APPENDIX C: Science in Support of the RECOVER Northern Estuaries Program and Performance Measure Development

1 Hypothesis Clusters

The MAP 2009 (RECOVER 2009) science strategy included the development of hypothesis clusters. Hypothesis clusters address the integration of the important stressor-response elements contained within a conceptual ecological model (CEM) to better capture and represent the complex stress or response relationships of the system (RECOVER 2006a; 2006b). A further benefit to the development of hypothesis clusters was the refinement in the types and numbers of Performance Measures (PMs) and metrics and their linkage to Interim Goals (RECOVER 2005). Salinity is a "stressor" in all the NE Hypothesis Clusters.

The ecological premise that underlies all CERP hypotheses in the NE is that prior to water management (i.e., landscape alteration and post inlet construction), natural landscapes, which included extensive, isolated wetlands, acted as a buffer and provided more natural patterns of freshwater inflow to the NE and sustained an ecologically appropriate range of salinity conditions with fewer high and low salinity extremes.

Water Quality CERP Hypothesis: The construction and operation of water storage and treatment facilities in the NE regions will improve the quantity, quality, and timing of flows into the estuaries, which will in turn provide a salinity envelope that avoids ecologically damaging high and low salinity extremes.

Oyster Health and Abundance CERP Hypothesis: Restoration of more natural freshwater inflows by retention of water in reservoirs, wetland rehydration, and changing delivery patterns; removal of fine-grained sediment; and introduction of artificial substrate into South Florida estuaries (all results of CERP implementation) should provide beneficial salinity and habitat conditions that promote the re-establishment of healthy Eastern oyster (*Crassostrea virginica*) beds. Detailed working hypotheses relating to oyster health and abundance can be found in the SSR 2006 (RECOVER 2006b).

Submerged Aquatic Vegetation CERP Hypothesis: The relationship between freshwater dynamics and SAV is complicated with outcomes dependent on the quantity, quality, and timing of freshwater releases coupled with the salinity requirements of individual species, ranging from freshwater-to-polyhaline, and their associated communities. In addition to the main hypotheses, a series of sub-hypotheses have been developed for use in understanding the synergistic nature of water delivery and SAV populations; these can be found in the Assessment Strategy 2006 for the MAP (RECOVER 2006a).

Benthic Infauna CERP Hypothesis: Benthic infauna are important indicators of water and sediment quality and are used to assess overall estuarine health and follow long-term trends in estuarine communities related to anthropogenic impacts. By examining shifts in the community and relating them to water management practices as depicted in the CEM, it is possible to obtain an understanding of the major environmental processes affecting the biota. This monitoring

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program can identify discrete zones in the estuary by community type, and rapidly identify and predict responses to flows. More details on the hypothesis cluster associated with infauna can be found in the MAP Part 2 (RECOVER 2006a).

Fisheries CERP Hypothesis: Several water quality/flow dynamics hypotheses have been developed and are described in detail in the MAP Part 2 (RECOVER 2006a). These hypotheses have been tested using several different technologies. Small fish and early developmental stages of larger species are more sensitive to adverse environmental conditions and therefore suffer the highest mortality rates. Many aspects of spawning, larval movement and development, juvenile growth, and predation have been directly linked to freshwater flow patterns.

1.1 Monitoring

MAP monitoring in the Northern Estuaries is based on the CEMs (Section 4.3 in the main Performance Measure Documentation Sheet) and Hypothesis Clusters. Oyster, SAV, and benthic infauna monitoring are currently being used to assess the status of the system and will be used to assess the progress of CERP as projects come online. The RECOVER System Status Report (SSR; published every five years) documents the measurement of ecological indicators and their application to assess conditions in the Everglades ecosystem. This information provides feedback to decision-makers on the ecological response to past restoration activities and informs the timing of planning for CERP projects yet to be implemented. This report also informs adaptive management actions and identifies uncertainties that need further study to assure restoration success.

2 Habitat Suitability Index Models

In support of the current update to RECOVER Interim Goals (due late 2020), quantitatively validated, GIS-based oyster habitat suitability index (HSI) models for the SLE and CRE were updated. Layers include salinity, temperature, and substrate type. HSI model outputs were first validated spatially using live oyster density data available from RECOVER monitoring data. A study by Chen et al. (*in prep*) showed that HSI values were significantly correlated with live oyster density in the CRE. HSI values <0.5 accurately indicated unsuitable habitat conditions where near zero live oyster density was observed. Both correlation and time series analyses suggest that the derived HSI output is a robust and accurate indicator of oyster habitat suitability in the estuary.

3 Mapping

Throughout the implementation of CERP, RECOVER aims to understand how flow and salinity conditions affect the Northern Estuaries over time by mapping the extent of oysters and SAV. If conditions in one location are no longer suitable for survival, organisms will settle and develop in other areas suitable to their physiological tolerances. Over time, scientists can capture change in environmental suitability by observing changes in oyster and SAV distribution as implementation of CERP projects progress. While monitoring of oyster reef and SAV health has taken place since the late 1990s in many of the subject estuaries, the extent and coverage of oyster reefs, SAV, and their current distribution in the estuaries requires updating every few years. Mapping these areas is essential since the spatial location, health, and bottom types (e.g.,

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sand, shell, silt, etc.) inhabited by oyster and SAV resources influences the natural expansion of those resources and the success of restoration activities. A detailed benthic habitat and substrate characterization mapping project for the Northern Estuaries was completed in 2011 (Dial Cordy and Associates Inc. 2011). The map products include habitat type (e.g., oyster reef and SAV) and substrate type throughout each of the estuaries including the Loxahatchee River Estuary and Lake Worth Lagoon. The estuaries were mapped in all areas up to the 9-foot bathymetric contour using side-scan sonar technology.

Oyster mapping produces an overall picture of oyster bed extent and is used to track landscape and patch-scale changes in oyster reef distribution. Since 2000, RECOVER has conducted routine monthly and quarterly *in situ* monitoring of oysters in the Northern Estuaries that includes density, disease prevalence and intensity, reproductive development, and juvenile recruitment, growth, and survivorship. Combined with mapping, these fitness indices improve our understanding of the overall condition of oysters in the region and how changes in the environment over time may trigger shifts in community structure and distribution. Re-mapping of these estuaries should occur approximately once every five (5) years as restoration efforts progress. The SLE and CRE were mapped again in 2019 (results and final deliverables pending) and the final map will be used to evaluate the best approach for continued mapping of oyster and other resources in the Northern Estuaries moving forward.

4 Future Tool Development and Needs

4.1 Duration and Return Frequency of Salinity/Flow Excursions

The CERP adage of "getting the water right" includes hydrologic restoration to improve the quantity (i.e., volumes of inflow in cfs), quality (i.e., salinity envelope), and timing (i.e., duration, return frequency, and seasonality) of freshwater inflows to the Northern Estuaries. The duration and return frequency of excursions outside the Optimum Salinity and Flow Envelopes are addressed to a degree (Section 3 in the main Performance Measure Documentation Sheet) based on supported data and deemed ecologically relevant to salinity stress and ecological indicator responses from past monitoring. Changes from the 2007 Salinity Envelope PM include the period in which target events are defined: namely, the modeled flows are over a 14-day moving average rather than a monthly mean for low flow targets in the SLE and all flow targets in the CRE.

The issue of duration, return frequency, as well as recovery periods of tolerable and optimum salinities for ecological indicator species, are further addressed in ongoing and planned studies (Section 4.3 and Section 4.4) whose results are expected to inform future revision of these targets.

Additional work is also necessary to establish targets for contributions of inflow from each estuary's watershed compared to Lake Okeechobee Regulatory Releases, and how to deal with "watershed-triggered" or "Lake Okeechobee-triggered" events. For example, supposing inflows from runoff in the SLE watershed is near but below the Optimum Flow Envelope high flow bound (e.g., 1350 cfs), and there are Lake Okeechobee Releases of 100 cfs, this would result in 1450 cfs, an excursion from the Optimum Flow Envelope by 50 cfs; this would be designated a Lake Okeechobee-triggered event. How to account for these events, and what relative

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contributions are acceptable from each source of freshwater needs to be addressed in future updates.

4.2 Climate Change and Sea-Level Rise

Future efforts need to directly address climate change, sea level rise (SLR), and increasing sea surface temperature (SST) projections in predictive modeling. El Niño Southern Oscillation (ENSO) events are inherently captured in the observed flows from the period of record used to model flow alternatives in this Salinity Envelope PM, but a more explicit approach to incorporating SLR boundary conditions into the CH3D hydrodynamic salinity model is warranted. It is expected that future SLR would influence salinity envelopes and provide an opportunity to study how flow regime would change to satisfy salinity criteria. Finally, increasing SST may act as a confounding variable affecting oyster populations as it relates to spawning periodicity and the combined stressors and interactive effects of temperature and salinity on oyster disease.

4.3 Ecological Models to Support RECOVER Tools and PMs

In addition to the oyster HSI model (Section 2) used for RECOVER Interim Goals, HSI models are currently in development for SAV species tape grass (*Vallisneria americana*) and shoal grass (*Halodule wrightii*) for the Northern Estuaries. One of the key drivers for SAV distribution and density is light availability, which is not addressed in this Salinity Envelope PM. Due to its importance, photosynthetically-active radiation (PAR) measurements and light attenuation coefficient calculations have been introduced to the updated RECOVER Northern Estuaries SAV Ecosystem Assessment (NESEA) program (Kahn 2019).

The oyster and SAV HSI models should be used to create updates for Northern Estuaries Oyster and SAV PMs. Other ecological models with more mechanistic approaches to evaluate and assess CERP activities such as those in Buzzelli et al. (2015) should also be further developed for these species-specific PMs by simulating ecosystem-level processes of freshwater inflow, salinity, light, and other environmental and biological parameters affecting estuarine ecology.

4.4 Ongoing Studies

4.4.1 Duration and Return Frequency of Salinity Excursions and Recovery Time for the Eastern Oyster

Regarding the need to better define the responses of ecological indicators to duration and return frequency of excursions outside the Salinity/Flow Envelope, the South Florida Water Management District (SFWMD) has an ongoing two-year contract with Florida Gulf Coast University and the University of North Carolina Wilmington to conduct a series of iterative mesocosm experiments exposing adult and spat Eastern oyster (*C. virginica*) to varying durations and return frequencies of stressful and damaging salinities (0–5), as well as duration at varying "recovery" salinities to simulate pulse release conditions and under different temperatures to reflect seasonality. The objective of the study is to generate data and decision support tools to inform water management operations when releases to the Northern Estuaries are deemed necessary. While driven especially for weekly operations, understanding the various tolerances

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of adult and spat oysters to volumes of freshwater inflows capable of reducing salinities of 0–5 for a given period can inform biological models used in setting PM targets. For example, it is expected that conclusions from this mesocosm study will inform hydrologic targets for consecutive excursion events and their durations can be refined in the next Salinity Envelope PM revision.

The first study hypothesis is that oysters will yield a higher probability of survival if high volume discharges (i.e., low salinities) are pulsed and interspersed with recovery periods of lower volume discharges (i.e., higher salinities). This is based on the premise that oysters may recover by opening their valves to eliminate waste, feed, and respire when tolerable salinities are available during these recovery periods. It is also hypothesized that longer recovery times between multiple exposure pulses will yield higher survival, and finally, that oysters will yield a higher probability of survival under low salinity conditions when temperatures are also lower (i.e., spring and winter, 18–24°C) compared to higher temperatures (i.e., summer and fall, ~30°C).

The mesocosm exposures should determine the effect of specific freshwater inflow regimes. Understanding the additive effect of temperature on tolerance of salinity is especially important during the summer rainy season when temperatures are elevated along with increased natural freshwater inflows. The results from the mesocosm experiments will expand on previous experimental results in peer-reviewed literature to provide a greater understanding of how inflows can be sustainably managed for Northern Estuaries oysters. One of the mesocosm study deliverables will include a "decision tree" (i.e., interactive flowchart) which can be used by water managers to assess ecological risks to oysters under different life history conditions (e.g., larvae or spat at settling stage, juveniles, adults beginning gametogenesis) across a wide range of salinities, and give recommendations to alternative inflow regimes.

4.4.2 Use of Biomarkers to Examine Salinity Stress in Tape Grass and Rangia Clam

A study by SFWMD Coastal Ecosystems scheduled to begin in early 2020 will further evaluate stress responses of both tape grass and the Rangia clam (*R. cuneata*) clam in mesocosm experiments using physiological biomarkers. The Rangia clam is a bivalve found in the mid-to-upper region of the CRE, a region which routinely experiences lower salinities and provides habitat for brackish to freshwater organisms (LaSalle and de la Cruz 1985; Wakida-Kusunoki and MacKenzie 2004; Wong et al. 2010). Measurement of physiological biomarkers provides a rapid and sensitive method of assessing the health of an organism and its stress responses. These responses can include protein accumulation, tissue nutrient or pigment concentration changes, or upregulation of antioxidant compounds. In both plants and animals, the generation of reactive oxygen species (ROS) occurs naturally and continuously during aerobic metabolism. Antioxidant systems are in place to detoxify and eliminate ROS. Changes in these antioxidant response mechanisms in marine organisms, including SAV and clams, have been observed as a result of salinity stress (Solé et al. 1999; Apel and Hirt 2004; Miller et al. 2010; Lauer et al. 2011; Carregosa et al. 2014). In SAV, osmoregulatory stress can also trigger changes in antioxidant carotenoid pigment compounds and affect chlorophyll *a* and *b* pigments (Thorhaug et al. 2006; Lauer et al 2011; Trevathan et al. 2011). The use of biomarkers such as antioxidant enzyme assays can be a vital tool in monitoring programs (Goldberg et al 1975; Obrea et al. 2002).

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The goal of the SFWMD mesocosm study is to understand stress responses of tape grass and Rangia clam to various salinity regimes (salinity treatments 0–18). Data gaps exist regarding an invertebrate benthic ecological indicator in the low salinity regions of CRE and in tape grass responses at the higher end of their salinity stress maxima. Unlike the physical metrics of presence/absence or density, physiological biomarkers provide a more sensitive assessment of organismal health to both short-term and chronic exposure to stressful environmental conditions, such as hypersalinity or hyposalinity. These mesocosm studies will be designed to examine multiple biomarkers in each organism under various ecologically relevant salinity regimes. RECOVER can further refine hydrologic targets for this and other PMs.

4.5 Ecological Indicator Species

Other ecological indicator species may be of interest to the RECOVER Northern Estuaries Program depending on new science within and external to RECOVER.

Previous MAP contracts in the Northern Estuaries included fisheries monitoring, although other agencies such as the Florida Fish and Wildlife Conservation Commissions Fish and Wildlife Research Institute (FWRI) continues seining and acoustic telemetry in the SLE and monitoring endangered smalltooth sawfish (*Pristis pectinata*) in the CRE. The way in which high freshwater inflows affect fish populations is of interest to RECOVER partners, managers, many stakeholder groups, and the public. Questions of inflow regime, estuarine geomorphology, and availability of habitat on local fish populations and their movements and responses to inflows could provide a useful addition to the suite of indicators for the Northern Estuaries dependent on study results. The RECOVER Southern Coastal Systems Program includes a Performance Measure for juvenile spotted seatrout (*Cynoscion nebulosus*) habitat quality (i.e., HSI) (RECOVER 2017).

Two Northern Estuaries projects with FWRI were identified and contracted by the SFWMD in late 2019 to analyze a backlog of seine sampling data from the St. Lucie Estuary (SLE) and Loxahatchee River Estuary (LRE), and to collect and analyze acoustic telemetry data of several large-bodied fishes in the SLE and Southern Indian River Lagoon including the spotted seatrout, common snook (*Centropomus undecimalis*), fat snook (*C. parallelus*), sheepshead (*Archosargus probatocephalus*), and goliath grouper (*Epinephelus itajara*). This project is leveraged by the FACT Network, a grassroots collaboration between marine scientists using acoustic telemetry and other technologies to track fish and sea turtles, which originated as the Florida Atlantic Coastal Telemetry Network but now includes partners from the Bahamas to the Carolinas (FACT Network 2019). The results may support reintroducing fisheries monitoring to the Northern Estuaries RECOVER monitoring program. Final deliverables for the seine data analysis and acoustic telemetry are due September 2020 and September 2021, respectively.

In addition, several sample sites included in the benthic infauna monitoring in the SLE and S-IRL have been backlogged: the samples are collected but not analyzed. A SFWMD contract was initiated in late 2019 to analyze these 216 backlogged samples. New to the benthic infauna monitoring program, genetic barcoding and representative photographs of each of 40 key invertebrate taxa will also be sent to taxonomists for conclusive morphological identification to develop a benthic infaunal database. This effort will be critical to identifying taxa tolerant to

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freshwater inflows and salinity excursions. Final deliverables for the sample processing and genetic barcoding is due in December 2020.

Finally, there are several suitable indicators of high flows (i.e., oysters and meso/polyhaline-adapted SAV), but only one SAV as an indicator of low flows (i.e., tape grass); the Rangia clam is a potential new indicator for low flows in the Caloosahatchee River Estuary (Section 4.4.2).

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