COMPARISON OF GROWTH BETWEEN COHORTS OF JUVENILE BLUEFIN TUNA (*THUNNUS THYNNUS*)

Steven Saul[1], Stephen C. Turner[2], David Die[1] and Audra Livergood[2]

SUMMARY

Vertebrae of juvenile bluefin tuna (Thunnus Thynnus) collected off of the East Coast of the United States are stained and aged. Estimated growth in age two and three individuals is compared across two different cohorts. Analysis of covariance indicates that growth rates are the same between the two cohorts sampled for these individuals. More work is needed to test this hypothesis across additional cohorts and for additional age groups.

KEY WORDS

Bluefin tuna, age determination, growth curves

[1] Rosenstiel School for Marine and Atmospheric Science, University of Miami. 4600 Rickenbacker Cswy. Miami, FL 33149, United States. E-mail: ssaul@rsmas.miami.edu.
[2] Southeast Fishery Science Center, National Marine Fisheries Service. 75 Virginia Beach Dr. Miami, FL 33149, United States. E-mail: steve.turner@noaa.gov

1. Introduction

This study compares the growth rates of two different cohorts of juvenile bluefin tuna ages two and three in the Atlantic Ocean off of the East Coast of the United States. Previous studies on age and growth of individuals have determined growth parameters for Atlantic bluefin tuna (*Thunnus Thynnus*) via direct aging of hard parts (Mather and Schuck 1960, Turner et al. 1991, Cort 1991, Mather et al. 1995).

Three calcified structures are typically considered when aging bluefin tuna: otoliths, vertebrae, and dorsal spines. The utility of each of these structures for determining age and the relative degree of precision and accuracy associated with using one structure over the other has been extensively compared in the literature (Nichy and Berry 1976, Berry et al. 1977, Prince et al. 1985, Lee and Prince 1991, Campana 2001, Rodriguez-Marin et al. 2006). For fish that are nine years of age or older, vertebrae tend to underestimate age due to the fact that the banding toward the outer portion of the centrum becomes less clear (Lee et al. 1983). To overcome this, a modified method for reading older "giant" bluefin tuna vertebrae, as well as increasing the precision and accuracy of aging juveniles, was determined by Prince et al. (1985). When compared to one another, vertebrae and otolith methods of aging provided estimates that were close to ages validated through tagging records, each with acceptable levels of precision. Despite this, however otolith age methodology yielded slightly higher error rates, most likely because growth zonations on the otolith are extremely vague, and the direction of growth patterns occurs in more than one plane, making measurements more subjective. Furthermore, vertebrae are easier and less costly to obtain from fishing operations, as well as easier to extract (Berry et al. 1977, Lee and Prince 1991). Comparison of vertebrae with spines for determining age has shown that there is little difference in age estimation for the age range sampled (ages six to 11) except for the tendency for vertebrae age nine and older to underestimate age by one year as aforementioned (Rodriguez-Marin et al. 2006).

Studies of Southern bluefin tuna from hard parts have generally found increased growth rates over the past four decades, which are hypothesized to be due to density-dependent response of the populations to historical exploitation (Polacheck, et al. 2004). No studies to date have specifically been done on bluefin tuna in the North Atlantic to determine whether temporal or spatial differences in growth rate occur between cohorts. In general, however various studies have been done on a variety of other fish species to determine whether differences occur in growth rates among cohorts (Wang and Tzeng 1999,Williams et al. 2007). The majority of these studies however have looked only at differences in growth between cohorts during the larval stages, rather than throughout the older periods of growth during their life history.

2. Materials and Methods

Individuals aged one through four were collected through United States National Marine Fishery Service sampling programs, by university scientists, and by other interested individuals in the Atlantic Ocean off of the East Coast of the United States from 1997 through 1999. The tail section of each juvenile containing the 35^{th} and 36^{th} vertebrae and first spine of the dorsal fin were removed according to Ruiz et al. (2004) and stored frozen. As adopted by previous researchers (Prince et al. 1985, Rodriguez-Marin et al. 2000) the sample was thawed, cleaned and then stained in alizarin red solution for three to five hours and stored dry before being aged (Berry et al. 1977). Age was estimated by counting the annual growth zones, where one annulus is represented by one ridge and one groove enhanced by the emersion in alizarin red. Each vertebra was read independently three times without prior knowledge of the morphometric data or the previous age determination, and counts started from the focus and moved outward toward the edge of the cone (Prince et al. 1985). Once each vertebra was read blindly three times, the three measured ages were compared:

- If the measured ages were different for each of the three readings, the sample was discarded.
- If the measured age was different for only one of the three readings, and the other two were the same, then a fourth reading was performed.
    - If the fourth reading matched the two measured ages that were the same, then only the deviant reading was discarded and replaced by the fourth reading
    - If the fourth reading did not match the two measured ages that were the same, then the sample was discarded.

For bluefin tuna, the portion of the von Bertalanffy growth curve that reflects juvenile growth (prior to the onset of sexual maturity) of the age groups in this study is largely linear (Turner et al. 1991). Furthermore, when plotting juvenile growth between only two ages, the curved characteristic of the von Bertalanffy is no longer present and the growth relationship is linear. Therefore, a linear relationship was used to model the growth of individuals between age two and three for each of the two cohorts that were sampled. An analysis of covariance was performed using R Statistical Software (Version 2.3.1) to test for differences between the growths of the two cohorts (Wang and Tzeng 1999, Dalgaard 2002).

3. Results

A linear regression model of each cohort separately for juvenile bluefin tuna ages two and three yielded the following equations (Figure 1; Table 2a and 2b).

1995 Cohort

$$L(cm) = 536.90 + 151.96t$$

$R^2 = 0.6422$

1996 Cohort

$$L(cm) = 536.46 + 148.46t$$

$R^2 = 0.4955$

A test for equal variance around the regression lines for the two cohorts shows that the variances are the same, a necessary assumption that must be fulfilled before constructing a joint linear regression model (Table 3). Once this was ascertained, a multiplicative model was set up that allows the relation between age and length to have different slopes and intercepts in the two cohort groups (Table 4). This model yielded the same equations for each of the cohorts as the regression analysis that fit each cohort separately (Table 2a and 2b).

1995 Cohort

$$L(cm) = 536.90 + 151.96t$$

1996 Cohort

$$L(cm) = 536.46 + 148.46t$$

Common $R^2 = 0.5716$

Fitting an additive model provides essentially the same equations for each of the cohorts as the joint multiplicative model and the regression fit of each cohort separately (Table 5).

1995 Cohort

$$L(cm) = 540.78 + 150.37t$$

1996 Cohort

$$L(cm) = 531.36 + 150.37t$$

Common $R^2 = 0.574$

This shows that the linear growth equations for each of the two cohorts are essentially parallel, however the average length of those individuals in cohort 1996 are 9.42 cm shorter than those in cohort 1995. The ANOVA table for the multiplicative model shows that the interaction between age and cohort is not significant, and consequently, the model can be reduced to be additive, thus corresponding to parallel regression lines (Table 6). The ANOVA tables for the additive model, regardless of the order in which the terms are added into the model, describe the same model as seen by the fact that the residual sum of squares are identical (Table 7 and Table 8). The partitioning of the sum of squares is not the same in each of the models and is dependent on the order in which the factors age and cohort are added to the model. Comparison of the previous covariance analysis with a simpler analysis using the Welch Two Sample t-test, in which the effect of age is ignored, confirms again that there is no difference in growth between cohorts (Table 9).

4. Discussion

Analysis of covariance shows that there is no difference in growth between cohorts for juveniles age two and three. Residual analysis indicates a large degree of variance in growth between individuals ages two and three (Figures 2 and 3). In particular, the diagnostics for the 1995 cohort suggests that this data may not be normally distributed (Figure 2). Length frequency analysis of the samples shows that length is not normally distributed for a given age (Figure 5). In addition, the departure from normally of the diagnostics is also due to the sample of age 2 fish for cohort 1995, which is skewed because of three very large fish (Figure 2). These three samples (Figure 2), in addition

to the samples of a large age 2 fish and two small age 3 fish from cohort 1996 (Figure 3) were reexamined and confirmed to be the appropriate ages.

The remaining variance in length and age was hypothesized to be explained by the fact that bluefin tuna exhibit rapid growth rates during the early years of life. As a result, fish at the upper and lower extremes of the residual distribution may have been collected during months that are away from the time of annual ring formation (i.e. birth date of the animal). Most studies describing spawning period for bluefin tuna in the western Atlantic suggest that birth occurs in the spring, and is assumed to occur in May (Mather and Schuck 1960, Brothers et al. 1983, Turner et al. 1990). Monthly adjustment of age for time of ring formation however did not provide an improved fit (Figures 6, 7, and 8). This is likely because all of the samples were collected during a short three month period in the summer, with the majority of the samples collected in July (Figure 4). Therefore, the variance in growth depicted in the model diagnostics seems to simply be due to the fact that bluefin tuna experience variable growth during the young years of their life, which is dependent on factors that can not be partitioned from this particular data.

More work is needed to test the hypothesis that differences in grow occur among cohorts. Additional cohorts that span a much longer time period, and additional age groups need to be included in such an analysis. If bluefin tuna cohort growth rates are compared on a more appropriate temporal and spatial scale, then differences may occur between at least some of the cohorts. Such differences in growth, as well as growth rate variability could correlate with years of environmental variability. Coupling of cohort growth rate information with environmental observations is needed to determine the effect of phenomena such as the El Nino Southern Oscillation and global climate change on growth rates of various cohorts of bluefin tuna in the Atlantic. Temperature and food supply are two important abiotic and biotic factors that are frequently altered by ecosystem regime shift and typically influence the growth rate of fishes (Crecco and Savoy 1985, Tsai et al. 1991, Rutherford and Houde 1995). Further analysis of differences between cohort growth rate across a longer time period and for additional cohorts may help us better understand the effects of environmental variability on the biota in the ocean.

5. Literature Cited

Berry, F.H., D.W. Lee, and A.R. Bertolino. 1977. Age estimations in Atlantic bluefin tuna: an objective examination and an intuitive analysis of rhythmic markings on vertebrae and in otoliths. Col. Vol. Sci. Pap. ICCAT 6(2): 305-317.

Brothers, E.B., E.D. Prince, and D.W. Lee. 1983. Age and growth of young-of-the-year bluefin tuna, *Thunnus thynnus*, from otolith microstructure. *In:* E. D. Prince and L. M. Pulos (Eds.). Proceedings of the International Workshop On Age Determination of Oceanic Pelagic Fishes: tunas, billfishes, and sharks. *NOAA Technical Report NMFS* 8: 49-59.

Campana, S.E. 2001. Accuracy, precision andquality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.

Cort, J.L. 1991. Age and growth of the bluefin tuna, *Thunnus thynnus(L.)* of the northeast Atlantic. Col. Vol. Sci. Pap. ICCAT 35(2): 213-230.

Crecco, V.A. and T.F. Savoy. 1985. Effects of biotic and abiotic factors on growth and relative survival of young American shad, Alosa sapidissima, in the Connecticut River. Can. J. Fish. Aquat. Sci. 42: 1640–1648.

Dalgaard, P. 2002. *Introductory Statistics With R.* New York: Springer Publications. 292pp.

La Mesa, M., M. Sinopoli, and F. Andaloro. 2005. Age and growth rate of juvenile bluefin tuna *Thunnus thynnus* from the Mediterranean Sea (Sicily, Italy). Scientia Marina 69(2): 241-249.

Lee, D.W. and E.D. Prince. 1991. Preliminary analysis of otoliths and vertebrae from nine recaptured Atlantic bluefin tuna (*Thunnus thynnus*). Col. Vol. Sci. Pap. ICCAT 35(2): 317-324.

Lee, D. W., E. D. Prince & M.E. Crow. 1983. Interpretation of growth bands on vertebrae and otoliths of Atlantic bluefin tuna, Thunnus thynnus, p. 61-70. *In:* E. D. Prince and L. M. Pulos (Eds.). Proceedings of the International Workshop On Age Determination of Oceanic Pelagic Fishes: tunas, billfishes, and sharks. *NOAA Technical Report NMFS* 8: 61-69.

Mather, F.J., J.M. Mason, A.C. Jones. 1995. Historical document: life history and fisheries of Atlantic Bluefin tuna. *NOAA Technical Report NMFS-SEFSC 370*. 165pp.

Mather, F.J., and H.A. Schuck. 1960. Growth of bluefin tuna of the western north Atlantic. U.S. Fish and Wildlife Service Fishery Bulletin 61(179): 39-52.

Nichy, F. and F.H. Berry. 1976. Age determination in Atlantic bluefin tuna. Col. Vol. Sci. Pap. ICCAT 5(2): 302-306.

Polacheck, T., J.P. Eveson, and G.M. Laslett. 2004. Increase in growth rates of southern bluefin tuna (*Thunnus maccoyii*) over four decades: 1960 to 2000. Can. J. Fish. Aquat. Sci. 61: 307–322

Prince, E.D., D.W. Lee, and J.C. Javech. 1985. Internal zonations in sections of vertebrae from Atlantic bluefin tuna, *Thunnus thynnus*, and their potential use in age determination. Can. J. Fish. Aquat. Sci. 42: 938-946.

Rodriguez-Marin, E., D. Olafsdottir, J. Valeiras, M. Ruiz, V. Chosson-Pampoulie, and C. Rodriguez-Cabello. 2006. Ageing comparison from vertebrae and spines of bluefin tuna (*Thunnus thynnus*) coming from the same specimen. Col. Vol. Sci. Pap. ICCAT 59(3): 868-876.

Ruiz, M., E. Rodriguez-Marin, and J. Landa. 2005. Protocol for sampling of hard parts for bluefin tuna (*Thunnus thynnus*) growth studies. Col. Vol. Sci. Pap. ICCAT 58(4): 1403-1419.

Rutherford, E.S. and E.D. Houde. 1995. The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, *Morone saxatilis*, larvae in Chesapeake Bay. Fish. Bull. 93: 315–332.

Tsai, C.-F., M. Wiley, and A.-L. Chai. 1991. Rise and fall of the Potomac River striped bass stock: a hypothesis of the role of sewage. Transactions of the American Fisheries Society 120: 1–22.

Turner, S.C., V.R. Restrepo, A.M. Eklund. 1991. A review of the growth of Atlantic bluefin tuna, *Thunnus thynnus*. Col. Vol. Sci. Pap. ICCAT 78: 271-293.

Wang, Y.-T. and W.-N. Tzeng. 1999. Differences in growth rates among cohorts of *Encrasicholina punctifer* and *Engraulis japonicus* larvae in the coastal waters off Tanshui River Estuary, Taiwan, as indicated by otolith microstructure analysis. Journal of Fish Biology 54: 1002–1016.

Williams, J.P., L.G. Allen, M.A. Steele, and D.J. Pondella II. 2007. El Nino periods increase growth of juvenile white seabass (*Atractoscion nobilis*) in the Southern California Bight. Mar. Biol. 152: 193-200.

6. Tables

Table 1. Number of fish sampled from each cohort and age group.

	COHORT	
AGE	1995	1996
2	43	24
3	35	48

Table 2a. Separate regression model for cohort 1995.

Coefficients	Estimate	Std. Error	t value	P(>|t|)
(Intercept)	536.9	32.18	16.68	<2e-16
Age	151.96	12.88	11.8	<2e-16

Table 2b. Separate regression model for cohort 1996.

Coefficients	Estimate	Std. Error	t value	P(>|t|)
(Intercept)	536.46	47.8	11.22	<2e-16
Age	148.46	17.65	8.41	3.20E-12

Table 3. F test results for homogeneity of variance around the regression line for each cohort.

F Calculated	F Tabulated	df numerator	df denominator	p-value
0.642	1.477	76	70	0.059

Table 4. Joint multiplicative regression model that allows relation between age and length to have different slopes and intercepts.

Coefficients	Estimate	Std. Error	t value	P(>|t|)
(Intercept)	536.899	36.2294	14.819	<2e-16
Age	151.9575	14.4992	10.48	<2e-16
Cohort1996	-0.4407	56.318	-0.008	0.994
Age:Cohort1996	-3.4991	21.5348	-0.162	0.871

Table 5. Additive joint regression model for the 1995 and 1996 cohort.

Coefficients	Estimate	Std. Error	t value	P(>|t|)
(Intercept)	540.783	27.133	19.931	<2e-16
Age	150.371	10.685	14.073	<2e-16
Cohort1996	-9.426	10.632	-0.887	0.377

Table 6. ANOVA table for the multiplicative joint regression model.

Model: Age*Cohort	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age	1	815312	815312	200.998	<2e-16
Cohort	1	3167	3167	0.7807	0.3784
Age:Cohort	1	107	107	0.0264	0.8711
Residuals	146	592221	4056		

Table 7. ANOVA table for the additive model where the factor age is added first and the factor cohort is added second.

Model: Age+Cohort	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age	1	815312	815312	202.339	<2e-16
Cohort	1	3167	3167	0.786	0.3768
Residuals	147	592328	4029		

Table 8. ANOVA table for the additive model where the factor cohort is added first and the factor age is added second.

Model: Cohort+Age	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Cohort	1	20408	20408	5.0648	0.0259
Age	1	798070	798070	198.06	<2e-16
Residuals	147	592328	4029		

Table 9. Welch two sample t-test between cohorts ignoring the effect of age

t estimated	t tabulated	df	p-value
-1.471	1.976	145.533	0.145

7. Figures

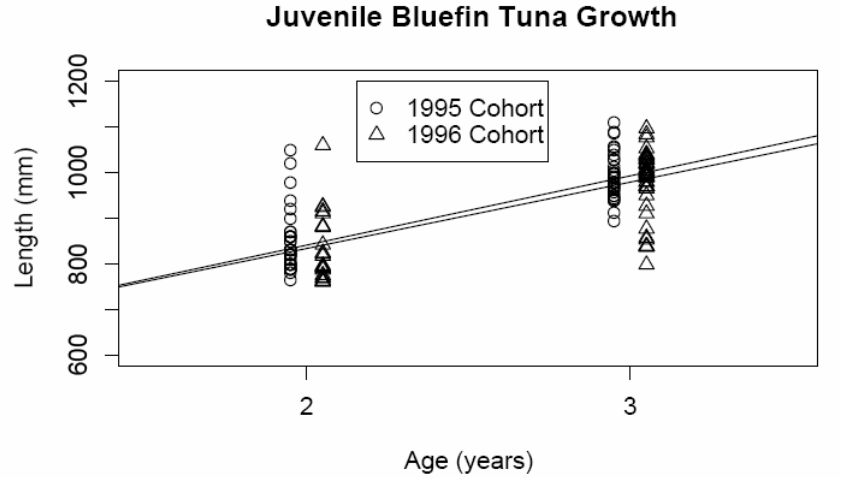

Figure 1. Linear regression fit to the length and age observations for ages one and two of each of the two cohorts. Each cohort has been offset to provide visual differentiation.

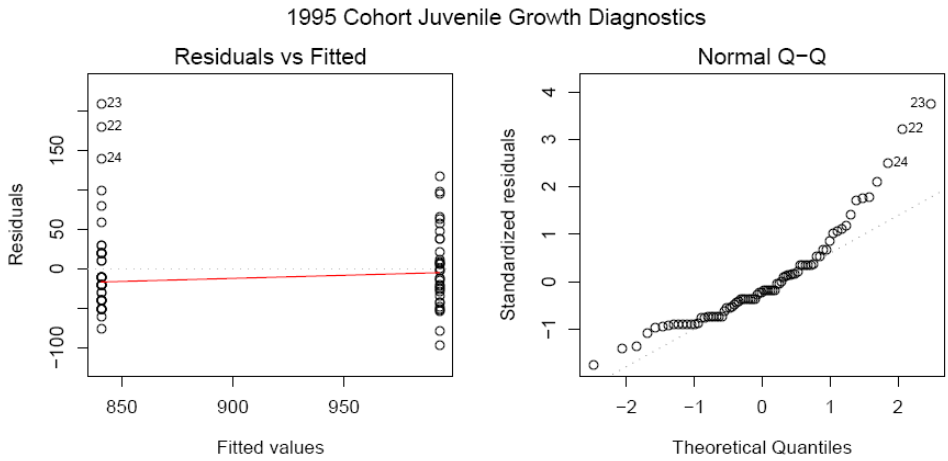

Figure 2. Model diagnostics for the length and age fit for the 1995 cohort.

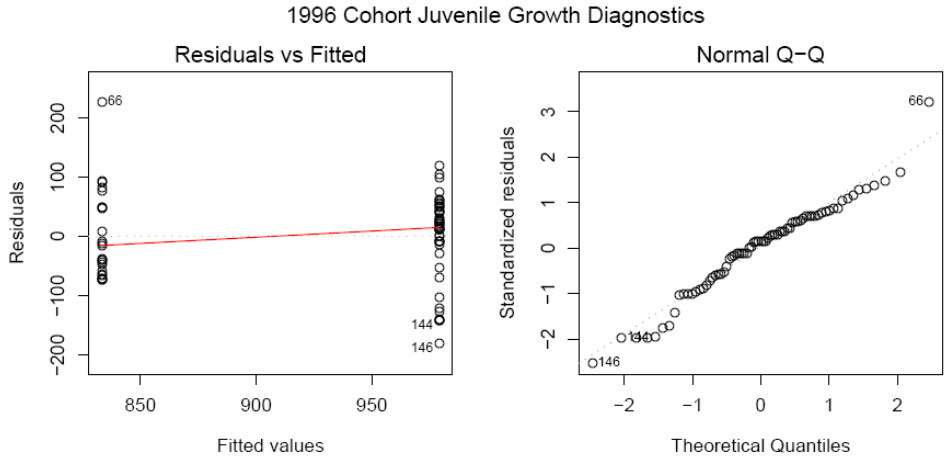

Figure 3. Model diagnostics for the length and age fit for the 1996 cohort.

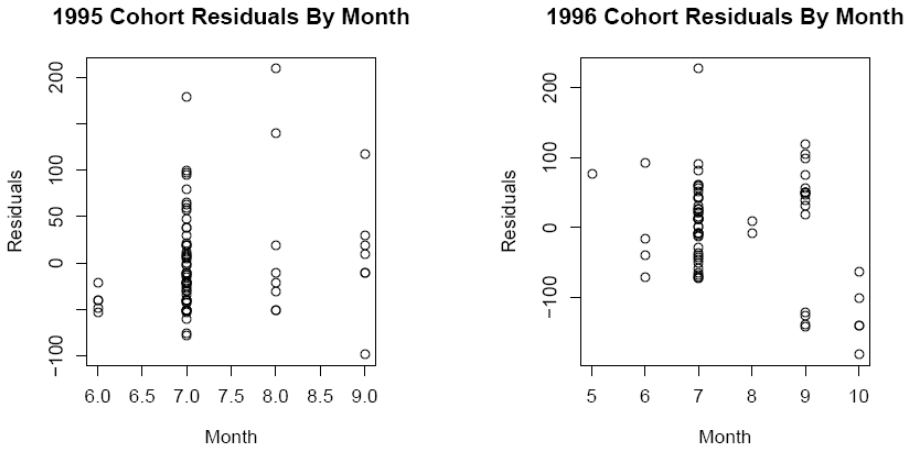

Figure 4. Residual distribution for the linear regression fit to the length and age observations by cohort and month.

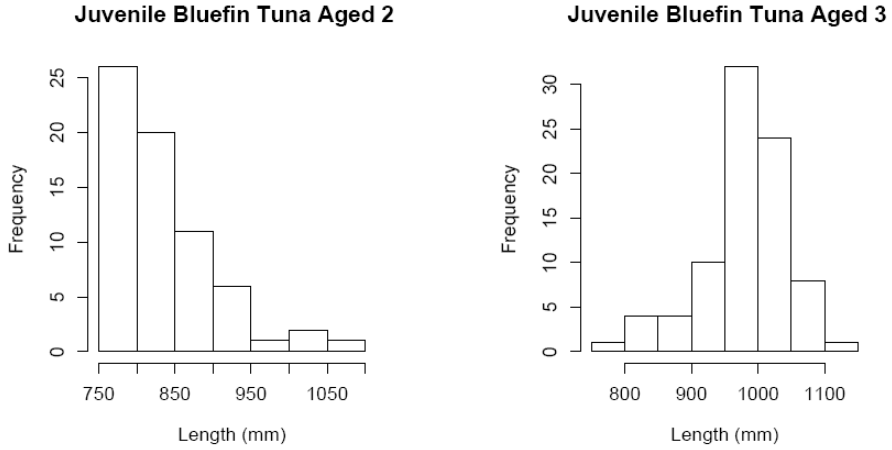

Figure 5. Length frequency distributions for samples age two and three.

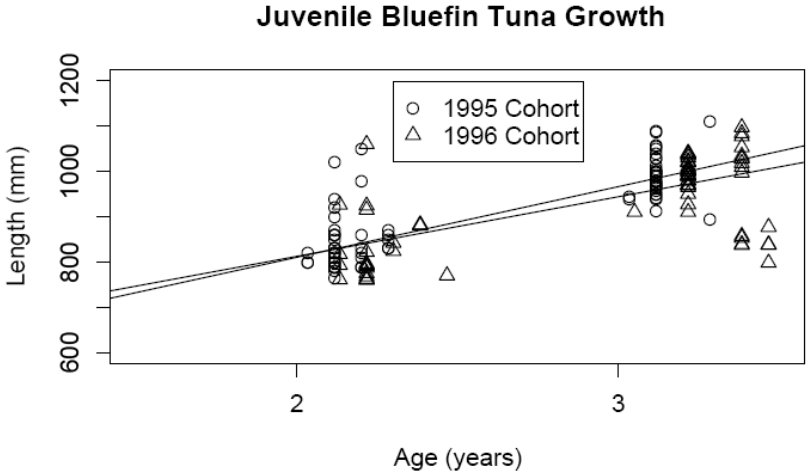

Figure 6. Linear regression fit to the length and age observations for ages one and two of each of the two cohorts with age offset assuming spawning and thus annual ring formation occurs in May.

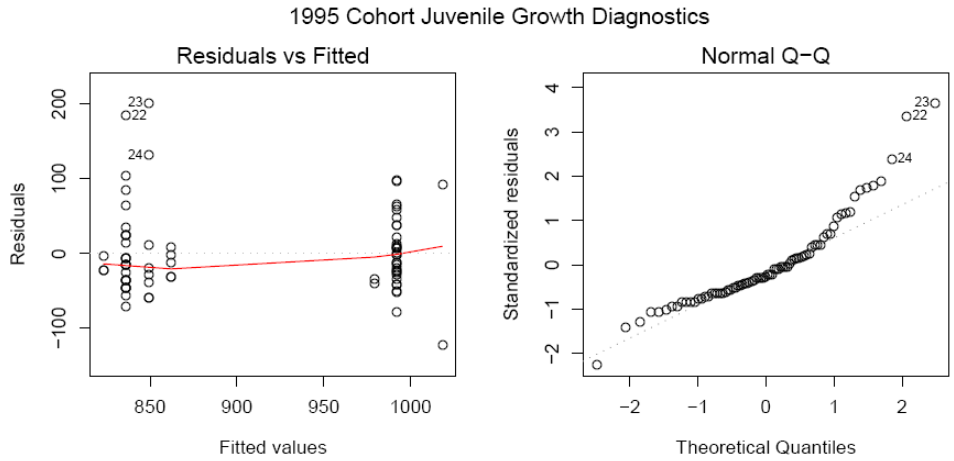

Figure 7. Model diagnostics for the length and age fit for the 1995 cohort with age offset assuming spawning and thus annual ring formation occurs in May.

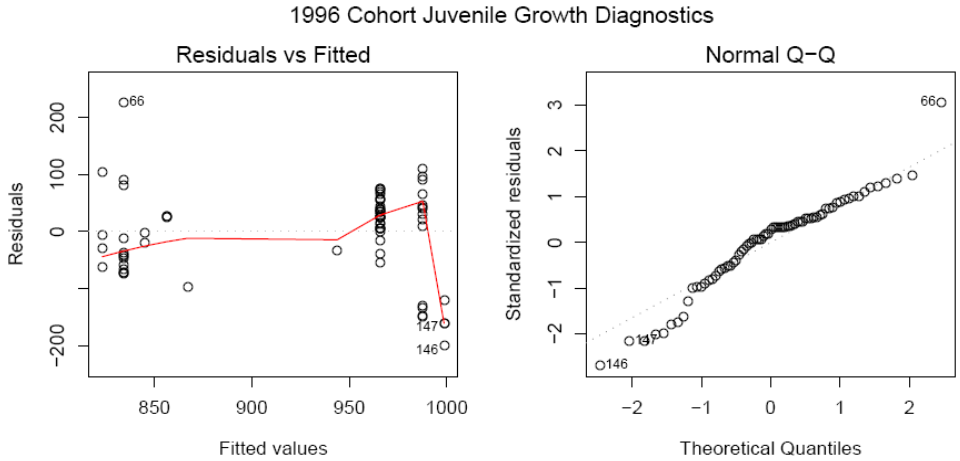

Figure 8. Model diagnostics for the length and age fit for the 1996 cohort with age offset assuming spawning and thus annual ring formation occurs in May.