UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

MEMORANDUM FOR: Nicholas Farmer and Adam Brame
Species Conservation Branch, Southeast Region Office

FROM: NOAA Genetics Group Members: Diana Baetscher (Alaska Fisheries Science Center), Devon Pearse (Southwest Fisheries Science Center), Jennifer Schultz (Office of Protected Resources), Eric Archer (Southwest Fisheries Science Center)

SUBJECT: Review of Waycott M, van Dijk K-j, Calladine A, Bricker E and Biffin E (2021) Genomics-Based Phylogenetic and Population Genetic Analysis of Global Samples Confirms *Halophila johnsonii* Eiseman as *Halophila ovalis*. Frontiers in Marine Science.

Upon request from the Southeast Regional Office (SERO), we, as representatives of the NOAA Genetics Group, reviewed Waycott *et al*. (2021) to address the following questions:

1) Are the genetic methods (laboratory and statistical) used in this research appropriate and sufficient for the main conclusions of the paper?
2) Does the research provided in this paper constitute the best available scientific (in this case, genetic) information on Johnson seagrass taxonomy?
3) From a genetics perspective (i.e., without additional information on taxonomy), do the data support that Johnson's seagrass (*Halophila johnsonii*) is synonymous with *H. ovalis* (i.e., not a separate species from *H. ovalis*)

We found that the laboratory and statistical methods used by Waycott *et al*. (2021) were appropriate and sufficient for the conclusions the authors present. The authors used several independent analyses to address the validity of the species, *H. johnsonii*, and its relation to *H. ovalis*. To evaluate phylogenetic (i.e., species-level) relationships, they evaluated 105 samples of Halophila spp. from 19 countries using plastid (17,999 base pairs (bp)) and nuclear (6,449 bp) DNA sequences derived from hybrid capture (also called RNA bait). They analyzed the plastid and nuclear DNA data separately to create independent maximum likelihood trees that shared the same general topology as a tree created using 990 genome-wide single nucleotide polymorphisms (SNPs) generated via double digest restriction-site associated digest sequencing (ddRAD-seq). ddRAD-seq was a reasonable method for obtaining a representative sample of loci from across the genome, and the filtering methods used (*de novo* clustering at 0.85, loci with < 20% missing data, and a single SNP per locus; lines 440-442) were appropriate. Thus, three independent genetic analyses confirmed that *H. johnsonii* nests within *H. ovalis*, with the greatest similarity to Antigua and East Africa samples. To evaluate population-level differences, they analyzed 48 samples from 13 populations at 990 SNPs and 294 samples at 10 microsatellite

DNA loci. The SNP and microsatellite data were analyzed using Principal Components Analysis and STRUCTURE to determine the most likely number of populations (K = 3 populations: eastern Australia, western Australia, and Indo-Pacific/Atlantic) within the species, *H. ovalis* (i.e., *H. johnsonii* was again nested within the Indo-Pacific/Atlantic clade). Thus, the methods used were robust to address the hypotheses, and we did not find any major flaws with the analyses.

The research provided in this paper constitutes the best available scientific (in this case, genetic) information on Johnson's seagrass. These new data are concordant with the data previously presented by Waycott *et al*. 2002. They also support previous analyses by Short *et al*. (2010), using internal transcribed spacer regions of 18–26S nuclear ribosomal sequences and morphology, that demonstrated that Antigua samples (n=9) belong to *H. ovalis* and were genetically identical to *H. johnsonii*. Short *et al*. (2010) also found that morphological characters diagnostic for *H. johnsonii* fell within the range of variation that occurs within *H. ovalis*, as previously suggested by Waycott *et al*. (2002), and were most similar to specimens from east Africa.

All genetic data presented by Waycott *et al*. (2021) support the conclusion that *H. johnsonii* is genetically indistinguishable from *H. ovalis*. The phylogenetic analyses of plastid and nuclear DNA sequences consistently resolved *H. johnsonii* within *H. ovalis*: *H. johnsonii* and Antigua *H. ovalis* shared same plastid genotype (patristic distance = 0), similar nuclear genotypes (patristic distance = 0.002), and were "not differentiated" by the SNP phylogenetic analysis (patristic distance = 0.002). Population genetic analyses of 990 SNPs and 10 microsatellite loci demonstrated genetic uniformity of all 132 *H. johnsonii* samples, indicating a lack of genetic diversity that is consistent with clonal (asexual reproduction) and a single colonization event. Furthermore, all 12 *H. johnsonii* and 1 Antigua *H. ovalis* samples genotyped with ddRAD loci shared the same multilocus genotype; and all 132 *H. johnsonii* samples and all nine Antigua *H. ovalis* shared 1 multilocus genotype at 9 microsat loci (one locus did not work in Antigua samples). In contrast, other *H. ovalis* populations generally have multiple multilocus genotypes and substantial genetic diversity, indicating that the genetic markers would have detected differences if they were present. The population-level analyses indicate that *H. johnsonii* is genetically indistinguishable from *H. ovalis*, clustering with samples from Antigua and east Africa, and that individuals from Australia are the most genetically distinct group within *H. ovalis*. Therefore, we agree with the conclusion of the authors that "lack of genetic diversity and the absence of sexual reproduction strongly indicates that the total range of *H. johnsonii* is actually one clone that is closely related to populations in Africa and Antigua…" This conclusion is further supported by the complete absence of male *H. johnsonii* plants, which suggests that it consists of a single female clone. The concordance of the results from multiple genetic data types and across complementary analysis methods provides strong support for this conclusion.