APPLICATION OF MIXTURE MODELS FOR ESTIMATING AGE AND GROWTH OF STREAM DWELLING BROOK TROUT[1]

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Abstract: Assigning fish age using mixture models of length distributions has been suggested as an alternative to using calcified structures; however, its use has been limited. Rather, the use of calcified structures is the method of choice for assigning age to individual fish. We assigned ages by using mixture models of 12 length frequency distributions for brook trout from southeast Minnesota streams and compared results to those from otoliths. We used the mixdist package in the software program R to fit finite mixture distribution models, comparing models with normal and lognormal length at age distributions, and with various constraints on standard deviations and age distributions. Neither normal nor lognormal distributions consistently provided the best fit. Likewise, there was no consistent best constraint on standard deviations; however, equal values most commonly provided the best fit. Modeled mean lengths at age differed from those based on otoliths. In particular, mean length at age-0 was smaller using modeled results compared to otoliths, because we did not have otolith samples from the smallest fish. Mixture models appear to provide a good alternative to calcified structures for age and growth information on brook trout in southeastern Minnesota.

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Examination of calcified structures for annuli is the most common technique used to identify age of fish. However, the quality of data this technique generates can be suspect. For example, only 27% of state fish agencies had confidence in aging older fish with scales (Maceina et al. 2007). Historically scales have proven to be inaccurate for estimating age of salmonids (Alvord 1953; Hatch 1961; Burnet 1969), but their use still persists by many state and federal agencies. Fisheries biologists for Minnesota Department of Natural Resources use scales as estimators of age for both brown and brook trout. Hining et al. (2000) found that only 32% of age 3 rainbow trout formed a third annulus and 0% of age 4 fish formed a fourth annulus. Alvord (1953) also found that up to 65% of age 3 and older brown trout failed to form an annulus. Other authors have reported that trout often do not form their first year annulus (Lentsch and Griffith 1987; Jensen and Johnsen 1982). Otoliths provide a better alternative to scales for most species, yet accuracy and precision of otoliths can still be low in some situations (Maceina et al. 2007). In addition, the use of otoliths requires fish to be sacrificed, which may be unacceptable for some populations. Also, the time and cost associated with aging fish using traditional methods and equipment (employee hours, microscope, Isomet saw, polisher, etc.) is substantial. Alternative methods for aging fish include examination of length frequency histograms or the use of mark-recapture data. Fish marked at a known age (usually age-0) can be recaptured at a later time to estimate growth and length at age. Mark-recapture is ideal because estimates of individual observed growth are possible; however, it is time consuming and requires additional effort of return sampling.

Length frequency analysis has been used to assign ages to fish since the late 1800's (Jackson 2007). While certainly the least expensive and time consuming, it can lead to inaccuracies when dealing with long lived and slow growing species. Several different techniques have been used for length frequency analysis (Macdonald 1987). The easiest is visually selecting modes from a length frequency histogram of sampled fish. When distinct modes are present, this method can produce satisfactory results. Another, more quantitative, technique involves using statistical models such as a mixed distribution model (Macdonald and Pitcher 1979). These are useful when the length frequency histogram does not have very distinct length groups, and can incorporate known-age data from other sources on some individuals. Several different software programs have been developed to analyze length frequency data (e.g., MIX, Macdonald and Green 1988; MULTIFAN, Fournier et al. 1990). Despite the early origins of length analysis and the advent of easy to use computer programs, its use is still secondary to ageing calcified structures.

Despite being a short lived species, brook trout are notoriously difficult to age with calcified structures. Several authors have proposed the use of otoliths for aging trout, given their increased accuracy and precision (Hall 1991; Hining et al. 2000); however, precision of age estimation from calcified structures can depend on geographic location, age structure, and maturity (Hoxmeier et al. 2001). For example, neither scales nor otoliths were found to reliably age brook trout in high-elevation Rocky Mountain streams in Wyoming (Kozel and Hubert 1987). Brook trout in southeast Minnesota are typically found in small numbers isolated in headwaters of streams. Some populations are unique to this region and may represent remnant stocks from pre-European settlement. Given the rarity of these populations, it is undesirable to sacrifice large numbers of fish for age and growth analysis.

To improve accuracy, reduce costs, and reduce the number of fish sacrificed for otoliiths, we sought an alternative to ageing brook trout with calcified structures. Specifically, we fit mixed distribution models of 12 length frequency distributions (six streams, two years) for brook trout and attempted to identify the best model structures for estimating individual ages, length at age distributions, and sample age distributions.

Methods

We sampled brook trout populations in six coldwater streams in southeast Minnesota in the fall of 2008 and 2009. Study streams included East Indian, Maple Creek, Coolridge Creek, Trout Valley, Trout Brook, and Garvin

Brook. Brook trout were collected by electrofishing with either a backpack or tow barge shocker depending on stream size. Each fish was measured for total length (nearest mm) and sagittal otoliths were removed from a random subsample of fish, except for the smallest fish. We did not take otoliths from brook trout under 80mm due to difficulty in extracting them without the aid of a microscope. Known age fish were developed by fin clipping age-0 brook trout identified by length in 2008, and recapturing these marked fish in 2009 as known age-1 brook trout for Trout Valley, Maple, Trout Brook, and Garvin. For East Indian, we fin clipped age-0 brook trout in the fall of 2007 and recaptured some at age-1 and age-2 in 2008 and 2009. In Coolridge Creek, brook trout were individually marked with PIT tags in fall 2006 and spring 2007 and recaptured at later dates as known age fish. The known ages of these fish were included in the dataset to inform the mixture model analyses.

Otoliths were read in whole view on a black background with reflected light. No information on length was available when ageing otoliths. Because not all fish were aged with otoliths, we assigned ages to the rest of the population using an age length key. We then calculated mean length at age based on observed and assigned ages to compare with mean length at age generated by mixture models.

Age was estimated using mixed distribution models developed from length frequency histograms seeded with "known age" fish (termed conditional data in mixdist). Known age fish were those marked at age-0 and recaptured as age-1, and from a second read of fish aged with otoliths. Otoliths were read a second time with access to length and year-class strength information, and ages were treated as known age fish for the length frequency analysis. Length frequency histograms were divided into 10-mm length groups. We used the mixdist package (Macdonald and Du 2010) in the software program R (R Development Core Team, 2009) to fit finite mixture distribution models to the length frequency histograms. Mixdist provides estimates for the age distribution (i.e., mixing proportions π), mean length at age (μ) and standard deviations of length at age distributions (σ). Mixing proportions are estimates of the relative abundance of each age group as a proportion of the entire measured sample. To the extent that trout were sampled by size-selective gear, mixture model estimates will be biased estimates of values of the wild population. The first step in fitting the distributions was to determine how many age classes were present in each sample. This was determined by otolith ages estimated from two reads and the maximum size of fish present in the sample. We used between 3 and 5 age groups among samples. We then input initial values for π_a, μ_a, and σ_a based on visual examination of the length frequency histogram. Constraints can be used on any of the three parameters (π_a, μ_a, σ_a) to reduce the number of parameters to be estimated. We tested several different constraints on standard deviation and compared model results. The best models were chosen based on the lowest χ^2 value. The constraints on standard deviations were as follows: None – attempts were made to estimate all σ_a, SEQ – all σ were assumed equal, CCV – assumes that σ_a increases with μ_a. We also tested between three length at age probability distributions: normal, lognormal, and gamma. Results with gamma distributions are not presented, as that distribution typically was not a better fit to the data. We did not constrain π_a for any of our tests except for Garvin Brook, where we set π_a to 0.05 for the 2007 year-class given that this year-class was almost absent. We did not constrain π_a for any of the other populations given that we had enough known age fish to show that the 2007 year-class was rare. A constraint that μ_a follow a von Bertalanffy growth curve was attempted on some initial distributions, but this constraint failed to converge on many of the initial examinations. Therefore, we dropped this constraint from further analyses. An example of the R code used for Garvin Brook 2009 is given in Box 1.

Results

Brook trout were collected in sufficient numbers in most streams and years, with the exception of Trout Brook in 2009 in where only 33 brook trout were collected. Macdonald and Pitcher (1979) recommend at least 50 fish for each age group; however, that is without an aged subsample. The number of brook trout collected was typically over 100 individuals and was as high as 820 in Trout Valley 2009 (Table 1). Most populations consisted of small fish less than 200 mm, but some had trout as large as 350mm. As such, most populations exhibited a young age structure comprising mainly of age-0 and age-1 fish. Strong and weak year-classes were evident, with 2007 being a weak year-class across most streams. We marked 783 brook trout as age-0 in 2008 and recovered 55 as age-1 to incorporate into our conditional dataset for three streams (Trout Valley n=39, Maple n=13, and Trout Brook n=3). We did not collect any marked fish in Garvin Brook. For East Indian, we marked 110 age-0 brook trout in the fall of 2007 and recaptured 19 in the fall of 2008 as known age-1 and two in the fall of 2009 as known age-2. In Coolridge Creek we tagged 251 brook trout with PIT tags and recaptured 27 in 2008 and 19 in 2009 as known age fish. A total of 295 otoliths were read from 6 streams and age length keys were developed from these data and applied to the unaged sample.

While most fish were aged at 3 years or less, there were a few exceptions. In Maple Creek, a 338-mm brook trout was aged at 6 years. Also, because we randomly selected fish to remove otoliths, we did not have many otolith samples from large fish given that they were rare in the population. If a concerted effort had been made to sacrifice all fish over 300-mm, we would have undoubtedly gotten more fish over age 3.

Most brook trout populations had a good fit except for Trout Valley in 2009. (Figure 1). Neither normal or lognormal probability distributions consistently provided the best fit (Table 1). Although there was not a single constraint on sigma that was the best across all streams, equal sigmas appeared to be most common (Table 1). Mean length at age differed between that derived from otoliths and from modeled estimates (Table 2). In particular, mean length at age-0 was smaller using modeled results compared to otoliths.

Discussion

Mixed distribution models provided similar results to that of an aged subsample in some situations but not others. Mixdist likely provided better estimates for the tails of the length distribution (small and large fish). Reasons for this could be because otolith reads were based on small sample sizes for really small and large fish. Age-0 were overestimated because we did not take otoliths on the smallest fish in proportion to their abundance, given the difficulty of removing otoliths from fish under 80mm. For larger fish, we did not remove many otoliths given their rarity in the population, and therefore we may have missed some older fish in the population.

Although otoliths have been suggested as the structure of choice for brook trout (Stolarski and Hartman 2008), we still found it difficult to interpret annuli on whole otoliths from brook trout. A subsample of otoliths were examined by other experienced individuals who also had difficulty determining age (D. Logsdon, MN DNR; D. Isermann, UW-Stevens Point). Because of this difficulty, there is still considerable error associated with “known age” fish from otolith reads in this study. Scales and fin rays were also examined for some brook trout populations, but they were more difficult to interpret than otoliths. Similarly, Kozel and Hubert (1987) had difficulty ageing brook trout with otoliths in a slow growing population in the Rocky Mountains. Difficulties in ageing brook trout with otoliths in the Driftless Area further increase the utility of assigning ages with mixed distribution models.

Larger fish were not sacrificed in high numbers for otolith removal because they were rare. These larger fish were likely older individuals (as opposed to fast growing younger fish), and therefore we likely underestimated the age structure in some populations. The oldest age groups should be interpreted as either age-3 and older or age-4 and older. However, given that most of the population was smaller fish less than 3 years old, these data likely reflect the majority of the population. Because most brook trout in southeastern Minnesota and Wisconsin appear to die after age-3 (Brasch et al. 1973), the lack of older fish makes it conducive for mixture analysis. Also, mixdist was useful in that estimates of mean length at age-3 and 4 could be calculated without sacrificing large numbers of older brook trout.

There was not a consistently best model structure that was a good fit for all populations. Both normal and lognormal probability distributions provided good fits to the data. Likewise, constraints on sigma that gave the best results varied. Equal sigmas gave the best results overall, suggesting that the range in growth remains consistent across ages. Although we did not find a model setup that was the best across all situations, starting with a lognormal distribution and a constraint on sigmas being equal should provide a good starting point.

A limitation to this study was the lack of more known age fish and otolith ages from older brook trout. An ideal comparison between age estimates from otoliths versus length frequency, would be to have all known age fish for each sample, however, sacrificing large numbers of brook trout is an unlikely scenario given their management and conservation importance. Also, because electrofishing can give a biased size distribution, results from mixture modeling will also be biased relative to the true population. However, this bias appears less than that derived from otolith estimates given the reasons described above.

Mixture modeling uses all length information, and any age information available to estimate mean length at age. In our experience, it performs best when you have information available about the range of ages in the sample of interest, about weak or missing year-classes, and about some known age individuals. Samples from two or more years are helpful for identifying the progression of strong and weak year-classes; however, mixdist only analyzes samples one year at a time. We knew about weak year-classes, individual growth differences, and relative size at age (from otoliths). This allowed us to start with meaningful parameters and for imposing constraints on sigma and π. While this incorporation of prior information can potentially introduce bias, it should give better results compared to blind reads of structures (especially scales).

References

Alvord, W. 1953. Validity of age determinations from scales of brown trout, rainbow trout, and brook trout. Transactions of the American Fisheries Society 83:91-103.

Brasch, J., J. McFadden, and S. Kmiotek. 1973. Brook trout: life history, ecology and management. Wisconsin Department of Natural Resources Publication 226, Madison.

Burnet, A. M. R. 1969. An examination of the use of scales and fin rays for age determination of brown trout (*Salmo trutta* L.). New Zealand Journal of Marine and Freshwater Research 3:147-151

Fournier, D. A., J. R. Sibert, J. Majkowski, and J. Hampton. 1990. MULTIFAN: a likelihood-based method for estimating growth parameters and age composition from multiple length frequency data sets illustrated using data for southern bluefin tuna (*Thunnus maccoyii*). Canadian Journal of Fisheries and Aquatic Sciences 47:301-317.

Hall, D. L. 1991. Age validation and aging methods for stunted brook trout. Transactions of the American Fisheries Society 120:644-649.

Hatch, R. W. 1961. Regular occurrence of false annuli in four brook trout populations. Transactions of the American Fisheries Society 90:6-12.

Hining, K. J., J. L. West, M. A. Kulp, and A. D. Neubauer. 2000. Validation of scales and otoliths for estimating age of rainbow trout from southern Appalachian streams. North American Journal of Fisheries Management 20:978-985.

Hoxmeier, R. J. H., D. D. Aday, and D. H. Wahl. 2001. Factors influencing the precision of age estimation from scales and otoliths of bluegills in Illinois reservoirs. North American Journal of Fisheries Management 21:374-380.

Jackson, J. R. 2007. Earliest references to age determination of fishes and their early application to the study of fisheries. Fisheries 32:321-328.

Jensen, A. J., and B. O. Johnsen. 1982. Difficulties in aging Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) from cold rivers due to lack of scales as yearlings. Canadian Journal of Fisheries and Aquatic Sciences 39:321-325.

Kozel, S. J., and W. A. Hubert. 1987. Age estimates of brook trout from high-elevation Rocky Mountain streams using scales and otoliths. Northwest Science 61:216-219.

Lentsch, L. D., and J. S. Griffith. 1987. Lack of first-year annuli on scales: frequency of occurrence and predictability in trout of the western United States. Pages 177-188 *in* R. C. Summerfelt and G. E. Hall, editors. The age and growth of fish. The Iowa State University Press, Ames.

Macdonald, P. D. M. 1987. Analysis of length-frequency distributions. Pages 371-384 *in* R. C. Summerfelt and G. E. Hall, editors. The age and growth of fish. The Iowa State University Press, Ames.

Macdonald, P. and J. Du. 2010. Mixdist: Finite Mixture Distribution Models. R package version 0.5-3. http://CRAN.R-project.org/package=mixdist**.**

Macdonald, P. D. M., and P. E. J. Green. 1988. Users guide to program MIX: an interactive program for fitting mixtures of distributions. ICHTHUS Data Systems, Ontario Canada.

Macdonald, P. D. M., and T. J. Pitcher. 1979. Age-groups from size-frequency data: a versatile and efficient method of analyzing distribution mixtures. Journal of the Fisheries Research Board of Canada 36:987-1001.

Maceina, M. J., J. Boxrucker, D. L. Buckmeier, R. S. Gangl, D. O. Lucchesi, D. A. Isermann, J. R. Jackson, and P. J. Martinez. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. Fisheries 32:329-340.

R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Stolarski, J. T., and K. J. Hartman. 2008. An evaluation of the precision of fin ray, otolith, and scale age determinations for brook trout. North American Journal of Fisheries Management 28:1790-1795.

Box 1. Example of R code used to estimate mean length at age for brook trout collected in Garvin Brook 2009.

```
library(mixdist)
data = read.csv("Garvin09ages.csv")
data=as.mixdata(data)
plot(data)
data

# lognormal, sigmas equal. set pi to .05 for 2007
# year-class
lnormSEQ = mix(data, mixparam(mu= c(105,185,195,250),
     pi = c(.6,.3,.01,.05), sigma= c(10,15,15,20)),
     "lnorm", mixconstr(consigma="SEQ", conpi="PFX",
     fixpi = c(F, F, T, F)), iterlim=200, usecondit=T)
summary(lnormSEQ)
#create black and white graph
plot(lnormSEQ, main = "Garvin 2009", sub = "",
     xlab= "Total length (mm)", ylab = "Relative
     frequency", bty=“o", BW=T)
```

Table 1. The goodness of fit (χ^2) of the best mixed distribution models fit to length frequency histograms from six brook trout populations. Models were fit using the MIXDIST package in the software program R. The constraints on standard deviations were: None – attempts were made to estimate all σ, SEQ - all σ were assumed equal, CCV – assumes that σ increases with μ. We also tested different data distributions: normal and lognormal.

Stream	Distribution	Constraints on σ	χ^2	P-value	Sample size
		2008			
Coolridge	normal	CCV	37.4	0.86	93
Maple	lognormal	none	25.5	0.88	259
East Indian	normal	SEQ	37.0	0.61	639
Garvin	lognormal	SEQ	16.8	0.95	147
Trout Valley	normal	SEQ	54.2	0.14	428
Trout Brook	lognormal	SEQ	33.9	0.56	343
		2009			
Coolridge	normal	SEQ	14.9	0.99	208
Maple	lognormal	none	50.1	0.24	767
East Indian	lognormal	SEQ	27.9	0.83	509
Garvin	lognormal	SEQ, fixed π	42.1	0.26	206
Trout Valley	lognormal	SEQ	124.4	<0.001	820
Trout Brook	lognormal	SEQ	17.3	0.94	33

Table 2. Mean total length at capture (in bold; sample size (n) and ±SE below) for brook trout caught in six southeastern Minnesota streams in fall of 2008 and 2009. Estimates were made from otoliths (aged) and from length frequency histograms fitted with a finite mixture model (Mix).

	Age-0		Age-1		Age-2		Age-3		Age-4	
	Aged	Mix	Aged	Mix	Aged	Mix	Aged	Mix	Aged	Mix
					2008					
Coolridge	**92.3**	**95.4**	**160.9**	**161.2**	**190.6**	**205.7**	**182.3**	**206.6**		
	(4) 8.2	2.0	(7) 7.3	10.5	(5) 11.3	4.7	(3) 3.5	14.3		
East Indian	**107.4**	**101.9**	**181.8**	**192.1**	**223.8**	**225.3**	**231.3**	**230.4**		
	(11) 2.9	0.8	(4) 4.6	3.5	(4) 10.3	6.7	(4) 9.9	18.2		
Garvin	**121.4**	**100.4**	**188.0**	**182.2**	**245.0**	**227.2**	**244.0**	**254.3**		
	(14) 5.6	1.3	(1) -	15.8	(10) 6.7	8.3	(1) -	7.8		
Maple	**114.2**	**102.9**	**195.4**	**190.2**	**234.3**	**219.7**	**252.0**	**259.2**		**316.8**
	(10) 2.9	0.8	(15) 5.0	6.2	(6) 17.5	8.7	(2) 7.0	9.3		6.9
Trout Brook	**135**	**106.6**	**194.0**	**174.1**	**228.7**	**213.3**	**-**	**255.4**		
	(12) 4.8	0.8	(3) 4.4	20.6	(6) 11.5	4.7	-	6.9		
Trout Valley	**139.9**	**114.5**	**223.0**	**163.8**	**236.0**	**243.9**	**270.0**	**274.6**		**311.4**
	(21) 3.5	1.1	(1) -	22.5	(3) 6.8	13.3	(3) 20.6	5.2		19.4
					2009					
Coolridge		**91.2**		**166.3**		**196.3**		**228.2**		
		1.2		3.8		4.8		8.4		
East Indian	**118.3**	**112.8**	**186.7**	**184.5**	**223.5**	**214.0**		**267.4**		
	(14) 5.2	0.9	(11) 5.8	2.1	(4) 8.5	4.6		12.9		
Garvin	**108.0**	**99.5**	**167.8**	**177.7**	**194.0**	**202.3**		**238.7**		
	(19) 3.1	1.1	(8) 4.3	2.1	(3) 25.5	10.1		5.9		
Maple	**103.6**	**97.7**	**154.7**	**156.6**	**224.0**	**201.0**	**270.0**	**282.2**		**339.4**
	(11) 6.3	1.1	(25) 3.9	1.4	(3) 7.8	3.5	(3) 12.0	4.9		13.5
Trout Brook	**105**	**103.5**	**168.1**	**165.4**	**193.8**	**172.5**		**203.8**		
	(2) 0.0	10.7	(11) 5.6	4.0	(5) 6.0	16.2		8.9		
Trout Valley	**129.9**	**124.1**	**169.7**	**191.3**	**228.2**	**219.1**	**297.0**	**270.3**		
	(17) 2.4	0.8	(3) 5.6	2.3	(5) 8.5	4.2	(1) -	11.4		

Figure 1. Length frequency histograms of brook trout caught in six streams in fall 2008 and 2009. Overlaying solid lines are best fitting mixture models. Mean length at age values are represented by triangles. Dotted curved lines are age groups.

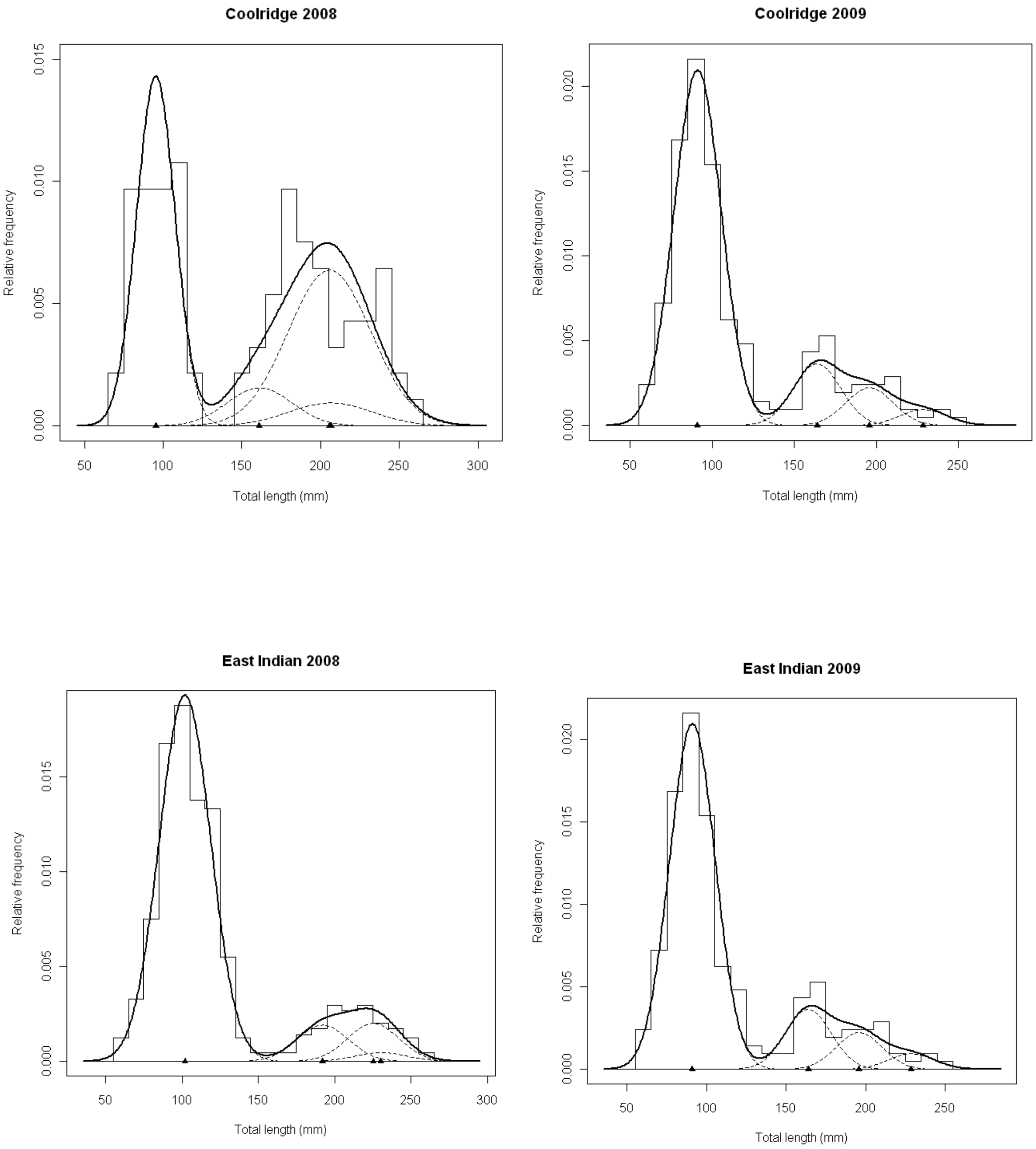


Figure 1 continued

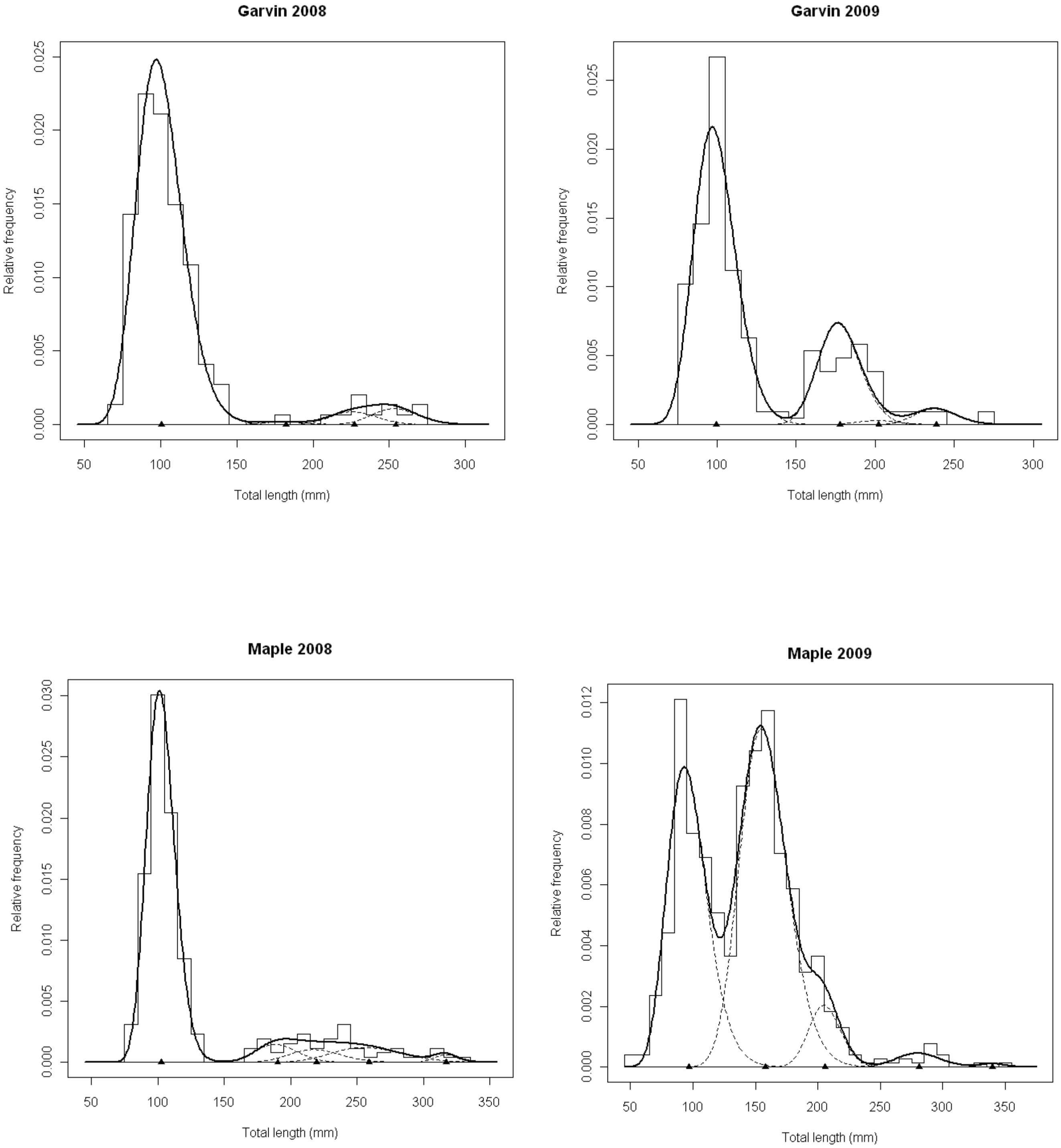
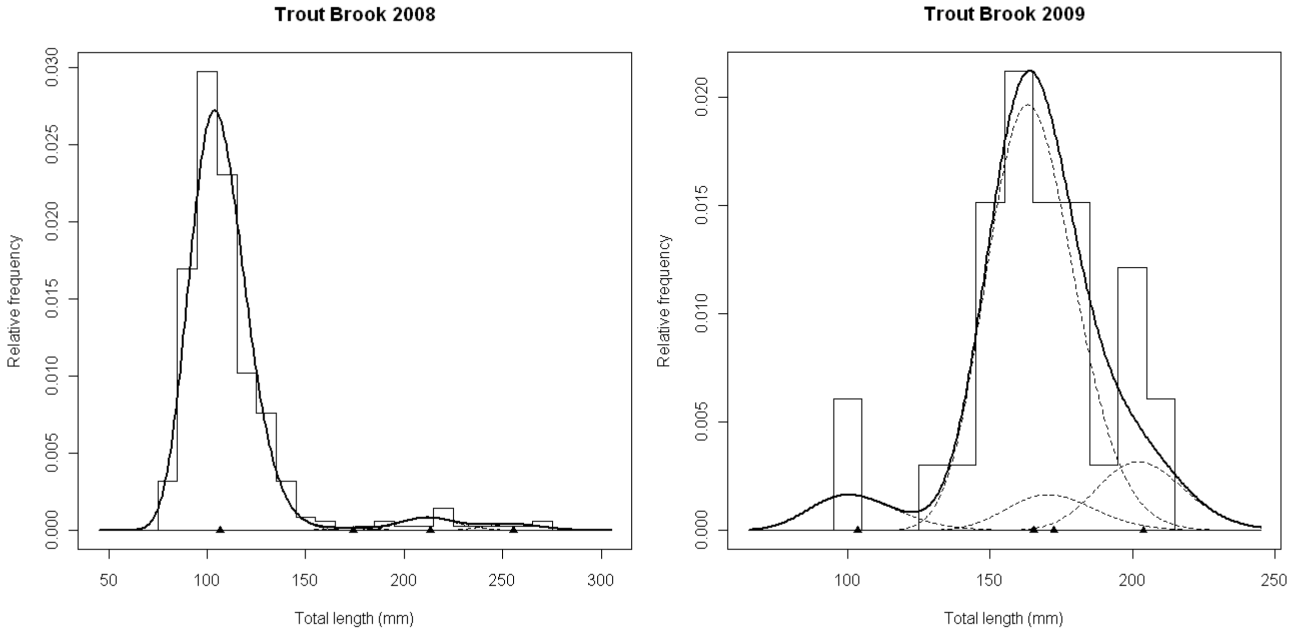
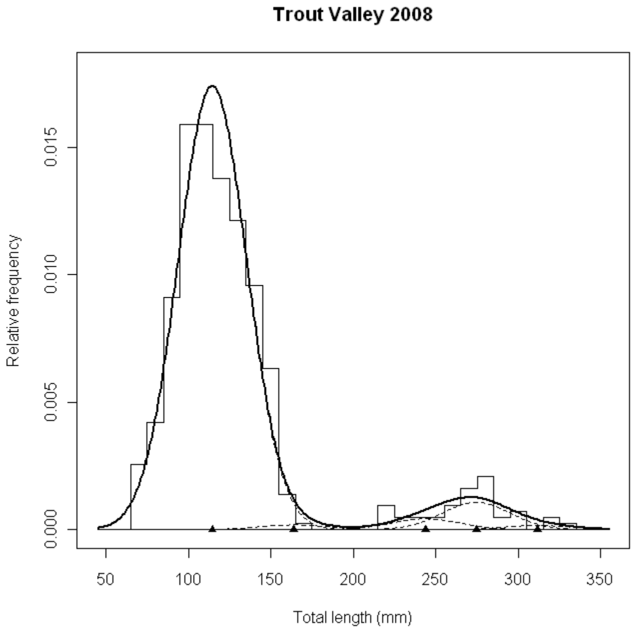


Figure 1 continued


Trout Brook 2008

Trout Brook 2009


Trout Valley 2008

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