

**A Critical review of the paper:  
“Stock identification of gag, *Mycteroperca microlepis*,  
along the southeast coast of the United States” by  
R.W. Chapman, G.R. Sedberry, C.C. Koenig and  
B.M. Eleby. *Marine Biotechnology* 1: 137-146, 1999**

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This review of Chapman *et al.*'s paper was prepared at the request of the Southeastern Fisheries Association Inc. to assist the Gulf of Mexico Fisheries Management Council in its reexamination of proposed management measures relating to gag grouper. It follows preliminary comments made to the Council's recent workshop in Panama City by Dr. Kenchington but was prepared independently. The conclusions below are my own.

Chapman *et al.* developed and used microsatellite DNA markers to analyze the genetic structure of the gag population(s) along the United States southeast coast. They found both allelic frequency differences among the samples and departures from Hardy-Weinberg (H-W) expectations in these samples. Chapman *et al.* concluded that this was most likely the result of inbreeding in limited populations.

The last decade has seen an increasing use of microsatellite DNA markers to discriminate among fish populations and to answer both fundamental and applied questions relating to population structure and fisheries management. The development of such DNA tools to help with the management of the gag resource is therefore quite promising. However, in my view, the paper by Chapman *et al.* suffers from two problems: 1) the data are quite weak and 2) the authors draw from their data strong conclusions that are both highly speculative and questionable.

The biggest problem with the data is the inadequacy of the sampling scheme, for which only vague information is provided in the paper. Samples were obtained from research surveys (presumably estuarine young of the year) and from sampling of commercial catches (presumably offshore post-juveniles). The samples were then pooled into convenient "populations" (e.g. North Carolina, South Carolina, etc.), with no particular rationale given for the pooling scheme. For example, no information is provided in the paper to explain why the offshore post-juveniles collected off the coast of South Carolina should be pooled with the estuarine young of year collected in Charleston rather than in those in Beaufort, apart from the fact that they were geographically closer to the former. Similarly: Why should all collections from eastern Florida be pooled into one population rather than 2 or more when these fish were a mixture of young-of-the-year and post-juveniles collected over quite a geographic range? In addition, the actual sample number per "population" is fairly low in some instances (e.g. 25 in North Carolina, 40 in Florida west coast), while it is not clear how many of these were young-of-the-year versus post-juveniles. It is unclear as well if all the young-of-the-year were even from the same collection year. To sum up, the "population" samples appear to be mixtures of fish from different year classes (young-of-the-year versus post-juveniles), which were often collected in different geographic locations. These "populations" were subjected to analyses to see if they were "distinct" from one another and if they conformed to expectations of Hardy-Weinberg equilibrium. It appears to me that such "population" samples are not even close to being adequate to address these questions.

The second problem with the data, although not as serious, was the very limited number of DNA markers that was used (3), particularly since they are not very informative (with only 6 to 8 alleles each).

The authors found allelic frequency differences among the various "populations" as well as deviations from Hardy-Weinberg equilibrium (heterozygote deficit) within these populations. Observations of heterozygote deficit compared to H-W expectation at fish/shellfish microsatellite loci are quite common (e.g. O'Connell and Wright, 1997; Gjetvaj *et al.*, 1997). Chapman *et al.* proceeded to

examine if these H-W disequilibria could be artifacts due to a number of typical technical problems, such as the presence of null alleles, difficulty in adequately scoring heterozygous individuals with alleles one single repeat apart and poor amplification of large alleles relative to small alleles. Overall, they made a reasonable case for the observed deviations from H-W not being a result of these problems. They did not, however, completely eliminate from my mind the possibility that partial null alleles could be, in part, responsible for their observations. In the Marine Gene Probe Laboratory, we have regularly come across these weakly-amplifying alleles in many fish and shellfish species. They cannot be detected by the approach that Chapman *et al.*, used to rule out true null alleles. This problem is outlined in O'Connell and Wright's (1997) review (p. 352).

Having ruled out the observed heterozygote deficits being artifacts resulting from technical problems, Chapman *et al.* also ruled out the possibility that these deficits could indicate strong population subdivision, on the grounds that "it is difficult to understand how gene flow could be restricted in this species". I find that I must disagree with this highly speculative contention. It appears to me that little is known about the mating/spawning/migrating behavior of this species. Many other fish species are known to mix in areas without obvious physical barriers and to migrate over very long distances and yet they exhibit strong population subdivisions (e.g. salmonids, inshore/offshore cod from Newfoundland: Ruzzante *et al.*, 1996a). Chapman *et al.* themselves provided some evidence of population subdivision in gag since most of their pairwise tests for gene frequency differences among their "populations" for the two loci that also detected deviations from H-W were significant.

After a fairly confusing discussion of the effect of large variance in reproductive output and the genetic sweepstakes hypothesis (Hedgewood, 1994; Ruzzante *et al.*, 1996b) Chapman *et al.* concluded that both the observed heterozygote deficit and the significant differences among population samples were most likely due to a serious population bottleneck and resulting inbreeding. Again this is highly speculative. I would suspect (although I have not tried to make exact calculations) that for inbreeding to result in the sort of observed heterozygote deficits, the effective population size would have to be reduced down to the low hundreds, which appears rather low given the reported gag landings.

In conclusion, I would suggest that the quality of the data in this study cannot support the strong statements that the authors have drawn from it. As previously mentioned, heterozygote deficits are commonly observed in many fish/shellfish microsatellite studies, including populations in which no easy explanation for them can be found. For example, adult scallops sampled from one large bank showed significant heterozygote deficiencies for 4 out of 7 microsatellite loci (Gjetvaj *et al.*, 1997), yet this large population is probably not inbred, does not exhibit assortative mating, and does not show any obvious barriers to gene flow. (It has a long pelagic larval stage.) In the Chapman *et al.* study, since the "population" samples are really pooled samples, I would suggest that the observed heterozygote deficits are probably simply a result of the heterogeneity of these pooled samples (Wahlund effect). Clearly, a much better sampling scheme must be devised before much conclusion can be drawn from microsatellite analysis for this species.

## References

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