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**GONADAL MATURATION, FECUNDITY, AND SPAWNING OF THE GREATER
AMBERJACK, SERIOLA DUMERILI (RISSO), IN HAWAIIAN WATERS WITH
REFERENCES TO CIGUATOXIN INCIDENCES**

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ABSTRACT

Reproductive biology of the greater amberjack, Seriola dumerili, was investigated to determine relationships with the irregular occurrence of ciguatoxic fish in Hawaiian waters. Incidence of toxic fish was independent of ovary maturity and spawning season. The spawning season is from February through June and peaks in March and April. Incidental spawning occurs year-round. Amberjack appears to be an intermittent spawner with three to four major spawnings in the relatively long protracted spawning season. Size at first maturity was 72-cm fork length and ripe ovaries were first observed in fish 78 cm long. Estimates of fecundity ranged from 1.3 to 4.2 x 10⁶ ova for the 83.0 to 118.6-cm size group. Sex ratio was 1.09:1 (males to females); males predominated among fish 60 to 79 cm in length and females among fish 100 cm in length.

ciguatera	<u>Seriola dumerili</u>
fecundity	spawning season
reproduction	greater amberjack

INTRODUCTION

Until May 1980, the greater amberjack, Seriola dumerili, comprised a substantial amount of the handline fishery landings in Hawaii, ranking third with an annual average catch of about 33,107 kg (DAR, 1980). Irregular occurrences of ciguatera poisoning (Halstead, 1970) which implicated the amberjack have virtually eliminated commercial sales.

In April 1979, a 2-year program was initiated by the Honolulu Laboratory, Southwest Fisheries Center, National Marine Fisheries Service (NMFS), with the University of Hawaii School of Medicine, and the United Fishing Agency (UFA) to develop a practical method to screen commercial fishes for ciguatera. Due to its history of implication in ciguatera poisoning, the amberjack was employed as a test species.

Previous workers (Hiyama, 1950; Watanabe, 1950; Cooper, 1964) have hypothesized that ciguatera poisoning is directly related to maturity and gonadal ripeness of the fish. A preponderance of toxic fish might occur during the spawning seasons. Banner et al. (1966) have found that elimination of the ciguatoxin in *Lutjanus bohar* was very slow such that after 30 months there was no detectable drop in toxicity level. They inferred that the incidence of toxicity would be higher in the larger, mature fish.

Of the few studies conducted on the reproductive biology of the greater amberjack, none has examined its relationship with ciguatera levels. Burch (1979) observed that peak spawning in the western Atlantic occurred in March through June with evidence of year-round incidental spawning. Similar fecundity and maturation studies of *S. dorsalis* off California have demonstrated that multiple spawning occurs from July to October (Baxter, 1960).

In this study, some aspects of reproduction of an exploited fish stock including (1) spawning season, (2) fecundity, (3) size at first maturity, and (4) sex ratio have been determined and compared with the occurrence of ciguatoxin.

MATERIALS AND METHODS

A total of 5,242 amberjack gonads were collected from April 1979 through April 1981 for ciguatera screening. Fish sampled were caught by commercial handline boats in the area between the island of Hawaii and Pearl and Hermes Atoll in the Northwestern Hawaiian Islands.

Fork length (FL), body weight (to the nearest 0.1 kg), date, and location of capture, when available, were recorded. Gonads and stomachs were removed and frozen for laboratory examination. Tissue samples extracted from various parts of the body were collected for ciguatoxin analysis using a radioimmunoassay technique developed by Hokama et al. (1977) at the John A. Burns School of Medicine, University of Hawaii.

Frozen gonads were weighed to the nearest gram and larger more developed ovaries were preserved in modified Gilson's fluid (Simpson, 1951) for fecundity estimates. Other ovaries were preserved in 4 percent formaldehyde solution. Testes were weighed and discarded.

To facilitate the penetrations of the Gilson's fluid, epithelium of the ovary was slit longitudinally on both lobes and inverted, exposing the sinuous germinal tissues. Daily agitations of samples over a 3 to 6-week period enhanced the digestion deterioration of the connective tissues and freeing of the hardened ova. Remaining connective tissue was sufficiently broken down so that any attached ova could be easily freed. Ova were separated from remaining tissue with a 1-mm mesh nylon screen, then collected and washed over a 0.183-mm mesh screen.

Subsamples for fecundity estimates were obtained by a volumetric method (Van Dalsen, 1977) with minor modifications. In place of a reversing magnetic stirrer, ova were initially suspended with a stirring rod. Then, an unidirectional magnetic stirrer was activated to create a counterflow. Prior to the formation of a vortex, two 5-ml aliquots were drawn with a pipette from the lower two-thirds of the mixture, and approximately 2 to 5 cm from the wall of the container.

Estimates of fecundity (F) were obtained by the following formula:

$$F = \frac{\sum_{n} N}{n} \cdot \frac{V}{5 \text{ ml}}$$

where

n = number of subsamples

N = number of ova in each subsample

V = total volume of the mixture in milliliters

Eight ovaries were sampled during the 1980 spawning season for fecundity estimates. Relationships of fecundity with FL, body weight (wt), and gonad weight (gw) were compared using Bartlett's "three-group" method for model II regression (Sokal and Rohlf, 1969; Ricker, 1973). Also from each of the subsamples, 300 randomly selected ova were measured and staged under a microscope (Kikkawa, 1980). Due to the irregular shape of the preserved ova, diameters were measured following the method of Clark (1934).

Earlier workers have customarily used ova diameter frequency distribution in spawning studies (Clark, 1934; Yuen, 1955; Otsu and Uchida, 1959). Although this method provides a good estimate of maturity, the technique is quite laborious. Due to the large number of samples, a simpler method was desirable. Bagenal and Braum (1968) suggested the use of gonad weight as an indicator of reproductive condition. Consequently a percent ratio of gonad weight to body weight (gw/wt x 100), termed the gonadosomatic index (GSI), was employed to represent maturity. For comparative purposes, the developmental stage of ovaries was also determined on the basis of the most advanced ova in the ovaries.

RESULTS

Developmental Stages of the Ovary

Seven stages of ova development could be identified based on physical characteristics: immature, early developing, developing, advanced developing, early ripe, ripe, and residual (Table 1). These stages were similar to those found by Uchiyama and Shomura (1974) in the swordfish, *Xiphias gladius*, and Kikkawa (1980) in the pink snapper, *Pristipomoides filamentosus*.

Ova diameter distributions of ova in various stages of maturity are shown in Figure 1. Primary or primordial cells occurred in all of the ovaries. These rudimentary cells were not measured.

Early developing ova made up the "developing" mode. They were considered unlikely to be released during the next spawning. The "ripening" category included ova in a wide developmental stage from "developing" to "early ripe" (Table 1). No running ripe ova were observed.

The developmental stage assigned to an ovary was based on the most advanced ova. Immature and recently spent females with only primordial cells in the ovaries were considered to have nondeveloping ovaries. Those with ova in the "developing" mode (Figure 1) were classified as developing. Ovaries were classified as "ripening" based on the presence of ova in "advanced developing" mode which included ova in the developing to ripe stage.

TABLE 1. DESCRIPTION OF THE OVARIES OF GREATER AMBERJACK AT VARIOUS STAGES OF DEVELOPMENT

Ova Maturity Stage	GSI Mean (\pm S.E.)	Physical Description of the Oocytes	Diameter $\mu\text{m} \times 10^2$	Ovary Maturity
I. Primordial	0.940 (0.059)	Oocytes prevalent in all ovaries; oocytes are usually ovoid and consist of uniform transparent gelatinous material.	--	Nondeveloping
II. Early	0.701 (0.052)	Oocytes are semi-transparent to translucent due to the formation of granular yolk matter. Oocytes are usually ovoid or wedgelike.	2.0-5.1	Developing
III. Developing	0.701 (0.279)	Oocytes are completely opaque with yolk material.	4.1-6.9	Ripening
IV. Advanced developing	2.80 (0.099)	Oocytes are usually spherical with uniform opaque yolk granules and encased in a transparent fertilization membrane.	4.6-7.4	Ripening
V. Early ripe	3.78 (0.55)	Oocytes are usually spherical. The yolk material is translucent; oil globules are present.	5.1-7.6	Ripening
VI. Ripe	4.86 (0.16)	Oocytes are usually almost transparent; oil globules are present.	--	Ripening
VII. Residual	3.80	Oocytes show signs of reabsorption. Cells are translucent and greatly reduced.	4.8-7.0	Ripening

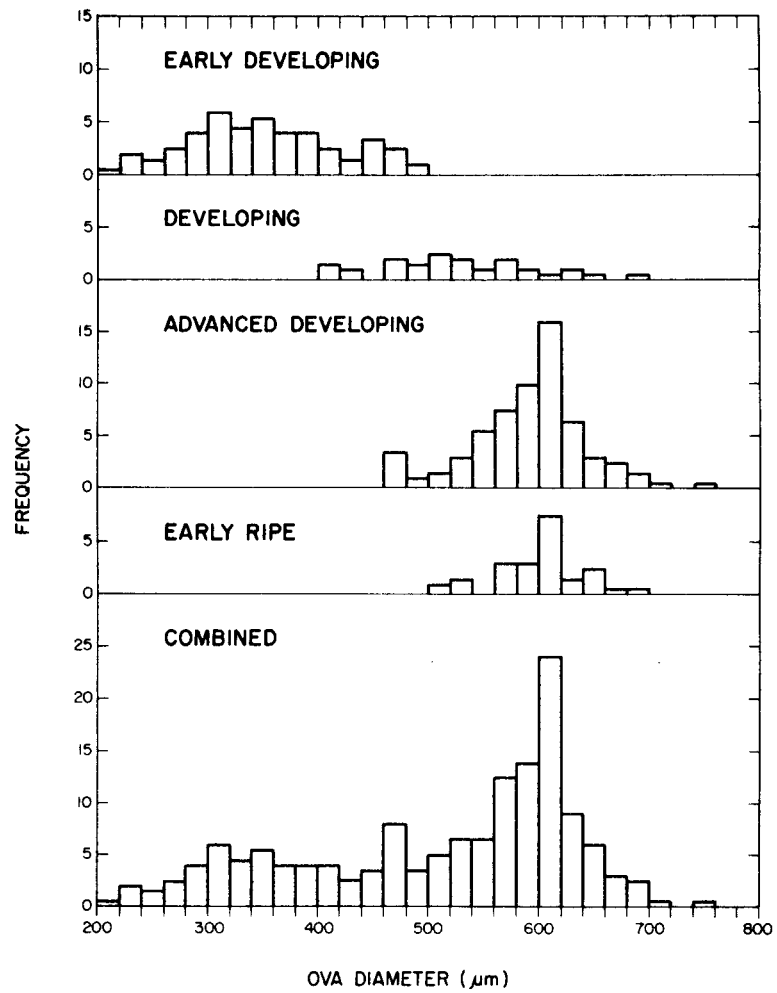


Figure 1. Size-frequency distribution of ova at various stages of maturation

Fecundity

In eight ripening females (83.0 to 118.6-cm FL), fecundity ranged from 1.3 to 4.2×10^6 ova. The relationships of fecundity to length, body weight, and gonad weight were linear. Bartlett's method for model II regression was used to describe the relationship:

<u>Relationships</u>	<u>d.f.</u>	<u>Results</u>
Fecundity and length	7	F = $-0.71 + 0.0025 \text{ FL}$ r* = 0.72
Fecundity and body weight	7	F = $0.97 + 0.05 \text{ wt}$ r* = 0.82
Fecundity and gonad weight	7	F = $-0.24 + 0.0036 \text{ gw}$ r* = 0.89

where

F = fecundity $\times 10^6$
FL = fork length in centimeters
wt = body weight in kilograms
gw = gonad weight in grams
r* = the unbiased correlation coefficient (Kendall and Stuart, 1967)

All developing and ripening ova in the most advanced mode were used to estimate fecundity. The fecundity was best correlated with gonad weight. Gonad weight, therefore, was considered the best predictor of fecundity.

Ovary maturity and gonadosomatic index

Females that (1) were too small to be sexually mature, (2) had ovaries containing only primordial cells, (3) had ovaries containing early developing oocytes, and (4) were recently spent had indices of about 1 percent. Females with indices 2.0 percent were considered to be sexually active and expected to spawn in the current spawning season. Fish with near ripe ovaries had indices 3.5 percent. The highest index for a female was 4.86 percent.

Spawning season

The seasonal distribution of GSI indicated that spawning began in February, peaked in March and April, and concluded by July (Figure 2). Mean GSI reached 2.5 percent for males and 3.0 percent for females in February and rose to 5 percent for males and 4.5 percent for females in March. At the conclusion of the spawning period, GSI declined to about 1.8 percent for both sexes. The occurrence of a few ripe ovaries and low mean GSI levels throughout the year indicates that incidental year-round spawning may occur.

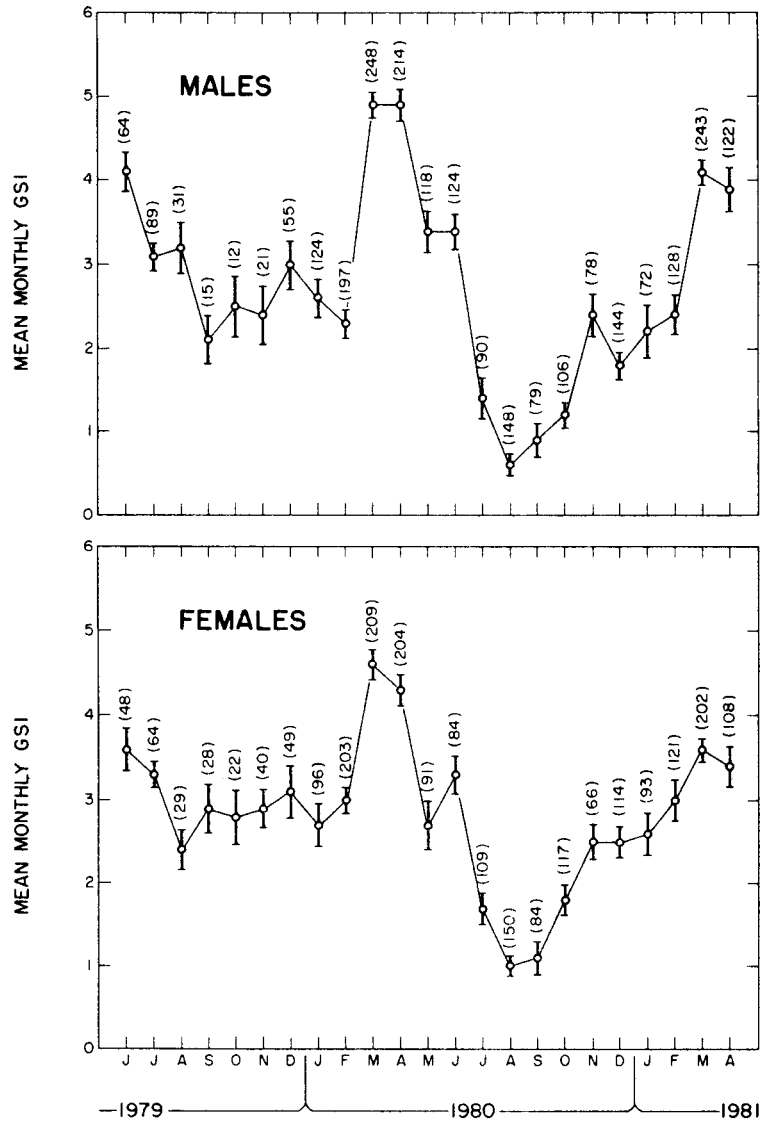


Figure 2. Mean monthly gonadosomatic index (GSI) for the greater amberjack. The vertical lines represent \pm standard error of the mean; (N) is sample size.

Size at maturity

The relationship between body length and the development of ovaries was determined from 144 randomly selected ovaries. The percentage of females with nondeveloping, developing, and ripening ovaries was plotted by 2-cm size classes to determine size at maturity. Females with developing ovaries were considered mature

and those with nondeveloping ovaries, immature. At 70 cm every female was immature; however, at 72 cm 40 percent had reached maturity. Thus, females were considered likely to reach maturity at 72 cm (Figure 3). The smallest female with ripe ovaries measured 78 cm and was estimated to be 2.5 years old (J.H. Uchiyama, 1983: personal communication).

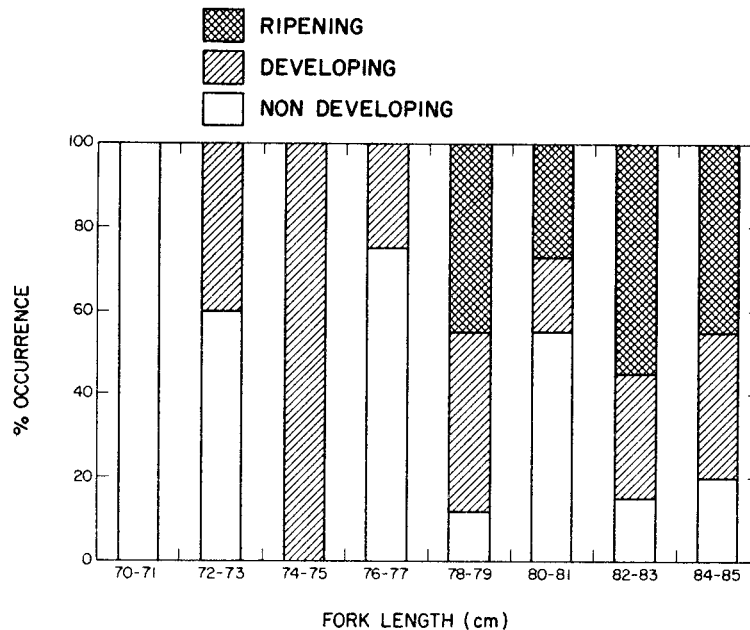


Figure 3. Percentage of distribution of ovaries in various stages of development by fish size

Sex ratio

Males predominated (1.09:1) in the 5,242 fish sampled for this study. Sex ratios by year were: 1979, 1.01:1; 1980, 1.11:1; and 1981, 1.10:1. The sex ratio for 1980 deviated most from the expected 1:1 ratio. Pooled by year, the sex ratio deviated significantly from 1:1 in March, June, and December (Table 2). For the 10-cm length classes, sex ratio deviated from 1:1 for most of the size categories (Table 3). Generally the males predominated in the larger sizes (≤ 100 cm). Among the smaller sizes (≤ 60 cm), no significant deviations from the expected were found.

TABLE 2. DEVIATION OF THE MALE TO FEMALE SEX RATIO FROM 1:1 IN GREATER AMBERJACK BY MONTH AND YEAR

Month	Year			Total a	d.f.	χ^2
	1979	1980	1981			
April	29:16	221:221	122:112	372:339	2	1.53
May	43:69	121:94	--	164:163	1	0.003
June	101:72	137:93*	--	238:165	1	13.22 [†]
July	89:64	92:111	--	181:175	1	0.10
August	31:29	150:151	--	181:180	1	0.003
September	15:28	83:87	--	98:115	1	1.36
October	14:26	108:119*	--	122:145	1	1.98
November	23:41	81:69	--	104:110	1	0.17
December	55:50	159:122	--	214:172	1	4.57
January	--	128:97	73:93	201:190	1	0.31
February	--	202:203	129:123	331:326	1	0.04
March	--	265:216	267:208	532:424	1	12.20*
Total b	400:395	1,747:1,583	591:536			
d.f.	8	11	3			
χ^2	0.03	8.08	2.68			

Note: Total a = the sex ratio pooled by month for all years; Total b = the sex ratio for each year

*P < 0.05

†P < 0.01

Incidence of ciguatoxic fish

Of the 5,227 amberjacks tested, 370 males and 372 females had high levels of ciguatoxin. The percentage of toxic males (13.6 percent) from that of females (14.9 percent) was not significantly different ($t = 1.6934$; d.f. = 5,226; $P > 0.05$).

TABLE 3. DEVIATION OF THE MALE TO FEMALE SEX RATIO FROM 1.09:1 IN GREATER AMBERJACK BY 100-MM SIZE CATEGORIES

Fork Length (mm)	Male	Female	n	χ^2
400	27 (39.7)	41 (60.3)	68	2.88
400-499	104 (46.0)	122 (54.0)	226	1.43
500-599	202 (52.3)	184 (47.7)	386	0.84
600-699	393 (57.4)	292 (42.6)	685	14.89*
700-799	835 (58.1)	601 (41.9)	1,436	38.13*
800-899	815 (54.1)	692 (45.9)	1,507	10.04*
900-999	282 (46.3)	327 (53.7)	609	3.33
1,000-1,099	40 (24.1)	126 (75.9)	166	44.55*
1,100-1,199	7 (13.0)	47 (87.0)	54	29.63*
1,200-1,299	1 (4.4)	22 (95.6)	23	19.17*
1,300	0 (0)	6 (100)	6	--

Note: percentages given in parentheses

*P < 0.01

A 2 x 3 contingency table of nontoxic and toxic females against stages of ovary development was constructed to show variations in the occurrence of toxic females by maturity ($\chi^2 = 9.517$; d.f. = 2; P < 0.01). A test of independence showed that toxic female amberjacks were most likely to have immature or ripening ovaries ($\chi^2 = 9.668$; d.f. = 2; P < 0.01) (Table 4).

TABLE 4. PERCENT DISTRIBUTION OF TOXIC AND NONTOXIC FISH AT VARIOUS DEVELOPMENTAL STAGES

Developmental Stage	Nontoxic	Toxic
Developing	86.0 (3,353)	14.0 (544)
Developing	84.8 (890)	15.2 (159)
Ripe	84.4 (464)	15.6 (86)
TOTAL	100.00 (4,707)	100.00 (789)

Note: Sample size is given in parentheses

No obvious trends in the relationship between the seasonal distribution of toxic fish and spawning could be detected. During 1980, occurrence of ciguatoxic fish peaked in January, March, June, August, and October. The mean percentage was 22 percent of the total month's catch.

DISCUSSION

In this study, fecundity is defined as the number of eggs in the female likely to be extruded at the next spawning (Bagenal and Braum, 1968). Ova in this category were those in the most advanced mode in the ova diameter frequency distribution. It was unlikely that the ova in the secondary mode would develop enough to be released with ova in the most advanced mode. Although the developmental rate of the ova was not determined and the advanced mode included ova in various developmental stages from developing to early ripe, it was assumed that at the time of spawning all of the ova in the most advanced mode would be extruded. Work on the jack mackerel off California demonstrated the occurrence of two major spawnings, i.e., the two modal groups of ova suggested a spawning with each mode.

The seasonal distribution of GSI did not indicate a second peak spawning during the relatively long breeding season, which was estimated to last from February to June, but the possibility of multiple spawning cannot be ignored. It is apparent from the multimodal distribution of ova diameters that the ova in the secondary mode would unlikely develop enough in size and maturity to be released with ova in the most advanced mode. Based on the continuous development of ova, it seems likely that the greater amberjack is a multiple spawner. Although there are no data to determine the number of spawnings per season, higher occurrences of juveniles (fish <45 cm and with sexually indistinguishable undeveloped gonads) in May, July, September, and October (Figure 4) could possibly reflect three or four major spawnings in the spring.

During the off-spawning season, the infrequent catches of ripe fish and a few juveniles indicate that incidental spawning may occur. Spawning would be closely associated with environmental conditions conducive to larval fish survival as was demonstrated for the Japanese horse mackerel, *Trachurus japonicus* (Chigirinskiy, 1970).

The spawning season for greater amberjack off the coast of Florida is from March to June, peaking in April and May. It was inferred from the infrequent catches of ripe fish that the amberjack exhibit low year-round spawning (Burch, 1979). Also, larval fish surveys in the eastern Gulf of Mexico and Straits of Florida tend to support the generalization that year-round spawning is typical of the *Seriola* spp. (Dooley, 1972; Munro et al., 1973; Prieto, 1974; Fahay, 1975).

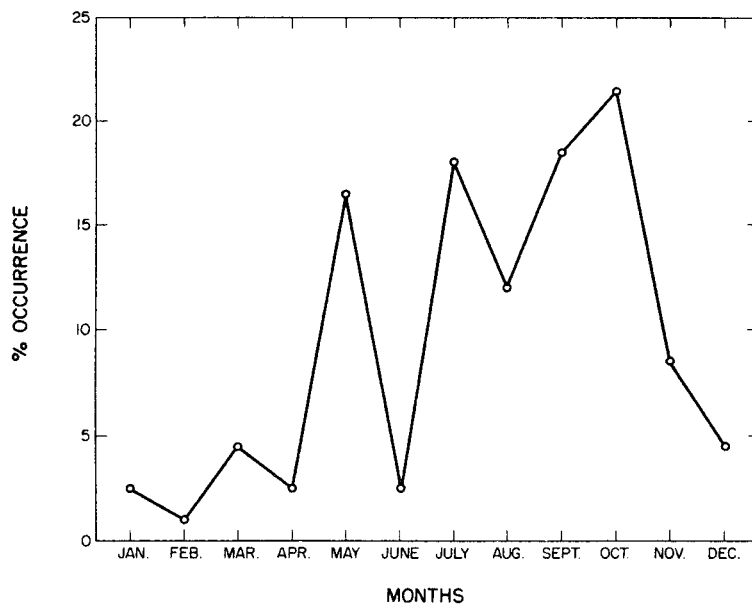


Figure 4. Percentage of distribution of juvenile amberjack ≤ 45 cm throughout the year

The literature on sex ratio provided little assistance in interpreting the significant variations from a 1:1 sex ratio. Deviations from the expected ratio could be due to sexual differences in longevity, growth, mortality, and behavioral patterns, or to sex reversal and migration of one sex out of the sampling area as suggested by Wenner (1972). Of these, only differences in mortality and longevity are supported by available evidence. The number of males in the midsize classes (60 to 90 cm) were significantly greater than the females. Conversely, fish ≥ 100 cm were predominantly females.

The occurrence of 12.2 percent immature and 16.9 percent ripening toxic fish is contradictory to the hypothesis that toxicity is related to spawning period and gonad development (Hiyama, 1950; Watanabe, 1950). No significantly higher number of toxic ripening fish was found. Investigators studying the acquisition and retention of ciguatoxin in the red snapper, *Lutjanus bohar*, have found that toxicity levels remained the same for about 30 months and that elimination of the toxin is very slow (Banner et al., 1966). Due to the persistence of the toxin, one would expect a higher percentage of toxic fish to have reached sexual maturity, but we did not find this to be so. A possible explanation of this anomaly is that at various stages of development, ciguatoxin could be concentrated in different organs. Higher levels of the fat soluble toxin (Scheuer et al., 1967) might be in tissues of high lipid concentrations such as the generative tissues at the onset of spawning. Substantiating

work on sardines, herring, and other clupeids have elucidated the increase of fats in the musculature, mesentery, and viscera preceding the onset of spawning (Blaxter and Holliday, 1963; Channon and El Saby, 1932). At the onset of spawning, fat reserves are channeled to the gonads as energy source and nutritive materials in the yolk (Shul'man, 1974). A close association of ciguatoxin with the homologous lipids (Lasker and Theilacker, 1962) might be expected and like the lipids, toxin might be concentrated in different organs due to physiological changes in the fish. During the prespawning period, movement of fat reserves to the reproductive organs would create a lipid and ciguatoxin gradient, thereby increasing the toxicity level in the viscera. Some ciguatera attacks were thought to be caused by the consumption of the more toxic viscera than the musculature (Halstead and Schall, 1958; Cooper, 1964; Helfrich et al., 1968). In immature or resting fish, higher concentrations of toxin would likely be in the musculature due to the rising fat reserves. Because only the musculature was tested by radioimmunoassay, this question remains unresolved.

SUMMARY

1. Greater amberjack gonad samples were collected from the Hawaiian commercial fishery from April 1979 through April 1981 as part of a ciguatera research program.

2. Fecundity estimates, based on counts of all ova in the most advanced mode, ranged from 1.32×10^6 to 4.2×10^6 for fish from 83.0 to 118.6 cm. Relationships of ova count to gonad weight, body weight, and length were best described by model II regressions. Gonad weight was the best predictor of fecundity.

3. Spawning season for the amberjack was February through July, peaking in March and April.

4. Infrequent occurrence of ripe ovaries and low mean GSI levels throughout the year indicated year-round incidental spawning.

5. Based on the occurrence of juveniles <45 cm and continuous ripening of the ova during the relatively long spawning period, it appears that the amberjack are intermittent spawners; it is possible that there may be three to four major spawnings in a single season.

6. Size at maturity was estimated to be 72 cm. Some fully ripe individuals were 78 cm.

7. The male to female sex ratio of the 5,242 amberjack sampled was 1.09:1, differing significantly from the expected 1:1 ratio. Males predominated among the midsizes (60 to 79 cm) whereas, females predominated in the larger sizes (≥ 100 cm).

8. The incidence of ciguatoxic fish was neither related to spawning season nor to sex.

9. There was a slightly higher rate of toxic females in the immature and ripening stages.

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