

**JIMAR, PFRP ANNUAL PROGRESS REPORT
FY 2001**

P.I. Name: E. Gordon Grau

Project Proposal Title: 656282 Developing Methods to Assess Sex and Maturation Stage of Bigeye Tuna (*Thunnus obesus*) and Swordfish (*Xiphias gladius*).

Funding Agency: JIMAR

1. Purpose of the project and indicative results.

The central aim of this proposal is to develop accurate, simple to use and economical tests to determine sex and maturational stage of Bigeye Tuna and Swordfish so that populations can be monitored on a regular basis and at low cost. We proposed the following objectives to achieve this goal:

- i) Develop and modify existing biotechnology to identify the sex and maturational status of individual fish in two species: bigeye tuna (*Thunnus obesus*) and swordfish (*Xiphias gladius*). Our approach will focus on developing genetic and immunoassays to detect sex and maturation specific compounds present in blood and muscle tissue samples.*
- ii) Validate the accuracy of this approach by 'ground truthing' the results with the standard method of staging maturity based on histological examination of the gonads.*
- iii) Transfer the new technologies to fishery biologists throughout the Pacific for use in constructing and monitoring maturity schedules for bigeye tuna and swordfish.*

2. Progress during FY 2001. Provide a thorough discussion of accomplishments and problems.

FIELD COLLECTIONS

A major problem encountered during the initial part of FY 2001, and as a carryover from FY 2000, was obtaining high quality blood serum samples needed to validate and establish the radioimmunoassay (RIA) for this project. Our initial attempt to use blood collected from fish previously caught by fishermen and sampled at the pier produced disappointing results. We discovered that high quality serum could only be obtained from fresh animals. Blood collected from animals that were caught more than 24 hours old could not be used for the initial validation of our assays. This eliminated the possibility of obtaining samples from fishermen and forced us to put more time, energy and money into collecting our own fresh fish for sampling. A cooperative agreement was

established with the Medical Foundation for the Study of the Environment (MFSE) and, with the assistance of JIMAR funds, several fishing expeditions were successfully conducted. Fishing locations were selected based on the highest probability of obtaining mature bigeye samples. A summary of our collections and total numbers of fish sampled are shown below in Table I.

Notes: (*) indicates unknown. Yellowfin tuna was added as an additional species to be studied in the project since they were often caught in much higher abundance than Bigeye tuna.

TABLE I.

Bigeye Tuna

Sample #	Date	Size (cm)	Location
1	11-7-00	< 60	C. Seamount
2	11-7-00	73	C. Seamount
3	11-7-00	115	C. Seamount
4	11-7-00	75	C. Seamount
5	11-7-00	57	C. Seamount
6	11-7-00	67	C. Seamount
7	11-7-00	87	C. Seamount
8	11-7-00	84	C. Seamount
9	11-7-00	119	C. Seamount
10	1-30-01	133	Palmyra
11	1-30-01	78	Palmyra
12	1-30-01	96	Palmyra
13	1-30-01	98	Palmyra

Swordfish

Sample #	Date	Sex	Size (cm)	Location
1	04-01-01	M	182	Mau Zone
2	04-02-01	*	87	Mau Zone
3	04-02-01	F	104	Mau Zone
4	04-02-01	*	81	Mau Zone
5	04-02-01	F	131	Mau Zone
6	04-02-01	F	134	Mau Zone
7	04-02-01	F	117	Mau Zone
8	04-02-01	M	103	Mau Zone
9	04-02-01	F	78	Mau Zone
10	04-02-01	*	114	Mau Zone
11	04-02-01	M	155	Mau Zone
12	04-02-01	F	162	Mau Zone
13	04-02-01	*	149	Mau Zone
14	04-02-01	F	114	Mau Zone
15	04-02-01	F	*	Mau Zone
16	04-02-01	F	170	Mau Zone
17	04-02-01	M	155	Mau Zone
18	04-02-01	M	118	Mau Zone
19	04-02-01	M	139	Mau Zone

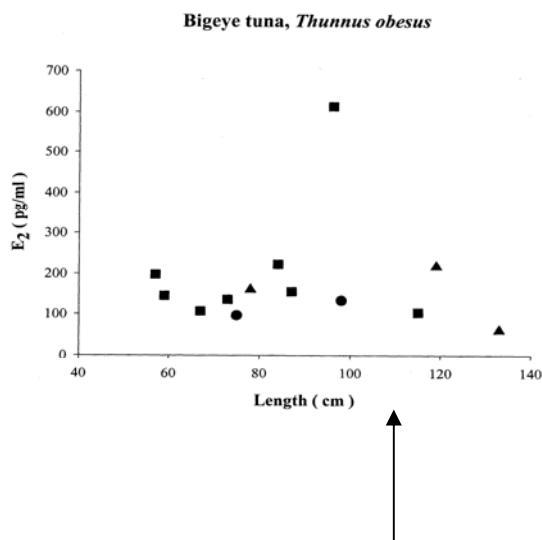
Yellowfin Tuna

Sample #	Date	Sex	Size (cm)	Location
1	11-07-00	F	66	C. Seamount
2	11-07-00	*	77	C. Seamount
3	11-07-00	*	83	C. Seamount
4	11-07-00	*	112	C. Seamount
5	01-30-01	M	130	Palmyra
6	01-30-01	*	71	Palmyra
7	01-30-01	M	85	Palmyra
8	01-30-01	*	76	Palmyra
9	01-30-01	*	88	Palmyra
10	01-30-01	F	116	Palmyra
11	01-30-01	F	104	Palmyra
12	01-30-01	M	83	Palmyra
13	01-30-01	M	125	Palmyra
14	01-30-01	M	132	Palmyra
15	01-30-01	F	130	Palmyra
16	01-30-01	M	113	Palmyra
17	04-11-01	M	*	Big Isle

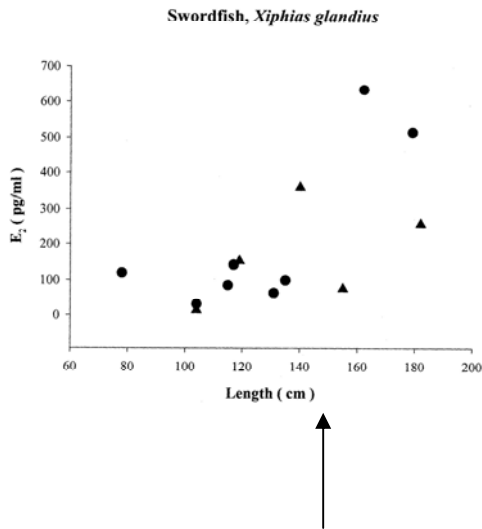
LABORATORY RESULTS

An estradiol radioimmunoassay (E2-RIA) has been optimized and established for all three species, Bigeye tuna, Yellowfin tuna and Swordfish. Representative E2-RIA results are shown below.

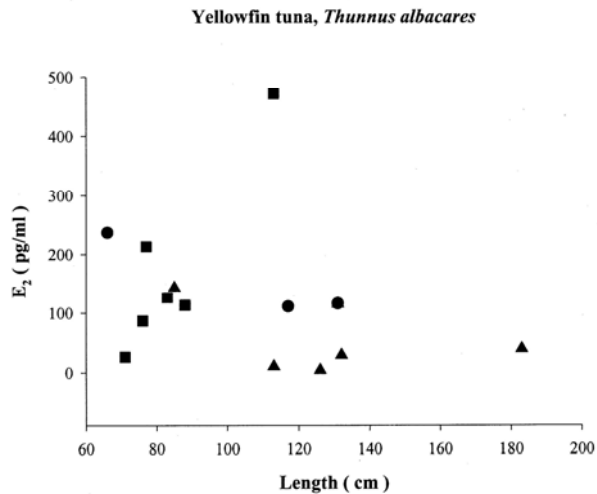
In each graph, levels of estradiol (measured in picograms per ml of serum) are plotted against the length (in centimeters) of each animal sampled. In all graphs, females are represented as closed circles; males are represented as triangles and undetermined sex (juveniles) is represented as closed squares.



Size at maturity for Bigeye tuna is 110cm (D. Itano, personal comm.)



Swordfish size of maturity has been shown to be 150cm.



It remains unclear as to whether estradiol can be used to determine the sex of either Bigeye tuna or Yellowfin tuna, since males express significant levels as compared to females. Additional samples of both mature male and female fish will need to be included before concluding the usefulness of estradiol in determining maturational stage of swordfish, bigeye or yellowfin tuna.

Efforts are ongoing to experimentally induce vitellogenin in juvenile swordfish. The challenge lies in capturing and maintaining juvenile swordfish for the duration of the experimental treatment. This work is being done in collaboration with a research team from Australia and is currently being conducted off the Kona coast of Hawaii. Vitellogenin will be purified from plasma collected from these experimental animals. To date, several animals have been captured and treated experimentally. Purified swordfish

vitellogenin is required to finalize the validation of the swordfish enzyme-linked-immunoassay (ELISA) to screen muscle samples for presence or absence of vitellogenin.

Genomic DNA has been obtained from every fish sampled. Efforts to identify a sex specific DNA marker using a RAPD (randomly amplified polymorphic DNA) approach have not produced any conclusive results thus far. We are currently pursuing genes that have been previously shown to be sex-linked in other vertebrates and remain cautiously optimistic.

3. Plans for the next fiscal year.

Sampling for swordfish, bigeye and yellowfin tuna will continue throughout the upcoming year. Plans are underway to optimize and establish a radioimmunoassay for 11-keto-tetosterone (11-KT), a male specific sex steroid in fish for each species to determine whether androgens will be a reliable indicator of sex in these animals. We anticipate that the swordfish ELISA for vitellogenin will be optimized and all swordfish muscle samples will be screened.