Application for Incidental Take Authorization (LOA) for U.S. Fish and Wildlife Service sampling procedures for threatened Gulf sturgeon and their effect on marine mammals

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The purpose of this Incidental Take Authorization (LOA) is to comply with statutory requirements to use the best scientific and commercial information available when assessing the risks posed to listed species by proposed federal actions.

1) Description of the Specific Activity:

The USFWS is working with NMFS, USGS, and other partners (Delaware State University, University of Southern Mississippi, Army Corp of Engineers) on several wide-ranging projects (across Gulf of Mexico) throughout critical habitat areas for the Gulf sturgeon which include both rivers and bays. These projects include: 1) a Natural Resource Damage Assessment (NRDA) project entitled "Mississippi Canyon 252 Assessment Plan for the Collection of Data to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon", 2) an annual summer and fall census and 3) fine-scale movement and habitat assessment within and nearby Choctawhatchee Bay. Sampling locations will occur throughout the Pearl, Pascagoula, Escambia, Yellow, Blackwater, Choctawhatchee, Apalachicola, and Suwannee rivers and their associated bays near the river mouths.

These four projects permitted under Florida Fish and Wildlife Conservation Commission (FWC) Special Activity License # SAL-11-0891-SRP and FWC general scientific collecting permit # FNW11-04 are briefly described below:

> <u>The Mississippi Canyon 252 Assessment Plan for the Collection of Data to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon</u>
>
> This is an ongoing NRDA project involving multiple collaborators, including U.S. Fish and Wildlife Service (Panama City Fish and Wildlife Conservation Office, Baton Rouge Fish and Wildlife Conservation Office) and NMFS, which involves monitoring the movements of the Gulf sturgeon in the bays and estuaries of north Central Gulf of Mexico and documenting the physical condition of Gulf sturgeon through visual observation, tissue collection, and photo-documentation as they enter the Gulf of Mexico during the annual fall migration and the subsequent migration back into freshwater rivers

in the spring. Eight rivers and their associated bays are being sampled which are part of the threatened Gulf sturgeon's critical habitat: Pearl, Pascagoula, Escambia, Yellow, Blackwater, Choctawhatchee, Apalachicola, and Suwannee. Data collection for this plan began in Fall 2010, is ongoing, and may be extended for another sampling year (Fall 2012 – Spring 2013), if deemed necessary. Collection of these data is considered a pre-assessment and post-assessment activity within the NRDA process for the MC 252 Spill. The NRDA process involves identifying the extent of natural resource injuries and the best way to restore those resources following an oil spill or hazardous substance release.

The main objectives of this plan are:

(1) Record physical condition of Gulf sturgeon during the fall and spring migrations through visual observation, photo-documentation, and physical measurements as listed in the Pre-Assessment Plan;
(2) Continue to document offshore movement and habitat use of 134 adult Gulf sturgeon that were part of the Pre-Assessment monitoring effort, and implant telemetry transmitters to monitor an additional 160 individuals.

Netting techniques used in the study are as follows:

Up to 160 (20 each from the Pearl, Pascagoula, Escambia, Yellow, Blackwater, Choctawhatchee, Apalachicola, and Suwannee rivers) adult Gulf sturgeon (> 1300 mm total length) will be collected in gill nets during the fall in the bays and rivers prior to their winter migration into the Gulf of Mexico, and again during the spring in the bays and rivers while the fish are migrating to spawning and summer resting grounds. Gill nets (single or multiple up to 6), both anchored and drifting as applies to the situation, are no longer than 300 feet long, and mesh size is no smaller than 12.7cm (5 inch) stretch mesh and no larger than 30.5cm (12 inch) stretch mesh. The large mesh gillnets target larger adult Gulf sturgeon which will be tagged in the study and avoids smaller juveniles. A 2-hour maximum soak time is permitted and all nets are to be continuously attended by field crews. Night fishing is acceptable.

For more information regarding the methodology of this study plan, please see Appendix A which is the latest work plan for the project entitled "Mississippi Canyon 252 Pre-Assessment Plan for the Collection of Data to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon."

<u>The summer and fall census</u>

This is a rotating census through the Florida panhandle rivers (freshwater areas only) that occurs from June through September. The rivers under rotation include those designated as critical habitat (68 FR 13370) Pearl, Pascagoula, Escambia, Yellow, Choctawhatchee,

Apalachicola, and Suwannee rivers. Each river already contains a known number of tagged fish, and will be sampled for two years in a row to obtain a population estimate. The rotating schedule is in place to avoid sampling of the same location multiple years in a row to avoid unnecessary stress on the fish. Gill nets (single or multiple up to 6) are no longer than 91.4 m (300 feet) long with large stretched mesh (12.7-30.5 cm), both anchored and drifting, deployed usually during the daylight hours. Limited night fishing, immediately after sunset with the final net set before dusk, may also occur. During the latter part of the summer, when water temperatures increase, fewer nets are deployed and they are checked more frequently to avoid stressing the fish unnecessarily. At times, when a large catch is anticipated, only one sinking or drifting gill net will be deployed and monitored. A 2-hour maximum soak time is required and all nets are to be continuously attended by field crews. There is no end date set for this census.

Assessment of fine-scale spatial and temporal core habitats of Gulf sturgeon within and nearby Choctawhatchee Bay, Florida

This project will provide resource managers with an increased understanding on the fine-scale relationship between Gulf sturgeon and habitat quality. Additionally, we will provide direct estimates on the fine-scale habitat requirements for Gulf sturgeon and benthic habitats including much needed estimates on the linkage between foraging and benthic habitat quality.

Sampling of juvenile Gulf sturgeon will be conducted in the fall in known concentration areas in the Choctawhatchee River. This sampling will be conducted using drift gillnets approximately above river kilometer 30 well above the head of the tide for the Choctawhatchee River. These locations have been selected based on past success in landing Gulf sturgeon as they are beginning their fall migration out of the river. The vast majority of in-river sampling is conducted with a single drift gillnet which has a maximum length of 121.9 m (400 feet) and stretch mesh ranging between 15.2 – 30.5 cm (6-12 inches). The drift nets vary in length from 3.7 – 7.3 m (12-24 feet) to allow for varying fishing conditions and flow regimes. In rare instances we may deploy a second gillnet which will be clearly visible from the vessel. The timing and location of sampling will be conducted in such a way as to minimize potential interactions with other species in addition to maximizing the probability of successful collections. Drift gillnets of varying mesh size will be used to collect a broad segment of the population likely to occupy estuarine waters. No more than three drift nets will be used in bays with a person on the boat dedicated to continuous monitoring of each net (up to three people). Nets will be tended at minimum hourly.

This project will rely primarily on passive acoustic telemetry although mobile tracking events will occur on a bi-weekly basis in both the primary study area and surrounding Bay waters following transmitter deployment through the entry of Gulf sturgeon into the Choctawhatchee River (April-May). During mobile tracking events, the locations of tagged fish will be determined through standard telemetry techniques, habitat parameters

(e.g. depth, salinity, dissolved oxygen, and temperature) will be recorded and a substrate sample collected.

A VR2W Positioning System (VPS) will be placed in core foraging habitats of Gulf sturgeon in Choctawhatchee Bay during periods of likely sturgeon residency (October-May) from 2011 through 2013. In the final year of the proposed project, the existing VPS array will be moved into near-shore marine waters close to the entrance of Choctawhatchee Bay where Gulf sturgeon are known to concentrate.

In the core habitat area we will reallocate the currently deployed acoustic receivers and add additional ones to develop the VPS grid system. Within this grid network sync acoustic transmitters and temperature loggers will be deployed to allow for the fine-scale estimation of positions. Outside of the core habitat area we will maintain an existing passive acoustic receiver network to allow for flexibility in study design in the 2nd and 3rd years of the planned project. For example, if our first year results indicate that Gulf sturgeon are not utilizing the central portions of Choctawhatchee Bay we may reconfigure the VPS system to provide broader spatial coverage of nearshore habitat areas. Receivers will be downloaded and maintained on a bi-monthly basis to ensure the functionality of the VPS system as well as minimizing the chances of data loss.

Concurrent with the telemetry component of this study we will conduct targeted sampling for Gulf sturgeon utilizing a stratified random design (e.g. region of bay and depth) on at least a monthly basis. To ensure adequate sample coverage in the VPS region 50% of all sampling efforts will take place in the core habitat area. Collected Gulf sturgeon will be sampled to assess foraging through gastric lavage. After being processed Gulf sturgeon will be returned to the location of collection and a substrate sample will be collected for infaunal and sediment analyses. Sampling in Choctawhatchee Bay and nearshore marine waters will be primarily conducted using drift gillnets. In situations where the depth exceeds net height (typically >15 feet) we may choose to utilize anchored gillnets. Choctawhatchee Bay has limited flow and a drift net which fishes the entire water column will essentially be anchored in place or move very slowly (<100m/hour) when allowed to drift passively. We will continuously monitor the nets for the presence of sturgeon and any other large animals through the float line for drift nets. In the case of anchored nets we will keep soak times to <1H to minimize the risk of bycath mortality since the sub-surface floats preclude detection of sturgeon from the surface. We plan to fish up to three nets concurrently in Choctawhatchee Bay and nearshore marine water. If necessary these nets may be tied end to end to facilitate rapid checking of the gear.

3) **Marine Mammal Species**

The following marine mammals may be affected by the proposed action:

Bottlenose Dolphin (*Tursiops truncates*)

Atlantic Spotted Dolphin (*Stenella frontalis*)

Pantropical Spotted Dolphin (*Stenella attenuata*)

Striped Dolphin (*Stenella coeruleoalba*)

These cetaceans were included because their range overlaps with Gulf sturgeon sampling efforts in the bays and estuaries from Louisiana to Florida. The Atlantic spotted dolphin generally occurs in deeper coastal or continental shelf habitats (20-250m), but Davis and Schmidly (1997) state that Florida populations may move into nearshore waters in late spring and summer, likely for feeding purposes. Pantropical spotted dolphins typically reside in habitat between 90 to 300m deep where they feed at or near the surface. Striped dolphins are often oceanic, found in deeper offshore habitats where they feed. A recent study examining temporal patterns of movement in the Atlantic spotted dolphin and the bottlenose dolphin off the West Florida continental shelf found significant seasonal variation in movement with no predictable patterns (Griffin and Griffin 2004). There was no clear temporal pattern observed, and prey patterns, current, and temperature, may be responsible for these less predictable patterns (Griffin and Griffin 2004).

4) Species descriptions

The bottlenose dolphin *Tursiops truncatus* inhabits temperate and tropical waters around the world. Coastal populations migrate into bay and estuarine environments, including river mouths. According to the 2007 National Marine Fisheries Service stock assessment report there were an estimated 4,191 bottlenose dolphins in the Northern Gulf of Mexico (GOM) coastal stock, and 2,239 bottlenose dolphins in the Northern Gulf of Mexico oceanic stock. There are several GOM strategic stocks including: Eastern GOM Coastal, Western GOM Coastal, Northern GOM Coastal, GOM Bay, Sound and Estuarine.

Current information regarding the GOM Bay, Sound and Estuarine stock was derived from the 2010 Bottlenose Dolphin Stock assessment report. The GOM Bay, Sound and Estuarine stock consists of individuals that largely remain in the bay, sound or estuary waters and are not likely to move through passes to the Gulf of Mexico. Potentially affected communities within the GOM Bay, Sound and Estuarine stock, with recent bottlenose dolphin abundance in parentheses, would potentially include: Bay Boudreau/Mississippi Sound (1,401), Mobile Bay/Bonsecour Bay (122), Perdido Bay (0), Pensacola Bay/East Bay (33), Choctawhatchee Bay (242), St. Andrew's Bay (124), St. Joeseph's Bay (81), Apalachicola Bay/St. Vincent Sound/St. George Sound (537), Apalachee Bay (491), Waccasassa Bay/Withlacoochee Bay/Crystal Bay (100), St Joseph Sound/Clearwater Harbor (37), Tampa Bay (559), and Sarasota Bay/Little Sarasota Bay (160).

Current population size of each stock is unknown for the majority of the stocks, except for the following: St Joseph's Bay, Apalachicola Bay, and Sarasota Bay, which have a minimum population size of 72, 498, and 160, respectively. Since there is a lack of recent mark-recapture of spotting data, population trends are unable to be determined for many of these stocks.

Potential biological removal (PBR), or the "maximum number of animals, not including natural mortalities, that may be removed from a marine mammal stock while allowing that stock to reach or maintain its optimum sustainable population" can only be given for stocks with recent population estimates. These were determined to be 0.7 dolphins for St. Joseph Bay, 5.0 dolphins for Apalachicola Bay, and 1.6 dolphins for Sarasota Bay. Total human caused mortality for these stocks over the past five years is currently unknown; however, in earlier estimates, the annual average number of removals never exceeded previous PBR levels.

The Atlantic spotted dolphin *Stenella frontalis* are usually found in groups of fewer than 50 individuals, but can be seen in larger groups. Inshore pods usually consist of 5-15 animals. They can be associated with bottlenose dolphins. These dolphins often approach vessels to "bowride." Atlantic spotted dolphins inhabit the tropical to warm temperate waters and generally occur in coastal or continental shelf waters. The Northern Gulf of Mexico stock is estimated at 24,500-31,000 animals.

The Pantropical spotted dolphin *Stenella attenuate graffmani* often occur in large groups of several hundred to one thousand individuals. They are known to school with other dolphin species. They appear to move inshore during the fall and winter months and offshore during the spring. Spotted dolphins inhabit shallower water during the day (300 to 1000 ft). Coastal spotted dolphins are found within 100 miles of the coast. The coastal stock is estimated to have a population size of 149,400 individuals (Gerrodette et al. 2005), with those in the Northern Gulf of Mexico having an estimated population size of 91,300 individuals.

The striped dolphin *Stenella coeruleoalba* is generally found in groups averaging between 25 to 100 dolphins, but can occur in much larger groups (thousands). This species rarely is found with any other species of dolphin or whale. This species lives in tropical to warm temperate waters and often located to upwelling areas and convergence zones. The Northern Gulf of Mexico stock is estimated at 4,500 to 6,500 individuals.

5) Type of incidental take authorization being requested and 6) amount of take requested

The incidental take is expected to be in the form of harassment, injury and/or death. This type of take could occur through use of set gill nets (rivers and bays) and drift nets (rivers and bays) to catch Gulf sturgeon. For the past 26 years, the USFWS and our partners have been using gill nets to sample Gulf sturgeon in the rivers and bays from the Pearl River, Louisiana to the Suwannee River, Florida. In over 19,500 hours of direct effort, which includes 192 days in the bays with 480 net sets, and 1,542 hours of effort, 19,991 Gulf sturgeon have been captured, but only two marine mammals (bottlenose dolphins) have ever been captured during USFWS sampling efforts (Table 1). The two bottlenose dolphin mortalities occurred by entanglement and subsequent asphyxiation in a drift net in Choctawhatchee Bay in spring of 2011 under the USWFS permit. In addition, one bottlenose dolphin was entangled and asphyxiated in a gill net

in 2006 during Gulf sturgeon sampling in the estuarine portion of the Mississippi River Gulf Outlet by the Corp of Engineers under their permit. No marine mammals have ever been captured during Gulf sturgeon sampling in freshwater. Most of the effort expended in the bays and estuarine areas has occurred since 2004, and in the past eight years only 3 marine mammal encounters have occurred. We are not aware of any other episodes of marine mammal take, due to Gulf sturgeon sampling or similar efforts, outside of these projects. For the next 1-2 years, we expect a similar level of effort to that of the past year, with approximately 40 hours of direct effort per river (360 hrs total) for the NRDA project, an additional 160 hours of direct effort for the summer/fall census, and 120 hours of effort for the Choctahatchee bay project. Following the conclusion of the NRDA project, we expect an annual effort of 160 hours for the summer/fall census, and a continued 120 hours of effort for the Choctahatchee bay project. If this pattern holds, we can anticipate a possible take of 0.4 marine mammals per year, or 1 marine mammal every 2.5 years. Although no other marine mammals have been taken in any other sampled Gulf bay (Mississippi Sound, Pascagoula Bay, Mobile Bay, Apalachicola Bay, and the Suwannee Sound), it is possible that additional take may occur in these locations. Therefore, the Service anticipates take of no more than two marine mammals each year as a result of this proposed action. We have only observed the take of bottlenose dolphin during sampling, but it is possible that take of any of the species of dolphin listed above could occur.

7) Anticipated impact of the activity upon the species or stock

According to recent National Marine Fisheries Service stock assessments there are about 4,191 bottlenose dolphins, with an estimated total abundance of 3,887 in the following at risk stocks Bay Boudreau/Mississippi Sound (1,401), Mobile Bay/Bonsecour Bay (122), Perdido Bay (0), Pensacola Bay/East Bay (33), Choctawhatchee Bay (242), St. Andrew's Bay (124), St. Joeseph's Bay (81), Apalachicola Bay/St. Vincent Sound/St. George Sound (537), Apalachee Bay (491), Waccasassa Bay/Withlacoochee Bay/Crystal Bay (100), St Joseph Sound/Clearwater Harbor (37), Tampa Bay (559), and Sarasota Bay/Little Sarasota Bay (160). In addition, there are 24,500-31,000 Atlantic spotted dolphin, 91,300 Pantropical spotted dolphin, and 4,500 to 6,500 striped dolphin in the Northern Gulf of Mexico (GOM) coastal stocks. We are requesting take of two marine mammals (any combination of bottlenose dolphin, Atlantic spotted dolphin, Pantropical dolphin, and striped dolphin) per year for five years. Although sampling activities for the Gulf sturgeon may affect bottlenose dolphin, Atlantic spotted dolphin, Pantropical spotted dolphin, and striped dolphin, we do not believe that our activities will impact the species or stock due to their large population sizes and the small (albeit potential) likelihood of encounter. Pantropical spotted dolphin, Atlantic spotted dolphin, and striped dolphin, primarily reside offshore in depths greater than that of the Gulf sturgeon. Sub-adult and adult Gulf sturgeon overwintering in Choctawhatchee Bay were generally found to occupy the sandy shoreline habitat at depths of 2-3 m (Fox *et al* 2002, Parauka *et al* 2001). Similarly, in a multi-year study, Ross et al. (2009) found Gulf sturgeon from both the Pascagoula and Pearl Rivers broadly overlap and use the shallow water

along the Gulf barrier islands as foraging grounds in the winter. These marine habitats utilized by the Gulf sturgeon were all less than 7 m deep, generally well oxygenated, and with relatively clear water; bottom substrates were mostly coarse sand and shell fragments or fine sand (Ross et al. 2009). Due to these facts, although actual rate of take suggests a lower estimate than two marine mammals per year, we are requesting take equal to the maximum take observed in any year.

8) The anticipated impact of the activity on the availability of the species or stocks of marine mammals for subsistence uses.

We are not aware of subsistence uses for these dolphin species but our sampling activities will not affect the availability of the species or coastal stock of these dolphin species for subsistence uses.

9) The anticipated impact of the activity upon the habitat of the marine mammal populations, and the likelihood of restoration of the affected habitat

Our sampling techniques are likely to have insignificant effects on the habitat of area dolphin populations. Drift netting and gill netting does not typically alter habitat, or if it does, the adverse effects are so minor that they cannot be meaningfully measured or evaluated.

10) The anticipated impact of the loss or modification of the habitat on the marine mammal populations involved.

Our sampling would not result in the loss or modification of habitat, and therefore, would have no effect on marine mammal populations in our sampling area.

11) The availability and feasibility (economic and technological) of equipment, methods, and manner of conducting such activity or other means of affecting the least practicable adverse impact upon the affected species or stocks, their habitat, or their availability.

Conservation Measures for the Above Gulf Sturgeon Projects

Despite a decent number of studies undertaken, the causes of marine mammal by-catch and behavioral sequences that lead up to by-catch and entanglement are poorly understood (Cox et al. 2003). For the most part, echolocating bottlenose dolphins can detect nets in enough time to avoid a collision (Kastelein et al. 2000), and many times when they approach a net will actively divert their course to avoid a net (Read et al. 2011). However, bottlenose dolphins will also work to depredate a net and a set gill net with fish can be attractive to dolphins (Read et al. 2011), which can make them susceptible to possible entanglement.

Acoustic pingers have been developed and tested to deter dolphins from fishing nets with mixed results (Barlow and Cameron 2003; Cox et al. 2003; Gazo et al. 2007). Although they can initially deter bottlenose dolphins (Barlow and Cameron 2003; Cox et al. 2003), they may not

entirely stop bottlenose dolphins from approaching nets (Gazo et al. 2007), and following habituation may even attract them. There are also likely interspecific behavioral differences (some populations of the same species may respond differently to pingers than others). Because of the disparity in the data, we do not propose use of acoustic pingers during work with Gulf sturgeon.

Due to bottlenose dolphin takes on 12 April 2011, NMFS developed conservation measures that are believed to reduce future potential for marine mammal interactions. These conservation measures were agreed to by USFWS personnel and will be used for all of the above investigations with a minor change to net soak times in the rivers and number of nets allowed in bay sampling.

Measures for all sampling regardless of location include:

1. Scanning the area, up and downstream of the net as far as can be accurately observed, visually for at least 30 minutes prior to setting the net (if a dolphin is spotted, gear may not be set at this location until the area is clear of marine mammals for at least 30 minutes).
2. Immediately responding to any net disturbance during deployment, fishing, and haul back.
3. If marine mammals are sighted, immediately haul gear onto the boat and do not reset gear until the area is clear of marine mammals for 30 minutes
4. Reducing the bag in the net by ensuring that proper anchor weight is keeping the net mouth open.
5. Reducing any floating lines or trailing lines with buoys.
6. The number, size, and color of the net floats shall be appropriate to maximize visibility of net activity.
7. Repair damaged nets.

The following measures will be taken when sampling in the bays, sounds, and river mouths:

1. Using only one net per dedicated person when sampling in the bays, not to exceed three nets.
2. Having a dedicated crew member to visually monitor the nets continuously before and during sampling.
3. The entire net shall be hand-checked once per hour.

The following measures will be taken when sampling in the rivers:

1. Continuously monitoring the net and float buoys during sampling.
2. The entire net shall be hand-checked once every two hours.

For a full description of the agreed-upon measures (minus the above-mentioned minor modifications), please see Appendix B entitled "Marine Mammal Conservation Measures and Recommendations for Gulf Sturgeon Surveys: Summer and Annual Fall Census."

12)Where the proposed activity would take place in or near a traditional Arctic subsistence hunting area and /or may affect the availability of a species or stock of marine mammal for Arctic subsistence uses, the applicant must either submit either a plan of cooperation or information that identifies what measures have been taken to minimize any adverse effects

N/A

13) The suggested means of accomplishing the necessary monitoring and reporting that will result in increased knowledge of the species, the level of taking or impacts on populations of marine mammals that are expected to be present while conducting activities and suggested means of minimizing burdens by coordinating such reporting requirements with other schemes already applicable to persons conducting such activity.

The following monitoring and reporting procedures will be in place in the event of a marine mammal capture during Gulf sturgeon sampling. These procedures are described in more detail in Appendix B (Conservation Measures for Marine Mammal Captures).

a. Marine mammal entanglements (live or dead) will be reported immediately to the stranding network at 1-877-WHALE HELP (1-877-942-5343). Research activities will cease until further coordination and discussion on the nature of the capture are discussed between USFWS and NMFS.
b. If there is a live entanglement, the animal will be freed from the net as quickly as possible while ensuring that the dolphin's blowhole is kept at the surface. Once the animal is free, it will be (1) photographed; (2) condition will be recorded including any injuries and swimming behavior; (3) details on the nature of the entanglement will be recorded, including by not limited to, gear characteristics, where in the net the animal was entangled, etc. This information will be sent to Stacey.Horstman@noaa.gov.
c. In the event of a mortality, the animal will be hauled aboard the vessel and retained for pickup by a stranding network member. Photos, measurements, and entanglement information will also be documented per "NMFS' Protocol for Dead Entangled Small Cetaceans." This protocol will is included in Appendix B.

14) Suggested means of learning of, encouraging, and coordinating research opportunities, plans, and activities relating to reducing such incidental taking and evaluating its effects

The means provided in 13) and the mitigation and conservation measures provided in 12) will facilitate and encourage coordination among the agencies and research activities related to reducing incidental take and evaluating its effects.

Conclusions

We have used gill nets to target Gulf sturgeon in the rivers and bays ranging from 4 to 26 years in each sampling location. We have captured two bottlenose dolphins in the past year using these methods. Due to this recent capture, mitigative measures were put in place adding restrictions to our sampling. Due to these more conservative methods, we find it even less likely that we will capture any additional dolphins; however, we are requesting take of two marine mammals ((any combination of bottlenose dolphin, Atlantic spotted dolphin, Pantropical dolphin, and striped dolphin) per year for 5 years.

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Table 1. History of Gulf Sturgeon Survey Efforts in the Florida Panhandle River Systems. Each river and bay was not necessarily surveyed annually. The Brothers River is considered a tributary to the Apalachicola river. The Ochlocknee and Blackwater have previously been sampled, do contain Gulf sturgeon but are not considered critical habitat.

Body of Water	Period surveyed (yrs)	Days surveyed	Net sets	Net set hours	Gulf sturgeon	Bottlenose Dolphins
Apalachicola River	26; 1978-2010	194	1,143	2,862	1,144	0
Brothers River	18; 1984-2010	84	504	756	944	0
Blackwater River	4; 2005-2010	14	49	11	164	0
Choctawhatchee River	19; 1988-2010	250	841	7,056	2,715	0
Escambia River	12; 1994-2010	130	480	1,200	667	0
Ochlockonee River	10; 1991-2010	25	100	150	123	0
Yellow River	10; 1993-2010	146	454	1,458	985	0
Suwannee River	25; 1986-2011	1,000	1,500	4,500	13,054	0
River total		**1,843**	**5,071**	**17,993**	**19,796**	**0**
Choctawhatchee Bay	9; 1972-2011	172	318	1,218	152	2
Pensacola Bay	4; 2004-2010	20	162	324	43	0
Bays total		**192**	**480**	**1,542**	**195**	**2**
Total		**2,035**	**5,551**	**19,535**	**19,991**	**2**

Appendix A

Mississippi Canyon 252

Year 2 Assessment Plan for the Collection of Data to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon

Mississippi Canyon 252

Year 2 Assessment Plan for the Collection of Data to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon

2011-2012

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For the

MC 252 NRDA Fish Technical Working Group

Deepwater Horizon/Mississippi Canyon 252 Trustees

SUMMARY

This Assessment Plan is a continuation of the Gulf Sturgeon Pre-Assessment Plan signed by the trustees and BP Exploration and Production, Inc. September of 2010 as part of the ongoing natural resource damage assessment (NRDA) for the Deepwater Horizon/Mississippi Canyon 252 (MC 252) Oil Spill. The Trustees will continue to monitor Gulf sturgeon (*Acipenser oxyrinchus desotoi*) movement and habitat use in north central parts of the Gulf of Mexico and collect blood from these and additional sturgeon to evaluate potential exposure and injury to fish from the MC 252 Oil Spill. Analytical pathways and procedures outline how habitat use will be used in conjunction with other available data to develop indices of spatial and temporal exposure to toxicants and potentially contaminated prey. Telemetry data will be integrated with cytogenetic and genomic biological endpoints. The goals of this study are to: 1) monitor movement of Gulf sturgeon; 2) evaluate habitat use by Gulf sturgeon and integrate this information with landscape-level attributes; 3) evaluate morphological characteristics of the sturgeon to assess overall health; 4) measure exposure of Gulf sturgeon through chemical analysis of blood; 5) evaluate genetic damage in Gulf sturgeon from PAHs and oil-related chemicals; and 6) evaluate toxicity to immune and hematological function through gene expression patterns in the blood of field-collected Gulf sturgeon. The collection of data on Gulf sturgeon outlined in this Plan to Determine Potential Exposure and Injuries of Threatened Gulf Sturgeon (Assessment Plan or Plan) is an assessment activity within the NRDA process for the MC 252 Oil Spill.

STUDY GOALS

This Plan focuses primarily on collecting data to facilitate the evaluation of potential exposure and injury to Gulf sturgeon as a result of the MC 252 Oil Spill. Specific field protocol details for collecting and handling sturgeon, collecting blood from sturgeon, deploying telemetry equipment, field safety, and data management are listed in the signed Pre-Assessment Work Plan, and activities associated with the Assessment Plan will continue under the those guidelines (Appendix 1). The signed 2010 Preassessment Plan is available at http://www.doi.gov/deepwaterhorizon/adminrecord/index.cfm

This Plan integrates Gulf sturgeon movement and habitat use with chemical exposure and measures of biological impairment (injury) from individual animals. Injury measurements are based on cytogenetic and genomic responses detected in peripheral blood samples. This Plan includes a laboratory exposure of sturgeon to MC 252 oil to calibrate expected cytogenetic and genomic responses. This controlled laboratory study will substantiate or confirm conclusions suggested by field collected samples (NOAA, 1996). The laboratory efforts of this Plan are designed to provide information

on the exposure of Gulf sturgeon to polycyclic aromatic hydrocarbons (PAHs) and any adverse effects of PAH exposure in field-collected individuals.

Tumors, immune suppression, and systemic toxicity have been associated with continuous low-level exposure to oil and related chemicals in water, sediment, and food web. This study will address each of these three possible conditions in Gulf sturgeon. Such effects directly translate into reduced survival of organisms and have direct implications on the structure and function of exposed populations. Fifteen PAHs are carcinogens (National Toxicology Program, 2002; Rice and Arkoosh, 2002), and many of the 4- to 7-ring compounds are mutagenic or teratogenic to a wide variety of organisms, including fish and other aquatic life (e.g. amphibians, birds, and mammals (Eisler, 1999)). This Assessment Plan is designed to obtain critical information on the exposure pathways and potential detrimental effects occurring in listed Gulf sturgeon as a result of PAH exposure from the MC 252 Oil Spill.

STUDY OBJECTIVES

The objectives of this Assessment Plan are to:

A. Record physical condition of Gulf sturgeon during the fall (2011) and spring (2012) migrations through visual observation, photo-documentation, and physical measurements as listed in the Pre-Assessment Plan;

B. Continue to document offshore movement and habitat use of 134 adult Gulf sturgeon radio tagged in the cooperative 2010 Pre-Assessment monitoring effort

C. Capture, radio tag, and monitor off shore habitat use of an additional 160 Gulf sturgeon captured in 2011 and 2012.

D. Collect blood from up to 160 Gulf sturgeon to evaluate PAH exposure and injury via quantification of cytogenetic and genomic responses.

E. Calibrate observed cytogenetic and genomic responses in field collected blood samples using controlled laboratory exposures of sturgeon to MC 252 oil.

PATHWAYS TO DETERMINE EXPOSURE:

Physical Fouling of Animals. Similar to the cooperative work conducted in 2010, photo-documentation will continue to establish a record of sturgeon morphological condition. Each collected fish will be photographed and released regardless of condition according to the protocol in Appendix 1.

Chemistry: Blood samples from each fish will be analyzed for select PAHs and associated petroleum hydrocarbons consistent with Fish and Chemistry Technical Working Groups and MC 252 Oil Spill NRDA recommendations. Baseline blood samples collected during the Pre-Assessment will be analyzed for select PAHSs, alkyl-PAH, and total petroleum hydrocarbons. The cycle of blood collection during the fall outmigration and spring spawning migration will be repeated in the fall of 2011 and spring of 2012.

Cell and Molecular Metrics: Gulf sturgeon exposure to oil and oil-related products will be documented by cytogenetic and genomic diagnostic measures. Measurement and quantification of DNA-adducts will serve as a direct measure of exposure and injury. Transcriptional genomic expressions also serve as xresponses able to classify an individual's PAH exposures.

Movement: Oceanic movement and habitat use across two consecutive winter periods will be evaluated as a means of estimating potential exposure duration to oiled substrate and contaminated invertebrate prey items. Information on currents, clean up activities and oil plume movement currently being collected through the MC 252 Oil Spill NRDA will provide spatial templates to assist in determining the duration and intensity of exposure to oil and contaminated prey, respectively.

PATHWAYS TO DETERMINE INJURY:

FIELD EFFORT:

Gulf Sturgeon Handling: Up to 160 (20 from each of the 8 rivers) adult Gulf sturgeon (> 1300 mm total length) will be collected in gill nets summer and fall of 2011 prior to their winter migration into the Gulf of Mexico. Blood will be collected and telemetry transmitters will be surgically implanted in each animal as described in Appendix 1. For this Plan, a second field effort will follow in the spring of 2012 to: 1) recapture and collect blood from previously captured sturgeon from either the 2010-2011 effort or the 2011-2012 Plans; and (2) to collect blood samples from up to 160 additional sturgeon (20 from each of the 8 rivers) as sturgeon return from the Gulf of Mexico to spawn in freshwater rivers after overwintering in the Gulf of Mexico. Six field teams will cover eight rivers using netting and handling guidelines that have been established through efforts by the Gulf Sturgeon Recovery Team, and these

guidelines will be observed in all field surveys (Appendix 1). The Ochlockonee River will be eliminated from the survey because no Gulf sturgeon were collected from that drainage during the Pre-Assessment Plan. Field records, data collection, and sample collection protocols will be the same as in the Pre-Assessment Plan (Appendices 1 and 2).

Telemetry Network: The total grid containing 135 Vemco® VR2w receivers will be re-deployed to the same offshore stations as outlined in the Pre-Assessment Plan and will utilize similar deployment methods. The array will be downloaded every 6 weeks and data management will follow the same procedure as outlined in the Pre-Assessment Plan.

LABORATORY EFFORT:

See Appendix 3 for specifics on blood sample processing.

Cytogenetics

Briefly, cytogenetic information will be obtained from blood samples collected in the field from individual Gulf sturgeon representing distinct populations along the Gulf coast. Response variable data to be collected includes: A) Number of BAP-DNA adducts; B) level of cell cycle inhibition; C) percentage of DNA fragmentation; and D) levels of DNA repair protein..

A. *DNA Adducts*

Metabolism of PAHs by mixed function oxidases (MFO) produces genotoxic intermediates that damage deoxyribonucleic acid (DNA) and lead to neoplasia in fish and other vertebrates. Formation of genotoxin-DNA complexes (*adducts*) are an indicator and metric of contamination and extensive data support their role in the initiation of chemical carcinogenesis (Phillips, 1983). In particular, the PAH benzo[a]pyrene (BaP) is a well-known carcinogen in humans and animals, and is particularly effective at inducing enzymes (Goksoyr and Forlin, 1992). BaP is metabolized to a reactive form (BPDE) which binds covalently to DNA, triggering mutations and neoplastic transformations, where cell cycle perturbations (Fimognari et al., 1999) occur in this kinetic mechanism. The BPDE reacts covalently with DNA to form BPDE-DNA adducts, which are detectable typically by ^{32}P-postlabeling (Harvey and Parry, 1998), as well as by flow cytometry (Shinozaki et al., 1998), as are other PAH-DNA adducts (Poirier, 1993; Mooney et al., 1995).

B. *Cell Cycle Inhibition*

Adduct presence is related to inhibition of cell cycle progression and is also detected by flow cytometric DNA fragmentation/integrity assays. Each of these perturbations is measurable.

C. *DNA Fragmentation*

DNA integrity is a measurable physiological biomarker. Noxious chemical compounds can have deleterious effects on DNA and cellular division, and often lead to mutagenesis induction and carcinogenesis (Fimognari et al., 1999). By virtue of the universality of DNA molecular structure and the physiological processes involved in maintaining its replicative fidelity for future generations, DNA biomarker assays provide assessments comparable across animal species, whether invertebrate or vertebrate. Consequently, agents that are genotoxic for one group of living organisms are typically genotoxic for other groups (Al-Sabti, 1985). DNA fractionation can be detected by using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) (Sailer et al., 1995) and enhanced binding of fluorescent dyes to DNA with detection by flow cytometry (Shapiro, 1993; Jenkins, 2008; Jenkins et al., 2011) and has been detected in killifish by BAP (Jung et al. 2009), among other studies.

D. *DNA Repair Protein*

DNA damage measurement results from the interaction between damaged DNA and repair processes. Double stranded DNA lesions are the most serious type of damage (Bradbury and Jackson, 2003), whereby these unrepaired double stranded (ds) breaks are the principle lesions leading to cell death or the formation of mutations and/or chromosomal aberrations (Belloni et al., 2005). DNA repair proteins are activated by ds breaks, and can be detected using antibodies against nuclear (poly[ADP-ribose]polymerase (PARP) (Bouchard et al., 2003) or DNA protein kinase (subunits Ku70 and Ku80) (Dynan and Yoo, 1998). The apoptotic process is involved in removing cells damaged by genotoxic insult (Darzynkiewicz et al 1992; Fimognari at al., 1999), and these functional stages are measurable, including increase in reactive oxygen species, mitochondrial dysfunction, and DNA strand breakage (Shapiro 1993).

Genomics

Briefly, individual gene expression profiles will be generated and evaluated by using both white and red blood cells from the same individual Gulf sturgeon samples being analyzed for chemistry and for cytogenetics. Hematology data will also be collected.

Gene Expression Profiles

Gene expression profiles in blood samples have been successfully developed and used to both classify exposures to specific PAH signatures and identify functional results of PAH-induced toxicity (Jung et al. 2011). Characteristic gene responses directly reflect the exposure to particular PAHs, and are predictive of toxicity to the organism as a result of the PAH exposure. In this study, the transcriptomic (gene expression) responses in the nucleated peripheral red and white blood cells of sturgeon exposed to oil and oil-related chemicals will be evaluated.

Defense mechanisms in animals are mediated by white blood cells. Neutrophils, a phagocytic cell type, in most vertebrates are elicited in response to stressors via the neuroendocrine physiological mechanism. This cell type can be differentiated using microscopy following staining a blood smear. Elevated percentages of neutrophils in whole blood are indicative of a stressed or immunologically compromised and challenged individual (Ainsworth et al. 1991). Such hematological staining of whole blood will contribute to data interpretation on animal condition and to genomics analyses.

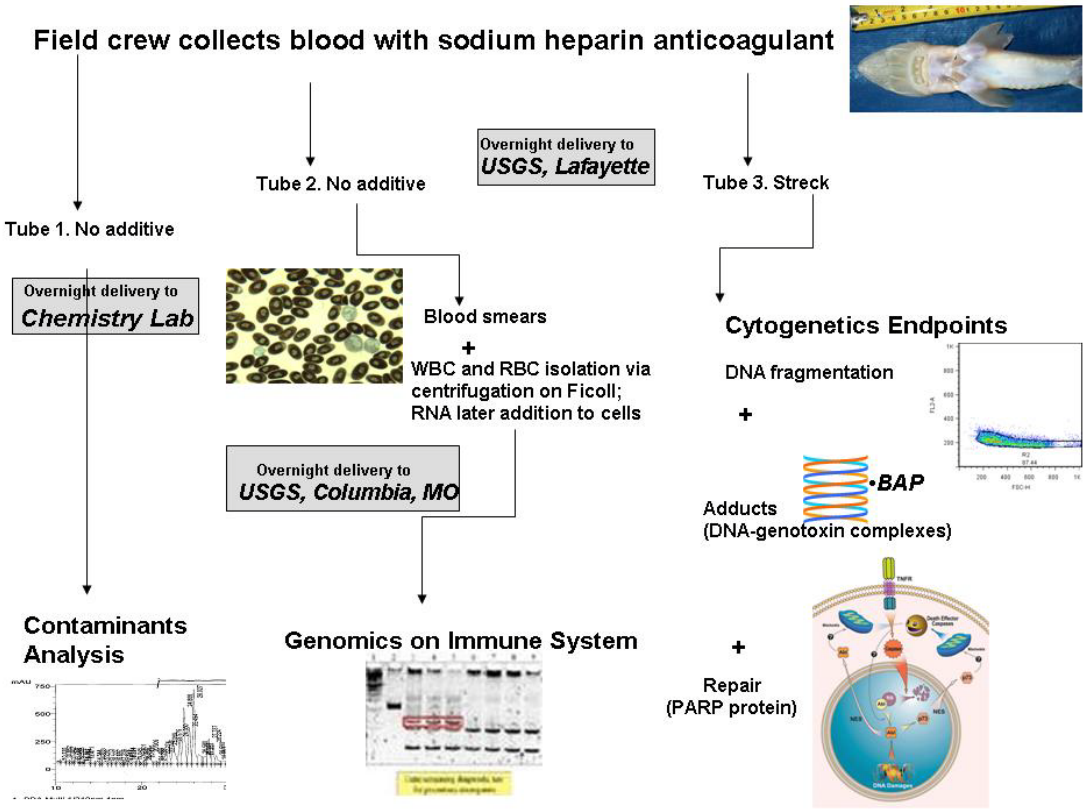

Figure 1. Gulf Sturgeon blood distribution for biological response assessments.

Positive Control Generation in Lab Studies

Shovelnose and/or Atlantic sturgeon, a surrogate for the Gulf sturgeon, will be exposed in the laboratory to defined concentrations of MC 252 oil. Exposure concentrations and preparation of the oil mixture will be defined by consultation with Fish and Toxicity Technical Work Group, coordinated with the MC 252 Oil Spill NRDA and include evaluation of the literature on known effects concentrations, and

review of available data from the MC 252 Oil Spill. Sturgeon will be exposed for a one month period, and blood samples will be collected after 10 days and 1 month. This will simulating acute and chronic exposure responses, respectively. Approximately 12 replicate sturgeon will be sampled at each time point of exposure to the oil.

The logistical coordination of blood samples (Figure 1) will not change from those defined in the field portions of this injury assessment.

PROJECT MANAGEMENT AND REPORTING

Glenn Constant (USFWS) is designated as Project Officer on all contracts originating from the Assessment Plan and will be responsible for ensuring dissemination and completion of all health and safety requirements, assigning tasks and verifying the complete execution of all field trips, consolidating and delivering all data.

SAMPLE AND DATA HANDLING

MC 252 NRDA chain-of-custody procedures will be observed at all times for all samples. All samples will be transferred with appropriate chain of custody forms and all samples that will undergo chemical or other analyses will be shipped to the appropriate laboratories for processing and analysis. .

All materials associated with the collection or analysis of samples under these protocols or pursuant to any approved work plan, except those consumed as a consequence of the applicable sampling or analytical process, must be retained unless and until approval is given for their disposal in accordance with the retention requirements set forth in paragraph 14 of Pretrial Order #1 (issued August 10, 2010) and any other applicable Court Order governing tangible items that are or may be issued in MDL No. 2179 In RE: Oil Spill by the Oil Rig "DEEPWATER HORIZON" (.E.D. LA 2010). Such approval to dispose must be given in writing and by a person authorized to direct such action on behalf of the state or federal agency whose employees or contractors are in possession or control of such materials.

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management

Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to BP (or Cardno/ENTRIX on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' DMT. Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to BP (or Cardno/ENTRIX on behalf of BP). Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC'd data shall be made available simultaneously to all trustees and BP (or Carno/ENTRIX on behalf of BP). Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Analytical Quality Assurance Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. In order to assure reliability of the consensus data and full review by the parties, no party shall publish consensus data until 7 days after such data has been made available to the parties. Also, the LADP shall not be released by the DMT, LOSCO, BP or Carno/ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and to BP (or Cardno/ENTRIX on behalf of BP).

Durable goods: Durable equipment (such as cameras, GPS, receivers) purchased by BP for this study will be returned to BP or their designated representatives at the conclusion of its use for this study unless otherwise agreed. Radio tags that are recovered or are not deployed will be returned to BP or its designated contractor at the end of this study, unless otherwise agreed.

Some equipment needed for this study may be in BP's existing inventory. BP-owned equipment will be used if available and when appropriate to the needs of the proposed work.

BUDGET

A detailed budget is attached. The total costs for this scope of work is $1,061,677. The Parties acknowledge that this budget is an estimate, and that actual costs may be higher. BP's commitment to fund the costs of this work includes any additional reasonable costs within the scope of this approved work plan that may arise. The trustees will make a good faith effort to notify BP in advance of any such increased cost.

Appendix 1: Field Protocol

SAMPLE PROCESSING

After completing all field sampling activities for a given day, the field team must deliver the collected samples, datasheets and electronic information (including photographs and GPS track log) to an appropriate NMFS or USFWS regional oversight location.

- Blood samples must be appropriately packaged and prepared for shipment to the receiving laboratory(ies).
- **Chain-of-custody** forms must be completed.
- All data from all field forms should be entered into the appropriate Excel file format (Forms or Flat version) either by the field sampler or a data management team member. Once the file is completed, it should be submitted to the data management team for incorporation into the database.
- All photographs must be archived, in accordance with the instructions in the **NOAA Field Photography Guidance** (NRDA_Field_Photographpy_Guidance.doc, available on the case FTP site).
- Synchronize the photos with the GPS track in accordance with the instructions in the **NOAA ARD-FAST Using GPS-Photo Link** instructions (GPSPhotoLink.doc, available on the case FTP site).
- Import the photos into the ORR PhotoLogger database. (This will allow the photos to be uploaded to ERMA.) See the document **NOAA PhotoLogger** for more information.
- All field data sheets will be scanned and originals stored in a secure location.

NETTING: Nets should be no smaller than 4 inch stretch mesh and no larger than 12 inch stretch mesh. A 1-hour maximum soak time for anchored nets is required in the Gulf sturgeon netting handling guidelines and all nets are to be attended by field crews. Start and end times will be recorded for all nets individually. Night fishing is acceptable, but no nets are to be left unattended overnight. GPS coordinates will be taken at the net location.

PROCESSING FISH: Fish are to be removed from nets immediately and handled in accordance with fish handling guidelines in the Gulf Sturgeon Recovery Plan (as amended). Total length and fork length will be measured for each fish in millimeters. Weight will be recorded in kilograms. Genetic samples (fin clips) will be collected and stored in ethanol for preservation and delivery to NOAA Fisheries Service Panama City Laboratory, Panama City, FL, 32408 (contact: Ivy Baremore 850-234-6541 ext 249).

Each fish will be electronically scanned for the presence of Passive Integrated Transponder (PIT) tags. Previously implanted PIT tag information will be recorded and new PIT tags will be injected in the soft tissue at the base of the dorsal fin if no PIT tag is found or if an existing tag is of a different frequency. PIT tags will be 134.2 kHz tags (12.5 mm x 2.07mm). All fish handled, including juvenile sturgeon. A Power Tracker V or VIII will be standard reader. Passive integrated transponder tags (PIT) and visible fin tags will be injected into each fish to provide a permanent unique identifier for individual fish for future evaluation. PIT tags are essentially coded devices encapsulated in glass that are injected into the fish and require the use of a scanning device to be detected. Each team will be required to carry a scanning device capable of detecting the PIT tags.

An ultrasonic acoustic transmitter will be implanted in 20 adult fish from each of the 8 river systems in the fall of 2011. Transmitters will be VEMCO® model V16-6H set to 90 second intervals and will be surgically implanted into the gastric cavity according to procedures listed by NMFS and FWS. Only adult fish (>1300 mm) will be implanted with transmitters.

All fish will be photographed from both sides while resting on the ventral surface, and then positioned to one side for a photograph of the ventral surface. Additional photographs will be taken inside each opercular cavity when it is opened during ordinary respiratory movements. Field crews should not attempt to hold open the opercular cavity to obtain photographs.

Blood samples will be drawn with sodium heparin as an anticoagulant and using a 10 ml sterile luer lock syringe (one per animal) fitted with a 27 – 21 gauge sterile needle (one per fish) and. After each animal has been photographed, measured, and tagged, the area posterior to the caudal fin should be swabbed with alcohol and the needle inserted into the caudal vein. Blood (10 ml) should be extracted and evenly dispensed to three separate sterile blood collection tubes. One tube will contain Streck cell stabilization reagent (Streck tube) for flow cytometry; the second tube will contain no additive; one tube will be for contaminants screen, and the third tube will be for white blood cell isolation for genomics. All tubes will be labeled with the PIT tag number of individual fish listed on the Gulf Sturgeon Catch Datasheet. All tubes will be immediately placed on ice and transported back to a NRDA designated laboratory where they will be stored at 4° C. See Figure 1 for simplified explanation of where samples will be sent and which analyses done

Appendix 2: Sturgeon Surgery Protocol

Tags and Receivers

1. Transmitters - VEMCO V-16 6H tags will be used programmed to transmit at 90 second intervals so that tags should last 6.4 years.

2. Receivers - VEMCO VR2W receivers will be used.

3. PIT tags and Readers:
    a. PIT tags – we will standardize to 134.2 kHz tags (12.5 mm x 2.07mm). All fish handled will be re-tagged with a new 134.2 PIT tag. PIT tags will be placed in the soft tissue at the base of the dorsal fin on either side of fish.
    b. A Power Tracker V or VIII will be standard reader.

4. T-bar tags will be placed in the pectoral fins. Each researcher will utilize their own tags with telephone numbers. Tag color and full number (including leading zeros) will be indicated on data sheet. T-Bar tags will be replaced as they are sloughed off or become fouled. It is suggested that the tag number appear twice on each t-bar tag (distal and proximal) in case part of tag becomes broken off.

Surgery

A. Supplies:

1. Tray for tools – use 70% EtOH to sterilize tools, tags and transmitters.

2. Vials for fin clips (ethanol)/gonad samples (10% formalin)

3. Sutures – Ethicon PDS II suture material CP1 -Chromic Gut, with cutting needle instead of tapered. Beware: suture material expires and disintegrates over time.

4. OTC – inject at 20mg/kg of body weight to mark hard parts.

5. Transmitters may be coated with polymer to make them biologically inert. Recommend MDX4-4210, Medical grade elastomer from Dow Corning http://www.factor2.com/MDX4_4210_Replacement_p/a-103.htm

6. Floy Tags

7. PIT tags

8. Tools
    a. Forceps
    b. Hemostats
    c. Scissors
    d. Scalpels
    e. Blades for scalpels
    f. Egg scooper
    g. PIT tag applicator
    h. FLOY gun
9. Other supplies:
    a. Towel
    b. Syringes/needles
    c. Latex gloves
    d. Betadine/Vaseline mix for topic antibiotic
    e. Measuring tape and scale
    f. Clipboard/datasheets
    g. Pencils

B. Procedure:

Surgical implantation of transmitters should be conducted using sterilized transmitters and equipment to help minimize post operative infection rates. Lay fish ventral side up on the sling. Using the scalpel make an incision about 3-5 scutes forward of the pelvic fins. Carefully cut through into the body cavity and elongate incision to accommodate transmitter. A retractor can be used to open the incision to examine internal organs. Insert the sterilized transmitters and close the incision using sterile resorbtive suture material. To ensure proper closure, a single interrupted suturing technique about 1 cm apart should be used with each suture individually tied.

Apply a thin layer of petroleum jelly mixed with topical Betadine over the incision areas to protect against infection. A mixture of betadine + petroleum jelly will increase longevity of application. The intent of the betadine ointment is to coat the sutures until the fish's mucous has a chance to coat them.

The betadine has antibacterial properties but also antifungal properties, which may be even more important.

Allow the fish to recover before release.

Appendix 3: Sturgeon Blood Processing

See Figure 1 for distribution.

Flow Cytometry - Fresh whole blood from Streck tube will be analyzed for DNA fragmentation and cell cycle. Fixed cells will be used for adduct and repair process analyes.

Cell Handling - Cells without additive placed in a given tube will be processed by standard lab techniques for differential staining and cell type identification, and a modified protocol for isolation of white cells using Ficoll (Jenkins and Ourth 1993). RNA later will be added to suspensions for use in genomics assays. Cells with Streck additive will be used for flow cytometric assays, and remaining cells will be pelleted and washed and stored in 2% paraformaldehyde.

Genomics Methods - We have developed a DNA microarray from shovelnose sturgeon (*Scaphirhynchus platorynchus*) tissues with selected targets identified for application on an Agilent format (Agilent Technologies). This array will be used to evaluate characteristic gene expression responses in red blood cells and in peripheral blood lymphocytes from oil-exposed sturgeon in the laboratory. Shovelnose sturgeon will be exposed to waterborne-oil at the Columbia Environmental Research Center (CERC) over an acute (4 day) and chronic (60 day) period. Blood samples will be taken at the end of each exposure period and separated on density-gradient materials into red blood cells and leucocytes, the later primarily consisting of lymphocytes (Tillitt et al. 1988). Blood samples from the field-caught Gulf Sturgeon will be processed according to protocols set forth in the existing study design for ecotoxicological monitoring of Gulf Sturgeon). Sub-samples of this blood will be taken for gene expression analysis and preserved for RNA extraction.

The extracted mRNA from each fraction of cells will be subject to microarray analysis for measurement of gene expression following reference design protocols (Richter et al. 2011). Paired control fish will serve as reference for the laboratory exposures and reference Gulf sturgeon will serve as the reference for the wild caught Gulf sturgeon blood samples. Several genes will be selected for confirmation analysis by qPCR in both shovelnose sturgeon and Gulf Sturgeon. Additionally, cDNA from three of the Gulf Sturgeon will be used for next-generation sequencing and comparison of sequence data from the shovelnose sturgeon. The sequence information from the Gulf sturgeon samples will be used to develop target sequences for a DNA microarray. The array will be a hybrid of sequences from both species, with special attention to genes associated with immune function. Gene expression data will be analyzed using standard techniques to identify differentially expressed genes, enriched gene ontology (GO) terms, hierarchical clustering, and pathway analysis (Richter et al. 2011). Enriched GO terms and pathways characteristic of the oil exposure will be identified.

Data Analyses and Reporting

Data from each laboratory-generated biological endpoint will undergo appropriate statistical analyses, correlations among parameters investigated. Attempts will be made at quantitative population modeling to evaluate the effects of alternations at the cell and molecular level on the sustainability of the gulf sturgeon (Capuzzo et al. 1988; Caswell 1989; McDowell 2005; Koojiman and Metz, 1984). Field data with spatial and temporal components will be applied in analyses.

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