SAW 47 Working Paper 9 (TOR 3) – Analysis of Sex Ratios

**Analysis of Trends in Sex Ratio,
Implications for Natural Mortality,
and Variation in Age-Length Keys
in Summer Flounder**

Eric N. Powell
Jason Morson

Haskin Shellfish Research Laboratory
Rutgers University

Introduction

The analyses described herein use the biological database for summer flounder. The goals of these analyses are to answer the following questions to the extent permitted by this database.

1. Does information exist in the sex-ratio data that would support the need to construct a sex-explicit model for summer flounder?
2. Does information exist in the sex-ratio data that would support the need to utilize regionally-specific sex-at-age keys?
3. Does information exist in the sex-ratio data that would support a differential natural mortality rate for male and female summer flounder or a nonlinear whole-stock natural mortality rate?
4. Does variation exist in the relationship of size and age that would support the need to utilize regionally-specific age-length keys?

Sex Ratio as a Function of Age

Methods and Results

Sex ratio data for young-of-the-year are not available prior to 1982; consequently analyses of sex ratio focus on 1982-2007. Due to data limitations, and regional variability in sex ratios as discussed in a subsequent section, we exclude data from southern New England north and also from Cape Hatteras south in this set of analyses. We also exclude all age-year combinations where the number of sexed summer flounder is less than 20. For some analyses, we have collected the data into six year groups with the central four being half-decadal. Year group 1 contains data from 1982-1985 and year group six for 2006-2007.

Table 1 shows the sex ratio by year and age for summer flounder. The year-group averages are in Table 2. Perusal of Table 1 elucidates two general trends.

First, the young-of-the-year are dominantly male. A female-biased sex ratio for young-of-the-year summer flounder occurs only thrice in 26 years, a frequency significantly different than the expected 50:50 split (binomial test, $P < 0.0005$). Furthermore, the fraction male for young-of-the-year fish frequently exceeds 0.6; in fact a sex ratio at least this divergent from 50:50

occurs in 17 of 26 years. The average number of age-0 fish sexed per year is 51.5. Given this average, a 50:50 male:female ratio by chance when measured would return a ratio of 60:40 or greater 7.56% of the time. The occurrence rate observed, 17 in 26, would not be expected to occur by chance (binomial test, $P < 0.0001$). Thus, young-of-the-year summer flounder are consistently over-represented by male fish.

Table 1. The fraction of summer flounder that are male at age for ages and years where the total number of summer flounder sexed was ≥20.

Year	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age8
1982	0.707	0.519	0.369	0.133					
1983	0.583	0.466	0.361	0.296					
1984	0.576	0.589	0.304	0.250					
1985	0.674	0.484	0.378	0.263					
1986	0.645	0.590	0.500	0.056					
1987	0.714	0.622	0.474						
1988	0.714	0.783	0.357						
1989	0.574	0.586	0.227						
1990	0.662	0.548							
1991	0.739	0.617	0.476						
1992	0.614	0.534	0.291	0.200					
1993	0.750	0.578	0.348	0.103					
1994	0.436	0.580	0.412	0.135					
1995	0.622	0.451	0.401	0.115					
1996	0.579	0.528	0.302	0.250					
1997	0.667	0.563	0.440	0.281	0.167				
1998	0.574	0.602	0.477	0.303	0.102	0.083			
1999	0.720	0.524	0.502	0.396	0.171	0.045			
2000	0.543	0.571	0.485	0.381	0.297	0.123	0.083		
2001	0.682	0.484	0.529	0.350	0.291	0.171	0.194		
2002	0.737	0.523	0.492	0.474	0.284	0.128	0.069		
2003	0.633	0.644	0.569	0.422	0.331	0.190	0.040	0.062	
2004	0.655	0.582	0.562	0.440	0.260	0.193	0.103	0.059	
2005	0.808	0.635	0.638	0.454	0.397	0.341	0.190		
2006	0.429	0.683	0.515	0.426	0.305	0.197	0.108	0.125	0.062
2007	0.286	0.581	0.666	0.441	0.250	0.196	0.250	0.067	

The second observation is a consistent change in sex ratio with age (Figure 1), such that male frequencies over 0.5 occur only 8 times in 25 cases in age-2 fish and ratios above 0.3 occur only 6 times in age-4 fish. In fact, of the sex ratios accumulated by year-group in Table 2, only one age and year-group pair was characterized by a sex ratio not significantly different from 50:50 male-to-female. Thus, summer flounder are consistently characterized by biased sex ratios regardless of age or half-decadal period within the time series and the direction of bias changes with age.

Percentage Male at Age
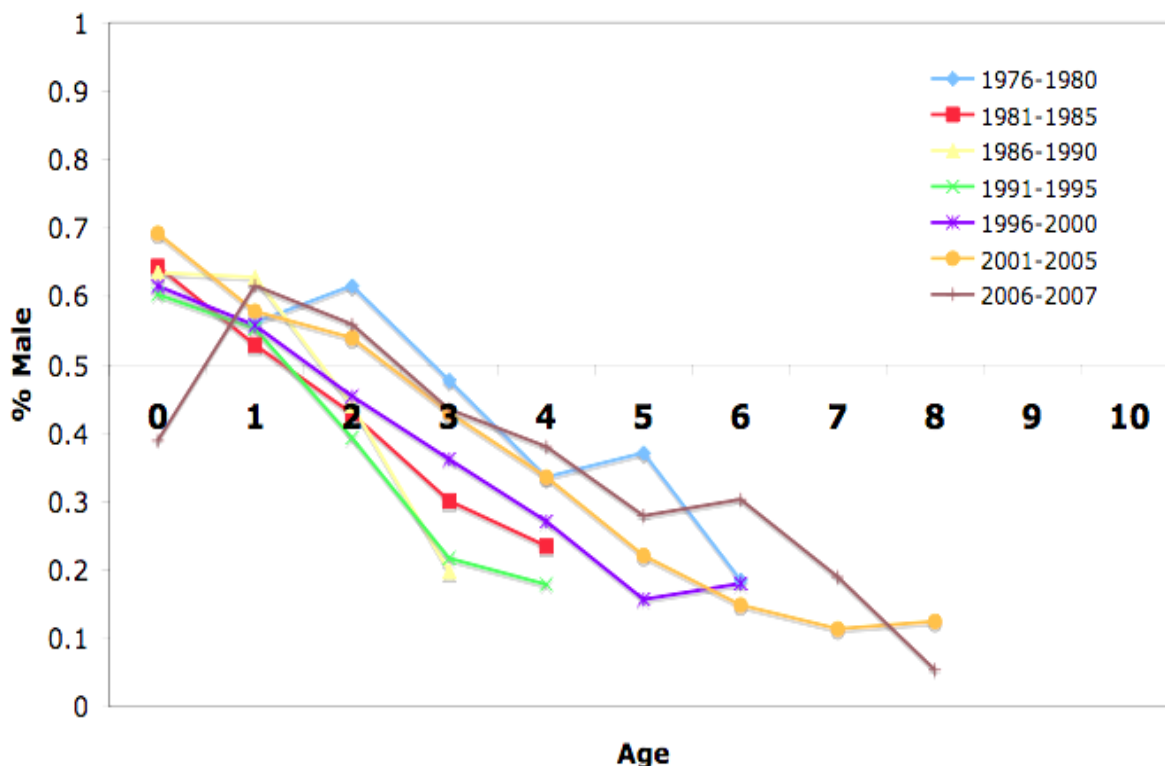
Figure 1. The percentage of the stock that is male by age, summarized by year-group.

Two additional observations are worthy of note. First, the three years where females predominate in age-0 fish include the last two years. This is unexpected from the time series record. However, the sex ratio for age-1 fish from the 2006 cohort conforms with typical age-1 sex ratios in being biased in favor of males. Thus, the aberrant 2006 young-of-the-year ratio is likely a sampling artifact. Second, the fraction of fish that are male at older age has increased over time, although remaining well below 0.5. This is particularly apparent for age-3 fish (Table 2). One explanation is that male fish are moderately more susceptible to the fishery at high fishing mortality rates, but two other explanations should first be considered. The same outcome would be obtained either if a reduction in natural mortality rate had occurred of if the originating sex ratio was biased to a greater degree in favor of males. Evidence in Table 1 does not strongly support the latter alternative. Evidence in a subsequent section does not support the penultimate option. The dispersion of males and females as the cohort ages, discussed in a subsequent section, might be interpreted to support the first alternative.

Table 2. The fraction of summer flounder that are male at age for ages and year groups where the total number of summer flounder sexed was ≥20. Parentheses indicate ratios not significantly different from an expected 50:50 split (binomial test, $\alpha = 0.05$).

Year Group	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8
1982-1985	0.644	(0.518)	0.357	0.222	0.167				
1986-1990	0.641	0.630	0.397	0.140					
1991-1995	0.601	0.543	0.370	0.133	0.074				
1996-2000	0.605	0.544	0.438	0.344	0.227	0.126	0.100		
2001-2005	0.690	0.568	0.550	0.423	0.302	0.192	0.117	0.071	0.158
2006-2007	0.386	0.621	0.588	0.436	0.277	0.197	0.164	0.097	0.042

Interpretation: Age-dependent Bias in Sex Ratio

A number of potential reasons exist for the male-dominated sex ratios seen in young-of-the-year summer flounder.

Females mature later than males. The observed females may under-represent the total number. The biological database records undifferentiated fish. Assigning all of these fish to the female sex, however, does not markedly change the data summarized in Table 2. Thus, maturity schedule cannot explain the male-dominated sex ratios observed for age-0 fish.

Young males may be more available to the survey. While this possibility cannot be excluded, the fact that females grow faster than males and that the male-biased sex ratios clearly are retained into age 2, albeit at diminishing intensity, suggest that availability is not an adequate explanation.

Protandry would produce the observed age-dependent sequence of sex ratios. Protandry, however, is not reported in flatfish, and would almost assuredly have been observed, were it to exist.

Biased sex ratios have been observed by others in summer flounder, however. Morse (1981) and Smith and Daiber (1977) found that younger, smaller fish were much more likely to be male and that this trend quickly reversed with increasing age. Morse (1981) offers that an initially male-dominated sex ratio is necessary to offset an apparently higher natural mortality rate in males, thus promoting a more nearly 1:1 sex ratio in the spawning stock.

The most viable explanation for biased sex ratios in young-of-the-year summer flounder is temperature-dependent sex determination. Temperature has been shown to influence sex ratios at the point of sexual differentiation in flatfish, not afterwards, and this influence of temperature seems to be a frequent characteristic of flounder species. For example, when barfin flounder, *Verasper moseri*, were reared at high temperatures (18°C for this species), all fish developed as males, whereas at 14°C, the sex ratio was close to 1:1 (Goto et al. 1999). Marbled sole, *Limanda yokohamae*, also show temperature-dependent sex determination; when the larvae were kept in 25°C water, the sex ratio was again strongly skewed towards males (Goto et al. 2000). Southern flounder, *Paralichthys lethostigma*, likewise develop a sex ratio skewed towards males when raised at higher (28°C) or lower (18°C) than optimal temperature, 23°C, which produces a 1:1 ratio (Luckenbach et al. 2003). Although little is known of the genetic determinants of sex, *Paralichthys olivaceus* uses an *XX* female/*XY* male system of genetic sex determination, yet high water temperatures (25-27.5°C) produce physiological males possessing an *XX* genotype.

Genetically determined males (*XY*) were never observed to develop into physiological females (Yamamoto 1999).

Among the flatfishes have been shown to exhibit temperature-dependent sex determination are two species of the same genus as summer flounder, the Japanese flounder or hirame, *Paralichthys olivaceus*,and the southern flounder, *Paralichthys lethostigma* (Yamamoto 1999; Luckenbach, et al.2003). Furthermore, in both cases, extreme temperatures result in a higher fraction of the young being male. This could potentially explain why a higher fraction of young-of-the-year summer flounder are found at the northern and southern edges of their range, as described subsequently.

Sex Ratio as a Function of Region and Depth

Methods and Results

For this analysis, we allocated strata to three depth zones (<25 fm; 25-50 fm; >50 fm). This division allocated sex-ratio data into approximately equivalent groups by data richness. Insufficient data were present to achieve a finer division of deeper-water strata. Strata were allocated to five regions: southern New England (we included Georges Bank strata in this grouping), the northern Mid-Atlantic Bight, Delmarva, and the strata south of Cape Hatteras. Finally, data were allocated to half-decadal year-groups: 1976-1980, 1981-1985, 1986-1990, 1991-1995, 1996-2000, 2001-2005, 2006-2007. We excluded all occurrences of age-year group, age-region, and age-depth combinations with sex ratios supported by a total count of males and females less than 30. ANOVAs were run by age using depth, year-group, and region as main effects. All interaction terms were included. Sex was implemented as a dependent variable by assigning a 0 to males and a 1 to females. Means, accordingly, were equivalent to the fraction female.

Table 3. Results of ANOVAs examining the impact of depth, region, and year-group on sex ratios at age. Blank cells indicate insufficient data. NS, non-significant at $\alpha = 0.05$. ×, an interaction term.

| Age	Region	Depth	Year Group	Depth × Region	Depth × Year Group	Year Group × Region	Year Group × Depth × Region
0	NS		≤.001			NS	
1	NS	NS	NS	≤.001	NS	NS	0.01
2	0.05	≤.001	≤.001	≤.001	NS	≤.001	0.005
3	NS	0.002	≤.001	NS	NS	NS	0.005
4	≤.001	NS	0.01	NS	0.03	NS	NS
5	≤.001	NS	0.006	NS	NS	NS	NS
6	NS	NS	NS	NS	NS	NS	NS
7	NS	NS	NS	NS	NS	NS	NS

Depth significantly influenced sex ratio in summer flounder, ages 2 and 3 (Table 3). The catch of age-0 fish was insufficient in the deeper depth zones to analyze. Whereas depth was only significant for ages 2 and 3 in the ANOVA, Tukey's studentized range tests identified depth differences in sex ratios for ages 1 through 4. The presence of significant interaction terms, however, limits the interpretation of this *a posteriori* test. For these younger fish, exclusive of age 1, a tendency exists for the sex ratios to be more nearly 50:50 offshore (Figure 2). Age-1 males are distinctly proportionately more common offshore. At ages 5 and older, depth no longer impacts sex ratios significantly in summer flounder.
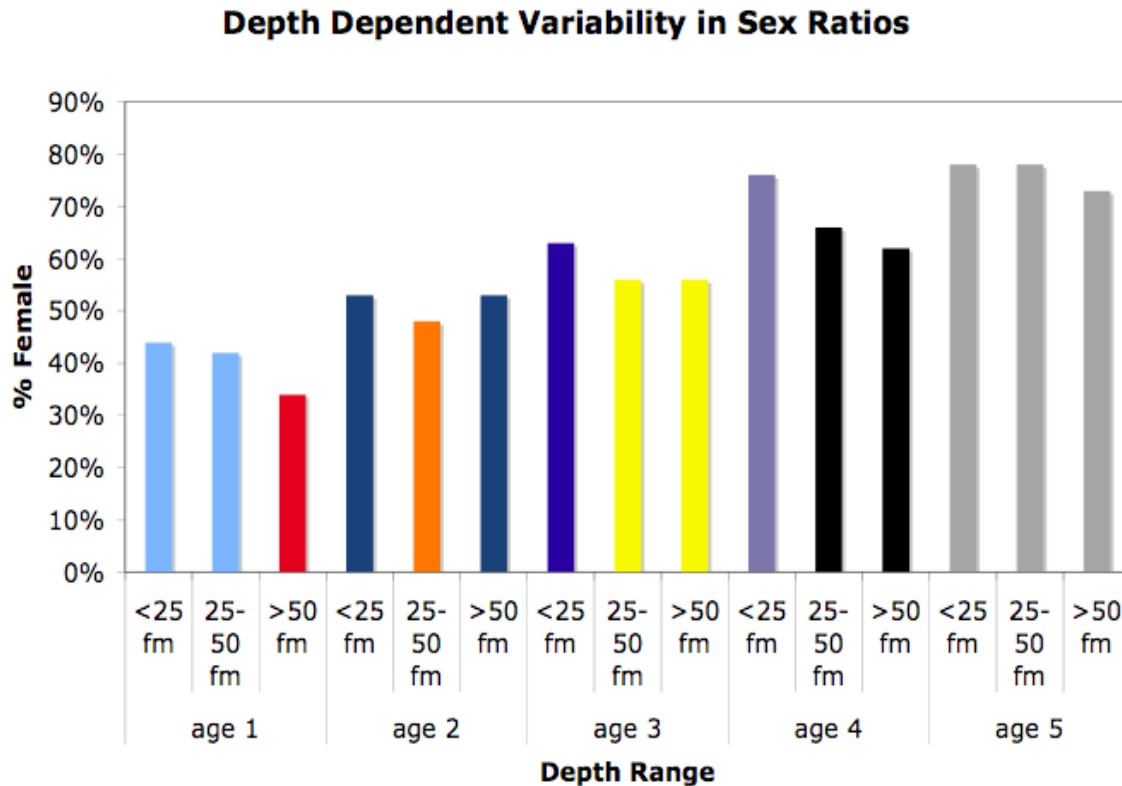
Figure 2. Mean percent female at age by depth. Bars within an age group with the same color were not significantly different in an *a posteriori* Tukey's test. ANOVA results are in Table 3

Sex ratios were significantly affected by region at age 2, but much more so for ages 4 and 5 (Table 3). The switch between depth and region as the dominant main effect between ages 3 and 4 is dramatic and suggests a differential segregation of the stock spatially as the fish age. Interaction terms were routinely significant between main effects for younger ages, but rarely significant after age 3. The change in significant main effect with age and the frequency of significant interaction terms including depth suggests that regionality in summer flounder sex ratios is not facilely explained simply in terms of depth and latitude. Tukey's studentized range tests document the tendency for the northern Mid-Atlantic and Delmarva regions to group together, while one of either the south Atlantic or southern New England regions differed significantly from the central region group (Figure 3). For the cases in which region was a significant main effect, a significantly lower fraction of females occurred in southern New England strata when compared to the Delmarva and northern Mid-Atlantic regions.

Year-group was highly significant for ages 0, 2, 3, 4, and 5. Tukey's studentized range tests clearly indicated a pattern for early years (1976-1980) and later years (2001-2007) to group together and for middle years (1981-1996) to group together (Figure 4). These groupings can be correlated with periods of low and high abundance. Furthermore, periods of lower abundance routinely had higher male ratios than periods of high abundance.
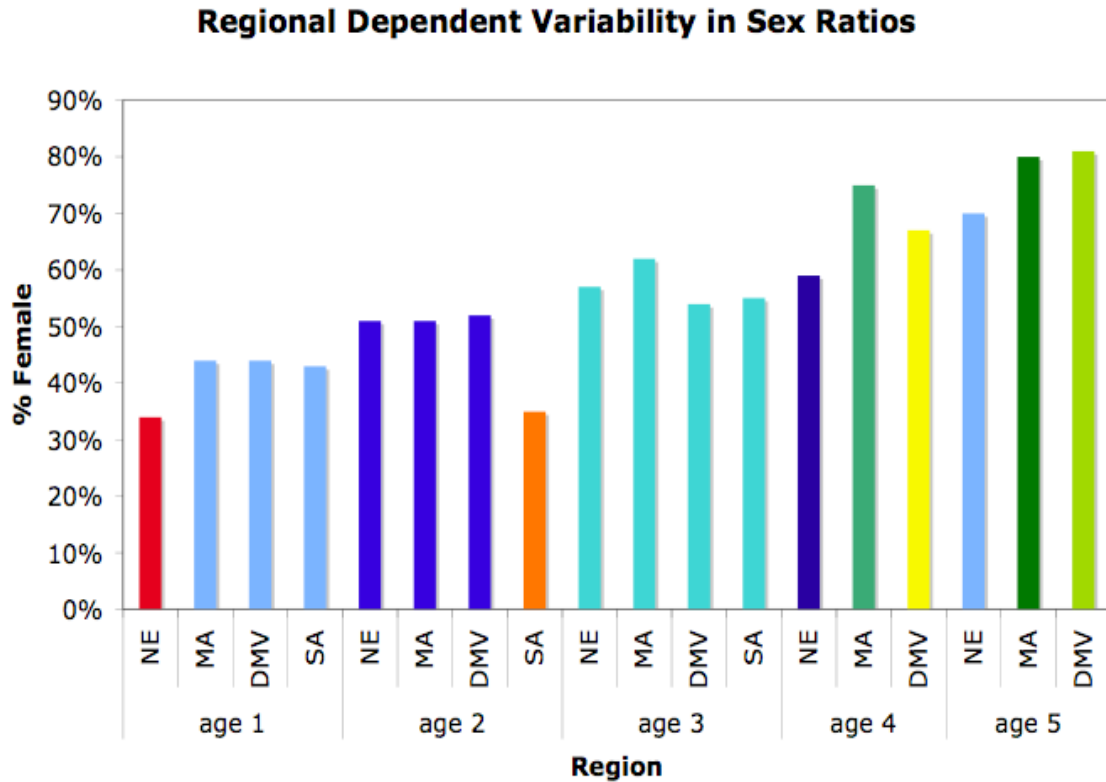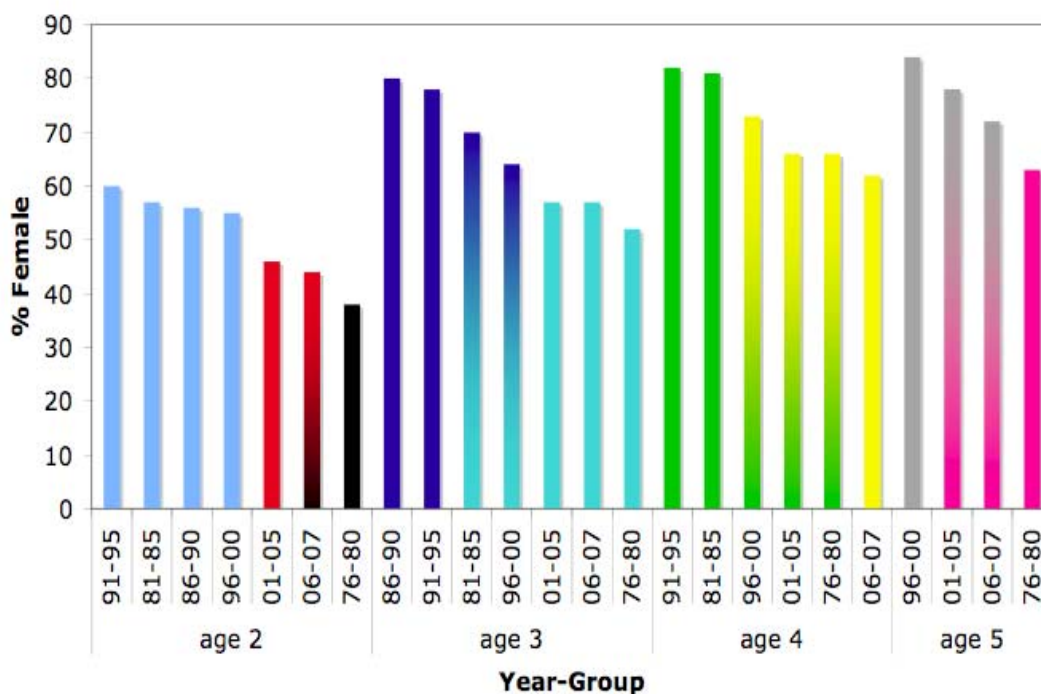

Figure 3. Mean percent female at age by region. Bars within an age group with the same color were not significantly different in an *a posteriori* Tukey's test. ANOVA results are in Table 3. NE, southern New England; MA, northern Mid-Atlantic; DMV, Delmarva; SA, south Atlantic.

Interpretation: Spatial Divergence in Sex Ratio

Examining the regional results for overall trends, it seems that the northern Mid-Atlantic and Delmarva regions have similar sex ratios regardless of age. In addition, the south Atlantic and southern New England regions have a tendency to be different from the Mid-Atlantic/Delmarva grouping, depending on age. When different, the southern New England and south Atlantic regions routinely have a higher fraction of males. This is precisely what would be expected from the temperature-dependent determination of sex that produces an increase in fraction male at the temperature extremes; however, sex determination in the first year of life militates against this explanation as the main effect of region is observed only later. Thus, alternative biological explanations or determinants from differential fishing mortality must be sought. The depth and year-group effects are, as yet, unexplained.

Age-Group Dependent Variability in Sex Ratios

Figure 4. Mean percent female at age by year-group. Bars within an age group with the same color were not significantly different in an *a posteriori* Tukey's test. ANOVA results are in Table 3.

Sex Ratio-imposed Requirement on Relative Mortality Rate

Methods and Results

The gradual shift in sex ratio from male-dominated to female-dominated with increasing age might accrue from differential mortality or differential availability. The latter would seem unlikely, as the trends in sex ratio are consistent across a wide range of age groups.

The differential rate of natural mortality can be directly calculated from the information provided in Table 2. The calculation is based on the following governing equation:

$$N_{1_m} + N_{1_f} = N_{0_m} e^{-Z_m t} + N_{0_f} e^{-Z_f t} \tag{1}$$

where N is abundance of males m or females f, Z is total mortality rate, t is time, and numerals designate consecutive time periods. The equation can be converted to ratio form by dividing through by N_1:

$$1 = \frac{N_{0_m}}{N_1} e^{-Z_m t} + \frac{N_{0_f}}{N_1} e^{-Z_f t} \tag{2}$$

Equation (2) can be solved iteratively under the conditions that the sum of the two terms on the right-hand side closely approximate 1, that $N_{1_m} \leq N_{0_m}$, and that $N_{1_f} \leq N_{0_f}$.

Table 4. Relative natural mortality rates of male and female summer flounder calculated from the changes in sex ratios with age, by year-group, Delmarva and northern Mid-Atlantic region only, from equation (2).

Year Group	Male (Z_m)	Female (Z_f)
1976-1980	0.46	0.12
1981-1985	0.82	0.42
1986-1990	0.51	0.13
1991-1995	0.53	0.30
1996-2000	0.25	0.22
2001-2005	0.53	0.22
All years	0.62	0.22

Table 4 demonstrates that the change in sex ratio over the lifespan of a cohort requires a differential natural mortality rate, whereby males die at a much faster rate than females. The calculation is robust in measuring the relative mortality rate. The mortality rate for males is often twice that of females. Considering the entire dataset, all years combined, the natural mortality rate for males is about three times the female rate (Table 4). The calculation is less robust in measuring absolute natural mortality rate as any mortality process distributed evenly among the sexes would not impact an estimate based on varying sex ratios. Nevertheless, the female natural mortality rate of 0.22 for all years is remarkably similar to the assumed female natural mortality rate derived from estimates of female lifespan.

Interpretation: Sex-specific Natural Mortality Rate

The change in sex ratio with age requires that male and female summer flounder be modeled separately as far as natural mortality rate. In lieu of a sex-explicit model, a whole stock mortality rate might be employed, although this is less satisfactory. The natural mortality rate, derived thusly, is not linear, however (Table 5). We calculated this age-dependent rate using the equation:

$$Z_{m+f} = -\log\left(\frac{N_{0_m}}{N_{0_{m+f}}} e^{-Z_m t} + \frac{N_{0_f}}{N_{0_{m+f}}} e^{-Z_f t} \right) \qquad (3)$$

Equation (3) was employed using age-independent mortality rates for males and females from Table 4, based on the observation of relatively constant mortality with age within sex inferred from Figure 5. Whole-stock natural mortality rates derived thusly vary from 0.45 for young-of-the-year summer flounder to 0.25 for fish age 7 and older (Table 5).

Table 5. Whole-stock mortality rate based on the age-dependency in sex ratio from 1976-2007 and the male and female natural mortality rates reported in Table 4.

Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7
0.447	0.424	0.394	0.362	0.316	0.286	0.262	0.245

A higher natural mortality rate in males could potentially be explained by some type of biological refuge for females. Female summer flounder are known to grow at a faster rate than males and may therefore be less prone to predation (Poole 1961). However, male and female growth rates are similar until age 2, so such an explanation would not be warranted when considering the apparent differential natural mortality in fish younger than age 2.
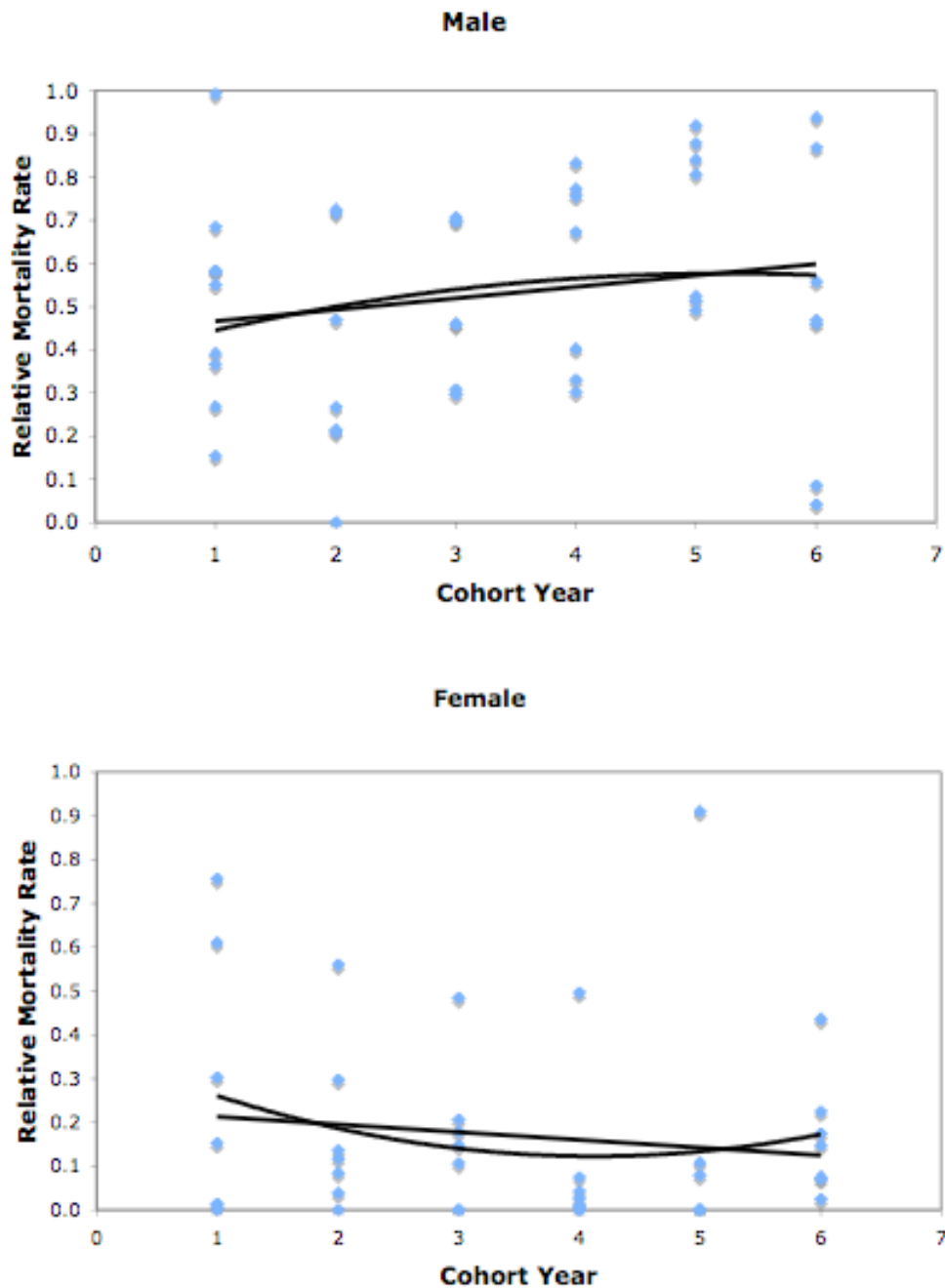
Some precedent exists for higher morality rates in male relative to female flatfish. Morse (1981) already proposed a higher natural mortality rate for males in summer flounder. Santos (1994) computed natural mortality rates for the four-spot megrim (*Lepidorhombus boscii*) by sex. The natural mortality rate for males was 0.41, and for females 0.34. Pearson and McNally (2005) also calculated mortality rates, using three different methods, for the sand sole, *Psettyichthys melanostictus*. The natural mortality rate for females ranged between 0.35 and 0.45, whereas the mortality rate for males was estimated to fall between 0.40 to 0.60.

Comparison of Age-Length Keys: Relationship of Length at Age with Region and Depth

Methods and Results

Tables 6 and 7 show the results of ANOVAs examining the relationship of depth, region, and year-group on length at age. For these analyses, the dependent variable, length, was ranked. Thus, the analysis is nonparametric. Depth significantly impacted length-at-age for males and females, ages 1 through 3 and age 4 for females. At age 0, summer flounder are only present in shallow waters, and at age 5 and older, depth no longer influences length-at-age, for the most part. Tukey's studentized range tests show that fish in deeper water are larger at a given age than fish in shallower water (Figure 6).

Length at age varied significantly with region for male and female summer flounder, ages 0-4, but not at older ages. Whereas Tukey's Studentized Range tests identified a tendency for some regions to group together at some ages, overall, fish are smaller in the south and get progressively larger at all ages, 0-4, northward (Figure 7). When regions did group together they did so in a north-central, south-central trend. In other words, the southern New England region never grouped with the south Atlantic or Delmarva regions, and the south Atlantic never grouped with the northern Mid-Atlantic and southern New England regions.

Male

Female

Figure 5. Trends in natural mortality rate by age for male and female summer flounder. Points are values from each of the years from 1976-2006 where sufficient data density permitted the estimate of within-cohort natural mortality rate at age. Neither polynomial nor linear curve fits transit a slope significantly at variance to zero.

Comparison of Age-Length Keys:Relationship of Length at Age with Region and Depth

Methods and Results

Tables 6 and 7 show the results of ANOVAs examining the relationship of depth, region, and year-group on length at age. For these analyses, the dependent variable, length, was ranked. Thus, the analysis is nonparametric. Depth significantly impacted length-at-age for males and females, ages 1 through 3 and age 4 for females. At age 0, summer flounder are only present in shallow waters, and at age 5 and older, depth no longer influences length-at-age, for the most part. Tukey's studentized range tests show that fish in deeper water are larger at a given age than fish in shallower water (Figure 6).

Length at age varied significantly with region for male and female summer flounder, ages 0-4, but not at older ages. Whereas Tukey's Studentized Range tests identified a tendency for some regions to group together at some ages, overall, fish are smaller in the south and get progressively larger at all ages, 0-4, northward (Figure 7). When regions did group together they did so in a north-central, south-central trend. In other words, the southern New England region never grouped with the south Atlantic or Delmarva regions, and the south Atlantic never grouped with the northern Mid-Atlantic and southern New England regions.

Disregarding the influence of fishing mortality on age-at-length, these trends indicate that summer flounder either grow at faster rates in deeper water and northern latitudes or that larger fish at age preferentially aggregate in these regions. Alternatively, in shallow waters and at southern latitudes larger fish may be more accessible to the fishery. While the fishery may not keep younger fish due to minimum size restrictions, younger fish may still be removed by the fishery as discard mortality. Whether it be a biological reason (e.g., differential growth rates) or a fishery-related reason (bigger fish at any age are more accessible in shallow/southern water), it seems clear that the average size of fish at age is larger in deeper/northern water than in shallow/southern water.

However, significant interaction terms also occur commonly in fish 4 years or less in age and these involve both depth and region with relatively equal frequency and intensity. The frequency of significant interaction terms including depth and region suggests that regionality in the trends in age at length for summer flounder cannot facilely be explained simply in terms of depth and latitude. A more complex mixture of biology and, perhaps, relative fishing impact is likely to be required. These trends are remarkably reminiscent of those observed earlier for sex ratio (Table 3).

Table 6. Results of ANOVAs examining the impact of depth, region, and year-group on male length for summer flounder. All regional data were included. Blank cells indicate insufficient data. NS, non-significant at $\alpha = 0.05$. ×, an interaction term.

Age	Region	Depth	Year Group	Depth × Region	Depth × Year Group	Year Group × Region	Year Group × Depth × Region
0	≤.001		≤.001			NS	
1	≤.001	≤.001	≤.001	≤.001	0.006	≤.001	NS
2	≤.001	≤.001	≤.001	NS	≤.001	≤.001	
3	≤.001	≤.001	≤.001	≤.001	NS	NS	NS
4	0.02	NS	≤.001	≤.001	NS	NS	NS
5	NS	NS	≤.001	NS	NS	NS	NS
6	NS	NS	NS	NS	NS	NS	NS
7	NS	NS	NS	NS	NS	NS	

Table 7. Results of ANOVAs examining the impact of depth, region, and year-group on female length for summer flounder. All regional data were included. Blank cells indicate insufficient data. NS, non-significant at $\alpha = 0.05$. ×, an interaction term.

Age	Region	Depth	Year Group	Depth × Region	Depth × Year Group	Year Group × Region	Year Group × Depth × Region
0	≤.001		≤.001			NS	
1	≤ .001	≤.001	≤.001	≤.001	NS	≤.001	0.003
2	≤.001	≤.001	≤.001	≤.001	≤.001	0.02	NS
3	≤.001	≤.001	≤.001	≤.001	0.03	0.02	NS
4	≤.001	0.008	≤.001	NS	≤.001	NS	0.02
5	NS	NS	≤.001	NS	NS	NS	NS
6	NS	0.01	≤.001	NS	NS	NS	NS
7	NS	NS	0.006	NS	NS	NS	
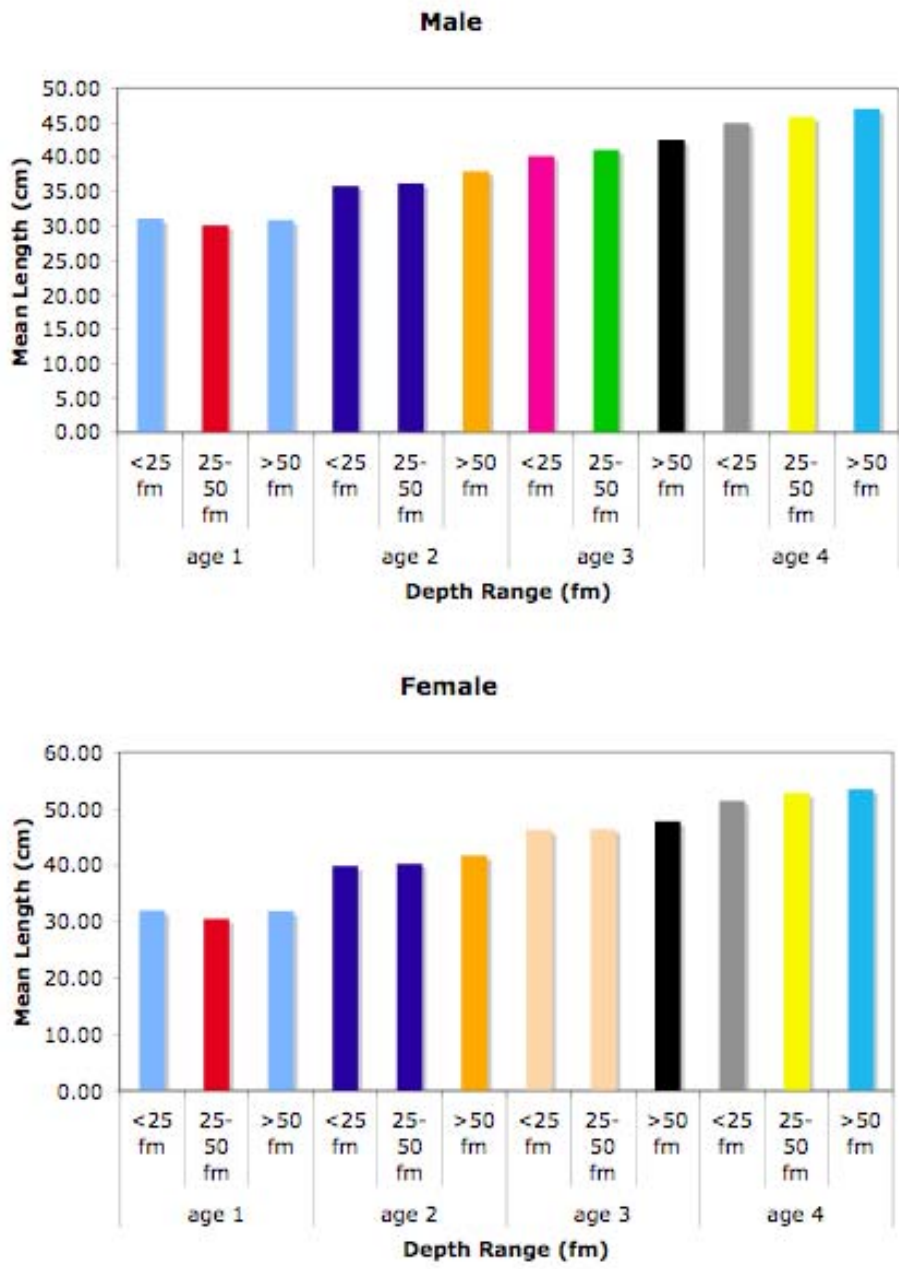
Figure 6. Mean length at age for male and female summer flounder. Bars within an age group with the same color were not significantly different in an *a posteriori* Tukey's test. ANOVA results are in Tables 6 and 7.

.
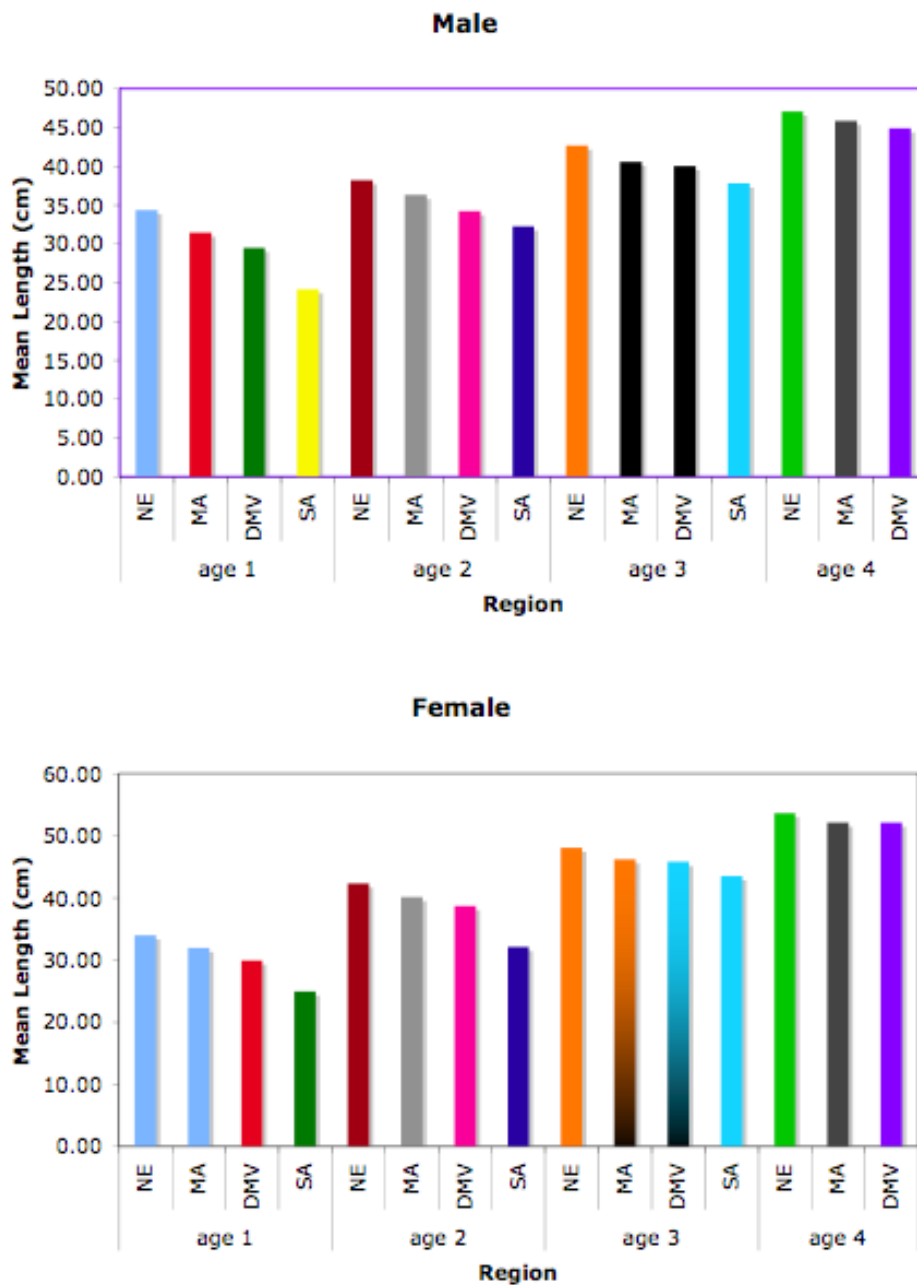
Figure 7. Mean length at age for male and female summer flounder. Bars within an age group with the same color were not significantly different in an *a posteriori* Tukey's test. ANOVA results are in Tables 6 and 7. NE, southern New England; MA, northern Mid-Atlantic; DMV, Delmarva; SA, south Atlantic

Disregarding the influence of fishing mortality on age-at-length, these trends indicate that summer flounder either grow at faster rates in deeper water and northern latitudes or that larger fish at age preferentially aggregate in these regions. Alternatively, in shallow waters and at southern latitudes larger fish may be more accessible to the fishery. While the fishery may not keep younger fish due to minimum size restrictions, younger fish may still be removed by the fishery as discard mortality. Whether it be a biological reason (e.g., differential growth rates) or a fishery-related reason (bigger fish at any age are more accessible in shallow/southern water), it seems clear that the average size of fish at age is larger in deeper/northern water than in shallow/southern water.

However, significant interaction terms also occur commonly in fish 4 years or less in age and these involve both depth and region with relatively equal frequency and intensity. The frequency of significant interaction terms including depth and region suggests that regionality in the trends in age at length for summer flounder cannot facilely be explained simply in terms of depth and latitude. A more complex mixture of biology and, perhaps, relative fishing impact is likely to be required. These trends are remarkably reminiscent of those observed earlier for sex ratio (Table 3).

Year-group consistently affected length at age for male and female summer flounder until age 6. Year-group no longer impacted length at age for male summer flounder at age 6 and older, but continued to do so for female fish. A few trends are clear in both males and females. First, summer flounder averaged much smaller at all ages in the period from 1976-1980 than any other year group. Additionally, year groups including years 1981-1985 and 2006-2007, generally group together as periods where size was smaller at age. Other year-groups are not consistently associated with small or large size-at-age groups. The former groupings are interesting because these periods are associated with relatively high abundance in comparison to the intermediate years. Additionally, a similar trend was noted in the sex ratio analyses.

Tables 8 and 9 repeat the ANOVAs of Tables 6 and 7, but restrict regional coverage to the two central regions, Delmarva and northern Mid-Atlantic. The frequency of significant main effects is much reduced in these ANOVAs relative to the earlier ones, although depth effects in particular continue to be present. Even for depth, significant main effects are less common, as are significant interaction terms, indicating that the central component of the stock offers a more spatially coherent picture than those portions near the northern and southern range limits.

Table 8. Results of ANOVAs examining the impact of depth, region, and year-group on male summer flounder length. Only the Mid-Atlantic and Delmarva regions were included. Blank cells indicate insufficient data. NS, non-significant at $\alpha = 0.05$. $\times$, an interaction term.

Age	Region	Depth	Year Group	Depth $\times$ Region	Depth $\times$ Year Group	Year Group $\times$ Region	Year Group $\times$ Depth $\times$ Region
0	≤.001		≤.001			NS	
1	NS	≤.001	≤.001	≤.001	≤.001	NS	NS
2	NS	≤.001	≤.001	NS	≤.001	NS	NS
3	NS	NS	≤.001	0.04	NS	NS	NS
4	NS	NS	≤.001	NS	NS	NS	NS
5	NS	NS	0.002	NS	NS	NS	NS
6	NS	NS	NS	NS	NS	NS	
7	NS	NS	NS				

Interpretation: Spatial Variation in Length-at-age

The ANOVAS of Tables 6-9 suggest that a single age-length key is not likely to be representative across all regions and in different depths. Furthermore, the differential with region and depth suggests that differential fishing pressure cannot be excluded as the mechanism generating these differences.

Table 9. Results of ANOVAs examining the impact of depth, region, and year-group on female summer flounder length. Only the Mid-Atlantic and Delmarva regions were included. Blank cells indicate insufficient data. NS, non-significant at $\alpha = 0.05$. $\times$, an interaction term.

Age	Region	Depth	Year Group	Depth $\times$ Region	Depth $\times$ Year Group	Year Group $\times$ Region	Year Group $\times$ Depth $\times$ Region
0	≤.001		≤.001			NS	
1	NS	≤.001	≤.001	≤.001	NS	0.005	≤.001
2	NS	≤.001	≤.001	NS	≤.001	NS	NS
3	NS	≤.001	≤.001	NS	0.01	NS	NS
4	NS	NS	≤.001	NS	0.004	NS	NS
5	NS	NS	≤.001	NS	NS	NS	NS
6	NS	NS	≤.001	NS	NS	NS	NS
7	NS	NS	0.04	NS	NS	NS	NS

Comparison of Age-Length Keys: Comparison of Key Structure Across Region and Depth

Methods and Results

These analyses focused on the age-length keys for the Delmarva and northern Mid-Atlantic region and, independently, on the three depth zones previously described, as these two regions were most similar in length at age. To compare keys efficiently, lengths were combined into 12 units, the central 10 being 5 cm intervals. Size 12 included fish ≥70 cm and size 1 included fish <20 cm. This yielded three age-length keys for the three depth zones and two for the two regions. Each of these returned a significant result from a by-region or by-depth chi-square test, and from a Cochran-Mantel-Haenszel test controlling for depth or region. Tests were conducted on doubly standardized arrays (columns and rows). These results are anticipated since the ages are not independently distributed with respect to lengths in these keys.

The commonest age at length translates a diagonal from the northwestern to the southeastern corner of the age-length array (age in columns, length in rows). This trend is consistent across keys. A same cell-to-same cell mapping evaluated by a Spearman's rank correlation test on doubly standardized arrays returned a significance level of $P < 0.0005$ or better for each pair-wise comparison (e.g., Delmarva vs northern Mid-Atlantic). This also is expected from the anticipated structure of the keys.

These two standard statistics are described because these approaches, plus the afore-described ANOVAs (Tables 6-9) represent typical statistical treatments of age-length data. None of them answer the query concerning equivalency of the age-length relationship documented by the distribution of ages at length in the two-dimensional array of the key.

To directly compare two keys, we used Geary's C and Moran's I statistics on the set of residuals obtained by calculating the expected key structure in one array from the observed key structure in the other. Each row was standardized, but a column standardization was not performed. Residuals were calculated for each array cell as $expected_{i,j} - observed_{i,j}$ where the expected values were obtained from the first of two paired arrays. The null hypothesis is that the residuals will be randomly distributed in x-y space. A statistical test revealing a non-random pattern in the residuals indicates that some portion of the two arrays under examination differ in the distribution of values among cells; that is, that the age-length relationship differs.

The test statistics were calculated following Cliff and Ord (1973) where:

$$Moran's\ I = \left(\frac{n}{W}\right) \frac{\sum_{\substack{i=1 \\ i \neq j}}^{n} \sum_{j=1}^{n} w_{ij} z_i z_j}{\sum_{i=1}^{n} z_i^2} \ ; \tag{4}$$

$$Geary's\ C = \left(\frac{n-1}{2W}\right) \frac{\sum_{\substack{i=1 \\ i \neq j}}^{n} \sum_{j=1}^{n} w_{ij} (x_i - x_j)^2}{\sum_{i=1}^{n} z_i^2} \tag{5}$$

and

$$W = \sum_{i=1}^{n} \sum_{\substack{j=1 \\ i \neq j}}^{n} w_{ij} \ ; \tag{6}$$

$$z_i = x_i - \bar{x}; \tag{7}$$

$n =$ number of samples; $x_i =$ datum of each sample i; and $w_{ij} =$ a weighting measure as described subsequently. Significance levels were calculated under the assumption of randomization (Jumars et al., 1977).

Calculation of Moran's I or Geary's C is contingent on the mathematical representation of the spatial relationship of the cell values (w_{ij}). We employed a King's moves weighting such that any two cells i and j located in the array at position k,l for i and at positions $k,l+1$, $k,l-1$, $k-1,l$, and $k+1,l$ for j were given $w_{ij} = 1.0$. For the remainder, $w_{ij} = 0$.

Moran's I is sensitive to the location of extreme departures from the mean ($x_i - \bar{x}$). The expected value of I for spatially randomly distributed samples is $-(n-1)^{-1}$, a number close to zero at high n (Cliff and Ord, 1973). High values of I occur if x_i and x_j are both, much above or much below the mean. Geary's C is sensitive to sample-to-sample variation ($x_i - x_j$). Values above 1.0 indicate negative spatial autocorrelation (i.e., neighboring values less similar than expected by chance), an even distribution. Values below 1.0 indicate positive spatial autocorrelation (i.e., neighboring values more similar than expected by chance), a patchy distribution.

Both statistics are provided; however the question at hand is the relationship of nearest neighbors in the array and thus a strong preference is given to Geary's C as the statistic best evaluating similarity between two age-length arrays.

An important question concerns the incorporation of zeros. The northeastern and southwestern corners of the arrays routinely contain zeros. These paired zeros increase the number of cells and thus bias the statistic in proportion to their importance, as they are a guaranteed characteristic of the array if formulated correctly. An additional concern is singleton zeros, as the residual obtained is less constrained than for cases with paired non-zero values. In our opinion, the most valid approach is to discount paired-zeros only. Results of comparison of three age-length keys for the three depths and two geographic regions are provided in Tables 10 and 11. These comparisons are based on the exclusion of paired-zero cells.

Table 10. Values of Geary's C and Moran's I and significance for the cases in which array cells characterized by paired zeros were excluded. Age-length keys compared were for males only. The first array in each array pair as listed is the parent array used to generate the expected values for the second array. Residuals were calculated for the second array. Significance values are Moran's I over Geary's C $\left(\dfrac{Moran's\ I}{Geary's\ C}\right)$. NS, not significant. For region, Delmarva was used as the parent array.

Array Pair	Moran's I	Geary's C	Significance
Depth 1 vs. 2	0.182	2.175	$P < 0.05$
			$P < 0.005$
Depth 1 vs. 3	-0.005	2.281	NS
			$P < 0.005$
Depth 2 vs. 3	0.164	0.932	$P < 0.005$
			NS
Region	-0.090	2.353	NS
			$P < 0.005$

Table 11. Values of Geary's C and Moran's I and significance for the cases in which array cells characterized by paired zeros were excluded. Age-length keys compared were for females only. The first array in each array pair as listed is the parent array used to generate the expected values for the second array. Residuals were calculated for the second array. Significance values are Moran's I over Geary's C $\left(\dfrac{Moran's\ I}{Geary's\ C} \right)$.

NS, not significant. For region, Delmarva was used as the parent array.

Array Pair	Moran's I	Geary's C	Significance
Depth 1 vs. 2	0.056	1.645	NS
			$P < 0.01$
Depth 1 vs. 3	0.174	1.392	$P < 0.05$
			$P < 0.10$
Depth 2 vs. 3	0.026	0.890	NS
			NS
Region	-0.013	1.573	NS
			$P < 0.05$

The trend with depth is exemplified by Table 12 in which is compared via residuals the arrays for depths 1 (<25 fm) and 3 (>50 fm). A negative residual indicates a higher value in the second array (>50 fm). Consistently, across size groups, the negative residuals occur at older ages indicating that male fish of a given size tend to be older at deeper depths. This is consistent with ANOVA results described earlier in Tables 6-9. Table 13 compares the male arrays for the two regions. In this case, the residual pattern is more complex; however, negative values tend to occur at younger ages for a given size, indicating that males tend to be older at size in the southern portion of the Mid-Atlantic Bight. This is also consistent with ANOVA results.

Table 12. Residual pattern for the comparison of and example age-length relationship in summer flounder males between arrays representing depths 1 and 3.

Length (cm)	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6
<20	54.43	-60.76	6.33				
20-<25	30.01	-40.91	10.90				
25-<30	23.04	-35.13	12.62	-0.60	0.07		
30-<35	6.92	14.68	-15.40	-5.69	-0.51		
35-<40	0.33	42.35	-31.37	-10.17	-0.69	-0.46	
40-<45		10.06	30.03	-28.95	-9.65	-1.49	
45-<50		1.00	18.93	12.20	-22.38	-7.77	-1.99
50-<55			4.42	18.67	-9.67	-2.05	-11.37
55-<60			1.91	-4.88	3.29	11.77	-12.09

Table 13. Residual pattern for the comparison of and example age-length relationship in summer flounder males between arrays representing Delmarva and the northern Mid-Atlantic Bight.

Length (cm)	Age 0	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6
<20	17.5540	-24.7482	7.1942				
20-<25	20.0380	-25.6052	5.5672				
25-<30	9.2232	-16.8604	7.6844	-0.2277	0.1805		
30-<35	0.4179	-2.9349	0.8727	1.4069	0.2375		
35-<40	-0.0198	-14.4489	7.3256	5.8648	1.2466	0.0317	
40-<45		-1.8036	-17.6687	12.4069	7.1451	-0.0799	
45-<50		-0.4988	-8.7182	-3.3502	12.3292	0.4147	-0.1767
50-<55		-1.6949	-2.6635	3.8740	6.7192	-6.2349	
55-<60		-4.0000	16.6667	13.3333	-31.3333	5.3333	

Interpretation

The male age-length relationships are more variable over depth and region than the female ones. Probably, this accrues from the higher natural mortality rates for the males which are also probably more variable spatially. The analyses suggest that a single age-length key may not be adequate, particularly for the males. However, comparison of model runs using different age-length keys would be needed to determine whether the observed variations are substantive for stock modeling. Regardless, the analyses again focus on the need to differentiate the two sexes and to investigate a spatially explicit model.

Conclusions and Recommendations

1.   Young-of-the-year summer flounder are dominantly male. Sex ratio changes gradually with age such that male frequencies over 0.5 occur infrequently by age 2 and rarely exceed 0.3 by age 4. The biased sex ratio at birth is likely the result of temperature-dependent sex determination (TSD).

2.   The age-dependency of sex ratio indicates the need to implement a sex-explicit model for summer flounder. Spatial variation in sex ratio suggests that a single sex-at-age key is not likely to be representative across all regions and in different depths.

3.   The change in sex ratio with age also requires that separate natural mortality rates be used for male and female summer flounder stock assessment models. In lieu of a sex-explicit model, a whole stock mortality rate might be employed, although this is less satisfactory. The natural mortality rate, derived thusly, is not linear, but varies from 0.45 for age-0 fish to 0.25 for fish age 7 and older.

4.   Higher natural mortality rate in male summer flounder is supported by published information on summer flounder and other flatfish.

5.   Spatial variation in length-at-age suggests that a single age-length key is not likely to be representative across all regions and in different depths. The differential with region

and depth suggests that differential fishing pressure cannot be excluded as the mechanism generating these differences.

6. The male age-length relationships are move variable over depth and region than the female ones, but each varies significantly. The analyses suggest that a single age-length key may not adequately describe the stock, particularly for the males.

References

Cliff AD, Ord JK. 1973. (*Spatial Autocorrelation)*. Pion Limited, London. 266 p.

Goto R, Mori T, Kawamata K, Matsubara T, Mizuno S, Adachi S, Yamauchi K. 1999. Effects of temperature on gonadal sex determination in barfin flounder (*Verasper moseri)*. Fish Sci 65: 884-887.

Goto R, Kayaba T, Adachi S, Yamauchi K. 2000. Effects of temperature on sex determination in the marbled sole (*Limanda yokohamae)*. Fish Sci. 66: 400-402.

Jumars PA, Thistle D, Jones ML. 1977. Detecting two-dimensional spatial structure in biological data. Oecologia *(Berl.),* 28: 109-123.

Luckenbach JA, Godwin J, Daniels HV, Borski RJ. 2003. Gonadal differentiation and effects of temperature on sex determination in southern flounder (*Paralichthys lethostigma*). Aquaculture 216:315-327.

Morse WW. 1981. Reproduction of the summer flounder, (*Paralichthys dentatus)* (L.) J Fish Biol. 19: 189- 203.

Pearson DE, McNally SVG. 2005. Age, growth, life history, and fisheries of the sand sole, *(Psettyichthys melanostictus)*. Mar Fish Rev. 67(4): 9-18.

Poole JC. 1961. Age and growth of the fluke in Great South Bay and their significance to the sport fishery. NY Fish Game J. 8: 1-18.

Santos PT. 1994. Growth and reproduction of the population of the four-spot me grim (*Lepidorhombus boscii* Risso) off the Portuguese coast. Neth J Sea Res. 32: 379-383.

Smith RA, Daiber FC. 1977. Biology of the summer flounder, (*Paralichthys dentatus)*, in the Delaware Bay. Fish Bull. 75: 823-830.

Yamamoto E. 1999. Studies on sex-manipulation and production of cloned populations in hirame, *(Paralichthys olivaceus)* (Temminck et Schlegel). Aquaculture 173: 235-246.