

NOAA Technical Memorandum NMFS



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HISTOPATHOLOGICAL MANUAL FOR MONITORING HEALTH OF STRIPED BASS IN RELATION TO POLLUTANT BURDENS.

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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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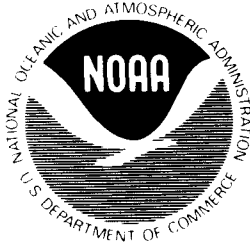
NOAA Technical Memorandum NMFS

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NOAA Technical Memorandum NMFS

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ABSTRACT

Since 1978, the Physiological Ecology Investigation staff at the Tiburon Laboratory has been examining the relationship of pollutants to the physiological condition or "health" of striped bass (Morone saxatilis). During the course of this investigation, a series of standard and new techniques were developed to quantitatively and qualitatively assess the health of prespawning striped bass while simultaneously sampling tissues for pollutant residues. Prespawning bass and their gametes were selected because they are relatively sensitive to the effects of pollutants at low chronic levels (Whipple, Eldridge and Benville, 1981).

The autopsy techniques were refined to include the measurements and characteristics that were 1) most indicative of pollutant stress and 2) also relatively easy to assess in a monitoring program.

This report includes histopathological techniques derived by the Cooperative Striped Bass Study (COSBS) and from a report on our recommendations to the State Water Resources Control Board for monitoring of striped bass in the San Francisco Bay ecosystem.

INTRODUCTION

Laboratory experiments performed at Tiburon Laboratory with striped bass eggs and larvae (1975-1977) revealed considerable variation in viability of eggs obtained from different females. We hypothesized that part of the variation might be related to pollutant stress on the prespawning females and that this stress might be quantified by relating measures of gametic egg condition to pollutant burdens in the livers and ovaries of the parental female. In an attempt to determine the source of this variation and to find out why so many of the eggs were inviable, we undertook sampling of prespawning females from the San Joaquin River off Antioch in 1978.

During this research a histopathological autopsy procedure was developed to examine the health of striped bass, emphasizing measurements of the egg and reproductive condition. Initial examinations revealed that many fish were in poor health. For example, many fish had skeletal abnormalities, open and healed lesions, high parasite burdens and gonads impacted by parasites, as well as necrotic eggs and ovaries. Consequently, the autopsy examination was expanded to include a number of additional measurements and subsamples of organs and tissues in order to quantify the relationship of pollutant burdens to parasites and to body, liver and egg condition.

An earlier version of this manual was printed in 1981 (Whipple and Jung, 1981) by the State of California Water Resources Control Board (SWRCB) as part of the Cooperative Striped Bass Study (COSBS). The present manual has been edited to include the most relevant measures and subsamples for indicating the health of striped bass. A list of these variables is in Appendix I. Selection of variables was based on observations and data analyses for more than 500 fish collected from 1978-1983. These results are reported in previous COSBS reports (Jung and Bowes, 1980; Jung et al., 1981; Whipple, Crosby and Jung, 1983) and in a paper by Whipple, Benville, Eldridge and MacFarlane (in prep.). This manual, therefore, includes the histopathological procedures and subsampling recommended to the California Department of Fish & Game (CDF&G) for monitoring striped bass during prespawning seasons in the future.

HISTOPATHOLOGICAL ASSESSMENT

I. PREPARATIONS PRIOR TO AUTOPSY

The following steps should be taken before examining the fish to reduce the time spent on each autopsy. There is no specific sequence to these steps.

A. Coding (Appendix II)

Post coding for the data sets above the autopsy table. Changes should be noted in large print or circled.

B. Labels (see page 23)

Complete experiment (EXP.) and location (LOC.) codes, FISH NO. and DATE entries on the set of labels. EXP. code represents a laboratory experiment or a year of field studies; LOC. is the place of capture. The FISH NO. is on the label tucked behind the gill cover of the fish. DATE is the date of your examination. See Coding for All Data Sets (Appendix II).

Cut out labels and place them on a clean surface to eliminate contamination of samples taken for pollutant analyses.

Enter codes for EXPERIMENT, LOCATION and METHOD at top of all data sets; use Coding for All Data Sets (Appendix II). Enter FISH NO. on all data sets; DISSECTION DATE and TIME (beginning of autopsy) on DATA SETS 2 and 6.

D. Scale Envelopes

For each fish, label a scale envelope clearly with EXPERIMENT code, LOCATION code, FISH NUMBER and DATE.

E. Photo Identification

Prepare an identification card for each fish--an index card with EXPERIMENT, LOCATION, FISH NUMBER and DATE written in large, clear numbers. This is used when photographing the fish.

F. Supplies

Lay out sample jars and lids, plastic freezer bags, scale envelopes, 4" x 4" squares of clean aluminum foil, and dissecting tools for easy access.

Check operation of all balances and cleanliness of weighing pans.

Check on supply of hexane-rinsed foil for PCB/pesticide samples and acid-washed containers for heavy metal samples. Procedures for preparing both are in Appendices IV & V.

Supplies for monocyclic aromatic hydrocarbon (MAH) analysis are found in Appendix VII.

Check supply of buffered 10%-formalin solution used as a preservative for histology samples. (Appendix III)

Filter paper and a Munsell chart for color determination should also be nearby.

II. EXAMINATION, AUTOPSY AND SUBSAMPLING PROCEDURES

The following procedures should be performed in the sequence presented.

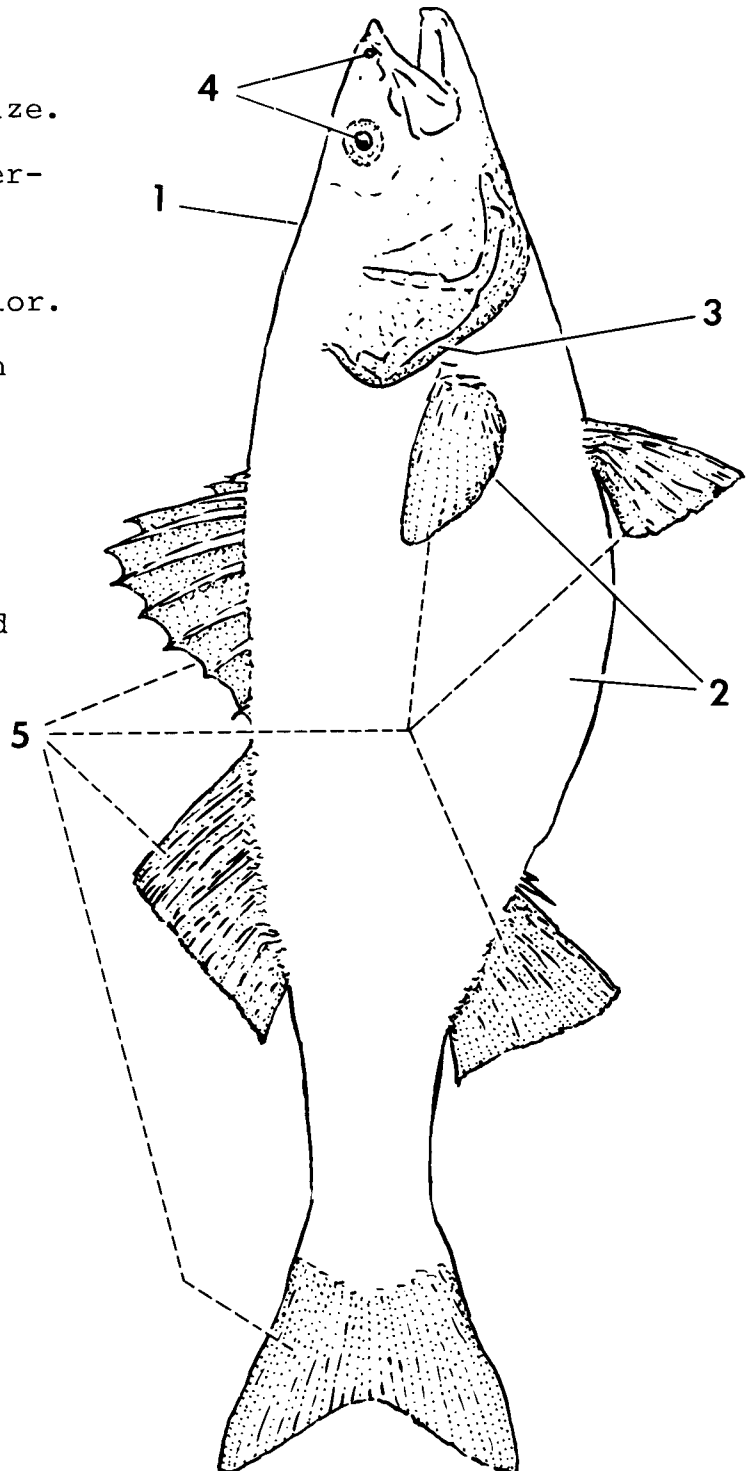
A. Exterior Examination

1. Check for the following (Figure 1: What to Look For) and use DATA SET 6 - DISEASE, PARASITES AND ABNORMALITIES and Coding for Data Set 6 to record your observations.
 - a. Skeletal abnormalities--fins, gill rakers, vertebral column, skull bones, etc.
 - b. Lesions, wounds, scars, reddening -- particularly on right ventrolateral surface or under pectoral fin. (Run your fingertips along these areas to feel for lesions which may have healed over; scale pattern is usually interrupted.)
 - c. Gill erosion, parasites, color
 - d. Eyes for opacity, and nares for occlusion
 - e. Fins for fin rot
 - f. Other

Figure 1. -- What to Look For.

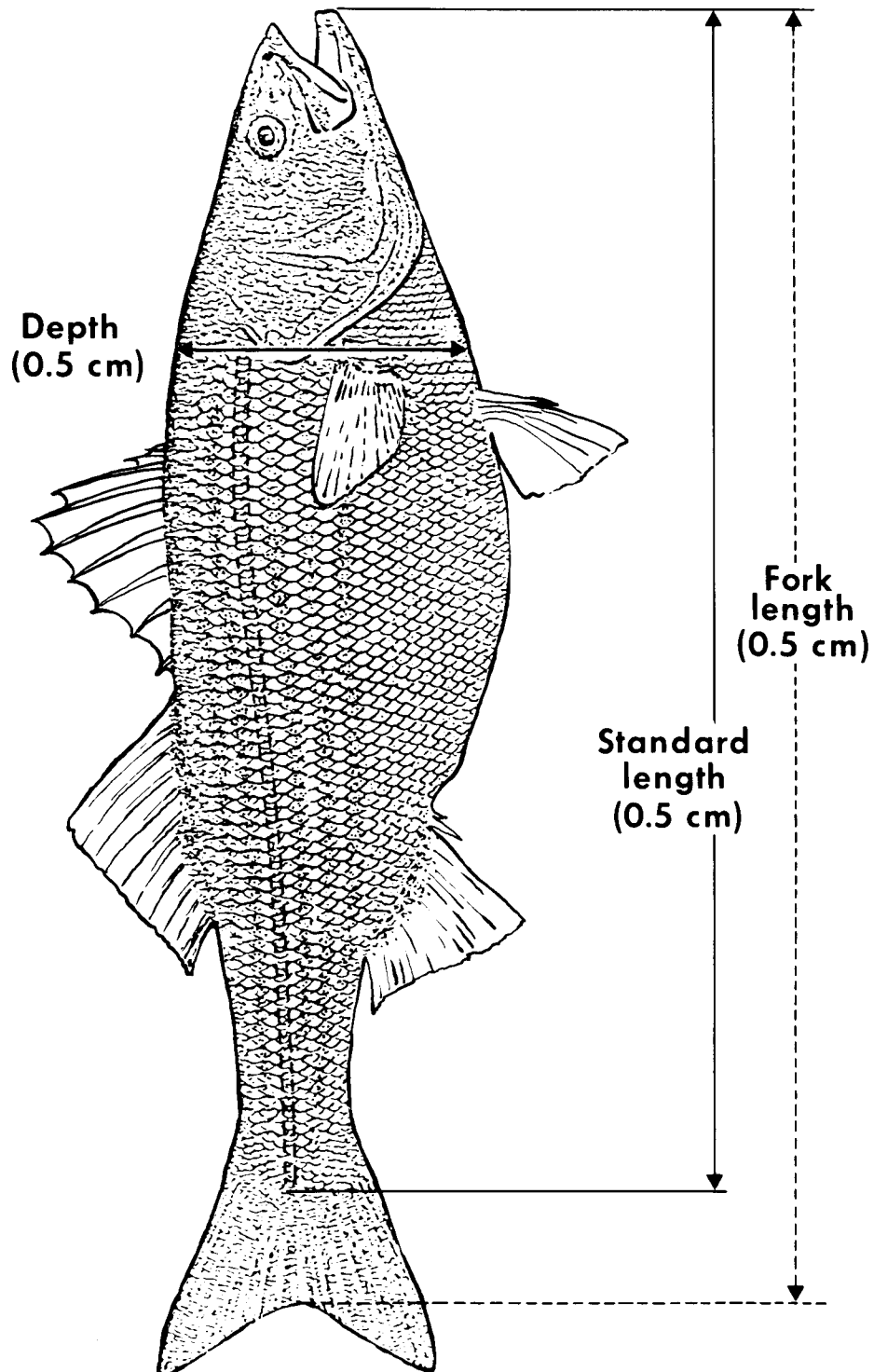
Check for the following:

- (1) Pugheadedness, scoliosis and other skeletal abnormalities.
- (2) Lesions, wounds -- measure size. Feel for them with your fingertips.
- (3) Gill erosions, parasites, color.
- (4) Opacity in eyes, occlusion in nares.
- (5) Fin rot and other fin abnormalities.
- (6) Other parasites, diseases and abnormalities.



2. Record the size of any wound or lesion under NOTES on the right-hand side of Data Set 6.
- B. Measurements (Figure 2: What to Count and Measure).
1. Complete all of DATA SET 2 - MERISTICS AND MEASUREMENTS except for AGE. Record measurements for the left side of the fish.
 2. Enter, to nearest 0.5 cm, FORK LENGTH and STANDARD LENGTH. DEPTH (width) is optional.
 3. Enter the weight of the fish to the nearest whole gram (1.0 g) under WET WT.
 4. Take 8-10 scales from just above the lateral line for age determination. Seal them in the pre-labeled scale envelope.

Figure 2.-- What to Count and Measure. Record Standard and Fork Lengths (mm) and Total Wet Weight of Fish (g). Take 8-10 scales from above lateral line for age determination.



C. Color Pattern Determination

1. Record your data on DATA SET 3 - COLOR PATTERN.

Refer to Figure 3: Color Pattern and use the Coding for Data Set 3. (Appendix II)

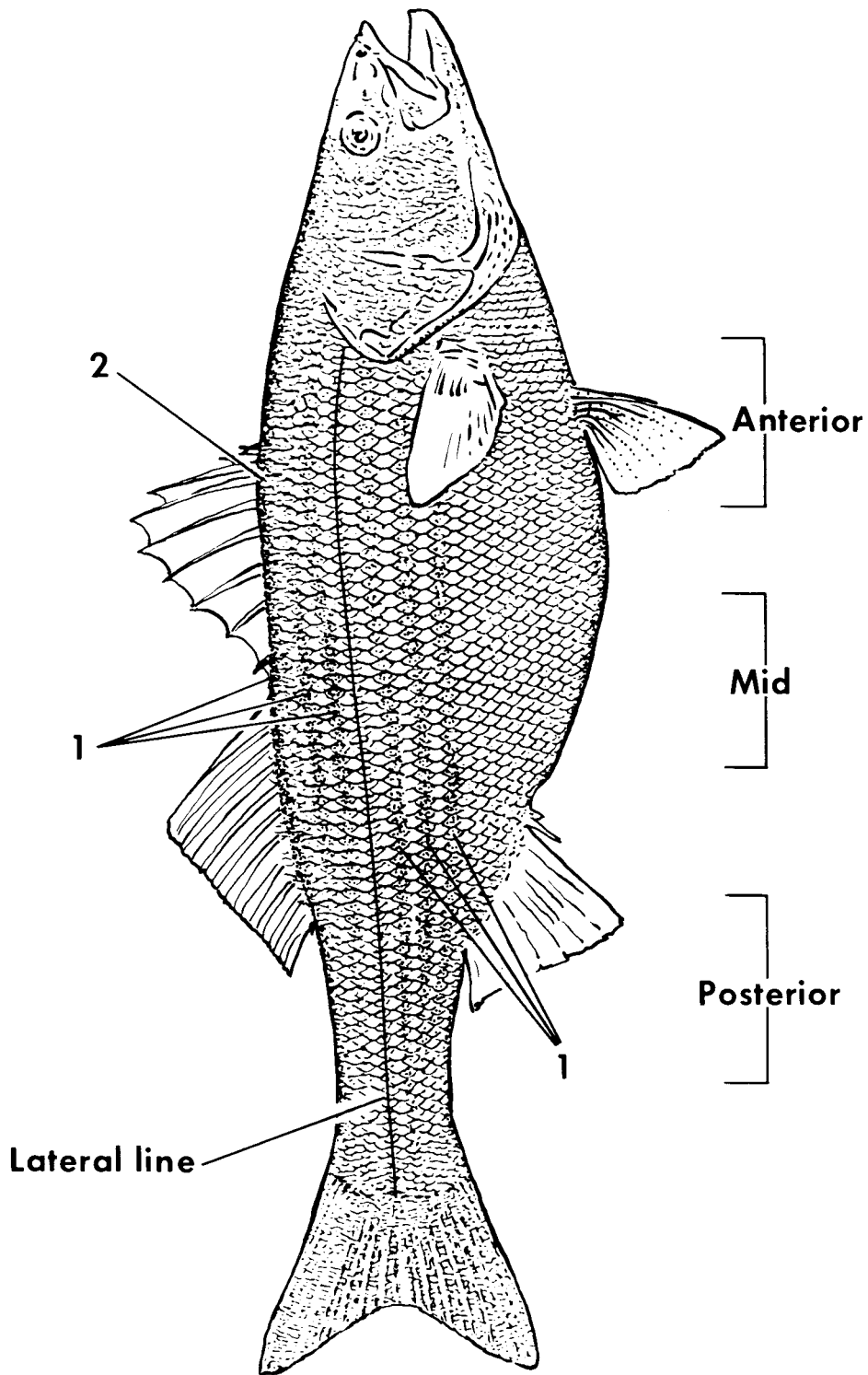
2. Record dorsal melanization code under COL PAT INT.

3. Starting with the left side of the fish, assess the degree of breakage in the striping pattern for the dorsal region. Record breakage codes for anterior, mid and posterior sections under STRIPING PATTERN BREAKAGE DORSAL A M P. Add values for DORSAL A, M and P and record under DORSAL TOT.

EXAMPLE: If dorsal stripes in the anterior section are broken, unbroken in the midsection, and broken in the posterior, record under DORSAL A M P TOT the following codes: 2 1 2 5.

4. Do the same for the ventral striping pattern and record under STRIPING PATTERN BREAKAGE VENTRAL A M P TOT.

Figure 3.-- Color Pattern. Record (1) degree of breakage in striping pattern for dorsal and ventral stripes (1 = solid stripes; 2 = broken stripes) and (2) color pattern intensity and rank.



5. Add values for DORSAL TOT and VENTRAL TOT and record under ALL STRPS TOTAL.
 6. Enter code for SIDE.
 7. Photograph left side of fish, with identification card prominently displayed, using black-and-white (Kodak Plus-X*, Panatomic*, etc.) or Polaroid* film. Record film roll and frame numbers under PHOTO ROLL/FRAME.
 8. Note general appearance of dorsal region and enter description of overall breakage (1/4, 2/4, or 3/4 broken) under DORSAL BREAKAGE.
 9. Repeat steps 3 - 8 for right side of fish.
- D. Dissection and Interior Abnormalities
1. Use DATA SET 6 - DISEASE, PARASITES AND ABNORMALITIES.

* Use of brand name does not imply endorsement by the National Marine Fisheries Service.

2. Open fish with ventral incision from vent to lower jaw. Lift gonads and check opening to exterior with blunt probe for any occlusions. Note on DATA SET 6 if blocked. Note any visceral adhesions or parasites which press on gonads and/or keep organs abnormally bound to the body wall or cause abnormal growth. Look for healed lesions in the body wall which may not have been visible on the outside. Note other parasites.

3. Tare balance with a pan lined with hexane-rinsed foil. Place gonads, stomach, intestines, liver, gall bladder and spleen (all organs except heart) in the pan. Record total gut weight to nearest whole gram (1.0 g) under WET WT VISCERA on DATA SET 6.

E. Subsampling

1. Left Ovary
 - a. Use DATA SET 4 - GONADS: MERISTICS AND FECUNDITY. Enter codes for SEX, gonad (GND) and maturity (MTR). Use one line for each gonad.
 - b. Record length (TL) and width (WI) of each gonad to nearest 0.5 cm.

- c. Enter weight of each gonad to nearest tenth gram (0.1 g) under WET WT. Record combined weights of both gonads under TOTAL WW (both lines).
- d. Examine for parasites, abnormalities, etc. and note on DATA SET 6.
- e. Take small portions of the anterior, mid and posterior sections for fecundity. Place in small vials with the appropriate labels (see page 23). Be sure anterior, mid and posterior sections are correctly labelled.
- f. Section remaining ovary for pollutant samples. FOLLOW CLEAN TECHNIQUES FOR EACH SAMPLE (Apps. IV, VI & VIII). Place POLL-I MAH sample into culture tube and process immediately to avoid losing the MAHs. Place POLL-I PAH sample for polycyclics in glass jar with foil-lined lid, shiny side of foil toward samples; freeze immediately on dry ice. Place POLL-II samples for heavy metals (HM) in acid-washed conventional polyethylene containers. Wrap POLL-III samples for chlorinated hydrocarbons (DDT, DDD, DDE) in foil prerinsed in nanograde hexane. Place labels securely on the outside of the containers with strapping tape.

2. Left Testis

Follow procedure for left ovary. No fecundity samples will be taken. Use the appropriate labels.

3. Right Ovary

- a. Weigh, measure, examine and note abnormalities, parasites, etc., as with the left ovary. Use DATA SET 4 - GONADS: MERISTICS AND FECUNDITY and DATA SET 6 - DISEASE, PARASITES AND ABNORMALITIES.
- b. Place an anterior sample for color on a piece of white filter paper. Match the egg colors to the Munsell color chart and enter on DATA SET 4 under MUNSELL HUE, VAL. and CHR.. Use Munsell codes and Coding for Data Set 4 to find HUE RANK I and II. Use same lighting (e.g. fluorescent) throughout examinations.
- c. Place an anterior portion in a petri dish with fresh water for examination of fresh eggs and measurement of egg diameters under microscope. See F, Egg Examination, p. 20. Another anterior section should be placed in a 10%-buffered formalin solution for histology.

Make sure sample is no larger than 2 cm circumference and is well covered with the formalin. Use appropriate label.

- d. (Optional). Take a midsection sample for parasitology. Seal in a plastic bag with label.

4. Right Testis

Proceed as for right ovary. The anterior sample for histology is first examined for sperm motility (See G., Sperm Motility, p. 21). Use appropriate labels.

5. Liver

- a. Examine liver and gall bladder for parasites, abnormalities, etc., and record your observation on DATA SET 6 - DISEASE, PARASITES AND ABNORMALITIES, using Coding for Data Set 6.
- b. Remove gall bladder carefully to avoid spilling bile on tissue to be analyzed for pollutants (Optional). Place gall bladder in a small vial and put into plastic bag to go with the liver parasitology sample.

- c. Weigh liver to nearest tenth gram (0.1 g).
Record weight on DATA SET 5 - LIVER, GALL
BLADDER AND STOMACH under LIVER SIZE WET
WT.
- d. Rank liver color according to Coding for Data
Set 5 and record under LIVER COLOR HUE RANK
I. If liver is red due to hemorrhaging be
sure to record on Data Set 6.
- e. Section liver for the following subsamples:
 - (1). Take a sample of the right lobe for
histology. Place in a vial with
10%-buffered formalin solution with
label.
 - (2). Take four middle samples (POLL-I MAH,
POLL-I PAH, POLL-II HM and POLL-III CH)
for pollutant analyses. Place carefully
in appropriate containers. (see 1 f.,
ovary pollutant sampling, p.15, and
Appendix VII). Use the appropriate
labels.
 - (3). Take a sample of the left lobe for
parasitology. Seal in a plastic bag with
the gall bladder vial and label.

6. Spleen

Examine spleen for parasites, abnormalities, etc.
Record your observation on DATA SET 6 - DISEASE,
PARASITES AND ABNORMALITIES.

7. Kidney

Examine for abnormalities and parasites. Record on
DATA SET 6 - DISEASE, PARASITES AND
ABNORMALITIES.

8. Other Organs

a. On DATA SET 5 - LIVER, GALL BLADDER AND
STOMACH, enter code for stomach contents
(STOMACH CNT); use Coding for Data Set 5.

On DATA SET 8 - SPLEEN AND OTHER ORGANS use
Coding for Data Set 8 to rank the amount of
mesenteric fat and condition of intestines.
Note any abnormalities on DATA SET 6 -
DISEASE, PARASITES AND ABNORMALITIES.

b. (Optional). Seal the stomach and intestines in
a freezer bag with the appropriate label for
parasitology.

9. Muscle

- a. Fillet left anterior dorsal muscle for pollutant analyses POLL-I MAH, POLL-I PAH, POLL-II HM and POLL-III CH). Use appropriate containers and techniques (see 1 f., p. 15 and Appendix VII) and the appropriate labels.
- b. Extra samples for pollutant analyses may be taken from right dorsal muscle.

F. Egg Examination

Record data on DATA SET 22 - GONADS: EGG DIAMETERS AND SPERM MOTILITY. Examine sample of eggs taken from anterior right ovary under 50X magnification on the dissecting microscope. Measure the diameter of five individual eggs, picked at random, to nearest 100 microns; record mean under MEAN EGGS FRESH. Note (1) any increase in perivitelline space, (2) if eggs are loose or clumped, and (3) if oil drops are dispersed or coalesced. Note the approximate maturation stage (MATUR) and any abnormalities (NOTES).

G. Sperm Motility (Optional)

Place a small portion of milt or ripe-running sperm on tip of pipette or blunt probe and stir into a 50-ml beaker of distilled water. Water temperature should be around 18 deg C or higher, but not higher than 25 deg C. Allow to sit for at least 5 minutes. Examine for signs of motility under 400X magnification on compound microscope. Dark-field phase shows spermatozoa very well. If spermatozoa are not motile after 5 minutes in water, they are probably still immature or abnormal; reexamine after 30 minutes. Record motility on DATA SET 22 - GONADS: EGG DIAMETERS AND SPERM MOTILITY.

H. Hematology (Optional)

Hematocrits and slides of blood smears. See Appendix VIII.

I. Specimen Archiving (Optional).

Replace fish number tag under gill cover. Place bags with parasitology samples (gonad, liver and gall bladder, stomach and intestines) in body cavity. Wrap entire fish tightly in a large plastic bag (trash bag), seal with strapping tape, attach label and freeze.

J. Storage of Samples

TISSUE	SAMPLE FOR	CONTAINER	PRESERVATIVE
L. Ovary	-Fecundity	Glass vial	Freeze
L. Gonad	-Pollutants I-MAH	Culture tube	Process immediately
	-Pollutants I-PAH	Glass jar, foil-lined lid	Freeze
	-Pollutants II	Acid-washed polyethylene	Freeze
	-Pollutants III	Hexane-rinsed foil	Freeze
R. Gonad	-Histology	Glass jar	10% formalin
	*-Parasitology	Plastic bag	Freeze
Gall bladder	*-Parasitology	Glass vial with liver sample	Freeze with fish
Liver	-Histology	Glass jar	10% formalin
	*-Parasitology	Plastic bag	Freeze with fish
	-Pollutants I-MAH	Culture tube	Process immediately
	-Pollutants I-PAH	Glass jar, foil-lined lid	Freeze
	-Pollutants II	Acid-washed polyethylene	Freeze
	-Pollutants III	Hexane-rinsed foil	Freeze
Stomach and Intestine	*-Parasitology	Plastic bag	Freeze with fish
Muscle	-Pollutants I-MAH	Culture tube	Process immediately
	-Pollutants I-PAH	Glass jar, foil-lined lid	Freeze
	-Pollutants II	Acid-washed polyethylene	Freeze
	-Pollutants III	Hexane-rinsed foil	Freeze
Tumors, Parasites (Other) (Unusual types)		Glass jar or plastic bag	10% formalin or freeze w/ fish

*Optional

K. Sample Labels

EXP.	LOC.	EXP.	LOC.	EXP.	LOC.
FISH NO.		FISH NO.		FISH NO.	
DATE		DATE		DATE	
L. OVARY-ANTERIOR		L. OVARY-MIDDLE		L. OVARY-POSTERIOR	
FECUNDITY		FECUNDITY		FECUNDITY	
(FREEZE)		(FREEZE)		(FREEZE)	
EXP.	LOC.	EXP.	LOC.	EXP.	LOC.
FISH NO.		FISH NO.		FISH NO.	
DATE		DATE		DATE	
L. GONAD-MIDDLE		L. GONAD-MIDDLE		L. GONAD-POSTERIOR	
POLL I-MAH		POLL I-PAH		POLL II-HM	
(PROCESS IMMEDIATELY)		(FREEZE W/FOIL ON LID)		(FREEZE IN POLYETHYLENE)	
EXP.	LOC.	EXP.	LOC.	EXP.	LOC.
FISH NO.		FISH NO.		FISH NO.	
DATE		DATE		DATE	
L. GONAD-POSTERIOR		R. GONAD-ANTERIOR		R. GONAD-MIDDLE	
POLL III-PCB,DDT/DDE		HISTOLOGY		PARASITOLOGY	
(FREEZE IN RINSED FOIL)		(10% FORMALIN)		(FREEZE W/FISH)	
EXP.	LOC.	EXP.	LOC.	EXP.	LOC.
FISH NO.		FISH NO.		FISH NO.	
DATE		DATE		DATE	
LIVER-R. LOBE		LIVER-MIDDLE		LIVER-MIDDLE	
HISTOLOGY		POLL I-MAH		POLL I-PAH	
(10% FORMALIN)		(PROCESS IMMEDIATELY)		FREEZE W/FOIL ON LID)	
EXP.	LOC.	EXP.	LOC.	EXP.	LOC.
FISH NO.		FISH NO.		FISH NO.	
DATE		DATE		DATE	
LIVER-MIDDLE		LIVER-MIDDLE		LIVER-L. LOBE & GALL-	
POLL II-HM		POLL III-PCB,DDT/DDE		PARASITOLOGY BLADDER	
(FREEZE IN POLYETHYLENE)		(FREEZE IN RINSED FOIL)		(FREEZE W/FISH)	

STRIPED BASS HEALTH INDEX

The following document is an example of applications for the "Histopathological Manual for Monitoring Health of Striped Bass in Relation to Pollutant Burdens."

The Striped Bass Health Index is extracted from the Guidance Document for Regional Effects Monitoring of the Aquatic Habitat Program developed for the California Regional Water Quality Control Board, Oakland, CA. It is included here to illustrate the use of this manual and is currently being used by the Aquatic Habitat Program in its 1984 Monitoring Study.

Recommended Components for Monitoring
the Condition of Striped Bass

R. Bruce MacFarlane and Jeannette A. Whipple

May, 1984

I. INTRODUCTION

A. ELEMENT

The striped bass (Morone saxatilis) is a major recreational species in the San Francisco Bay-Delta estuary. Mature, prespawning females will be sampled annually in April and May at two locations downstream of their spawning areas in the San Joaquin and Sacramento Rivers. Specimens will be subjected to biological and pollutant analyses to determine the health of adults and eggs. Analyses will be performed which assess the physiological and reproductive conditions of the fish that are associated with pollutant exposure.

B. RATIONALE (For the Element)

The striped bass is an important member of the Bay-Delta community. Assessing the physiological condition, or health, of this species can provide valuable information relating to both the pollutant status of the ecosystem and the welfare of the animal. Considerations that justify monitoring the condition of the striped bass include:

- (1) It is an ecologically significant species. Since it is a tertiary carnivore, the striped bass is at, or near, the top of the Bay-Delta trophic structure. Because of this position, its life span (to 30 years) (Setzler et al., 1980), and its relatively high lipid content, (Gadbois and Maney, 1983), the striped bass has a high bioaccumulation potential for pollutants. In fact, pollutant analyses of Bay-Delta striped bass tissues performed by several laboratories have shown higher concentrations of many classes of pollutants (petrochemicals, PCBs, pesticides, metals) than striped bass, and several other species, from estuaries on the East and West coasts of the United States (Whipple et al., 1983; Gadbois and Maney, 1983).
- (2) The striped bass ranges throughout the Bay-Delta, thereby acting as an integrator of the pollution status of the estuary. However, the population occurs predominantly in the northern portion of the bay and in the delta, thereby being more affected by water quality in these areas than in other portions of the estuary.
- (3) The species is economically and recreationally significant. An estimated \$20 million are spent annually by the recreational fishery for striped bass in the Bay-Delta (based on consumer price index projections of Stanford Research Institute's 1966 estimate).

Additionally, the species is viewed as an indicator of the well-being of the estuary and enjoys high public visibility and concern. Within this context, this species is of interest from the perspective of public health. Studies have shown that Food and Drug Administration(FDA) Action Levels have been approached or exceeded in striped bass flesh for several contaminants (PCB's, total DDT, toxaphene, aldrin/dieldrin, heptachlor, chlordanes) (Whipple et al., 1983). Mercury contamination of the fish prompted the State Department of Health to issue an advisory in 1972 against its consumption by pregnant women and children and the warning is still in effect.

- (4) The striped bass population structure is monitored annually by the Department of Fish and Game (CDFG). Also, the National Marine Fisheries Service (NMFS), the State Water Resources Control Board (SWRCB) and the University of California have established a five-year data base for the physiological and reproductive conditions of the population. The data base was produced in a Cooperative Striped Bass Study (COSBS),

which also identified impaired condition parameters associated with elevated pollutant burdens in the fish (Whipple et al., 1983; Whipple et al., in prep.). The combination of the previous sampling program with an ongoing quantitative assessment of the population will provide a better evaluation of the striped bass in the Bay-Delta and its recruitment potential.

II. OBJECTIVES

The primary objective of this element is to provide indices of the physiological and reproductive conditions as well as the pollutant burdens of the striped bass that reflect the extent of anthropogenic contamination in the Bay-Delta system. A secondary objective is to provide an assessment of the physiological health of the striped bass population that could be used by regulatory agencies charged with protecting this valuable resource.

III. GENERAL METHODS

We recommend that mature, prespawning females be collected in conjunction with the CDFG tagging program in April and May of each year in the San Joaquin River at Antioch and in the Sacramento River at Clarksburg. If the tagging program should be terminated in the future, sampling should still be conducted in these areas during April and May even though additional expense will be incurred (approximately \$10,000/year). Prespawning females provide several advantages for monitoring compared to other life stages. First, this stage is among the most sensitive to and diagnostic of pollutant effects (Whipple et al., 1981). Secondly, measures of reproductive condition can be more easily evaluated and used to estimate potential pollutant-related effects to recruitment into the population.

Sampling at the specified locations has several advantages over other collection strategies. These locations are already being sampled by CDFG; therefore, collection is much easier and cheaper. Both locations are natural bottlenecks in the rivers, immediately downstream of the spawning areas, which makes sampling easier and ensures that specimens with ripe gonads will be available. Both rivers should be sampled because data indicate there may be two distinct (genetic?) "stocks" which spawn in the two locations (Whipple et al., 1983). The Sacramento River "stock" appears to contain a different pollutant profile qualitatively and quantitatively, and exhibits different types and degrees of physiological impairments than the San Joaquin River "stock." Sampling from only one area may bias the results. Also, both rivers provide input into the Bay-Delta and should be represented.

Mature females, (as a guide, \geq 50 cm. standard length), provide more information relating to pollutant exposure and reproductive potential than males; however, if more funds become available, males should also be evaluated since they demonstrate pollutant-related impairments that are somewhat different than females (Whipple et al., 1983).

To provide an acceptable evaluation of the species health, we recommend that a minimum of 20 females be collected at each location, and that the sampling extend over the two month period to ensure an adequate temporal representation of the spawning run

and to correct for exposure to episodic pollution (i.e., spills) in the migration path. We suggest that four females be sampled per week for five weeks during April and May at each site.

Upon collection, fish should be placed on ice, returned to the laboratory and analyzed as soon as possible. Ancillary data such as temperature, salinity (conductivity), dissolved oxygen and Secchi depth should be recorded at each collection.

In the laboratory, each fish will be autopsied and subsampled according to procedures detailed in the striped bass autopsy manual. Briefly stated, each fish will be systematically examined, dissected and tissues subsampled for parameters that characterize each specimen and have been determined by integrated COSBS field and laboratory studies to be associated with pollutant exposure (Whipple et al., 1983). The recommended measurements also take into consideration time, manpower, expertise, and complexity constraints. These analyses are divided between biological measurements and pollutant analyses.

The biological measurements include:

- (1) External examination and meristics -- age (via scales), length, weight, body condition, color pattern, parasites, deformities, abnormalities, etc.

- (2) Internal examination and morphology -- (a) Liver: size, weight, color, LSI(Liver somatic index); (b) Ovaries: size, weight, eggs/gram, fecundity, egg color, GSI (gonadosomatic index), egg stage, ovary maturity stage, histology (including degree of egg resorption) and (c) Other: mesenteric fat, skeletal deformities.
- (3) Internal parasites and parasitic damage -- roundworm and tapeworm larvae, tapeworm lesions, parasite locations, severity of parasite damage (adhesions, necrosis, scars), host reaction to parasites.

Factor analysis of data from field specimens revealed a correlation between extent of parasite damage and host reactions with the concentration of pollutants, primarily monocyclic aromatic hydrocarbons (MAH) in tissues (Whipple et al., 1983; Whipple et al., in prep.). The cestode larva (Lacistorhynchus tenuis) is a common parasite in fish; however, Bay-Delta striped bass are heavily infested and exhibit a unique, severe host reaction (immune response) (Moser et al., 1984). This response, coupled with the parasite damage, may produce extensive morphological and physiological trauma to liver, gonad and other tissues.

Recommendations for the types and extent of pollutant analyses are based on several factors: correlations between the contaminants and reduced physiological and reproductive conditions determined by data analyses from field specimens; laboratory exposure studies; analytical time and complexity, and cost. Although other contaminants which have not been as extensively measured or evaluated toxicologically may be contributing to the reduced health of the species, we believe that the most significant classes of pollutants impacting physiological and reproductive processes, or reflecting the presence and effects of other contaminants, are the MAHs (benzene, xylene isomers, ethylbenzene, toluene) and the alicyclic hexanes (cyclohexane, methylcyclohexane, dimethylcyclohexane isomers) (Whipple et al., in prep.). Perhaps of lesser significance, but thought to contribute to physiological decline and found in high concentrations in some specimens, are PCBs (Whipple et al., 1983) and certain heavy metals including copper, zinc, mercury, cadmium and chromium (Whipple et al., in prep.).

We recommend MAH and alicyclic hexanes be measured firstly in liver tissue. Although there is a relationship between reproductive condition and concentration of these compounds in ovaries, a stronger association exists between reproductive fitness and liver concentrations. Also, MAH and alicyclic hexanes

analyses are simpler and less affected by analytical interferences in liver. If sufficient funds are available, after these analyses are completed, the next priority is heavy metal analysis. Pollutant analyses procedures are detailed in previous sections of this manual.

IV. ACTION LEVELS

Establishing reasonable action levels for environmental quality assessment is a formidable task that almost always requires subjective decisions and diligent review as new information is acquired. Accordingly, the following are suggested as initial criteria upon which regulatory action is warranted, and are based on the best judgment of COSBS personnel:

- (1) Whenever more than 5% of the mature, prespawning female striped bass sampled exhibit resorption or damage of the entire ovary. Factor analysis of data from Bay-Delta striped bass revealed a strong association between the extent of ovary and/or egg resorption and the concentration of pollutants in ovary and liver tissues.

- (2) Likewise, whenever more than 5% of all the eggs from all the mature prespawning females sampled, excluding those with complete ovary resorption, are resorbing.
- (3) Whenever there is a 20% or greater differential between the percentage of fish sampled with open, unhealed, cestode larvae-induced external lesions and fish with healed, external lesions (Moser and Sakanari, in prep.). An example of this case would be when 10% of the fish sampled have healed lesions and 30% of the fish have open lesions. The COSBS study determined conclusively that the "red sores" and lesions exposing the peritoneal cavity are produced in response to cestode larvae infestation. Furthermore, data analysis revealed a strong correlation between the extent of parasite infestation and host reaction with the pollutant body burden (Whipple et al., in prep.).
- (4) Whenever more than 10% of the liver tissue samples subjected to pollutant analysis contain contaminants exceeding National Academy of Sciences (NAS) (1973) maxima for aquatic life, or FDA (1981) Action Levels. (For comparison to FDA Action Levels, pollutant concentrations are converted to estimated concentrations in muscle using appropriate tissue differential ratio.)

- (5) Whenever there is a failure to procure at least 20 mature, prespawning females at either location that can not be explained by lack of effort or climatological factors.

V. RECOMMENDED ACTION BY REGULATORY AGENCIES

Depending upon which factor or factors "send up the red flag", the following regulatory actions may be appropriate and are recommended:

- (1) When FDA Action Levels are exceeded, human and/or animal health advisories should be announced. Human health advisories concerning mercury contamination and roundworm infestation (Anasakis sp.), already in place, should be maintained until these conditions no longer exist.
- (2) When FDA Action Levels or NAS guidelines for aquatic life are exceeded, vigorous efforts should be made to determine the source and extent of the contamination, and to mitigate its occurrence.
- (3) When the cause of the red flag is unknown, but suspected to be pollutant-related, a research project should be initiated to determine the causative agent and the extent of impact to the ecosystem. This may include intensive pollutant analysis of the archived samples.

- (4) When the causative agent (pollutant) is identified, regulatory action should follow to control or eliminate the agent.

VI. ADDITIONAL RESEARCH/RECOMMENDATIONS

The decline in the abundance and condition of striped bass in the San Francisco Bay-Delta community warrants the establishment of a concerted, multi-disciplinary research effort to study the problem. The study should be maintained until the primary contributory factors, pollutant-related and otherwise, are definitively resolved and corrected. We also recommend support for further research to possibly refine or revise the monitoring procedure and data analysis. The proposed monitoring program will provide a component of the assessment of water quality; however, it is not the only or ultimate program.

VII. DATA ANALYSIS

All data will be entered on data sheets provided in the autopsy manual. These data should be entered as the fish are examined, when appropriate. Data from calculations of derived variables (e.g., GSI, LSI) and from subsequent analyses (e.g., pollutant analyses, histological analyses, etc.) should be entered on the data sheets as soon as available. These data sheets have been designed for direct transposition onto computer

cards, computerized data files, or whatever means are used for entry into the computer. Prior to, or within, computerized statistical analyses (i.e., multivariate analysis, discriminant function analysis, etc.) the means and standard errors will be calculated and recorded for all variables from individual sampling sites as well as for both areas combined.

The cost for data analyses will depend upon which hardware and software are used. If a macrocomputer such as the one at the Lawrence Berkeley Laboratory is used, computer analytical costs will be minimal. Final recommendations for data analytic procedures are in preparation and will be provided to the California State Water Resources Control Board when completed.

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APPENDIX I-- VARIABLE LIST FOR FACTOR ANALYSES #3: MONITORING

A). AUTOPSY MEASUREMENTS

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
EXPERIMENT NO.	ALL
LOCATION	ALL
METHOD OF CAPTURE	ALL
ENVIRONMENTAL DATA:	
FISH NUMBER	ALL
DAY	1
MEAN TEMPERATURE	1
MEAN SALINITY	1
MEASUREMENTS & MERISTICS:	
AGE	2
FORK LENGTH	2
STANDARD LENGTH	2
DEPTH	2
WET WEIGHT FISH	2
CONDITION (KFL) *	2
CONDITION (KSL) *	2
CONDITION (K3) *	2

*CALCULATED: $KFL = (\text{wet weight fish} / \text{fork length}) \times 100$; $KSL = (\text{wet weight fish} / \text{standard length}) \times 100$; $K3 = (\text{wet weight fish} - \text{wet weight viscera}) / \text{standard length} \times 100$

APPENDIX I--(CONT.)

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
COLOR PATTERN:	
DORSAL LEFT	3
VENTRAL LEFT	3
DORSAL RIGHT	3
VENTRAL RIGHT	3
TOTAL LEFT	3
TOTAL RIGHT	3
TOTAL DORSAL	3
TOTAL VENTRAL	3
TOTAL BREAKAGE-ALL	3
GONADS: MEASUREMENTS	
SEX	4
TOTAL LENGTH-LEFT	4
WIDTH-LEFT	4
TOTAL LENGTH-RIGHT	4
WIDTH-RIGHT	4
WET WEIGHT-LEFT	4
WET WEIGHT-RIGHT	4
TOTAL WET WEIGHT	4
EGGS PER GRAM	4

APPENDIX I-- (CONT.)

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
GONADS: MEASUREMENTS (CONT.)	
FECUNDITY	4
COLOR HUE-EGGS	4
COLOR VALUE-EGGS	4
COLOR CHROMA-EGGS	4
EGG COLOR RANK 2	4
GONADOSOMATIC INDEX (GSI ₁) *	4
GONADOSOMATIC INDEX (GSI ₂) *	4
LIVER: MEASUREMENTS	
TOTAL LENGTH	5
WIDTH	5
WET WEIGHT	5
COLOR HUE	5
COLOR VALUE	5
COLOR CHROMA	5
LIVER COLOR RANK 2	5
LIVER SOMATIC INDEX (LSI ₁) *	5
LIVER SOMATIC INDEX (LSI ₂) *	5

* CALCULATED: $GSI_1 = (\text{wet weight gonads} / \text{Total wet weight fish}) \times 100$;
 $GSI_2 = \text{wet weight gonads} / (\text{Total wet weight fish} - \text{wet weight viscera}) \times 100$;
 $LSI_1 = (\text{wet weight liver} / \text{Total wet weight fish}) \times 100$; $LSI_2 =$
 $\text{wet weight liver} / (\text{Total wet weight} - \text{wet weight viscera}) \times 100$

APPENDIX I--(CONT.)

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
PARASITES:	
ROUNDWORM LARVAE	6
TAPEWORM LARVAE	6
TAPEWORM LESIONS	6
TOTAL LOCATIONS	6
TOTAL TYPES	6
TOTAL SEVERITY	6
TOTAL HOST REACTION	6
SEVERITY SKELETAL ABNORMALITIES:	
GILL RAKER, FIN RAYS	6
HEAD BONES	6
VERTEBRAL COLUMN	6
OTHER ORGANS: MEASUREMENTS	
RANK MESENTERIC FAT	8

APPENDIX I--(CONT.)

B.) SAMPLE MEASUREMENTS

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
HEAVY METALS (DRY WEIGHT=DW) :	
SAMPLES ARCHIVED	10
PETROLEUM HYDROCARBONS-**	
MONOCYCLIC AROMATICS,	
ALICYCLIC HEXANES	12
(LIVER AND GONAD) :	
BENZENE	12
MONOCYCLICS	12
TOTAL MONOCYCLICS	12
TOTAL CYCLOHEXANES	12
TOTAL HYDROCARBONS	12
CYCLOHEXANE	12
METHYLCYCLOHEXANE	12
1, -DIMETHYLCYCLOHEXANE	12
1, -DMCH2	12
1, -DMCH3	12
1, -DMCH4	12
1, -DMCH5	12
TOLUENE	12

**ALL PETROLEUM COMPOUNDS IN PPM WET WEIGHT.

APPENDIX I--(CONT.)

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
ETHYLBENZENE	12
<u>p</u> -xylene	12
<u>m</u> -xylene	12
<u>o</u> -xylene	12
CHLORINATED HYDROCARBONS - PCBS:	
SAMPLES ARCHIVED	13
CHLORINATED HYDROCARBONS - DDE, DDD, DDT:	
SAMPLES ARCHIVED	14
PETROLEUM HYDROCARBONS - POLYCYCLIC AROMATICS:	
SAMPLES ARCHIVED	15
LIVER: HISTOLOGY:	
SAMPLES ARCHIVED	35-36
TESTES: HISTOLOGY:	
SAMPLES ARCHIVED	37

APPENDIX I--(CONT.)

<u>VARIABLE NAME</u>	<u>DATA SET NUMBER</u>
OVARIES- HISTOLOGY:	
GONAD MATURITY	38
STAGE EGG	38
MEAN MAX DIAMETER	38
% FOLLICLE CELLS	39
% PERINUCLEOLUS	39
% YOLK VESICLE	39
% PRIMARY YOLK	39
% SECONDARY YOLK	39
% TERTIARY YOLK	39
% MIGRATORY NUCLEUS	39
% EARLY COALESCENCE	39
% LATE COALESCENCE	39
% EGGS RESORBED	39
% EMPTY FOLLICLES	39

APPENDIX II--CODING FOR DATA SETS

Coding for All Data Sets

EXPERIMENT

(Field)

1 = 1978	5 = 1982
2 = 1979	6 = 1983
3 = 1980	7 = 1984
4 = 1981	

LOCATION

1 = San Francisco Bay, off Tiburon
*2 = San Joaquin River, off Antioch
3 = Sacramento River, off Knights Landing
4 = Sacramento River, off Clarksburg
*5 = Sacramento River, off Clarksburg - Traps
6 = Chipps Island
7 = Carquinez Strait, Mothball Fleet
8 = Carquinez Strait, Martinez
9 = San Joaquin River, off Antioch
10 = San Pablo Bay, Corte Madera Creek
11 = Umpqua/Smith River(s), OR

*Main CDF&G sampling areas

APPENDIX II--(CONT.)

- 12 = Coos River, OR
- 13 = Lake Mead, NV
- 14 = Cherokee Reservoir, TN
- 15 = Montezuma Slough
- 16 = Spawned Elk Grove, reared at Steinhart Aquarium and Tiburon Laboratory (Exp. 18-20)
- 17 = San Pablo Bay, Crockett to Rodeo
- 18 = Gill-netted off Tiburon Pier, held 6 months at Tiburon Laboratory (Exp. 15)
- 19 = Suisun Bay
- 20 = West San Pablo Bay
- 21 = Spawned Elk Grove, reared at Tiburon Laboratory by Eldridge (Exp. 3 - 5)
- 22 = Hudson River, NY
- 23 = All over San Francisco Bay-Delta (Exp. 61)

METHOD (of Capture)

- | | |
|----------------------|----------------------------|
| 1 = Gill net | 6 = Hook and line |
| 2 = Shocker | 7 = Dip net |
| 3 = Diversion screen | 8 = Mixed methods |
| 4 = Traps | 9 = Combined methods above |
| 5 = Trawl | |

APPENDIX II--(CONT.)

Coding for Data Set 2

DISSECTION DATE = Month/day

DISSECTION DAY = Julian; refer to perpetual calendar

DISSECTION TIME = beginning of autopsy; 24-hr clock

SEX

1 = Female

4 = Predominantly male

2 = Male

hermaphrodite

3 = Predominantly female

hermaphrodite

Coding for Data Set 3

COL PAT INT (Color pattern intensity: dorsal melanization)

1 = Light

2 = Medium

3 = Dark

STRIPING PATTERN BREAKAGE

1 = Not broken

2 = Broken

SIDE

1 = Left

2 = Right

APPENDIX II--(CONT.)

Coding for Data Set 4

SEX

1 = Female	4 = Predominantly male
2 = Male	hermaphrodite
3 = Predominantly female	
hermaphrodite	

GND (Gonad)

1 = Left	2 = Right
----------	-----------

MTR (Maturity)

1 = Immature	5 = Resting (recovering)
2 = Maturing	
3 = Spawning, ripe-running	6 = Resorbing (abnormal)
4 = Spent	

EGG COLOR RANK

<u>Munsell Hue =</u>	<u>I</u>	<u>II</u>
2.5 R	1	1
5.0 R	1	2
7.5 R	1	3
10.0 R	1	4

<u>Munsell Hue =</u>	<u>I</u>	<u>II</u>
2.5 YR	2	5
5.0 YR	2	6
7.5 YR	2	7
10.0 YR	2	8

APPENDIX II--(CONT.)

Coding for Data Set 4 (cont.)

EGG COLOR RANK

<u>Munsell Hue</u> =	<u>I</u>	<u>II</u>	<u>Munsell Hue</u> =	<u>I</u>	<u>II</u>
2.5 Y	3	9	2.5 GY	4	13
5.0 Y	3	10	5.0 GY	4	14
7.5 Y	3	11	7.5 GY	4	15
10.0 Y	3	12	10.0 GY	4	16

Coding for Data Set 5

LIVER COLOR HUE I

- | | |
|----------------------------------|------------------|
| 1 = Deep red | 4 = Yellow |
| 2 = Deep red with yellow patches | 5 = White-yellow |
| 3 = Yellow with red patches | |

STOMACH CNT (Contents)

- | | |
|-----------|----------|
| 1 = Empty | 2 = Full |
|-----------|----------|

STOMACH CNT TYPE

Normally empty during prespawning stage. (Codes not developed, but can be added.)

APPENDIX II--(CONT.)

Coding for Data Set 6

DISSECTION DATE = Month/day

DISSECTION TIME = Beginning of autopsy, 24-hr clock

DISEASE/PARASITE LOC (Location)

Head

- 1 = Snout
- 2 = Mouth and jaw
- 3 = Operculum
- 4 = Upper jaw
- 5 = Lower jaw
- 6 = Skull bones
- 7 = Gill rakers
- 8 = Gills
- 9 = Eyes

Body

- 10 = General on external body,
on all fins
- 11 = Dorsal fins
- 12 = Pectoral fin
- 13 = Pelvic fin
- 14 = Anal fin
- 15 = Caudal fin
- 16 = External body musculature
- 17 = External body near vent
- 18 = Internal body musculature
- 20 = Body cavity
- 21 = Vent

APPENDIX II (CONT.)

Coding for Data Set 6 (cont.)

Organs

- | | |
|--|----------------------|
| 22 = Heart | |
| 23 = Kidney | 32 = Swim Bladder |
| 24 = Spleen | 33 = Adrenal Glands |
| 25 = Gallbladder | 34 = Colon, rectum |
| 26 = Intestinal mesenteries,
fat, or fascia | 35 = Urinary bladder |
| 27 = Intestine | 36 = Peritoneum |
| 28 = Stomach | 37 = Pyloric Cecae |
| 29 = Gonad | |
| 30 = Liver | |
| 31 = Brain | |

Tissues

- | |
|-------------|
| 38 = Muscle |
| 39 = Blood |
| 40 = Bile |

DISEASE/PARASITE TYPE*

- | | |
|---|---|
| 1 = None | 5 = Copepods |
| 2 = Effects probably due to
handling, net damage, gaff,
hooks, etc. | **6 = Roundworm larvae |
| 3 = Blisters (usually inside
operculum, mouth) | **7 = Cestode larvae, small
brown cysts |
| 4 = Isopods | 8 = Roundworm cysts, hyaline
brown "rafts" |

*Refer to Dr. Michael Moser, University of California-Santa Cruz,
for questions about parasites; ** Most commonly occurring.

APPENDIX II (CONT.)

Coding for Data Set 6 (cont.)

- | | |
|---|--|
| 9 = Acanthocephalans, knobs
in gut (Hudson River fish) | 16 = Fin rot |
| 10 = Lamprey wound | 17 = Cirrhosis of liver(?) |
| 11 = Seal or bird bite/wound | 18 = Effect probably due to
benzene exposure |
| 12 = Possible heavy metal
effect | 19 = Papillomas |
| 13 = Suspected bacterial or
viral infection | **20 = Cestode raft (often causes
lesions) |
| 14 = Tumor-unspecified cause | 21 = Adult nematode |
| 15 = Unknown cause: | 22 = "Pepper spots," dead eggs,
individual sacs |
| a. Heart fat | 23 = Roundworms in Oregon fish |
| b. Abnormal ovaries | 24 = Roundworm cysts in Oregon
fish |
| c. Internal hemorrhaging | 25 = Rafts, unknown origin,
in Oregon fish |
| d. Abnormal intestinal
waste | 26 = Monogenetic trematode |
| e. Wounds | 27 = Larval digenetic trematode |
| f. Clear lumps or eggs | 28 = Leech |
| g. Opacity | 29 = Experimental puncture |
| h. Abnormal shape or size | 30 = Missing part |
| i. Abnormal color | 31 = <u>Lymphocystis</u> |
| j. Sacs of dead eggs, etc. | 32 = Adult digenetic trematode |
| k. Abnormal texture | 33 = Clam larva (glochidia) |

** Most Commonly Occuring

APPENDIX II--(CONT.)

Coding for Data Set 6 (cont.)

DISEASE/ PARASITE SEV/ABND (Severity/Abundance)

- | | |
|-------------------|------------------------|
| 1 = None | 4 = Many |
| 2 = Few or slight | 5 = Very many or heavy |
| 3 = Average | |

DISEASE/PARASITE HOST RCTN (Reaction)

- | | |
|----------------------------------|--|
| 1 = No reaction obvious | 18 = Pressure necrosis |
| 2 = External hemorrhaging | 19 = Visceral adhesions |
| 3 = Healed external wound | 20 = Fatty; white or yellow |
| 4 = Open wound | 21 = Generalized necrosis
(internal organs) |
| 5 = Excess mucus | 22 = Black/brownish/white spots |
| 6 = Clear fluid | 23a = Not developed |
| 7 = Clouding | 23b = Underdeveloped |
| 8 = Tissue soft | 23c = Abnormal shape |
| 9 = Bright red | 24 = Dead eggs |
| 10 = Reddened | 25 = Abnormal tissue growth |
| 11 = White, pale or no color | 26 = Constriction |
| 12 = Greenish or yellowish fluid | 27 = Cracking |
| 13 = Tissue hardened | 28 = Irregular scale pattern |
| 14 = Abnormally green | |
| 15 = Tissue erosion | |
| 16 = Reddened sores | |
| 17 = Hemorrhaging | |

APPENDIX II--(CONT) .

Coding for Data Set 6 (cont.)

ABNORMALITY LOC (Location) = same as for DISEASE/PARASITE LOC

ABNORMALITY TYPE-(Skeletal only)

- | | |
|----------------------------------|---------------------------|
| 1 = Abnormal coloration | 5 = Pugheaded |
| 2 = Enlarged size | 6 = Scoliosis or lordosis |
| 3 = Reduced size | |
| 4 = General skeletal abnormality | |

ABNORMALITY SEV/ABND-(Severity/Abundance)

- | | |
|-------------|-----------------|
| 1 = None | 4 = Severe |
| 2 = Slight | 5 = Very severe |
| 3 = Average | |

Coding for Data Set 8

MESENTERIC FAT

- | | |
|----------------------|--------------|
| 1 = None | 3 = Average |
| 2 = Slight or sparse | 4 = Abundant |

INTESTINE

- | | |
|-----------|----------|
| 1 = Empty | 2 = Full |
|-----------|----------|

APPENDIX II--(CONT.)

Coding for Data Set 12

TISSUE ID:

1 = Gonad 3 = Blood
2 = Liver 4 = Muscle

COMPOUND ID:

5	Cyclohexane	40	1,2 DMCH
10	Methylcyclohexane (MCH)	50	Toluene
20	Benzene	60	Ethylbenzene
32	<u>Trans</u> 1,4 & 1,1 DMCH*	70	<u>p</u> -xylene
33	<u>Cis</u> 1,4 DMCH	80	<u>m</u> -xylene
35	1,4 DMCH	90	<u>o</u> -xylene

Totals:

350 Total MCH-Tissue
360 Total CH-tissue
370 Total MCH & CH-tissue
380 Total MCH-all tissues
390 Total CH-all tissues
400 Total MCH & CH-all tissues

*DMCH = dimethylcyclohexane

APPENDIX II--(CONT.)

Coding for Data Set 22

SEX

1 = Female	4 = Predominantly male
2 = Male	hermaphrodite
3 = Predominantly female	
hermaphrodite	

GONAD

1 = Left	2 = Right
----------	-----------

MATUR(ity)

1 = Immature	5 = Resting, (recovering)
2 = Maturing	6 = Resorbing (abnormal)
3 = Spawning, ripe-running	
4 = Spent	

SPERM MOTIL(ity)

1 = Immotile	2 = Motile
--------------	------------

MEAN FRESH

(See Text, ignore columns for preserved eggs).

APPENDIX II--(CONT.)

Coding for Data Set 38:

MAGNIFICATION: At which eggs were microscopically evaluated.

MICROMETER: Conversion factor for ocular micrometer determined from stage micrometer.

AREA SCANNED/AREA SUBSAMPLED: Approximate area in square mm.

AGE: Ages from Data Set 2.

MAT: Ovary maturity stage(1-6). See Appendix Table A.

RANK EGG COLOR: Values in Coding for Data Set 4.

COND: Slide condition. 1= Very good; 2= Average; 3=Poor; 4=Terrible

AGE TISS: Hours from capture to preservation of ovarian subsample.

NO. EGGS EXAM.: Number of eggs at max. maturation stage examined.

EGG STG: See Appendix Table A.

MEAN EGG DIAM: Mean diameter of 5 eggs at maximum stage and size.

STAGE W/ VAC: Egg stage that vacuoles are observed.

RANK OIL: Rank amount of oil in eggs:

1= lower than average; 2= average; 3= higher than average; 4= abnormally large amount. Ranks 3 and 4 indicate egg resorption.

Oil is bright red from Oil Red-O lipid stain.

STAGE RSRBD: Egg stage of resorbing eggs.

DLY?/NCRT?: First space for delayed code; second for necrotic code:

1=No; 2= Yes.

BOX/SLIDE: Box and slide box number.

PHOTO: Number of roll and exposure number.

APPENDIX III --Preparation of Buffered Formalin
(For Preservation of Eggs and Testes)

Add 0.340 g potassium phosphate, monobasic (KH_2PO_4) and 0.355 g sodium phosphate, anhydrous, dibasic (Na_2HPO_4) per 100 ml formalin.

Check pH and adjust to 7.0 if necessary with a few drops of 10% HCl or 1N NaOH.

APPENDIX IV--

Glassware Cleaning Procedure for Trace Metal Analyses

1. Rinse in Milli-Q (or distilled/deionized) water.
2. Place in Micro Bath solution for 24 hours.
3. Rinse well with tap water (3 times).
4. Rinse with Milli-Q water.
5. Soak in 6N HCl for at least 24 hours.
6. Rinse 5 times with Milli-Q water.
7. Soak in 2N HNO₃ for at least 24 hours.
8. Rinse three times with Milli-Q water.
9. Dry in oven at about 50 deg C (not >70 deg C).
10. Store in dust-free area wrapped in assembly wipes.

Reference: G. A. Knauer, Moss Landing Marine Laboratory (personal communication).

APPENDIX V

Preparation of Clean Foil for PCB Samples

Perform the following under a laboratory hood.

1. Use nanograde hexane to rinse a metal pan. Put the used hexane in a container marked "used hexane".
2. Cut pieces of heavy-duty aluminum foil into pieces approximately 8" by 10". Handle the foil with rinsed metal tongs.
3. Dip the foil in the rinsed metal pan that contains fresh nanograde hexane.
4. Allow the rinsed foil to drip-dry in a covered glass cylinder.
5. NOTE: A quality control measure of PCB's can be done on the used hexane.

Reference: Dr. Donald Gadbois, NMFS, Gloucester, Mass.

Modified by Dr. Gerald Bowes, State Water Resources Control Board, Sacramento, CA. (personal communication).

APPENDIX VI

Trace Metal Analysis of Fish Liver

1. Precleaning procedure
 - A. Clean all glassware, plasticware, etc., prior to sample preparation as per written procedure.
2. Sample preparation
 - A. Weigh frozen liver sample on tared piece of weighing paper. Record as sample wet weight.
 - B. Homogenize tissue in Potter-Elvehjem homogenizer for three minutes, or until homogeneous, whichever occurs first. NOTE: May have to add a small volume (less than one ml) of Milli-Q or distilled/deionized water (dd water).
 - C. Quantitatively transfer to labelled, preweighed 30-ml beaker by rinsing residue from homogenizer (including pestle) with about 1 to 2 ml Milli-Q or dd water.
 1. Cover with watch glass.
 2. Place in oven at 70 deg C for 72 hours.
 3. Weigh beaker plus dried sample.
 4. After correcting for beaker weight, record as sample dry weight.
 - D. Under the hood, add 5 ml concentrated HNO_3 ; cover with watch glass.
 - E. Under hood, place beakers on hot plate, bring to slow boil carefully, and reflux for 3 hours.

APPENDIX VI--(CONT.)

- F. Reduce heat to below boiling and take samples to dryness.
 - G. Place in muffle furnace at 400 deg C overnight; let cool to room temperature.
 - H. Dissolve in 5 ml concentrated HNO₃.
 - I. Add dropwise H₂O₂ until no further oxidation is observed. There should be no darkening of solution or residue at this point. If there is, bring solution to a slow boil again until solution is clear, or slightly yellow.
 - J. Heat solution to just below boiling; slowly evaporate until residue is only slightly moist.
 - K. Allow to cool.
 - L. Add 20 ml 1% HNO₃.
 - M. Transfer to 25-ml volumetric flask.
 - N. Make up to volume with 1% HNO₃.
 - O. If analyzing same day, can aspirate directly from volumetric; if analyzing later, transfer into acid-cleaned conventional polyethylene vial.
3. Sample preparation blanks
- A. For each analytical batch of sample, prepare duplicate blanks according to sample preparation procedure, except no tissue placed in homogenizer or beakers.

APPENDIX VI (Cont.)

4. Zinc Standards (as example of a trace metal).
 - A. Prepare stock solution of zinc at a concentration of 500 ppm Zn^{++} , freeze in conventional polyethylene, Teflon or quartz vials in 1.0 ml aliquots.
 - B. Take frozen stock solution of zinc from freezer and let thaw. $Zn^{++} = 500$ ppm.
 - C. Add 0.800 ml stock solution to 200-ml volumetric flask, make to 200 ml with 1% (v/v) HNO_3 , mix thoroughly. $Zn^{++} = 2$ ppm.
 - D. Take 25.0 ml of 2 ppm Zn^{++} standard, place in 50-ml volumetric flask, make to 50 ml with 1% HNO_3 . $Zn^{++} = 1.0$ ppm.
 - E. Take 12.5 ml of 2 ppm zinc standard, place in 50-ml volumetric flask, make to 50 ml with 1% HNO_3 . $Zn^{++} = 0.5$ ppm.
 - F. Take 6.25 ml of 2 ppm zinc standard, place in 50 ml volumetric flask, make to 50 ml with 1% HNO_3 . $Zn^{++} = 0.25$ ppm.
 - G. Have 0.5 to 1.0 liters 1% HNO_3 available for standard blank determinations.

APPENDIX VI (Cont.)

Reference: Stephenson, M. D., M. Martin, S. E. Lange, A. R. Flegal and J. H. Martin. California mussel watch 1977-1978. Volume II: Trace metal concentrations in the California mussel, Mytilus californianus. Water quality monitoring report No. 79-22. State Water Resources Control Board, Sacramento, CA. November 1979.

Procedure modified by Dr. R. Bruce MacFarlane, NMFS, Tiburon, CA.

APPENDIX VII--

Processing Tissue Samples for Mono- and Polycyclic Aromatic
Hydrocarbon Analyses by Gas Chromatography

Monocyclics

1. Put 10 g tissue (wet weight) on piece of B-2 weighing paper.
2. Cut the tissue into 1 cm. pieces and place into a 20 x 150 mm screw-cap culture tube with a Teflon liner (33 ml capacity).
3. Add 6 ml of 4N NaOH (0.6 ml per gram sample).
4. Add 4 ml of TF Freon that contains a recovery standard of 10 ppm nonane (0.4 ml/gram sample).
5. Tightly cap tube, shake and place in oven or waterbath for 18 hours at 35 deg C (make sure NaOH has penetrated all of the sample).
6. Remove from oven and shake tube for 1 minute.
7. Centrifuge at 2000 rpm for 15 min.
8. Freeze sample for at least 30 minutes.
9. Recentrifuge at 2000 rpm for 15 minutes.
10. Transfer clear Freon layer to a screw-cap vial. If not clear, add dilute H_2SO_4 and recentrifuge.
11. Measure Freon in test tube at room temperature; mark level.
12. Sample is ready for injection. If you cannot inject immediately, freeze samples.

APPENDIX VII.--(CONT.)

Dicyclics or Polycyclics

1. Follow steps 1, 2 and 3 of monocyclics procedures.
2. Place in oven for 18 hours at 35 deg C.
3. Remove tube from oven and cool.
4. Add 15 ml peroxide-free diethyl ether.
5. Recap tube, and shake for 1 minute.
6. Centrifuge at 3000 rpm for 10 minutes.
7. Transfer the ether layer to a 25-ml concentrator tube. Add 10 ml of peroxide-free diethyl ether and repeat steps 5 and 6.

NOTE: If a large emulsion layer forms, try freezing sample and recentrifuging as in the monocyclic procedure.

8. Combine the 15 -ml and 10-ml extracts in the concentrator tube.
9. Concentrate to 2 ml in the Kontes tube heater. Then add 2 ml of hexanes and reconcentrate to 1 ml.

Data Entry: See Appendix II and use Appendix Fig. H.

Reference: Nunes, Pepsi and Pete E. Benville. 1978. Acute toxicity of the water-soluble fraction of Cook Inlet crude oil to the Manila clam. Mar. Poll. Bull. 9:324-331.

Warner, J. S. 1976. Determination of aliphatic and aromatic hydrocarbons in marine organisms. Anal. Chem. 48:578-583.

Procedures modified by Pete E. Benville, NMFS, Tiburon, CA.

APPENDIX VIII

Procedures for Hematology

1. Obtaining blood specimens
 - A. Heart punctures using vacutainers.
 - 1) Stick the needle (22 gauge 1-1/2") in about 3 or 4 scales down from the anterior isthmus until you feel the heart muscle.
 - 2) For whole blood, use a 4-cc lavender top tube (contains EDTA).
 - 3) For serum, use a 7-cc red-top tube (sterile).
 - B. Heart punctures using a plastic syringe
 - 1) Use a tuberculin or 3-cc syringe with a 23-26 gauge needle (depending on the size of the fish).
 - 2) For whole blood, use a syringe rinsed in 3% EDTA.
 - 3) For serum, do not use EDTA. Transfer blood to a sterile tube. Spin the blood for 15 minutes at 4000 rpm. Use a pipette to separate the serum.
 - C. Caudal tail cuts
 - 1) Cut the caudal tail with a scalpel.
 - 2) Use capillary tubes coated with heparin to determine hematocrits or to obtain plasma.
 - 3) If serum is desired, use noncoated capillary tubes.

APPENDIX VIII.--(CONT.)

- 4) Spin the capillary tubes for 7 minutes in a microcentrifuge.
 - 5) Separate the serum or plasma immediately after centrifuging.
2. Hematocrit determinations
- A. If a lavender vacutainer was used, use the plain capillary tubes; otherwise use the heparinized tubes.
 - B. Fill the tubes, leaving about 0.5 cm of space at one end. Seal the tubes with critoseal.
 - C. Spin the tubes in the microcentrifuge for 7 minutes.
 - D. When determining the hematocrit values, note the buffy coat layer and the cherry-red layer of the tubes.
3. Blood smears
- A. Place a drop of whole blood (from a lavender tube) at one end of a clean slide.
 - B. Place another clean slide at a 45-degree angle to the first slide. Barely touch the drop of blood, allowing the blood to spread across the width of the angled slide. Smoothly and evenly push the blood across the slide.
 - C. Air dry the slide and place in methanol for 3-5 minutes.
 - D. Stain the slide in Wright's stain for 3-10 minutes.
 - E. Place the slide in sodium phosphate buffer solution (pH 6.7) for 3-5 seconds.

APPENDIX VIII.--(CONT.)

- F. Wash the slide in distilled water and blot dry with Whatman filter paper No. 42.
- G. NOTE: The times of the staining procedure will vary with individual fish and with age of the stain.

Reference: Seiverd, Charles E. 1958. Hematology for the medical technologist, second edition. Lea and Febiger, Philadelphia.

Modified by Brian Jarvis, NMFS, Tiburon Laboratory, Tiburon, CA.

APPENDIX IX
Egg Histology

A. PREPARATION

1. SUBBED SLIDES -- Dissolve 1g gelatin in 1 litre hot dH₂O.
Cool and add 0.1g chromium sulfate. Store in refrigerator.
Dip slides several times, drain and dry in vertical position.
Store in dust-free box.
2. GLYCERINE JELLY--Allow 8.0g gelatin to soak 1-2hr in 52 ml
H₂O. Add 50.0 ml glycerine and 0.1g phenol (or merthiolate).
Heat 10-15 min. (not above 75 deg. C). Stir until homogenous.
Store in refrigerator.
3. OIL RED O (C.I. 26125)--
Oil Red O.....0.7g
Absolute Isopropanol.....200ml.
Shake solution well and leave overnight. Filter. Dilute 180 ml of
Oil Red O solution with 120 ml dH₂O. Leave overnight at 4 deg. C.
Filter. Let stand at room temperature 30 min. Filter. Stable for
6-8 months.
4. SCOTT SOLUTION
Sodium Bicarbonate.....2.0g
Magnesium Sulfate.....20.0g
dH₂O.....1000.0 ml
Add a pinch (0.1g) phenol or thymol.

APPENDIX IX.--(CONT.)

B. CRYOSTAT PROCEDURE

1. If cryostat has been "down" for a long time, see instruction manual for routine maintenance.
2. Always do daily maintenance (oiling) on microtome before starting cryostat.
3. Turn machine on and allow chamber to cool to pre-set temperature.
4. While cryostat is cooling, cut formalin preserved ovary and place in water for 10-20 minutes.
5. Cover bottom of cryomold with OTC compound and place drained tissue on top. Cover tissue with OTC compound and place chuck support on top. Place on Cold bar in cryostat.
6. After tissue is frozen, remove cryomold from frozen block and tighten in chuck jaws of microtome.
7. 10-15 sections are placed on subbed slides and allowed to dry.
8. Follow appropriate staining procedure:

APPENDIX IX.--(CONT.)

C. STAINING PROCEDURE

- a.) Mount frozen sections (10-15) on subbed slides. Allow to dry 5-10 minutes.
- b.) Rinse in 60% isopropanol.....30 sec.
- c.) Stain in Oil Red O.....10-min.
- d.) Rinse in 60% isopropanol for a few seconds.
- e.) Wash in running water 2-3 min.
- f.) Stain in Harris Hemotoxylin.....2-3 min.*
- g.) Wash in tap water.....3-min.
- h.) Blue in Scott's Solution.....3 min.
- i.) Wash in tap water.....3 min.
- j.) Mount in glycerine jelly.

D. EGG STAGES (See Appendix Table A.)

E. DATA ENTRY (See Appendix II and use Appendix Figs. I, J & K).

Procedures modified by Michael Bowers, NMFS, Tiburon Laboratory, Tiburon, CA. Staining modified from G.L. Humason, Animal Tissue Techniques, 3rd edition. W.H. Freeman and Company. San Francisco, CA. 641 p.

*Staining times will vary with age of the stains.

APPENDIX TABLE A.--Working codes for Ovary Maturation and Egg Maturation.* For Data Sets 38 and 39 - Histology: Ovaries.
(Appendix Figs. J - K)

OVARY MATURATION		EGG MATURATION	
STAGE		STAGE	
NUMBER	DESCRIPTION	NUMBER	DESCRIPTION
1	Immature Female (never spawned)	1	Follicle cells
		2	Perinucleolus stage Early Late-cytoplasm paler
		3	Yolk vesicle

2	Maturing Female (green eggs, but not yet ready to spawn)	1,2,3 and 4 and 5 or 6	<u>Size and yolk increasing</u> Primary yolk stage Oval Secondary yolk stage Round Tertiary yolk stage Rectangular or irregular

* Procedures for analyses of egg condition developed by Jeannette A. Whipple, NMFS Tiburon Laboratory.

APPENDIX TABLE A.--(Cont.)Codes for Ovary and Egg Maturation.

OVARY MATURATION		EGG MATURATION	
STAGE		STAGE	
NUMBER	DESCRIPTION	NUMBER	DESCRIPTION
3	Late Maturing or Prespawning Female (spawning or near spawning)	1,2,3 and 7 or 8 or 9	Migratory nucleus (irregularly shaped, not in center, can see nucleoli) Fused yolk and oil, coalesced into droplet,polar nucleus (chromatin appears in nucleus ?) Completed fusion of yolk & oil-chromosome of nucleus in metaphase
4	Spent Female	1,2,3 (sometimes 4) and 10 11	Some resorbing eggs (eggs degenerating and being resorbed). Mostly collapsed or empty egg follicles.

APPENDIX TABLE A.--(Cont.)Codes for Ovary and Egg Maturation.

OVARY MATURATION		EGG MATURATION	
STAGE		STAGE	
NUMBER	DESCRIPTION	NUMBER	DESCRIPTION
5	Resting Mature Female or Recovering Ovary (nonspawning season)	1,2,3 and 11 less obvious	See above. Sometimes Stage 3 more developed than in immature fish.
6	Abnormal or Prematurely Resorbing Ovary	1,2,3 and 10	Often abnormal eggs, necrosis and "dark crystals" in eggs. Resorbing eggs are usually stages 4,5,6; rarely 7 or 8

APPENDIX FIGURES

Appendix Figure A.-- Data Set 1: Capture and Field Samples

Appendix Figure B.-- Data Set 2: Meristics and Measurements

Appendix Figure C.-- Data Set 3: Color Pattern

Appendix Figure D.-- Data Set 4: Gonads-Meristics and Fecundity

Appendix Figure E.-- Data Set 5: Liver, Gall Bladder and Stomach

Appendix Figure F.-- Data Set 6: Disease, Parasites and
Abnormalities.

Appendix Figure G.-- Data Set 8: Spleen and Other Organs

Appendix Figure H.-- Data Set 12:Pollutants-Petroleum Hydrocarbons

Appendix Figure I.-- Data Set 22:Gonads-Egg Diameters and Sperm
Motility

Appendix Figure J.-- Data Set 38:Gonads-Histology; Ovaries-Maximum
Maturity

Appendix Figure K.-- Data Set 39:Gonads-Histology; Ovaries
II-Relative Number Stages

(NOTE: See page 4 for instructions on entering data)

STRIPED BASS - POLLUTANTS: PETROLEUM HYDROCARBONS

DATA SET 1 2
 1 2
 EXPERIMENT 3 4 5 6

LOCATION 7 8
 METHOD: 9

FISH NO.	DISSECT	DATE	GC ANALYS	TISSUE AMOUNT		VOLUME		MULTIPL FACTOR	COMP ID	CONCENTRATION (ppm = mg/kg, μ l/l)	NOTES								
				ID (g, ml)	AMOUNT	EXTRACT (ml)	INJ (μ l)												
10-12	13	17	18	22	23	24	25	28	29	33	34	35	36	37	40	41	43	50	
									5										
									1 0										
									2 0										
									3 0										
									3 2										
									3 3										
									3 5										
									4 0										
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									6 0										
									7 0										
									8 0										
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									3 5 0										
									3 6 0										
									3 7 0										
									3 8 0										
									3 9 0										
									4 0 0										

Appendix Figure H.

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