

An Updated Biological Status Review

and

Summary of the Proceedings of a Workshop to Review the Biological Status
of the Seagrass, *Halophila johnsonii* Eiseman

by

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SUMMARY

Since the original status review (Kenworthy, 1993) was published in the Federal Register in September 1993 (58 FR. 48326), additional biological, ecological and distributional information regarding *H. johnsonii* has been obtained by a number of sources. This report summarizes a workshop convening these sources as well as additional information provided by other scientists and resource agencies obtained between 1993 and the present.

There have been no confirmed reports of *H. johnsonii* outside of the originally reported geographical range. Johnson's seagrass is known to occur only between Sebastian Inlet and northern Biscayne Bay on the east coast of Florida, USA. Johnson's seagrass has one of the most limited geographical distributions on any seagrass known. Qualitative and quantitative benthic resource surveys in the Indian River Lagoon, Lake Worth Lagoon, and elsewhere in Palm Beach County confirmed the regional and vertical distribution of *H. johnsonii* as originally described by Kenworthy (1993). Surveys of fixed transects, individual patches, and analyses of vegetative growth rates indicate the dynamism of *H. johnsonii* distribution and abundance. Since no male flowers have been observed, nor has seedling recruitment been confirmed, the distribution and abundance of Johnson's seagrass appears to rely solely on vegetative means of reproduction and growth. The appearance and disappearance of *H. johnsonii* on fixed transects and in disturbed areas can be explained by high densities of apical meristems, rapid rates of horizontal growth, and a fast leaf turnover.

The enormous potential for vegetative expansion, a perennial and intertidal growth habit, and a relatively high tolerance for fluctuating salinity and temperature enable Johnson's seagrass to colonize

and thrive in environments where other seagrasses cannot survive. The range of these environments include the flood tidal deltas of the inlets, the intertidal zone, and in areas with poor water quality. The persistence and abundance of *H. johnsonii* in Lake Worth Lagoon and elsewhere in Palm Beach County suggests that this plant can survive in conditions less than optimum for its' congeners.

Additional molecular genetic information supports distinguishing *H. johnsonii* as a separate species from *H. decipiens*. Preliminary studies of genetic variation using RAPD-PCR methods at one site near Jupiter Inlet indicates a surprisingly high amount of genetic diversity, given the lack of evidence for sexual reproduction. More detailed and extensive phylogenetic studies are needed to determine the origin of this species and the source of genetic diversity.

For the first time, quantitative evidence of faunal community diversity and abundance in *H. johnsonii* meadows were reported. Samples obtained from drop nets near Sebastian Inlet revealed that faunal communities in Johnson's seagrass patches are more similar to other seagrass habitat than they are to bare sand. During a one year bimonthly sampling program a total of 320 organisms representing 26 species were collect in *H. johnsonii* meadows.

I. INTRODUCTION

In September 1993 the National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce, proposed adding the seagrass *Halophila johnsonii* (Johnson's seagrass) to the United States List of Endangered and Threatened Plants (58 FR. 48326). As a follow-up, in August 1994 NMFS proposed designation of critical habitat for Johnson's seagrass (59 FR. 39716). Since the original proposal to list *H. johnsonii*, there

have been new field and laboratory observations as well as additional research on species distribution, ecology, utilization, genetics and phylogeny. In order to update the original status report (Kenworthy, 1993), NMFS conducted a workshop on the biology, distribution, and abundance of *H. johnsonii*. The workshop, held in St. Petersburg FL on November 14 & 15 1996, was coordinated and supervised by Dr. Herb Kumpf, NMFS, and hosted by Dr. Michael Durako, Florida Marine Research Institute (FMRI), Florida Department of Environmental Protection (FDEP). Scientific participants included: Dr. W. Judson Kenworthy, Beaufort Laboratory, NMFS, Beaufort, NC; Dr. Robert Virnstein and Lori Morris, St. Johns River Water Management District (SJRWMD), Palatka FL.; Dr. Walter Nelson and Shelly Heidelbaugh, Florida Institute of Technology (FIT), Melbourne FL.; Dr. Jeri Jewitt-Smith, Texas A&M International University (TAMIU), Laredo, TX.; Drs. Michael Durako and Margaret Hall, FMRI, St. Petersburg, FL.; Jeff Brown, Southeast Regional Office, NMFS, St. Petersburg, FL.; and Gregg Miller, Office of Protected Resources, NMFS, Silver Spring, MD. The objectives of the workshop were to: 1) review the most recent distributional studies and scientific research on *H. johnsonii* since publication of the status review in September 1993, and 2) revise the status report according to the new scientific information.

During the first session the workshop was organized into four topics: 1) a review of surveys on the distribution and relative abundance of *H. johnsonii* in the Indian River Lagoon (IRL) (Virnstein and Morris), 2) an update and discussion of research on the genetics, phylogeny, and status of *H. johnsonii* as a viable species (Jewitt-Smith), 3) studies of faunal abundance and patch dynamics in *H. johnsonii* beds near Sebastian Inlet (Heidelbaugh and Nelson), and 4) general synthesis of species distribution, biology, and ecology (Kenworthy et al.). The participants also discussed the distribution and

abundance data provided by FDEP surveys in Palm Beach County. During the second session the workshop participants began revising a draft biological status summary prepared by Herb Kumpf.

The general objectives of this report are to: 1) summarize the information provided by the scientists attending the workshop, and 2) update the original biological status review (Kenworthy 1993) with new scientific information and observations on the distribution and abundance of *H. johnsonii*. In the original status review five factors were considered critical to listing Johnson's seagrass as threatened: 1) the species has a limited geographical range, possibly the smallest range of any seagrass in the world (strong endemism), 2) within the species geographical range it is relatively uncommon compared to the other six species, and occurs in a disjunct and patchy distribution; 3) due to the absence of male flowers the species lacks a known sexual life cycle and therefore, dispersal and regeneration of populations by seed are not likely, 4) absence of a sexual life cycle would indicate that regeneration of populations relies solely on vegetative growth which can have severe spatial constraints as well as negative genetic implications (e.g., limited adaptability through out crossing); 5) the species small size and limited energy and nutrient storage capacity makes it vulnerable to disturbance and prolonged adverse environmental conditions (e.g., water turbidity and physical disruption). In this report, I review the scientific information presented at the workshop to determine the need to reconsider or modify any of these factors supporting the proposal to list *H. johnsonii* as a threatened species.

II. STATUS REVIEW

A. Distribution

Geographical Range

Since 1993 NMFS staff have conducted seagrass distributional surveys and diver observations in coastal water bodies ranging from the known southern geographical limit of *H. johnsonii* in Biscayne Bay through Florida Bay, the Florida Keys, outer Florida Bay (Gulf of Mexico), Puerto Rico, and the Virgin Islands. Based on these surveys, we conclude there is no evidence for the presence of *H. johnsonii* any further south or west in the continental United States than was originally reported (Eiseman and McMillan, 1980). We have also interviewed scientists working on the east and southeast coasts of Florida, the northern Indian River Lagoon, Banana River, Mosquito Lagoon, and in the Bahamas, Puerto Rico, and the Virgin Islands. None report or confirm sightings of *H. johnsonii* beyond its currently known geographical range, which includes the inland marine and estuarine waters between Sebastian Inlet (approximately lat. 27°50'N) and Virginia Key, FL. (approximately lat. 25°45'N) (Figure 1).

Local Distribution

Until recently, the local areal distribution of *H. johnsonii* was not well described anywhere within its known geographical range. Detailed benthic surveys in Jupiter and Hobe Sounds (Kenworthy, 1993), and near Fort Pierce and Sebastian Inlets (Kenworthy, 1993; Gallegos and Kenworthy, 1996), indicated that *H. johnsonii* was one of the least frequently encountered of all the seagrasses found

within its geographical range, and had a patchy and disjunct distribution. The largest patches of *H. johnsonii* were found on the flood-tidal deltas just inside the inlets with decreasing abundance along axes from the inlets to the more interior regions of the lagoon (Kenworthy, 1993).

In an effort to expand the information base for seagrass distribution and abundance in the Indian River Lagoon, in 1994 the SJRWMD initiated an extensive benthic transect survey (Virnstein and Morris, 1996). Lagoon-wide seagrass mapping of distribution and abundance and ground truth checks, which began in 1986 and continued in 1989 and 1992 were expanded in 1994, 1995 and 1996 to incorporate 75 permanent transects, ranging in length from 25 to 500 m, throughout the lagoon. These fixed transects were nondestructively sampled at 10 m intervals for seagrass species presence and percent cover (Virnstein and Morris, 1996). Sampling on these transects occurred in summer 1994, winter 1995, summer 1995, winter 1996, and summer 1996. The results of these surveys corroborated previous information that: 1) *H. johnsonii* does not occur further north than Sebastian Inlet (occurrences in 17 out of 75 total fixed transects throughout the IRL), and 2) areal distribution is patchy and disjunct from Sebastian Inlet to Jupiter Inlet (occurrences in 17 out of 34 fixed transects within the known range of the species in the IRL). Additionally, these transects confirmed that *H. johnsonii* occurs over a depth range extending from the intertidal down to approximately -2 m mean tidal height. These transects also indicated that Johnson's seagrass can be found growing with other large-bodied species of seagrass (*Halodule wrightii* and *Syringodium filiforme*), if the canopy of the larger species is relatively sparse. In the transects where it was observed, the distribution of *H. johnsonii* was disjunct, occurring at the upper and lower edges of many transects. *Halophila johnsonii* appeared to be excluded from areas of moderate to dense coverage of the larger species

such as *H. wrightii* and *S. filiforme*. Average percent cover of *H. johnsonii* per transect ranged from a minimum of 0.2% in winter 1996 to 8.5 % in summer 1994. There was a wide variation in percent cover per transect as well as an indication of seasonal variation, with the maximum coverage in summer and minimum coverage in winter. On some transects Johnson's seagrass disappeared in the winter and reappeared the following summer. Relative to the other six species that occur in the lagoon, *H. johnsonii* comprises < 1.0 % of the total abundance of seagrasses. The transect data corroborates previous intensive surveys in Jupiter and Hobe Sounds, and near Fort Pierce Inlet (Kenworthy, 1993; Gallegos and Kenworthy, 1995).

The SJRWMD survey found *H. johnsonii* in a wide range of environmental, water quality, and salinity conditions. It was observed on sandy shoals, turbid muddy basins, and near the mouths of canals with fluctuating salinity and water color. It also occurred on channel margins near inlets with very high current velocities, in areas of the lagoon with the poorest water transparency and in highly eutrophic conditions. These observations are similar to previous descriptions of the species distribution and abundance (Dawes et al., 1989; Dames and Moore and Palm Beach County DERM, 1990; Kenworthy, 1993; Gallegos and Kenworthy, 1995), and suggest that *H. johnsonii* is relatively more "eurybiotic" than its congener, *H. decipiens*. *Halophila johnsonii*'s broader range of tolerance for light, temperature and salinity demonstrated in an earlier physiological study (Dawes et al., 1989) are supported by observations of the species distribution and abundance.

Interviews with local scientists and resource managers, and a resource inventory by the Palm Beach County Department of Environmental Resources Management show that *H. johnsonii* is present in Lake Worth Lagoon (LWL) (Dames and Moore and Palm Beach County DERM, 1990). The DERM

seagrass survey indicated that mixed communities of seagrasses comprising *H. wrightii*, *H. decipiens* and *H. johnsonii*, covered between 35 and 40% of LWL (approximately 800-1000 acres).

Unfortunately, the qualitative manner in which the surveys were conducted make it impossible to separate the amount of *H. johnsonii* coverage from that of the other species.

A more recent qualitative survey of potential marina sites along the Atlantic Intracoastal Waterway (AICW) and in LWL by FDEP staff confirms that *H. johnsonii* occurs in a patchy and disjunct distribution throughout Palm Beach County (Mary Morris, FDEP, Tallahassee, FL, personal communication and unpublished report). Of the 52 sites where seagrass species presence was noted, 36 contained *H. johnsonii*. Of these 36, 25 were categorized as patchily distributed and 12 as having contiguous beds of *H. johnsonii*. The coverage of the 25 areas in the patchy distribution category was further delineated into 15 sites with sparse coverage, six with moderate coverage, three with dense coverage, and one as undetermined. Of the 11 contiguous beds, three were classified as moderate coverage, seven were considered dense, and one as undetermined. Overall, 15 sites were categorized as sparse, nine as moderate, 10 as dense and two undetermined. In this survey the observers recorded a 30-acre patch of *H. johnsonii* located just north of Bingham Island between the ICW and the barrier Island. This was the largest contiguous patch of *H. johnsonii* reported in any of the surveys. In this patch, *H. johnsonii* was intermixed with sparse coverage of *H. decipiens* and *H. wrightii*.

To date, there are no extensive or intensive surveys of *H. johnsonii* between LWL and Biscayne Bay. As with LWL and the IRL, the distribution of *H. johnsonii* in North Biscayne Bay is disjunct and patchy (Kenworthy and Schwarzschild, unpublished field observations; Lee Hefty, Dade County DERM, personal communication).

All of the surveys documenting the distribution and abundance of *H. johnsonii* confirm that its distribution is patchy and disjunct. Patch sizes varied more than seven orders of magnitude from 0.05 m² (Kenworthy, 1993) to 150,000 m² (Kent Smith and Mary Morris, FDEP, Tallahassee, FL, personal communication). Recently, Heidelbaugh and Nelson (this workshop) found that patch sizes of *H. johnsonii* may be quite dynamic. They studied the temporal sequence of patch size within three individual patches of *H. johnsonii* on the flood-tidal delta just inside Sebastian Inlet. Three patches of different sizes (16.3 m², 82.2 m², and 418.3 m²) were monitored from June 1995 to June 1996. Between June 1995 and February 1996 the smallest bed grew from 16.3 to 58.7 m², an increase of 277%. The medium size patch grew to 170.3 m² by June 1996, an increase of 206%. The largest bed grew to 460.0 m² by February 1996 and eventually merged with the smallest patch, and together formed a larger patch covering 815 m² in June 1996. It is not known whether this magnitude of patch growth occurs throughout the range of the species. This rapid growth may reflect the plant's response to the physical dynamics associated with the flood delta at Sebastian Inlet and the optimum water quality conditions found near the inlet. Recently, observers have described large patches of Johnson's seagrass which occur well outside the direct physical influence of an inlet; including a 30-acre meadow in LWL (Kent Smith, FDEP, personal communication), and other patches on the order of hundreds of m² near Vero Beach and Wabasso in the IRL (Lori Morris and Bob Virnstein, this workshop).

Vertical Distribution

The maximum lower depth distribution of *H. johnsonii* coincides with *H. decipiens* and is generally deeper than the maximum depth distribution of *H. wrightii*, *S. filiforme*, and *Thalassia testudinum* in the IRL (Kenworthy, 1993; Gallegos and Kenworthy, 1995; Kenworthy and Fonseca, 1996). Even though *H. johnsonii* grows deeper than the larger bodied species and may photosynthesize more efficiently at lower irradiances (Dawes et al. 1989), it is sensitive to water transparency and the availability of PAR. For example, near the center of its range in Hobe and Jupiter Sounds, the percent coverage of *Halophila* spp. declines from 38% to about 2% along a 14 km gradient of declining optical water quality from Jupiter Inlet to the interior of Hobe Sound (Kenworthy, 1993). Since the availability of photosynthetically active radiation (PAR) affects the lower depth limits and overall abundance of seagrasses (Kenworthy and Fonseca, 1996), optical water quality contributes to the patchy and disjunct distribution of *H. johnsonii* in deeper portions of the lagoons.

Halophila johnsonii can be found surviving in environments that would be marginal or limiting to other species. This is partly due to the fact that the upper depth distribution of *H. johnsonii* is much shallower than those of *H. decipiens* and *S. filiforme* and, like *H. wrightii*, Johnson's seagrass can survive in the upper subtidal and intertidal zone. In the 17 transects where *H. johnsonii* was observed by the SJRWMD there were several instances of disjunct vertical distribution where *H. johnsonii* occurred alone or in a very sparse canopy of *H. wrightii* at the upper and lower edges of the transects (Morris and Virnstein, this workshop). A similar distribution was seen in Lake Worth where, for decades, optical water quality and sediment conditions have been marginal for seagrass growth. The higher relative abundance of Johnson's seagrass observed in LWL may be explained by the fact that the lagoon experienced many years of poor water quality and sediment deterioration which limited the

larger species from developing density and coverage comparable to most of the IRL. If *H. johnsonii* is excluded from the denser canopies of the larger species, as suggested by the survey data in the IRL (Kenworthy, 1993; Morris and Virnstein, this workshop), then either the absence or sparse coverage of the larger species in Lake Worth would have opened up space for Johnson's seagrass to occupy. This would provide an explanation for the relatively higher abundance of Johnson's seagrass in LWL.

General Comments on Distribution and Abundance

The recent information on *H. johnsonii* distribution and abundance confirms our original ranking of this species. Johnson's seagrass has one of the most restricted geographical ranges known for any seagrass in the world, and is one of the least abundant where it occurs. Within the distributional range of Johnson's seagrass, the least abundant species is *H. engelmannii*, however, *H. englemannii* has a much wider geographical range, including the Bahamas, Florida, Cuba and the Gulf of Mexico.

The results of the new surveys confirm the possible importance of patch size and patch dynamics for Johnson's seagrass. Throughout its range *H. johnsonii* distribution is patchy, vertically disjunct, and it is excluded from areas of dense coverage of the larger bodied species. The ability of *H. johnsonii* to grow in the shallow intertidal zone uniquely distinguishes it from *H. decipiens*, and may facilitate the occurrence of Johnson's seagrass in shallow water environments where fluctuating temperature, salinity, and optical water quality conditions prevent colonization by either *H. decipiens* or the larger species, *T. testudinum*, *S. filiforme*, or *H. wrightii*.

B. Growth and Reproduction

Posluszny and Tomlinson (1990) suggested *H. johnsonii* was dioecious, but there are still no confirmed sightings or descriptions of the male flower to support this classification (Jewett-Smith et al., in press). In cases where female flowers have been observed, they often occurred on each node associated with a leaf pair along the entire length of rhizome segments (Kenworthy, 1993), indicating that a patch may be an entire female clone. Frequently, within a few meters of an isolated patch with abundant females, patches were found with no flowers. Possibly these flowerless patches of Johnson's seagrass are male clones with ephemeral flowers.

The female flowers observed in Jupiter Sound and Sebastian Inlet had three well-developed stigmas per flower and the stigmas were nearly as long as the leaves (1.6-2.1 cm). Flowers with unfertilized ovules were common in these patches of Johnson's seagrass indicating a substantial allocation of reproductive effort to the formation of female sex organs (Kenworthy, 1993). It seems unusual that a plant would devote so much effort to sexual reproduction if the male flowers were not present.

Johnson's seagrass frequently grows with *H. decipiens* in deep (IRL) or turbid waters (Lake Worth) leaving the possibility open that the two species could hybridize. In Jupiter Sound, male flowers of *H. decipiens* were observed in the same patch as female flowers of Johnson's seagrass on two occasions, June and August 1991 (Kenworthy, 1993).

On one sampling occasion, three seeds were found beneath a patch of *H. johnsonii*, however, these seeds were morphologically indistinguishable from those of *H. decipiens* (Jewett-Smith et al., in press). Numerous efforts to find seeds in the sediments beneath patches of Johnson's seagrass have failed to locate any which subsequently germinated and developed into *H. johnsonii* plants (Jewett-Smith et al.,

in press). Additionally, none of the surveys conducted throughout the range of the species have reported seeing germinated seedlings of Johnson's seagrass in the field.

Since no male flowers were known, nor was there any evidence for a sexual life cycle outside of the presence of female flowers, Eiseman and McMillan (1980) and McMillan and Williams (1980) suggested that Johnson's seagrass may reproduce by apomixis. To date, this form of reproduction has never been confirmed for any seagrass species. The fact that male flowers haven't been observed doesn't mean that they don't occur. When *H. engelmannii*, *H. hawaiiiana*, and *H. stipulaceae* were first monographed their male flowers were unknown (den Hartog, 1970). Eventually, the male flowers of *H. hawaiiiana* (Herbert, 1986) and *H. stipulaceae* (Lipken, 1975) were shown to be highly ephemeral, and Herbert (1986) demonstrated that anthesis of *H. hawaiiiana* occurs only at night. This explains the potential difficulty in observing and describing the male flowers of these two species and also may explain why male flowers of *H. johnsonii* have yet to be observed.

Alternatively, vegetative growth and rhizome branching may be the only means of propagation for *H. johnsonii*. Vegetative growth occurs all year (perennial growth habit) (Kenworthy, 1993; Heidelbaugh and Nelson, this workshop). In the IRL this growth strategy is different from *H. decipiens*, which forms meadows annually by a combination of seed germination (annual recruitment) and vegetative reproduction. The patch growth rates of Johnson's seagrass measured by Heidelbaugh and Nelson (this workshop) indicate a high rate of vegetative growth. Taking the growth rate of the smallest patch between June and October (from 16.3 to 43.2 m²), and assuming a circular shape, the patch expanded at about 1 cm * d⁻¹ during the summer and early fall period. This same patch also continued to expand between October and February, confirming a perennial vegetative growth form, even at the northern

limit of the species geographical range. This measured growth rate is consistent with known rates of leaf formation (a new leaf pair every 3 to 4 days) and rhizome growth for *H. johnsonii*, and for the genus *Halophila* in general (Herbert, 1986; Kenworthy et al., 1989; Kenworthy, 1993; Bolen 1997). Bolen (1997) recently estimated rhizome elongation rates between 0.35 and 0.53 cm * apex⁻¹ * day⁻¹ for a population of *H. johnsonii* in northern Lake Worth Lagoon. Likewise, Kenworthy (1993) estimated a growth rate of $\approx 0.5 \text{ cm} * \text{apex}^{-1} * \text{d}^{-1}$ for *H. johnsonii* at several sites in the Indian River Lagoon. These values are about half the rate of expansion estimated for patch growth at Sebastian Inlet. In the absence of any known form of sexual reproduction for *H. johnsonii*, the dynamic growth of patches and the appearance and disappearance of the species on fixed transects must be partially explained by this rapid vegetative growth rate. Apical meristem densities for *H. johnsonii* average between 500 and 1100 apicals * m² (Kenworthy, 1993; Bolen, 1997). This is one to two orders of magnitude higher than most other larger bodied species of seagrass and provides an enormous vegetative growth potential. While apical meristems are producing new leaf pairs, they spread horizontally at the front or outside perimeter of a patch. At the same time, older leaf pairs and their rhizomes, either at the center of a circular patch or the rear of a migrating patch, die and disintegrate. Even though an individual leaf pair is not motile and has a relatively short life span, the growing apex of a clone continues to move along the landscape. And therefore, at any given point in space and time *H. johnsonii* may or may not be present. Given the reported apical densities, the rapid elongation rates, and the short turnover time of leaf pairs, it is not surprising that there is large spatial and temporal variation in the distribution and abundance of *H. johnsonii*. Fixed transects and one-time sampling of individual sites could actually provide misleading information about the distribution and abundance of Johnson's seagrass.

It seems difficult to explain the disappearance and reappearance of *H. johnsonii* on some of the transects sampled by the SJRWMD without observing sexual reproduction (Virnstein and Morris, this workshop). However, if one considers the potential rates of areal expansion from vegetative growth (Heidelbaugh and Nelson, this workshop), we might be able to explain the seasonal and annual fluctuations on the fixed transects. Mortality, or the disappearance of patches, can occur by a number of processes, including burial from bioturbation, sediment deposition, or herbivory (Williams, 1988; Fonseca et al., 1994). The *H. johnsonii* canopy is only 2-5 cm tall and may be easily covered by sediment transported during storms or redistributed by macrofaunal bioturbation during the feeding activities of rays and other benthic organisms. As suggested by *H. johnsonii*'s perennial growth habit, some apical meristems may overwinter in the vicinity of the fixed transects where patches were previously recorded, or even where patches weren't observed inside the quadrats. During subsequent periods of favorable environmental conditions, these plants may colonize the permanent quadrat positions (as described in the previous paragraph). Because of the generally low water visibility in the IRL, it is possible for an observer to overlook plants of *H. johnsonii* growing within 2 or 3 meters of a fixed transect. This could leave the impression that the plants were absent and reestablished by seed, when in fact, colonization of the quadrats may have resulted from vegetative growth and encroachment by adjoining undetected patches.

C. Genetics

Enzyme polymorphism has been used to complement morphological and ecological information, and to evaluate the degree of divergence between species of seagrasses (McMillan and Williams, 1980; McMillan, 1980; Waycott, 1995). For example, Capiomont et al. (1996) demonstrated small intra- and interpopulation isozyme variability in the Mediterranean seagrass *P. oceanica*, suggesting that the populations were primarily clonal. The conclusion that *P. oceanica* may be a single clone throughout the Mediterranean was supported by an independent study using alternative genetic methods; DNA fingerprinting, minisatellites, and random amplified polymorphic DNA techniques (Procaccini et al., 1996). Similar methods are now being used to examine genetic variability and phylogeny in the genus *Halophila*. Differences in banding patterns for three of seven enzymes systems tested (acid phosphatase, phosphoglucomutase, and glutamate oxaloacetate transaminase) were used to support the proposal that *H. johnsonii* and *H. decipiens* are separate species (Eiseman and McMillan, 1980). The isozyme data complemented the morphological characteristics which were used to distinguished between the two *Halophila* species: 1) differences in leaf shape (linear vs. oblong-elliptic), 2) differences in leaf margins (entire vs. serrulate), 3) leaf hairs (absent vs. present on one side, and 4) differences in angle of cross veins (60° vs. 45°). This biochemical data was important in distinguishing the species, because prior to its' published description (Eiseman and McMillan, 1980) Johnson's seagrass was mistaken as a morphological variant of *H. decipiens* (Zieman, 1980), or misidentified as *H. baillonis* (Phillips, 1960).

Using the randomly amplified primer DNA-polymerase chain reaction (RAPD-PCR), Dr. Jerilyn Jewitt-Smith (this workshop and Jewitt-Smith et al., in press) identified 25 polymorphic bands extracted from total genomic DNA of the three North American *Halophila* spp. The DNA banding

patterns for *H. johnsonii* and *H. decipiens* indicated they shared only 28% of RAPD generated bands (Jewitt-Smith, this workshop). This additional molecular genetic information provides support for distinguishing Johnson's seagrass from *H. decipiens* as a separate species. Moreover, the isozyme data combined with ecological and morphological information suggests a much closer relationship between *H. johnsonii* and *H. minor* than with *H. decipiens*.

Clearly, the sexual characteristics, morphology, physiological ecology, and genetic analyses provide a solid base of scientific information for recognition of Johnson's seagrass as a distinct species.

Whether *H. johnsonii* is reproductively isolated from the other two North America *Halophila* spp. remains unknown. The sometimes prolific female flowers and long stigmas found in patches of *H. johnsonii* mixed with *H. decipiens* suggest the possibility that cross fertilization between the two species is at least physically reasonable. Using pollen tube fluorescence and photo microscopy Bolen (1997) examined approximately forty female flowers of *H. johnsonii* and found indirect evidence indicating the presence of either pollen tubes or ovules in the female flowers that may have undergone pollination. The author did not, however, report finding direct evidence of pollen associated with the female flowers and cautioned that the fluorescence could have been phloem or vascular tissue (Bolen, 1997).

Without a complete description of the male flowers it is difficult to use floral characteristics for a comprehensive taxonomic classification. The absence of male flowers also raises a number of concerns about how the species maintains viable genetic structure and diversity. Surprisingly, preliminary analyses of banding patterns from RAPD-PCR for five samples of *H. johnsonii* at Jupiter Inlet indicated there was more diversity (similarity index = 0.45) than expected at one site, considering there

may be either limited or no sexual reproduction (Jewett-Smith et al., in press). Les (1988) hypothesized that because of limited sexuality in some aquatic plants, there should be less variation within populations and higher variation between them. The relatively high variation for Jupiter Inlet could result from sexual recombination or a founder effect where the initial colonizing population had relatively high diversity. Recalling the potentially high rates of vegetative growth known for this genus and species (Heidelbaugh and Nelson, this workshop; Herbert, 1986; Kenworthy et al., 1989; Kenworthy, 1993), it is reasonable to assume that the rapid clonal expansion of a few successful genotypes could result in high intra-population variation (Laushman, 1993). This amount of genetic diversity, and ultimately the genetic structure among and between the disjunct patches and populations, may be important for the persistence of this species (Alberte et al., 1994; Williams and Davis, 1996). Future studies, encompassing larger within and between site sample sizes, are needed to evaluate the overall genetic structure and diversity of *H. johnsonii* (see Williams and Davis, 1996).

D. Faunal Utilization

Despite the global distribution and relatively high diversity of *Halophila spp.* (estimated ≥ 12 worldwide), there has been very little work done on their faunal communities and the utilization of *Halophila* meadows. Results are limited to a few studies in remote green turtle (*Chelonia mydas*) feeding areas of Oman (Ross, 1985; Jupp et al., 1996) and in Moreton Bay, Australia where dugongs graze *H. ovalis* (Preen, 1995). Never-the-less, these studies provide compelling evidence that even the small *Halophila* species are utilized by large herbivores. Their rapid growth, high turnover rates, and labile tissues make *Halophila* species a good source of nutrition for herbivores (Kenworthy et al., 1989; Lanyon, 1991; Preen, 1995; Bolen, 1997). In Florida we have observed manatees feeding in *H.*

johnsonii patches near Jupiter Inlet and there is one report of the stomach contents of a juvenile green turtle with *H. johnsonii* (Paul Mikkelsen, personal communication, DERM, Palm Beach County, FL.).

Heidelbaugh and Nelson (this workshop) presented the first quantitative evidence of faunal community abundance in *H. johnsonii* meadows. During a one year period, bimonthly 1 m² drop net samples were collected from three locations on the flood tidal delta just inside Sebastian Inlet, FL. Samples were obtained in 1) Johnson's seagrass patches, 2) *H. wrightii* patches, and 3) bare sand areas. A total of 320 organisms representing 26 species were collected in the *H. johnsonii* patches, 690 organisms covering 30 species in the *H. wrightii*, and only 78 organisms from 9 species in the bare sand. These results suggest that *H. johnsonii* patches are more similar to other seagrass habitat than they are to bare sand and have considerable ecological resource value.

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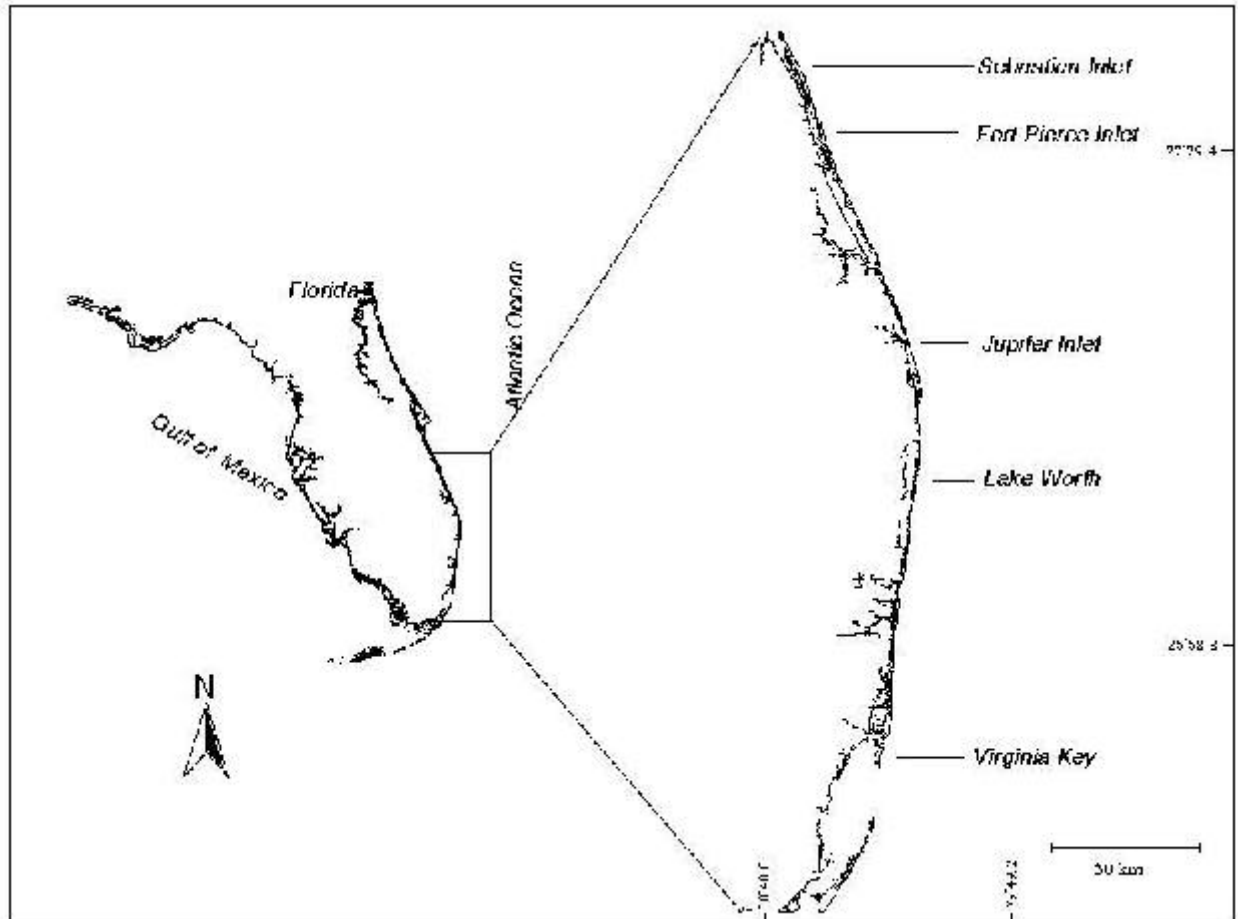


Figure Legends

Figure 1. Map of the Florida coastline showing the geographical range of *Halophila johnsonii* between Sebastian Inlet, Indian River Lagoon and Virginia Key, Biscayne Bay.