Age and growth of totoaba, Totoaba macdonaldi (Sciaenidae), in the upper Gulf of California

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The totoaba, Totoaba macdonaldi (Gilbert), also known as Mexican giant bass, is found only in the Gulf of California. This species during 1934–45 supported one of the most important sport and commercial fisheries in the Gulf, with total annual landings exceeding 2,000 metric tons (Rosales-Juárez and Ramírez-González¹). At present, it is considered endangered (Flanagan and Hendrickson, 1976; NMFS²) as a result of 1) a high mortality of juveniles in shrimp trawl nets, 2) past overexploitation, 3) current illegal fishing during its reproductive season (early February to early May), and 4) ecological alterations of its spawning and nursery grounds. Flanagan and Hendrickson (1976) suggested that there was a high probability that this species would become extinct by 2000 AD. In 1975 the Mexican government declared a moratorium on fishing totoaba.

This paper reports on the age and growth of totoaba as determined from sectioned-otolith readings and contrasts current population age composition with what was known about the early population. Previous studies have reported ages based on nonvalidated scale readings (Nakashima, 1916; Berdegué, 1955; Molina et al.³).

Materials and methods

Totoaba were sampled in 1986–91 from the northern part of the upper Gulf of California between 31° and 32° N Lat. and 114° and 115° W Long. (Fig. 1). In 1989–91, juveniles were collected from shrimp trawl nets. Adults were sampled with gill nets during their reproductive season (Feb–Apr) of 1986, 1987, and 1989–91.

After determining individual standard (SL) and total length (TL) in millimeters and weight in grams, we extracted otolith (sagittal) pairs from 118 fish and embedded them in epoxy resin. For comparison with other age studies of totoaba, a linear regression was performed for converting total length into standard length. Lowerre-Barbieri et al. (1994) reported that sectioned otoliths were the best structure for ageing weakfish, *Cynoscion regalis*.

To permit data recovery when only severed heads were available, 118 otoliths were weighed (OW; +/-0.001 g) and measured to determine their relation with SL (Pauly, 1984); only whole individuals were used in the present study. Maximum otolith length (OL; +/- 0.05 mm) was measured from rostrum to postrostrum margins (anteriorposterior), and maximum otolith thickness (OT; +/- 0.05 mm) from the dorsal to ventral margins (distal-proximal plane).

A transverse section was made from 101 otoliths with an Isomet low speed saw following a technique described by Beckman et al. (1990), Lowerre-Barbieri et al. (1994), and Secor et al.⁴ and the otolith ring counts were read to determine age. Each thin section was read three times with transmitted light in a bright field by the same person. Following the criteria of Beamish and Fournier (1981), we calculated an index of average percent error for the single reader.

Three different axes (Fig. 2) were explored to measure the otolith radius (OR) of 94 thin sections. Annuli were most clearly counted and measured along axis 1; thus otolith radius (OR) was defined as the distance from the center of the core to the otolith outer edge along the ven-

- ¹ Rosales-Juárez, F., and E. Ramírez-González. 1987. Estado Actual del Conocimiento Sobre la Totoaba (*Cynoscion macdonaldi*), Gilbert 1890. Secretaría de Pesca, México, 41 p. ISBN 968-817-086-0. [In Spanish; available at CICESE library.]
- ² NMFS (National Marine Fisheries Service). 1991. Endangered Species Act Status Review, totoaba (*Cynoscion macdonaldi*). Prot. Spec. Manage. Admin. Rep. SWR-91-01, 9 p.
- ³ Molina, D., M.A. Cisneros-Mata, R. Urias, C. Cervantes y M. A. Márquez. 1988. Prospección y evaluación de la totoaba (*Totoaba macdonaldi*) en el Golfo de California. Tech. Report, 18 p. [In Spanish, available from Cisneros-Mata, CRIP-Guaymas, Calle 20, No. 605 Sur, Guaymaa, Sonora, Mexico c.p. 85400.]
- ⁴ Secor, D. H., J. M. Dean, and E. H Laban. 1990. Manual for otolith removal and preparation for microstructural examination. Electric Power Research Inst., The Belle W. Baruch Inst. for Marine Biology and Coastal Research, 85 p.

Manuscript accepted 27 February 1997. Fishery Bulletin 95:620–628 (1997).

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Figure 1

Location of sampling sites in the northern upper Gulf of California. (\blacktriangle) = adults; juveniles were collected within the 40-m depth contour.

tral arm of the sulcal groove. The relation between otolith radius and fish length was fitted by using the Gompertz function (Ricker, 1979) and the computer program Fishparm 3.0 (Prager et al., 1989). To determine size at ages that were not collected, we backcalculated past ages following Bagenal and Tesch (1978) and Jerald (1983), using the Gompertz relation between OR and SL, not the otolith length to fish length regression.

A von Bertalanffy growth model (VBGM) was fitted to the observed SL at the midpoint of each age group represented in our sample (n=101) and also to the back-calculated sizes (n=346) determined from 81 otolith thin sections, by using the computer program Fishparm 3.0 (Prager et al., 1989). The growth equation was calculated for pooled sexes because juveniles can be sexed only by using histological techniques; age and length differences between males and females have not been reported in the literature.

Three juveniles captured in trawl nets during July and August 1989 were kept alive and transferred to the Centro Ecológico de Sonora Research Aquarium (Hermosillo, Sonora, Mexico); (see Almeida Paz et al. [1990] for more details). One fish died after almost 12 months of captivity; the other two were sacrificed 24 months after capture. Lengths and weights of these fish were taken every month beginning five months after capture. These lengths and the ring counts found in the otoliths of these fish were used to validate annual otolith ring deposition.



	Table 1	
Meristic relations between totoaba sta otolith weight (OW). For otolith measu	andard length (SL) and total length (TL), otolith length (OL), otolith urements, $n = 118$.	thickness (OT), and
Parameter Equation		r^2
Fish total length (TL)	SL = 0.91TL - 26.34 (n = 951)	0.99
Otolith length (OL)	$SL = 16.91 \exp(4.7(1 - \exp(-0.96 \times OL)))$	0.99
Otolith thickness (OT)	$SL = 8.53 \exp(5.4(1 - \exp(-0.17 \times OT)))$	0.98
Otolith weight (OW)	$SL = 788.8 \times OW^{0.4518}$	0.99

Results

From shrimp trawl nets, 1,125 juvenile totoaba (100– 600 mm SL, mean=223, SD=65 mm SL) and 157 adults were collected (600–1,850 mm SL, mean =1,360, SD=89 mm SL). Figure 3 shows the lengthfrequency distribution for the specimens collected.

Totoaba sagittal otoliths are large and beanlike, as are most sciaenid otoliths (Secor et al.⁴). Table 1 shows the linear equation describing the relation between SL and TL, the Gompertz relations between SL and otolith length (OL), otolith thickness (OT), and the allometric equation for the SL and otolith weight (OW) relation (Fig. 4). Otolith growth in weight changes in relation to fish growth in length and is an allometric, not isometric relation (b=2.176, H_0 : b=3.00, student's *t*-test (0.025, 111)=21.41). Transverse sections of the totoaba otolith (Fig. 5) typically showed clear, opaque, and translucent zones and when the otolith is sectioned exactly through the focus, the core appears as a dense opaque zone, next to which the first annulus is found. The distance between the core and the first annulus is variable but is typically greater than increment sizes between the remaining annuli. The relation between otolith radius (OR) along radius 1 and SL (Fig. 6) was fitted to a Gompertz function:

$$SL = 30.92 \times \exp(3.86(1 - \exp(0.99 \times \text{OR}))),$$

[$r^2=0.98, n=94$].

We found specimens representing 15 year classes between 0 and 24 years (Table 2). Of the 101 readable otolith sections, 66 fish were young-of-the-year (110-377 mm SL). Ten juveniles were of age class 2

(378–620 mm SL), and one was of age class 3 (740 mm SL). The remaining specimens were adults between age classes 4 and 24. The index of average percent error was 16.10% for the single reader.

Observed lengths and otolith ring counts were used to fit the von Bertalanffy model to obtain the growth curve for the totoaba population in the Gulf of California (Fig. 7); the fit was good $(r^2=0.98, n=101)$. Past ages (n=346) were backcalculated from the thin-section otolith radius (OR) to fish length relation of 81 fish, and the von Bertalanffy growth model was also determined (Fig. 7). Table 2 compares the observed standard lengths with those calculated from both growth models. Figure 8 shows the relation between maximum whole otolith length and age as an exponential function.

In the case of the fish that died after a year of captivity (11 mo 21 d), its otoliths showed only one ring; the second fish held for two years, had two rings. The otoliths from the third specimen were decalcified and readings, unfortunately, were not possible. Fish held in captivity were captured in the same trawls as the rest of the juveniles used for age determination in this study. Otoliths of juveniles sacrificed at the time of capture did not present any rings or marks similar to those detected in otoliths of the individuals kept in captivity.

Discussion

The relation between otolith dimensions and fish length can be used to obtain data from the totoaba heads commonly found on the beaches of the northern Gulf. This relation is particularly important when one considers the restrictions and the potential impact of sampling an endangered species. Otolith growth and fish growth are proportional re-

gardless of how growth rate changes with time; in early stages, both fish and otoliths increase faster than in adult stages after maturity is reached. Barbieri et al. (1994) reported for Atlantic croaker (*Micropogonias undulatus*) that age has an important effect on the otolith dimension to fish length relation. For totoaba, however, fish length alone described over 98% of the variability in otolith size. This growth pattern is common for other sciaenid species (Ross, 1988; Murphy and Taylor, 1989).

The relation between standard length and otolith radius in sciaenids has been fitted to several growth equations. Maceina et al. (1987) and Blake and Blake





(1981) found good fit with a linear model, but Barger (1985) found the best fit with a power function. For totoaba in our study, the best fit was found with a Gompertz model for radius along axis 1. The wide range of radii at the maximum fish length (shown in Fig. 6), supports our assumption that annuli are formed throughout the life of the fish, as is also suggested by the power function fitted to the otolith length and age relationship (shown in Fig. 8). Barbieri et al. (1994) also reported the formation of annuli throughout the life of the Atlantic croaker. Although the otolith length to age relation could be used to estimate fish age, the fit is not as good as that between standard length and



age. No "Lee's phenomenon" (Ricker, 1969) was observed, and the von Bertalanffy growth model resulting from back-calculated data was very similar to that derived from observed data (Fig. 7; Table 2).



Gauldie and Nelson (1990) commented that otolith growth is typically repressed on the ventral plane because this part of the otolith is in direct contact with the skull, which restricts otolith growth; al-

> though otolith growth ceases in the ventral plane, it continues to grow in the sulcular region. For totoaba, otolith growth seems to continue on the proximal side along the sulcal groove. The ventral arm of the sulcal groove has been reported as the best area for otolith reading in other sciaenids (Beckman et al., 1990; Lowerre-Barbieri et al., 1994).

> Major increases in length occur during the first and second years, diminishing once totoaba reach their sixth or seventh year, the age at first maturity. After this stage, the growth curve reaches an asymptote from about the twelfth to fourteenth year. The observed adult mean standard lengths at age in our study are very close to those calculated by VBGM from observed data and also from back-calculated data.

> In a comparison of age determinations with scales (Nakashima, 1916; Berdegué, 1955;

Table 2

Observed and predicted mean standard length at year-class midpoints. n = sample size. SD = standard deviation. VBGM = von Bertalanffy growth model. BC = back-calculated model.

Year class midpoint (yr)	n	Observed SL: mean (mm)	Observed SD	Predicted VBGM (mm)	Predicted VBGM BC (mm)
0.5	66	218	66	216	3191
1.5	10	503	75	525	595
2.5	1	740		750	797
3.5	1	1,080	-	914	945
4.5	0	_	_	1,034	1,053
5.5	0	_	_	1,121	1,133
6.5	0	-	_	1,184	1,192
7.5	1	1,260	_	1,231	1,234
8.5	0	—	_	1,264	1,266
9.5	0		_	1,289	1,289
10.5	1	1,271	_	1,307	1,306
11.5	0	_		1,320	1,318
12.5	2	1,363	38	1,329	1,327
13.5	5	1,318	88	1,336	1,334
14.5	4	1,309	102	1,341	1,339
15.5	5	1,340	24	1,345	1,342
16.5	1	1,390	_	1,348	1,345
17.5	1	1,300	_	1,350	1,347
18.5	0	_	_	1,351	1,348
19.5	1	1,280	-	1,352	1,349
20.5	0	_	_	1,353	1,350
21.5	1	1,490		1,354	1,350
22.5	0	_	_	1,354	1,351
23.5	0	-		1,354	1,351
24.5	1	1,453	<u> </u>	1,354	1,351
Total	101				

¹ Otolith core to margin measurements along ventral arm of sulcal groove were used for year-class 0, and predicted lengths were derived from the resulting equation.

Flanagan, 1973; Molina et al.¹), versus otoliths, as used in our study (Table 3), the greatest difference is found with Nakashima (1916). He estimated the maximum age for a fish of 1,980 mm (SL) to be nine years; our study shows that this fish could be older than 24 years. The von Bertalanffy parameters we estimated are very similar to those reported by Berdegué (1955) and Flanagan (1973). In the Molina et al.¹ study there was a large underestimation of maximum age in comparison with our results, reflected in the K value. This difference could be due to our use of a greater range of year classes from young-of-the-year to adults, whereas Molina et al.¹ used only adult fish. Juveniles or young-of-the-year should be included to fit the von Bertalanffy growth curve because if only adults are used, there is a tendency to obtain low K values (Beckman et al., 1990).

Scales often result in the underestimation of age (Beamish and McFarlane, 1987) owing to difficulty in reading, especially in the outer rings which are very close. Furthermore, there is a possibility of using regenerated scales in which the first rings that were formed are not included. Authors working with sciaenids have mentioned that reading scales is easier for short-lived species, like some species of *Cynoscion* (Villamar, 1972; De Vries and Chittenden, 1982).

On the basis of growth and otolith marks observed in two juveniles held in captivity, we suggest that ring formation in totoaba from the Gulf of California is annual; annual deposition patterns have been reported for other sciaenids (Beckman et al. 1990, Murphy and Taylor 1991), including species of *Cynoscion*, a closely related genus (González, 1977; Blake and Blake, 1981; Shlossman and Chittenden, 1982; Barbieri et al., 1994; Lowerre-Barbieri et al., 1994). The well-defined seasonality in temperature in the Gulf of California (Alvarez Borrego et al., 1973; Paden et al., 1991) also suggests that the marks seen in totoaba otoliths are annual because such temperature changes are an important factor in ring deposi-



calculated (---) data of age (number of rings) and standard length (the year-class midpoint was used for age). Open triangles are observed data.



tion (Brothers, 1978; Beckman et al., 1990). Berdegué (1955) considered scale rings to be annual on the basis of the migratory pattern and reproductive period of totoaba.

The recent creation of the Upper Gulf of California and Colorado River Delta Biosphere Reserve will enhance conservation efforts for totoaba by protecting important spawning and nursery habitat. Furthermore, fishing pressure from commercial shrimp trawls and gill nets will be greatly decreased.

> Barrera-Guevara (1990) reported that 92% of young-of-the-year totoabas were killed in the commercial shrimp fishery. In our study we were not able to sample organisms between ages 5 and 11 because they were not available to trawls and gill nets and because we did not sample in areas where prerecruit totoaba concentrate. These areas are difficult to sample because of their depth: the Guaymas basin reaches more than 200 m depth. It has been suggested that the summer migration of totoaba is toward deep waters in the central Gulf of California (Berdegué, 1955; Arvizu and Chavez, 1972; Flanagan and Hendrickson, 1976). These fish, however, are accessible to hook and line fishing, and "catch and release" sport fishing practices should be encouraged.

> It is clear that the current available habitat for totoaba will not allow significant population increase. Nevertheless, we found a population age-structure similar to that existing during the early 1890's (assuming that fish of ages 3-11 years exist but were unavailable to our sampling as previously described), and we suggest that continued conservation efforts should allow for the survival of a stable but small population of totoaba in the Gulf of California. Estimates of adult survival proposed by Cisneros-Mata et al. (1995) before and after the 1975 moratorium also support evidence of stability in the current population age-structure.

Acknowledgments

We thank the fishermen of the Gulf of Santa Clara and Puerto Peñasco for their collaboration in sampling adult totoaba, especially Heriberto Amaya, Rosario Angulo, and their families. We thank personnel of the Centro Regional de Investi-

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Table 3 Comparison of von Bertalanffy growth parameters for dif ferent studies of totoaba.					
Author	K	<i>L</i> _∞	t _o	Max. age (yr)	
Present observed					
data	0.3162	1,355	-0.0499	24	
Present back-					
calculated data	0.3103	1,352	-0.3679	24	
Nakashima (1916)		1,980		9	
Berdegué (1955)		1,330		15	
Flanagan (1973)	0.16	1,467		20	
Molina et al. ¹	0.271	1,373	-2.264	19	

gación Pesquera en Guaymas for assistance in the field with sampling juveniles. The Fish and Wildlife Foundation is acknowledged for support of field work during 1989. Sampling was carried out under Fisheries Research Permit No. 1229, Secretary of Fisheries, Mexico. We acknowledge the valuable suggestions of two anonymous reviewers.

Literature cited

Almeida-Paz, M., G. Morales-Abril, and

M. J. Román Rodríguez.

1990. Aspectos sobre aclimatación y crecimiento de juveniles de totoaba, *Totoaba macdonaldi*, (Gilbert) (Pisces: Sciaenidae) en condiciones de cautiverio. Ecológica (Mexico) 2(1):7-12. [In Spanish.]

Alvarez-Borrego, S., L. A. Galindo-Bect, and

B. P. Flores-Baez.

1973. Hidrología. In Estudio Químico sobre la contaminación por insecticidas en la desembocadura del Río Colorado. Reporte final de la segunda etapa, a la dirección de acuacultura de la secretaría de recursos hidraúlicos, p. 5-177. Univ. Autón. Baja California, Ciencias Marinas, tomo I, sec. I. [In Spanish.]

Arvizu, J., and H. Chávez.

1972. Sinopsis sobre la biología de la totoaba, *Cynoscion macdonaldi* Gilbert 1890. [In Spanish.] FAO Fish. Synopsis 108.

Bagenal, T. B., and F. W. Tesch.

1978. Age and growth. In T.B. Bagenal (ed.), Methods for assessment of fish production in freshwater, 3rd ed., p.101– 136. Blackwell Scientific Publication. Oxford, England.

Barbieri, L. R., M. E. Chittenden Jr., and C. M. Jones.

1994. Age, growth, and mortality of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. Fish. Bull. 92:1-12.

Barger, L. E.

1985. Age and growth of Atlantic croakers in the northern Gulf of Mexico, based on otolith sections. Trans. Am. Fish. Soc. 114:847–850.

1990. The conservation of Totoaba macdonaldi (Gilbert),
(Pisces: Sciaenidae), in the Gulf of California, México. J.
Fish. Biol. 37(suppl. A):201-202.
Beamish, R. J., and D. A. Fournier.
1981. A method for comparing the precision of a set of age
determination. Can. J. Fish. Aquat. Sci. 38:982–983.
Beamish, R. J., and G. A. McFarlane.
1987. Current trends in age determination metho-
dology. In R. C. Summerfield and G. E. Hall (eds.), Age
and growth of fish, p.15–42. Iowa State Univ. Press.
Beckman, D. W., A. L. Stanley, J. H. Render, and
C. A. Wilson.
1990. Age and growth of black drum in Louisiana waters
of the Gulf of Mexico. Trans. Am. Fish. Soc. 119:537–544.
Berdegué, A. J.
1955. La pesquería de la totoaba (Cynoscion macdonaldi)
en San Felipe, Baja California. Rev. Soc. Mex. Hist. Nat.
16(1-4):45-78. [In Spanish.]
Blake, C., and B. F. Blake.
1981. Age determination in six species of fish from a Mexi-
can Pacific coastal lagoon. J. Fish Biol. 18(4):471–478.
Brothers, E. B.
1978. Exogenous factors and the formation of daily and sub-
daily growth increments in fish otoliths. Am. Zool. 18:631.
Cisneros-Mata, M. A., G. Montemayor-López, and
M. J. Román-Rodríguez. 1995. Life history and conservation of Totoaba macdo-
naldi. Conserv. Biol. 9(4):806–814.
De Vries, D. A., and M. E. Chittenden Jr.
1982. Spawning, age determination, longevity and mortal-
ity of silver sea trout, Cynoscion nothus in the Gulf of
Mexico. Fish. Bull. 80(3):487–500.
Flanagan, C. A.
1973. Study of the feasibility of modeling the totoaba fish-
ery of the northern Gulf of California, with preliminary
estimation of some critical parameters. Univ. Arizona,
Biol. Sci. Rep. 249, 58 p.
Flanagan C. A., and J. R. Hendrickson.
1976. Observation on the commercial fishery and reproduc-
tive biology of the totoaba Cynocion macdonaldi, in the
northern Gulf of California. Fish. Bull. 74(3):531–544.
Gauldie, R. W., and D. G. A. Nelson.
1990. Otolith growth in fishes Comp Biochem Physiol

1990. Otolith growth in fishes. Comp. Biochem. Physiol. 97A(2):119-135.

González, L. W.

Barrera Guevara, J. C.

 1977. Aspectos técnicos de preparación de otolitos para estudios de edad de algunas especies del genero Cynoscion (Pisces: Sciaenidae). Lagena nos. 39-40:43-48. [In Spanish.]
Jerald, A., Jr.

- 1983. Age determination. In L. A. Nielsen and D. L. Johnson (eds.), Fisheries techniques, p. 301-324. Am. Fish. Soc., Bethesda, MD.
- Lowerre-Barbieri, S. K., M. E. Chittenden Jr., and

C. M. Jones.

1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. Fish. Bull. 92:555-568.

 Maceina, M. J., D. N. Hata, T. L. Linton and A. M. Landry Jr.
1987. Age and growth analysis of spotted sea trout from Galveston Bay, Texas. Trans. Am. Fish. Soc. 116: 54-59.

Murphy M. D., and R. G. Taylor.

1989. Reproduction and growth of black drum *Pogonias* cromis, in northeast Florida. Northeast Gulf Sci. 10(2): 127-137.

^{1991.} Direct validation of ages determined for adult red

drums from otolith sections. Trans. Am. Fish. Soc. 120:267–269.

Nakashima, F.

1916. Cynoscion macdonaldi, Gilbert. Copeia (37):85–86. Paden, C. A., M. R. Abbott, and C. D. Winant.

1991. Tidal and atmospheric forcing of the upper ocean in the Gulf of California: 1: Sea surface temperature variability. J. Geoph. Res. 96:18,337-18,359.

Pauly, D.

1984. Fish population dynamics in tropical waters: A manual for use with programmable calculators. ICLARM Studies and Reviews (8):1-325.

Prager, M. H., S. B. Saila, and C. W. Recksiek.

1989. FISHPARM: A microcomputer program for parameter estimation of nonlinear models in fisheries science, second ed. Old Dominion Univ. Oceanogr. Tech. Rep. 87-10. Ricker, W. E.

1969. Effects of size-selective mortality and sampling bias

on estimates of growth, mortality, production, and yield. J. Fish. Res. Board Can. 26:479–541.

- 1979. Growth and rate models. Chapter 11 in W. S. Hoar, D. J. Randall and J. R. Brett (eds.), Fish physiology, vol. 8, p. 301–324. New York Academic Press.
- Ross, S. W.
 - 1988. Age, growth, and mortality of the banded drum, Larimus fasciatus (Sciaenidae) in North Carolina. Northeast Gulf Sci. 10(1):19-31.

Shlossman, P. A., and M. E. Chittenden Jr.

1982. Spawning, age determination, longevity and mortality of the silver sea trout, *Cynoscion nebulosus*; red drum, *Sciaenops ocellata*; and the black drum, *Pogonias cromis* in south central Louisiana. La. Dep. Wild. Fish. Contrib. Mar. Res. Lab. Tech. Bull. 31:41-48.

Villamar, A.

1972. Age determination in fishes of the family Sciaenidae. J. Ichthyol. 13(4):550-561.