Confirmation of some infectious diseases (viral, bacterial, or fungal) is made using pathogen isolation techniques: cell culture – using fish cell lines – for viruses and rickettsial bacteria, and plate agar for bacteria and fungi. Some viral, rickettsial, and sporozoan (e.g., myxosporidian and microsporidian) diagnostics are also done via polymerase chain reaction (PCR) assays performed on fresh or frozen tissue.

The UCD typically does all the virology, rickettsial isolation, and PCR assay assessments.

The fish health management program is supported by an on-going effort to survey wild stocks of white seabass. The goal of this disease assessment program is to determine which pathogens and diseases are “naturally-occurring” among wild white seabass. Toward this end, blood and tissue samples from wild white seabass are collected, preserved, and analyzed. When a new pathogen is discovered in cultured seabass, the goal is to identify it and then determine if it occurs in wild white seabass. Initial characterization of new pathogens/diseases is done by documenting gross lesions with photography and then using histology to define microscopic features and associated pathology. Morphologic characterization is further refined using transmission electron microscopy (TEM). Attempts are also made to propagate new pathogens on plate agar or fish cell lines so as to simplify identification and characterization.

Although morphologic techniques are useful in the initial characterization of a new pathogen or disease among cultured fish, they are of limited value in surveying wild fish stocks. The reason for this is simply that wild fish with diagnostic lesions (i.e., those with moderate to severe infections) rarely survive to be captured and assessed. Sick wild fish either die quickly, or weaken and are consumed by predators. Diagnostic tools used to assess wild fish need to be more sensitive and geared toward detection of fish with: 1) latent infections (i.e., fish that are carriers, but asymptomatic); 2) mild infections (i.e., fish with mild, sublethal infections); or 3) no infections (i.e., fish that were exposed to a pathogen and were either immune, or developed an infection and were able to clear the pathogen). Currently, the two assays with the greatest application to disease assessment among wild fish stocks are the PCR assay and the enzyme-linked immunosorbent assay (ELISA).

The PCR assay is a molecular diagnostic tool based on the detection of pathogen DNA. Major advantages over morphologic techniques include a higher level of precision (e.g., positive PCR results can only rarely be confused) and sensitivity (e.g., only a few strands of DNA are necessary for PCR detection). The ELISA is a hematological assay and in contrast to the PCR assay that detects pathogen *infection*, ELISAs are used to determine level of pathogen *exposure*. Pathogen exposed fish develop pathogen specific antibodies and these antibodies can persist for months to years in peripheral blood. ELISAs therefore have the distinct advantage of detecting not only fish that are currently infected, but being capable of detecting exposure in fish that have already cleared the pathogen.

PCR and ELISA assays are both time consuming and difficult to develop. Both are also dependent on being able to culture the pathogen artificially (on fish cell lines) or in purifying pathogen antigens in sufficiently large quantities. Dr. Ron Hedrick's lab at UCD has been instrumental in the development of PCR and ELISA assays for a number of viral and rickettsial pathogens of white seabass. Once the appropriate diagnostic tools have been developed, blood and/or tissue samples from wild fish are tested to determine if the new pathogen is present, or if wild fish have been exposed to the

pathogen. Final disposition of infected or exposed hatchery fish depends on the results of these tests and follows the objectives described above.

BMPs for fish health management

- Require fish health inspections before transfer to a growout facility and prior to release into the wild
- Require fish health inspections when daily mortality increases or fish behavior changes
- Allow only the transfer or release of healthy, asymptomatic white seabass
- Do not allow the release of fish that have been infected with a highly contagious lethal disease not known to occur in wild white seabass
- Allow an abbreviated health inspection and an early release to help minimize loss when xenobiotic exposures occurs at a growout facility

7.2 Non-infectious diseases

Non-infectious diseases have a major impact on hatchery production of cultured white seabass by killing fish outright, and by increasing the percentage of fish culled (removed due to disease or deformity) from the population. Major categories of non-infectious diseases include: GSS disease, larval mass mortality syndrome, developmental deformities, cannibalism, and exposure to xenobiotic chemicals or red tide (dinoflagellate bloom).

7.2.1 Gas supersaturation disease

Prior to hatchery system and procedural changes in 2007 and 2008, GSS disease had been the most important non-infectious disease affecting cultured white seabass. Losses from GSS-associated eye lesions had been in the thousands, annually, but have decreased at least 10 fold in 2007 and 2008. There are many causes of GSS, but within Agua Hedionda Lagoon major influences are: 1) daily fluctuations in water temperature; and 2) photosynthetic activity of plants within the inner portion of the lagoon. Plant photosynthesis puts huge amounts of dissolved oxygen into the water column, and when warm water, heated in the shallow confines of the inner lagoon, hits the colder ocean water (during an outgoing tide), the water in the outer lagoon becomes supersaturated. Gas saturation levels as high as 110 percent total gas pressure (TGP) have been recorded for Agua Hedionda Lagoon on a consistent basis. This supersaturated water is subsequently pumped into the hatchery and severely impacts cultured white seabass.

Additional potential sources of GSS within the hatchery include: 1) ozone treatment of ambient Agua Hedionda Lagoon "make-up" water (ozone is used to kill microorganisms and break down complex organic compounds); 2) hydrogen peroxide therapy used to treat external parasites; 3) some pieces of equipment (e.g., protein skimmers); and 4) aeration or oxygen supplementation using gas diffusers.

GSS can cause a variety of problems, but with white seabass, the primary target organs are the eyes. Gas slowly accumulates within the eyes, and there is progressive loss of eye sight and eventual blindness. Secondary bacterial and fungal infections are common. GSS eye lesions are an obvious negative survival trait (blind fish do not survive very long in the wild), and fish with lesions are culled on a regular basis.

Smiley (2004) studied the effects of GSS on juvenile white seabass. Major findings included: 1) that smaller/younger (50 to 60 dph) white seabass were *less* susceptible than larger/older (110 to 120 dph) white seabass; 2) ocular lesions were worse in fish exposed in warmer (23°C; 73°F) versus colder (18°C; 64°F) water; and 3) the prevalence and severity of eye lesions increased with increasing TGP exposure. Ocular lesions included: corneal emphysema, orbital emphysema, iridial hemorrhage, subretinal hemorrhage, perineural hemorrhage (surrounding the optic nerve), and inflammation of the iris and subretinal areas. Surprisingly, ocular lesions were *not* similar to those routinely observed in hatchery fish. Experimentally-exposed fish consistently developed corneal emphysema, while hatchery fish typically develop intraocular emphysema (gas within the globe and not within the cornea).

There is no treatment for most forms of GSS-related eye damage. Fish with small gas bubbles could theoretically be placed in deep (5 to 10 m) tanks or net pens, which would allow hydrostatic pressure to shrink lesions, but this is not practical with the physical constraints of the hatchery. Fortunately, there are some management practices and system design alterations that can help reduce GSS levels and prevent eye lesions.

The hatchery began implementing a series of changes in 2006 and 2007 to reduce GSS exposure. The most significant change in 2007 was that the hatchery began rearing larval and young juvenile fish in cooler waters (18 to 20°C versus 23°C; 64 to 68°F versus 73°F). Rearing fish in colder water increases the gas carrying capacity of water, at the same time minimizing the thermal expansion of gas pockets that do develop in the fish's eye. The second major alteration was that the J2 system was completely overhauled, with installation of new pumps, plumbing, and degassing towers. Exposure of J2 system fish to GSS was markedly reduced when the system was re-plumbed so that water from the protein skimmer and ozone-treated make-up water were diverted through the new degassing tower prior to reaching the grow-out tanks. The third change was to minimize use of all of the hatcheries eight raceways. The combination of these three changes has significantly reduced the incidence of GSS-related eye disease, in addition to improving cold tolerance.

7.2.2 Larval mass mortality syndrome

Prior to 2003, Larval Mass Mortality Syndrome (LMMS) was one of the greatest causes of losses at the Carlsbad hatchery. LMMS is characterized by sudden loss of 80 to 100 percent of an incubator’s population or, in some cases, loss of an entire spawn. Losses typically occur over a one to three day period, with tens of thousands of larvae dying with little or no clinical signs. Newly hatched larval white seabass, from 1 to 10 dph, are

the most common age group affected. Wet mount preparations of dead and dying fish have occasionally revealed bacterial or protozoal infections, but often there are no grossly visible lesions or pathogens.

The etiology of LMMS is unknown, but one likely explanation is acute toxicity from organophosphate pesticides (OPP). OPPs (e.g., diazinon and chlopyrophos) are commonly used in both commercial and residential applications. OPPs are neurotoxins and are designed to kill insects via chemical inhibition of acetylcholinesterase (an important neurotransmitter in both invertebrates and vertebrates). Unfortunately, larval fish are also highly susceptible to OPP poisoning (Hamm 1997, Hamm et al. 1998, Hamm and Hinton 2000, Hamm et al. 2001). It is hypothesized that runoff from residential homes and commercial businesses into Agua Hedionda creek and lagoon carries with it enough OPP residue to impact newly hatched larval white seabass.

There is circumstantial, toxicologic, and pathologic evidence to support the OPP hypothesis for LMMS in cultured white seabass. Circumstantial evidence hinges on the fact that LMMS is typically more common in the spring. Spring is when the surrounding agriculture areas are planted and when residential pesticide use is high; spring runoff following from heavy rainfall events may also be a factor. Both diazinon and chlorpyrophos have been detected, in part per billion (ppb) levels, in water samples taken from Agua Hedionda Lagoon, and some larvae have had retinal and CNS lesions (single cell necrosis) consistent with OPP toxicity.

The absence of LMMS events at the hatchery since 2003 is presumptively attributed to the installation of an ozone treatment system for all the make-up water at the hatchery. Treating OPPs with ozone effectively oxidizes many OPPs, including diazinon (Lenntech 2007).

7.2.3 Developmental deformities

Developmental deformities are important non-infectious diseases of cultured white seabass. Some developmental defects are congenital (present at the time of hatch), but many manifest themselves when larvae reach a certain age or size. The most common developmental deformities are those involving the curvature of the spine (scoliosis, lordosis, kyphosis) and jaw (prognathisms or brachygnathism). Other deformities include: defects in scale patterns (a peculiar swirling pattern develops posterior to the pectoral fins) or scale loss, spiked “horns” developing on the dosum of the head, incomplete caudal fin development (the tail assumes an oval shape), opercular defects (missing and/or malformed operculae), and “spinal fusion” (a general lack of elongation, resulting in short stumpy fish, possibly caused by fusion of vertebral bodies).

Although there had been a general decrease in the number of fish with developmental defects from 2001 to 2006, there has been a sharp resurgence in the prevalence of deformed fish starting in late 2007. Jaw – maxillary and mandibular – deformities continue to be major reasons for culling fish prior to tagging. In addition, the “horn

head" phenomenon has become extremely common among hatchery fish. In some 2007 to 2008 spawns, the percentage of culled fish was as high as 60 percent.

Although the general trend of decreasing developmental defects, from 2001 to 2006, was largely attributed to improved nutrition, the abrupt increase in deformities since 2007 does not appear to be diet related. Jaw deformities are often discovered as early as five to seven dph when fish are examined for gut contents; the "horn head" deformity has been observed as early as 16 dph. In both cases, deformities arose prior to the start of exogenous feeding with a prepared diet.

Several changes in hatchery operations have been made to attempt to identify the source of the developmental abnormalities but with limited success. The incubation tanks have been retrofitted to isolate them from the rest of the hatchery to prevent airborne pathogens from entering the system. In addition, using UV filtration in place of ozonation to eliminate potentially hazardous byproducts (bromates) from that process has not significantly decreased the incidence of deformities. Survival rates have increased by raising larvae to 18 to 20 dph at HSWRI's Mission Bay facility and then transporting them back to the hatchery; however, this situation is only temporary as the Mission Bay lab is not an OREHP production facility and has its own research and facility needs.

Investigations are currently focused on poor water quality associated with exposure to exogenous chemicals. The water quality hypothesis is based on anecdotal and experimental evidence that larval white seabass do better when reared in water other than Agua Hedionda Lagoon water, and the elimination of other major causes of developmental deformities. Potential sources of chemical mutagens include diatom/dinoflagellate blooms (producing biotoxins) and pesticide and herbicide runoff into the lagoon. Research into the cause or causes of developmental deformities are ongoing and are of highest priority for the OREHP.

7.2.4 Xenobiotic chemical exposure

Prior to 2007, losses of older juvenile cultured white seabass from exposure to xenobiotic contaminants had been rare. The two most well documented cases were: 1) losses at the Marina Del Rey growout facility in 2002; and 2) a large fish kill at the Quivira Basin (Mission Bay) growout in 2003. The Marina Del Rey incident was traced to a leaking "pump-out station" located on the dock adjacent to the net pen. The pump-out station functions to assist boaters in emptying their chemical toilets, and the one next to the net pen had been observed leaking prior to and throughout the three to four day period when fish were dying. Several hundred juvenile white seabass died before the leaking pump-out station was repaired. Moribund fish were grossly normal, but histology revealed severe hepatic necrosis. Chemical toilets utilize a variety of noxious compounds, including formalin, and fish were probably killed because of a combination of direct toxicity and multiple organ failure.

The second example of a chemical spill killing cultured white seabass occurred in 2003 at the Quivira Basin, Mission Bay growout facility. The pen is located in an open boat slip; a nearby boat either accidentally spilled a large quantity of diesel fuel, or purposely pumped contaminated bilge into the water. A metallic sheen was noted on the water, and there was a prominent smell of diesel fuel in and around the net pen at the time fish began dying. Over 1,000 juvenile white seabass died within a three to four day span. The major clinical finding was pale gills; histologically, there was severe necrosis of gill epithelium.

In recent years, the Southwestern Yacht Club growout facility, located in San Diego Bay, has experienced a series of chemical exposures that have resulted in the loss of hundreds of juvenile (four to six month old) fish. Most incidents were fuel spills, but at least one was a municipal sewage spill. The OREHP has instituted a quick response policy that includes an abbreviated health inspection and early release to help minimize loss when xenobiotic exposures occur.

Xenobiotic chemical exposure to larval white seabass at the Carlsbad Hatchery is also of concern. The hatchery obtains its water from Agua Hedionda Lagoon. Agua Hedionda Lagoon is bordered on the north and west by a densely packed urban environment. Herbicides, insecticides, and fungicides are used annually and drainage is either directly into Agua Hedionda Lagoon or into Agua Hedionda Creek, which subsequently flows into the lagoon. Potential links with larval mass mortality syndrome (see section 7.2.2 above) have already been described. Chemical contaminants, that escape ozone neutralization, may also be associated with immunosuppression and bacterial enteritis, a major killer of larval white seabass 7 to 21 dph.

7.2.5 Cannabilism

Cannibalism is a major cause of fish loss and injury among cultured white seabass. Larger fish will frequently eat smaller fish, especially if fish are underfed. Unsuccessful attacks are characterized by fish with a whitish ring of superficial lesions around the head (“ring head”). Bite wounds frequently involve the eyes, resulting in bilaterally symmetrical cloudy corneas. When severe, head injuries can become complicated by secondary bacterial infections. Ensuring that fish are well fed and frequent “grading” (sorting of fish according to size) can help to control cannibalism.

7.2.6 Red tides

Dinoflagellate blooms (i.e., red tide) periodically occur along the coasts of Central and Southern California. Although some dinoflagellate species have been associated with domoic acid toxicity to marine mammals and some fish species, there have not been problems with cultured white seabass directly linked to red tides. If blooms are severe, dinoflagellates can become a problem by mechanically impeding respiration (via clogging gill filaments). Heavy blooms have also been associated with a drop in dissolved oxygen, which can stress or kill fish in crowded tanks or raceways. Ensuring

good water flow by keeping the end screens clean is the best defense against reduced oxygen levels.

7.3 Infectious diseases

Infectious diseases of cultured white seabass include those caused by viruses, bacteria, rickettsia, fungi, and sporozoans (spore-forming protozoan pathogens). Infectious diseases are usually considered the most dangerous of diseases (compared with non-infectious and parasitic diseases) because they have the greatest potential for spread to wild fish stocks, and are generally more lethal and difficult to treat.

7.3.1 Viral pathogens

7.3.1.1 Viral nervous necrosis

Viral nervous necrosis (VNN) is caused by a single-stranded RNA virus, the viral nervous necrosis virus (VNNV). VNNV is classified as a nodavirus and predominantly affects central nervous system (CNS) tissue; it is analogous to the human poliovirus. Among cultured white seabass, VNN usually affects larval fish between the ages of 20 and 40 dph. Fish older than 60 dph appear to be resistant to VNN, although some infections do occur.

Primary target organs in white seabass (and the majority of other fish species) are retina of the eye, brain, and spinal cord. Larval white seabass with VNN are usually found at the surface of water, floating on their sides, and appear paralyzed, with loss of swim bladder control. Tanks with symptomatic fish usually have high mortality, with up to 50 percent losses. Losses generally continue until fish grow out of the susceptible age range.

VNN is a progressive, lethal disease and there is no treatment. The OREHP policy, prior to 2002, was to euthanize all VNN infected *and* exposed fish – even if exposed fish were clinically healthy and asymptomatic. This sometimes results in euthanizing an entire production run of fish. The OREHP's wild fish disease surveillance program was initiated early in 2002, with emphasis on collecting as many blood samples from wild white seabass as possible. VNN-infected cultured white seabass were also sampled and an ELISA was developed to detect the presence of VNN-antibodies in wild white seabass. The results of ELISAs run on the blood collected from wild fish revealed that 18 percent (14 of 78) of subadults, and 53 percent (9 of 17) of adult wild white seabass had been exposed to VNNV. These results allowed the OREHP to eventually release thousands of white seabass that had been exposed to VNNV in 2002, but which were clinically healthy, per the fish disease policy. There have been no VNNV outbreaks since 2002.

7.3.1.2 Viral hemorrhagic septicemia

Viral Hemorrhagic Septicemia (VHS) has not been identified in white seabass. It is primarily a disease of cultured salmon. However, VHS is of concern because the causative rhabdovirus, VHS virus, has been isolated from asymptomatic Pacific sardines (*Sardinops sagax*) and Pacific mackerel (*Scomber japonicus*) taken from the coastal waters of Southern California in 2001.

At the present time, the risk to cultured and wild marine fish stocks appears to be minimal. We know that the VHS virus is present in Southern California waters, but there has not been a confirmed case of VHS (i.e., an infected fish with disseminated virus and lesions) in any fish from Southern California, including those baitfish from which the virus was isolated. Additionally, thousands of pounds of frozen baitfish (total estimate = 250,000 tons) have been shipped to Australia since the mid-1990s, and VHS has never been confirmed in any fish from Australian waters.

Although the risk appears small, the OREHP will continue to monitor cultured white seabass for clinical signs of VHS. Work in developing ELISA and PCR diagnostics will continue. Should an outbreak of VHS in cultured white seabass be confirmed, these fish would be euthanized, per the fish disease policy, unless ELISA and/or PCR diagnostics can show that it occurs in wild white seabass.

7.3.1.3 *Herpesviru enteritis*

Herpesviruses (family *herpesviridae*) are common pathogens of teleost fish, so it was not too surprising that a herpesvirus (presumptive diagnosis based on TEM morphology) was detected in cultured white seabass in 2002. The majority of herpesviruses has strict host specificity and rarely produces disease in other species.

Among cultured white seabass, there have been three confirmed (via TEM and PCR) outbreaks of herpesvirus at the Carlsbad Hatchery. The first outbreak was in November of 2003; the second was in October of 2005; and the third was in February 2009. All three outbreaks occurred in raceway fish exposed to untreated water from Aqua Hedonia Lagoon, and all were in young juvenile fish between 120 and 240 dph. Peak mortality for the epizootics was hundreds to over a 1,000 dead per day.

Although no other outbreaks have been confirmed in cultured white seabass, it is believed that herpesviral epizootics have been an annual occurrence, possibly dating as far back as 1995 when the hatchery was first constructed. Throughout the 10-year life of the hatchery, spikes in raceway mortality have consistently occurred during the colder winter months (November through February) when water temperatures drop as low as 10^{o}C. The combination of smaller/younger fish and cold water appears to trigger the disease. Typically, mortalities spike when smaller juvenile white seabass, 90 to120 dph, are moved from the 23^{o}C recirculating J2 system into the raceway system, which is on ambient flow-through water. It is believed that smaller, younger white seabass are not immunocompetent, and that lower water temperatures further impair their immune systems.

Primary clinical signs for herpesviral epizootics include: 1) a sudden increase in mortalities among smaller juvenile fish; 2) well fleshed (i.e., not emaciated) fish with no external lesions; and 3) moribund fish that go into a terminal spiral, in mid-water or near the surface, just prior to death.

The virus has proven difficult to culture and as a result no ELISA assay has been developed to test for herpes exposure. A PCR assay has been developed, but only indicates the presence/absence of active virus. To date, no wild white seabass have tested positive for herpesvirus using the PCR assay. Although we believe that herpesvirus is a "normal" pathogen of white seabass, we have no hard evidence and must assume that this virus is a novel pathogen to wild white seabass. As such, the OREHP policy is to quarantine any fish that test positive for herpesvirus via PCR. These fish must be retested one month after mortality levels have returned to normal levels. If and when an ELISA is developed for white seabass herpesvirus, and if wild fish serum samples demonstrate herpesvirus exposure to wild white seabass, the OREHP can then go to the more permissive policy of euthanizing infected fish, but releasing healthy exposed fish.

7.3.1.4 *Necrotizing hepatitis* (suspect viral hepatitis)

A new disease of cultured white seabass was discovered in the summer of 2004. The disease was initially observed in yearling white seabass held at HSWRI's growout facility in Catalina Harbor on Santa Catalina Island and has since been confirmed in fish held at the Catalina Seabass Fund (CSF) net pen (also in Catalina Harbor), and at the Dana Landing growout facility in Mission Bay, San Diego. Affected fish were discovered as a by-product of routine health inspections, and were clinically healthy – eating well, well muscled, and swimming strongly. The only common denominators between the Catalina fish (both HSWRI and CSF fish) and the Mission Bay fish were: 1) they were cultured hatchery fish; 2) they were older (>365 dph) white seabass; and 3) they had similar liver lesions.

Grossly, livers were often smaller than normal, but the most prominent finding was a "mottled" appearance. Mottling was a consequence of randomly distributed colors, consistency, and "elevations." Color varied from tan to brown to cream and maroon. Darker areas were firmer (consistency) and usually depressed ("elevation") below the capsular surface. Both left and right liver lobes were affected and the pattern was random. There were no obvious abscesses or granulomas, and the capsular surface was usually smooth (i.e., no peritonitis).

Histologically, livers were characterized by multifocal coagulation necrosis, with variable mononuclear inflammation, vacuolar degeneration, hepatocellular regeneration, and occasional pancreatic metaplasia. Samples from the HSWRI pen were sent to UC Davis for virology, and consisted of pooled samples of liver and spleen from 20 fish. Pooled samples were homogenized and inoculated onto several fish cell lines. Additionally, paraffin histology blocks were subsampled and processed for TEM. Virology and TEM were both negative for a viral pathogen. Although a viral etiology

cannot be completely ruled out, the liver damage observed at these three growout facilities was likely a result of exposure to some aqueous hepatoxic agent – either manmade or natural. There are numerous compounds in existence that can result in hepatic necrosis with subsequent degeneration, regeneration, and potential pancreatic metaplasia.

7.3.2 Bacterial pathogens

7.3.2.1 *Flexibacter maritimus*

Flexibacter infections are not uncommon among cultured white seabass. *Flexibacter* are long, thin gram-negative bacilli. Infections occur primarily on the skin, although occasional involvement with gills has been observed. In addition to being a primary obligate pathogen, *Flexibacter* can also occur as a secondary invader and often complicate cutaneous lesions initiated by protozoan or metazoan infestations. *Flexibacter* infections coincident with *Uronema marinum*, a ciliated protozoan parasite, are common.

Flexibacter infections are most common among young juvenile white seabass (60 to 90 dph) and occur when the fish are transferred from the warm water, recirculating J2 system into the ambient water raceways or growout facilities. If this move is made during the winter months, when water temperatures range as low as 10^{o}C, then fish can break out with *Flexibacter* infections. *Flexibacter* outbreaks also occur in the hatchery raceways, or in net pen systems, when water temperatures fluctuate rapidly. Outbreaks occur under these circumstances because: 1) smaller/younger fish have less well-developed immune systems; 2) smaller/younger fish have been exposed to fewer pathogens; 3) the lower water temperature probably results in vasoconstriction to peripheral organs (fins and skin), reducing immune surveillance; and 4) there is a thermal limit for the immune response, and when ambient temperature dips below that limit, the immune system becomes severely impaired.

Control of *Flexibacter* infections is largely manageable by good husbandry, proper temperature acclimation, and timing transfer of smaller fish to minimize handling and temperature stress. When outbreaks do occur, treatment with medicated feed usually resolves the problem. Although both Romet© (sulfadimethoxine & ormetoprim sulfa) and oxytetracycline feeds have been used, Romet© is generally preferred because of greater palatability and efficacy. Fish are treated at 3 percent of body weight for 10 days.

7.3.2.2 *Vibrio*

Vibrio are small motile, gram-negative bacteria and are common pathogens of marine fish. Among juvenile cultured white seabass, *Vibrio* are usually associated with lesions involving skin or fins. In most cases, *Vibrio* infections are secondary to damage caused by protozoan or metazoan parasite infestations. At the hatchery, *Vibrio* infections commonly occur as secondary infections of *Uronema marinum* infestations; at net pen

sites, *Vibrio* infections often occur with skin fluke infestations. Mixed infections involving *Vibrio*, *Flexibacter*, parasites, and fungi are also relatively common. Disseminated infections are less common but do occur.

Diagnosis of Vibriosis is made via wet mount preparations and visualization of motile bacteria with phase contrast or dark field microscopy; bacilli are short rods and Gram-negative. Infections are typically mixed with *Flexibacter*, protozoa, and sometimes metazoan parasites. Treatment of secondary cutaneous *Vibrio* infections involves elimination of the primary pathogen. Protozoan and metazoan infestations are managed with aqueous hydrogen peroxide (H_2O_2). Once the parasites are gone, *Vibrio* infections often resolve spontaneously. With disseminated infections, treatment with a ten day course of antibiotic feed (usually Romet©) is recommended.

Vibrio enteric infections have emerged as the most significant killer of cultured larval white seabass in 2007 and 2008. Vibrio enteritis has been responsible for the deaths of millions of larval white seabass between 5 and 20 dph. Onset is associated with the start of exogenous feeding with live artemia at five dph. Clinically, there is extremely high mortality, with incubator losses ranging from 80 to100 percent. Grossly, fish are small, thin, and dark. Dead fish float at the surface of the water, in large rafts, prior to sinking. Histologically, infected fish have moderate to massive numbers of bacteria in the stomach, intestines, or throughout the gastrointestinal tract. Bacteria cultured from infected larvae have been tentatively identified as *Vibrio vulnificus* by the California Animal Health and Food Safety Lab (Davis, CA). Additional isolates have been sent to the Washington Aquatic Animal Disease Diagnostic Laboratory (Pullman, WA) for confirmation of genus and species ID.

Vibrio enteritis in larval white seabass is believed to be related to poor water quality, poor egg quality, or some combination. The poor egg quality hypothesis is based on the fact that the hatcheries broodstock population is aged. Egg quality, as well as genetic diversity, should improve when younger broodstock fish are introduced into the hatchery. Newly caught wild adult and subadult white seabass will be gradually metered into the four existing broodstock tanks in accordance to the broodstock replacement plan (Section 5.2.3).

The water quality hypothesis is based on experimental evidence (experiments done in 2008) that have shown that larval white seabass have higher survival and growth rates when reared in water derived from sources outside Aqua Hedionda Lagoon. Improvements – some dramatic – have been observed when larval white seabass have been reared; 1) at the HSWRI Mission Bay facilities; 2) at a hatchery in Ensenada, Mexico; and 3) in purified Long Beach Harbor water obtained from a commercial source. Interest is currently focused on the possibility that the ozone purification system, at the Carlsbad hatchery, has been improperly used and that toxic bromates are at least partially responsible for the high prevalence and severity of *Vibrio* enteritis among larval white seabass.

The causative relationship between exposure to toxic ozone by-products and bacterial enteritis is thought to be indirect, with bromate exposure resulting in sublethal toxicity and possibly immunosuppression. It is also possible that bromate exposure could simply be improving conditions for bacterial infection and growth by damaging and killing intestinal mucosal epithelium.

Other sources of xenobiotic exposure – the commercial agriculture fields and the Carlsbad municipal golf course – have also not been ruled out with respect to involvement with the recent surge in bacterial enteritis. Some of the myriad of chemicals used at these businesses could be escaping destruction by the hatchery's ozone system. Alternatively, some xenobiotic compounds could be converted into more reactive and dangerous chemicals following ozonation. Larval fish are at a highly sensitive stage of development and exposure to even minute (part per billion or part per trillion) quantities of xenobiotics could result in immunosuppression, sublethal injury, or developmental deformities.

7.3.3 Rickettsial bacteria

Rickettsia are bacteria that have evolved to live intracellularly. The two pathogens known to infect cultured white seabass are: *Epitheliocystis* sp. (a benign gill pathogen); and *Piscirickettsia salmonis* (a lethal systemic pathogen which primarily targets liver and kidney).

7.3.3.1 *Epitheliocystis*

Epitheliocystis is a common pathogen of both marine and freshwater teleost fish, and has been reported in a variety of cold and warmwater fish species, both cultured and wild (AFIP 2004). This intracellular organism commonly infects gills, nares, and GI tract; it has only rarely been observed in internal organs. Intracellular replication results in massive hypertrophy of individual cells; infected cells eventually rupture to release and disseminate the bacteria.

Among cultured white seabass, *Epitheliocystis* is a common, but benign pathogen. It primarily develops in older (>120 dph) raceway fish, or in older net pen fish. The source of the infection is unknown, but is suspected of being resident wild fish in the lagoon, or living in and around net pen sites. The primary target organ for *Epitheliocystis* in white seabass is the gill and diagnosis is made via wet mount examinations of gill scrapings. Characteristic lesions observed with dark field illumination, using a compound microscope, are cystic bacterial aggregates that range from 50 to 300 microns in diameter. Cysts are round to oval, with smooth surfaces, and are uniformly filled with thousands of short, non-motile bacilli. Occasionally, infections can be severe, with thousands of cysts lining gill filaments. The presence of massive numbers of *Epitheliocystis* cysts would appear to severely impair respiration, but no mortality is associated with infection and fish spontaneously shed cysts (or cysts rupture) if held for a long enough period of time (several weeks to months). Experimental treatment with oxytetracycline (in medicated feed) had no effect on the recovery of a group of raceway

fish in 2002. Consequently, fish are not treated and the disease is allowed to run its course; healthy, asymptomatic fish are cleared for release.

7.3.3.2 *Piscirickettsia salmonis*

Piscirickettsia salmonis (*P. salmonis*) is small gram-negative bacillus. The bacterium is a lethal, obligate, intracellular pathogen of many anadromous and marine fish species (Fryer and Hedrick 2003). The initial outbreak occurred in 1998 in the hatchery's raceways. It was subsequently found at Newport Harbor, Channel Islands Marine Research Institute, Santa Barbara Harbor, and Dana Point Harbor. Once a positive diagnosis was made and samples collected, all fish were euthanized. The initial *P. salmonis* outbreak is documented in detail by Chen et al. (2000). Samples collected from the first outbreak were used to develop an ELISA diagnostic for wild fish assessment. The results of the wild fish assessment did not detect evidence of *P. salmonis* in wild white seabass. Thus, any time there is a *P. salmonis* outbreak the white seabass will have to be euthanized.

A second outbreak occurred in April of 2005 at a land-based facility in King Harbor, Redondo Beach. The initial diagnosis was based on gross and histologic findings. *Piscirickettsiosis* was subsequently confirmed via culture (on chinook salmon embryo cells), PCR, and polyclonal FAT (fluorescent antibody testing). All 3,000 fish were euthanized.

7.3.4 Fungal infections

Fungal infections among cultured white seabass are relatively uncommon. The three types of fungal infections seen are: ocular, cutaneous, and disseminated. The ocular form is most common and is believed to be a terminal form of severe intraocular emphysema caused by GSS disease. It is believed that fungi colonize necrotic ocular tissue following damage induced by gas infiltration. Affected eyes are characterized by partial to complete infiltration with friable, brown-yellow, coarsely granular necrotic debris.

The cutaneous form had been rare prior to 2004. Cutaneous fungal infections have been an increasingly common phenomenon among the hatchery's raceway fish. The juvenile white seabass had been previously infected with *Flexibacter* and *Hexamita*, and this may have predisposed fish to a higher than normal prevalence of cutaneous mycoses.

Disseminated infections, involving multiple internal organs (liver, kidney, spleen) are rare, but have been observed in a few hatchery and growout facility white seabass. The major finding at necropsy is multifocal nodular masses in parenchymal organs. These lesions are similar to fish with chronic *Piscirickettsiosis*, however, *P. salmonis* is almost always associated with high mortality, while disseminated mycosis is almost always an incidental finding in one or two fish among a larger healthy population of fish.

Diagnosis of fungal infections is made with wet mount preparations and visualization of fungal hyphae under dark field illumination. Fungi have also been successfully isolated on Sabouraud Dextrose agar. Histologic assessment of lesions consistently reveals massive granulomatous inflammation. Fungal hyphae may be difficult to identify on standard HE slides; use of special stains (e.g., GMS or giemsa stains) can enhance detection. Precise identification of the species involved has not been determined, but is suspected of being the same pathogen that occasionally affects cultured California halibut (*Paralychthys californicus*). There currently is no treatment; vigorous culling of affected fish is recommended to reduce the pathogen load in the water column and to help minimize transmission from fish to fish.

7.3.5 Sporozoans

Sporozoans are spore-forming protozoan pathogens that often have complex life cycles associated with more that one host. Although they can broadly be grouped with other external protozoan parasites based on similarities in certain life stages, they are phylogenetically distinct and have significantly different reproductive strategies. The two major classes of sporozoans are microsporidians and myxosporidians. No microsporidian pathogens have been identified for cultured or wild white seabass.

7.3.5.1 Renal Myxosporidians

Unidentified *Myxosporidian* parasites have been observed in the collecting and mesonephric ducts of numerous hatchery and net pen white seabass. Infected fish had no clinical signs and no increase in daily mortality. Various life stages of the parasites have been found in both the lining epithelium, and in the lumen. There is little or no inflammatory response to these parasites and they appear to be an incidental finding and harmless. No treatment has been attempted.

7.3.5.2 Unidentified renal sporozoan pathogen

There have been four epizootics involving this new, lethal, highly contagious, sporozoan pathogen: two at Southwestern Yacht Club (2005 and 2007); one at Catalina (2006); and one at the Carlsbad Hatchery (2007). Total losses have been over 106,000. Clinically, the disease is characterized by a gradual increase in daily mortality that eventually tops out at 20 to 50 dead per day in smaller pens with one to 3,000 fish. In larger net pens, losses can be in the hundreds per day. Fish have no external lesions and are usually well muscled (i.e., not thin).

Early kidney lesions are presented as multifocal, unencapsulated masses. Histologically, renal lesions were large granulomas and diffuse granulomatous inflammation. The majority of granulomas and macrophage aggregates contained one to 20 unidentified pathogens – possibly a sporozoan or coccidian parasite. Pathogens were characterized by a spherical to oblong shape with variable numbers of dark basophilic, blunt-ended, rod-like structures in the cytoplasm. Nuclei were not observed. The smallest organisms were 5 microns in diameter with six to ten of the basophilic

rods. The largest pathogens were 14 microns in diameter and contained up to 100 of the basophilic internal structures. The basophilic rod-like structures were two to four microns long and about one micron in diameter. The rod-like structures were uniformly purple-blue (deeply basophilic).

Older kidney lesions were typified by large, coalescing, cystic lesions, as renal tubules and ducts were blocked and filled with fluid. Granulomatous lesions have also been found in the gills, heart, liver, and pyloric cecae. Gill lesions were linear foci of pallor in the filaments. Severe liver lesions can grossly mimic *Piscirickettsia salmonis* infection, but severe liver lesions are uncommon.

This new disease is thought to have originated from one or more wild marine fish species that routinely congregate around net pen facilities. At this time, it is unknown whether or not this is a naturally occurring disease of wild white seabass. As such, any hatchery fish discovered with this disease will be euthanized. UC Davis is assisting with development of a PCR assay and ELISA to help screen wild fish for infection or exposure. Histology, cytology, and TEM will be used to characterize and identify this new pathogen.

7.3.6 Metazoan parasites

Metazoan parasites affecting teleost fish include both flukes (trematodes) and tapeworms (cestodes). No tapeworms have been identified in cultured white seabass, but several fluke species are known to infest white seabass. Flukes infesting white seabass have all been monogenetic trematodes – that is they only need one host to complete their lifecycle.

7.3.6.1 *Anchoromicrocotyle guaymensis*

Anchoromicrocotyle guaymensis is a monogenetic trematode primarily affecting white seabass at the two Catalina Harbor growout facilities, and Dana Point Harbor. This fluke had been previously classified as *Cynoscionicola pseudoheteracantha*, but this was corrected in 2005. The disease is usually a problem in the summer and fall months. Small numbers of wild juvenile and subadult white seabass, captured from Mission Bay, have also been found to have this gill parasite. White seabass afflicted with *Anchoromicrocotyle guaymensis* are treated with multiple H_2O_2 treatments. Tarps are used to surround the containment net and fish are treated with 100 ppm H_2O_2 for one hour for three consecutive days.

Clinically, heavily infected fish are thin, listless, and anorexic. Severely affected fish will have pale gills (anemia) and flukes will stand out as dark black-brown linear forms. Individual flukes will appear as pairs of dark streaks (these are macroscopically visible gonads), with the largest worms measuring 7 x 0.25 x 0.2 mm; immature flukes are detectable only with light microscopy. Fresh dead fish (recovered from the surface or bottom of the pen) will often have the best gross lesions as the flukes will stand out sharply against the pale washed out gills. Immersion of euthanized (or anesthetized)

fish in a shallow clear or white container, filled with seawater, will also be helpful in assessing all four sets of gills.

Eggs are ovoid and symmetrical, with tapered ends attached to long, coiled, thread-like structures. The eggs appear to be designed to entangle structure following expulsion by adults. It is likely that eggs entangle and attach to gill lamellae, and that newly hatched larvae directly infect host fish. Alternatively, eggs could become entangled in containment netting, thereby avoiding treatment when fish are immersed in hydrogen peroxide in separate treatment containers. The eggs of *C. pseudoheterocantha* are reportedly more resistant to treatment than either larvae or adults, and are probably the cause of rapid recurrence if fish are not given multiple treatments.

7.3.6.2 *Gyrodactylus*

Gyrodactylus is a common skin pathogen of freshwater fish, and has been reported in several marine fish species (Noga 2001). It has only been seen in cultured white seabass at two growout facilities. *Gyrodactylus* flukes have also been occasionally seen in gill samples from adult broodstock at the hatchery; flukes have never been observed among larval or juvenile hatchery white seabass.

Gyrodactylus in white seabass have been limited to the gills and are characterized by large attachment hooks, absence of eye spots, and the presence of embryonated eggs. Flukes can be controlled with H_2O_2 at the growout facilities or either H_2O_2 or formalin at the hatchery. Treatment consists of a bath of 100 ppm H_2O_2 for one to two hours for three consecutive days.

7.3.7 Protozoan parasites

A wide variety of protozoan parasites are known to infect fresh and marine fish species. Protozoan parasites can be subdivided into three groups: the ciliates, the flagellates, and the sporozoans. Ciliates are small, motile unicellular organisms characterized by the presence of large bands or sheets of short cilia, which beat in synchrony for locomotion. Flagellates are also motile, unicellular organisms, but use a smaller number of long flagella for motility. Both ciliates and flagellates reproduce by binary fission.

7.3.7.1 *Ciliates*

Three species of ciliated protozoan parasites have been found in cultured white seabass. The most common and benign is *Trichodina*; the most dangerous and lethal is *Uronema marinum*. A third as yet unidentified species has been observed in a few fish.

7.3.7.1.1 *Trichodina*

Trichodina are small disc-shaped unicellular protozoan parasites that range in size from 30 to 60 microns. *Trichodina* have an inner circular ring of denticles (used for feeding) and a peripheral outer ring of cilia (used for locomotion). They move in a characteristic circular fashion and can be found both on the skin and in the gills. These parasites are very common in both hatchery and net pen fish, but are largely harmless, grazing on the surface debris and never invading into deeper tissues. On rare occasions, when massive numbers of *Trichodina* are present, treatment with H_2O_2 is required.

7.3.7.1.2 *Uronema marinum*

Uronema marinum is the most dangerous external protozoan parasite affecting cultured white seabass. This lethal pathogen is responsible, annually, for the loss of thousands of hatchery and net pen fish. Typically, epizootics occur in older raceway or net pen juveniles that are on ambient water; *Uronema* has not been observed in smaller larval or juvenile white seabass, those located within the main hatchery building, during the past three years.

Clinically, *Uronema* epizootics are characterized by high mortality and large numbers of fish with hemorrhagic cutaneous ulcers on skin and fins. Ulcers have irregular margins and are usually deep, extending down into the underlying musculature. An even more virulent strain of *Uronema* has been observed on a few occasions and is characterized by ocular and central nervous system lesions, with protozoa invading the eyes and sometimes extending through the cranial vault into the brain. *Uronema* skin ulcers are frequently complicated by secondary infection with *Flexibacter* and/or *Vibrio* bacteria; older lesions can be mixed with other protozoa and fungi.

Diagnosis of the cutaneous form of *Uronema* is made with skin scrapings and examination of wet mount preparations with dark field microscopy. Highly motile *Uronema* are unicellular protozoa characterized by relatively large size (15 x 40 microns to 40 x 90 microns), elliptical amoeba-like shape, and cilia covering entire outer surface. The ocular form of *Uronema* can be identified by typical gross appearance and wet mount examinations of ocular aspirates. The central nervous system form of *Uronema* can be confirmed with histology. The most recent form of *Uronema* is a brachial form which attacks primarily the gill filaments.

Uronema epizootics are managed with three one hour, 75 ppm H_2O_2 bath treatments. With ocular and central nervous system forms of *Uronema*, higher concentrations of peroxide have been used (up to 150 ppm). Unfortunately, the more virulent forms of *Uronema* have been resistant to H_2O_2 treatment. Resistance is probably related to sequestration and, therefore, protection of organisms in the eye and brain. Aggressive culling of fish with eye lesions and moribund fish, used in combination with H_2O_2 baths, is recommended when the ocular or central nervous system forms of *Uronema* are encountered.

7.3.7.1.3 *Cryptokaryon irritans*

Cryptokaryon irritans is a common and dangerous pathogen of marine fish but has rarely been encountered with white seabass. The only epizootic to occur over the past seven years was an outbreak that occurred at the King Harbor growout facility in August of 2008. The epizootic was restricted to one of two above ground tanks and fish were treated with hydrogen peroxide prior to release. Treatment with peroxide, just prior to release, is recommended because some organisms penetrate into the skin or branchial mucosa and are able to survive H_2O_2 therapy. Surviving organisms inevitably result in recurring infections when fish are housed in flow-through systems used at all growout sites.

7.3.7.1.4 Unidentified ciliates

A small number of unidentified ciliates have occasionally been observed in moribund white seabass with cutaneous lesions. Almost all of these cases have occurred with mixed infections involving other protozoa and bacteria. Some fish with unidentified ciliated protozoans have had non-inflation of the swim bladder, or were severely moribund, and were in contact with the bottom of raceways. The unidentified ciliates are presumed to be opportunistic pathogens that normally live in the bottom detritus.

One unidentified ciliate that has been seen on several occasions is an elongate protozoan with a distinctive baleen-like structure. This baleen-like structure is lined by cilia and is probably the opening of the oral cavity. This motile parasite is longer and slimmer than *Uronema*, and measures 15 x 60 microns. This parasite has been seen three or four times at the Carlsbad Hatchery, and has always been observed mixed with *Uronema marinum*.

7.3.7.2 Flagellates

7.3.7.2.1 *Ichthyobodo*

Icthyobodo are small (7 to10 microns), oval, flagellated protozoan parasites that are found in both gills and skin of cultured white seabass. *Ichthyobodo*, also know as *Costia*, are common parasites of both raceway and net pen fish. Similar to *Trichodina*, they are relatively benign parasites and usually not associated with clinical signs, or with increased mortality. Diagnosis is made with skin scrapings and visualization with dark field microscopy. *Ichthyobodo* are minimally motile and have a characteristic flickering (as in the flickering of candle light) motility. With gill samples, *Ichthyobodo* are best observed in thin preparations; look for organisms at the periphery of the smear, in areas with large numbers of red blood cells (RBCs). Although *Ichthyobodo* are small (similar in size to RBCs), parasites can be detected at low magnification (4 and 10X objective fields), by scanning large areas and looking for movement among RBCs. Slides are best checked immediately as *Ichthyobodo* are fragile and die quickly if slides dry out; dead parasites are almost impossible to detect because they are small and stop moving. Under most circumstances, *Ichthyobodo* infestations are not treated. Occasionally, heavy infestations will require treatment with H_2O_2.

7.3.7.2.2 *Cryptobia*

Cryptobia is a small flagellated protozoan parasite that is occasionally observed in cultured white seabass. *Cryptobia* are small (1.5x the size of *Ichthyobodo*; 5 x 10 microns), oval to pear-shaped, and had two long flagella, one at each pole.

7.3.7.2.3 *Hexamita*

Hexamita is another relatively new pathogen of cultured white seabass. The pathogen is very similar in size and morphology to *Spironucleus*, and the two are difficult to distinguish. Among freshwater fish, *Hexamita* is a relatively common enteric pathogen. *Hexamita* is also the cause of Hole-in-the-head disease – a disfiguring cutaneous disease of a select group of teleost fish species (e.g., discus). *Hexamita* has reportedly been associated with enteritis in cultured white seabass, but written descriptions have not been located and occurrence in white seabass comes only through anecdotal reports. Fish with *Hexamita* were successfully treated with H_2O_2.

Affected fish have discrete round to oval ulcers. Some ulcers were located over the flanks, but many were centered over the head region – cranial to the first dorsal fin, above and between the eyes, and above the operculum. Ulcers were full thickness, with complete loss of scales and skin, and were pale white-yellow. Ulcer margins were sharply delineated and these “cookie cutter” lesions in white seabass were consistent with descriptions of Hole-in-the-head disease of freshwater discus.

Skin scrapings and wet mount preparations revealed the presence of large numbers of flagellated protozoan parasites. Parasites were highly motile, oval to oblong, and slightly larger (1.5 to 2x) than *Ichthyobodo*, measuring 7 x 15 microns. The organisms had four paired sets of long flagella: three pair on the anterior end, and a single posterior pair.

7.3.7.2.4 Unidentified flagellates

Small numbers of unidentified flagellate protozoan parasites have been observed in a few raceway fish at the hatchery. The pathogens were characterized by two tufts of short flagella (or long cilia) on opposite poles (12 and 7 o’clock) and a distinctive hopping type of motility. The unknown organisms were similar in size to *Uronema*.

7.3.8 Isopods

Parasitic isopods have only been encountered in cultured white seabass held in net pens in Huntington Harbor. Isopods were grey with white horizontal striations; size varied from one to two cm long by three to six mm wide. Since occurrence was very rare, no treatment was required. Parasites can be manually removed from affected fish.

7.3.9 Copepods

Parasitic copepods are relatively common among wild white seabass and California sheephead but have only rarely been encountered among cultured white seabass. The only epizootic occurred at the Channel Island Habor growout facility (Oxnard, CA) in June of 2008. Juvenile white seabass were infested with moderate to large numbers of *Caligus* sp. copepods. The epizootic was associated with moderately elevated mortality. Treatment with hydrogen peroxide was moderately effective in reducing the number and severity of infected fish.

Chapter 8. Regulatory Considerations

8.1 Permit and permissions

The permit and permissions process for the OREHP often involves consultation with the Department and outside agencies. Section 8.1 lists the permits and permissions required to operate hatchery and growout facilities. This process is site and project specific so not all permits or permissions are required for the hatchery or growout facilities.

8.1.1 California Environmental Quality Act

The Department, within the Resources Agency, is the lead State agency responsible for managing living marine resources. The Department is charged with protecting and managing the public's fish and wildlife resources of the State. The Department is a Trustee Agency and a Responsible Agency pursuant to the California Environmental Quality Act (CEQA), Sections 15386 and 15381, respectively. As a Trustee Agency for the State's fish and wildlife resources, the Department has jurisdiction over the conservation, protection, and management of fish, wildlife, native plants, and the habitat necessary for biologically sustainable populations of such species. In this capacity, the Department administers the California Endangered Species Act (CESA), the Native Plant Protection Act, and other provisions of the California Fish and Game Code that afford protection to the State's fish and wildlife public trust resources.

A Mitigated Negative Declaration (MND) covering the OREHP has been prepared and will be adopted by the Department in 2010. Preparation and adoption of a CEQA document is necessary to obtain the permits required to operate the hatchery and growout facilities. The MND covers many of the same issues included in the WSEP, including benthic quality and genetic concerns. Each time the permits are renewed the MND should be reviewed.

8.1.2 California Endangered Species Act

The CESA is administered by the Department and parallels the federal Endangered Species Act (ESA). The CESA policy is to conserve, protect, restore, and enhance any endangered or threatened species and its habitat. Under the CESA, an “endangered species” is defined as a species of plant, fish, or wildlife that is “in serious danger of becoming extinct throughout all, or a significant portion of its range” and is limited to species or subspecies native to California. The CESA prohibits the “taking” of listed species, including species petitioned for listing (i.e., State candidates), except as otherwise provided in State law. State lead agencies are required to consult with the Department to ensure that any action it undertakes is not likely to jeopardize the continued existence of any endangered or threatened species or result in destruction or adverse modification of essential habitat.

The Department has reviewed the location of the growout facilities and determined that they will not adversely affect any State listed endangered or threatened species.

8.1.3 National Pollution Elimination Discharge Permit

Waste discharges from finfish culture operations in marine environments are regulated through the National Pollution Discharge Elimination System (NPDES) permit process when they produce more than 45,454 kg (harvest weight) of warm water fish annually (Agency 2008). In California, a NPDES permit is issued by the local Regional Water Board and has monitoring requirements consistent with the type of discharge.

Neither the hatchery nor the growout facilities exceed the 45,454 kg threshold requirement; therefore, they are not required to obtain NPDES permit coverage. The hatchery, however, is required to monitor intake and effluent flow volumes and pollutant levels. In addition, they are required to submit an Annual Monitoring Report by Feburary one of each year.

Although NPDES permits with monitoring conditions are not required for the growout facilities, the OREHP voluntarily began monitoring benthic conditions in 2004. The purpose of the benthic monitoring is to determine if there are negative impacts to the benthos caused by the growout facilities. Between 2004 and 2006, thirteen of the growout facilities were sampled. The land-based growout facility at King Harbor, Redondo Beach was not sampled. A description of the benthic monitoring program can be found in Section 9.1. The Los Angeles Regional Water Quality Control Board has requested periodic water quality testing for the four facilities within their jurisdiction (Channel Islands Harbor, Marina del Rey, and the two Catalina Harbor growout facilities).

8.1.4 Coastal Development Permit

The CCC is responsible for administering the California Coastal Act (Coastal Act) and the federally approved California Coastal Management Program pursuant to the Coastal Zone Management Act. The Coastal Act policies, implemented by the CCC, address issues such as public access and recreation, natural resource protection, agricultural operation, coastal development projects, port activities, and energy production. Jurisdiction is within the 1,100-mile-long coastal zone, which encompasses 1.5 million acres of land, and up to five miles inland from the mean high tide line. This jurisdiction also extends into the ocean to the Federal waters' limit through the CCC's federal consistency authority under the Coastal Zone Management Act. Development activities in the coastal zone generally require a CDP from either the CCC or the local government.

The hatchery and each growout facility had to obtain a CDP from the CCC. The land-based growout facility in King Harbor growout facility is not required to have one because it operates under a CDP for SEA Lab, a science education center operated by Los Angeles Conservation Corps. Normally, CDPs are issued for development and are

not renewed once the development is completed. The OREHP is unique in that the CCC requires the permits to be renewed every five years because development (release of fish) is ongoing. The Department and the growout facility operators are co-applicants on the CDPs for the growout facilities. The Department is also a co-applicant with HSWRI on the hatchery's CDP. The CDPs for the growout facilities and the hatchery should be renewed in 2010.

8.1.5 State Lands Lease

The California State Lands Commission (SLC) has jurisdiction over all of California's tide and submerged lands, and the beds of naturally navigable rivers and lakes each of which are sovereign lands, swamp, overflow lands, and school lands (proprietary lands). Management responsibilities of the SLC extend to activities within submerged land and those within three nautical miles of shore. A lease may be required for activities that occur on state tide and submerged lands including recreational piers, marinas, industrial wharves, tanker anchorages, oil and gas, and geothermal development.

Most of the waters in which the growout facilities reside have been leased or purchased by the local city or county and a State Lands Lease is not required. The exceptions are the two growout facilities at Catalina Harbor, which are owned by the State and leased to the Catalina Island Conservancy. The terms of that lease did not include fish culture, therefore the two the OREHP growout facilities had to obtain separate State Lands Leases. The Department was a co-applicant on both the leases.

8.1.6 Section 401 and 404 of the Clean Water Act Permit Requirements

Section 404 of the Clean Water Act requires applicants for any project that may result in a discharge of dredge or fill material into jurisdictional waters of the United States to obtain a Section 404 permit from the U.S. Army Corps of Engineers (USACE). Section 10 of the Rivers and Harbors Act requires applicants to obtain authorization from the USACE for projects that involve construction, excavation, or deposition of materials, or for any activities that affect the location and navigable capacity of waters of the United States. The USACE can authorize any of these activities by a standard individual permit, letter of permission (LOP), nationwide general permit, or regional permit.

Applicants receiving a permit from the USACE are required under Section 401 of the Clean Waters Act, to obtain Section 401 water quality certification from the local Regional Water Board. This ensures that any discharge will meet State surface water quality standards. Section 401 water quality certification is not required under an LOP because LOPs can only be issued if there is no discharge or fill. When necessary, issuance of these permits requires the USACE to consult with NOAA Fisheries Service and the U.S. Fish and Wildlife Service (USFWS) for ESA issues. Additionally, NOAA Fisheries Service must be consulted with respect to Essential Fish Habitat (EFH) issues.

No Section 401 certification is required because the facilities are too small to warrant certification. Eight of the growout facilities obtained individual LOPs when the facilities were built. A provisional group LOP, dated April 3, 2006, has been granted for the five growout facilities that did not previously obtain an LOP. The provisional LOP will be finalized once the CDPs have been renewed.

The USACE determined that the hatchery's outflow structures and associated intake structures comply with the terms and conditions of Nationwide Permit 7. Nationwide Permit 7 covers outfall structures and associated intake structures where the effluent from that outfall is authorized, conditionally authorized, or specifically exempted, or is otherwise in compliance with regulations issued under the National Pollution Discharge Elimination System program (Section 402 of the Clean Water Act).

8.1.7 Private Aids to Navigation Permit

A private aid to navigation is a buoy, light, or daybeacon owned and maintained by any individual or organization other than the U.S. Coast Guard (USCG). These aids are designed to allow individuals or organizations to mark privately owned marine obstructions or other similar hazards to navigation. Permission to place a private navigational aid must be obtained from the USCG and the type of aid shall be determined by the USCG. Before applying to the USCG, permission to build any structure used as a private aid to navigation must be granted by the USACE. Installation and maintenance of the aid is the responsibility of the owner or operator.

Seven of growout facilities are attached to a dock and do not require any navigational aids to warn vessels of their presence. The other five facilities are not attached to a dock (Agua Hedionda Lagoon, Newport Bay, Santa Barbara, and the two Catalina Harbor facilities). The growout facility located in Agua Hedionda Lagoon does not require any navigational aids because vessels are not allowed within the lagoon. The Newport Bay and Santa Barbara growout facilities are located within permanent mooring fields that are well documented on navigation charts, thus no navigational aids are needed. The Catalina Harbor growout facility operated by Catalina Seabass Fund is located in a temporary mooring field in Catalina Harbor with the facility moving between two mooring sites seasonally. The USCG determined that the facility needed one white light located amidships, flashing at a four second interval. The larger Catalina Harbor growout facility operated by HSWRI is moored near the mouth of Catalina Harbor, and the USCG determined that the facility needed four white flashing lights, one on each corner of the facility, flashing at a four second interval.

8.1.8 Section 7 Endangered Species Act Consultation

The U.S. Fish and Wildlife Service (USFWS) and NOAA Fisheries Service grant at-risk species and stocks protection under the federal ESA with endangered, threatened, and depleted status designations. NOAA Fisheries Service is charged with the implementation of the ESA for marine and anadromous species, while the USFWS implements programs and regulations for terrestrial and freshwater species. The ESA

requires NOAA Fisheries Service and the USFWS to develop recovery plans for species added to the list of Threatened and Endangered (T&E) species. The plans describe necessary conservation measures to ensure recovery of the species so that it becomes appropriate to remove the species from the T&E list. Section 7 of the ESA of 1973 requires that Federal agencies insure that their actions are not likely to jeopardize the continued existence of endangered or threatened species or result in the destruction or adverse modification of the critical habitat of such species.

The OREHP receives funding from the SFRA, which places a tax on fishing gear and fuel. Because of this, the OREHP undergoes Section 7 consultations annually in order to receive the SFRA funds.

8.1.9 MMPA designation

In addition to the ESA, the federal Marine Mammal Protection Act (MMPA 1972, amended 1994) also provides designations for at-risk marine mammal stocks. A species or a stock of a species is designated as depleted when it falls below its Optimum Sustainable Population (OSP) or if the species is listed under ESA. The MMPA also lists a stock as strategic if: 1) it is listed as a T&E species under ESA; or 2) the stock is declining and likely to be listed as threatened under the ESA; or 3) the stock is listed as depleted under the MMPA; or 4) the stock has direct human-caused mortality which exceeds that stock's Potential Biological Removals (PBR) level. The term PBR is defined as "the maximum number of animals, not including natural mortalities that may be removed from a marine mammal stock while allowing that stock to reach or maintain its OSP". NOAA Fisheries Service develops estimates of PBR's for each marine mammal stock in U.S. waters.

Under Section 118 of the MMPA, NOAA Fisheries Service classifies all U.S. commercial fisheries into one of three categories (I, II, III) based on the level of incidental serious injury and mortality of marine mammals that occurs in each fishery. The categorization of a fishery determines whether fishery participants will be required to comply with certain provisions of the MMPA, such as registration, observer coverage, and take reduction plan requirements. Participants in Category I or II are required to be registered under the MMPA. Category III fisheries may incidentally take marine mammals without registering for or receiving an authorization from NOAA Fisheries.

In 2004, the OREHP was designated a Category III fishery. There have been only eleven incidences of lethal take of a marine mammal (California sea lion) since the first growout facility became operational in 1992. Any take of marine mammals is reported immediately to NOAA Fisheries, the Growout Facility Coordinator, and the OREHP Coordinator (Section 7.6).

8.1.10 The Magnuson-Stevens Fishery Conservation and Management Act and Essential Fish Habitat

The Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) of 1976 governs the conservation and management of ocean fishing. The MSFCMA establishes sole U.S. management authority over all living resources within the 200-nautical mile exclusive economic zone (EEZ) of the U.S. The 1996 amendments, termed the Sustainable Fisheries Act (SFA) of 1996, designated and conserved Essential Fish Habitat (EFH) for species managed under a Fisheries Management Plan to minimize any adverse effects on habitat caused by fishing or non-fishing activities and to identify other actions to encourage the conservation and enhancement of such habitat. EFH is defined as "those waters and substrate necessary for spawning, breeding, feeding, or growth to maturity".

Federal agencies are required to consult with NOAA Fisheries for any action authorized, funded, or undertaken, or proposed to be authorized, funded or undertaken that may adversely affect any EFH.

NOAA Fisheries staff has reviewed the location of the growout facilities and determined that they will not adversely affect any EFH (primarily eelgrass habitat).

8.1.11 United States Environmental Protection Agency

The United States Environmental Protection Agency (EPA) was established to perform basically two functions: 1) research and development; and 2) abatement and control of pollution through a combination of research, monitoring, standard-setting, and enforcement activities. Although the EPA has no direct ocean resource management responsibilities, it administers and enforces various environmental protection statutes of general application, including the Federal Insecticide, Fungicide, and Rodenticide Act, under which it registers and regulates the use of pesticides or approves State plans for that purpose. The products regulated include tributyltin, a component of ship bottom antifoulant paints, which has an adverse effect on nontarget marine life.

The OREHP does not utilize any insecticides, fungicides, or rodenticides, nor does it use any regulated antifoulants, thus the OREHP did not consult with the EPA.

8.1.12 Local Authority Permissions

Local authorities include cities, counties, harbor departments, and private land owners (e.g., marina owners, power plants, Catalina Island Conservancy). The permissions can be a formal permit or lease, or simply a letter of permission from the local authority stating their approval of the growout facility. Information for the local authority varies and may include certification or permits mentioned above. For the purposes of the CDP approval, any permission from a local authority that leases the land from another entity must include the lease agreement.

The growout facility operators have all obtained permission for their facilities. The hatchery has a lease agreement with NRG Cabrillo Power II LLC, the local landowner

(as well as a conditional use permit from the City of Carlsbad, a wastewater discharge permit, and a regulated stormwater management plan).

Chapter 9. Environmental Considerations

9.1 Benthic monitoring program

The purpose of the benthic monitoring program is to ensure the growout facilities do not negatively impact the benthos. Salmon farming has been well studied and documented regarding effects on benthic communities, and consequently provides useful examples for the OREHP. Changes in free sediment sulfide concentrations are used in the Pacific Northwest salmon farming industry as a proxy for changes in the benthic community (Brooks and Mahnken 2003a). Other elements from aquaculture operations, such as zinc and copper, may also impact benthic communities, so the OREHP is voluntarily monitoring those too. Benchmarks have been set by some regulatory bodies outside of California for sulfide, zinc, and copper, and are used to minimize impacts of salmon farms to the benthic community. If the benchmarks are exceeded, the salmon farm is required to lie fallow until the benthos has remediated or returned to pre-farm conditions.

9.1.1 Chemical remediation

Brooks et al. (2004) defined the term chemical remediation as the reduction of accumulated organic matter with a concomitant decrease in free sediment sulfide ($S^{=}$) concentrations and an increase in sediment redox potential under and adjacent to salmon farms to levels at which more than half the reference area taxa can recruit and survive.

The time required for chemical remediation is influenced by the availability of sulfate; dissolved oxygen in the benthic boundary layer; bottom current speeds; temperatures; the composition of the natural macrobenthic community; and the depth of organic deposits (Brooks 2006). In general, it appears that chemical remediation requires a few months when the depth of organic deposits is less than a few centimeters. The longest documented chemical remediation took seven years (Brooks et al. 2004). Given the low biomass and short growout cycle, chemical remediation is expected to occur in a matter of months at the OREHP growout facilities.

9.1.2 Biological remediation

Brooks et al. (2004) defined biological remediation as the restructuring of the infaunal community to include those taxa whose individual abundance equaled or exceeded one percent of the total invertebrate abundance at local reference stations. Recruitment of rare species representing less than one percent of the reference abundance was not considered necessary for complete biological remediation.

There is a lag between chemical and biological remediation as the latter occurs in stanzas characterized by macroinvertebrate feeding guilds. For quickly remediating

sites in temperate latitudes, biological remediation also depends on the season in which chemical remediation is complete. Many taxa spawn seasonally and new recruits are available for a limited period of time. In those cases where chemical remediation occurs in the fall, biological remediation may not be complete until the next spring and summer (Brooks 2006). Given the low biomass and short growout cycle in the white seabass growout facilities, biological remediation is expected to occur one season after chemical remediation is complete.

9.1.3 Materials and methods

These facilities are located in shallow water and hold small maximum biomasses of fish. The benthos at these sites has not previously been monitored and an attempt to find appropriate local reference locations was made part of the benthic monitoring program. Acceptable reference locations should have depths equal to the depth under the growout facility (±1 percent) and a proportion of sediment fines (silt and clay) equal to that found under the growout facility (± 20 percent).

The study design relies on a regression approach to identify trends in sediment free sulfides, Total Volatile Solids (TVS), redox potential, zinc, and copper as a function of distance from the growout facility perimeter and at the reference station allowing for an inferential test of the significance of differences.

The survey uses a stainless steel bottom grab to collect samples of the sediment. Various qualitative and quantitative parameters are analyzed for each sample. A detailed description of the sample collection and various analyses is available in Brooks (2006).

Each growout facility will be sampled on a 3-year cycle for two more cycles. The results of the benthic sampling will be reviewed at the end of the third cycle to determine if monitoring needs to continue. If it is determined that monitoring will continue, the cycle and benchmarks will also be reviewed and adjusted where appropriate.

9.1.4 Sources of organic carbon

Chemical changes in sediments are associated with biological oxygen demand (BOD) rather than organic carbon. The causes of organic enrichment at salmon farms are wasted feed and feces (Brooks and Mahnken 2003a) although salmon mortalities and fouling communities may also contribute. BMPs for salmon net pen facilities require the daily removal of carcasses along with the use of antifouling compounds on the nets which would reduce this contribution.

Feed

Salmon feed contains 40 percent protein, 30 to 35 percent lipids, and about 10 percent digestible carbohydrates (necessary to bind the pellets) (Nash 2001). These high energy diets more closely resemble the natural prey of salmon. The amount of wasted feed is based on feeding efficiency. Early estimates stated that up to 30 percent of feed

was lost (Beveridge et al. 1991). Rosenthal et al. (1995) noted even higher loss rates (up to 35 percent) for wet feeds. In other studies, the amount of wasted dry feed was closer to 5 percent (Weston 1986, Gowen and Bradbury 1987, Findlay and Watling 1994). Food conversion rates (FCRs) are used to monitor the waste of feed. A review by Brooks and Mahnken (2003a) revealed that less than 5 percent of the dry feed delivered to salmon net pens in British Columbia was lost to the environment.

The OREHP utilizes a marine fish food that contains 50 percent protein, 14 percent fat, and has Vitamin C and proteinated zinc incorporated into it (Curtis 2005, Drawbridge et al. 2007). Biological FCRs for the growout facilities range between 3.0 and 9.0, and average 5.0 (Brooks 2006). These values are likely inflated because they do not take into account actual feeding levels but rather a standardized three percent body weight per day, which is unadjusted for reduced feeding activity and growth during cold water periods. Under controlled laboratory conditions, Lopez et al. (2006) reported FCR values of 0.7 to 1.0 for juvenile white seabass. Under field conditions at the growout facilities where food was precisely measured, Buhr et al. (2006) reported FCR values of 0.91 to 2.45. Additionally, growout facility operators are required to monitor feeding behavior to ensure the fish are fed to satiation but not excessively. As a result lost feed from these facilities should be minimal and should not contribute significantly to organic matter in the benthos below the facilities.

Feces

Weston (1986) estimated that 25 to 33 percent of the feed eaten by salmon in net pens would be ejected as feces, while a more recent study by Nash (2001) reports that approximately 12.5 percent of the feed weight would be ejected in feces. Of the feed ingested, subtract 87.7 percent for digested protein and 8.25 percent for ash, leaving about four percent to be ejected as organic matter in the feces. Add this to the uneaten feed (five percent) and the result is that about 8.8 percent of the labile organic carbon compounds delivered as feed are discharged from the net pen structure in particulate form, contributing to biological oxygen demand (BOD) in the sediments (Nash 2001, Brooks and Mahnken 2003a).

The OREHP growout facilities are placed in areas with good tidal flow to minimize the buildup of feces below the facility. Operators of raceway facilities are required to vacuum the raceways daily to prevent the build up of feces and feed in the raceway. Department divers observed the benthos under the Huntington Harbor facility in 2003, two months before 2,000 fish were released (Valle and Wertz 2003), and found little to no difference under the facility compared to the surrounding area.

Fish mortality

Winsby et al. (1996) reviewed and analyzed salmon mortality at British Columbia net pens in 1994. Their data suggest approximately nine percent (2,000 t) of the total salmon production (22,000 t) died at the farms. Winsby et al. (1996) concluded that most of the salmon carcasses were removed to approved disposal locations. BMPs of salmon farms require daily physical removal of any carcasses, and therefore do not contribute to any biological loading on the environment.

Growout facility operators are required to observe their pens daily and remove dead fish as soon as they are seen. It is much easier to find dead fish in these small facilities compared to commercial aquaculture pens, thus it is likely that very few dead fish are allowed to decompose and fall through the nets.

Biofouling
Biofouling is a significant factor in coastal environments and can weigh down nets and restrict water flow. Weston (1986) concluded that biofouling organisms on the net pens and the debris released during cleaning were not significant sources of organic input to sediment beneath salmon net pens. No literature was found that quantitatively describes the mass of fouling organisms on net pens at salmon farms.

Biofouling can be an issue at all growout facilities. Nets and raceways are usually cleaned in situ and may produce short-term increases in organic matter under the facilities. Cleaning nets and raceways on a regular basis prevents organisms from building up so that it does not accumulate under the facility.

9.1.5 Sediment-free sulfides ($S^{=}$)

Previous studies have found that macroinvertebrate community characteristics are highly correlated with free sediment sulfides ($S^{=}$) and redox potential (Brooks 2001, Nash 2001, Brooks et al. 2002, Brooks and Mahnken 2003a, b, Brooks et al. 2003, Brooks et al. 2004). British Columbia's marine finfish culture waste regulations rely on free sediment sulfides as a regulatory tool (Brooks and Drawbridge 2005). Free sediment sulfides were chosen because they do not exhibit some of the testing problems that other sediment components do. For example, tests cannot distinguish between samples of total volatile solids and total organic carbon with woody debris, and those without. Redox potential is difficult to consistently measure with sufficient accuracy due to contamination of the probe, making it impractical for regulatory programs (Wildish et al. 2004).

Brooks and Mahnken (2003a, b) were able to show a relationship between the number of taxa present in the macrobenthic community in the sediment and the free sulfide concentration of the sediment. Sensitive infauna are excluded from sediments when sulfides exceed several hundred µM. Other taxa, particularly annelids, proliferate in sediments at sulfide concentrations as high as 15,000 µM (Brooks 2006). Brooks and Mahnken's (2003a) work demonstrates that on average, half the taxa are excluded at sulfide concentrations of 960 µM. Thus, measuring sulfide concentration ($S^{=}$) around the growout facilities can be used to determine potential effects on the benthos.

9.1.5.1 Results of sulfide sampling

Initial sulfide sampling at the OREHP growout facilities revealed that six growout facilities had high levels of sulfides in the sediment at the facility perimeter (paired t-test, $\forall = 0.05$) (Table 9-1). Of these facilities, four also had high levels of sulfides at the reference station. Huntington Harbor and Agua Hedionda Lagoon growout facilities had

a higher, but not significantly higher, sulfide concentration at the facility perimeter when compared to the reference station. Five growout facilities had high $S^{=}$ concentrations at 10 m from the facility perimeter; of these, one (Newport Bay) had higher $S^{=}$ concentrations at 10 m from the facility perimeter when compared to the facility perimeter.

Table 9-1. Sulfide concentrations (µM) at the OREHP's growout facilities (from Brooks 2007).

Growout facility	Facility perimeter	10 m from perimeter	Reference station	t-value	*df*	p-value
Santa Barbara	182	69	110	1.221	5	0.277
Channel Islands Harbor	1,686[1]	1,008	928	1.678	5	0.154
Marina del Rey	1,342	629	1,227	0.617	5	0.564
Catalina Harbor: HSWRI	230	20	57	1.580	5	0.175
Catalina Harbor: CSF	22	129	224	-1.858	5	0.122
Huntington Harbor	752	736	314	1.898	5	0.116
Newport Bay	586	1,178	112	2.453	3	0.091
Dana Point	152	120	264	-1.953	4	0.122
Agua Hedionda Lagoon	658	335	410	1.229	5	0.274
Mission Bay: Dana Landing	637	288	510	0.336	5	0.751
Mission Bay: Quivera Basin	1,990	1,229	1,206	2.471	5	0.056
San Diego Bay: Grape Street	380	72	0	1.857	5	0.122
San Diego Bay: SWYC	148	209	376	-1.471	4	0.215

Notes: 1. Values that likely significantly affect macrobenthic communities are highlighted in red.

Four of the marina-based growout facilities sampled had elevated sulfide concentrations both at the facility perimeter and the reference station. All the marina reference locations likely had altered benthic communities adapted to the stressful conditions documented there (Brooks 2006). Of the four open water growout facilities sampled, Agua Hedionda Lagoon had elevated sulfide concentrations (658 µM $S^{=}$) at the facility perimeter; the reference station was much lower. The remaining facilities had low sulfide concentration levels both at the facility perimeter and the reference station.

Fish are stocked in the growout facilities based on a maximum stocking density at release of 12 to 18 kg/m^3. A growout facility may not be at the maximum stocking density at the time of release for many reasons, with the two most common reasons being that not enough fish were available to transport to the facility when it was stocked; and high mortality due to a disease outbreak reduced the stocking density. Table 9-2 reveals that seven of the 13 growout facilities have stocking densities of 10 kg/m^3 or greater at the facility when benthic monitoring occurred. It is at these facilities that the greatest impacts would be expected to occur; however, only two facilities had higher $S^{=}$ concentrations at the facility perimeter than at the reference station. One growout facility had high $S^{=}$ concentrations at both the facility perimeter and reference station, while the four remaining facilities had low $S^{=}$ concentrations at both the facility perimeter and the reference station. Three of the growout facilities had relatively low stocking densities (6 to 7 kg /m^3), yet had high $S^{=}$ concentrations at both the facility perimeter and reference station, indicating that some other agent is probably the cause of the high $S^{=}$ concentrations around the facility.

Table 9-2. Release and sample information at the time of benthic monitoring (from Brooks 2007).

Growout facility	Release date	Sample date	Release biomass (kg)	Days before/after release	Stocking density at release (kg/m^3)
Santa Barbara	8/29/2005	9/28/2005	734	30 – after	10[1]
Channel Islands[2]	**9/15/2005**	**9/29/2005**	**1,220**	**14 – after**	**7**
Marina del Rey	**10/9/2005**	**9/27/2005**	**644**	**12 – before**	**12**
Catalina Harbor: HSWRI	10/28/2004	9/15/2004	2807	43 – before	10
Catalina Harbor: CSF	10/29/2006	11/28/2006	1025	30 – after	6
Huntington Harbor	**11/15/2006**	**9/12/2006**	**181**	**64 – before**	**7**
Newport Bay	8/15/2006	9/13/2006	130	29 – after	2
Dana Point	1/6/2006	11/7/2005	305	60 – before	8
Agua Hedionda	**12/13/2004**	**9/14/2004**	**3,210**	**90 – before**	**16**
Mission Bay: Dana Landing	**9/9/2005**	**10/12/2005**	**222**	**33 – after**	**24**
Mission Bay: Quivera Basin	**9/13/2005**	**9/21/2005**	**382**	**8 – after**	**6**
San Diego Bay: Grape Street	11/1/2004	9/13/2004	1270	49 – before	13
San Diego Bay: SWYC	5/12/2005	10/20/2005	222	161 – after	10

Notes: 1. Red indicates growout facilities at or near maximum stocking density at the time of benthic monitoring.
2. Bold indicates growout facilities with high $S^=$ concentrations at the facility perimeter and/or reference station.

Typically an OREHP growout facility receives two batches of juvenile white seabass that are raised at the facility for two to six months and then released. The facility is usually fallow for two to four months before receiving additional fish. The median size of the OREHP growout facilities is 0.86 t (range 0.13 to 33.6 t). This is very different from commercial aquaculture cage systems such as the Pacific Northwest salmon farms which typically hold salmon for 18 months with a system size around 1,500 t (Nash 2001).

There are some special circumstances that provide additional fallow periods (and potential remediation) for some of the growout facilities. Santa Barbara is the only exposed open ocean location, and because of winter storm surge the facility is removed from the water each fall and allowed to lie fallow until the following spring. The Catalina Harbor CSF growout facility is moved each spring to an “outer” mooring that is closer to the harbor mouth. In the fall, the facility is moved to an “inner” mooring that has more protection from winter storms, providing a fallow period for each site. The Newport Bay growout facility usually does not operate during the winter months, due to freshwater runoff from winter storms. The Agua Hedionda Lagoon growout facility is moved every two to three years so that the lagoon can be dredged because of sediment build-up resulting from power plant operations in the lagoon.

9.1.5.2 Sulfide benchmarks for measuring changes in the benthic community

British Columbia has set free sulfide ($S^{=}$) benchmarks for soft bottom at 1300 µm at 30 m beyond the net pen perimeter. If this benchmark is exceeded, the facility has to lie fallow until the sulfide levels are below the benchmark. This closure allows the site to remediate.

Reference Station Mean Sulfide Concentration Less Than 1000 µM $S^{=}$
The OREHP has developed an interim benchmark for sediment sulfide concentration of 1000 µM $S^{=}$ at 10 m from the facility perimeter for growout facilities with reference station sulfide concentrations less than the benchmark. Should the mean concentration at 10 m from the facility perimeter exceed this benchmark, the facility will have to lie fallow for a minimum of three months. After three months, sampling for sulfides will be repeated monthly until the mean value at 10 m is less than 750 µM $S^{=}$. Once sulfide levels subside, the facility can be restocked.

Reference Station Mean Sulfide Concentration Greater Than 1000 µM $S^{=}$
Since there are three growout facilities with high perimeter and reference station sulfide concentrations, a separate benchmark for those sites has been developed. Should the mean concentration at 10 m from the facility perimeter exceed 1300 µM $S^{=}$, the facility will have to lie fallow for a minimum of three months. After three months, sampling for sulfides will be repeated monthly until the mean value at 10 m is less than 1000 µM $S^{=}$. Once sulfide levels subside, the facility can be restocked.

9.1.6 Redox potential

Oxygen is delivered to sediments by diffusion from the overlying water column, and by mechanical infusion of water into sediments (Brooks and Mahnken 2003a). In sediments with high organic content, bacterial catabolism of organic matter can create significant BOD. As organic matter increases, oxygen levels drop, and the sediments become reducing – leading to the exclusion of some taxa (Brooks and Mahnken 2003a). Studies have shown that redox potential can be highly variable (Brown et al. 1987, Hargrave et al. 1993, Hargrave et al. 1995, Wildish et al. 1999) making it difficult to use in regulatory programs (Wildish et al. 1999, Wildish et al. 2004).

9.1.6.1 Results of redox potential sampling

The OREHP has collected data on redox potential (Table 9-3) at the various growout facilities. Five of the growout facilities had negative redox potential at the facility perimeter; of these, only Mission Bay: Quivera Basin's redox potential was positive at the reference station. Three of the growout facilities have significant differences ($\forall$ = 0.05) between the facility perimeter and reference station. Only one (Marina Del Rey) of the three facilities had lower redox potential at the facility perimeter compared to the reference station. In each case, differences in the sediment grain size between the facility perimeter and reference location may have contributed to the differences.

Table 9-3. Redox potential values (mV) at the OREHP's growout facilities (from Brooks 2007).

Growout facility	Mean at growout facility perimeter	Mean at reference station	t-value	*Df*	p-value
Santa Barbara[1]	106.75	93.50	0.583	5	0.585
Channel Islands Harbor[1]	-117.85[1]	-57.83	-1.929	5	0.112
Marina del Rey[1]	**-99.80**	**-15.93**	**-3.771**[2]	**5**	**0.013**
Catalina Harbor: HSWRI	86.25	40.00	2.364	5	0.064
Catalina Harbor: CSF[1]	**130.00**	**11.80**	**4.816**	**5**	**0.005**
Huntington Harbor[1]	-12.40	-3.13	-0.756	5	0.484
Newport Bay[1]	4.00	37.43	-2.374	3	0.098
Dana Point[1]	**42.50**	**-9.43**	**2.910**	**4**	**0.044**
Agua Hedionda Lagoon	-31.50	-75.00	1.805	5	0.131
Mission Bay: Dana Landing[1]	-6.35	-70.10	1.144	5	0.304
Mission Bay: Quivera Basin[1]	**-53.45**	**3.73**	**-3.446**	**5**	**0.018**
San Diego Bay: Grape Street	27.00	31.00	-0.166	5	0.874
San Diego Bay: SWYC[1]	33.93	1.67	1.232	4	0.286

Notes: 1. Samples with negative redox potential are in red.
2. Statistically significant ($\forall$ = 0.05) differences between reference conditions and perimeter stations are bolded.

9.1.6.2 Benchmarks for redox potential

While redox potential can be predictive of changes in the macrobenthic community, it is difficult to measure with precision. As a result, Brooks (2000c) recommended to British Columbia that sulfide benchmarks be used in managing the salmon farms rather than redox potential. No redox potential benchmarks were found in the literature.

The OREHP will continue to collect redox potential as part of its benthic monitoring program; however, the Department will not set any benchmarks for redox potential because of the known problems with accurately measuring redox potential.

9.1.7 Total Volatile Solids

There is diverse literature describing changes in sediment chemistry near salmon farms (Ye et al. 1991, Holmer and Kristensen 1992, Johnsen et al. 1993, Hargrave et al. 1995, Lu and Wu 1998, Karakassis et al. 1999). These case studies demonstrated consistent, but variable, increases in sediment carbon under and immediately adjacent to salmon farms. The studies also suggest that organic deposits are patchy with significant variability in replicates from the same sample station.

Except for a few very high rates observed during the early days of salmon farming, it appears that salmon farms have typically contributed between 12 and 62 g TVS/m^2 per day under or on the perimeter of the net pens (Brooks and Mahnken 2003a). However, Total Volatile Solids (TVS) and Total Organic Carbon (TOC) are not reliable indicators of benthic effects because the analyses do not discriminate between labile forms of organic matter which have a high BOD and refractory forms, such as eelgrass or

macroalgae detritus or woody debris which have low BOD (Brooks and Drawbridge 2005).

9.1.7.1 Results of TVS sampling

The OREHP has collected data on TVS levels (Table 9-4) at the various growout facilities. In 2005 and 2006, 10 growout facilities were sampled and the TVS was transformed [ArcSin(Sqrt(proportion))] for the analysis (Brooks 2006, Brooks 2007). TVS samples collected in 2004 were not transformed (Brooks 2004). Six of the growout facilities had elevated TVS levels at both the facility perimeter and the reference station, with four facilities being significantly different between the facility perimeter and reference location.

Table 9-4. TVS values (percent difference between dry and combusted weight of the sediment) at the OREHP's growout facilities (from Brooks 2007).

Growout facility	Mean at growout facility perimeter	Mean at reference station	t-value	*df*	p-value
Santa Barbara[1]	**0.122**[2]	**0.143**	**-4.269**	**5**	**0.008**
Channel Islands Harbor[1]	**0.248**[3]	**0.226**	**6.218**	**5**	**0.002**
Marina del Rey[1]	0.318	0.299	0.646	5	0.547
Catalina Harbor: HSWRI	6.238	3.217	1.131	5	0.309
Catalina Harbor: CSF[1]	0.0	0.0	1.809	5	0.130
Huntington Harbor[1]	0.070	0.060	0.800	5	0.460
Newport Bay[1]	0.046	0.040	1.011	3	0.386
Dana Point[1]	0.242	0.246	-0.504	4	0.641
Agua Hedionda Lagoon	2.620	1.970	1.378	5	0.227
Mission Bay: Dana Landing[1]	**0.141**	**0.260**	**-4.738**	**5**	**0.005**
Mission Bay: Quivera Basin[1]	**0.328**	**0.270**	**3.823**	**5**	**0.012**
San Diego Bay: Grape Street	7.898	6.400	2.294	5	0.070
San Diego Bay: SWYC[1]	0.114	0.239	-2.712	4	0.053

Note: 1. TVS was transformed for the analysis ArcSin (Sqrt(proportion)).
2. Values that likely significantly affect macrobenthic communities are highlighted in red.
3. Statistically significant ($\forall$ = 0.05) differences between reference conditions and perimeter stations are bolded.

9.1.7.2 TVS benchmarks

Brooks (2000c) reported that TVS was a stable endpoint in same sample measurements; however, TVS was not by itself an adequate physiochemical surrogate for predicting biological response because it was observed in both refractory and labile modes. No TVS benchmarks were found in the literature.

The OREHP will continue to collect TVS data as part of its benthic monitoring program; however, the Department will not set any benchmarks for TVS because TVS sampling does not distinguish between high BOD TVS and low BOD TVS.

9.1.8 Sedimented zinc

Zinc is an essential trace element for fish nutrition, and it is added to fish feeds by the manufacturer as part of the mineral supplement. Sediment concentrations of zinc are typically increased near salmon farms; although the form of zinc incorporated into feed has been changed by the manufacturers in recent years to a more bioavailable form of proteinated zinc or zinc-methionine analog (Brooks 2006). This change appears to have reduced increases in sedimented zinc near salmon farm net pens (Brooks and Mahnken 2003b). Long-term studies have demonstrated that zinc concentrations return to background levels during chemical remediation, leaving no evidence of a long-term buildup.

9.1.8.1 Results of zinc sampling

The OREHP uses a proteinated form of zinc in the feed (Skretting 2007) to minimize the addition of zinc in the sediments surrounding its net pens. Benthic monitoring at the growout facilities revealed no significant difference between sedimented zinc concentrations at the growout facility perimeter compared to reference stations (paired t-test, $\forall = 0.05$) except in cases where the reference station was significantly higher (Table 9-5). Four growout facilities (Marina Del Rey, Dana Point, Mission Bay: Quivera Basin, San Diego Bay: Grape street) have zinc concentrations that are likely to significantly affect macrobenthic communities at both the facility perimeter and the reference stations. Only one growout facility, Channel Islands Harbor, had an elevated zinc concentration at the net pen perimeter while the reference station concentration was lower. Given this, the growout facilities are most likely not the cause of the elevated zinc concentrations found at some of the facilities.

Table 9-5. Zinc concentration (µg/g sediment) at the OREHP's growout facilities (from Brooks 2007).

Growout facility	Mean at growout facility perimeter	Mean at reference station	t-value	*df*	p-value
Santa Barbara	30	28	2.407	5	0.061
Channel Islands Harbor	387[1]	144	0.885	5	0.417
Marina del Rey	480	414	2.432	5	0.059
Catalina Harbor: HSWRI	70	89	-2.363	5	0.064
Catalina Harbor: CSF	74	77	-1.245	5	0.268
Huntington Harbor	**427**[2]	**341**	**2.695**	**5**	**0.043**
Newport Bay	130	139	-0.473	3	.0668
Dana Point	**248**	**351**	**-17.355**	**4**	**0.000**
Agua Hedionda Lagoon	52	41	2.427	5	0.060
Mission Bay: Dana Landing	**43**	**118**	**-4.680**	**5**	**0.005**
Mission Bay: Quivera Basin	273	234	1.735	5	0.143
San Diego Bay: Grape Street	273	225	1.468	5	0.202
San Diego Bay: SWYC	**61**	**55**	**-9.952**	**4**	**0.001**

Notes: 1. Values that likely significantly affect macrobenthic communities are highlighted in red.
2. Statistically significant ($\forall = 0.05$) differences between reference conditions and perimeter stations are bolded.

9.1.8.2 Benchmarks for monitoring zinc

Washington State is the only jurisdiction that has developed Marine Sediment Quality Standards for metals (WAC 173-204-320) (Brooks 2006). These standards are based on the Apparent Effects Thresholds (AET). The Florida Department of Environmental Protection has developed Threshold Effects Levels (TEL) and Probable Effect Levels (PEL) (MacDonald 1994), while Long et al. (1995) developed an Effects Range-Low (ER-L) and Effects Range-Moderate (ER-M). The State of California has not developed zinc benchmarks. The zinc benchmarks are summarized in Table 9-6.

The OREHP has already mitigated for sedimented zinc by using proteinated zinc in the fish feed. Additionally, initial benthic monitoring indicates that the source of zinc around the growout facilities probably comes from other sources rather than the facilities. As a result, the Department will not set zinc benchmarks at this time. The results of the first benthic sampling will be compared to subsequent samples, and should there be significant changes in zinc deposition the Department will reconsider setting zinc benchmarks.

Table 9-6. Published sediment zinc and copper benchmarks (µg/g dry sediment).

Contaminant	ER-L	ER-M	(ER-L + ER-M)/2	TEL	PEL	(TEL + PEL)/2	WA State AET
Zinc	150	410	260.0	124	271	197.5	270.0
Copper			152.0	18.7	108	63.35	390.0

9.1.9 Sedimented copper

Copper is another micronutrient added to fish feeds (Chow and Schell 1978). Copper is also used in the anti-fouling treatments (e.g., Flexguard XI) for the nets. The latter use is most likely the cause of increased copper levels surrounding salmon net pen facilities. Brooks (2000a) developed a model to estimate water column concentrations of copper surrounding treated net pens. The results of Brooks' (2000a) monitoring efforts resulted in recommendations for BMPs that include washing copper-treated nets in upland facilities and annual monitoring of copper at growout facilities using copper-treated nets.

9.1.9.1 Results of copper sampling

Benthic monitoring at 10 of the OREHP growout facilities revealed that four facilities had significantly different concentrations of sedimented copper between the facility perimeter and reference station (paired t-test, $\forall = 0.05$) (Table 9-7); however, only one (Marina Del Rey, a raceway facility that does not use copper as an antifoulant) had a higher copper concentration at the facility perimeter. The other three sites had significantly higher copper concentrations at the reference site. Five of the growout facilities had elevated copper concentrations at the facility perimeter that likely significantly affect macrobenthic communities. Only one, Marina Del Rey, was significantly different (higher) than the reference station. This indicates that the marinas

or bays are already in a degraded state and that the effects are not from the use of copper-treated nets but from other inputs to the system.

In the past, the OREHP used Flex Guard XI to treat nets at many of its facilities; however, that practice was discontinued in 2006 in response to concerns about potentially increasing the sediment copper loading in the benthos under facilities that are already impacted from other copper sources. Because copper-treated nets are no longer in use, there should be little to no increase in sedimented copper around the growout facilities.

Table 9-7. Copper concentration (µg/g sediment) at the OREHP's growout facilities (from Brooks 2007).

Growout facility	Mean at growout facility perimeter	Mean at reference station	t-value	*Df*	p-value
Santa Barbara	6	6	1.782	5	0.135
Channel Islands Harbor	103[1]	120	-1.457	5	0.205
Marina del Rey	**396**	**337**	**2.955**[2]	**5**	**0.032**
Catalina Harbor: HSWRI	29	34	-2.547	5	0.051
Catalina Harbor: CSF	27	25	2.087	5	0.091
Huntington Harbor	147	136	0.946	5	0.387
Newport Bay	62	57	0.519	3	0.640
Dana Point	**280**	**474**	**-9.643**	**4**	**0.001**
Agua Hedionda Lagoon	**22**	**11**	**2.666**	**5**	**0.045**
Mission Bay: Dana Landing	**23**	**96**	**-4.952**	**5**	**0.004**
Mission Bay: Quivera Basin	258	261	-0.104	5	0.921
San Diego Bay: Grape Street	198	144	1.079	5	0.330
San Diego Bay: SWYC	**276**	**214**	**-11.777**	**4**	**0.000**

Notes: 1. Values that likely significantly affect macrobenthic communities are highlighted in red.
2. Statistically significant ($\forall$ = 0.05) differences between reference conditions and perimeter stations are bolded.

9.1.9.2 Benchmarks for monitoring copper

Washington State is the only jurisdiction that has developed Marine Sediment Quality Standards for metals (WAC 173-204-320) (Brooks 2006). These standards are based on AET. The Florida Department of Environmental Protection has developed TEL and PEL for copper (MacDonald 1994), while Long et al. (1995) developed an ER-L and ER-M for copper. The State of California has not developed copper benchmarks. The copper benchmarks are summarized in Table 9-6.

The OREHP has mitigated for sedimented copper by discontinuing the use of copper-treated nets. Additionally, initial benthic monitoring indicates that the source of copper around the growout facilities probably comes from other sources rather than the facilities. As a result, the Department will not set copper benchmarks at this time. The results of the first benthic sampling will be compared to subsequent samples, and should there be significant changes in copper deposition the Department will reconsider setting copper benchmarks.

9.2 Water quality monitoring

Water quality monitoring is usually required by the Regional Water Boards through the NPDES permit. Since none of the growout facilities are required to obtain NPDES permits, water quality monitoring for most facilities has not been required. The Los Angeles Regional Water Quality Control Board (LARWQCB) has requested water quality sampling for facilities within their jurisdiction (Channel Islands Harbor, Marina Del Rey, Catalina Harbor: HSWRI, and Catalina Harbor: CSF). Water quality monitoring includes biannual collection of water temperature, ammonia, and dissolved oxygen levels inside the facility and just outside the facility perimeter. Additionally, each year divers shall make a visual inspection of the bottom to look for adverse conditions. The Department shall submit an annual report to the LARWQCB summarizing the results of the water quality monitoring.

Although the hatchery does not operate under a NPDES permit, the SDRWQCB does require water quality monitoring. Influent sampling includes monthly sampling for salinity, pH, temperature, settleable solids, total suspended solids, total Kjeldahl nitrogen, organic nitrogen, ammonia, unionized ammonia, nitrate, nitrite, phosphorus, orthophosphate; quarterly sampling for zinc and copper; annual sampling for acute toxicity; and one-time sampling for chronic toxicity and California Toxics Rule (CTR) priority organic and inorganic pollutants. Effluent sampling includes daily sampling for flow rate; monthly sampling for salinity, pH, temperature, settleable solids, total suspended solids, total Kjeldahl nitrogen, organic nitrogen, ammonia, unionized ammonia, nitrate, nitrite, phosphorus, orthophosphate; quarterly sampling for zinc and copper; annual sampling for acute toxicity; and one-time sampling for chronic toxicity and CTR priority organic and inorganic pollutants. Sand filter backwash is sampled weekly for total suspended solids. Hatchery staff is required to maintain self-monitoring reports and to submit annual reports to the SDRWQCB.

9.3 Bird and mammal interactions

Each growout facility takes precautions to prevent the take of marine mammals, birds and other fish. In areas where marine mammals are present, growout facilities utilize raceway systems to provide rigid protection for the white seabass and prevent intrusion of birds and marine mammals. Raceway facilities are generally covered by chain link fencing that has shade cloth stretched over it. This prevents birds from becoming entangled or preying upon the fish and provides shade for the fish.

The net pen facilities utilize brightly colored, large mesh nets underwater to surround the smaller containment net. There is generally a one m space between containment net and predator barrier. The predator barrier is held taut by anchors to prevent any entanglement. Above the water, chain link fence surrounding the walkways prevents the haul-out of marine mammals. Shade cloth or bird-netting covers the facility and prevents birds from preying upon the fish. The bird netting is also kept taut to prevent entanglement.

The land-based facility is located within a Quonset hut-type building covered by a heavy tarp that provides shade for the fish and protection from birds and other animals.

NOAA Fisheries has categorized the white seabass growout facilities as Category III fisheries under the Marine Mammal Protection Act. Category III designates fisheries with a remote likelihood or no known serious injuries or mortalities to marine mammals. Owners of vessels or non-vessel gear in Category III fisheries may incidentally take marine mammals without registering for or receiving a marine mammal authorization.

9.4 Effects on sensitive habitats

The OREHP growout facilities generally occur within marinas or established vessel mooring fields; thus, the effects on sensitive habitats should be minimal. NOAA Fisheries staff reviewed the location of the growout facilities and determined that none were located in an area that would impact eelgrass beds, although some facilities are close to eelgrass beds. Dive surveys conducted annually will assure that the effects of the growout facilities, if any, remain localized so that it would not be likely to impact eelgrass or other sensitive habitat.

Chapter 10. Genetics

10.1 Genetic considerations

Beyond the technical aspects of maintaining brood fish is the concern that genetic variability of the wild population could be diminished by releasing large numbers of hatchery fish. If the effective population size (number of broodstock participating in spawn events) in the hatchery is small, important alleles may be lost in the hatchery progeny; rare alleles would be especially vulnerable to loss. Should the hatchery progeny grow and reproduce with wild fish, this could change the genetic diversity of the wild population, by reducing the frequency of these alleles. Diminishing genetic variability due to selective breeding and survival within the hatchery is an important consideration. These concerns are driven largely by observations made of some adverse interactions between wild and hatchery populations of salmonids.

Tringali and Bert (1998) used the Ryman-Laikre model (1991) to compare the genetic risks associated with stock enhancement for the marine species, red drum (*Sciaenops ocellatus*) a Sciaenid species, and the anadromous species, Gulf sturgeon (*Acipenser oxyrinchus desotoi*). For red drum, they found that the lack of genetic substructure in the red drum population and the large population size offset the risks to single-locus and quantitative genetic variation. Tringali and Bert (1998) noted that was true as long as there were adequate numbers of effective breeders and the per-generation contribution was modest. On the other hand, Gulf sturgeon breeding populations are very small, making the hatchery contribution quite large (proportionally). Tringali and Bert (1998) found that almost all combinations of the three parameters effective breeders, population size and hatchery contribution could result in a substantial loss of single-locus and polygenic variation and a reduced adaptive potential.

Although the study of genetic resources described for salmonids has greatly advanced the field of applied population genetics and has provided an efficient tool for the management of valuable salmon populations, using anadromous salmonids as a general model for the conservation and utilization of genetic resources of many marine species should be done cautiously because their life history strategies are radically different.

10.2 Conserving genetic diversity

Genetic quality assurance has been a priority for the OREHP since the early years of the program. Studies to examine the genetic characteristics of wild white seabass were initiated in the mid-late 1980's and ran parallel to the culture and assessment research (Bartley and Kent 1990).

Work by Bartley et al. (1995) developed the protocols for conservation of genetic diversity of white seabass by the OREHP. The suggested protocols address three main factors: 1)

the genetic structure of the wild population, 2) the genetic structure of the broodstock, and 3) monitoring of both the wild population and the hatchery population.

10.2.1 Genetic structure of the wild population

Initial work conducted by Soulé and Senner ([Date unknown]) focused on finding one or more genetic loci that could be used in determining the population structure of white seabass. Samples were taken from Baja California, Mexico fish and processed using electrophoresis. Polymorphism was detected in only two enzyme systems (acohol dehydrogenase and phosphoglucomutase). Heterozygocity levels ranged from 0.009 to 0.043.

A survey of the Southern California Bight (Bartley and Kent 1990) revealed no stable population sub-structuring in the area. Bartley et al. (1995) estimated that gene flow was approximately nine migrants per generation and therefore sufficient to homogenize the genetic structure of the population. The study evaluated 22 enzyme systems representing 33 distinct loci in 13 different samples that varied spatially and temporally ($\Sigma N=510$ fish). Average heterozygosity values ranged from 0.033 to 0.064, genetic identity was greater than 99 percent in all pair-wise comparisons and only three percent of the genetic variation was attributed to between sample differences. In highly mobile species such as white seabass (Vojkovich and Reed 1983), gene flow among localities is apparently sufficient to homogenize the genetic structure. However, since several gene loci possessed rare alleles (frequency < 2 percent) that contributed to genetic diversity, Bartley et al. (1995) recommended that a hatchery replenishment program should strive to conserve this allelic diversity.

A subsequent study by Franklin (1997) looked at the population structure of white seabass from Point Conception, California to Magdalena Bay, Baja California, Mexico and the northern Gulf of California. Additionally, Franklin analyzed samples from one of the white seabass growout facilities (Channel Islands Harbor). He used microsattellite DNA from eight polymorprhic loci and randomly selected 12 fish from each area (N = 120) for the study. The results of Franklin's study indicate three major natural spawning groups that are physically segregated by ocean currents and a geographic barrier. On the outer west coast of California and Mexico, the northern spawning group is centered off the Southern California Bight and central Baja California, while the southern spawning group is located off southern Baja California. These two spawning groups are separated by the Southern California Gyre. The third spawning group is located in the Gulf of California. While this group is separated from the other groups by the Baja California peninsula, Franklin's (1997) results showed some mixing between the southern Baja California and the Gulf of California spawning groups.

Franklin's (1997) analysis of the hatchery-raised fish from the Channel Islands Harbor growout facility revealed a reduction in genetic diversity compared to the wild population, which is expected because the fish came from only one or two spawn events, with each spawn event consisting of one to two females and two to four males.

10.2.2 Effective hatchery population size

Bartley et al. (1995) also determined the size of the broodstock population necessary to maintain genetic diversity by looking at the presence of rare alleles and allelic diversity. In order to have the rare alleles present in the fish produced at the OREHP hatchery, it is necessary to collect enough broodstock so that rare alleles are sampled. Binomial sampling theory describes the probability of collecting an allele of frequency *p* as:

(1) $$N = \frac{\ln(1 - \alpha)/\ln(1 - p)}{2}$$

where *N* is the number of fish required and *α* is the confidence level. Therefore to be 95 percent certain of collecting broodstock that possess rare alleles (2 percent frequency), a minimum effective population size of approximately 74 brood fish are needed. Therefore, a founding effective population size of 74 fish will represent 99 percent of the heterozygosity of the source population.

However, allelic diversity is more sensitive to small population size than heterozygosity (Allendorf and Ryman 1987). Allelic diversity in a founding population is given by:

(2) $$n' = n - \sum(1 - P_j)^{2N}$$

where *n'* is the effective number of alleles remaining after establishing a population with *N* founders, *n* is the original number of alleles, and P_j is the allele frequency. For a simplified two allele model with various allele frequencies in the source or wild population, over 93 percent of the allelic diversity due to rare alleles (2 percent in this example) will be conserved if the effective size of the founding population exceeds 50 fish. Theoretically, the strategy of utilizing an effective population size of 74 fish as broodstock appears to be sound and will conserve over 90 percent of the natural genetic variability in the region, as measured by heterozygosity and allelic diversity.

Effective population size (N_e) is one of the primary determinants of genetic diversity. In order to avoid problems associated with founding hatchery populations from a restricted genetic base, as has occurred in tilapia transplanted to Asia (Eknath et al. 1993), the effective number of broodstock will be optimized for the OREHP white seabass project. To satisfy the genetic conservation goal of the program, an N_e of 74 fish is required.

Effective population size is influenced by sex ratio and variance in reproductive output, and is usually lower than actual population size (*N*). Bartley et al. (1992), using linkage disequilibrium data from allozyme genotypes, showed that the effective population size of a mass spawning group of white seabass broodstock was about 50 percent of the actual population size. Therefore, using the conservation goals stated above, a total of 150 (2 x 74 = 148) adult brood fish was originally recommended. In practice and to be even more conservative, the Carlsbad Hatchery was designed to accommodate 200 adult fish that are evenly divided among four breeding pools. Each broodstock tank maintains 50 adult white

seabass in a 1:1 ratio of male to female fish. Deviations necessitate that more broodstock are maintained according to the expression:

(3)
$$N_e = \frac{4(N_m * N_f)}{(N_m + N_f)}$$

where *m* and *f* are the numbers of males and females, respectively. A schedule for annually rotating 20 percent of the male brood fish among breeding pools was originally proposed in order to increase the diversity in progeny by increasing the number of different matings per broodstock.

10.3 Genotyping

10.3.1 Broodstock source and genotyping

To help ensure that the genetic diversity of hatchery-released progeny will be similar to wild populations, broodstock are collected only from the northern component of the white seabass population range from Point Conception, California to central Baja California, Mexico. No captive-bred progeny are used as brood individuals. At the time of capture, all white seabass broodstock have tissue samples (fin clips) taken to genotype the fish.

Much of the early work was done by an OREHP contractor, who changed the analytical equipment used during the contract causing calibration problems. Additionally, genotyping was not consistent for all broodstock (some broodstock were genotyped at seven loci, while others were genotyped at fewer loci). These differences resulted in some progeny appearing to have more than two parents which, of course, is not possible. Due to the problems with early genotyping efforts, all hatchery broodstock were genotyped again by a new geneticist hired by HSWRI in collaboration with researchers at the NOAA Fisheries Southwest Fisheries Science Center located in La Jolla, California.

10.3.2 Progeny genotyping

Samples for genotyping of spawning events and release batches were collected sporadically from 1997 to 2000 and regularly between 2000 and the present. Early genotyping was conducted by the same contractor that processed the broodstock. Tissue collection was required for a subset of ≥200 yolk sac larvae (YSL) from every spawn and 96 juveniles from every release batch.

Current tissue collection protocols for genotyping are experimental. These protocols will be modified based on the results of the experiments, currently being conducted by the new HSWRI geneticist, to determine how many offspring must be sampled to accurately estimate proportional per parent contribution to a single spawn.

10.4 Research

10.4.1 Coykendall's genetics research

Recently, Coykendall (2005) completed a study of white seabass that examined wild stocks, hatchery releases, and breeding stocks. This study used the same eight microsatellite DNA loci as Franklin (1997). The executive summary of Coykendall's dissertation (Coykendall and Hedgecock 2006) that was provided to the Department is supplied below. Coykendall's study focused on the potential problem that stocking of large numbers of hatchery fish, with different levels of genetic diversity from wild populations, could reduce the genetic resources of enhanced populations. This is known as the *Ryman-Laikre effect* or *model.*

> *We employed the Ryman-Laikre model of genetic impact of hatchery supplementation to wild populations. The model requires estimates of three parameters: hatchery effective population size (or in this case effective number of breeders), the effective size of the wild population, and the contribution that the hatchery fish make to the overall reproduction of the population. Estimates of these three parameters, caveats associated with them, and our general conclusions are addressed below.*
>
> ***Hatchery effective size, N_{eh}*** *– To understand the biology of hatchery spawns, we used two different methods of estimating the genetic output of the hatchery systems. The first method looked at several spawning events individually. We used data from four spawning events in 1998, one in 1999, and five from 2001. These spawning events came from tanks B1, B3, and B4. Using genotypes of the broodstock and a subset of the spawns from 4 – 7 microsatellite loci, we assigned offspring to parents to divulge the reproductive success of each broodstock. This led to an estimate of the effective number of breeders per spawning event of 2 to 8 individuals. We ascertained that the limiting factor in most spawning events is the number of contributing females to each spawn (anywhere from one to seven). Furthermore, we found evidence of repeat spawning by both males and females.*
>
> *Caveats: Given the information that we had from the work that GIS did, not all offspring could be assigned to a single parental pair. Broodstock in Tank 4 were genotyped at seven loci, but broodstock in other tanks were genotyped at fewer loci. This reduces the power of assignment tests. Offspring that were not successfully assigned a single parental pair were excluded from this analysis. In addition, we discovered a few genotyping errors of the broodstock. It is vitally important for parentage analyses that the parental genotypes are accurate. We were able to correct some inaccurate genotypes but others may not have been detected.*

In order to obtain an estimate for an entire hatchery release, we used a method whereby we could combine the data from all spawning events from a single year. By using this method, we were able to use all of the information available to us (even if we were not able to assign a single parental pair to a particular offspring) and obtain confidence intervals. Our estimation of the effective number of breeders for the 2001 hatchery release was 34.6 (95% CI: 20.6 – 76.5). Note that this differs a little from the 56 (95% CI 28 – 159) that Dennis Hedgecock reported to the Joint Panel in June 2004.

Caveats: Not all of the data from the 2001 release was available to us. In fact, 1.4% of the spawn groups were not sampled. Also, some spawning in the Catalina net pens contributed to the 2001 release, but those individuals are not in our genotype database. This could lead to an underestimate of N_{eh}. We are also assuming that the results for the 2001 release are an indication of the level of genetic diversity across generations. To confirm this assumption, these estimations should be performed across an entire generation and averaged for a more accurate estimate.

Wild effective population size, N_{ew} *– We estimated N_{ew} using both a moment-based method and a pseudo-likelihood estimator of genetic drift based on temporally-spaced changes in allele frequencies. The moment-based technique yields a mean of 5,679, and a 95% confidence interval of 3,977 – 7,678. The pseudo-likelihood method provides a mean of 6,087 and a 95% confidence interval of 2,384 – 57,310.*

Caveats: The wild samples we used do not constitute a random sample because juveniles were not included. This could bias our results either way. We also assume that the changes we observe in allele frequencies over time are due to random processes and not migration, mutation, population subdivision, etc., although previous geographic surveys and our own analyses suggest that population structure in the white seabass is very weak and not likely a source of error. The methods we employed work best for temporal samples that span at least one generation of the organism, but since the white seabass generation length is so long, we were unable to capture an entire generation length in our samples. According to simulations on other studies, this could result in overestimating N_{ew}.

Contribution of the hatchery to overall reproduction, x_h *– This estimate came from juvenile-targeted tag-recapture studies. Allen et al. (2003) reported that their juvenile-targeted tag-recapture study yielded a hatchery contribution of 6.6% based on the 2001 to 2002 sampling period. This number represents the percent of tagged fish for all white seabass that were caught for*

four months of sampling. However, cumulative data from 1997 – 2003 percentages of tagged fish vary depending on sampling site (Mike Shane, pers.comm.). There was a 1.4% recapture rate along the mainland coast of southern California, 14.6% in mainland bays, 35.9% along the Catalina Island coast, and 78.0% in Catalina Harbor, leading to an overall percentage of tagged fish for this time period of 7.2%. Moreover, five times as many gill nets were set on the mainland coastal sites and bays than at Catalina Island, but the area differential between these two sampling sites is such that the catch per unit effort along the mainland was probably less than at Catalina Island (Mike Shane, pers.comm.). Based on these sites, we used the average of 6.6 percent and 7.2%, 6.9% as our hatchery contribution estimate.

Caveat: Our estimate represents the very upper limit of hatchery contribution because the estimate was obtained from a juvenile-targeted tag-recapture study. We expect that there is a significant amount of mortality of the hatchery-produced fish before they become sexually mature. Therefore, for current consideration of white seabass genetic diversity, 6.9%should be treated as an upwardly biased value. Further analyses of the white seabass hatchery effect on genetic diversity should include new estimates of x_h because the yearly releases have been composed of increasingly older fish in order to maximize survivorship prior to release and this trend is continuing to rise, which would lead to a higher contribution of the hatchery fish to the whole population's reproduction.

Estimate of the genetic impact of hatchery enhancement: *All combinations of estimated N_{eh} and N_{ew} coupled with a proportional contribution from the hatchery to the total reproduction of 0.069 from tag-recapture studies result in negative effects on the genetic diversity of the wild population ranging from 1.5 – 92.9%. If N_{eh} is as high as 76.5 (upper 95% confidence interval value) and N_{ew} is as low as 2383.6 (lower 95% confidence interval value), then supportive breeding will decrease the total effective population size by 1.5%. More substantial negative change would result if N_{eh} is 20.6 or 34.6 and N_{ew} is as large as 57,310. In these cases, 88.6 – 92.9% reduction in the effective population size for the entire population would ensue. However, this summary must be tempered by the uncertainty in the underlying estimates. Uncertainty could be reduced by further research. Negative impacts could also be alleviated by increasing the effective size of the hatchery population, using genetic analysis to assess reproductive success of broodstock and to find ways to decrease its variance, for example, by rotating out fish that are not performing.*

Coykendall provided a useful approach to analyzing the genetic impact of hatchery production on wild populations, but the analysis did not take into consideration the specific sampling, breeding, and release protocols used by the ORHEP. As such, the

genetic diversity of white seabass produced by the hatchery could be underestimated and therefore their impact on wild populations is overestimated.

Department review of Coykendall's (2005) work found that her results were based on statistical estimates of the hatchery broodstock size, the wild broodstock size, and the relative contribution of the hatchery fish to the wild stock. The estimates of these three parameters have large margins of error. These errors are the result of several factors including the use of too few genetic markers (microsatellite loci) for hatchery parentage assignment, lack of information on the age demographics of the wild fish used to calculate wild effective population size, and the typical issues associated with mark-recapture sampling for collecting released hatchery fish (Rodzen pers. comm.). Another assumption of the Ryman-Laikre model is that the released hatchery fish are actually reproducing; this is unknown with the white seabass.

Subsequent review of Coykendall's analysis by HSWRI staff, including the new fishery geneticist, has provided more specific information on the hatchery spawning and sampling practices that could have influenced Coykendall's results (Appendix B). Overall, the results have the potential to significantly underestimate the actual genetic diversity of the white seabass produced at the Carlsbad hatchery and, therefore, overestimate the reduction in effective population size of the enhanced population. Coykendall acknowledges the wide error bars in assessing the hatchery's potential impact on wild populations.

Additionally, the figure used to define the contribution of hatchery fish to wild populations may have been overestimated further exaggerating the genetic impact on wild populations. It is possible that the juvenile tag recovery surveys are returning proportions of wild and hatchery juveniles after both have experienced significant (and possibly differential) mortality. If so, the tag recovery survey results might not provide true estimates of the proportion of wild and hatchery juveniles produced by a given group of adults within a time period because of mortality. Because the Ryman-Laikre model estimates the effective size of the current combined spawning adults, an accurate estimate of their offspring output, not a survival estimate of those offspring some time in the future, is needed. Therefore, using the juvenile recruitment survey to estimate hatchery contribution is probably not the optimum Ryman-Laikre model input because it could bias the estimate of parental offspring output that the model is designed to use.

Given the above considerations and ongoing genetic analysis that is informed by current hatchery protocols, there is good justification to set the hatchery output to 350,000 individuals. The issues and objectives of the enhancement are clearly defined and a research strategy is in place to gather genetic information. A key component of the research is adaptive management that takes into account newly acquired information. Numbers of released fish and broodstock management can be adapted to guard against reduction in the genetic diversity of wild populations.

10.4.2 Current and future genetics research

In 2007, HSWRI began working with a fish genetics researcher to establish a working operational plan for replenishment of the white seabass population whereby the genetic integrity of the wild stock is not compromised. This genetic research plan is focused on four primary goals: 1) understanding spawning patterns (specifically, the relative reproductive contribution of individuals among spawning populations at the hatchery), 2) identifying parent-offspring relationships among fish that are released, 3) comparing genetic diversity of released fish to that of the wild stock, and 4) studying the possibility of culture-induced selection in the hatchery and growout environments. The plan is designed to be adaptive, and information gained from the above research will allow HSWRI to evaluate and, if necessary, refine breeding protocols for white seabass to ensure that the stockable fish produced for enhancement match as best possible the genetic diversity of the wild population.

In order for adaptive management to provide a useful framework for the ORHEP, target and limit reference points on levels of genetic diversity and effective population size in hatchery populations could be established (see FAO 1997 for general discussion of reference points in a precautionary approach to fisheries). Genetic reference points are not well established, but could include targets for levels of genetic diversity, effective population size and number of alleles in hatchery fish, or limit reference points for percent reduction in effective population size or percent contribution of hatchery fish to wild populations. These reference points will provide guidance for monitoring programs so that management can be adapted, i.e. hatchery procedures modified or not, when reference points are reached.

Until the genetics questions are more adequately addressed and reviewed, HSWRI is currently maximizing the genetic diversity of the parental contributions within the annual release total to the fullest extent practical. The current operational protocol for the hatchery is to utilize one to three female equivilants (one female equivilant equals ~2 million eggs) per run for a total of 28 to 32 spawns per year. Approximately 12,500 fish will be released per run totaling 350,000 fish released per year. This protocol is based on the results of HSWRI's recent genetic work and their observed reproductive behavior within the hatchery.

10.5 Monitoring of the enhanced white seabass population

Systematic monitoring of the enhanced (natural and hatchery) populations is essential in evaluating the effectiveness of the enhancement program. The OREHP has until recently (2004) focused efforts on juvenile white seabass because releases have been too low to effectively assess the adult population. Since 2001, the OREHP has released over 100,000 hatchery-raised fish annually. These fish have already recruited to the recreational fishery and have started to recruit to the commercial fishery. Detailed information on current and future monitoring efforts can be found in Chapter 11 of this document.

Part 3 - Evaluation of the Ocean Resources Enhancement and Hatchery Program

Chapter 11. Current Research and Future Needs

11.1 Juvenile gill net survey

11.1.1 Sample design

From 1988 to 2008, researchers, under contract with OREHP, conducted a standardized gill net sampling survey designed to capture 1- to 4-year-old juvenile white seabass in shallow waters from Santa Barbara south to Imperial Beach off San Diego. Initially, the survey focused on determining the distribution of young fish, but switched in 1996 to look at recruitment of 1-year-old fish and recovery of tagged fish.

From 1988 through 1994, San Diego State University (SDSU) and HSWRI were contracted by the OREHP to establish and carry out the field surveys for wild and hatchery reared white seabass. It was during this time that many of the protocols for the gill net sampling program were established, including gear, and spatial and temporal definitions which maximized the catch of white seabass.

In 1995, the juvenile gill net sampling was modified to reduce bycatch while targeting juvenile white seabass. Additionally, sampling duties were split between SDSU and HSWRI researchers who sample the southern portion of the Southern California Bight, and California State University, Northridge (CSUN) and Vantuna Research Group (VRG) of Occidental College researchers who sample the northern portion of the Southern California Bight (Table 11-1). Beginning in FY 2005-06, only CSUN researchers conducted sampling in the northern portion of the Southern California Bight. In FY 2006-07, sampling in the southern portion of the Southern California Bight was conducted by HSWRI researchers only.

Table 11-1. Juvenile gill net sampling sites, FY 1995-96 to 2007-08.

Coastal Sites	CSUN/VRG	SDSU/HSWRI
Santa Barbara	X	
Ventura	X	
Malibu	X	
Catalina Island – West	X	
Catalina Island – East[1]	X	
Palos Verdes	X	
Seal Beach	X	
Newport Beach	X	
Oceanside		X
Carlsbad		X
La Jolla		X
Point Loma		X
Silver Strand/Imperial Beach		X

Table 11-1. Juvenile gill net sampling sites, FY 1995-96 to 2007-08.

Coastal Sites	CSUN/VRG	SDSU/HSWRI
Embayment Sites		
Marina del Rey	X	
Catalina Harbor	X	
Newport Bay		X
Agua Hedionda Lagoon		X
Mission Bay		X
San Diego Bay		X

Notes: 1. Catalina Island – East station was dropped in FY 2004-05 due to budget constraints.

The sampling protocol employed two types of gill nets. The main type was the same monofilament gill nets that were employed in the OREHP surveys since 1992. These Type 1 nets were 45.7 m (150.0 ft) in length and 2.4 m (8.0 ft) in depth, consisting of six 7.6 m (25.0 ft) panels of three different mesh sizes: two each of 25.4, 38.2, and 50.8 mm square mesh (1.0, 1.5, and 2.0 in.). A second type of net (Type 2), first used in FY 1996-97, was employed in an effort to increase the catch of potentially tagged white seabass in coastal areas. These nets had the same dimensions as the Type 1 gill nets but consisted of mesh sizes that had proven to be most effective in past sampling years at capturing juvenile white seabass (three panels each of 25.4 and 38.2 mm (1.0 and 1.5 in.) square mesh).

Beginning in 1995, each coastal site was set with six replicate Type 1 gill nets. In addition, two replicate, Type 2 gill nets were set. All gill nets were set randomly within designated coastal locations, which included sand/rock, reef/kelp habitat. All nets were set perpendicular to shore (or kelp line) in water 5 to 14 m Mean Lower Low Water (MLLW) in depth where prior sampling established that juvenile white seabass were most abundant.

In embayments, six Type 1 nets were set in a minimum depth of 2.5 m (MLLW). Within each embayment the six nets were randomly distributed within the outer, middle and inner areas, resulting in coverage of the different types of available habitats. Comparisons between the pairs of embayment and coastal sites were made using only Type 1 net catches.

Sampling was conducted in April, June, August, and October. Initially, these months coincided with releases of hatchery-raised fish. However, once OREHP began releasing fish at different times of the year, the releases did not necessarily coincide with sampling. In recent years, lack of funding forced the OREHP to reduce sampling to two months each fiscal year. Table 11-2 shows the sample coverage over time.

Table 11-2. Juvenile gill net sampling schedule FY 1995-96 to 2007-08.

	North (CSUN/VRG)				South (SDSU/HSWRI)			
Year	**Aug**	**Oct**	**Apr**	**Jun**	**Aug**	**Oct**	**Apr**	**Jun**
1995/96	x[1]	x	x	x	x	x	x	x
1996/97	X	x	x	x	x	x	x	x

Table 11-2. Juvenile gill net sampling schedule FY 1995-96 to 2007-08.

	North (CSUN/VRG)				South (SDSU/HSWRI)			
Year	**Aug**	**Oct**	**Apr**	**Jun**	**Aug**	**Oct**	**Apr**	**Jun**
1997/98	X	x	x	x	x	x	x	x
1998/99	X	x	x	x	x	x	x	x
1999/00	X	x	x	x	x	x	x	x
2000/01	X	x	x	x	x	x	x	x
2001/02	X	x	x	x	x	x	x	x
2002/03	X	x	x	x	x	x	x	x
2003/04	X	x	x	x	x	x	x	x
2004/05[2]	X	x	x		x	x	x	x
2005/06[3]		x		x	x	x		
2006/07[3]		x		x	p[4]	x		x
2007/08	X	x	x	x	x	x	x	x

Notes: 1. "x" indicates all stations were sampled.
2. To stay within their budget, VRG contractors had to drop one month (June) of sampling.
3. Sampling was reduced to 2 months due to budget constraints.
4. "p" indicates that only partial sampling (La Jolla and Mission Bay) was conducted.

The date and time of deployment and retrieval, and a unique collection number was recorded for each net set. In addition, latitude and longitude coordinates (using Global Positioning System: GPS), and surface and bottom temperatures were recorded just prior to retrieval.

The species identity and total length (to the nearest mm) were recorded for all individual fish taken. These records will be referenced by the collection number for the net, and the mesh size and replicate panel number in which the fish was caught. In addition to this information, individuals of target species (i.e. white seabass) were assigned a unique identification number, measured for standard length (to the nearest mm), weighed (to the nearest g), and a necropsy was performed to determine the sex, identify stomach contents, and remove otoliths. Sagittal otoliths were extracted from each fish and were used to determine the age of each specimen. Each white seabass was also scanned for the presence of a CWT – indicating hatchery origin. If a CWT was detected, the specimens were left intact and frozen for processing by HSWRI. White seabass marked with Floy tags (1996 – 1998) were processed similarly and turned over to the Department following CWT extraction and post-mortem examination by HSWRI.

11.1.2 Results of the juvenile gill net surveys

Since July 1988, 1,400 hatchery-raised juvenile white seabass have been recovered in the juvenile gill net studies (11 percent of the fish caught, N = 12,657). Overall tag returns have increased significantly over time; however, when looking at tag returns from embayments vs. coastal sites, the increase in tag returns in embayments was not significant while tag returns at the coastal sites have steadily increased (Allen et al. 2005).

11.1.3 Current and future work based on juvenile gill net survey data

The data collected during the gill net surveys is currently being used to evaluate the best time of year to release hatchery-raised white seabass, as well as size and release modality (e.g. direct verses from acclimation pens). Recent research suggests that hatchery-raised white seabass have a higher chance at survival when released from a growout facility during the spring and summer months versus other times of the year (Shane pers. comm.). HSWRI has contracted with a population biologist to try to determine a population estimate from mortality rates observed during gill net surveys. A stock assessment for white seabass should be completed prior to evaluating the OREHP program and would validate the model.

11.2 Adult surveys

HSWRI researchers began development of an adult head collection program in June 1998 (Kent et al. 1999). Work began by identifying commercial fish markets that purchase white seabass and determining if large numbers of fish can be scanned quickly. In FY 1998-99, a head length-total length conversion was developed. In addition to scanning for a CWT and measuring head length, otoliths were removed for ageing and information on when and where the fish was caught was collected.

A local fishing tournament provided the first opportunity to sample recreational catch in 1998 with 61 adult white seabass scanned for the presence of a CWT. In addition to this tournament, recreational fishermen turned in another 339 heads for scanning. As a result, the first CWT-tagged adult white seabass was recovered from the recreational fishery in June 1999 (Kent et al. 1999).

Since then, HSWRI researchers have continued to opportunistically scan commercially-caught white seabass at the commercial markets. For the recreational fishery, HSWRI has relied on anglers donating their white seabass heads for scanning. A highly successful tournament targeting the CPFV fishery, conducted by HSWRI, has increased the number of recreationally-caught white seabass heads kept and stored for scanning for the presence of a CWT. The tournament began in 2004 with only 12 percent of the CPFV-caught white seabass scanned. The proportion of heads saved for scanning has generally increased over time (from 39 percent in 2005 to 41 percent in 2008) (Shane pers. comm.). In 2008, five hatchery-raised white seabass were detected in the 1,835 fish heads saved by the CPFV fleet. In addition to HSWRI's tournament, Marina del Rey Anglers and San Diego Oceans Foundation have sponsored contests aimed at recovering white seabass heads from all sectors of the recreational fishery. These tournaments and contests have cash prizes as incentives for turning in the heads. Freezers have also been placed at many of the southern California sportfish landings so that anglers (private boat, dive, and CPFV) can drop off their heads.

In June 2008, CRFS samplers in southern California began scanning and measuring white seabass. CRFS is a multi-part survey to estimate the total catch and fishing effort of marine recreational anglers in California. Field sampling is conducted at publicly

accessible sites during daylight hours, and alternate methods are used to estimate the catch for nighttime and private access fisheries. Data are collected by an access point field survey. Samplers intercept anglers that have completed fishing trips on piers, jetties, beaches, public launch ramps, and other locations along the coast where the public has access to fishing. They also conduct sampling at sea on CPFVs. The samplers ask anglers questions about their fishing activities that day and examine their catch to determine the number and species of fish caught. In most cases, the sampler also measures and weighs the fish. A telephone survey of licensed anglers is conducted to collect information on effort when field observations of effort are not feasible, such as fishing at night and fishing from boats that return to private marinas. A telephone survey of CPFV operators is also conducted to improve effort estimates for this component of the fishery. The data gathered from field sampling, the telephone survey of licensed anglers, sport fishing license sales, and the telephone survey of CPFV operators are combined to estimate catch and effort.

The Department began a random sampling program for the commercial fishery as well in June 2008. This program builds on the Department's previous opportunistic sampling program for white seabass length-frequencies and covers the major commercial markets in the Southern California Bight.

The various recreational sport and commercial sampling programs conducted by HSWRI, the Department, and CRFS, are used to estimate the number of hatchery-raised white seabass caught by both the recreational and commercieal fisheries. As of December 2008, a total of 125 tagged adult white seabass (legal-size) have been recovered from both the recreational and commercial fisheries (Shane pers. comm.) (Figure 11-1). In recent years, several older hatchery-raised white seabass (10 to 13 years old) have been recovered (Figures 11-2 and 11-3). This information will be used to evaluate the program.

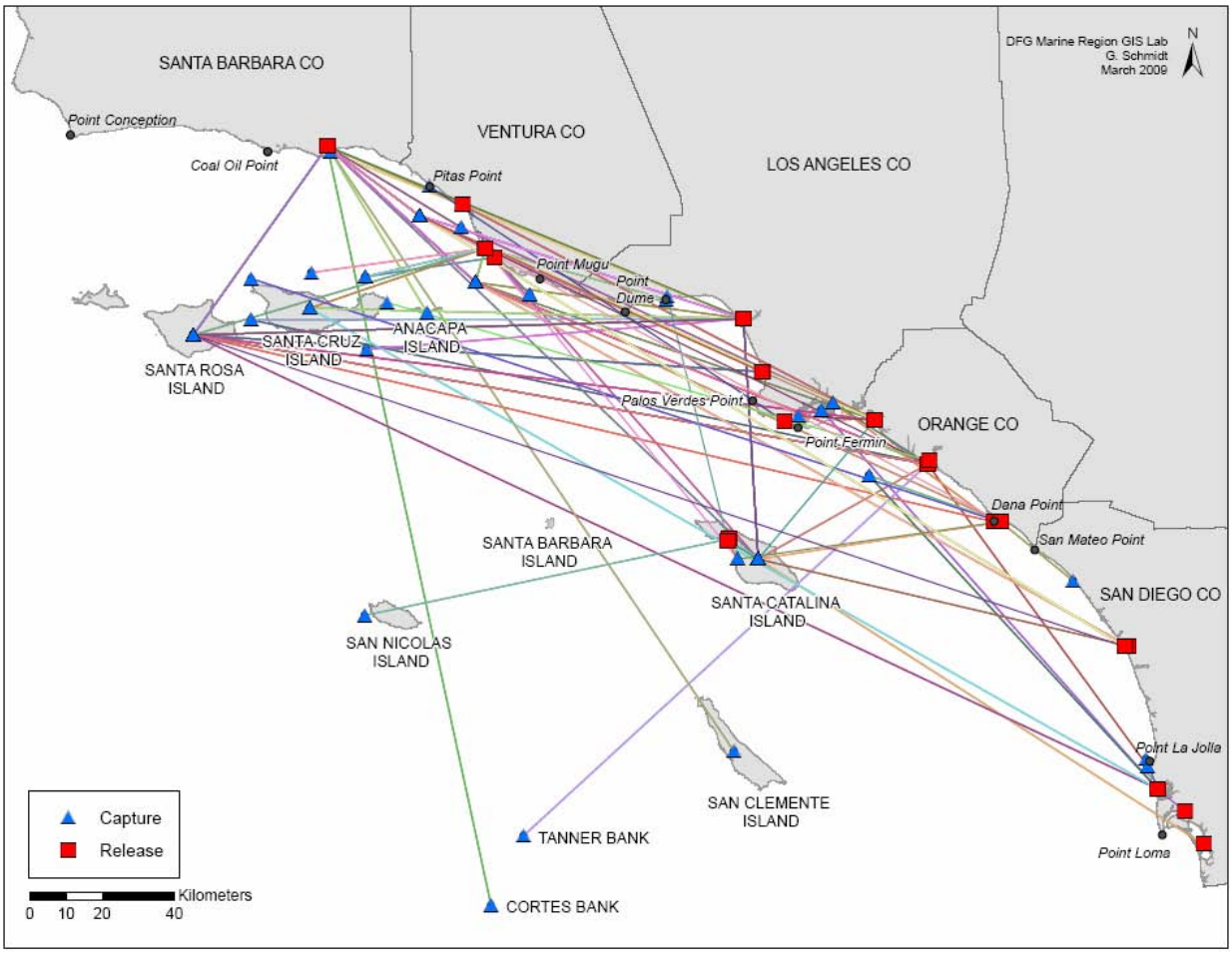

Figure 11-1. Release and capture locations of recovered tagged white seabass 1992 to 2008.

Note: Each line corresponds to an individual fish and is meant only to show location of release and final capture point and does not show route of travel.

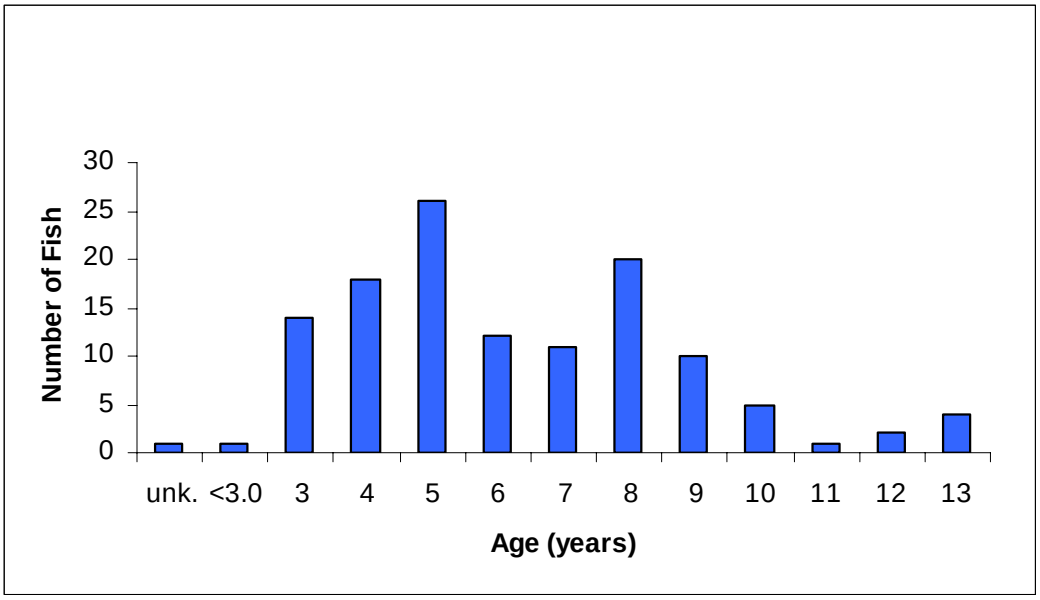

Figure 11-2. Number of tagged white seabass recovered per age group from 1992 to 2008.

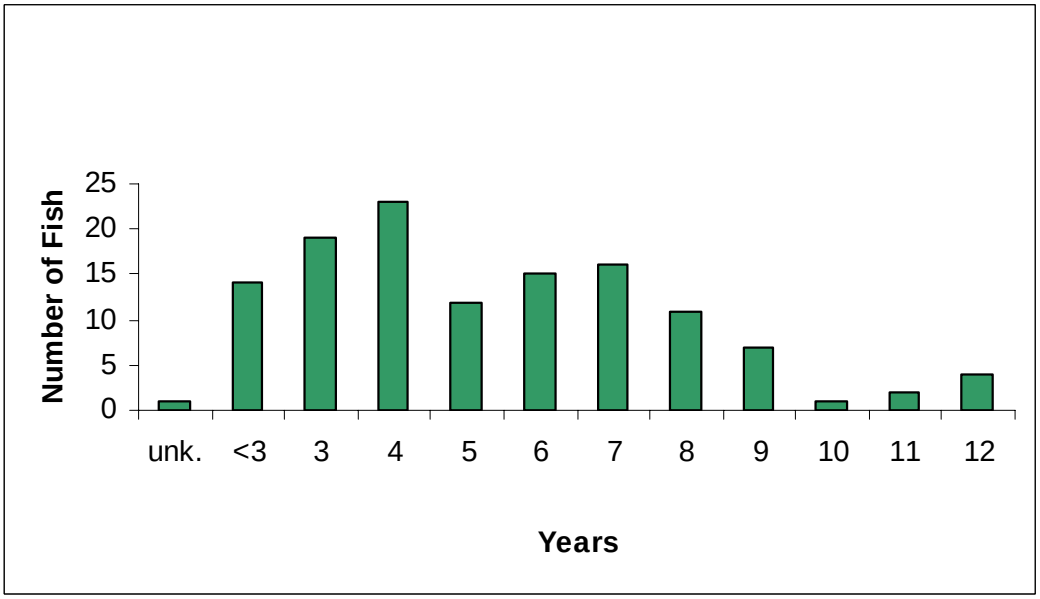

Figure 11-3. Number of years tagged white seabass released from 1992 to 2008 remained at liberty until recovery.

11.3 Acoustic studies

In 2001, HSWRI began acoustic tracking studies of juvenile white seabass. Initial studies focused on actively tracking individual fish movements of hatchery-raised juvenile white seabass. In 2003, 10 juvenile white seabass with sonic tags were released in Mission Bay. Five individuals were raised entirely at the hatchery and five

spent several months at a growout facility in Mission Bay. Prior to releasing the fish, 12 hydrophones were submerged in strategic positions in Mission Bay as well as along the adjacent coastal waters. Results of this study revealed that individuals from both groups emigrated from the bay within a few days. Individuals that did not emigrate had low survivorship, as evidenced by the fact that tags were recovered in the bay for all but one individual that did not leave the bay (Drawbridge et al. 2004).

In June 2004, 19 juvenile cultured white seabass were implanted with acoustic transmitters and released from a growout facility in Mission Bay along with 4,059 other cultured white seabass. Eight fish emigrated from the bay; seven did so within three days post release. There were five presumed mortalities (based on tag recoveries), likely due to predation. Researchers were unable to determine the disposition of the six remaining fish (Drawbridge et al. 2005).

In November 2004, 25 acoustically tagged juvenile white seabass were released from the Agua Hedionda Lagoon growout facility as part of a release of almost 10,000 white seabass. Underwater hydrophones were deployed in the lagoon and along the coastline adjacent to the lagoon entrance. By day five, 14 individuals had emigrated from the lagoon. Upon leaving the lagoon, the hydrophones detected an even dispersion of fish moving to the north and to the south. There were four mortalities due to predation during the first five days based on tag recoveries. Another four fish were likely entrained in the cooling water intake for the power plant in the lagoon based on their last location in the lagoon (near the intake) before the tags went silent. The fate of the three remaining fish is unknown (Drawbridge et al. 2005).

The results of the 2004 studies in Mission Bay and Agua Hedionda Lagoon reveal that almost half (48 percent) of the juvenile white seabass emigrated from the embayment within a week of their release, and they all left at night on an ebbing tide (Drawbridge et al. 2006) (Figure 11-4). Fish that did not emigrate from the embayment were likely preyed upon by octopods, birds, or marine mammals.

Further acoustical studies have been placed on hold while HSWRI researchers determine whether marine mammals, particularly harbor seals, can hear the pinging of the transmitters. If marine mammals can hear the transmitters, this may bias the observed mortality patterns of the tagged fish and limit this approach as an assessment tool.

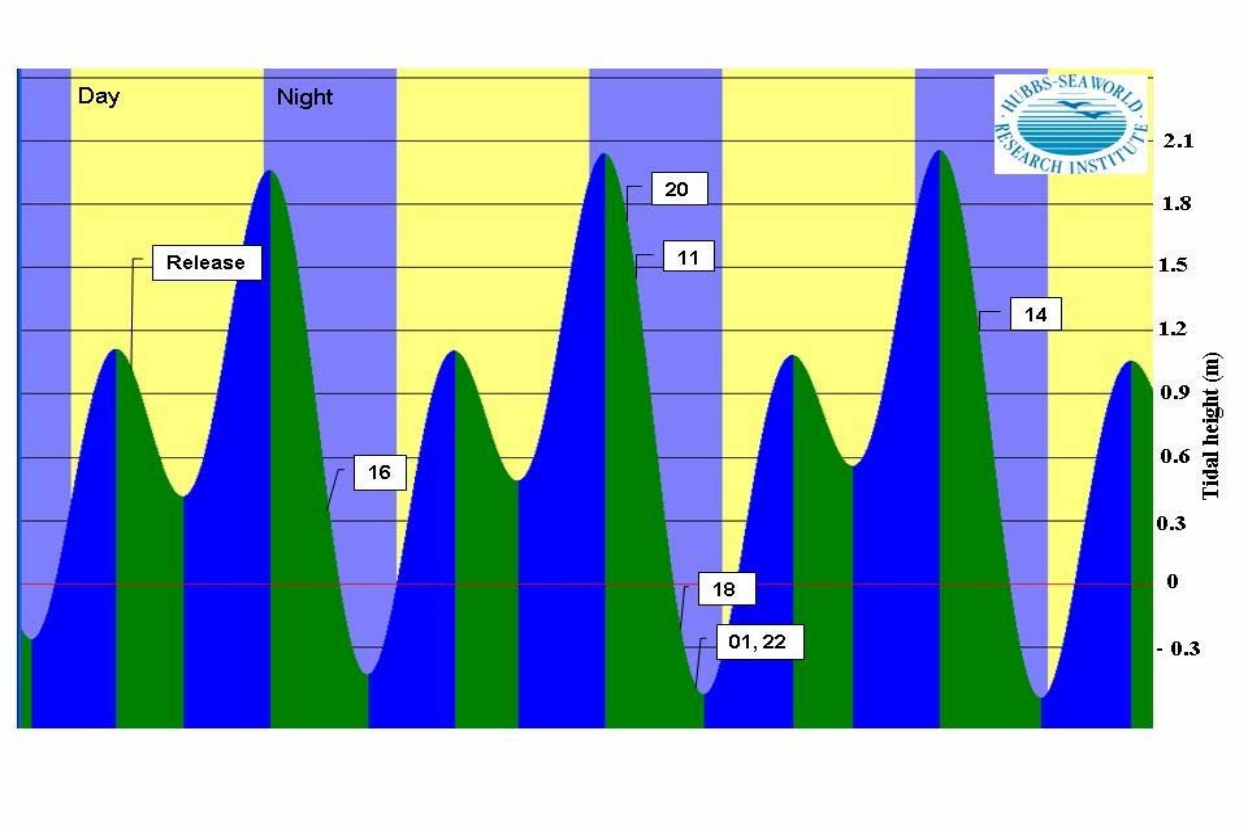

Figure 11-4. Diurnal and tidal cycles during which hatchery-raised white seabass with implanted acoustic pingers emigrated from Mission Bay in San Diego. Boxes represent individual fish identification codes, with lines showing when they were detected at buoy stations outside the bay (Drawbridge et al. 2006).

11.4 Nutrition studies

In 2009, HSWRI, in collaboration with researchers from the United States Department of Agriculture and the University of Idaho, began a study to identify alternative sources of fish meal and oil that can be incorporated into the diets of marine fish. The primary goal of the three year study is to reduce the fish meal and fish oil content of feeds for white seabass and California yellowtail by 75 percent and 50 percent for fish meal and oil, respectively, without a reduction in fish performance.

The first objective was to determine appropriate dietary inclusion levels for combinations of proteins by measuring fish growth, survival, nutrient retention, and feed efficiency. Candidate proteins included both plant-based meals (soybean, corn-gluten) and terrestrial animal by-product meals (blood, meat, bone, feather and poultry by-product). These alternate ingredients were blended to create a high-performance amino acid profile in substitution for fish meal.

The first experiment with white seabass tested a series of diets set at 42 percent protein and 12 percent lipid. The source of protein was varied among treatment groups to include a 52 percent fishmeal control diet and a series of diets reducing fishmeal from

20 down to 0 percent of the diet. Results from the first trial showed that one of the protein blends coupled with only 10 percent fish meal outperformed all other diets including the 52 percent fish meal control diet. This high performing treatment yielded an average survival >90 percent, weight gain of > 500 percent, and food conversion rate of <1.0.

Recently, two additional trials were completed with white seabass testing a series of 0 percent fish meal diets made with a high quality chicken by-product protein, and corn protein concentrate with *Spirulina* and liver meal as palatability enhancers. White seabass did very well on these diets, outperforming fish that were fed both a fish meal and a commercial control diet. The diet with *Spirulina* seemed to be accepted by the fish more quickly at the beginning of the trial than the other diets, suggesting that *Spirulina* may be a palatability enhancer.

Chapter 12. Program Evaluation

Stock enhancement programs are increasing worldwide; however, many early programs lacked this final, critical element – program evaluation. Blankenship and Leber (1995) cite the lack of evaluation as a major obstacle of early stock enhancement efforts. The lack of effective fish-tagging systems and the inability to culture marine fishes past the early life stages contributed to the inability to evaluate stock enhancement efforts. The OREHP has overcome these and other hurdles making program evaluation possible.

12.1 Scientific Advisory Committee

To assist the Department in evaluating the OREHP, the Department will establish a Scientific Advisory Committee (SAC) made up of experts in Croaker (white seabass) biology, population dynamics, genetics, environmental quality, economics, and fish pathology. The SAC will develop science-based criteria, based on the goals and objectives of the OREHP, to help evaluate the success of the program. In addition the SAC will review proposed research aimed at evaluating the OREHP, review the Adaptive Management Plan (AMP) (Section 12.2), assist in the Program evaluation and recommend changes.

SAC members would be appointed by the Director to advise the Department in the areas of future research, methodology for program evaluation, genetics, benthic monitoring, and changes to current practices outlined in this Plan, the CHP, and GPM. The Department would consider the SAC's recommendations for changes to current Program practices and future research. The SAC will include:

- One member with demonstrated expertise in the area of fish genetics;
- One member with demonstrated expertise in fish pathology;
- One member with extensive experience in marine aquaculture;
- One member with demonstrated expertise in population biology or dynamics;
- One member with demonstrated expertise in the area of benthic and/or water quality;
- One member with demonstrated expertise in the area of Croaker (white seabass) research;
- One member from the California Coastal Commission;
- One member of the OREAP, nominated by the OREAP who has expertise or significant knowledge of or experience with habitats of white seabass, genetics, aquaculture, fisheries management, water quality, or research; and
- One member from the Department.

12.2 Adaptive Management Plan

An Adaptive Management Plan (AMP) provides a mechanism to continuously evaluate the OREHP. The AMP, which includes monitoring and experimentation to address critical questions, is the process by which information on key uncertainties will be generated, analyzed, disseminated, and incorporated into project decision-making. The result will ultimately be a better informed and improved white seabass replenishment project.

The AMP should specifically:

- Identify performance standards and measures for achieving the OREHP's goals and objectives based on the best existing baseline/reference conditions;
- Identify monitoring activities to track stock replenishment progress and targeted research (applied studies) to test hypotheses related to adaptive management decisions;
- Include applied studies that can be initiated in the planning phase, which will be during the next CDP cycle;
- Identify specific adaptive management questions and related monitoring/experiments;
- Include processes for identifying applied studies for later phases; and,
- Define a process for synthesizing data from adaptive management studies and incorporating that information into decision-making to improve current phases and design future phases.

The four critical issues surrounding the OREHP that must be included in the AMP are: 1) maximizing the contribution of potential of stocked fish through optimized culture and release strategies, 2) maintaining genetic diversity, 3) managing disease, and 4) minimizing impacts to the environment from the hatchery and growout facilities. The WSEP lays out interim steps to ensure that the OREHP has every opportunity of successfully demonstrating the potential for using stock replenishment as a management tool, while avoiding negative impacts to the environment. Additional research is needed to determine if these interim steps are appropriate and necessary and can be incorporated into the AMP, or if they need to be changed to better protect the population and/or the environment. For example, under the benthic monitoring program, growout facilities have to maintain sulfide levels less than 1000 µM in the sediments around the facility. Should a growout facility exceed the benchmark during the triennial survey, then it must lie fallow until testing shows that the sediment is below the benchmark. Under adaptive management, the benchmark could change (higher or lower), there could be different benchmarks for already impacted areas (marinas, harbors) and more pristine areas (open coast, Catalina harbor), or the periodicity of the survey could change.

The OREHP is currently operating under a self-imposed release cap at Catalina Island (30,000 fish/year). The cap was put in place because of concerns that too many fish released at Catalina Island may result in negative effects caused by inter and/or intraspecific competition. Data from the juvenile gill net studies indicate that fish released at Catalina Island stay at Catalina and do not disperse as quickly as they do along the mainland coast. It has been hypothesized that the narrowness of the shelf around the island results in limited juvenile white seabass habitat causing the fish to remain close to shore. It would be beneficial to conduct a study of the dispersion rate at Catalina Island to determine if the cap should remain, and if so at what level. This information could then be included in the AMP.

The Department intends to develop the AMP within the next five years to be approved by the SAC. The AMP would then be incorporated into the WSEP. Additionally, the Department may need to adopt regulations to implement the AMP.

12.3 Evaluation of the OREHP

The evaluation of marine stock enhancement programs has varied widely. In Texas, evaluation of the red drum enhancement program was conducted by comparing gill net and sport-boat fisherman catches in stocked and unstocked bays. Results of this evaluation showed that the number of fish harvested in bays that have been stocked almost doubled over historic mean harvest rates in those systems (McEachron et al. 1993). In Japan, commercial landings were surveyed to evaluate the effectiveness of the flounder stock enhancement program. The results showed that over a 3-year period the recovery rate was 0.15, and the total income and the benefit estimate was $260,000 and $63,000 U.S., respectively (Kitada et al. 1992).

The OREHP has three key elements that will make evaluation of its program easier than other programs. First, since 1990, all white seabass have been tagged with CWTs so that they are identifiable. Second, white seabass husbandry issues have been resolved, and the hatchery is able to consistently raise fish to release size, allowing for larger scale releases. Since 2001, the OREHP has successfully released more than 100,000 fish each year (Figure 12-1). Given that it takes four years for white seabass to reach legal size (710 mm; 28 in.), fish released in 2001 should have entered the fishery in 2005. Third, HSWRI has been collecting data on juvenile and adult recoveries for over 10 years, and the Department implemented its own adult recovery program for the recreational and commercial fisheries in 2008 (Sections 11.1 and 11.2).

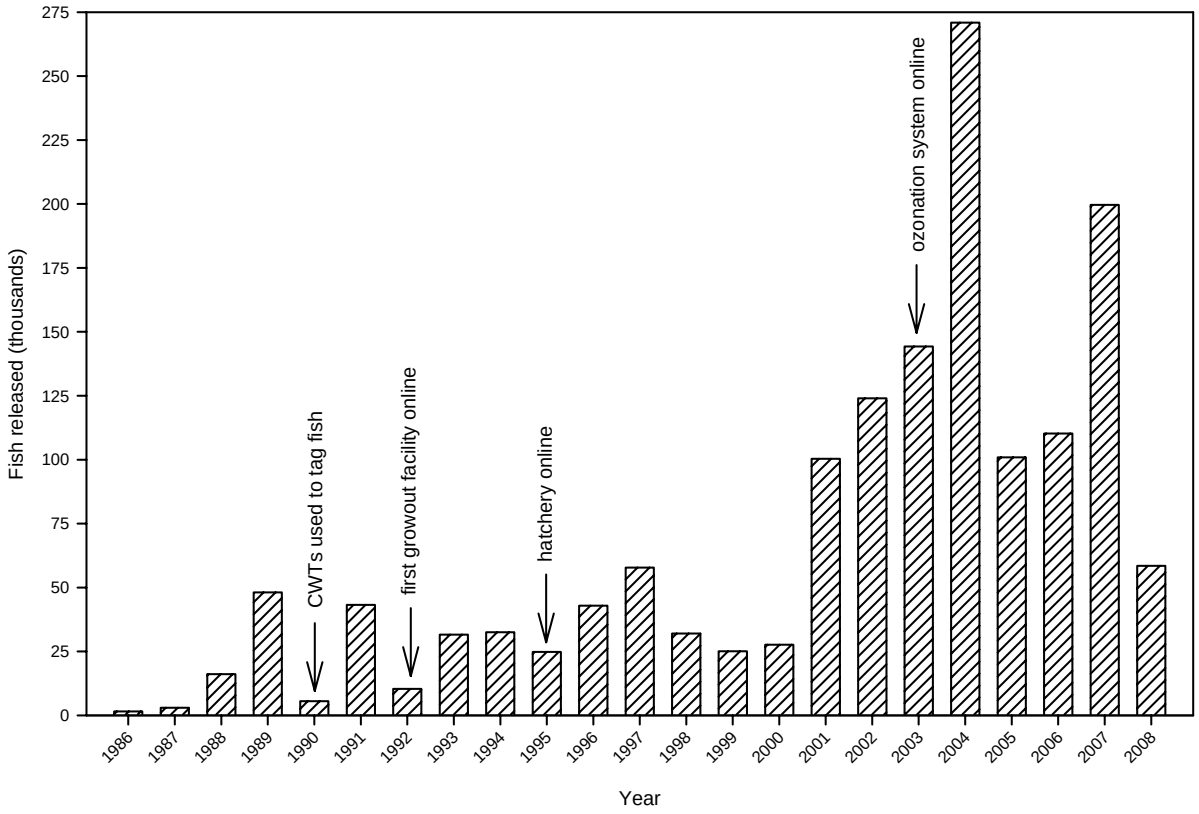

Figure 12-1. The OREHP white seabass releases 1986 to 2008.

There are also key elements that will make evaluation more difficult. First, the hatchery releases very few fish compared to other enhancement programs. Second, white seabass move around much more than other species such as flounder. Third, intrinsic water quality at the hatchery appears to be degraded due to urbanization and agricultural runoff in the watershed, thus negatively impacting hatchery operations. All of these elements will complicate overall program evaluation. In addition, low/modest tag returns make it difficult to draw conclusions with statistical significance. This will be critically important when making a decision about release caps.

The Department is planning on a program evaluation during the next CDP cycle. Prior to the evaluation, the SAC will need to develop quantitative criteria to evaluate the program's success based on the goals and objectives of the OREHP (Section 1.4). Key components of the program evaluation include:

12.3.1 Stock assessment

A stock assessment is critical to determining if the OREHP is enhancing the white seabass population. Ragen (1990) estimated the pre-fishery biomass of white seabass between 1.5 and 2.6 million fish using records of white seabass landings from the

Avalon Tuna Club. At the time of publication, there has been no stock assessment of white seabass in California. Any such stock assessment should include recreational and commercial fisheries landings, life history information, mortality rates, age and growth data, including recent work done by HSWRI, data from the juvenile and adult studies, information on changes in relative abundance over time, and other sources of data. Data gaps should be identified and prioritized, and efforts should be made to fill those gaps.

12.3.2 Adult sampling programs

Both the Department and HSWRI are sampling the commercial and recreational fisheries for hatchery-raised white seabass (Section 11.2). This data is also critical because it will help determine the ratio of hatchery-raised to wild fish. For the recreational fishery, HSWRI uses fish count information to determine how many white seabass were caught, what proportion of the catch were scanned, and how many were hatchery-raised fish. The Department will use expansion calculations that are part of the CRFS program to obtain the same data. For the commercial fishery, HSWRI can determine the proportion of fish scanned that were hatchery-raised, but cannot easily determine how many fish were landed because the information collected from fish processors is in pounds rather than number of fish. The Department's program will attempt to determine how many white seabass were caught in the commercial fishery, what proportion of the catch were scanned, and how many were hatchery-raised fish. The end result of these analyses should be a recovery rate for each fishery.

12.3.3 Bioeconomic model

Some enhancement program evaluations look at the economics of the enhancement program, such as Japan's flounder enhancement program (Kitada et al. 1992). A bioeconomic model was developed in the early stages of the OREHP (Botsford et al. 1988); however, it needs to be updated to reflect current information. If the model cannot be updated, a new bioeconomic model needs to be developed. Inputs to the bioeconomic model include the costs associated with raising white seabass to release size, fishing levels to determine commercial and recreational proportions, life history parameters, and the recovery rate for each fishery (from the adult sampling programs). Outputs from the program may include the costs per fish, value to each fishery, and/or a cost to benefit ratio and can be used to evaluate the efficacy of the program.

12.3.4 Genetics research and benthic monitoring

Genetic risk is another factor that should be reviewed during the program evaluation. Tringali and Bert (1998) examined the genetic risks associated with stock enhancement of two species and found that the genetic risks varied greatly due to differences in biology. Application of the Ryman-Laikre model (1991) can be used to evaluate the genetic effects of enhancement plans. Additional genetic research is being conducted by HSWRI, and the results should be available for the program evaluation. If changes to current hatchery protocols are needed to ensure that there are no negative effects on

genetic diversity, based on the review of genetic risks to the wild population, then they should be evaluated by the SAC as well and included in the CHP.

Studies have shown that salmon farming pens can affect the benthos, resulting in changes to the macrobenthic community as well as the chemical composition of the sediments (Brooks 2000a, d, c, b, Nash 2001, Brooks and Mahnken 2003a, b, Brooks et al. 2003). The OREHP began sampling the benthos surrounding the growout facilities in 2004 and will continue those efforts according to the BMPs listed in this document. The OREHP's evaluation should include a review of the benthic monitoring program to determine if the growout facilities are having a negative effect on the benthos.

12.3.5 Other data sources

The OREHP contractors and other researchers have conducted studies on different aspects of white seabass biology. The results of these studies can be used during the program evaluation. For example, HSWRI has collected data relative to the release and recapture of hatchery-raised white seabass. Analysis of this data can be used to determine the optimum size at release, optimum release time and release location to minimize mortality and maximize the fishes' chance of surviving to recruit to the fishery. Ageing studies have been conducted by HSWRI, the Department, and others and could be used as inputs into the stock assessment and bioeconomic model.

12.4 White Seabass Enhancement Plan review

The SAC could be used to conduct a review of the WSEP, at least every five years, to determine the effectiveness of the OREHP and suggest changes if needed, particularly to the BMPs and the AMP.

12.5 Plan amendment

The WSEP is designed to be flexible and adaptable to a wide range of future conditions and intended to function without the need for frequent amendment. Minor changes to the BMPs can simply be made by revising the other guidance documents for the OREHP, mainly the CHP and GPM. However, future research, environmental, biological, or economic changes may create a need to revise the WSEP to ensure that the enhancement of white seabass is conducted in a responsible manner. Examples of actions that might require a WSEP amendment include:

- Changes to the goals and objectives of the OREHP; and
- Changes to the AMP.

The Commission will be asked to approve an amended Plan.

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Appendix A. Glossary of Terms and Abbreviations

Appendix A. Glossary of Terms and Abbreviations

Adaptive Management - In regard to a marine fishery, it means a scientific policy that seeks to improve management of biological resources, particularly in areas of scientific uncertainty, by viewing program actions as tools for learning. Actions shall be designed so that even if they fail, they will provide useful information for future actions. Monitoring and evaluation shall be emphasized so that the interaction of different elements within the system can be better understood.

Bag limits - The total amount of fish that may be captured per person per day by law.

Benthic - On or relating to the region at the bottom of a sea or ocean.

Biological Oxygen Demand (BOD) - Chemical procedure for determining how fast biological organisms use up oxygen in a body of water.

Biological remediation - The restructuring of the infaunal community to include those taxa whose individual abundance equaled or exceeded 1 percent of the total invertebrate abundance at local reference stations.

Broodstock – A group of sexually mature individuals of a cultured species that is kept separate for breeding purposes.

California Toxics Rule (CTR) - An Environmental Protection Agency rule that establishes numeric water quality criteria for priority toxic pollutants and other provisions for water quality standards that are to be applied to waters in the State of California.

Central Nervous System (CNS) - Part of the nervous system that functions to coordinate the activity of all parts of the bodies of multicellular organisms.

Chemical remediation - The reduction of accumulated organic matter with a concomitant decrease in free sediment sulfide ($S^{=}$) concentrations and an increase in sediment redox potential under and adjacent to salmon farms to levels at which more than half the reference area taxa can recruit and survive.

Coded Wire Tag (CWT) - A sequentially-numbered, small (1.1 mm long by 0.25 mm diameter), magnetized, stainless steel wire tag.

Commercial fishing - The act of fishing with the intent of selling the catch.

Commercial Passenger Fishing Vessel (CPFV) - A licensed fishing vessel that takes recreational anglers fishing in return for a fee. The vessel operator must follow certain requirements such as providing the Department with a log that, among other things, includes the number of anglers and an enumeration of the catch.

Enzyme-linked Immunosorbent Assay (ELISA) - A biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

Essential Fish Habitat (EFH) - Those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity.

Exclusive Economic Zone (EEZ) - A zone created by the Magnusen-Stevens Fishery Act, extending from 3 nautical miles to 200 miles offshore the United States and its territories, over which the United States has management jurisdiction of natural resources including fisheries, oil, and minerals.

Fecundity - The potential reproductive capacity of an organism or population, measured by the number of eggs.

Fluorescent Antibody Testing (FAT) - A laboratory test that uses antibodies tagged with fluorescent dye to detect the presence of microorganisms.

Food Conversion Rate (FCR) – A measurement for determining appropriate feeding levels. FCR is calculated as the weight of food fed divided by the weight gain of fish for a specified time period.

Gas Supersaturation (GSS) – A noninfectious disease, which can develop in cultured fish, that is associated by poor water quality and is caused by elevated total dissolved gas in the water.

Genotype – Genetic makeup of an individual; determines the hereditary potentials and limitations of an individual.

Gentoyping - The process of determining the genotype of an individual by the use of biological assays.

Gill net - A single wall of webbing, bound at the top by a float line and at the bottom by a weighted line and used for entangling fish.

Hook-and-line - Any fishing line with attached hooks (e.g., longline, troll and stick gear, among others).

Landings - The number or poundage of fish unloaded at a dock by commercial fishermen or brought to shore by recreational fishermen for personal use. Landings are reported at the points where fish are brought to shore.

Larval Mass Mortality Syndrome (LMMS) – A lethal syndrome, believed to be caused by exposure to organophosphate pesticides, which is characterized by the sudden loss of 80 to 100 percent of an incubator's larval population or, in some cases, loss of an entire spawn.

Letter of Permission (LOP) – Letter issued by the U.S. Army Corps of Engineers that authorizes projects that involve construction, excavation, or deposition of materials, or for any activities that affect the location and navigable capacity of waters of the United States.

Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) - Created by Congress in 1976, a 200-mile federal fisheries zone and eight regional councils to oversee the U.S. fisheries, which operate under the authority of the U.S. Department of Commerce.

Microsatellites - Loci (or regions within DNA sequences) where short sequences of DNA (nucleotides; adenine - A, thiamine - T, guanine - G, cytosine - C) are repeated one right after the other.

MS-222 – White powder used for anesthesia, sedation, or euthanasia of fishes.

Otolith - One of a number of tiny calcium-containing granules in the inner ear; provides sensory information on the position and movement of the head in space. Patterns of otolith growth provide information on fish age.

Organophosphate Pesticides (OPP) - Neurotoxins that are designed to kill insects via chemical inhibition of acetylcholinesterase, an important neurotransmitter in both invertebrates and vertebrates.

Optimum Sustainable Population (OSP) –The number of animals which will result in the maximum productivity of the population or the species, keeping in mind the carrying capacity of the habitat and the health of the ecosystem of which they form a constituent element.

Passive Integrated Transponder (PIT) - A type of tag applied to or incorporated into an animal for the purpose of identification and tracking using radio waves.

Pathogen - An agent that causes disease.

Polymerase Chain Reaction (PCR) – A technique used to amplify specific regions of a DNA strand.

Potential Biological Removals (PBR) - The maximum number of animals, not including natural mortalities that may be removed from a marine mammal stock while allowing that stock to reach or maintain its OSP.

Size limit - The minimum size a fish or other organism must be for it to be possessed.

Stock - A species, subspecies, geographical grouping, or other category of fish capable of management as a unit.

Stock Structure - Any description of the population attributes of a stock (age, size, sex), usually within a spatial context. This commonly refers to the spatial distribution of breeding groups or genetically-related organisms.

Total Dissolved Gas (TDG) - A measure of the sum total of all gas partial pressures (including water vapor) in water.

Total Gas Pressure (TGP) - The sum of the partial pressures of each individual gas in the mixture. Partial pressure is defined as the pressure which the gas would have if it alone occupied the volume.

Total Organic Carbon (TOC) - The amount of carbon bound in an organic compound and is often used as a non-specific indicator of water quality.

Total Volatile Solids (TVS) – The percent difference between the dried and combusted weights of sediment samples collected from the growout facility parameter.

Transmission Electron Microscopy (TEM) - A microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

Trawl – A large bag net that is tapered and forms a flattened cone. The mouth of the net is kept open while it is towed or dragged over the sea bottom.

Viral Hemorrhagic Septicemia (VHS) - A deadly infectious fish disease caused by the viral hemorrhagic septicemia virus.

Viral Nervous Necrosis (VNN) – See **Viral Nervous Necrosis Virus**

Viral Nervous Necrosis Virus (VNNV) – A single-stranded RNA virus, which predominately affects the central nervous system of larval and juvenile fish and causes Viral Nervous Necrosis (VNN).

Appendix B. Review of Coykendall's Dissertation

Review of Chapters Three and Four from Coykendall

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INTRODUCTION

At the request of the California Department of Fish and Game (DFG), and the California Coastal Commission (CCC), we reviewed the PhD dissertation completed in 2005 by D.K. Coykendall under the advisorship of Dr. Dennis Hedgecock formerly at the University of California, Davis and currently at the University of Southern California. The dissertation is entitled “Population structure and dynamics of white seabass (*Atractoscion nobilis*) and the genetic effect of hatchery supplementation on the wild population.” Because of the importance of genetics to the quality assurance components of the Ocean Resources Enhancement and Hatchery Program (OREHP), new genetic information is vitally important. This is especially true today, when the OREHP is immersed in a CEQA review and simultaneously developing an enhancement plan as mandated by new state law (SB 201). In this regard, the Coykendall work is being viewed as an important document for the OREHP because of the scope of what is covered and the fact that it is contemporary.

This document has been previously reviewed separately by two biologists from the DFG (see J. Rodzen 2006 and M. Lacy 2006). The difference between their review and the review presented here is that HSWRI has working knowledge of the genetic sampling program, including its history. Furthermore, HSWRI is intimately familiar with general spawning patterns and partitioning of cohorts into release batches.

Here we review Chapters Three and Four from Coykendall. Our original plan was to solely critique conclusions put forth in Chapter Four, which focuses on the potential impacts to the wild WSB population via the Ryman-Laikre model (Ryman and Laikre 1991). In reviewing Chapter Four, however, Chapter Three, which estimates the breeding effective size of the broodstock population per spawn event and per total annual release, came under scrutiny since the results are carried over into Chapter Four. It may be necessary in the future to evaluate Chapters One and Two of Coykendall, as well, but we feel the original purpose of the review – to evaluate our WSB breeding practices in the context of their effect(s) on the wild population and to justify maintaining an annual release target of 350,000 juvenile WSB – has been fulfilled by our critique of Chapters Three and Four alone.

CHAPTER THREE

Background

A primary goal of the hatchery is to maximize the genetic diversity of the juvenile white seabass (WSB) released into the wild in order to minimize the potential negative genetic impact (e.g. reduction in diversity) on the mixed (wild + captive-bred) population. One way to evaluate genetic diversity in a population is to estimate the genetically effective population size (N_e). N_e is a theoretical concept defined as the size of an ideal population (non-overlapping generations, random mating, equal sex ratios, and Poisson distribution of reproductive variance; Wright 1931) having the same amount of random genetic drift as real population (Hartl and Clark 1997). The concept is applicable to both captive-breeding programs, where we are interested in estimating and maximizing the effective number of breeders (N_b) from the parental (broodstock) generation contributing to the hatchery-bred progeny, and wild populations.

Discussion

Coykendall estimated N_e in the hatchery population three ways by evaluating 254 broodstock and their purported offspring at two levels (spawn events and release batches[1]):

1. the variance and inbreeding effective sizes (N_{eV} and N_{eI}, respectively) were calculated for each of ten different spawning events (50-100 offspring per five events from 1998 and 1999 and 85-100 offspring per five events from 2001), [2] and
2. the effective number of breeders (N_b) was calculated for the entire 2001 release (250 offspring proportionally divided among 32 of 46 total release batches). [3]

The genetic markers used to establish parentage and perform subsequent analyses of the effective sizes were a subset of seven of the microsatellite loci described in Franklin (1997).

Experiment 1: Demographic estimation of N_b per spawning event

The purpose of this experiment was to determine the variance (N_{eV}) and inbreeding (N_{eI}) effective sizes for each spawn event. N_{eV} assesses the rate of change in allele frequencies over time due to genetic drift and N_{eI} assesses the rate of increase in inbreeding. The samples included yolksac larvae from four spawn events from 1998 and one from 1999 and fin clips from five release groups from CY2001 supplied by

[1] Coykendall refers to release cohorts or batches as "spawning groups", which is confusing relative to single spawning "events." "Release batch" is the preferred terminology.
[2] It should be noted that the 1998-99 samples were yolksac larvae, while the 2001 samples were fin clips from juvenile fish. The implications of this may require further evaluation.
[3] By our accounting (of the text in Coykendall page 53) the number should actually be 36 of 46 total release batches.

HSWRI. All samples were originally collected by HSWRI and submitted to Genetic Identification Services (GIS).

We are concerned about several aspects of this experiment. First, all parental assignments appear to have been made using the same set of 254 brood fish. The genotypes of the 254 samples were provided by GIS along with the genotypes for yolksac larvae from 1998 and 1999 spawn events. It is unclear how the 254 brood fish relate chronologically to the offspring being analyzed. For example, in 2001 there were only 178 brood fish in all four breeding pools combined. At no single point in time (or year-long period) were there 254 fish spawning in hatchery pools; the broodstock management plan calls for 200 fish total (50 fish per each of four breeding tanks). In addition, the five 2001 release batches that Coykendall sampled were from only two of four breeding tanks (B3 and B4; see Tables 3.1 and 3.2 from Coykendall), meaning that ~100 brood fish, not 254, are actually representative of the 2001 spawn events chosen. This type of error is carried over into the results reported on page 55 when male proportional contributions 0.03 to 0.46 are calculated from numerators of 1 to 16 (males contributors per spawn event). In fact, if a maximum of 25 males exist in any one breeding pool, then the proportional range should be 0.04 to 0.64. It is not clear, but it appears likely, that Coykendall used all males existing in the tanks over a three year time frame and not a more appropriate instantaneous per spawning event approach. The female contributory analysis is similarly flawed.

Second, to calculate N_{eV} and N_{el} per spawn event, the samples should have been collected from single spawn events (e.g. the yolk sac larvae from GIS). However, at least one (2001rel34) of the "spawn events" chosen by Coykendall was actually a release batch comprised of two separate spawn events (see Tables 3.1 and 3.2 from Coykendall). Only juvenile fin clips were used for tissue samples at the time of release because spawn groups were mixed early in the culture process. To choose these release batches comprised of multiple spawn events and label them as individual spawn events introduces a fundamental error, which may be due Coykendall's own confusion over the nomenclature issue regarding spawn events, groups, and/or batches mentioned previously.

Additionally, to further emphasize the impact of this apparent confusion, on page 57 Coykendall states that "only two males contributed to more than one spawn in 2001. BS228M provided the majority of spawning in all three 2001 spawning events and had a total contribution of 52% (Table 3.3c). If the effective number of breeders is calculated as a combination of the spawns within the same breeding tank, then Nbv of Breeding Tank 1 would be 6.0, Nbv of Breeding Tank 3 would be 9.3, and Nbv of Breeding Tank 4 would be 3.8, which are all above the average Nbv's from their respective tanks (Table 3.1)." When she says "spawning events" she really means release batches. Of the three release batches (her "spawning events") from pool B4 in 2001 that she is describing (#16=JUL1401B4; #7=AUG2900B4; and #31=AUG2900B4), two are the same spawn AUG2900B4! This would clearly have a significant effect on all of her calculations. As another example, on page 60 of the discussion she writes "If repeat spawning was not a factor, we could calculate Neh of the 2001 release by summing the

number of all of the spawning groups scaled by the number of spawning events composing each of them, then multiply that by the mean of the spawning events (or as many as have been estimated). In 2001 release, there were 29 spawning groups consisting of one spawning event, ten consisting of two events, six consisting of three and 60 one consisting of four (Table 3.2). The harmonic mean of the five demographic Neh estimates from the 2001 spawning events is 3.09, so (29x1)+ (10x2)+ (6x3)+ (1x4) =71 x 3.09=220. Therefore, repeat spawning lowered the potential 2001 Neh from 220 to 35, a reduction of 84%." This analysis is flawed by the fact that all the spawning events are not different (i.e. some of the same spawns are mixed among release batches). In other words, there were not 71 separate spawn events.

Third, Coykendall does not explicitly discuss how she chose the specific spawn events or release batches used in her analyses. Each spawn event is a "snapshot in time," and the N_b per spawn event may change significantly over the course of a single spawning season in a single breeding tank. For example, using relative egg output as an indicator of contributing females per spawn, it is apparent that few brood fish usually contribute to the beginning and end of a season, but the number of contributors tends to rise toward the middle of a season when water temperatures are warmer. One or two spawning events occurring during one or two nights in a 4-5 month spawning season are unlikely to be a good general proxy for all spawn events within a breeding tank. Moreover, Coykendall extrapolates in a very confusing manner the contribution results for individual brood fish in those very few spawn events to an entire year's worth of production. On page 56 Coykendall states, "Over all three spawning events from Breeding Tank 1, BS135F contributed 66% and BS147F contributed 33% to the reproduction over the entire year." This sentence is contradictory and confusing relative to the inferred extrapolation. On one hand, the text says "over all three spawning events," but "over the entire year" is used in the same sentence. Each group of broodstock spawns between 60-90 times per year during a given 4-5 month season. Three spawns represents at most 5% of the spawning events, hardly a large enough proportion to extrapolate over the entire year. On page 57, a similar extrapolation is made where it is said, "Only two males contributed to more than one spawn in 2001." This conclusion is irresponsible as stated (and likely very erroneous) given the very small group of samples Coykendall analyzed (i.e. five batches from only two of four spawning groups). The sentence should read, "Only two males contributed to more than one of the five spawn events sampled in 2001." Finally, in the discussion on page 60, Coykendall attributes an 84% reduction in the potential N_b as being due to repeat spawning. This conclusion is extrapolated as an effect for the entire year even though it is based on only five of 36 sampled release batches.

Experiment 2: Allele rarefaction estimation of N_b for the 2001 release

A set of 250 fin clips was chosen proportionately from an available 3,456 – 96 fin clips were taken from each of 36 release batches for 2001 – and used in allele rarefaction analyses to estimate N_b for the entire 2001 release of 101,318 fish. An additional 10 release batches for a total of 46 release batches in 2001 were not sampled by HSWRI.

To illustrate choosing samples proportionately from the release batches: if release batch 40 contributed 7% of the total 2001 release, then 18 (or 7%) of the 250 samples were chosen from release batch 40. Unlike the previous experiment, the individuals in this experiment were genotyped by Coykendall, not GIS.

First, the sample set chosen may not be representative of the actual 2001 release. Ruzzante et al. (1998) determined that sample sizes of ~50 are required to accurately estimate the allele frequency profile of a population independent of the census size when using microsatellites, and this paper is widely cited regarding proper sample choice in molecular population genetic studies that use microsatellites. Samples were chosen by Coykendall from all 36 available release batches, and Coykendall likely equated the entire 2001 release to a population, in which case genotyping 250 individuals should be sufficient. The point was to estimate allelic richness in the entire 2001 release, but we must consider that a population is defined as a group of individuals within a species that can reproduce with one another and exist in the same place at the same time. With that in mind, the 2001 release is actually the product of four separate populations represented by each of the breeding tanks [aside: failing to partition the broodstock and offspring into "family" groups may have contributed to parental assignment problems in the above experiment]. Coykendall could have genotyped as few as 200 individuals, with release batches pooled according to source tank (release batches from multiple source tanks excluded) and 50 samples chosen randomly from each.

Coykendall's work focused on the release batch level, but it is questionable whether choosing samples proportionally was legitimate. In attempting to elucidate her actual sampling scheme, it appears that she may have used approximately 40-50 individuals per breeding tank, which is good, although it was probably not by intent. However, several of the release batches were comprised of spawning events from more than one tank, so it is unknown how the samples were partitioned among those events. The proportionality requirement also implies that the 17 of 36 release batches (47%) that contained <2000 fish, representing <2% of the 2001 release total of 101,318 fish, would have been represented by <5 individuals. Moreover, up to 14% (5 of 36) of release batches may have been represented in analyses by only one individual. Even though 250 total individuals were genotyped, it seems the allelic richness of the smaller release batches may be significantly under-represented, and it would not be surprising if the total allele count of 65 reported by Coykendall for the 2001 release is somewhat low. It may have been more powerful to genotype an equal number of individuals from each of the 36 release batches than to rely on such small samples sizes for nearly 50% of the release batches.

Second, the larger problem may be that the difference in allelic richness of 14 between the broodstock and 2001 total release may, at best, be high or completely wrong if the incorrect broodstock were included. We are again faced with the fact that all analyses appear to have been made assuming 254 brood fish, which may be only partially applicable to the spawn events as discussed for the demographic experiment. The total 2001 release was comprised of spawn events from broodstock in all four breeding

tanks, but there are never more than 50 brood fish per tank and, in fact, there were only 178 brood fish at HSWRI during 2001.

Third, Coykendall then used a numerical fitting procedure in order to estimate N_b for the entire 2001 release. The method apparently takes into account the sample sizes and allele frequency profiles of the parental and offspring groups and the differences between them. It does not require establishing parentage; all the broodstock and their potential offspring can be used in the analysis. Basically, it can be assumed that the more alleles each group contains and/or the smaller the difference between the two groups, the higher the relative genetic diversity and the higher the relative N_b. Coykendall estimated N_b to be 34.59, but because of the issues we pointed out above regarding sample choice, this number may misrepresent the diversity in and contributing to the 2001 release.

It follows that there are errors in both the numerator and denominator of the N_b to census size (N) ratio, calculated by Coykendall to be 0.14 (or 34.59/254 as stated on page 57). Obviously, Coykendall again uses the 254 brood fish. If N_b = 34.59 is an underestimate and N = 254 is overestimated, then the N_b/N ratio is biased low.

Conclusions

In essence, this chapter is confusing and often times misleading. Typographical errors made discerning what was actually done difficult (e.g. on page 53, “32” should be “36”; on page 55, spawn event “37” should be “31”; and in Table 3.1, the numbers 1 and 2 are transposed for #males and #females for 2001rel31, when based on data from Table 3.3c). Salient information (e.g. how and why particular samples were chosen for the demographic experiment) was left out of the text, and poor wording and division of subsections made it difficult to discern that there were actually two experiments being performed on two different sets of offspring samples.

The most significant problem we found was Coykendall’s apparent confusion in the makeup of a release batch, which she referred to confusingly/erroneously as “spawn groups”. There were also apparent failings in appropriate sample choice that carried over into subsequent analyses. Also problematic was the tendency of Coykendall to extrapolate the results from her limited samples to the production over the course of an entire year. Overall, the results in this chapter have the potential to significantly underestimate the actual genetic diversity of the WSB produced at the Carlsbad hatchery.

We also found it curious in this chapter (and the dissertation in general), that Coykendall does not cite the work of Bartley et al. (1995), who developed the broodstock management plan currently being implemented.

Clarification from the author on the questions raised in this review is needed before utilizing the results of this chapter in any meaningful capacity.

CHAPTER FOUR

Background

An important goal for WSB enhancement has been to determine the optimal per year hatchery release of juvenile WSB. A useful method to estimate this number is again based on the concept of N_e. The impetus here is to avoid or minimize the Ryman-Laikre effect, or the potential negative impact of a drop in N_e experienced by a mixed population due to hatchery supplementation of the wild population (Ryman and Laikre 1991; Ryman et al. 1995; see also Figure 1 below). A higher proportion of offspring from hatchery broodstock survive earlier life stages than do offspring from wild individuals (although, some of the skew in the variance may be mitigated by higher relative mortality in hatchery-bred juveniles after release into the wild). Stocking of hatchery-bred progeny can cause a reduction in genetic diversity as a result of these large differences in reproductive success, especially if the hatchery broodstock are a small fraction of the wild population. The ultimate concern is that less genetic diversity due to long-term stocking may result in a mixed population that is less responsive to stochastic environmental change.

Discussion

In order to estimate optimal release and control to our best ability the Ryman-Laikre effect, we must estimate four parameters:

1) initial wild effective size (N_{e0});
2) hatchery effective size (N_b);
3) the threshold, or baseline, mixed effective size (N_t); and
4) the current percent hatchery contribution to the natural population (*x*).

Using a subset of six of the microsatellite loci described in Franklin (1997), Coykendall genotyped 297 wild WSB collected by HSWRI. In this chapter, potential error due to sampling scheme effects (e.g. sample size) was taken into account through corrections in the estimation of F-statistics, which are used in the subsequent estimation of N_{e0}. Coykendall then estimated N_{e0} to be ~6000 (95% confidence intervals (CI) depended on the mode of estimation). An N_b = 34.6 was calculated in Chapter Three for the 2001 release (this N_b estimate is questionable as discussed in the review above, but we will use it here as we have no other estimate available). Coykendall then applied the Ryman-Laikre model to evaluate the effect of the WSB enhancement program on the effective size of the mixed stock after supplementation (Ryman and Laikre 1991).

Coykendall determined that long-term hatchery supplementation may reduce N_e by 2-93%. However, this conclusion included a large range of possible N_{e0} estimates, spanning 3,700 via moment-based and ~55,000 via pseudo-likelihood analyses between upper and lower 95% CI. The most dramatic reduction in N_e (89-93%) would result from the pressure of $N_b \leq 34.6$ on an N_{e0} = 57,310 (the upper 95% CI value from the pseudo-likelihood estimate). However, the pseudo-likelihood distribution of N_{e0}, if

unimodal, must be highly skewed as the mean of 6,087 and lower 95% CI of 2384 are both an order of magnitude smaller. Additionally, the total range for the moment-based analysis of N_{e0} was from 3,977 to 7,678. Although the moment-based approach may be less reflective of WSB life history, the distribution does not appear skewed and the estimates are all below 10,000, lending support to an N_{e0} of substantially less than 57,310. We cannot propose a more realistic N_{e0} without reanalyzing the data (which is unavailable), but we suggest that a negative genetic impact is likely to be much closer to 2% than 93%, especially in light of information discussed next on the preliminary mark-recapture data used by Coykendall.

Included in the above calculations, and more problematic to an accurate and realistic data interpretation, was Coykendall's use of a 6.9% hatchery contribution (*x*) to the natural breeding stocks that came from mark-recapture data for juvenile fish (Figure 1). A hatchery contribution of 6.9% will decrease N_{et+1} (where N_{et+1} is the first generation following N_{e0} for which hatchery-bred fish may have contributed to the gene pool) below N_{e0} by some amount between the 2-93% mentioned above. However, this value of *x* is not reflective of individuals that actually have the potential to contribute to successive generations of the mixed stock and was rightly stated by Coykendall to be upwardly biased. Current mark-recapture data gathered by M. Shane of HSWRI puts *x* for reproductively-mature hatchery-bred WSB at <1%. According to Ryman-Laikre calculations, a 1% hatchery contribution to the mean pseudo-likelihood estimate of N_{e0} = 6,087 with an N_b = 34.6 actually raises N_{et+1} to 6,101. In fact, for all $N_{e0} \leq$ 6,910 with $N_b \geq$ 34.6, any hatchery contribution of ≤1% should raise N_{et+1} above N_{e0}. Regardless, because *x* is currently low (<1%), it is highly unlikely that hatchery supplementation to date has had a significant negative impact on genetic diversity of the wild population. It is unclear why Coykendall used 6.9% and not a more accurate number (~1%) that was also available from the OREHP data.

Conclusions

The magnitude of the negative genetic impact stated in Chapter Four of Coykendall is likely to be an overestimation. In fact, the small hatchery contributions thus far may have had the potential to even increase diversity in the mixed population due to the fact that broodstock that may have otherwise not successfully reproduced in volume in the wild are given the chance in a hatchery setting.

In addition, the point of the Ryman-Laikre model is not necessarily to couch the results in a negative light as a purely detrimental reduction in genetic diversity as Coykendall has done. Reproduction in the wild without supplementation has the potential to naturally reduce (or increase) genetic diversity through random genetic drift, as well. Because the commonly assumed outcome is a reduction in diversity through supplementation, the objective should be to set a lower acceptable limit for N_e in the mixed population that will maintain a sufficient level of genetic diversity such that the population is still able to withstand stochastic environmental changes without significant risk of severe depletion or extinction. That number (N_e) has been empirically defined at

≥500 (Tringali and Bert 1998; see also Figure 1), although its applicability to a species such as the WSB with Type III survivorship is yet to be determined.

As concluded in the previous chapter, clarification from the author on the questions raised in this review is needed before utilizing the results of this chapter in any meaningful capacity.

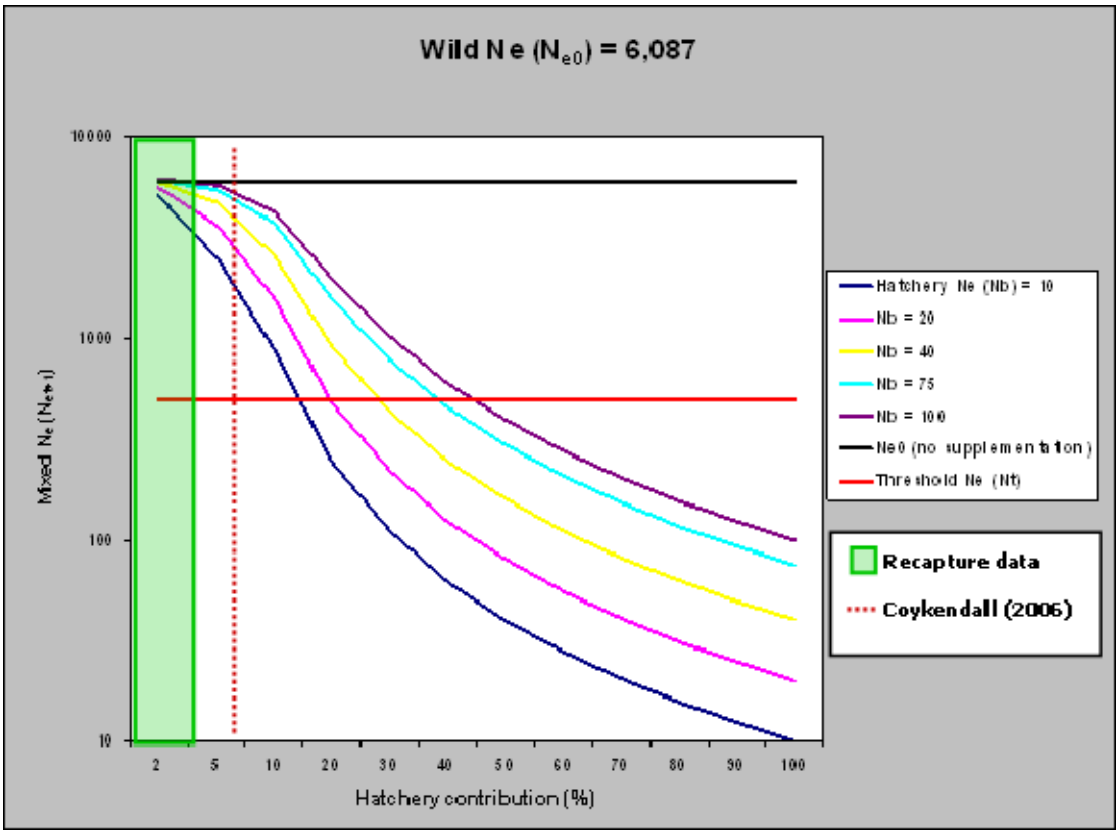

Figure 1: Ryman-Laikre model predictions for the reduction in N_e of the mixed stock due to hatchery supplementation of the wild population. Original N_{e0} prior to stocking (horizontal black line) is the pseudo-likelihood estimation of mean N_{e0} = 6087 by Coykendall (2006). The threshold (horizontal red line) corresponds to the baseline N_t = 500 described in the literature (e.g. Tringali and Bert 1998; Taniguchi 2003). Curved N_b lines represent various estimates of broodstock contribution to the hatchery gene pool; Coykendall (2006) estimated N_b = 34.6 (which would fall just below the yellow curve). Finally, *x* is the percent hatchery contribution to the wild stock as determined by mark-recapture data, with the vertical dotted red line representing the 6.9% estimation from Coykendall (2006) and the green shaded box representing a potential span of *x* from recaptured reproductively mature WSB.

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