Project Title: Identifying and Characterizing Physical and Biological Features Critical to Atlantic sturgeon and Estimating and Validating Multiple Methods of Assessing Adult Population Abundance within the Pamunkey River.

Project Duration: July 2018 – November 2020

Applicant Names:
Pamunkey Indian Tribe
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<u>Project Summary</u>

Atlantic sturgeon were listed as Threatened or Endangered under the Endangered Species Act throughout the United States in 2012. At the time of listing, it was believed that a single distinct population segment (DPS) was native to the Chesapeake Bay and its origin was the James River. In 2013, Atlantic sturgeon were found spawning in the Pamunkey River (Hager et al. 2014) and their genetic composition determined to be distinct from that found in the James River (Tim King, personal communication). Sturgeon were an invaluable cultural and economic resource and historically, the sturgeon held great spiritual significance for the Pamunkey Tribe. Annual ceremonies involving the single-handed capture of large sturgeon served as a way for young men to prove their manhood. Once obtained, the sturgeon would be introduced to the children of the tribe as a tangible symbol of the strong and yet gentle nature of god of the river. Through such activities, the sturgeon bound the tribe to the river and the river to the tribe in a truly mutually beneficial relationship.

Population models based on mark recapture research from 2013-2017 suggest that the Pamunkey River population is very small (Kahn et al. 2014), potentially the smallest in existence. In 2016, gravid females and milting males, some previously tagged in the Pamunkey River, were found in the Mattaponi River. Thus, adult fish pass back and forth between these two rivers inter-annually presumably spawning in both rivers. One of the greatest management needs as a result of listing is to determine how many spawning populations exist and to estimate the abundance of these populations. This research must proceed without negative impacts resulting from the course of the research. It is also imperative that the critical habitats with respect to spatial, temporal and physical characteristics are delineated, recognized and protected.

There are four overarching and interconnected goals of this proposed research. The first is to begin to create a more comprehensive ecological picture of the Pamunkey and Mattaponi Rivers through data gathering and assimilation and create a Pamunkey River Riverkeeper position that can continue this work into the future and foster improved stewardship. The second is to improve physical models of the Pamunkey and Mattaponi systems to better understand the water quality factors inherent to preferred Atlantic sturgeon spawning habitats. The third is to calculate the spawning population sharing these rivers and the last is to determine if off-the-shelf side scan sonar units can be used to enumerate Atlantic sturgeon.   Due to budget constraints, not all activities can occur equally in every year, nor is it necessary that all activities occur in every year. For more detail see the budget and timeline.

Project Description

Introduction

Approximately seven million pounds of sturgeon, Atlantic and shortnose, were harvested annually between 1890 and 1905 when the coastal fishery for Atlantic sturgeon was newly developed and at its apex (Hildebrand and Schroeder 1928). Some of these were being harvested in the Pamunkey and Mattaponi systems. These fisheries at the turn off the century were not the traditional localized fishery that had existed since European colonization, but were industrialized efforts that moved north, sequentially targeting varied river populations with fishing overlapping with spawning efforts to optimize roe harvest (Smith 1985). Due to this highly effective approach, by 1920, the coast-wide population of Atlantic sturgeon had fallen to less than 1% of the level in the 1880s (IUCN 2012). The impact of this fishery on shortnose sturgeon is unclear since at that time, there was no differentiation between Atlantic and shortnose sturgeon in harvest records. Since the Atlantic sturgeon fishery in United States waters was not closed until 1998 (ASMFC 1998), important historic harvest records exist that may help clarify certain aspects of early fisheries.

Even though sturgeon were no longer targeted by commercial fisheries they continue to be removed from the population through human-mediated mortalities and environmental degradation (ASSRT 2007). Economically driven decisions, have resulted in Atlantic and shortnose sturgeon being selectively removed from many traditional habitats along the Atlantic Coast and today anthropogenic factors (bycatch, habitat loss, etc.) continued to limit recovery (ASSRT 2007). Recognizing a true threat of extinction, the National Marine Fisheries Service (NMFS) listed shortnose sturgeon as endangered under the Endangered Species Preservation Act in 1967. Fourteen years after the fishery was closed for Atlantic sturgeon, NMFS listed five distinct population segments (DPSs) of Atlantic sturgeon under the Endangered Species Act (ESA) on February 6, 2012 (77 FR 5880, 77 FR 5914).

Hope, however, looms on the horizon. When Atlantic sturgeon were listed under the Endangered Species Act in 2012, the James River contained the only known spawning population in the Chesapeake Bay (ASSRT 2007), suggesting only one spawning population between North Carolina and Delaware. Likewise, there are no known spawning populations of shortnose sturgeon in that range. Six Chesapeake Bay systems, the Susquehanna, Potomac, James, York, Rappahannock, and Nottaway Rivers, historically supported Atlantic sturgeon spawning populations (ASSRT 2007), totaling as many as 20,000 females prior to the beginning of the commercial fishery (Secor 2002). In 2013, a spawning population of Atlantic sturgeon, once thought extirpated, was identified in the Pamunkey River (Hager et al. 2014), a tributary to the York River, which is only approximately 27 kilometers north of the James River. In 2016, gravid and milting fish were found in the Mattaponi River the more northern tributary of the York River system. Evidence of an Atlantic sturgeon spawning population in Maryland in the Nanticoke River was discovered in the fall of 2014 (Blankenship 2014), but the others are assumed to be extirpated. The discovery of these persistent populations of Atlantic and shortnose sturgeon speaks to the species' resilience.

During the past five years Chesapeake Scientific, the U.S. Navy (USN), and NMFS have been conducting a tagging, tracking and population assessment of the Pamunkey River's spawning population. Despite considerable effort, we have yet to attain an adult in spawning condition during spring sampling but over 160 different adults have been collected during the four years of annual fall collections. No tagged adults have returned in the spring either, which stands in stark contrast to findings in the James River where fish tagged in the spring or fall may return in either season (Hager, 2011). Through collaborative

efforts with the USN, many of the spawning adult fish obtained were implanted with Vemco 69khz transmitters (n>74). This allows them to be tracked for the next ten years through the York River System, including the Pamunkey and Mattaponi Rivers using a network of receivers. In addition to this tracking research, benthic side scan assessments have been conducted by the NOAA Chesapeake Bay Office that have improved our understanding of the types of potential spawning sediments that exist in the Pamunkey River, their location and their spatial extent. These, however, require more ground-truthing and analysis to be of greater use. We also lack important data on the physical attributes of the York River's tributaries, which are known to be import to successful spawning (Secor et al 2002, Bushnoe et al. 2005).

Concurrent, with specimen collection, tagging, and tracking efforts, we have calculated annual and inter-annual population estimates based on mark recapture techniques. However, due to a unique life history where all adults do not return to spawn every year, calculations must continue in order to gain a more precise estimate. We have conducted sufficient tagging, annual and cumulative population estimations, and geological assessments in the upper York River watershed to begin to understand its functionality and ecology as it pertains to Atlantic sturgeon better than we understand the sturgeon's use of most systems they inhabit. Due to these efforts and the reduced dimensional geology of the Pamunkey River, there exists a fortuitous yet advantageous chance to test and validate newly demonstrated side-scan sonar technology (Flowers and Hightower 2015, Kaeser and Rueter 2015).

Though several papers have been published using side scan technology to estimate sturgeon populations, this method has not been scientifically validated. All side scan technology is not created equally. Side scan sonar units vary in frequencies and wattage, important factors that directly effect a unit's ability to detect a target. In order for side scan to become a scientifically valid tool each unit's ability to detect real targets must be scientifically validated. Once its ability to detect a real target is established through repeated controlled tests and its accuracy and precision mathematically quantified and its error accurately assessed, side scan has the potential for unprecedented accuracy and precision of abundance estimates without interaction. Information acquired and validated in marine environments is unprecedented and has important implications and possibilities for the preservation of the species as a whole. Beyond ecosystem-based biological information, the result would be a controllable canvas to develop harmless methodological assessment tools that can then be validated and standardized to benefit the species wherever they occur. The never-before-achieved confidence intervals that have been attained on the Pamunkey River's total spawning population for the past four years are an example of the quality of data that such small river research can provide (Kahn et al. 2014, Kahn and Hager unpublished data). Due to these past research efforts and the large number of sturgeon carrying transmitters that return every spawning season, the Pamunkey is the ideal environment in which to develop side scan sonar based population assessment research applications that can subsequently be used in larger systems and elsewhere along the coast once validated.

Goals and Objectives

The Pamunkey Indian Tribe proposes to work in collaboration with the Chesapeake Scientific to accomplish several objectives that will benefit the tribe, Virginia, the USN, and NMFS. The tribal portion of this tribal/state grant partnership will 1) establish a native Riverkeeper for the York River, which like the other 150+ Riverkeepers will be a member of the Waterkeepers Alliance, 2) establish long term water quality monitoring stations in the Pamunkey, and Mattaponi Rivers, 3) assist in conducting mark-recapture sturgeon abundance estimates using PIT tags and tissue samples under Dr. Hager's permit and guidance, 4) conduct side-scan and benthic sampling to ground truth benthic imaging in the Pamunkey

River, 5) better describe sturgeon spawning grounds and habitats critical for other life stages based on physical and tracking data, and 6) validate and mathematically asses the use of side scan sonar as an Atlantic sturgeon identification tool.

Methods

Riverkeeper Position. The Pamunkey Tribe has a vested interest in protecting the Pamunkey and Mattaponi rivers and ultimately the York River system. The James River has benefitted greatly from having a Riverkeeper present and watching over the river full time. The James River Riverkeeper, Jamie Brunkow, has offered to support, train, and nominate a York River Riverkeeper position. This grant will begin to train a member of the Pamunkey Indian Tribe to fill this position, which makes sense given the historically symbiotic relationship between the Pamunkey Tribe and the Pamunkey River. This individual will be critical not only to this research team, but will provide a leader for the Pamunkey's environmental conservation efforts beyond the scope of this project.

The Pamunkey Tribe, in addition to a York River Riverkeeper, will provide a source of historic environmental knowledge that has not previously been explored. Such knowledge can be gathered and records of that information archived in the tribal library for future generations of the tribe and for scientists who want to better understand the system's historic function and productivity. Sources of such productivity are supplied through tribal records, personal journals, and even a few remaining eye witness accounts. State and Federal records of fish sales originating from both rivers and extensive records on the Tribal shad hatchery exist and will be documented. Historic/primary sources are often ignored by scientists who do not understand their value, however, such records were crucial in the discovery of the Pamunkey River's remnant Atlantic sturgeon population and with further effort will likely provide guidance as to what a restored system looks like and how to attain it.

Water Quality Monitoring. Three long-term water quality monitoring stations will be established on each river in the upper, middle, and lower portions of the Pamunkey and Mattaponi Rivers (six stations). The United States Geologic Survey (USGS) currently maintains one water quality monitoring station in the Pamunkey River near Hanover, VA and one in the Mattaponi River near Beulahville, VA. Proposed stations will be established around the existing USGS stations to provide more thorough coverage of these systems. The stations established by this grant will monitor salinity, temperature, and dissolved oxygen, which are the primary stressors of Atlantic sturgeon in freshwater habitats. The water quality monitoring stations will also monitor a variety of other conditions such as pH, conductivity, flood stage, flow velocity, and turbidity. The tribal Riverkeeper will download the data from these stations once every other month and make the water quality data available publicly. Following the completion of this grant, the York River Riverkeeper will continue to monitor these stations. Data from these stations can be used to define any areas of environmental concern, establish baseline dynamics for the river and help define critical habitats for various species.

Mark-Recapture Models. The Pamunkey Tribe in continued coordination with Chesapeake Scientific will monitor Atlantic sturgeon spawning abundance in the Pamunkey and Mattaponi Rivers using a number of different independently validated models. The first method of population monitoring is traditional mark-recapture monitoring. This method will involve 30 sampling days in the Pamunkey River, which is the sampling intensity required to collect sufficient numbers of Atlantic sturgeon to achieve 95% confidence that the accuracy of the estimate is within 5% of the true population abundance of spawning adults in the York River system. These levels of confidence are much higher than is normally achieved when sampling natural populations of animals. It is not necessary to collect fish in the Mattaponi to

determine the total abundance of spawning adults in the population because fish pass freely between the systems inter-annually. Though it would be necessary to sample an additional 30 days in the Mattaponi each year to determine the river's annual spawning abundance the reduced budget of this grant prevents us from collecting this valuable data.

Upon capture all fish are measured and sex determined from expression of gametes if possible. Fork length is used as a measurement because total length is often altered over time and the heterocycle tail tends to be measured differently by individual investigators. To attain information on abundance, fish are marked with two different tags, one internal (passive integrated transponder or PIT tag), one external (T-bar tag) and a DNA sample is taken. Kocik et al. (2013) suggest using double marks at the time of initial capture also but only record a recapture if both tags are still present upon recapture. However, between 2013 and 2017, we noted the loss of both PIT and T-bar tags from some fish and therefore consider a fish recaptured if it only has one of the two original marks still present. While tag based mark-recapture studies are the simplest and most expedient, we also conduct mark-recapture studies using DNA tissue samples. These samples are collected from the caudal fin and sent to USGS in Leetown, West Virginia for storage and analysis. Therefore, any sturgeon that by chance shed both a PIT tag and T-bar tag and was thought to be a first capture rather than a recapture would result in an over-estimate of the actual population. Since a sturgeon's DNA doesn't change, mark-recapture using DNA can't be completed until the samples are processed and analyzed, but provide the most reliable mark-recapture estimates.

Two different mark recapture models are used to calculate the abundance of adults on the spawning run. First, a Schumacher-Eschmeyer model (Ricker 1975) is used to calculate within year closed population estimates of spawning Atlantic sturgeon in each river. This method is most useful for within year population estimates because it is simple to calculate and provides a reliable estimate. The second equation to calculate abundance is the Huggins Robust model, which allows for periods of closed population estimates, followed by periods of death and emigration, before more collections of closed populations and open periods. This equation is more complicated and while it provides a more accurate understanding of the total number of adult Atlantic sturgeon using each river over time, the closed population estimates obtained from the Schumacher-Eschmeyer model for each closed period are very similar (for instance, Kahn et al. 2014 reports a 2013 spawning abundance of 75 individuals using Schumacher-Eschmeyer, while the Huggins Robust model estimates the spawning abundance that year was actually 81 individuals). This is likely due to the fact that sampling populations in these rivers provides very high levels of confidence that cannot be obtained in other systems.

Total Population Validation. To validate the mark-recapture estimates, which have been tested through time in other systems and on other species, we will conduct the analyses using both DNA samples and tag returns as noted above. Furthermore, the data obtained from 2018 through 2020 can be combined with the data from 2013 through 2017 to determine the probable total number of sturgeon in the population. After that time period, we would be highly confident in the total population based on the fact that males return every one to three years and females return every three to five years (Smith 1985, Peterson et al. 2008). Furthermore, current robust population estimates for the entire population (periods of closed, then open populations for a total adult abundance estimate), which include individuals that were marked but haven't returned to be recaptured (over-estimating the true abundance) suggest the population is between 275 and 425 individuals, of which over 130 have been marked to date. Given the large proportion of the total population that has been marked both with tags and with tissue samples and the rate at which new individuals are marked each year, we believe it is very likely we can mark almost every fish in the population by the end of this three-year grant cycle,

revealing the true population abundance or a very close approximation, no model or calculation required.

Benthic Groundtruthing. In 2016 NOAA Chesapeake Bay Office side scanned and classified benthic composition through delineation of image types. This method provides reasonably accurate classification of side scan image types previously delineated by computer which separates different types spatially but does not actually identify what the composition of each image type is. Some groundtruthing of these types has been done through benthic grab sampling but benthic grab methodology has numerous weaknesses and is not as accurate as personal observation (i.e. dive sampling), therefore type characterizations and spatial delineations can be much improved. Our groundtruthing will improve upon the drop box benthic sampling methods used by NOAA Chesapeake Bay in the 2016 side scan sonar imagining pilot study. Dive sampling will be conducted by Dr. Hager and incorporated into maps of benthic composition. Benthic samples will be attained and recorded at each promising location and the extent of potential spawning substrate improved using GPS located on the vessel that records the location of each dive. Samples on each dive will be attained and pictures will also be attained if possible.

This study will occur congruently with all the other work during the sampling season (August – September). This timing will also allow us to observe any sturgeon activity occurring in each sample site and potentially provide egg samples. Eggs are permitted under our current permit and thus no additional permitting required. The extent of the study will be dictated by the amount of time that has been funded since there is no way of predicting how much time will be required to improve our understanding of each site. Sites will be selected in collaboration with NOAA and prioritized based on which areas they feel were not sampled well with drop box methodology as well as which are most frequently occupied by spawning fish based on current and historical telemetry records. The study area covers the entire middle and upriver spawning reaches (over 30 miles) so it is not possible to predetermine where dive sampling will occur at his time. Dive locations will be prioritized based on NOAA's confidence in their drop box sampling and our tracking data combined. This aspect of the study is to be determined through future discussions with NOAA's benthic side scan mapping experts and ouor team. We also plan on using active telemetry while diving to increase our chances of documenting spawning behavior.

In addition to these data, Dr. Berquest (College of William and Mary, Va. Division of Geology and Mineral Resources) constructed benthic maps for the Pamunkey River based on sample collections alone. These can be incorporated into our efforts to further define the extent of suitable anadromous fish spawning habitats. Over the long-term, correlation between water quality and benthic conditions and various research projects concerned with the status of sturgeon or any other species may help identify limiting factors for ESA listed species like Atlantic sturgeon as well as tribally important species such as American shad and herring.

Spawning Habitat Assessments and Validations. One of the key means we will use to verify sturgeon habitat preference that may be slightly less than obvious will be through the years of detections of telemetry tags that have been recorded in the Pamunkey since 2013 and the Mattaponi since 2015. We intend to combine previous years of tracking data with groundtruthed sonar maps and water quality station data to describe the physical characteristics Atlantic sturgeon spawning habitats. Congruently, we will be collecting data on benthic habitats being occupied by Y-O-Y fish that have not yet been collected or tagged. Thus, our efforts will guide future collection efforts for this life stage based upon

habitat assessment and other published work on where such life stags have been found in other systems.

Side Scan Sonar Atlantic sturgeon image Validation. Though papers are being written (Flowers and Hightower 2015, Kaeser and Rueter 2015) claiming that off the shelf side scan sonar units (600-1200khz) can be used to identify and then estimate Atlantic sturgeon abundance no one has actually validated this approach. We intend to do exactly that using numerous captive sturgeon provided through netting methodologies being employed during the above described mark recapture operations. This approach provides sturgeon of different sizes throughout the entire sampling season that are not overly stressed due to holding, which can severely alter behavior (Musick and Hager 2007). Fish will be selected for validation study based on their health and released if stress is indicated so there is no way of knowing how many fish will be required to complete the sample design described in Table 1 and 2. No fish with transmitters will be used so that normal behavior of these marked fish will persist in telemetry array. A sub-sample of fish of varied sizes, known because they were just worked up when obtained through mark recapture netting will be intentionally placed in the side scan beams of several different units under the controlled conditions (**Table 1**). Fish will be physically held within the side scans' beam between large floats so that the exact position of each fish will be indicated since air filled floats will show up on sonar. Nets completely obscure a side scans ability to function creating a wall that the sound waves cannot pass through and thus cannot be used. Through this repetitive and controlled approach, we can mathematically determine how accurate such an approach is and some of the variables that alter its precision. Fish will be scanned at three different angels, three different distances and for five replicates for a total of 30 samples (**Table 2**). Angles of 0˚, 45˚ and 90˚and distances of 10, 35 and 60 meters (**Table 1**) will be used. A nested ANOVA will subsequently be applied to determine statistical differences in fish identification/detectability (**Table 2**).

Table 1.

3 different angles	0	45	90		
3 different distances	10	35	60		
replicates	1	2	3	4	5

Table 2.

Replicates	0			45			90		
	10	35	60	10	35	60	10	35	60
1									
2									
3									
4									
5									

nested ANOVA with equal sample size
a (group, number of angles) = 3
b (subgroup, number of distances) = 3
n (number of replicates per subgroup) = 5

While it is true that these conditions listed above are not the only variable that may affect a side scan's function and its ability to detect and identify a sturgeon one cannot test all variables that might. And this approach is not applicable to the varied systems in which this methodology may be employed. The intent of our study is to validate whether the approach can be applied under normal field conditions and how accurate it is under those conditions. It is not to define which conditions affect outcomes and then mathematically calculate how each condition affects the results.

Benefits or Results Expected

Management benefits:
1) Establish a York River Riverkeeper position to protect the river system and to establish long-term water quality monitoring stations in the Mattaponi and Pamunkey Rivers.
    a. Establish correlation between changes in water quality and changes in fish populations or fish behavior/movement.
    b. Identify potentially problematic water quality conditions or sources of water quality degradation in the York River system to identify projects to aid in the recovery of Atlantic sturgeon.
2) Assemble historic records of the tribe and commercial fishery landings in the York River to estimate historic abundances of various fishes and thus provide a frame of reference for recovery of the York River.
3) Identify the total abundance of Atlantic sturgeon in the Pamunkey River using data gathered from 2013-2017 coupled with additionally tagged and tissue sampled individuals from 2018-2020.
    a. With the knowledge of the size of the population and recapture rates seen in the Pamunkey River, we will also be able to compare calculated mortality rates to those calculated in the South Atlantic distinct population segment.
    b. We can also determine the rate of return of both males and females in the Pamunkey River system to see how they compare with generally accepted rates of return used by other researchers on the coast (Smith 1985).
    c. We can use the size of the population to determine the true ratio of effective population size to actual population size.
4) Combine tracking data with groundtruthed sonic maps and physical water quality characteristics to identify and fully describe spawning regions within the Pamunkey. These maps will also provide important ecosystem tools identify regions likely to be essential to other species as well as other life stages of Atlantic sturgeon. Thus, this work will provide guidance to assist other research efforts into the future.

Need for Government Assistance

From 2013 through 2017 the bulk of the funding for Pamunkey and Mattaponi research has come from Chesapeake Scientific LLC., a private, for-profit company. Though transmitters have been paid for by the USN and the Navy funds five receivers in the Pamunkey River annually, Chesapeake Scientific has paid for the placement and maintenance of the other 24 seasonal stations in the Pamunkey and Mattaponi Rivers as well as all the specimen collection costs (gear, travel, vessels, staff, overhead, etc.). The USN does provide some volunteer staff each season and since 2014 NMFS has assigned an employee for seasonal field help. It is not private industries' responsibility to preserve endangered species and we need assistance to continue this valuable research.

Federal, State, tribal and local government activities.

A great deal has been accomplished by Chesapeake Scientific LLC and through collaborative work with the USN and NOAA. This largely privately funded work as previously described above provides the basis upon which proposed research can be accomplished. However, the proposed grant would allow the tribe to play a much more important role in current research efforts and provide a platform for the tribe to become the leader in Pamunkey River conservation efforts. This is a continuation of ongoing private and Federal research efforts as described in the above. Links to the most recent USN reports can be found at Chesapeake Scientific.com

Environmental Impacts

No negative environmental, biological, social, political or economic impacts have thus far been realized through the past five years of ongoing research efforts and none are anticipated through proposed work. In fact, we anticipate a much-welcomed positive affect on social, political, and economic attributes if NOAA funding replaces private funding. Only positive impacts on listed species and/or their critical habitat are anticipated as a result of the proposed activities. All capture and surgical techniques will duplicate those we have used for the past five years as described in Kahn and Mohead 2010. Anticipated takes are already allocated under NOAA permit 19642 and an annual Virginia state permit currently 060198.

Project Management

The Pamunkey Indian Tribe will administer the grant funds while the Principal Investigator (Chris Hager, Chesapeake Scientific) will be responsible for completing the project milestones on time and within the budget. Thus, the majority of proposed Federal budget costs will be allocated to Tribal Personnel to manage the grant and implement the project. In addition, we have budgeted two Tribal Field Technicians to aid in proposed sturgeon survey and water quality data collection.

Data Sharing Plan

It is the intent of the Pamunkey Tribe to fully share all sturgeon detections with the United States Navy (USN) so that an archive of this effort can be maintained by an entity that will endure beyond the individuals involved in this effort. This is also a suitable partner since the USN and its contractor Chesapeake Scientific LLC currently lead the investigations in the York River watershed, the Bay and inshore ocean waters. All tracking data will be sent to the USN and all data on individual specimens given to the investigator who implanted the tag. All data that was funded by NOAA through this grant will also be given to an appropriate entity at NOAA so that a single federal data base on NOAA funded research efforts in the Chesapeake can begin to evolve. The Pamunkey Tribe will construct a River Keepers site where river parameters will be made available. We will work with the NOAA Chesapeake Bay Office to inform them of improvements to side scan sonar based maps through expanded groundtruthing so that these may be incorporated into their data.