AGE, GROWTH, AND REPRODUCTION OF THE NORTHERN PUFFER, *SPHOEROIDES MACULATUS*[1]

JOANNE L. LAROCHE[2,3] AND JACKSON DAVIS[4]

ABSTRACT

Rings on vertebral centra of the northern puffer, *Sphoeroides maculatus*, were shown to be true year marks. Age groups 0 to V were found in 1,128 specimens collected from Chesapeake Bay. Females were significantly larger than males at each age. Most growth in length took place during the first growing season from June through October. Sexual maturity was reached at age I. Peak spawning occurred in June and July.

The northern puffer, *Sphoeroides maculatus* (Bloch and Schneider), occurs on the Atlantic coast of North America from Newfoundland to Flagler County, Fla. (Shipp and Yerger, 1969). It is the only tetraodontid that is abundant along the middle Atlantic coast and that comprises a large part of the spring and fall commercial catches in Chesapeake Bay. During the period 1962 to 1970, total annual landings from Chesapeake Bay ranged from 1 to 12 million pounds. The steady northward progression of commercial landings in the spring from North Carolina to Chesapeake Bay and the southward progression of catches in the fall suggest an annual coastal migration.

There is little published information on the life history of the northern puffer. Welsh and Breder (1922) reported the food habits, larval development, and weight-length relationships of puffers from New Jersey. Shipp and Yerger (1969) reviewed the taxonomy of *S. maculatus*.

This paper describes a technique for determining the age of the northern puffer and reports the rate of growth, weight-length relationships, age at sexual maturity, and spawning time.

This is the first published report of age determination for a tetraodontid.

MATERIALS AND METHODS

Age and growth determinations were based on 123 fish captured during November 1969 and 1,005 fish collected from April through November 1970 from lower Chesapeake Bay, Mobjack Bay, and the mouths of the York and Rappahannock rivers, Va. A homogeneous puffer population in the area sampled is assumed. Fish were primarily obtained from commercial pound nets and haul seines but also with a push net, beach seine, and trawl and from commercial crab pots.

Total length was measured from the tip of the snout to the center of the flared caudal fin. Fish with a distended ventral sac were not weighed because water was retained in the sac. Fish were classified as immature, gravid, running ripe, or spent by gross inspection of the gonads.

In a preliminary study, otoliths, cleithra, opercles, jaw bones, fin rays, and all the vertebrae were removed from a small number of fish. Afterwards only vertebrae No. 2-No. 7 were removed from the fish and were disarticulated and then stained in a solution of 4-8% KOH and Alizarin Red S. The stain reduced glare from the centrum surface and increased contrast

[1] Contribution No. 525, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

[2] This paper is adapted from a thesis submitted in partial fulfillment of the degree of Master of Arts, College of William and Mary, Williamsburg, Va.

[3] Virginia Institute of Marine Science, Gloucester Point, VA 23062; present address: Ira C. Darling Center, University of Maine, Walpole, ME 04573.

[4] Virginia Institute of Marine Science, Gloucester Point, VA 23062.

Manuscript accepted April 1973.
FISHERY BULLETIN: VOL. 71, NO. 4, 1973.

between growth bands while KOH removed adherent flesh. Staining techniques of Hollister (1934), Galtsoff (1952), Daiber (1960), and La Marca (1966) were not effective in staining growth marks differentially. Puffer vertebrae have deeply concave centra and had to be sliced lengthwise in order to measure straight-line distances. Vertebrae No. 4 and No. 5 with the neural spines removed were placed dorsal surface up in a V-shaped slit in a wood block and were sliced with a hand-held razor blade. All vertebral measurements were made on vertebra No. 4 using an ocular micrometer in a dissecting microscope at 15× under reflected light. The marginal width or distance between the last annulus and the centrum edge was measured on the posterior segment of vertebra No. 4.

AGE DETERMINATION

Vertebrae and saccular otoliths exhibited growth marks, although only the growth marks on vertebral centra were both distinct and consistent. Growth marks on otoliths were ambiguous and could not be distinguished in larger fish. Cleithra, opercles, jaw bones, and fin rays exhibited no discernible growth marks.

Growth marks on northern puffer vertebrae formed distinct steps on the centrum surface which are best seen when a vertebra is rested on one end at a 45° angle from the vertical and is viewed looking down on the centrum face. The step is more opaque and its surface texture appears coarser under magnification in reflected light than the rest of the centrum. A narrow, dark, translucent ring appears along the inner edge of each step. The step and narrow dark band form a continuous ring on the centrum and constitute the annulus. Broad opaque bands with uniform surface texture lie between the narrower annuli. Together these features represent 1 yr's growth. Season marks on black bullhead, *Ictalurus melas*, vertebrae are narrow, translucent bands associated with a depression in the centrum surface and alternating with broad, opaque bands (Lewis, 1949). In contrast, narrow dark annual bands on channel catfish, *I. punctatus*, were usually associated with a ridge (Appelget and Smith, 1951).

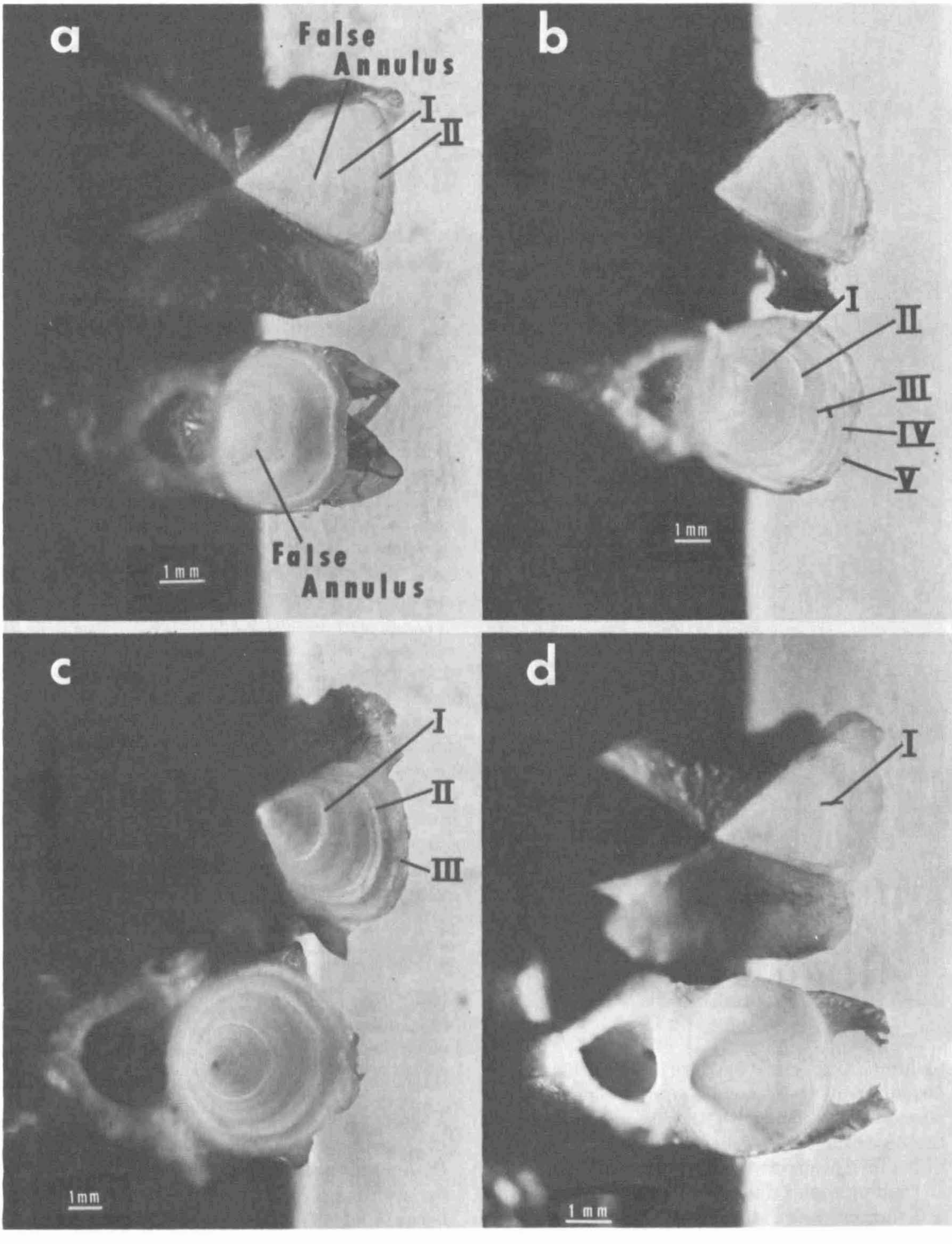
Five age groups of northern puffer were found by considering the marks on vertebrae to be annuli.

There was excellent agreement among vertebrae No. 3-No. 7 in the number and position of annuli. Age could not be assigned because of disagreement in less than 1% of the fish. However, age could not be determined in 7% of the fish because of false annuli, crowding of annuli at the centrum edge, and changes in the surface texture of centra in older fish. False annuli were faint dark bands which were not associated with a distinct step. Another extraneous mark was a shallow depression forming a continuous ring on the centrum which was not a definitive step. This feature was found on black bullhead vertebrae (Lewis, 1949). False annuli were most frequent in the first growth zone (Figure 1a). With increasing age, annuli became crowded at the centrum edge (Figure 1b), and the surface texture of centra became more coarse and irregular, especially near the edge (Figure 1c). However, there was no indication that annuli at the centrum edge were obscured by crowding in the 58 age III and 17 age IV fish examined.

VALIDATION OF THE ANNULUS

The annular step on vertebrae of the northern puffer met four of Hile's (1941) criteria for validating the annulus as a true year mark. First, a single annulus was formed each year. No annuli were present on vertebrae of yearlings in April and early May, but a distinct ring became visible at the outer edge of the centrum late in May when growth resumed (Figure 2). Annulus formation began in May, and by August most yearlings had formed an annulus (Figure 3). The fish taken in April with a single annulus and wide marginal width

FIGURE 1.—Vertebrae of the northern puffer: (a) vertebrae from a 2-yr-old northern puffer showing a false annulus adjacent to the first annulus, (b) vertebrae from a 5-yr-old northern puffer on which the third annulus appears double because of the expanded step, (c) vertebrae from a 3-yr-old northern puffer, (d) expanded annular step on the vertebrae from a 1-yr-old northern puffer.


a
False
Annulus
I
II
False
Annulus
1 mm
b
I
II
III
IV
V
1 mm
c
I
II
III
1mm
d
I
1 mm

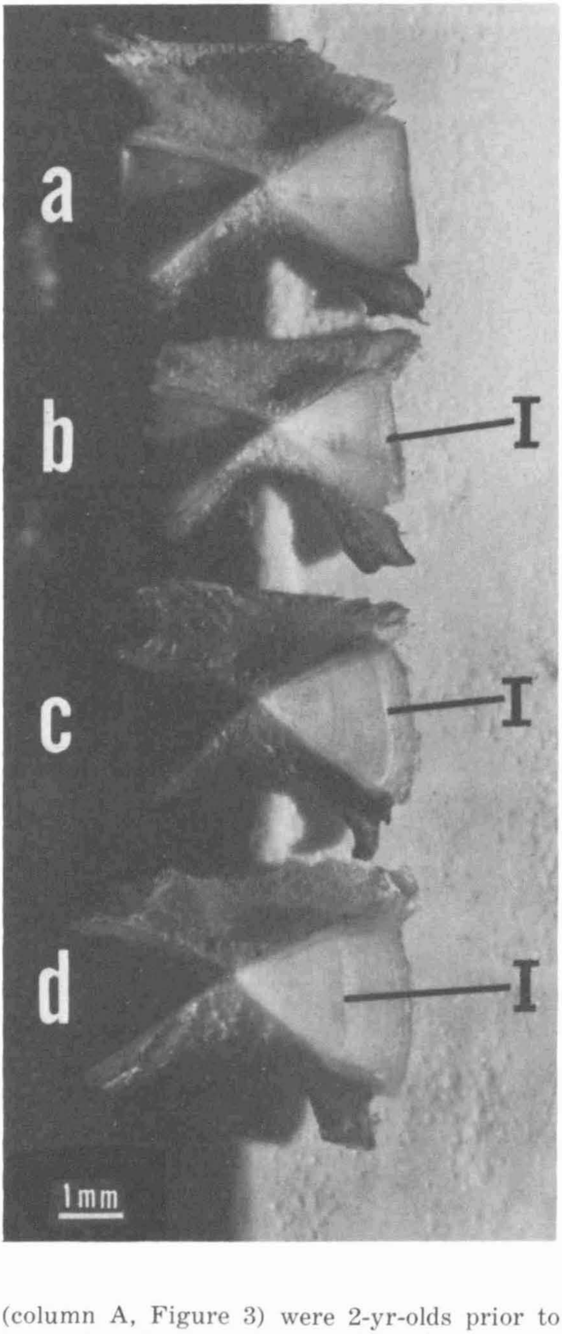


FIGURE 2. (left)—Vertebral sections of the northern puffer showing the increase in margin width after formation of the first annulus: (a) young-of-the-year female captured in November, (b) female captured in May with the first annulus just visible at the centrum edge, (c) age I male captured in September, (d) age I male captured in November.

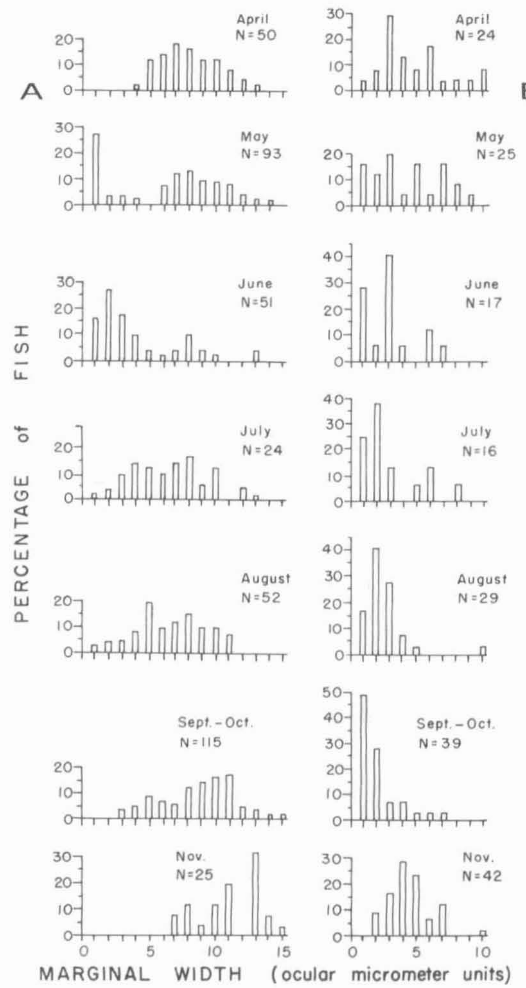


FIGURE 3.—Frequency distributions of margin widths (distance between the last annulus and the centrum edge) of vertebral centra from the northern puffer: (A) with a single annulus, (B) with two annuli.

(column A, Figure 3) were 2-yr-olds prior to formation of the second annulus. The greatest percentage of fish with the second annulus at the centrum edge occurred in September. Annulus formation on the centrum may take longer than on scales and otoliths because vertebrae are larger and structurally more complex. Yasuda (1940) showed that annuli on vertebrae of *Scombrops* sp. were formed 1.5 mo later than on otoliths. In older fish the length of time for annulus formation may be extended further because of the decreasing annual addition to

TABLE 1.—Calculated total length at end of each year of life of male and female northern puffer.

Age group	Number of fish		Mean length at capture (mm)		Mean calculated total length (mm) at end of year of life							
					1		2		3		4	
	M	F	M	F	M	F	M	F	M	F	M	F
I	190	315	181	200	133	151	—	—	—	—	—	—
II	88	99	204	235	125	141	183	208	—	—	—	—
III	22	32	224	256	125	128	178	194	208	237	—	—
IV	7	9	236	274	146	137	185	194	213	238	227	259
Weighted mean length					131	147	182	204	209	237	227	259
$S_{\bar{x}}\ t_{.05}$					±2.5	±2.2	±3.5	±4.2	±6.8	±6.1	±18.4	±15.6
Length increment					131	147	52	58	27	33	19	22
Calculated length range					93-191	90-203	140-231	161-260	172-246	197-279	201-250	236-289
Measured length range					120-225	125-259	161-232	181-264	199-256	223-292	211-263	246-295

vertebral length as body length increments decrease.

The second criterion was met by the agreement of measured lengths with length at each age as calculated by the Lee method (Lagler, 1956) (Table 1). Mean weighted lengths at each age coincided closely with the measured length for fish 1 yr younger because most fish were taken in the fall after the growing season and in late spring before the growing season. Total vertebral length and distance between annuli across the center of the vertebra were used to calculate lengths. Measurements were made along the shortest straight line connecting the approximate midpoints of annuli and the centrum edge (Figure 4). The distance between annuli could not be measured with certainty in 5% of the fish because the annular step was expanded into a broad, opaque shelf without a distinct end point (Figure 1d). The plot of body length on vertebral length was approximately linear, and the regression equation was $L = 27.52 + 3.28V$ with a significant correlation coefficient of 0.9827 where L is total length in millimeters and V is vertebral length in ocular micrometer units.

The third and fourth criteria were met by the correspondence of peaks in the length-frequency plots for each age group with peaks in the plot of the entire sample and by the regular increase in number of annuli with increasing body length (Figure 5). Overlap in mean body length among age groups was caused by early summer annulus formation in yearlings coupled with an extended period of annulus formation in older age groups. The wide range of lengths attained in the first growing season also contributes to the observed overlap of body lengths. The biomodal distribution of females in age group I was caused by the great number of fish larger than 210 mm taken in April and May from culled and sorted commercial pound net catches. Throughout the rest of the sampling period, the majority of fish came from unculled pound net and haul seine catches.

Therefore, the annulus on northern puffer vertebrae has been validated based on agreement with these criteria.

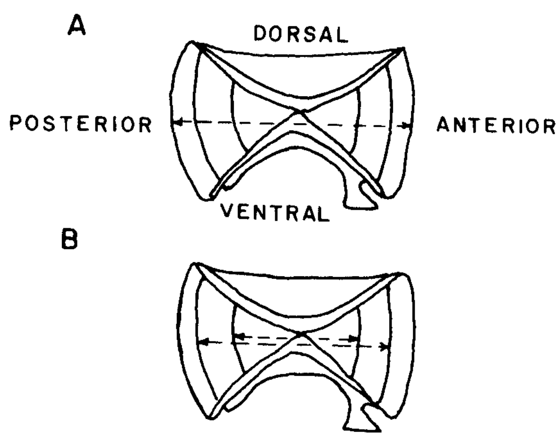


FIGURE 4.—Measurements taken on vertebral sections which were used in calculating length at age: (A) vertebral length, (B) distance between annuli across center of vertebra.

GROWTH

Young-of-the-year which were first collected in June from shallow eelgrass (*Zostera marina*) beds in the York River averaged 28 mm in length. Young-of-the-year in the fall of 1970 ranged from 88 to 184 mm (Table 2). The November sample came from a culled pound net catch; therefore, 160 to 184 mm is not a true indication of the length range after the first growing season. A better estimate is 83 to 195 mm based on lengths of yearlings without an annulus taken in April and May with a trawl and from unculled pound net catches. This corresponds closely to the calculated range of lengths at age I for all fish, 90-203 mm (Table 1). In December a live 37-mm juvenile was found floating at the surface near the mouth of the York River. Several fish of this size were collected in October on the east side of Chesapeake Bay during 1968 and 1969 by personnel of Virginia Institute of Marine Science. It is not known whether these small fish were hatched late in the season in Chesapeake Bay or were southern migrants from more northern spawning stocks.

The von Bertalanffy mathematical expression of growth as outlined by Ricker (1958) closely fits the weighted mean length at each age for age groups I to IV (Figure 6). The weighted mean length for age group I was calculated from age groups II, III, and IV because commercial gear is selective for the larger fish of age group I. Females were larger than males at all ages. Covariance tests (Ricker, 1958; Mottley, 1941; Snedecor, 1956) on the Ford-Walford lines for males and females indicated a significant difference in the lengths attained by males and females in the first growing season, but the difference in growth after the first season was not significant at the 5% level. The decrease in yearly increment of length was similar for both sexes (Figure 6).

REPRODUCTION

Differences between testes and ovaries in vascularization and size became apparent in

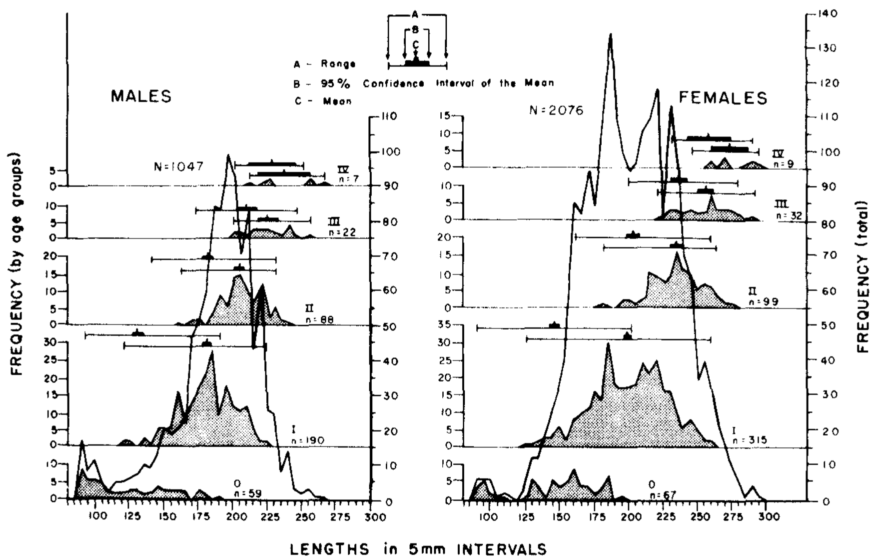


FIGURE 5.—Length-frequency distributions of the northern puffer (shaded polygons for each age group; unshaded polygon represents fish from the entire 1970 collection; distribution characteristics for each age group are shown above polygons with calculated lengths above measured lengths).

TABLE 2.—Length-frequency of young-of-the-year northern puffer.

Total length (mm)	June sex unknown	July sex unknown	Aug. M	Aug. F	Sept. M	Sept. F	Oct. M	Oct. F	Nov. M	Nov. F	Dec. sex unknown
18- 22	1										
23- 27	6										
28- 32	3										
33- 37	1										
38- 42											1
43- 47											
48- 52											
53- 57											
58- 62		1									
63- 67		1									
68- 72		2									
73- 77											
78- 82											
83- 87											
88- 92		2			5	4					
93- 97					2	6					
98-102		3	1		4	2					
103-107					1	1					
108-112			1			1					
113-117					1	1					
118-122					1						
123-127					1						
128-132					1	3					
133-137					2	3					
138-142					2	3					
143-147					1	4	1	1			
148-152						1					
153-157											
158-162									1	1	
163-167						1		1		1	
168-172										2	
173-177										1	
178-182										1	
183-187										2	
N	11	9	2		21	30	1	2	1	8	1

young-of-the-year by early September. Both sexes reached sexual maturity by the second growing season. In the spring the gonads of fish as small as 88 mm contained either milt or ova. Running ripe males were first obtained during the last week of May. Females were gravid during May but from June through the first week of July they were found in all three conditions, gravid, ripe, and spent. Few running ripe females were taken. Males were running ripe longer than females and may spawn a greater number of times.

By late July gonads of most fish taken from the mouth of the York River, Mobjack Bay, and lower Chesapeake Bay were spent and in recovery stages. However, males taken from the mouth of the Rappahannock River in mid-August were still running ripe, and many females were gravid. Welsh and Breder (1922) took ripe females along the New Jersey coast from July 30th to August 27th. Wheatland (1956:296-297) reported northern puffer spawning from late May until August in Long Island Sound. Time and duration of northern puffer spawning appears variable within Chesapeake Bay and along the middle Atlantic coast. By September gonads of puffers from lower Chesapeake Bay were in various stages of recovery. Neither milt nor developing ova were visible during October and November.

The ratio of males to females in May and November was approximately 1:1. However, females were consistently more abundant throughout the summer and early fall. During this period the ratio of males to females was 1:3. Males were least abundant during the peak of spawning in June and July. This change in the sex ratio may be related to reproductive behavior.

WEIGHT-LENGTH RELATIONSHIP

Logarithmic functions describing the exponential increase in weight with increasing length according to sex and gonadal condition were calculated (Figure 7). These plots indicate that logarithmic transformation of weight and length give a linear relation. About 89 to 99% of the variation in weight is associated with variation in length of pre- and post-spawning puffers of each sex. Puffers of both sexes were heavier after recovery from spawning (late July to November) than fish of comparable length before spawning (April to early July). Covariance tests (Mottley, 1941) indicated that this difference in weight was not significant in females but was in males at the 5% level. A large increase in muscle and liver tissue occurred during the growing season but was not quantified. By November fish appeared more robust and had larger livers than in April.

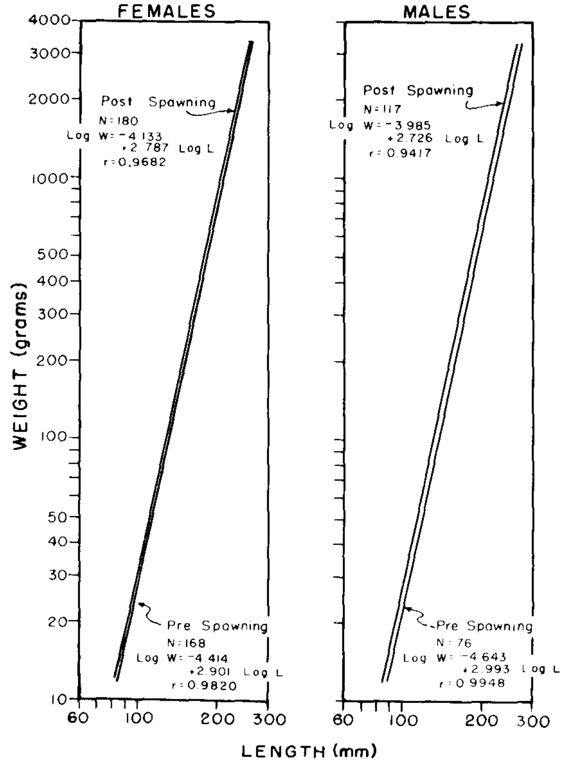


FIGURE 7.—Weight-length relationships for northern puffers in postspawning condition (late July to November) and in prespawning condition (April to early July).

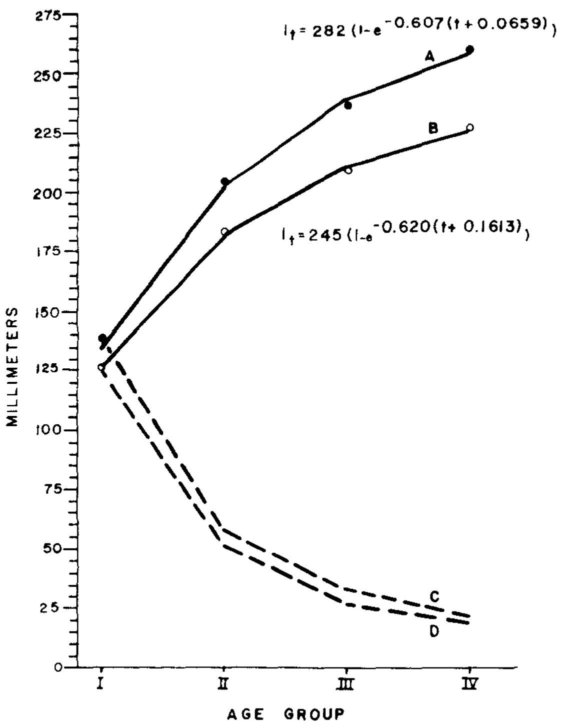


FIGURE 6.—Growth curves of female (A) and male (B) northern puffer fitted by the von Bertalanffly growth equation and plots of increments in length of females (C) and males (D) (circles represent calculated lengths).

ACKNOWLEDGMENTS

The authors express their appreciation to John V. Merriner, Mark Chittenden, Jr., Joseph G. Loesch, George C. Grant, and John J. Norcross for their counsel and criticism of the manuscript.

LITERATURE CITED

APPELGET, J., AND L. L. SMITH, JR.
1951. The determination of age and rate of growth from the vertebrae of the channel catfish, *Ictalurus lacustris punctatus*. Trans. Am. Fish. Soc. 80: 119-139.

DAIBER, F. C.
1960. A technique for age determination in the skate, *Raja eglanteria*. Copeia 1960:258-260.

GALTSOFF, P. S.
1952. Staining of growth rings in the vertebrae of tuna *(Thunnus thynnus)*. Copeia 1952:103-105.

HILE, R.
1941. Age and growth of the rock bass, *Ambloplites rupestris* (Rafinesque), in Nebish Lake, Wisconsin. Trans. Wis. Acad. Sci. Arts Lett. 33:187-337.

HOLLISTER, G.
1934. Clearing and dyeing fish for bone study. Zoologica (N.Y.) 12:89-101.

LAGLER, K. F.
1956. Freshwater fishery biology, 2d ed. Wm. C. Brown Co., Dubuque, Iowa, 421 p.

LA MARCA, M. J.
1966. A simple technique for demonstrating calcified annuli in the vertebrae of large elasmobranchs. Copeia 1966:351-352.

LEWIS, W. M.
1949. The use of vertebrae as indicators of the age of the northern black bullhead *Ameiurus m. melas* (Rafinesque). Iowa State J. Sci. 23:209-218.

MOTTLEY, C. McC.
1941. This covariance method of comparing the lead-lengths of trout from different environments. Copeia 1941:154-159.

RICKER, W. E.
1958. Handbook of computations for biological statistics of fish populations. Fish. Res. Board Can., Bull. 119, 300 p.

SHIPP, R. L., AND R. W. YERGER.
1969. Status, characters, and distribution of the northern and southern puffers of the genus *Sphoeroides*. Copeia 1969:425-433.

SNEDECOR, G. W.
1956. Statistical methods applied to experiments in agriculture and biology. 5th ed. Iowa State College Press, Ames. 534 p.

WELSH, W. W., AND C. M. BREDER, JR.
1922. A contribution to the life history of the puffer, *Spheroides maculatus* (Schneider). Zoologica (N.Y.) 2:261-276.

WHEATLAND, S. B.
1956. Oceanography of Long Island Sound, 1952-1954. VII. Pelagic fish eggs and larvae. Bull. Bingham Oceanogr. Collect. Yale Univ. 15:234-314.

YASUDA, H.
1940. On the rings formed on scale, vertebral centrum and otolith of the same individual fish. *Scombrops chilodipteroides* and *Theragra chalcogramma* (Pallas). [In Jap., Engl. summ.] Bull. Jap. Soc. Sci. Fish. 8:298-300.