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Age, Growth, Food Habits, and Reproduction of Bonefish, *Albula vulpes*, in South Florida Waters

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Marine Research Laboratory

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GERARD E. BRUGER

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ABSTRACT

Bruger, G. E. 1974. Age, Growth, Food Habits, and Reproduction of Bonefish, *Albula vulpes*, in South Florida Waters. Fla. Mar. Res. Publ. No. 3. 20 p. Life history and biology of bonefish, *Albula vulpes*, were studied from 170 specimens collected from anglers in the Lower Florida Keys between February 1970 and June 1972 and from 36 museum specimens. Of 205 sets of scales examined for age evaluations, 183 sets (89.3%) were readable. The oldest fish encountered was a 12 year old female, 620 mm fork length (24.4 inches), weighing 4196 grams (9.25 pounds). Ages greater than 12 years may be expected. The relationship of scale radius to standard length is linear, r=0.988. Use of marginal increments to determine time of annulus formation was inconclusive. No single time can presently be fixed for annulus formation of the population; only for single individuals. Back-calculations of standard lengths for Age Classes I to IX were computed. Agreement between empirical and backcalculated lengths is shown, although empirical lengths are consistently larger since collections do not coincide with the precise time of annulus formation. Length-weight relationships were calculated for 169 fish (77 males, 88 females and 4 fish of undetermined sex). The relationship for all fish is expressed by the equations

$$W = 2.799 \times 10^{-5} SL^{2.9321} \quad \text{or} \quad \text{Log } W = 2.9321 \text{ Log } SL - 4.5529.$$ Sexes were also considered separately and their equations presented.

Scale radius-standard length, length-weight and backcalculation data all suggest a change in growth rate at approximately 300-400 mm standard length (Age Class III-IV). Standard and fork lengths are highly correlated (r = 0.999); the relationship is expressed by the equation $FL = 1.07 \ SL$.

Crustaceans, principally penaeid and alpheid shrimp and portunid and xanthid crabs, found in 73% of stomachs, predominated as food items. Noncrustacean items, including fish and fish remains (present in 15%), mollusks (present in 28%) and miscellaneous material contributed little either numerically or volumetrically. Nineteen stomachs (15%) were empty or contained no recognizable food items.

Stomachs of many bonefish were parasitized by acanthocephalid worms tentatively identified as *Dollfusentis heteracanthus* (Cable and Linderoth).

Microscopic examination of histologically prepared gonadal sections was used to describe oogenesis and spermatogenesis and to thereby determine spawning time. Ripe and near-ripe males and females are present throughout the year, indicating that year-round spawning may occur.

CONTENTS

INTRODUCTION

Dynamics and life history of the bonefish, *Albula vulpes* (Linné), have received little attention, although this species is distributed worldwide in tropical and subtropical waters and is avidly pursued by anglers seeking to test their skills against the elusive, often frustrating, but always spectacular "ghost-of-the-flats" (Figure 1). Most anglers seek the bonefish for sport only, and unless a fish is to be a trophy mount, or inadvertently dies from exhaustion during the arduous fight, it is revived and returned alive. Anglers hold this fish in such esteem that conservation of this resource is practiced voluntarily.

Investigations to date have dealt primarily with the processes of the metamorphosis of the transparent, ribbon-shaped leptocephalid larvae (Figure 2) into juveniles by "growing through shrinking." Dr. C. H. Gilbert, the first to identify larval bonefish as such, did not publish his findings, but Jordan (1905) and Gill (1907) made use of his 1889 drawings. Hollister (1936) described larval metamorphosis from a specimen collected in Bermuda and kept alive for ten days throughout the transition. Fitch (1950) briefly described gross morphological changes during metamorphosis. Rasquin (1955) made detailed histological, histochemical, and morphological observations on metamorphosing larvae, concluding that larvae derive nutrition during metamorphosis by reabsorbing the large gelatinous core separating the developing notochord and visceral organs. Alexander (1958), in an extensive discussion of larval development, presented information on previously unrecorded premetamorphic larvae (7.8-65.0 mm), vertical distribution of larvae in the water column, and geographical distribution as limited by the 20°C
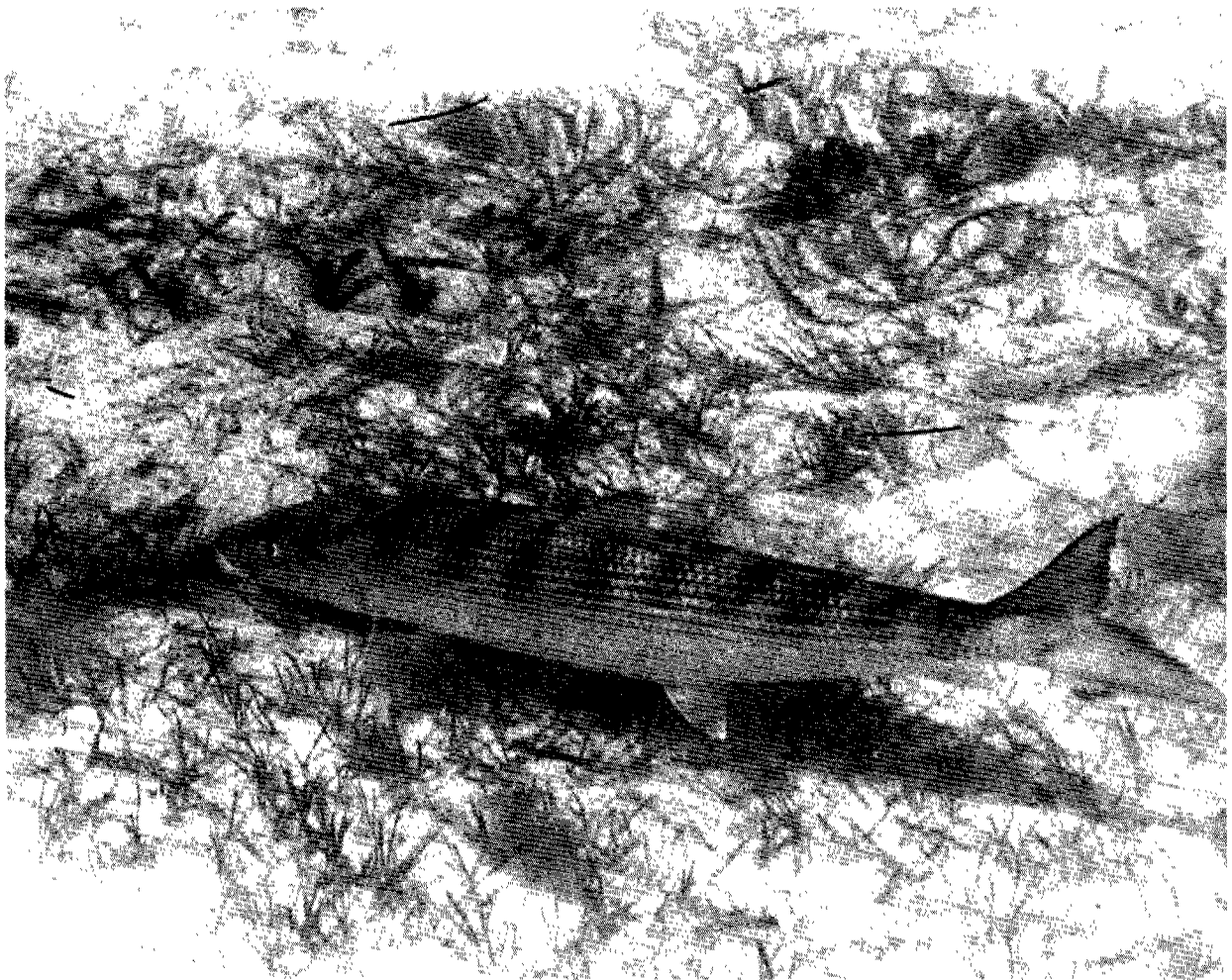

Figure 1. Bonefish, *Albula vulpes* (Linné), feeding on a typical south Florida flat. (Photo courtesy of Lefty Kreh.)

isotherm, and demonstrated that spawning occurs throughout the year in West Indian waters.

Few investigations have dealt with aspects of life history after metamorphosis. Erdman (1960) described food habits, relative and seasonal abundance, and factors limiting abundance of Puerto Rican bonefish stocks, and Warmke and Erdman (1963) reported on marine mollusks eaten by Puerto Rican bonefish. Alperin and Schaefer (1964) described the unprecedented collection of 108 recently metamorphosed juveniles (33-81 mm fork length) in Great South Bay (Long Island), New York during August and September 1962. Specimens in this size range are extremely rare. Occurrence of juveniles in New York indicates the potential dispersal of larvae by oceanic currents. References concerning adult fish are limited to faunal lists (e.g., Bean, 1888; Jordan and Evermann, 1896; Beebe and Tee-Van, 1928; Longley and Hildebrand, 1941), popular magazine accounts, and books written about bonefishing (e.g., Babson, 1965).

Therefore, my study was begun early in 1970 to evaluate age, growth, food habits, and reproduction of bonefish in Florida waters where the fish support an extensive sports fishery.

METHODS AND MATERIALS

SOURCE OF MATERIALS

Bonefish specimens collected for this study were donated primarily by numerous anglers and guides in the lower Florida Keys. Such collections, although often sporadic, were for several reasons considered the best sources:

1) Limitations imposed by concurrent research programs made collections by our staff impractical;
2) The elusive nature of bonefish, which are caught almost exclusively on hook and line, demands skill, patience, time, and thorough knowledge of the fish's behavior. An angler uneducated in the ways of bonefish will generally engage a guide for a day's fishing, usually at considerable expense. It is unlikely that a novice fisherman will catch bonefish unaided;

3) Bonefish are not commonly sought as table fare in Florida and are thus unavailable from commercial sources.

From February 1970 through April 1971, 155 specimens were procured from anglers and guides between Marathon and Key West. Fish were either frozen or refrigerated until collected by research personnel. Precise capture location and depth were obtained when possible. Upon return to the Key West Laboratory, standard, fork, and total lengths (SL, FL, TL) were measured to the nearest millimeter according to methods described by Lagler (1952). Weights were recorded in pounds and ounces, later converted to grams. All length and weight data were recorded from completely thawed fish. Topp (1963) showed that dehydration and freezing of specimens ultimately caused permanent decrease in body length. Since many specimens were frozen or refrigerated for varying periods before collection, an experiment was conducted to determine percentage decrease in body length attributable to freezing and desiccation, the resultant value being applied as necessary to compensate for shrinkage.

Scale samples for age analysis were consistently taken from the left side, three scale rows dorsal to the lateral line and 21-27 scales posterior to the first anterior scale.

Sex and gonadal development state were judged macroscopically. Gonads were then removed, weighed to the nearest gram, and a small section (approximately one cm^3) placed in Bouin's fixative and returned to the St. Petersburg laboratory for histological preparation and sectioning at a thickness of 6 μm. Gonadal sections were then stained with Papanicolaou hematoxylin (Harris)-eosin Y.

Stomach and intestinal contents were preserved in 10% formalin and retained for analyses.

Additional material examined includes 15 specimens collected from Biscayne Bay and the Marathon vicinity in April and June 1972. Gonadal sections were not taken from four of these specimens. Other specimens came from collections at the Rosenstiel School of Marine and Atmospheric Sciences (RSMAS), Miami; National Marine Fisheries Service Southeast Fisheries Center, Miami Laboratory (NMFS/ TABL), Miami; Louisiana State University (LSU), Baton Rouge; and also specimens kindly donated by Mr. Frank Lund of Jupiter, Florida. Table 1 lists pertinent data on these specimens.

Figure 2. Premetamorphic bonefish leptocephalid larva 33.5 mm SL (after Eldred, 1967).

TABLE 1. SOURCES OF SUPPLEMENTAL BONEFISH SPECIMENS AND PERTINENT COLLECTION DATA.

Source	Collection Number	Collection Date	Location	Number	Size Range (mm FL)
Rosenstiel School of Marine and Atmospheric Sciences (RSMAS)	UMML 16775	24 Sept. 1963	Everglades National Park, Flamingo-Buttonwood Canal Bridge; Monroe Co., Fla.	22	139-181
National Marine Fisheries Service Southeast Fisheries Center, Miami Laboratory (NMFS/TABL)	TABL 100716	24 Sept. 1963	Same as above		
RSMAS	UMML 21866	25 Sept. 1964	Bear Cut, Virginia Key; Dade Co., Fla.	1	52
RSMAS	UMML 49:966	20 June 1956	Key Biscayne, S. of Crandon Park; Dade Co., Fla.	1	58
RSMAS	UMML 9132	No data		1	98
NMFS/TABL	TABL 67-43	23 March 1967	Bear Cut, Virginia Key; Dade Co., Fla.	1	60
Louisiana State University	LSU 470	3 May 1967	Hobe Sound; Martin Co., Fla.	6	45-86
Mr. Frank Lund	FL: 1-4	Spring-Summer 1962	Hobe Sound; Martin Co., Fla.	4	42-65
Florida Department of Natural Resources Marine Research Laboratory		29 Apr. 1972	Biscayne Bay; Dade Co., Fla.	3	597-622
Florida Department of Natural Resources Marine Research Laboratory		12-16 June 1972	Marathon Key; Monroe Co., Fla.	12	497-680

RESULTS AND DISCUSSION

AGE AND GROWTH

Before beginning any analysis of age and growth in bonefish it is necessary to realize that there exist two virtually distinct segments of this fish's life: the leptocephalid larval stage characteristic of elopomorph (*sensu* Greenwood et al., 1966) fish and postmetamorphic stages when adult form is assumed. Since duration of the leptocephalus stage is unconfirmed for bonefish, but may be extensive (perhaps 5-6 months; Erdman, 1960), all ages reported in this study are understood to be postmetamorphic.

Scales used for age evaluations were immersed in glycerin in a black-bottomed watch glass and examined using direct light under a low power binocular dissecting microscope (12-50X). Radial measurements were made from the focus to the angular margin of the ventral anterior field of the scale (Figure 3) using an ocular micrometer, and recorded in ocular micrometer units (1 omu = 0.833 mm). Readings were made from at least two scales per fish, independent of bias introduced by reference to length, weight, or sex. Of 205 sets of scales examined, 183 sets (89.3%) were readable, while 22 (10.7%) were not. The oldest fish aged was a 12-year-old female, 620 mm FL (2.03 ft), weighing 4196 g (9.25 lb). This is by no means the largest specimen collected, suggesting that bonefish may reach ages considerably higher than reported here.

VALIDITY OF SCALE AGING METHOD FOR BONEFISH

Van Oosten (1929) established the following criteria upon which the validity of the scale aging technique is based:

1. That the scales remain constant in number and [retain their] identity throughout the life of the fish.
2. That the annual increment in the length (or some other dimension which must then be used) of the scale maintains, throughout the life of the fish, a constant ratio with the annual increment in body length.
3. That the annuli are formed yearly and at the same time each year [or that some other discoverable relation exists between their formation and increment of time].

Otoliths are considered to be valid aging structures. Scales bear annular marks corresponding to those of otoliths and therefore also are considered valid aging structures. Otoliths of bonefish, although certainly present in premetamorphic larvae, do not begin ossification until metamorphosis has begun (Rasquin, 1955). Upon completion of metamorphosis, at a standard length of 20-29 mm, ossification is complete. Scalation begins at approximately 35 mm and is complete at 40-45 mm (Hildebrand, 1963). Therefore, scales and otoliths develop fully at nearly the same time; differences are not appreciable for the purposes of this study.

Further evidence is derived from comparison of scales and otoliths of the same adult fish; both correspond in "annular" markings (Figure 4). Constancy in the number of scales on individual fish is assumed. Scale regeneration necessarily invalidates scale integrity, but regenerated scales were obvious and rejected from analysis.

To demonstrate proportionality between scale radius and standard length, regression lines were fitted to the entire set of 204 plotted points of scale radius-standard length data, and to those portions above and below a point of apparent change in rate of growth (scale radius 7.0-8.0 omu). These lines (Figure 5) are expressed by the equations:

$$\hat{Y} = 94.84 + 30.56X \text{ (scale radius} > 7.0),$$
$$\hat{Y} = 20.89 + 45.34X \text{ (scale radius} < 7.0), \text{ and}$$
$$\hat{Y} = 68.11 + 32.62X \text{ (all data)},$$

where X is scale radius measured in ocular micrometer units and $\hat{Y}$ is standard length in mm. Calculated correlation coefficients are sufficiently high ($r = 0.985$, $r = 0.936$, and $r = 0.988$, respectively) to demonstrate proportionality between scale radius and standard length.
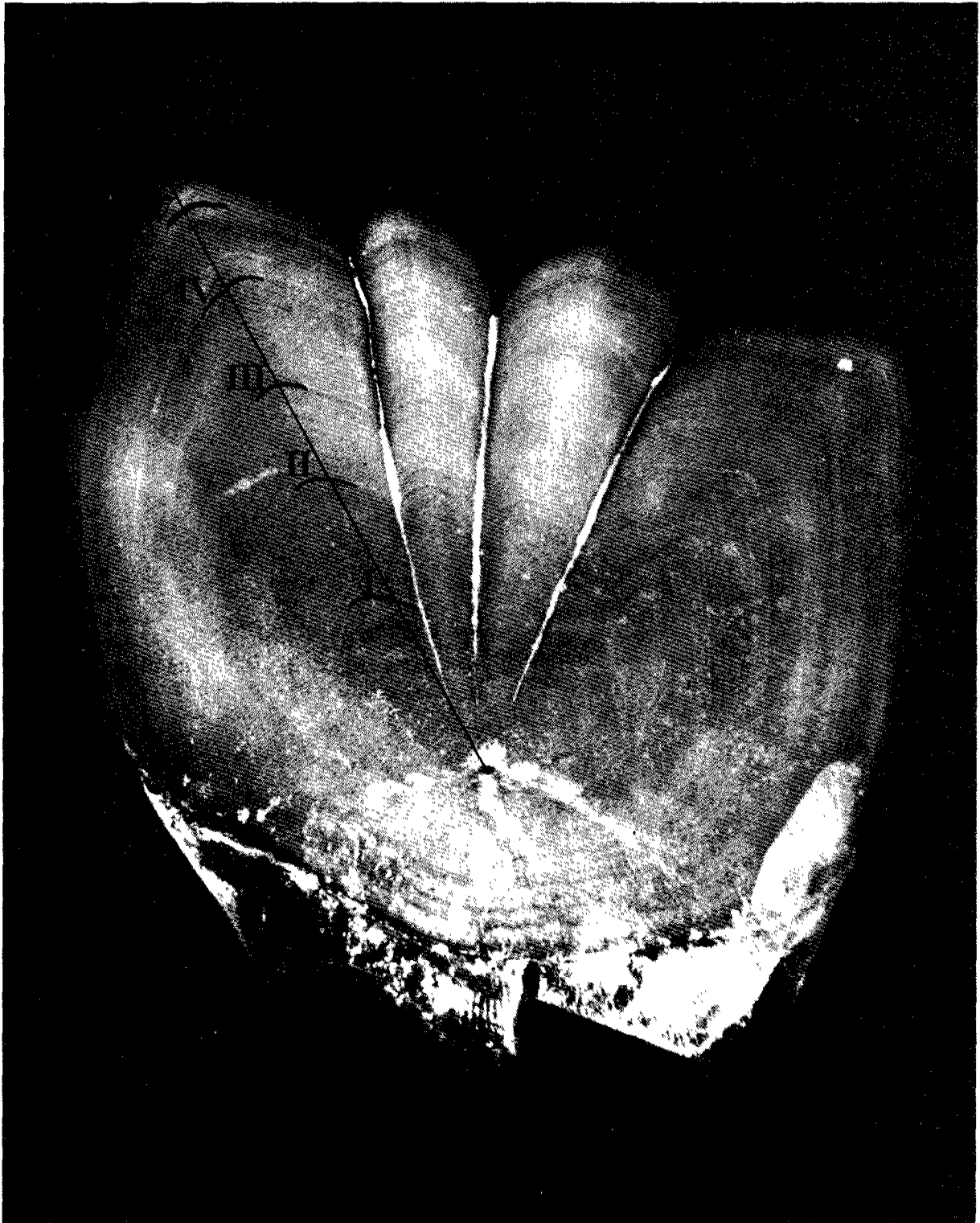
Figure 3. Bonefish scale showing radial measurement and locations of five annuli.
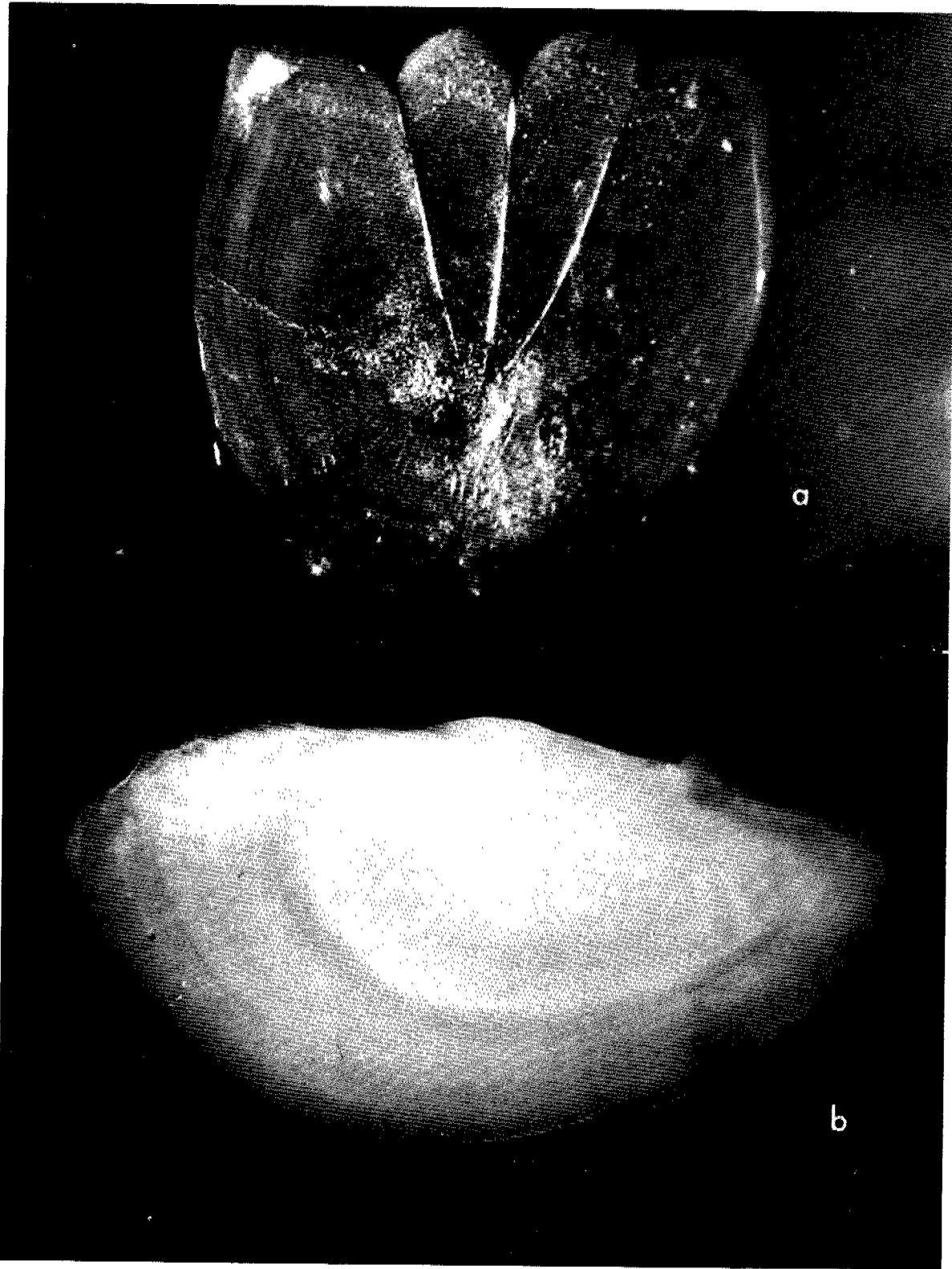
Figure 4. Scale (a) and otolith (b) from three-year-old male bonefish. Both show same "annular" markings.
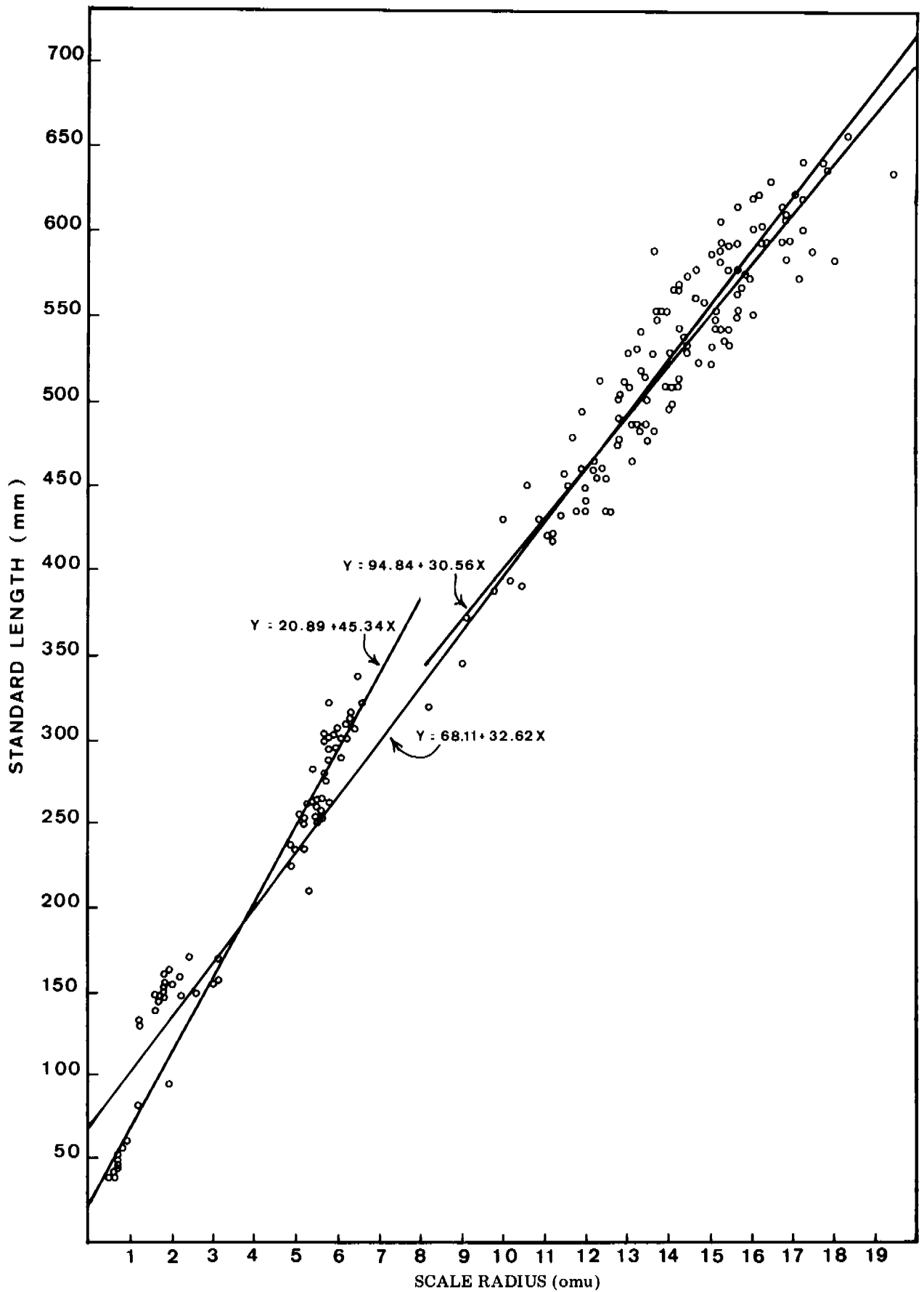
Figure 5. Relationship of scale radius to standard length for 204 bonefish.

ANNULUS FORMATION

Although bonefish inhabit subtropical and tropical waters essentially free from extensive thermal variations usually associated with annulus formation of fishes in temperate waters, "annular" marks on bonefish scales (and otoliths) are quite distinct. Explanations for annulus formation in tropical species are unfortunately not so distinct. Chevey (1933) felt that annual temperature variation of "only 4 to 5°C seems sufficient to cause a slowing up of growth in fish and evidence of this derangement in nutrition appears as markings on the scales." De Sylva (1963) assumed that annual temperature changes of only 6.5°C are sufficient to cause annulus formation on scales of great barracuda, *Sphyraena barracuda*, in south Florida waters.

Menon (1953), on the other hand, theorized there was "an inherent physiological rhythm as a more possible causative factor [than temperature] in the formation of growth checks." Moe (1969), in a thorough documentation of annulus formation in otoliths of red grouper, *Epinephelus morio*, from the Gulf of Mexico, suggests that annulus formation is a result of physiological changes associated with spawning time, increased photoperiod, and rising bottom water temperatures in spring. He further states that temperature may not significantly influence annulus formation because fluctuations are only 5 to 6°C annually. Annulus formation in king mackerel, *Scomberomorus cavalla*, seems to be related to gonadal maturation (Beaumariage, 1973).

Many fishes in tropical waters have protracted spawning seasons (Voss, 1953; Voss, 1954; Clancey, 1956; de Sylva, 1963; Beardsley, 1967) making correlation between spawning time and annulus formation difficult (Moe, 1969). Alexander (1958) postulated that bonefish spawn year-round in West Indian waters. Bonefish leptocephalid larvae have been collected in Puerto Rico every month of the year (Erdman, 1960 and personal communication), further indicating extended spawning. If these suppositions are correct, then no single time can be fixed for annulus formation of the bonefish population; only for single individuals. Marginal increments have been used extensively to determine time of annulus formation in many species (Tabb, 1961; de Sylva, 1963; Moe, 1969; Beaumariage, 1973). Limited sample sizes in many months preclude using increments conclusively in this study, but the data point out differences in time of annulus formation (Table 2).

TABLE 2. MONTHLY MARGINAL INCREMENTS FROM BONEFISH SCALES.

Month	Mean Increment	Range	No. of Fish Examined
Jan.	0.75	0.4-1.5	8
Feb.	0.80	0.2-1.9	10
Mar.	0.90	0.2-2.8	22
Apr.	0.93	0.1-2.0	29
May	0.76	0.4-2.1	5
June	0.73	0.2-1.7	23
July	0.92	0.2-1.8	5
Aug.	0.70	0.5-1.1	3
Sept.	0.97	0.5-1.4	7
Oct.	0.79	0.2-2.3	27
Nov.	1.19	0.9-1.9	6
Dec.	0.70	0.4-1.0	2

BACKCALCULATION OF STANDARD LENGTH

Table 3 presents backcalculated standard lengths of age classes I through IX as derived from 140 sets of scale readings. Fish older than age IX were few in number and therefore not used in these calculations. Calculated lengths were determined by a modified direct proportion method described by Tesch (1968), using the equation

$$SL_a = \frac{SR_a \, (SL_c - C) + C}{SR_c}$$

where SL_a = calculated standard length, SR_a = radius to any annulus, SL_c = standard length at capture, SR_c = scale radius to margin and C is a constant (68.11) derived from the scale radius-standard length regression. The calculated constant (C) is not implied to be the length at which scales first develop. Empirical lengths are consistently larger than backcalculated lengths

TABLE 3. BACKCALCULATED STANDARD LENGTHS OF BONEFISH, AGE CLASSES I THROUGH IX.

Age Class	Mean SL at Capture	N	I	II	III	IV	Annulus V	VI	VII	VIII	IX
I	271	29	228								
II	375	9	251	322							
III	430	10	231[1]	313	381						
IV	478	29	226[2]	311	384	446					
V	519	19	218	298	371	450	496				
VI	544	21	213	289	360	431	486	523			
VII	576	9	217	305	377	445	497	536	561		
VIII	591	7	216	306	385	467	515	545	564	578	
IX	602	7	227	309	383	450	493	530	553	574	587
Number of backcalculations		140	137	111	102	92	63	44	23	14	7
Grand mean backcalculated standard length			224	305	376	445	495	530	559	576	587
Average annual increment			224	81	71	69	50	35	29	17	11
Range of backcalculated standard lengths			173-322	247-394	298-452	363-540	424-559	463-596	500-620	529-621	538-634
Standard deviation			27.7	307.5	379.1	449.0	30.1	28.3	30.9	27.7	35.5
Confidence intervals $t_{.05}$			171-279	†	†	†	438-556	479-589	495-623	516-636	500-674

[1] Two annuli indistinct at first year mark, therefore backcalculations not performed for this age.

[2] One annulus indistinct at first year mark, therefore backcalculation not performed for this age.

† Because of excessive standard deviation at this age, confidence intervals were not computed.

for any age class since collections do not coincide with the precise time of annulus formation.

LENGTH-WEIGHT RELATIONSHIP

Length-weight relationships were calculated for 169 fish ranging from 210-656 mm SL (8.4-26.4 in.) and 227-6010 g (0.5-13.2 lb). Seventy-seven males, 88 females and four fish of undetermined sex were used in these calculations. The length-weight relationship for all fish is expressed by the equations

$$W = 2.799 \times 10^{-5} SL^{2.9321} \text{ or}$$

$$Log W = 2.9321 Log SL - 4.5529$$

Sexes were also considered separately to evaluate possible differences. The length-weight relationship for males is expressed by

$$W = 1.418 \times 10^{-5} SL^{3.0433} \text{ or}$$

$$Log W = 3.0433 Log SL - 4.8483$$

and for females by

$$W = 4.6526 \times 10^{-5} SL^{2.8492} \text{ or}$$

$$Log W = 2.8492 Log SL - 4.3323$$

(Figure 6). At the $\alpha = .05$ level (t = 1.96, ∞ df) a significant difference is apparent between sexes (t = 3.12).

STANDARD LENGTH-FORK LENGTH RELATIONSHIP

Standard and fork lengths are very highly correlated (r = 0.999) and this relationship can be expressed by the equations FL = 1.07 SL, or SL = 0.93 FL.
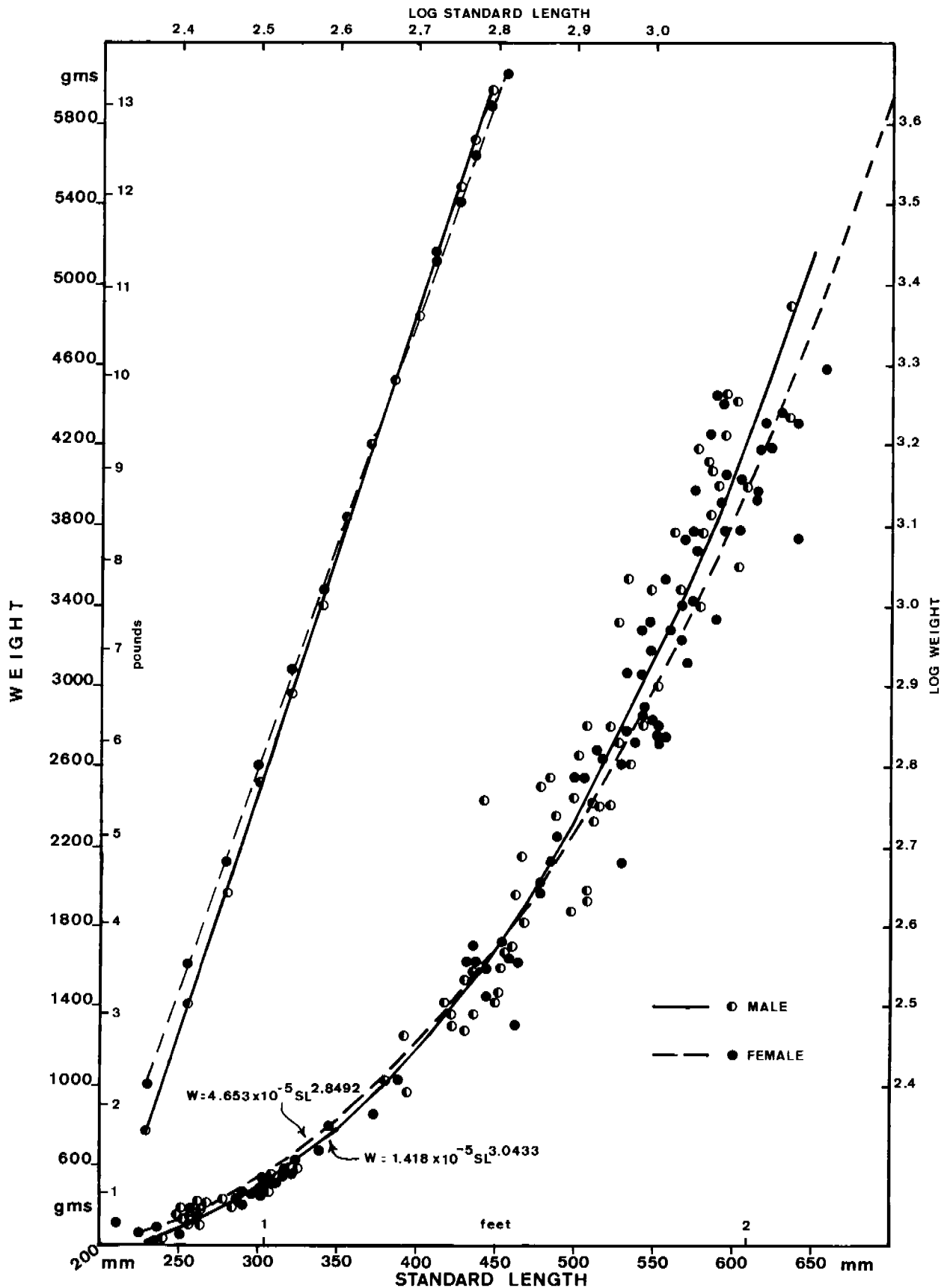
Figure 6. Relationship of length to weight of 77 male and 88 female bonefish.

FOOD HABITS

Stomachs and intestines of 129 fish were examined to determine food habits. Identifications were made to species when possible, but food items were often crushed beyond recognition by the powerful, well-developed pharyngeal teeth.

Crustaceans, principally penaeid and alpheid shrimp and portunid and xanthid crabs, found in 73% of the stomachs, predominated as food items (Table 4). Noncrustacean items, listed in Table 5, including fish and fish remains (present in 15% of the stomachs), mollusks (present in 28%), and miscellaneous material contributed little either volumetrically or numerically. Nine-

teen stomachs (15%) were either empty or contained no recognizable food items.

Anglers and guides occasionally broadcast shrimp and crab parts in likely areas to attract fish and encourage feeding. Consequently, the relative proportion of such crustaceans found during this study may have been biased to some extent by this practice. Despite this possible bias, crustaceans are thought to comprise a substantial portion of the bonefish diet.

Erdman (1960) found clams (mainly *Codakia costata*) comprising 40% of stomach contents and crabs 30% (by frequency of occurrence) in bonefish from Puerto Rico. The dissimilarity of diets between Florida and Puerto Rican stocks is probably related to

TABLE 4. FREQUENCY OF OCCURRENCE OF CRUSTACEAN FOOD ITEMS FOUND IN 129 BONEFISH STOMACHS, EXPRESSED AS NUMBER OF STOMACHS CONTAINING A PARTICULAR FOOD ITEM AND AS A PERCENTAGE OF TOTAL NUMBER OF STOMACHS EXAMINED.

Food Item	Number Stomachs Containing Items	Percentage
Stomatopoda		
Pseudosquilla ciliata	5	3.9
Decapoda		
Nantantia		
Penaeidea		
Penaeidae—shrimps	23	17.8
Penaeus sp.	2	1.6
Penaeus duorarum	2	1.6
Caridea	3	2.3
Palaemonidae	1	0.8
Pontonia sp.	1	0.8
Alpheidae—snapping shrimp	45	34.9
Reptantia		
Macrura		
Callianassidae	3	2.3
Brachyura		
Calappidae—shameface crabs	3	2.3
Calappa sp.	2	1.6
Protunidae—swimming crabs	22	17.1
Portunus sp.	12	9.3
Portunus depressifrons	5	3.9
Callinectes sp.	1	0.8
Xanthidae	21	16.3
Panopeus sp.	24	18.6
Pilumnus sp.	1	0.8
Goneplacidae—walking crabs	1	0.8
Grapsidae	1	0.8
Ocypodidae		
Uca sp.	1	0.8
Uca speciosa	1	0.8
Oxyrhyncha—spider crabs	11	8.5
Majidae		
Pitho sp.	7	5.4
Labinia sp.	2	1.6
Parthenopidae		
Parthenope sp.	1	0.8

relative availability of food items, and demonstrates that bonefish are generalized carnivores capable of utilizing a large variety of food items.

Stomachs of many bonefish were parasitized

to varying degrees by acanthocephalid worms tentatively identified by Dr. W. L. Bullock (University of New Hampshire, personal communication) as *Dollfusentis heteracanthus* (Cable and Linderoth).

TABLE 5. NONCRUSTACEAN FOOD ITEMS FOUND IN BONEFISH STOMACHS

Mollusca

Bivalvia	Gastropoda
Arcopsis adamsi	*Astraea* sp.
Argopecten gibbus	*Cerithium eburneum*
A. nucleus	*C. muscarum*
Cardita domingensis	*Conus jaspideus*
C. floridanum	*Cylichna* sp.
Chione cancellata	*Diodora cayenensis*
Codakia sp.	*Fasciolaria hunteria*
C. obicularis	*F. tulipa*
Lima pellucida	*Hyalina avena*
Lucina blanda	*Natica canrena* (?)
L. pensylvanica	*Olivella* sp.
Lyonsia hyalina	*Prunum apicinum*
Nuculana acuta	*Retusa* sp.
Tagelus sp.	
Tellina sp.	
Tellina similis	
Transennella sp.	

Merostomata

Xiphosura sp.—horseshoe crabs

Pisces

Ophichthidae—snake eels
Ophidiidae—cusk-eels and brotulas
Syngnathidae—pipefishes and seahorses
Opisthognathidae—jawfishes
Gobiidae—gobies
Scorpaenidae—scorpion fishes
Triglidae—searobins
Balistidae—triggerfishes and filefishes
Miscellaneous unidentifiable fish parts

Miscellaneous

Sipunculid worms
Anemones
Thalassia
Sand

REPRODUCTION

Microscopic examination of histologically prepared gonadal sections was used to describe oogenesis and spermatogenesis and to thereby evaluate spawning time in bonefish collected in this study.

OOGENESIS

Descriptions of oogenetic stages follow Moe (1969). Oocytes were measured across the largest dimension of a cell, since cells were not always circular. Care was taken to measure only those oocytes sectioned through the center and

containing nuclear material.

Primary stage—oogonia; stage 1—early oocytes; stage 2—resting, previtellogenic oocytes, (Figures 7, 8): oogonia were not often seen and for this reason any that were discerned are grouped with stages 1 and 2. Discrimination between late stage 1 and early stage 2 oocytes was also difficult as development during this time is rapid. Therefore, evaluations of size and development of these stages is approximate.

Stage 1 oocytes (Figure 7) range from about 18 to 65 μm in diameter, with oocytes being round to oval and often angular. Nuclei are relatively large, approximately 50% the cell diameter, with a well-defined, basophilically staining nuclear membrane. The nucleus typically contains one prominent nucleolus and several less prominent ones. Cytoplasm is strongly basophilic, containing large, less basophilic inclusions.

Stage 2, resting previtellogenic oocytes (Figure 8), range to approximately 150 μm. Oocyte shape varies considerably during this stage. Nuclear diameters range from 25 to 50% of the major axis. The nucleus is well defined, granular, and contains many nucleoli, one of which is dominant in size. Both cytoplasm and nucleus stain basophilically, cytoplasm very strongly, and nuclei less so.
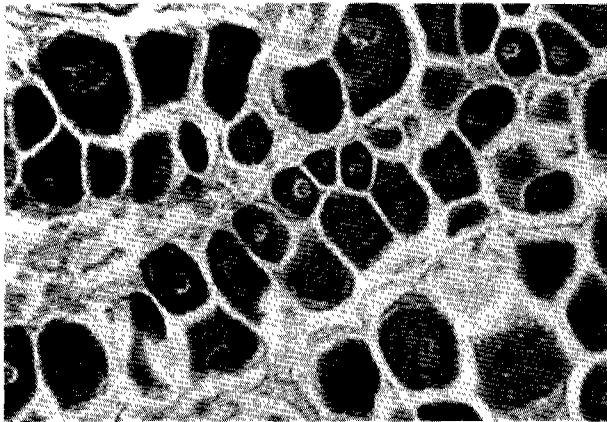

Figure 7. Photomicrograph of stage 1 oocytes (center). Taken from the gonad of a 553 mm SL five-year-old female captured in June 1970.
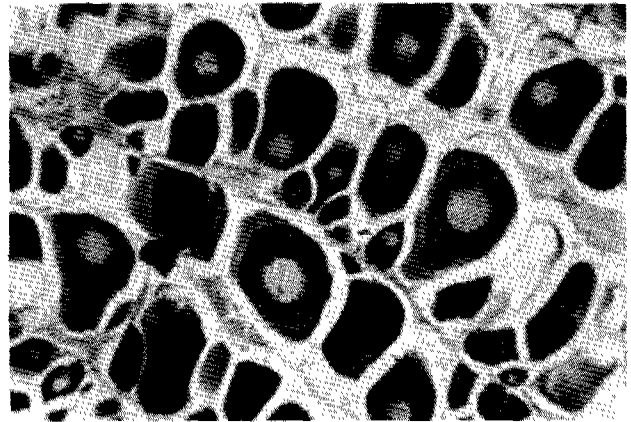

Figure 8. Photomicrograph of oocytes in stage 2 (center) and stage 3 (right). This gonad was taken from a 445 mm SL three-year-old female in June 1970.
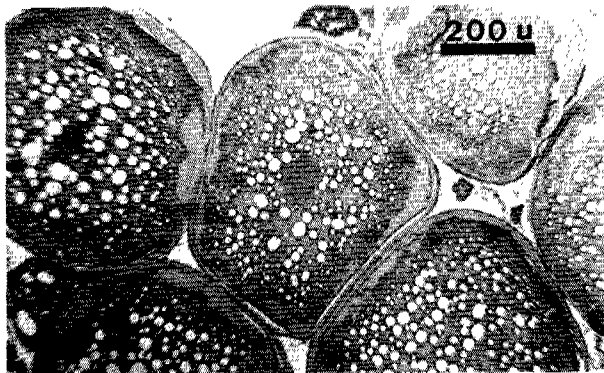

Figure 9. Photomicrograph of oocyte in mid to late stage 4. This gonad came from a 586 mm SL seven-year-old female collected in April 1971.
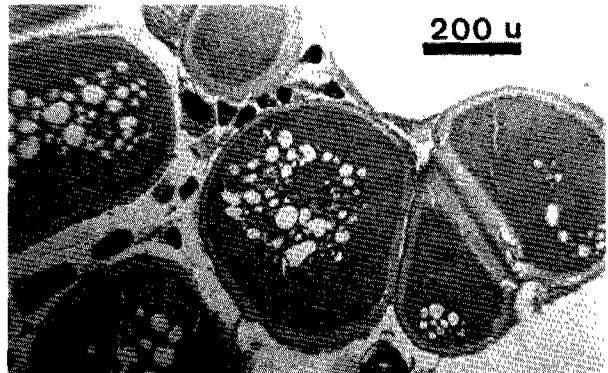

Figure 10. Photomicrograph of oocytes in late stage 4. Yolk vesicles have started to coalesce around the nucleus. Taken from a 263 mm SL one-year-old female in October 1970.

Stage 3—early vitellogenic oocytes; Plate I(a): this stage ranges from about 75 to 250 μm in diameter, with a mean of 146 μm (based on 332 measurements). Oocytes become rounded as they increase in size. Nuclei range from about 34 to 42% of the cell diameter, with the nuclear periphery surrounded by many nucleoli, none of which are dominant, and a distinguishable basophilic nuclear membrane. Cytoplasm has taken on a very fine granular appearance and stains basophilically, but has lost the strong affinity for hematoxylin that was apparent in stage 2. The zona radiata has become apparent and stains acidophilically.

Stage 4—vitellogenic oocytes; Plate I(b), (Figures 9, 10): rapid growth of oocytes begins in stage 4 when oocytes attain maximum size before spawning. Diameters range from approximately 165 to 700 μm, with a mean of 394 μm (from 506 measurements). Early in this stage, the nucleus is well defined and bordered by numerous basophilic nucleoli. Gradually the cytoplasm is replaced by acidophilic yolk globules. Yolk vesicles are abundant throughout the cytoplasm and as the stage progresses they coalesce near the nucleus. The acidophilic zona radiata thickens as the stage progresses. During the final phase of stage 4 yolk globules coalesce to give the oocyte a completely smooth acidophilic appearance broken only by a few yolk vesicles.

Figure 11. Photomicrograph of oocyte in early stage 5. Few examples of this stage were found. Collected in October 1970 from a 251 mm SL one-year-old female weighing 327g (11.5 oz.)

Stage 5—egg stage; (Figure 11): few representatives of this stage were available since this is the final stage before spawning, maturation and ovulation apparently taking place very rapidly. This stage is characterized by a very lightly staining, acidophilic yolk mass broken only by a scattering of yolk vesicles. Oocytes in this stage often lose rotundity and assume amoeboid shape, possibly caused by histological processes.

SPERMATOGENESIS

Spermatogenesis is divided into five stages: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. For purposes of this study it was neither desirable nor necessary to thoroughly document precise origins and development of spermatogenesis of bonefish. Rather, only the relative abundance of various stages was considered, thus providing an index of the degree of ripeness. The varying degrees are covered in the following section.

GONAD DEVELOPMENT CLASSES

Nine classes have been established as a basis for evaluating seasonal gonadal development and spawning time. These classes correspond roughly to those of Moe (1969) and are briefly summarized here.

Class 1—immature female: this class is characterized by lack of evidence indicating previous spawning. Only stage 1 and 2 oocytes are present; no atretic bodies are apparent. The ovary is rather small in cross section and very compact.

Class 2—mature resting female: this class is defined by ovaries which have undergone vitellogenesis but have returned to a resting state. Oocytes of stages 1, 2, and 3 are present, but stage 2's predominate. Atretic bodies may be present. There are no indications that vitellogenesis is advancing.

Class 3—mature active female (prespawning): this class is described by ovaries undergoing extensive vitellogenic activity. Oocyte stages 1 through 4 are present, with stages 3 and 4 forming the mass of developing oocytes.

Class 4—spent female (postspawning): this class contains those ovaries showing evidence of recent spawning. Any unshed stage 3 and 4 oocytes are degenerating and the incidence of atretic body formation is high.

Class 5—immature male; (Figure 12): this class is characterized by small compact testes
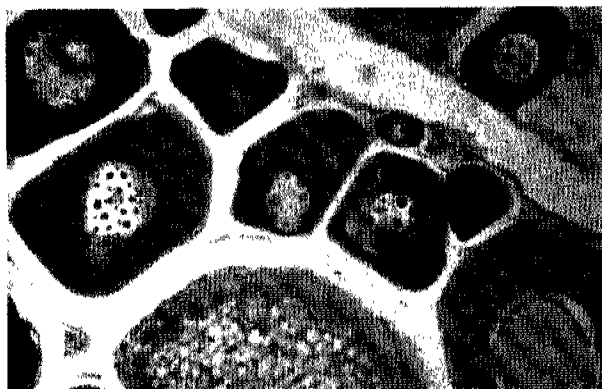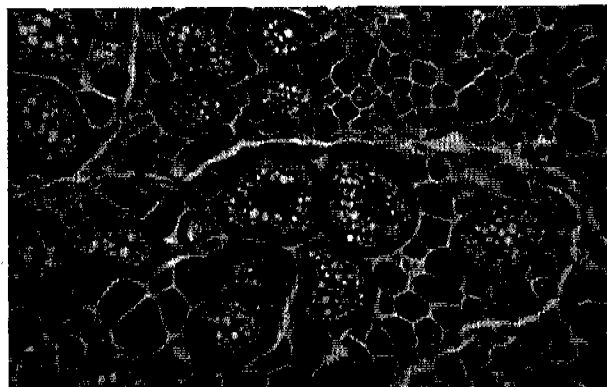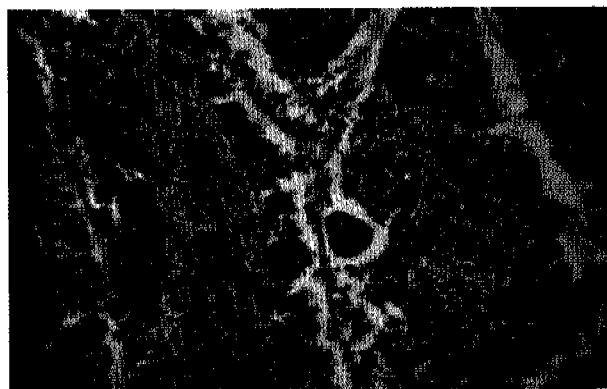
Plate I. Photomicrographs of oocyte and spermatozoa development in gonads of bonefish. a) Oocytes in early stage 2 and stage 3. Gonad was collected from a 543 mm SL age 4 female in April 1970. b) Oocytes in early stage 4 (center). Oocytes in stages 1, 2, and 3 are also seen throughout the gonad. Taken from a 533 mm SL five-year-old female in June 1970. c) Male gonad in class 6. Early stages of spermatogenesis indicated by presence of spermatogonia, primary and secondary spermatocytes. From a 512 mm SL four-year-old male collected in April 1970. d) Male gonad in class 7. Primary and secondary spermatocytes, spermatids, and some spermatozoa are present. Collected from a 553 mm SL age 6 male taken in October 1970.

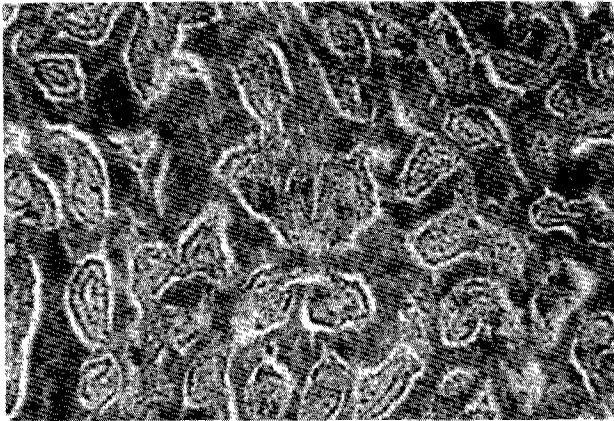containing only spermatogonia. Crypts are tight-ly packed and there is no evidence of previous spawning.

Figure 12. Photomicrograph of immature male gonad. Spermatogonia are just beginning to develop. Taken from a 320 mm SL three-year-old male collected in June 1970 (class 5).

Class 6—mature inactive male; Plate I(c): testes are characterized by the presence of early stages of spermatogenesis. Spermatogonia and primary and secondary spermatocytes are present in crypts.

Class 7—ripening mature male; Plate I(d): spermatogonia are rarely present in this class. Primary and secondary spermatocytes have developed into spermatids and spermatozoans. The latter have begun to collect in the central portion of the crypts.

Class 8—ripe male; (Figure 13): this class is characterized by an almost complete absence of any stage other than tailed sperm. Secondary spermatocytes and spermatids are occasionally present but their development into spermatozoa is quickly forthcoming. Crypts have ruptured and the entire gonad is filled with tailed sperm.

Class 9—spent male (postspawning); (Figure 14): testes of this class show evidence of recent spawning and are returning to an inactive resting state (Class 6). New spermatogonia are develop-ing and any unshed sperm from the previous spawning season may be dispersed throughout the gonad.

Figure 13. Photomicrograph of ripe male (class 8). Spermatozoa fill the entire gonad. This gonad was taken from a 578 mm SL seven-year-old male collected in April 1972.
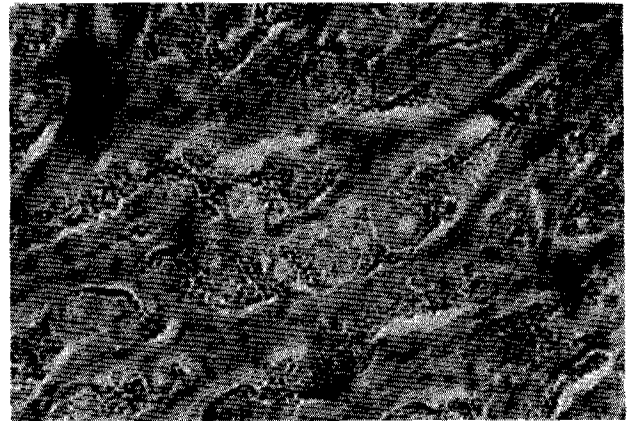

Figure 14. Photomicrograph of spent gonad (class 9) from a 487 mm SL five-year-old male collected in October 1970. Connective tissue predominates but there is evidence that this gonad is returning to a resting, inactive state (class 6).

Figure 15 presents data on the monthly occurrence and distribution of gonadal states of 80 females and 68 males collected from 1970 to 1972. Only those fish for which an accurate assessment was made were used in these evalu-ations. Although monthly collections were usually small, data definitely indicate that ripe and near-ripe males and females are present throughout the year in Florida waters. Spawn-ing may therefore occur year-round as hypothe-sized by Alexander (1958) for West Indian stocks.
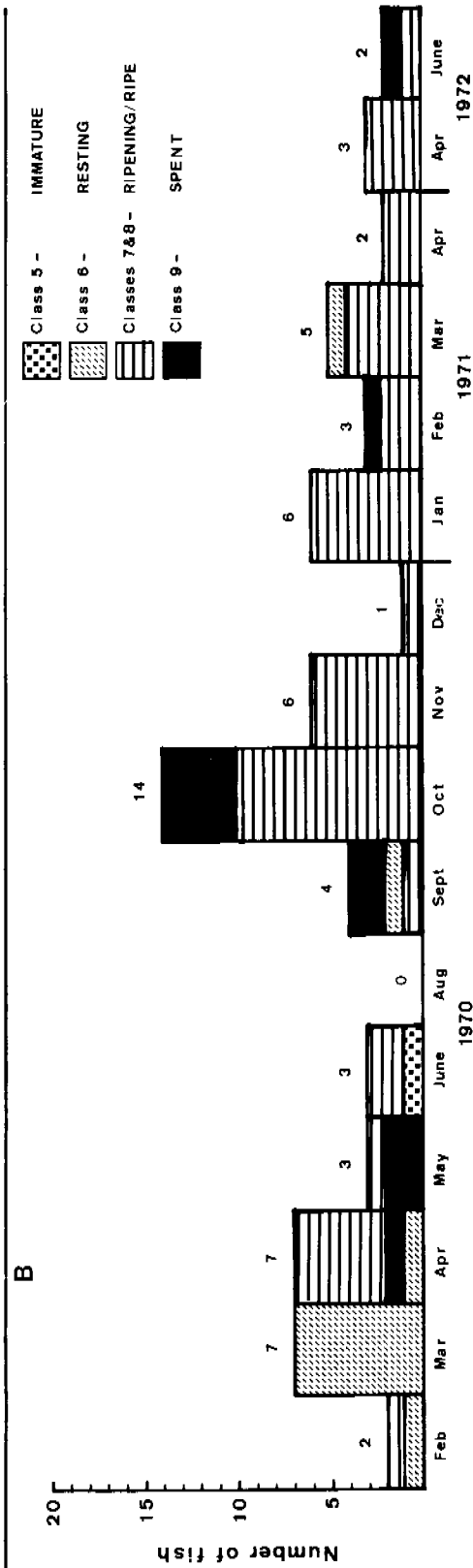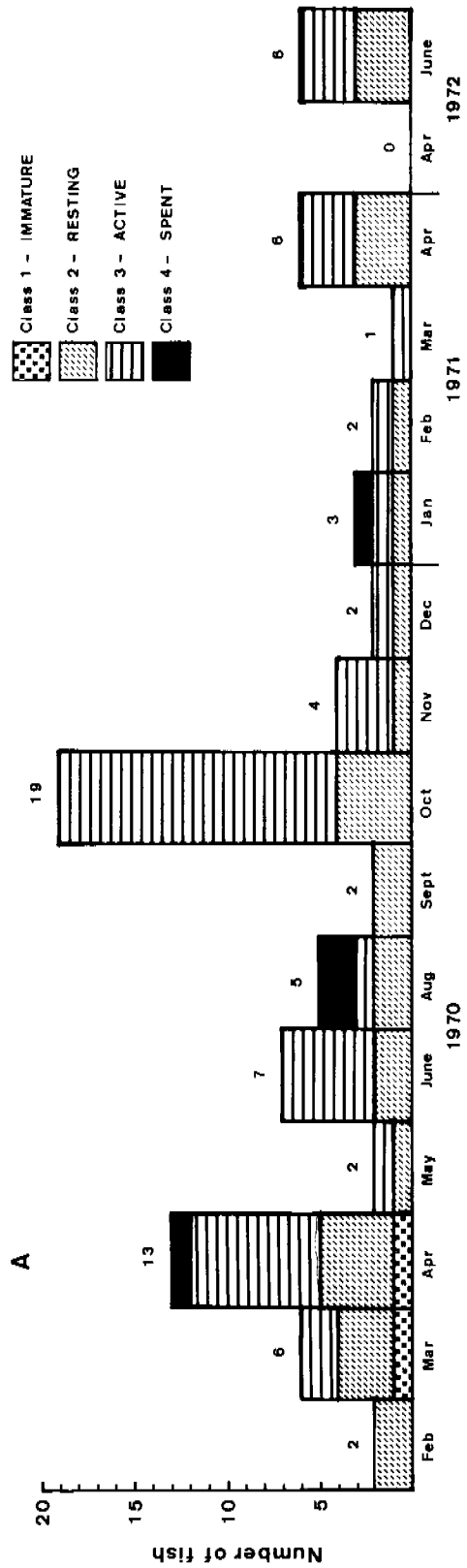
Figure 15. Number of bonefish in each gonadal development class collected from Florida Keys 1970-1972. Numbers at top of columns indicate number of fish per month for which determination was possible. (A) Female. (B) Male.

SPAWNING

Occurrence of sexually mature fish, barely one year old, 210 to 338 mm SL (8.3-13.3 in.), weighing 227 to 680 g (0.5-1.5 lb) was unexpected. Gonads from females among these fish contained mid to late stage 4 oocytes almost exclusively, and also the only examples of stage 5 oocytes encountered (Figure 13). Extensive meristic and morphometric comparisons have shown these specimens to be conspecific with *Albula vulpes* (Topp and Beaumariage, personal communication). All these small fish were collected during April, August, October, November, and December on the coastal side of reefs separating the Keys from the Florida Straits, at depths of 9.1 to 12.2 meters (30-40 ft). Among larger females, those containing advanced stage 4 oocytes were also taken in deeper waters. Alexander (1958) postulated that bonefish eggs "are spawned either offshore or in areas where currents would take the eggs offshore" and Meek (1916) felt albulid fishes spawned at sea where the larvae developed (after Alexander, 1958). Since spawning of these fish may be imminent within a very short time, there are two alternatives as to spawning location: 1) spawning could occur on the inside of boundary reefs; however, the relative lack of larvae collected by extensive sampling in this area of Florida's waters (Eldred, 1967) suggests that 2) ripe fish are moving over the reefs into the Straits of Florida where depths quickly drop to many hundreds of feet and where currents would quickly disperse both eggs and developing larvae.

SUMMARY AND CONCLUSIONS

1. One hundred fifty-five bonefish, *Albula vulpes* (Linné), were collected from February 1970 through April 1971 from sport-fishermen in the lower Florida Keys. Scale samples, standard, fork, and total lengths, gonadal section, and stomach contents were taken for each fish. An additional 54 samples were obtained in April and June 1972 and from various museum collections.

2. Scales were used for age determinations and as a basis for backcalculating standard lengths at previous ages. They were cleaned, immersed in glycerin, and examined for annual marks. Assessments were made on 183 of 205 sets of scales examined (89.3%). Age classes 0 through XII were represented among those fish aged and there are indications that ages higher than XII may be attained.

3. Criteria established by van Oosten (1929) regarding validity of the scale aging method are discussed and have tentatively been satisfied. Relationship between scale raduis and standard length was demonstrated by fitting regression lines to the data; the resultant correlation coefficients were sufficiently high (r = 0.985, r = 0.936, r = 0.988) to demonstrate proportionality between scale radius and standard length.

4. Causes and time of annulus formation are discussed. No single time can presently be established for annulus formation in bonefish. Use of marginal increments to establish time of annulus formation was not conclusive because collections were often sporadic. Further sampling and evaluation are necessary in this area.

5. Agreement between mean empirical and mean backcalculated lengths is shown. These data indicate a distinct change in rate of growth between ages III and IV.

6. The exponential equation
$$W=2.799 \times 10^{-5} \ SL^{2.9321}$$
expresses the length-weight relationship of bonefish. Sexes were also considered separately and their equations calculated. Dimorphism is evident from the length-weight relationship.

7. Standard length-fork length relationship is expressed by the equations SL = 0.935FL or FL = 1.07SL, and is highly linear.

8. Decapod crustaceans, especially penaeid and alpheid shrimp and portunid and xanthid crabs formed the majority of food items encountered. Fish and mollusks were only occasionally found.

9. Sections of gonadal material were fixed in Bouin's fluid, later sectioned at six microns and stained with Papanicolaou hematoxylin (Harris)-eosin Y.

10. Microscopic analysis of gonadal sections was used to evaluate oogenesis and spermatogenesis and to determine spawning time.

11. The presence of ripe and near-ripe females and males throughout the year was evidence that there is at least some year-round spawning in the species.

12. Sexual maturity may be reached at an age of only one year, and a near-ripe female may be as small as 326.6 g (11.5 ounces) and 251 mm SL (9.9 inches).

13. Spawning may occur in deep waters adjacent to the Florida Keys where developing eggs and larvae would quickly be dispersed by currents. Additional evidence of this must await further sampling.

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