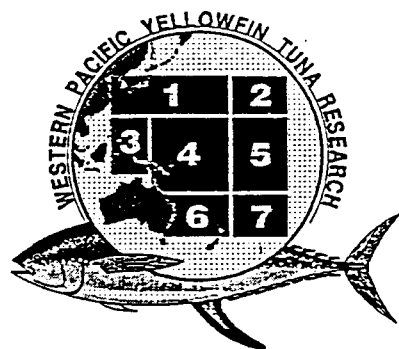


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**Progress report on a large-scale investigation on the  
reproductive biology of yellowfin tuna in the central and western  
Pacific region**

David Itano  
University of Hawaii  
Pelagic Fisheries Research Program  
P.O. Box 1346  
Kaneohe, HI 96744



# Progress report on a large-scale investigation on the reproductive biology of yellowfin tuna in the central and western Pacific region

## INTRODUCTION

The second meeting of the Western Pacific Yellowfin Tuna Research Group (WPYRG2), held in Honolulu, Hawaii in 1992, identified the need for improved reproductive studies on yellowfin to more accurately define and contrast areal, temporal and vertical variations in spawning activity, sex ratio and maturity. During the WPYRG3 meeting held in Pohnpei during 1993, different studies on the reproductive biology of yellowfin tuna having many common goals were described to the meeting and the possibility of combining these projects was discussed and eventually endorsed by the meeting.

The combined project was approved for U.S. Department of Commerce funding under the University of Hawaii administered Pelagic Fisheries Research Program and combines the research capabilities and objectives of Japan's National Research Institute of Far Seas Fisheries (NRIFSF), the South Pacific Commission (SPC), the Micronesian Maritime Authority (MMA), the U.S. National Marine Fisheries Service and the University of Hawaii. In addition to the above mentioned agencies, data and sample collection efforts have been significantly enhanced by the Philippine government Bureau of Fisheries and Aquatic Resources in (BFAR), the Research Institute for Marine Fisheries in Indonesia and the Forum Fisheries Agency.

This investigation essentially began in mid-December 1994 with the hiring of a project coordinator based in Hawaii who maintains communication and sampling coordination among project collaborators at the NRIFSF (Dr. S. Tsuji), SPC (Dr. W.J. Hampton) and the MMA (Mr. C. Heberer).

## OBJECTIVES

The objectives of the study are to:

- (i) define the spatio-temporal and size related patterns in reproductive parameters for yellowfin tuna in the main region of spawning in the equatorial western Pacific and a representative sub-tropical region with seasonal spawning activity (Hawaii) and;
- (ii) to compare the reproductive parameters of fish taken by surface and sub-surface fisheries that may influence interaction between fisheries through temporal variation in vulnerability to different gear types.

## PROCEDURES

The project will run for a two-year period to allow some degree of comparison between years and to provide better information on spawning areas and seasonality. Yellowfin between 50 and 150 cm will be sampled for the study from both surface (troll, purse seine) and sub-surface (deep handline, longline) fisheries. All fish will be identified to sex and measured to fork length with the ovary samples from females being weighed and portion removed for subsequent analysis. Additional environmental and fishery related data will be collected with each samples, e.g. capture location, date, time, school type, school association, capture depth, sea surface temperature, etc.



Whenever possible, ovary sampling will be conducted by observers or fishermen at sea allowing the collection of fresh material and accurate fork length and catch data. Sampling will be conducted during the same time periods from longline, purse seine, troll and handline fisheries from a broad region encompassing the Hawaii Island chain, south to an area ten degrees north and south of the Equator, extending westward to the Philippines and eastern Indonesia. Some port sampling of gonad material will be conducted where single-day or short trip fisheries, i.e. troll and handline, land fresh yellowfin for sashimi markets.

Purse seine samples are being collected by observers of the Forum Fishery Agency on U.S. flag vessels and MMA observers on Japanese, Taiwanese, Korean, FSM and U.S. purse seiners. Longline samples are being collected on Japanese longline training vessels through coordination with the NRIFSF and by MMA observers on Asian longline vessels basing out of Micronesian ports. Handline samples are being collected in the Philippines and Indonesia where large handline fisheries exist for large, sashimi grade yellowfin and bigeye tuna. The main source of ovary samples for Hawaii will be from small scale handline and troll fisheries.

Analysis of spawning frequency will be conducted by histological examination of fresh ovarian tissue preserved in 10% neutral buffered formalin and mounted for viewing with light microscopy. Batch fecundity estimates will be made from a selected subsample of whole ovaries using the hydrated oocyte method. Data useful for the determination of length at 50% maturity, sex ratio by length and spatio-temporal patterns in spawning distribution will be collected at the time of sample collection. All samples and collected data will be entered on a relational database for storage and analysis.

## PROGRESS SUMMARY

The project became active at the start of 1994 with the establishment of a project coordinator with an office and access to a laboratory and telecommunication facilities. A thorough literature review on the subject of tuna reproduction was conducted and planning carried out among the project collaborators. Sampling gear was sourced, purchased, assembled into field sampling kits and shipped to main centers of sample collection in American Samoa, the Federated States of Micronesia, Guam, the Philippines, Indonesia and the main islands of Hawaii.

The Project Coordinator conducted several visits to sampling areas to determine optimal sampling strategies, establish sampling programs and make arrangements for sample storage and shipment to Hawaii. Field observers and fishermen were trained in sample and data collection for the project using sampling materials developed during the site visits. Sampling commenced in April 1994 with samples being shipped to Hawaii for histological preparation by a commercial laboratory. Project collaborators and cooperating agencies have been kept informed as to project status and a thorough progress report was presented to the 45th Annual Tuna Conference (May 23-26, 1994, Lake Arrowhead, California).

## RESULTS

### Sample Collection

As of 31 July 1994, sampling has been or is being conducted on purse seine vessels of the U.S.A. (9 trips), Marshall Islands (1 trip), Japan (2 trips), Taiwan (3 trips) and Korea (3 trips). Longline sampling has been or is being conducted on vessels of Japanese (2 trips), Taiwanese (3 trips), Korean (1 trip), Mainland Chinese (1 trip) and U.S. (1 research trip). Most of the purse seine trips so far have only collected between 25 and 50 samples per trip and some collected zero samples

as the entire load consisted of skipjack and/or small yellowfin or the observers experienced opposition to the sampling from the vessel operators. The longline sampling is proceeding at a much better rate with around 50 to 85 usable samples being collected per trip. Most of the purse seine and longline sampling has been conducted in the waters of the Federated States of Micronesia, Papua New Guinea and Kiribati. Handline sampling in the Philippines has resulted in 100 samples collected per month and approximately 60 to 80 samples per month at the sampling site in Indonesia. Over 50 troll caught ovary samples have been received in Honolulu with approximately 50 still to be collected from the islands of Hawaii and Kauai. As of 31 July, no samples have been analyzed but 150 samples were in the process of being stained and mounted by the histology lab.

*Size distrib.*

#### Evaluation of preservation method on ovarian tissue

The effect of preservation method on the quality of the final histological section made from a yellowfin ovary was evaluated during July 1994. A control sample was removed and preserved in phosphate buffered 10% formalin solution approximately seven hours after the fish had been killed. Sub-samples from this single, mature ovary from a reproductively active fish were frozen at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$ , to simulate fish harvested and stored by tuna purse seine and longline vessels<sup>1</sup>. Other sub-samples from the same ovary were kept in sealed plastic bags stored in ice brine maintained at temperatures ranging between  $0.3^{\circ}\text{C}$  to  $5.1^{\circ}\text{C}$  in an attempt to simulate fish storage conditions that exist in many handline and troll fisheries. Other samples were kept in a refrigerator at temperatures ranging between  $7.0$  and  $13.0^{\circ}\text{C}$ .

Samples measuring approximately  $10 \times 10 \times 5$  mm were removed and preserved in 10% formalin at 24 hour intervals from the refrigerator and ice brine trials for periods of one and three weeks respectively. These samples were placed in 10% formalin buffered with sodium phosphate (mono and dibasic) or seawater (37 ppm) or not buffered (cut with distilled water only) to test the effect of buffering on the prepared slides. All trials were then treated with standard H & E staining, sectioned to  $6\mu\text{m}$ , with three serial sections mounted per slide.

Preliminary results indicate that storage in a commercial refrigerator for any length of time allowed too much cell destruction or decomposition for reliable determinations of gonad stage or reproductive activity. The samples kept in ice brine at temperatures ranging between  $0.3^{\circ}\text{C}$  to  $5.1^{\circ}\text{C}$  fared much better with very little degeneration of the tissue during the first week followed by slow and gradual deterioration of sample quality for the remainder of the trial. Post ovulatory follicles were in good condition and suitable for spawning frequency work in samples up to approximately the sixth day post mortem and were clearly visible and in fair condition through the 14 day. Samples older than 17 days became completely useless for reproductive work. Post ovulatory follicles were visible in the samples kept at  $-20$  and  $-80^{\circ}\text{C}$  but had been damaged by the freezing process and were clearly inferior to the control sample or the ice brine samples.

Sodium phosphate buffering and no buffer both produced excellent results and were difficult to distinguish. Samples preserved in seawater buffered formalin showed a great deal of cell disruption in the oocyte structure although post ovulatory follicles were still well preserved and reasonably easy to distinguish.

*- change to sampling kits  
- use distilled water/Na Ph*

<sup>1</sup> A freezer set to  $-50$  to  $-60^{\circ}\text{C}$  which would have more closely simulated conditions on an ULT asian longliner was not available at the time of this study.