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Mapping of Pathogenic Vibrio spp. Densities in the Gulf of Mexico and Their Relationship to Fecal Indicators Used in Oyster Harvest Regulation

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By

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Abstract

Vibrio vulnificus and *Vibrio parahaemolyticus* are halophilic gram-negative bacteria found throughout estuarine environments. Pathogenic strains of these *Vibrio* species can infect surrounding waters, sediment, oysters, and, ultimately, humans, causing food poisoning or even death. Sediment, oyster, and water samples from six sites along a salinity gradient in Breton Sound were analyzed for *Vibrio spp*. concentrations during varying months during 2011. The data was then compiled into monthly maps indicating the density of both *Vibrio vulnificus* and *Vibrio parahaemolyticus* at each specific site. This study seeks to map the differing concentrations of pathogenic *Vibrio spp.* and investigate alternative indicator species to be used in the testing of water and oyster samples by the Louisiana Department of Health and Hospitals (DHH) to determine the status of the oyster harvesting areas surrounding the coastline of Louisiana. The goal of this study is to further corroborate recent findings that indicate fecal indicators are not effective indicator species when testing for non-cholera *Vibrio*, as well as to elucidate post-harvest treatment methods that can increase the safety of Louisiana oysters for raw consumption purposes.

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1.1 Introduction

Vibrio parahaemolyticus was discovered in 1950 by Tsunesaburo Fujino, while *Vibrio vulnificus* was discovered much later in 1976, with its first reported case in 1979 (Shinoda, 2011). Upon the first discovery of *Vibrio parahaemolyticus*, it was categorized as a Pasteurella because there were structural differences to that of *Vibrio*, indicated by the straight cell form of the isolate; whereas *Vibrio* cell forms are curved (Martinez, Urtaza & Baker-Austin, 2013). Yet when new taxonomic characterizations were established in 1960, *Vibrio parahaemolyticus* was reexamined and identified as being a member of the *Vibrio* genus, where it is currently classified (Martinez et al., 2013). Surprisingly, both species can grow in clean and clear waters, contingent upon a few abiotic factors; because both *Vibrio* species are halophilic, they grow best in brackish waters within specific salt concentration, pH, and temperature ranges.

1.2 Toxicology of *Vibrio* spp.

1.2.1 Background

Both *Vibrio* species have been found to oscillate highly within seasons, with the highest population levels established when temperature exceeds 20° C and has a salinity range of 5-25 ppt (Randa et al., 2004). Studies have found that there are even certain warm-water and cold-water *Vibrio* populations, varying by two orders of magnitude in summer and winter months according to some studies (Randa et al., 2004). While temperature has a larger effect on the growth of the species than salinity, there is debate

whether the variance in population is due to temperature or to the population entering a viable but nonculturable state (Randa et al., 2004).

Vibrio outbreaks have been reported not only within the Gulf of Mexico, but all over the world. Outbreaks in North America alone differ in severity and strain type, with some specific to the Pacific Northwest and others to the Gulf of Mexico (Martinez et al., 2013). Spain and Japan both had outbreaks in 1999 and 1950, respectively (Martinez et al., 2013). The outbreak in Spain (1999) led to 51 reported cases, 9 of which were confirmed by laboratory tests (Martinez et al., 2013). The study of the unique outbreak in Spain suggested that the strain of *Vibrio parahaemolyticus* found near Galicia, Spain differed from both the Asian and North American pandemic isolates. The outbreaks in North America and Asia in the late 1990s were caused by a newly emerged O3:K6 clone, first detected in India in 1996. It is believed that the strain in Europe diverged from the parent clone (O3:K6) by alteration of the genes associated with the O and K antigens (Martinez et al., 2004). The outbreaks in North America were large and near important international seaports, leading scientists to believe that the O3:K6 clone spread to Spain where it then mutated.

1.2.2 Biochemical Analysis

1.2.2a *Vibrio Vulnificus*

With 459 cases of infection in the United States and carrying one of the highest mortality rates of any bacterial pathogen, understanding the mechanisms and makeup of *Vibrio vulnificus* are extremely important in aiding the diagnosis and treatment of

infected individuals. First isolated in 1976, the bacteria is Gram-negative, motile, curved and rod shaped. Because it is Gram-negative, the LSP (endotoxin) produced by the bacteria is thought to play a key role in the development of fever and septic symptoms in infected hosts (Bross et al., 2007). *Vibrio vulnificus* strains are classified into three distinct biotypes, with biotype 1 being responsible for the majority of human infections, biotype 2 strains being primarily eel pathogens, and biotype 3 being a mixture of biotypes 1 and 2, causing human wound infections limited to people handling tilapia with most reported cases in Israel (Jones & Oliver, 2009).

As stated earlier, the strains of *Vibrio* differ greatly throughout the world, and therefore a link between genotypic or phenotypic information with virulence has been largely unsuccessful (Bross et al., 2007). Yet detection of a Type B (Type A is mostly nonclinical isolates) strain under biotype 1 based on a 17-bp nucleotide sequence of the 16S rRNA gene has indicated a positive correlation between the cause of human infection and the Type B genotype (Bross et al., 2007). In biotype 1, distinct heterogeneous LPS types were observed; whereas homogenous LPS types were found in biotype 2 (Bross et al., 2007). While the significance of these observations is still unclear, it is clear that the presence of a capsule occurs in virulent strains, and not in nonvirulent.

Environmental changes such as heavy metals or oxidizing agents, high osmolarity, pollutants, starvation, exposure to low temperature, or interaction with eukaryotic hosts affect the heat shock proteins that *Vibrio vulnificus* produces. Chaperonins DnaK and GroEL, along with Clp and Lon proteases, are induced by the

environmental stresses through a process called *cross protection*; these proteins improve the bacterium's thermotolerance, salt tolerance, UV exposure tolerance, and more (Bross et al., 2007). This response is a major link between bacterial ecology and pathogenesis, and the stress may cause genomic differences that can increase the chances of survival of the bacteria when it moves from water to oyster to human.

Virulence of *Vibrio vulnificus* can be determined by multiple properties as stated earlier, as well as the production of alternate sigma factors, SSR repeats, motility, quorum sensing, and numerous extracellular enzymes, including proteases, coolagenase, mucinase, esterase, chondroitinase, hyaluronidase, DNAase, and sulfatase (Bross et al., 2007). A member of the RTX toxin family has been recently identified as a determinant of virulence as well, with the toxin causing "pore formation in red blood cells, necrotic death of Hep2 cells, and depolymerization of actin in HeLa cells" (Bross et al., 2007).

A virulent strain of *Vibrio vulnificus* is able to affect its host through a variety of evasive measures. The acidity of the stomach is a natural defense against bacteria, but the Gram-negative *Vibrio* neutralizes this environment by using lysine decarboxylase to break down lysine to form cadaverine, an acid neutralizer (Jones & Oliver, 2009). Cadaverine also acts as a "superoxide radical scavenger," suggesting a link between acid and oxidative stress tolerance, also linked to a decrease in superoxide dismutase (SOD) activity, suggesting that the connection between the two pathways is what helps the bacteria survive in the human gut at such a low pH level (the bacteria does poorly in low pH in laboratory settings) (Jones & Oliver, 2009).

1.2.2b *Vibrio parahaemolyticus*

While an estimated 4500 cases of infection occur (not necessarily reported) in the United States, *Vibrio parahaemolyticus* has a lower lethality rate and lower virulence levels than *Vibrio vulnificus*, yet the infection can cause damage and painful symptoms. The major virulence factors of *Vibrio parahaemolyticus* are the thermostable direct hemolysin and the hemolysin-related hemolysin, encoded by the *tdh* and *trh* genes (Martinez, 2004). The hemolysin causes beta-hemolysin of human erythrocytes, known as the "Kanagawa phenomenon" (Martinez et al., 2004).

Both the TDH and TRH genes are the best overall predictors of potential virulence in *Vibrio parahaemolyticus* currently. In 2003, a genome sequencing project also found that T3SS2 (secretion system 2) was also linked to pathogenicity of the strain, regardless of whether or not the strain was TDH/TRH positive, and is also considered a strong predictor in virulence of strains (Baker-Austin et al., 2010). While the majority of strains contain at least one of the hemolysin genes, only a small portion of the strains contain the virulent gene markers and can actually cause gastroenteritis in human beings.

Increases in *Vibrio parahaemolyticus* infections around the world have been associated with the serotype O3:K6 since the mid-1990s. After 1996, a sudden emergence of infections triggered the epidemic expansion of the clone, rapidly spreading it through most of south-eastern Asian countries in two years (Baker-Austin et al., 2010). In November of 1997, infections caused by this same clone were detected for the first time outside of Asian in South America, triggering the first pandemic

expansion of this pathogen (Baker-Austin et al., 2010). Large outbreaks of *Vibrio parahaemolyticus* around the world are linked to non-pandemic strains, however.

For both *Vibrio vulnificus* and *Vibrio parahaemolyticus*, a strong connection to iron has been indicated, as withholding iron from the bacteria by the host has been reported to play an important role in resistance, and designated as a type of "nutritional immunity"; increasing the availability of iron can induce increased microbial growth and pathogenecity (Wright, 1981).

1.2.3 Symptoms & Treatment

Both species of *Vibrio* infect their human host either by way of ingestion or through contact with an open wound in contaminated waters. *Vibrio parahaemolyticus* may not be as lethal as vulnificus, but it is a leading cause of seafood-associated gastroenteritis globally (Martinez et al., 2013). Symptoms from both species are similar, but *Vibrio parahaemolyticus* specifically has symptoms such as water diarrhea, often with abdominal cramping, nausea, vomiting, and fever, with less common wound, soft tissue, or bloodstream infections. Treatment for *Vibrio parahaemolyticus* is normally not necessary, as there is no evidence that antibiotic treatment will decrease the length of the illness. Drinking liquids to replace lost fluids through diarrhea is most common, but in severe cases, antibiotics such as tetracycline or ciprofloxacin can be used depending on the antimicrobial weaknesses of the organism (Su, 2007).

Vibrio vulnificus symptoms usually develop within 16 hours of consumption of contaminated seafood, with similar symptoms being vomiting, diarrhea, and abdominal pain, yet unique to *Vibrio vulnificus* is the development of bullous skin lesions. The bacterium invades directly from the open wound or GI tract to cause septicemia and cellulitis and rapid development to ecchymoses and bullae, with severe cases resulting in necrotizing fascitis (Bross et al., 2007). Within 24 hours of infection, more than half of patients seen had developed the skin lesions associated with severe cellulitis (Bross, 2007). Fatality rates are greater than 50 percent for primary septicemia and about 15 percent for wound infections. This percent rises to 80 percent lethality rate in patients with liver disease, as iron overload increases the growth and lethality of the strain. The amount of oysters consumed did not affect the outcome; eating just one infected oyster is enough to initiate disease in predisposed individuals (Jones & Oliver, 2009). Most who acquire the infection have at least one predisposing immune-compromising condition.

Treatment of *Vibrio vulnificus* infections is commonly 100mg intravenously or orally of doxycycline (Vibramycin) twice a day, plus 2g intravenously of ceftazidime (Fortaz) every eight hours. Other therapies include 2g intravenously of cefotaxime (Claforan) every eight hours, or 750mg orally/400mg intravenously of ciprofloxacin (Cipro) twice a day (Bross et al., 2007). Regarding infection by an open wound, aggressive and prompt care is essential, including but not limited to surgical incision, drainage of abscesses and amputation. Because *Vibrio vulnificus* infections are commonly fatal in individuals with hepatic problems, recovery is determined by the

speed and accuracy of treatment based on early diagnosis. When treatment was delayed by as little as 24 hours in patients with septicemia, mortality rates rose from 33 to 53 percent, with 100 percent mortality rate in patients not treated within 72 hours (Bross et al., 2007).

1.2.4 Detection Methods & Prevention

Not all strains of *Vibrio vulnificus* or *parahaemolyticus* cause illness, yet 35 states have implemented a reporting system to state public health officials and the Center for Disease Control in the hopes of lowering fatality rates resulting from large outbreaks (cases of *Vibrio vulnificus* were up 43% in 2013 as opposed to 2006). Detection of the bacterium usually occurs through a process of isolations and PCR replications, leading to gene isolation and subsequently the apparent (or not) gene expression in the sample. The main method of detection for *Vibrio*-infected water, sediment, or seafood samples is sampling waters with an indicator species. If the water column or seafood is found to have the pathogenic strains present, then the area can be shut down for oyster harvesting production, but only after results have been confirmed; therefore, infected seafood could still be reaching the public before an actual laboratory analysis has decided that the area of harvest needs to be shut down.

In Louisiana, the Molluscan Shellfish Program (Section 1.3.1) tests for harmful bacteria by using fecal coliform indicator species. Other methods of detection approved by the Environmental Protection Agency include the indicators *E. coli* and enterecocci, both of which are proven to be more accurate estimates of *Vibrio* counts within water

columns (EPA). These indicators, coupled with fecal coliform, are within a larger indicator species known as total coliforms, proven to be more sensitive to pathogenic *Vibrio* counts (Kaneko & Colwell, 1973).

While many people enjoy their seafood raw, cooked seafood is one of the primary methods of prevention. Keeping product frozen from harvest until it reaches the restaurant can also prevent pathogenic strains of *Vibrio* bacteria in the seafood. Those with compromised immune systems, liver illness, or recovering alcoholics should also avoid raw seafood. Cooking the product will get rid of the bacteria as well; shellfish should be boiled until they open, then boiled for another five minutes, and shucked oysters should be boiled for at least three minutes (Bross et al., 2007). People open or exposed wounds should avoid warm seawater or contact with seafood at the site of the wound. Any seafood juices released while cutting the food should be rinsed away and the equipment sterilized. Again, freezing or putting leftover seafood in the fridge will also inhibit the growth of the bacteria.

1.3 Louisiana Department of Health & Hospitals

The Louisiana Department of Health and Hospitals (DHH) mission statement is "to protect and promote health and ensure access to medical, preventative, and rehabilitative services for all citizens of the State of Louisiana," as printed on their website (http://www.dhh.louisiana.gov/index.cfm/page/2/n/4). Under the state government, DHH is in charge of public health relations for the state, including finances such as Medicaid. DHH houses the Molluscan Shellfish Program of Louisiana,

dedicated to the regulation of oyster harvesting waters along the Louisiana Gulf Coast (LeBlanc, 2014).

1.3.1 Molluscan Shellfish Program of Louisiana

As previously stated, the Molluscan Shellfish Program is responsible for the regulation of oyster harvesting waters within Louisiana. These areas are set forth by the Louisiana Sanitary Code and the National Shellfish Sanitation Program (NSSP), a federal/state cooperative program recognized by the U.S. Food and Drug Administration (USFDA) and the Interstate Shellfish Sanitation Conference (ISSC) for the sanitary control of shellfish produced and sold for human consumption (LeBlanc, 2014). The program reclassifies oyster harvesting areas as "open for harvest" or "closed for harvest" into two seasons with the four periods: November-February, March-April, May-August, and September-October (LeBlanc, 2014). In order to reclassify the areas and create a baseline for harvestable oysters, the Program utilizes fecal coliform as an indicator and adverse observations exclusively to test for harmful bacteria in the water column and some oyster meat samples (Lemaire, 2013). DHH also had ten Registered Sanitarians attend a seafood sensory training program at the International Food Protection Training Institute, training individuals how to detect taint in seafood by smell after the BP oil spill. This skill is "critical in helping Louisiana determine whether to open or close molluscan shellfish harvest areas," and is part of a partnership with the National Oceanic and Atmospheric Association's National Marine Fisheries Services and the USFDA (LeBlanc, 2014).

1.3.2 Fecal Indicator & *Vibrio* Correlation

Since the pathogens appear intermittently in natural waters at low concentrations, and detection and quantification are labor intensive, the routine microbiological water analyses are based on detection of indicator organisms, which share the same habitats (Savichtcheva, 2006). Fecal coliform is a subset of total coliform bacteria and is utilized as an indicator in testing water quality for sewage contaminants, including the possible presence of pathogenic bacteria, viruses, and protozoans (USEPA, 2006). The fecal coliform indicator is more fecal-specific in origin, and therefore is a strong indicator of bacteria (pathogenic or not) that live in human and animal digestive systems. Because fecal coliform is approved by the Environmental Protection Agency as a measure for testing water quality, a few states, including Louisiana, still use this indicator to test for pathogenic *Vibrio* spp. in water and shellfish samples due to its low cost, as opposed to upgrading to more precise indicators (USEPA, 2006). As stated earlier, however, fecal coliform is not as strongly correlated to pathogenic *Vibrio* spp. in the water, sediment, or oyster samples as the newly approved indicators of enterococci or *E. coli*. Even though enterococci and *E.coli* have been approved as stronger indicators than fecal coliform, they still have little to no correlation to *Vibrio* densities overall, as proven in a recent study published the University of North Carolina (Wetz et al., 2012).

Some studies have shown that there is little to no correlation and at times a negative correlation between fecal coliform and pathogenic *Vibrio vulnificus* and *Vibrio parahaemolyticus* (Savichtcheva, 2006). Kaniko and Coldwell attribute the lack of

correlation between the fecal coliform density and *Vibrio* density to the ecology of the organism (Kaneko, 1973). The Environmental Protection Agency (EPA) approved fecal coliform as an indicator to be used in drinking water sanitation tests, and while the correlation and precision of the indicator toward pathogenic *Vibrio* varies within bodies of water, its approval as a water test standard enables it to be used for shellfish contamination testing as well. Because fecal coliform can test for a multitude of bacteria and viruses, it is more economically feasible to use it additionally to test waters and oysters for *Vibrio*, even though other indicators (enterococci and *E.coli*) are recommended by the EPA to test for *Vibrio* (Environmental Protection Agency, 2006). As previously stated, however, the indicators still do not have a strong correlation to *Vibrio* densities in oysters, and a supplemental indicator should be used outside of fecal indicators.

1.4 Louisiana Oyster Bans

Due to the amplified effects of *Vibrio* infection in those with compromised immune systems, the Food and Drug Administration (FDA) is in the process of pursuing a policy that would prevent any raw Gulf of Mexico oysters from being sold during the warmer months from April 1 to October 31 (Scott, 2009). This national shellfish rulemaking body refused to endorse the plan for the second time during the 2009 year. The proposed ban was successfully initiated in California as a pilot run in 2003, where cases of *Vibrio vulnificus* fatalities and reported illnesses were down from 5.5 median cases from 1991-2002 to 0 from 2003-2010; however, cases of *Vibrio*

parahaemolyticus increased in 2004, fluctuating slightly within recent years (Vugia et al., 2013).

Because Louisiana provided 70% of harvested oysters to California, the economic impact was an export loss of nearly $20 million for Louisiana (Bell & Schexnayder, 2003). Those who oppose the ban cite the economic impact and job loss in Gulf states as prime reasons to deny the proposed ban, as well as the fact that only those with compromised immune systems, who should not be eating any raw seafood anyways, are the only ones with severe health risks. Besides an outright ban of Gulf Coast oysters, there are currently two approved post-harvest treatment methods (low-temperature pasteurization and high pressure processing) that are approved and accepted by the state of California, therefore allowing Gulf Coast oysters to be imported during warmer months (Bell & Schexnayder, 2003).

CHAPTER 2: MATERIALS & METHODS

2.1 Data Collection

In a previous study conducted by Nabanita Bhattacharyya and Dr. Aixin Hou, 293 environmental isolates were taken from multiple sites in Breton Sound and Barataria Bay. The sites chosen were also tested by the Molluscan Shellfish Program, and include: sites 4, 8, 11, 12,15,16,18 from Breton Sound and sites 2, 8, 16, and 23 from Barataria Bay (Bhattacharyya, 2013). The sites in Breton Sound represent the following corresponding areas: Site 4 (Lake Lery), Site 8 (Grand Lake), Site 16 (Black Bay), Site 18 (Gallega Islands), Site 11 (Oak River Bay), Site 12 (Four Horse Lake), and Site 15 (Bayou Terre aux Boeufs) (Bhattacharyya, 2013). At each site, 100ml-water grab samples (less than 5 meters deep) were taken to be further analyzed for *Vibrio.*

After collection, the isolates were tested, as recorded in the study "A Pentaplex PCR Assay for Detection and Characterization of *Vibrio vulnificus* and *Vibrio parahaemolyticus* isolates" (Bhattacharyya, 2013). The isolates were then analyzed using real-time PCR, and then recorded in excel based on their genetic identification. It is from this data set and sampling list that the maps of *Vibrio vulnificus* and *Vibrio parahaemolyticus* for the year 2011 in Breton Sound were created.

2.2 Geographic Information Systems Mapping

Using the data compiled in the previous section, ArcGIS software was utilized to create GIS maps of *Vibrio* concentrations in Breton Sound for each sampling period

throughout 2011. Each map reflects the densities of either *Vibrio vulnificus* or *Vibrio parahaemolyticus* per each sampling period, at all tested sites. With nineteen sampling periods, thirty-eight maps were created, one for each bacteria species. The baseline map downloaded to ArcCatalogue was from the United States Geological Survey as a Multi-Resolution Land Cover map. The map zone is GCS_North_American_1983, with the datum being D_North_American_1983. The log of the data samples was calculated in Excel and linked to ArcMap. Under the data properties, the varying-size pie distribution was used as the symbol to indicate the varying data densities.

CHAPTER 3: RESULTS

For both *Vibrio vulnificus* and *Vibrio parahaemolyticus*, the overall trend indicated higher densities in the warmer months, with the highest densities varying depending on the sample type (water, sediment, or oyster). The highest densities within water samples occurred during the June 27, 2011 testing for both *Vibrio vulnificus* and *Vibrio parahaemolyticus*. For the sediment samples, the October 13, 2011 sampling indicated the highest concentration of bacteria present. Lastly, the oyster meat had two dates with high densities: August 23, 2011, and September 15, 2011. The lowest overall densities occurred in the colder months, from November through March, as expected based on the bacteria's temperature tolerances. Also, generally higher concentrations were present in sediment samples than in water samples, especially in March when water temperature was lower. The station with the lowest salinity (<1 ppt) had the lowest concentrations among the sampling stations. The results indicate the impacts of temperature and salinity on *Vibrio* densities. Comparing this data to the Department of Health and Hospitals Oyster Harvest Guideline, some of the concentrations fall within coordinates of sites marked as "green" or safe. At the end of this chapter, Figures 1 and 2 indicate the distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* during March 2011, respectively. Figure 3 is the DHH map for March 2011, and the sites near station 18 and 16 are still indicated as green/open, even after finding measurable levels of *Vibrio. (*See the Appendix for all thirty-eight maps and distributions.)

The table below indicates a portion of the data utilized to create the maps, specifically the March 2011 map at the end of this chapter. The numbers listed in these

two tables are the raw data sets, and not the log of the numbers, which was utilized when making the maps. Data for all maps can be found in the Pentaplex PCR study conducted by Nabanita Bhattaryya (2013).

Table 1. Vibrio parahaemolyticus density for March 2011

Water (CFU/100ml)	Oyster (CFU/g meat)	Sediment (CFU/g sediment)
0	NC	0
0	NC	0
123	181	98
47	NC	22
103	NC	69
217	66	495
NC	NC	NC

NC: No Count
TMTC: Too Many To Count

Table 2. Vibrio vulnificus density for March 2011

Water (CFU/100ml)	Oyster (CFU/g meat)	Sediment (CFU/g sediment)
0	NC	0
0	NC	0
497	731	396
190	NC	91
419	NC	280
844	268	2001
NC	NC	NC

NC: No Count
TMTC: Too Many To Count

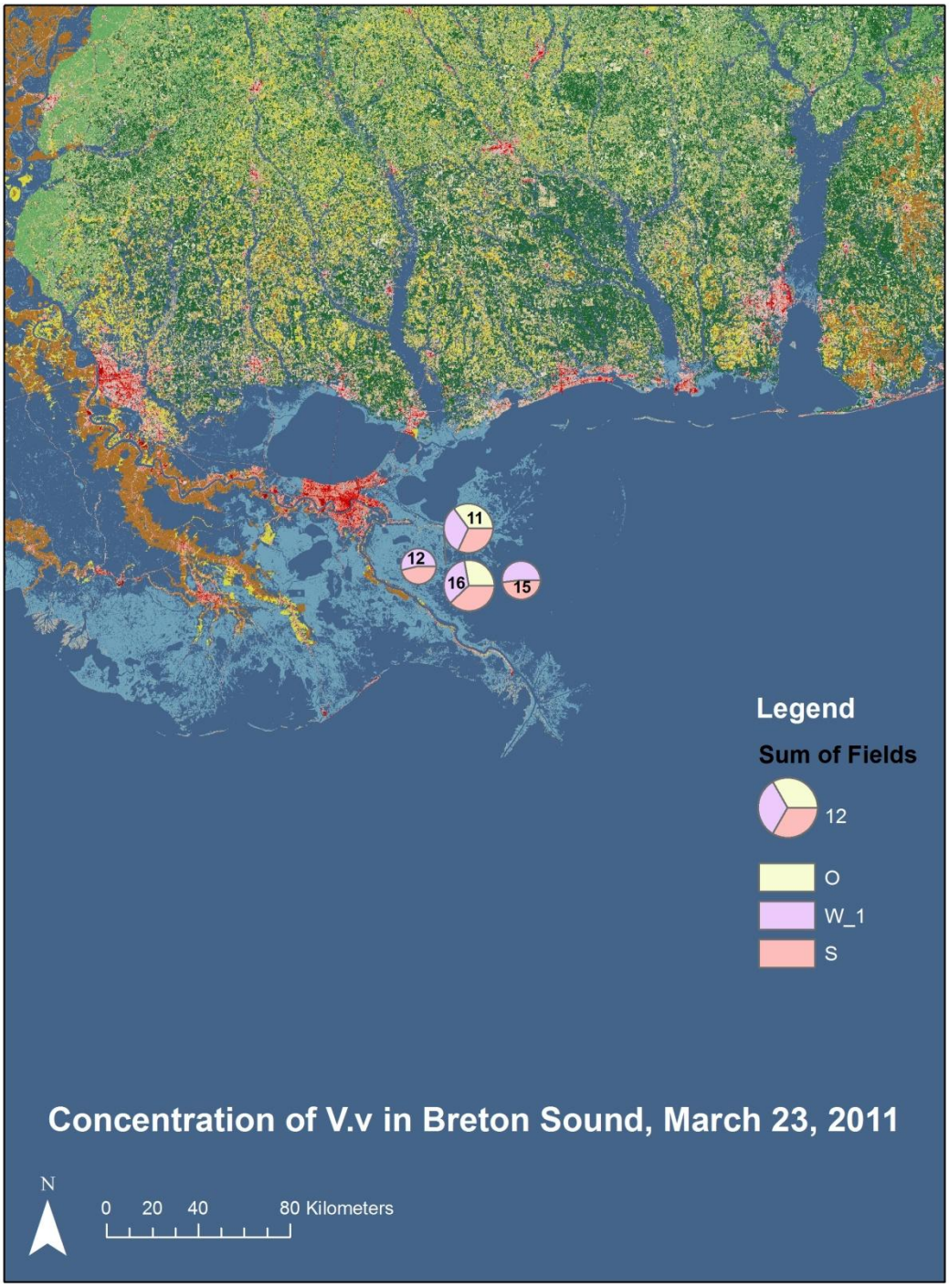


Figure 1. Vibrio vulnificus density, March 23, 2011.

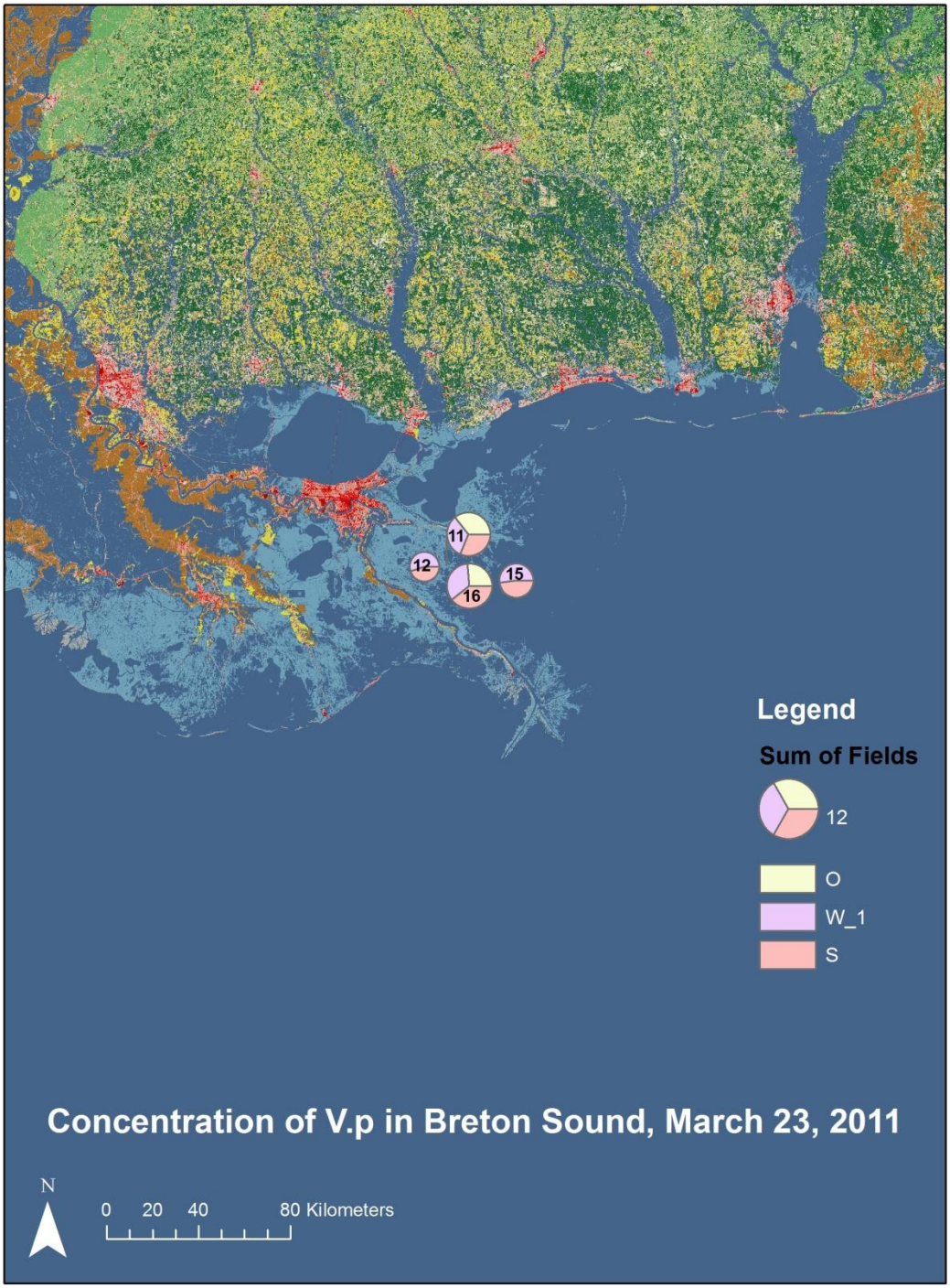


Figure 2. Vibrio parahaemolyticus density, March 23, 2011.

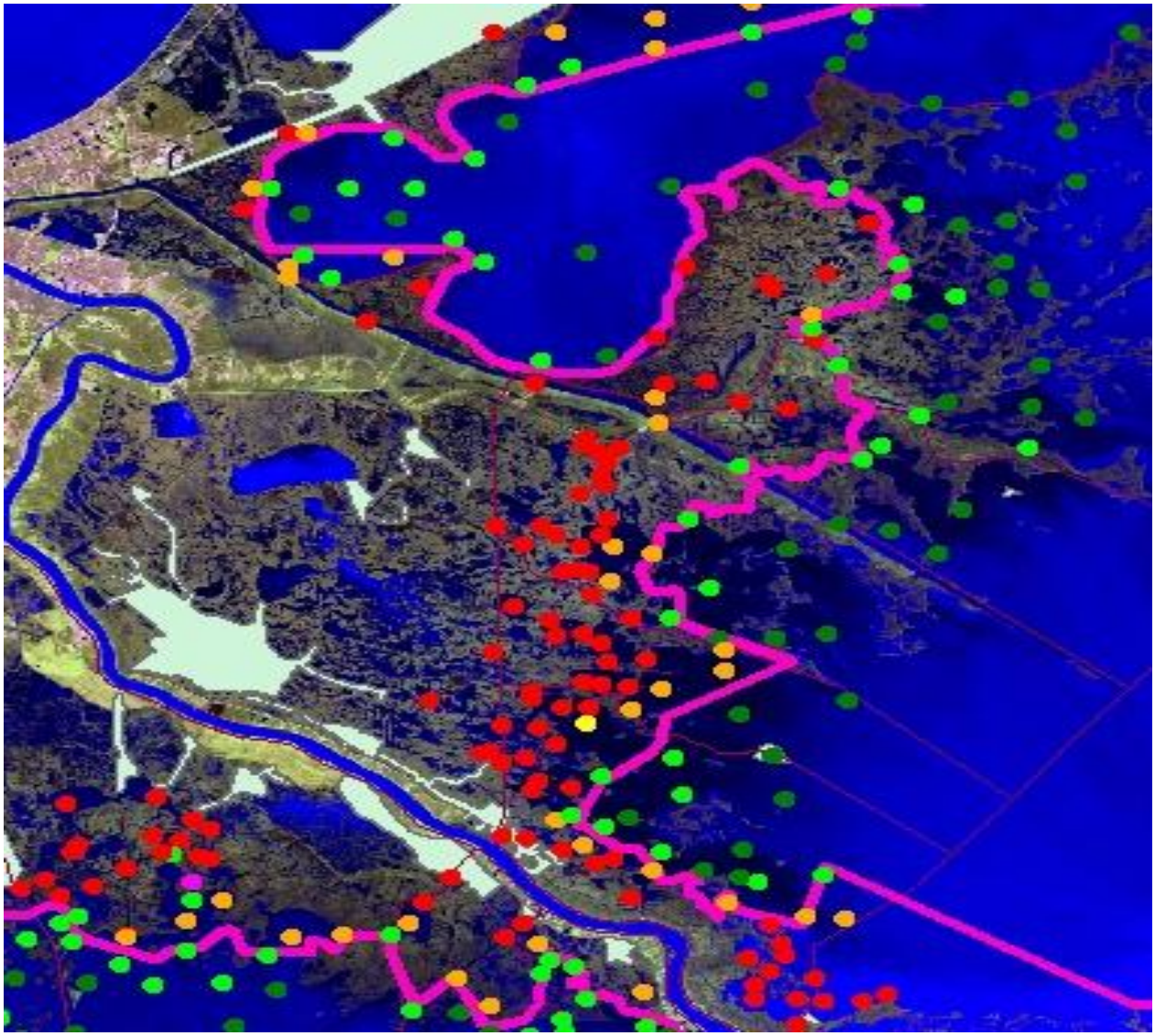


Figure 3. Department of Health and Hospitals March 2011 Site Closure Map.

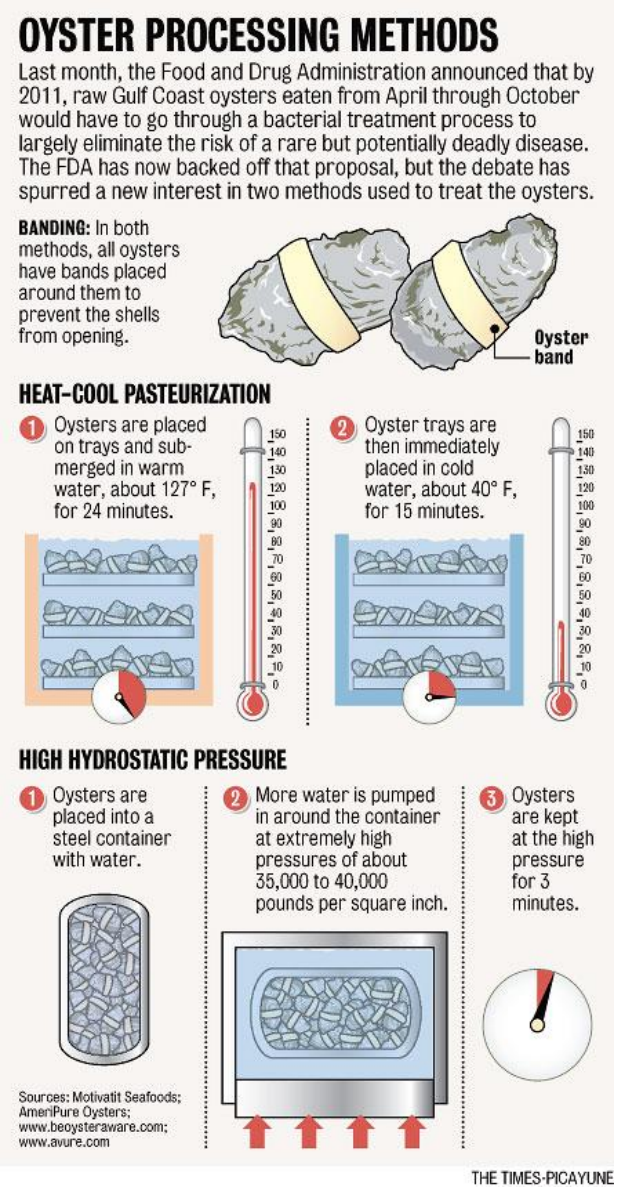
CHAPTER 4: DISCUSSION & CONCLUSION

While the U.S. Environmental Protection Agency has approved fecal coliform as an indicator for the testing of drinking water, it is not precise enough to be used to test for non-cholera *Vibrio* in oysters. The fact that it is a low-cost test makes it appealing to states to continue using; however, the loss of buyers (as in the case of the California ban on Louisiana oysters) comes at a higher price than shifting to a more precise test would cost in the long-term. Based on the DHH maps of open and closed oyster harvesting areas, the indicator is not as accurate regarding densities of *Vibrio* present in a water column; this is in part due to the poorly understood dormant state of the bacteria, in which instance it would not show up as pathogenic on screening. Better indicators including enterococci and *E.coli,* are being used by multiple states in lieu of fecal coliform, and should be considered as supplemental indicators in Louisiana to fecal coliform. As stated previously however, it is important to remember that even these "better" indicators have been proven to have little to no correlation to *Vibrio* densities, and more accurate indicators need to be researched and approved.

In order to prevent states such as California from creating long-term bans on Louisiana raw oyster products, as well as the recent controversial ban proposed by the USFDA, post-harvest measures should be taken in Louisiana to allow the oysters to be exported to California during banned export months. The measures, as discussed above, include low-temperature pasteurizing and high pressure processing. Low-temperature pasteurizing of raw oysters includes pasteurizing at 50 degrees C for up to 15 minutes; one study concluded that pathogenic *Vibrio* were reduced from >100,000 to

non-detectable levels in oysters in ten minutes of processing alone (Andrews et al., 2000). In 1997, Ralph Nader's Center for Science in the Public Interest called for a federal requirement that all oysters be heat treated. This method is used by at least one Louisiana processor with some success, however most processors do not treat oysters post-harvest (Andrews et al., 2000).

A more efficient method-- high pressure processing-- is an alternative method that has not only been proven to lower microbial count, and therefore extend shelf life, but also creates a "perfectly" shucked oyster in the process. High pressure processing (HPP) subjects the food of choice (oysters) to elevated pressures, with or without heat, to inactivate vegetative bacteria or to maintain natural freshness. Regarding Pacific oysters, the optimum pressure range to decrease microbial densities, while also keeping desired oyster qualities, was between 240 and 275 MPa (Adams et al., 2006). In 1999, Ernie Voisin of New Orleans discovered the art of HPP and its automatic shucking capabilities (McGill, 1999). While Voisin intended on financing a machine for his



oyster company entitled Motivatit Seafood Inc., the cost to process 300 pounds (750 oysters) is steep at $500,000 (McGill, 1999). Unlike post-harvest heating methods, such as low-temperature pasteurizing, high pressure processing maintains the salinity, taste, and most moisture in an oyster; however, the HPP post-harvest method is expensive, and may not be financially feasible for many processing companies in Louisiana.

Because of the drawbacks associated with each post-harvest processing method, producers in Louisiana feel confident in selling unprocessed, raw oysters. One producer, Anthony Uglesich stated, "[I've] never had a complaint of an illness from [my] non-processed oysters. Vibrio vulnificus is not a problem for healthy people and oyster-borne bacteria are rarely a problem if you are careful about where you get your oysters" (McGill, 1999). This sentiment, however, means reactive medical treatment for individuals who are sick instead of proactive sanitation methods, whether by better indicators or post-harvest treatments.

While there are better indicator species that the DHH can use to increase the precision of their tests, oyster harvest companies can also use post-harvest treatment methods proven to reduce non-cholera *Vibrio* to undetectable and healthy levels. With only two seafood companies (Motivatit Seafoods and AmeriPure Oysters) utilizing post-harvest treatment methods in Louisiana, only 15-20% of the Gulf coast oyster production is being treated (Kirkham, 2009). If policies were implemented by the state to urge such changes, then the cost would be offset by the exported oysters now available in markets with stricter health and sanitation codes. Changing the standards

of oyster harvesting and treatment methods should not only be a concern for export reasons, but also for the sake of Louisianans as well.

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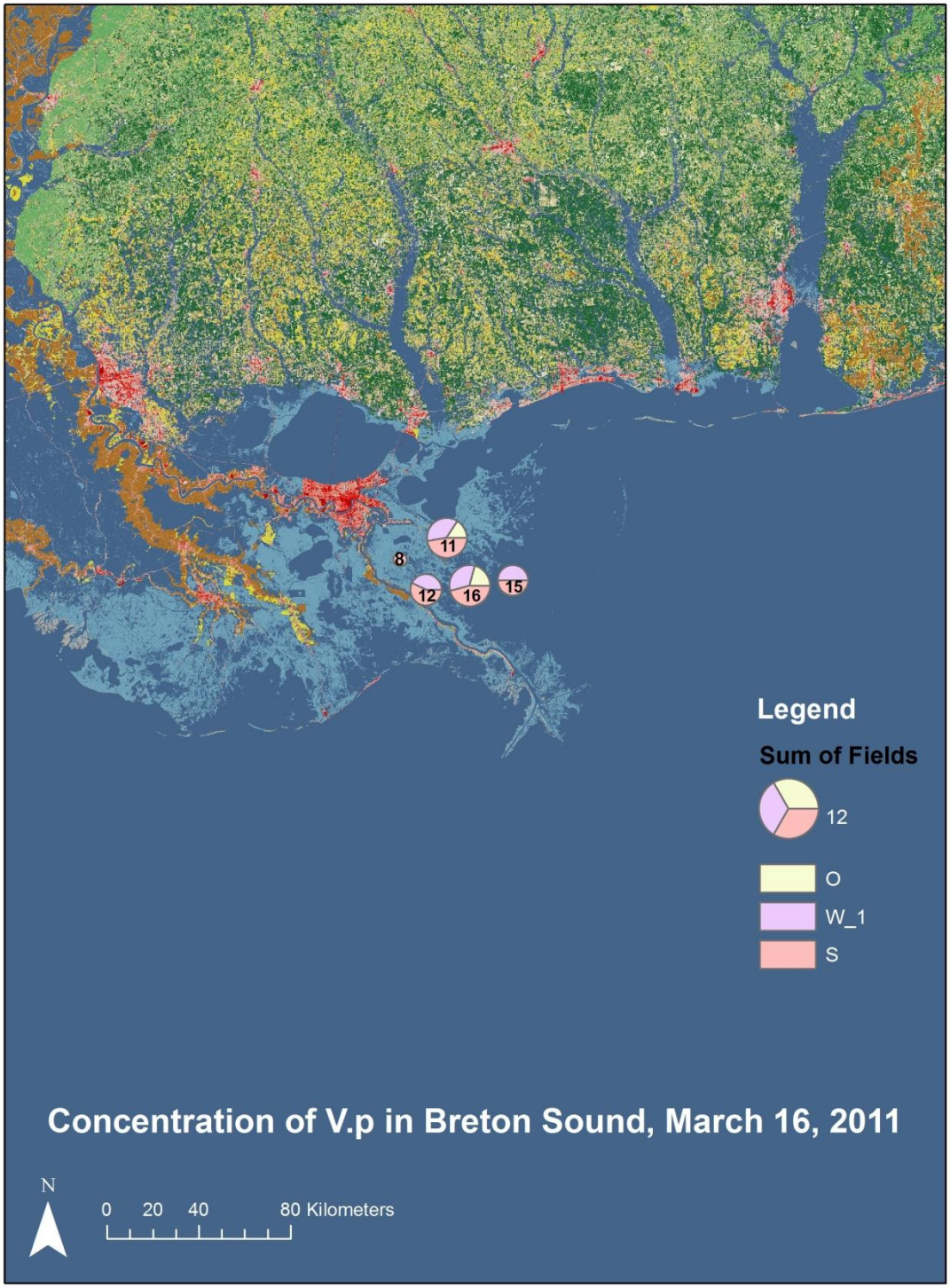
APPENDIX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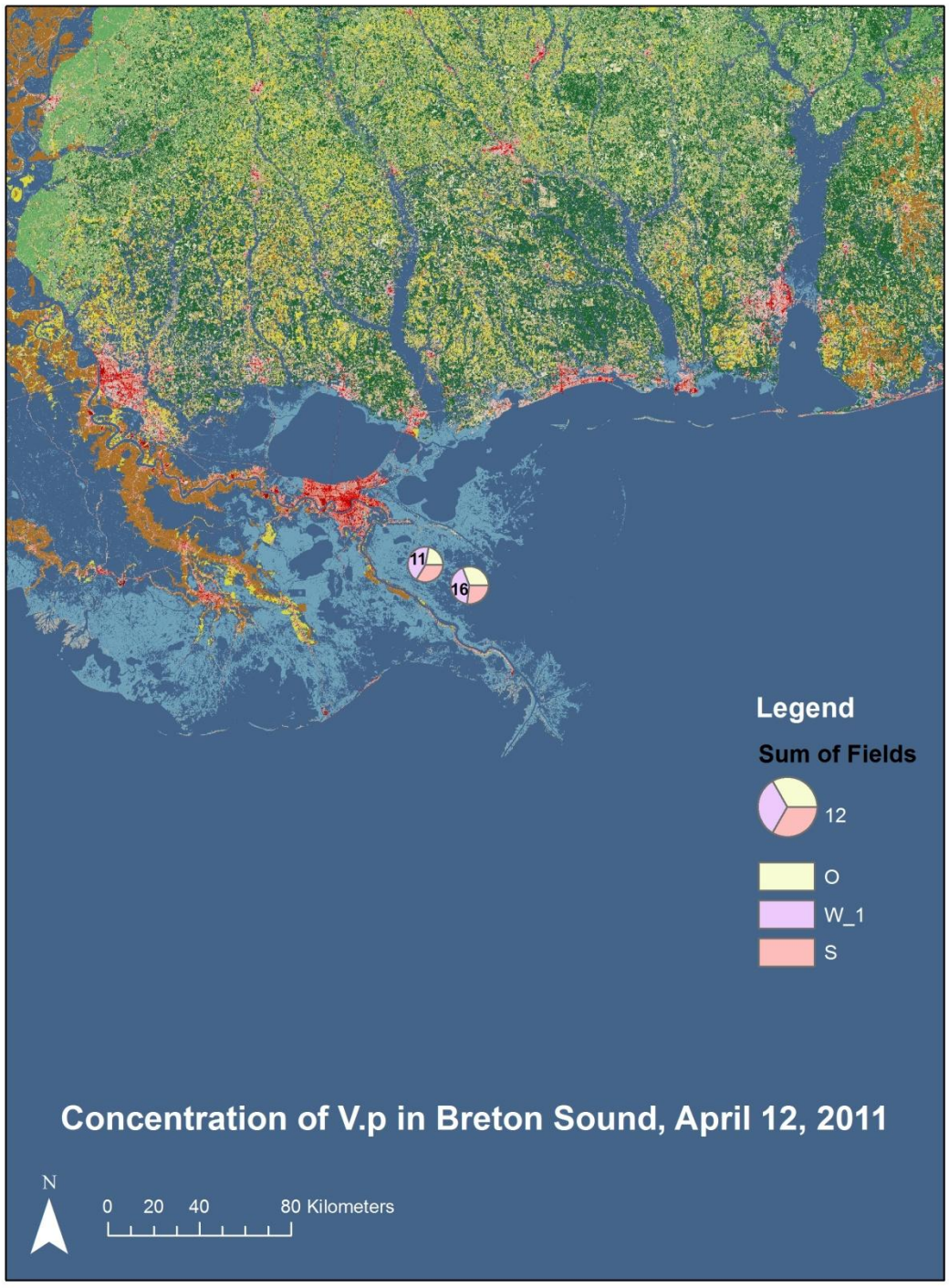
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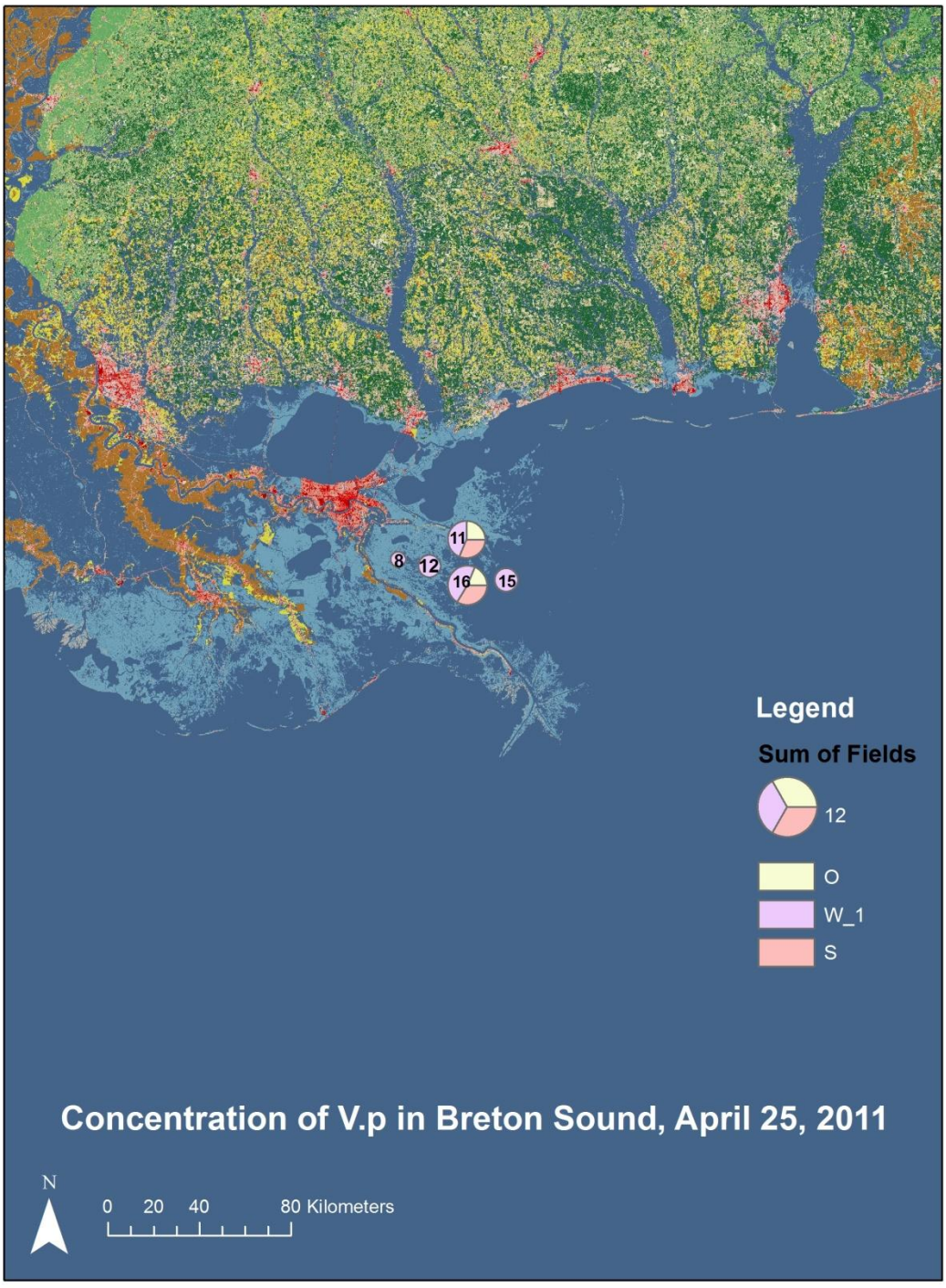
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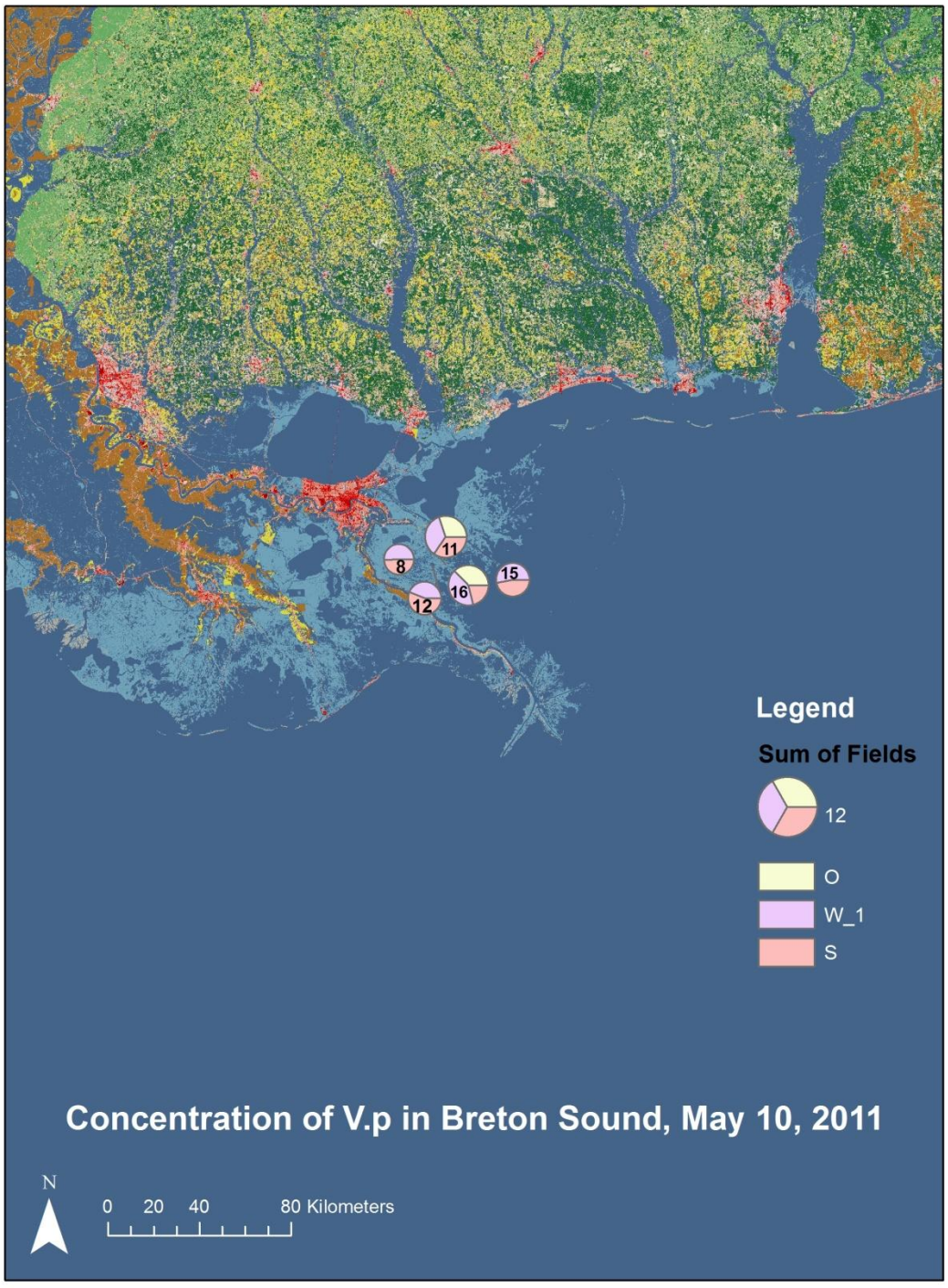
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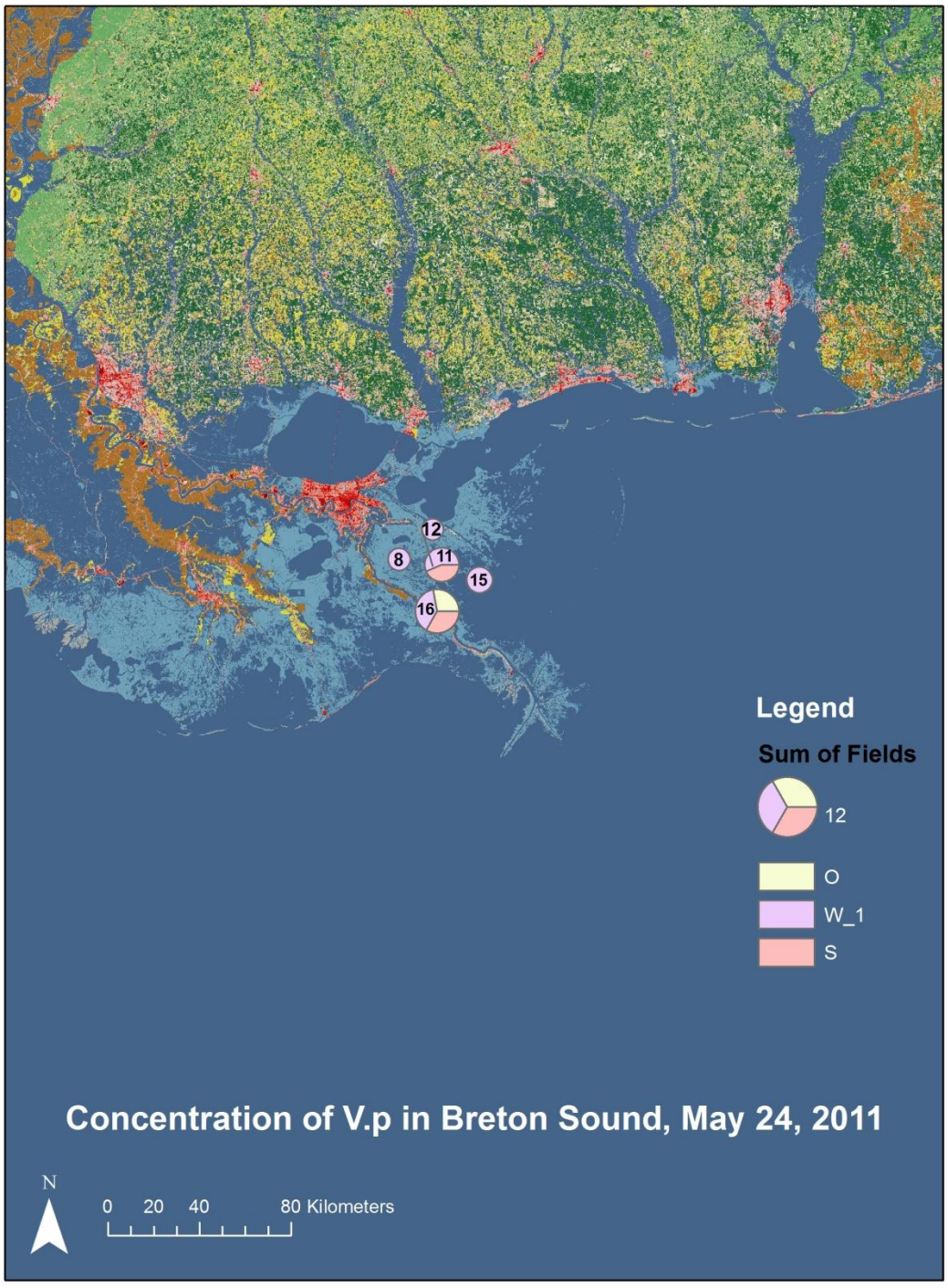
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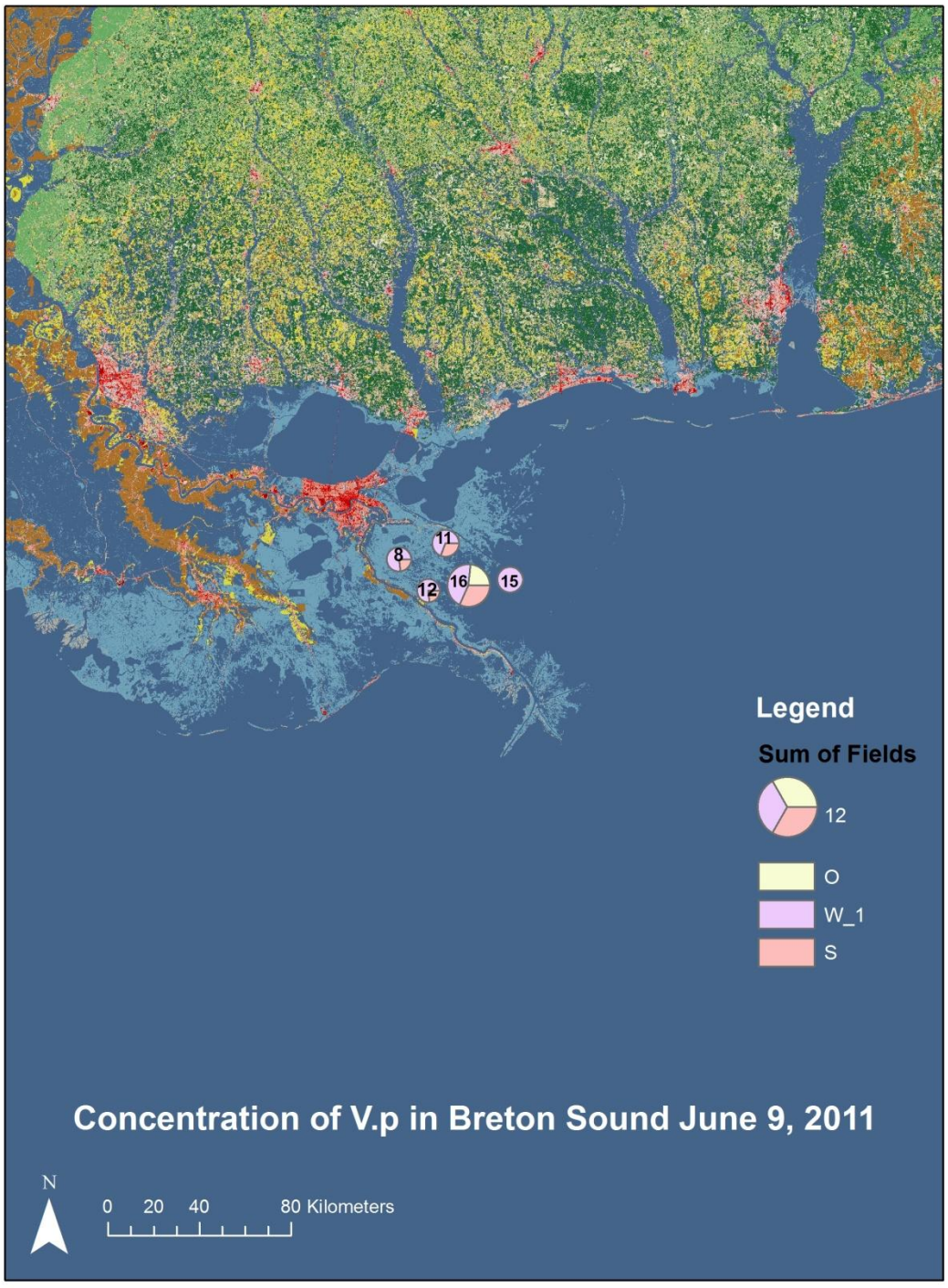
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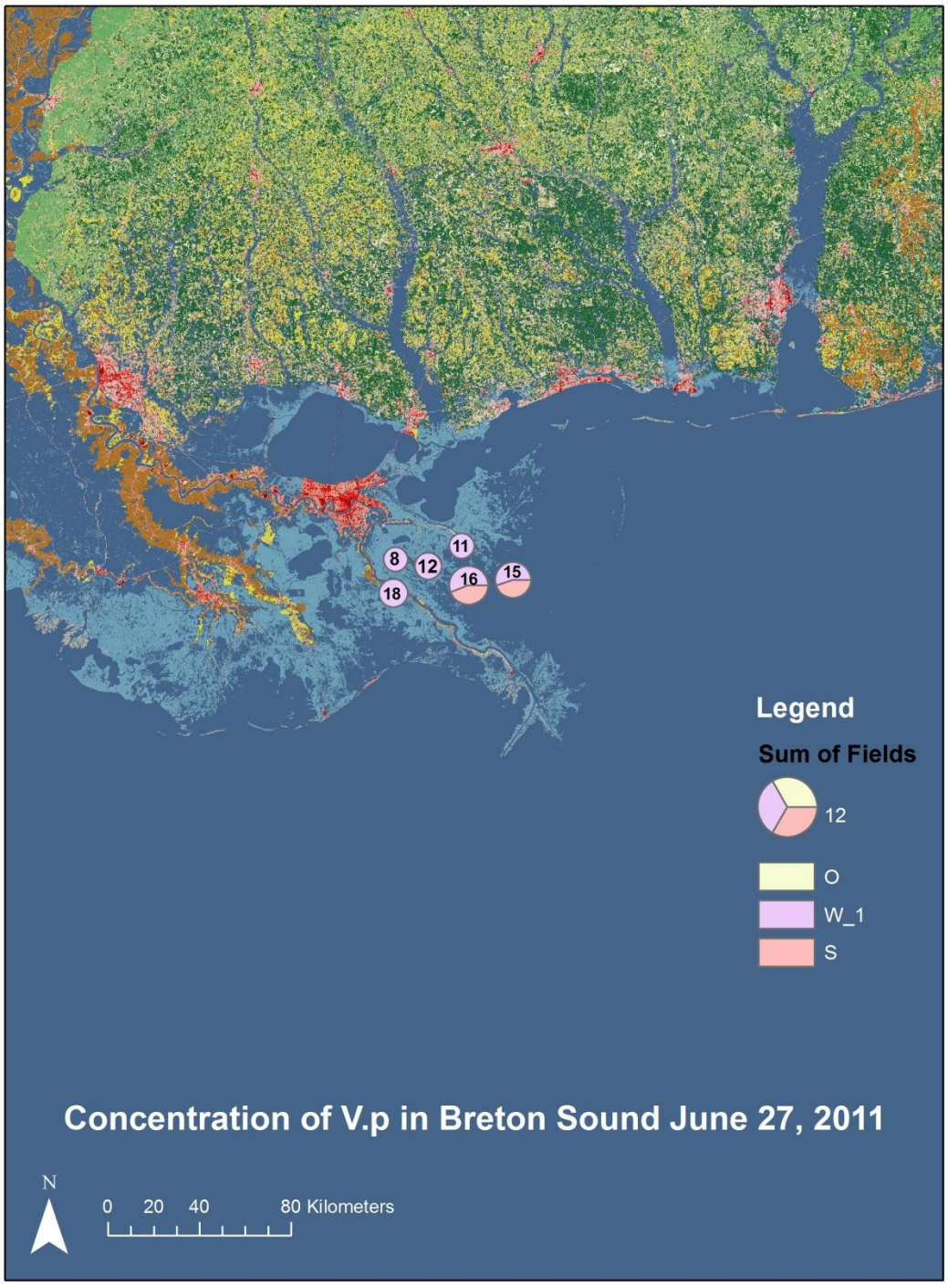
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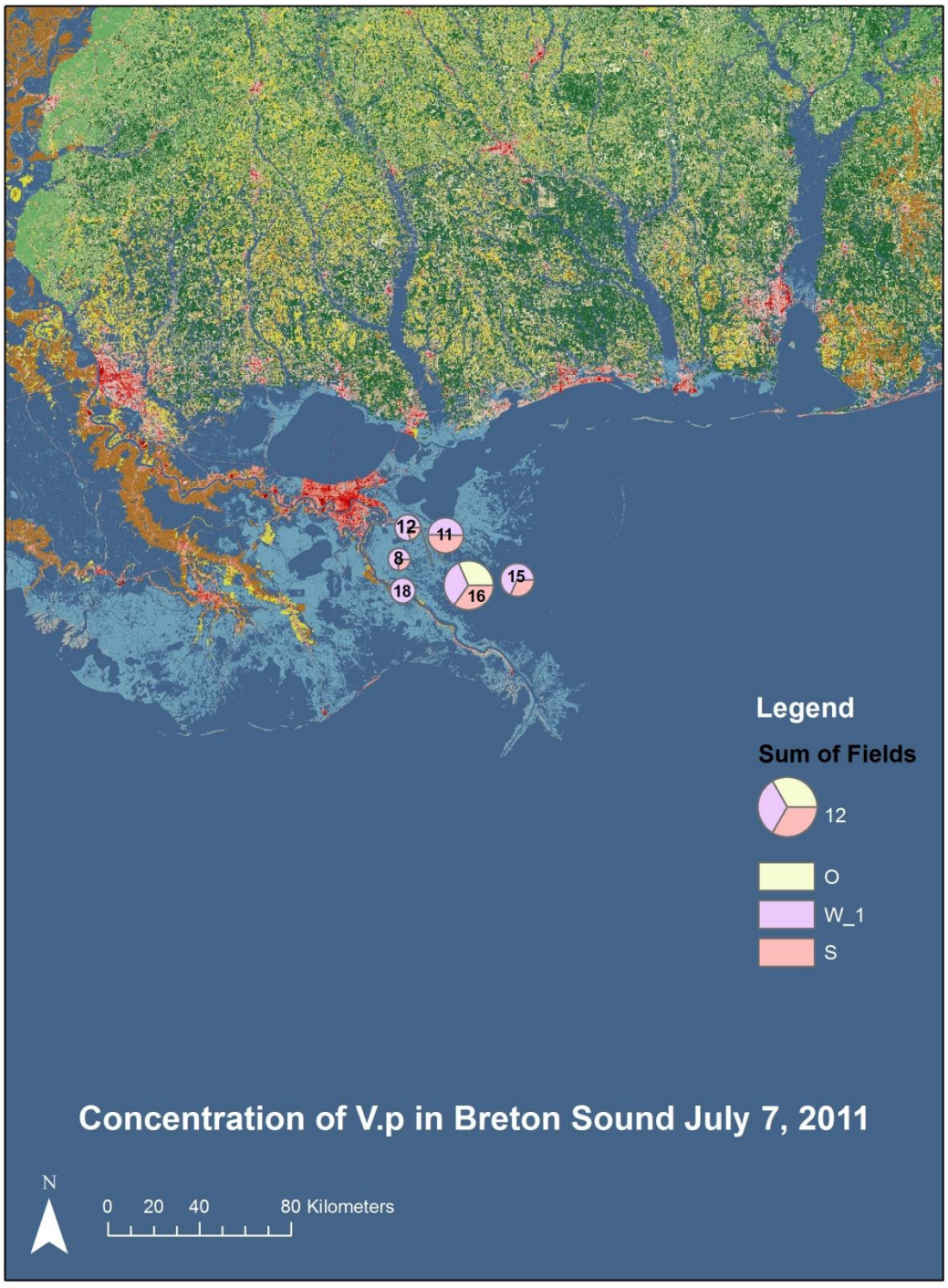
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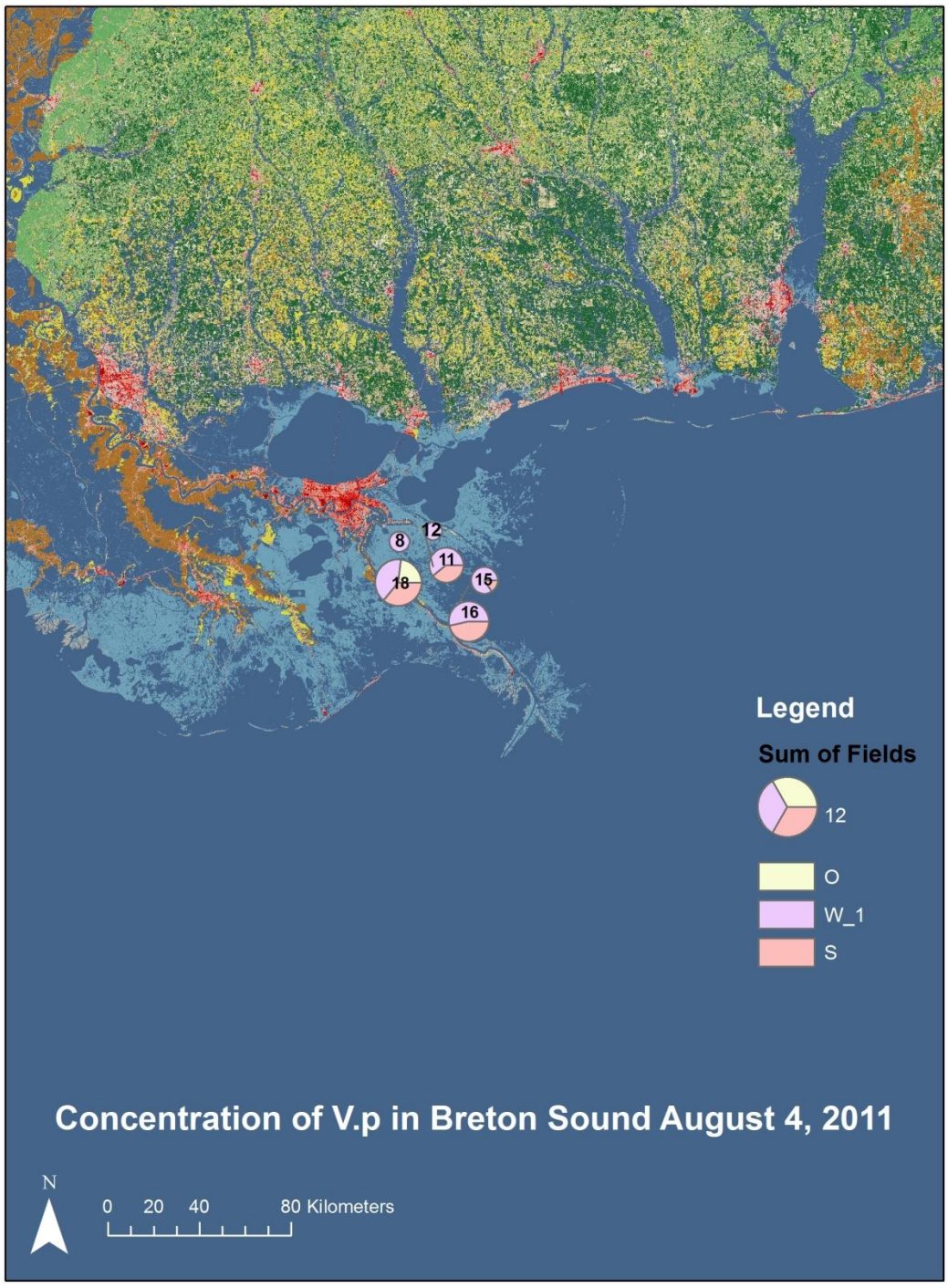
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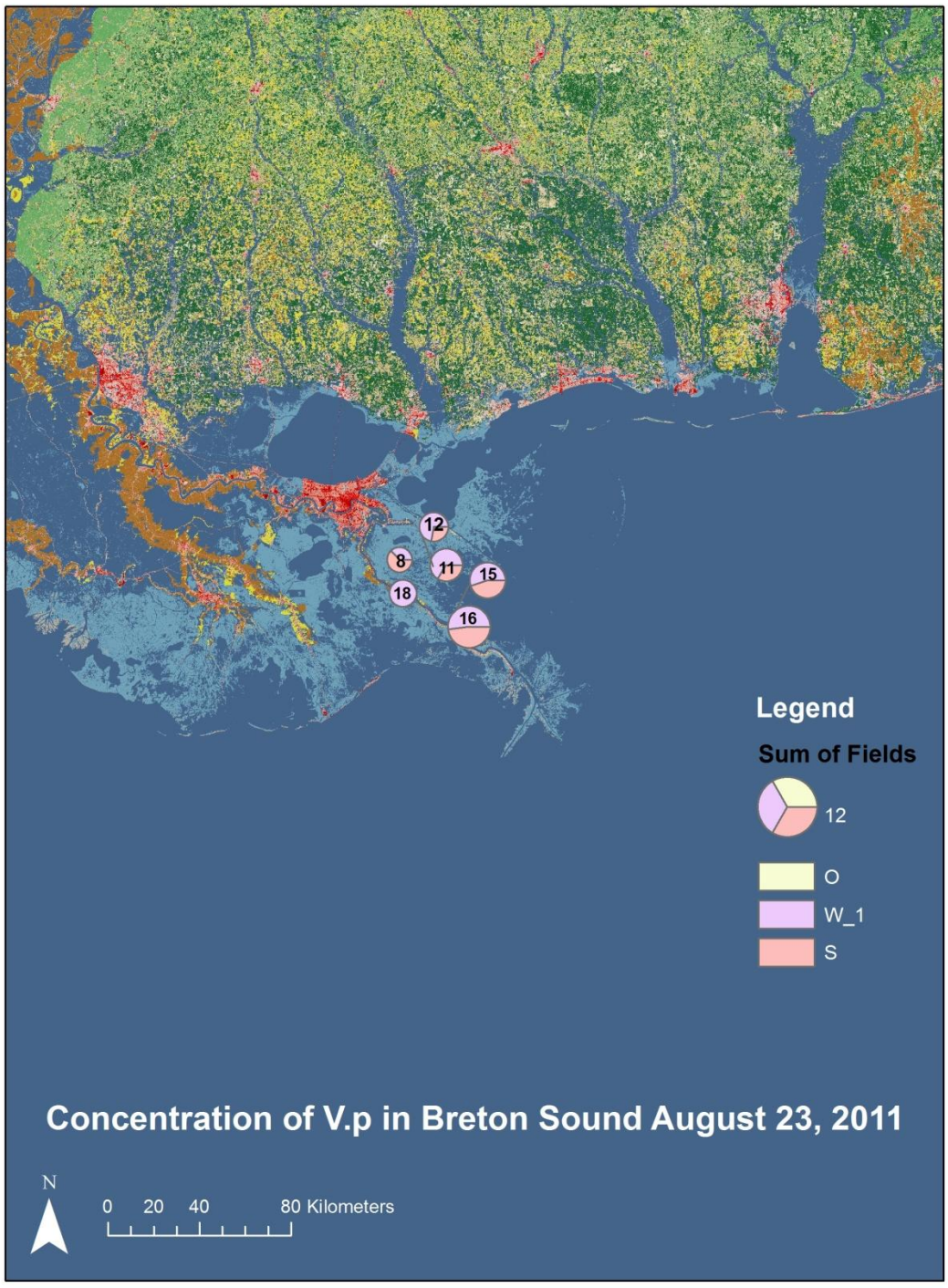
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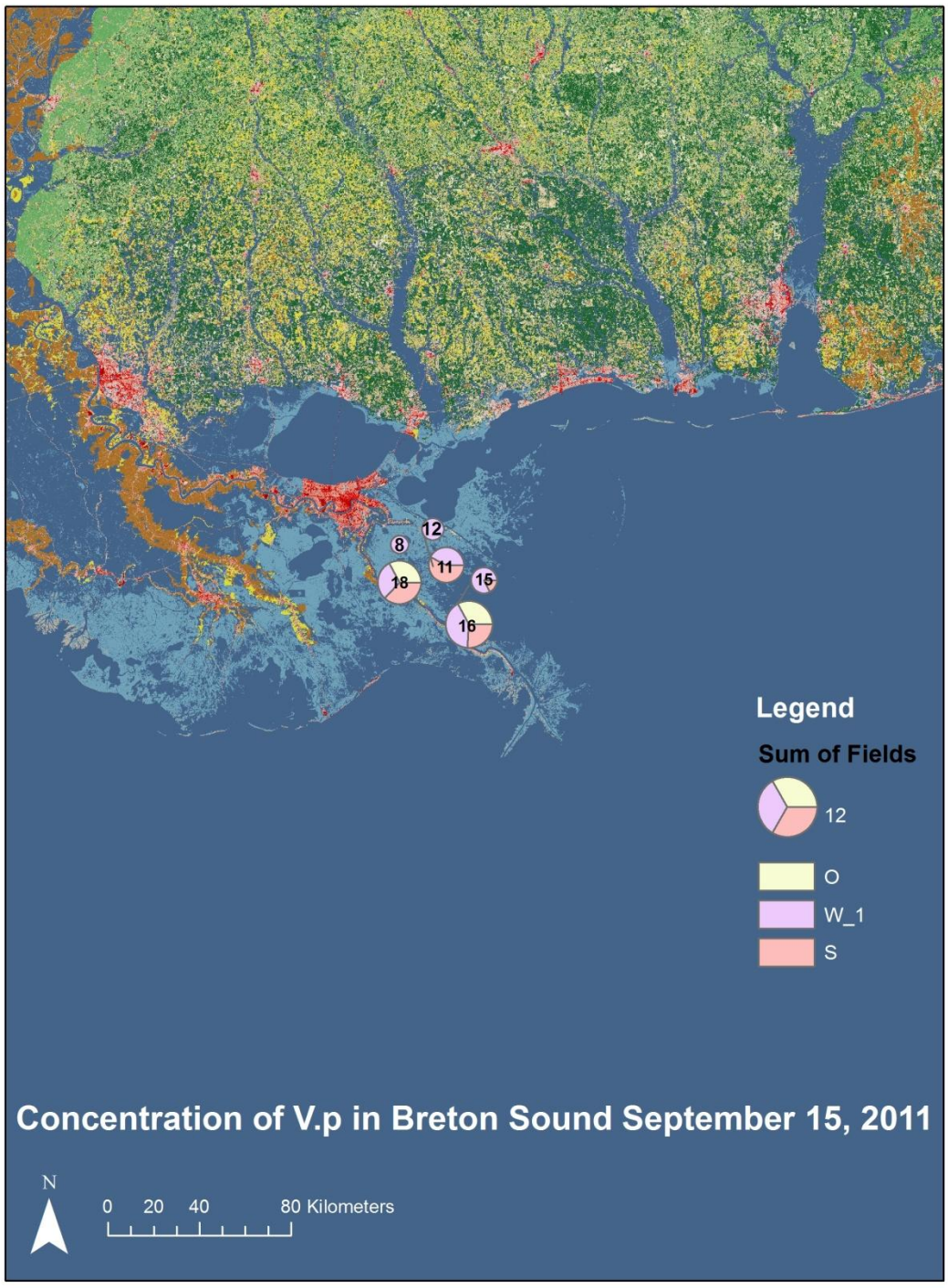
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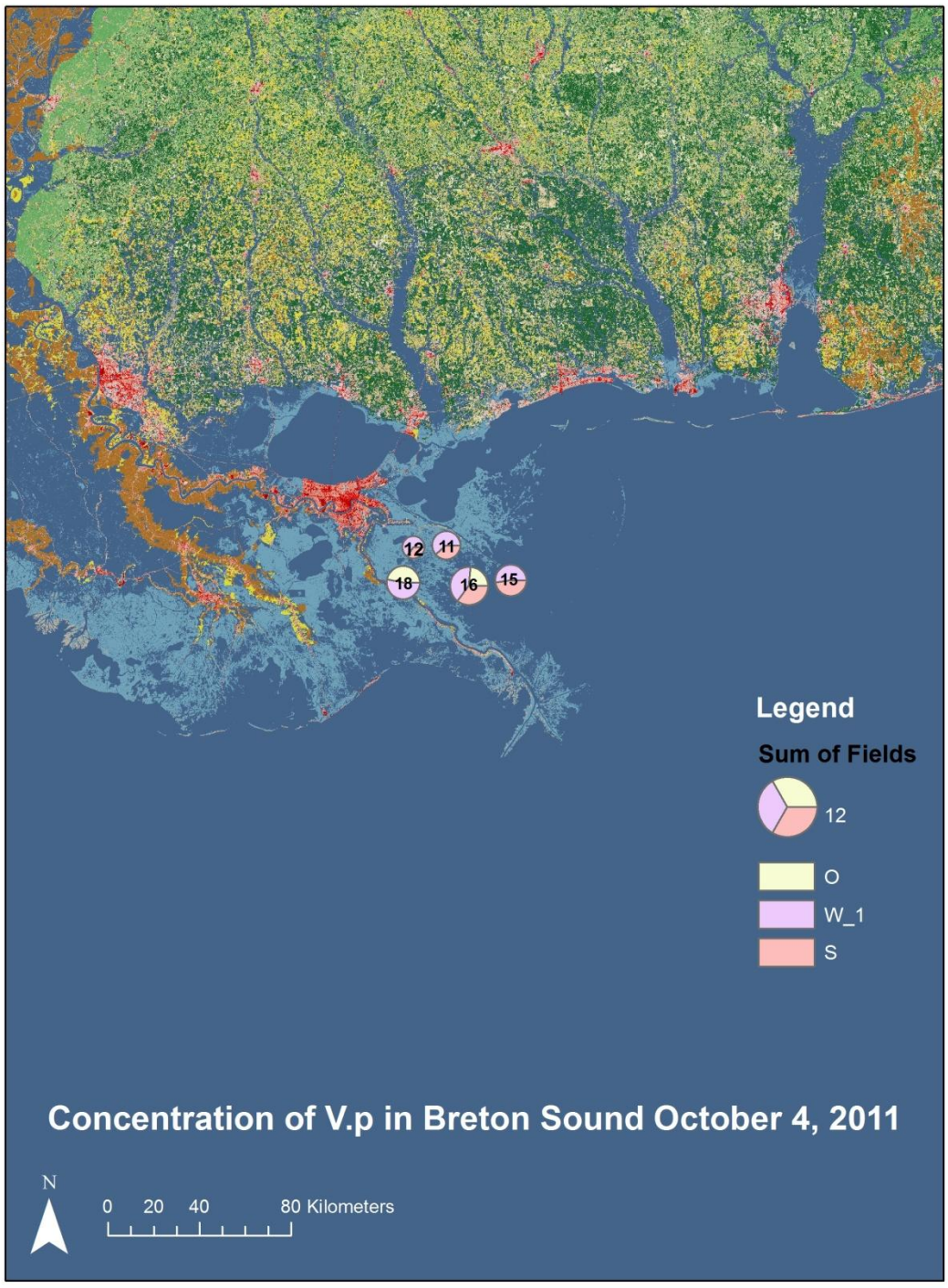
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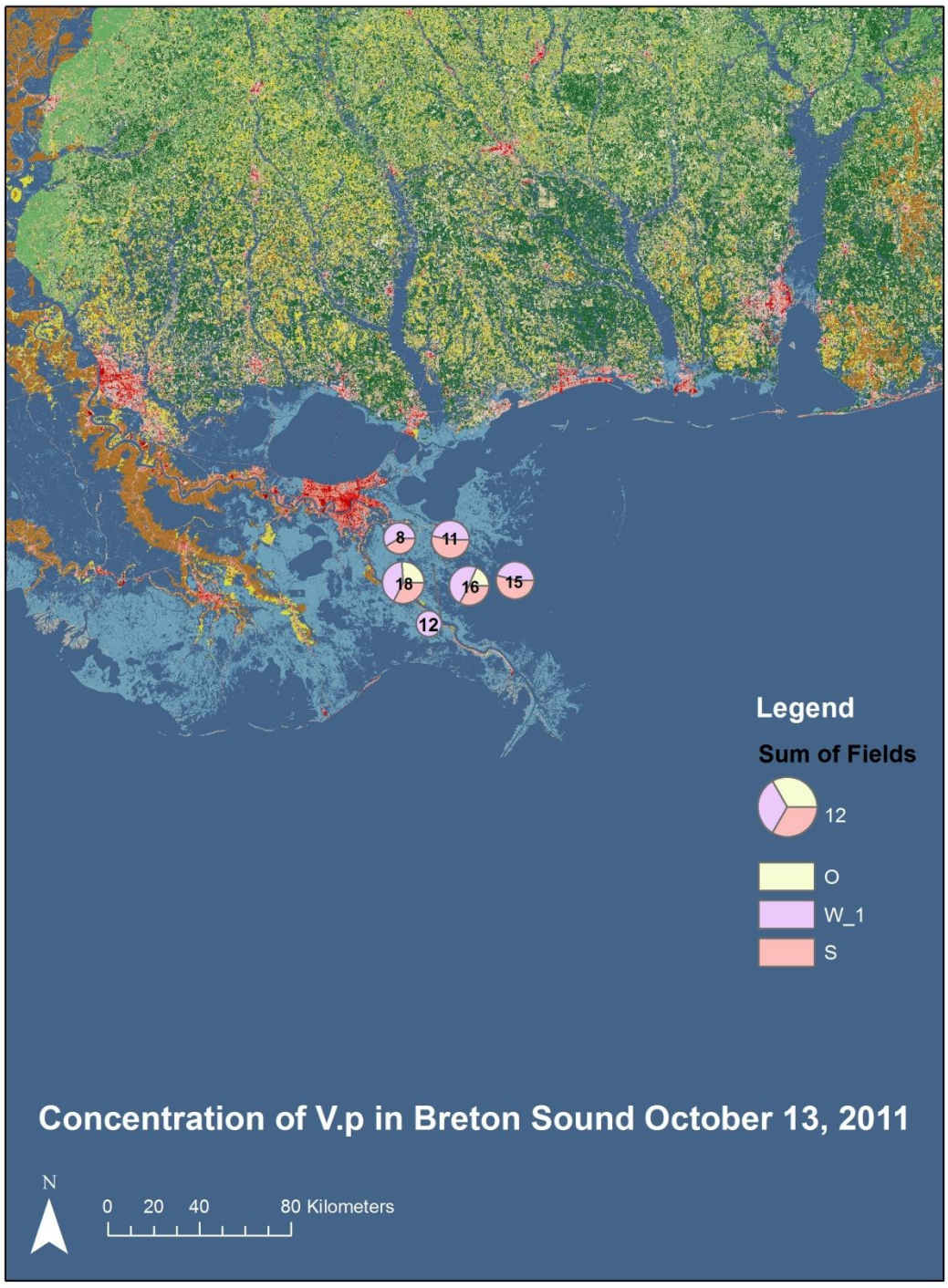
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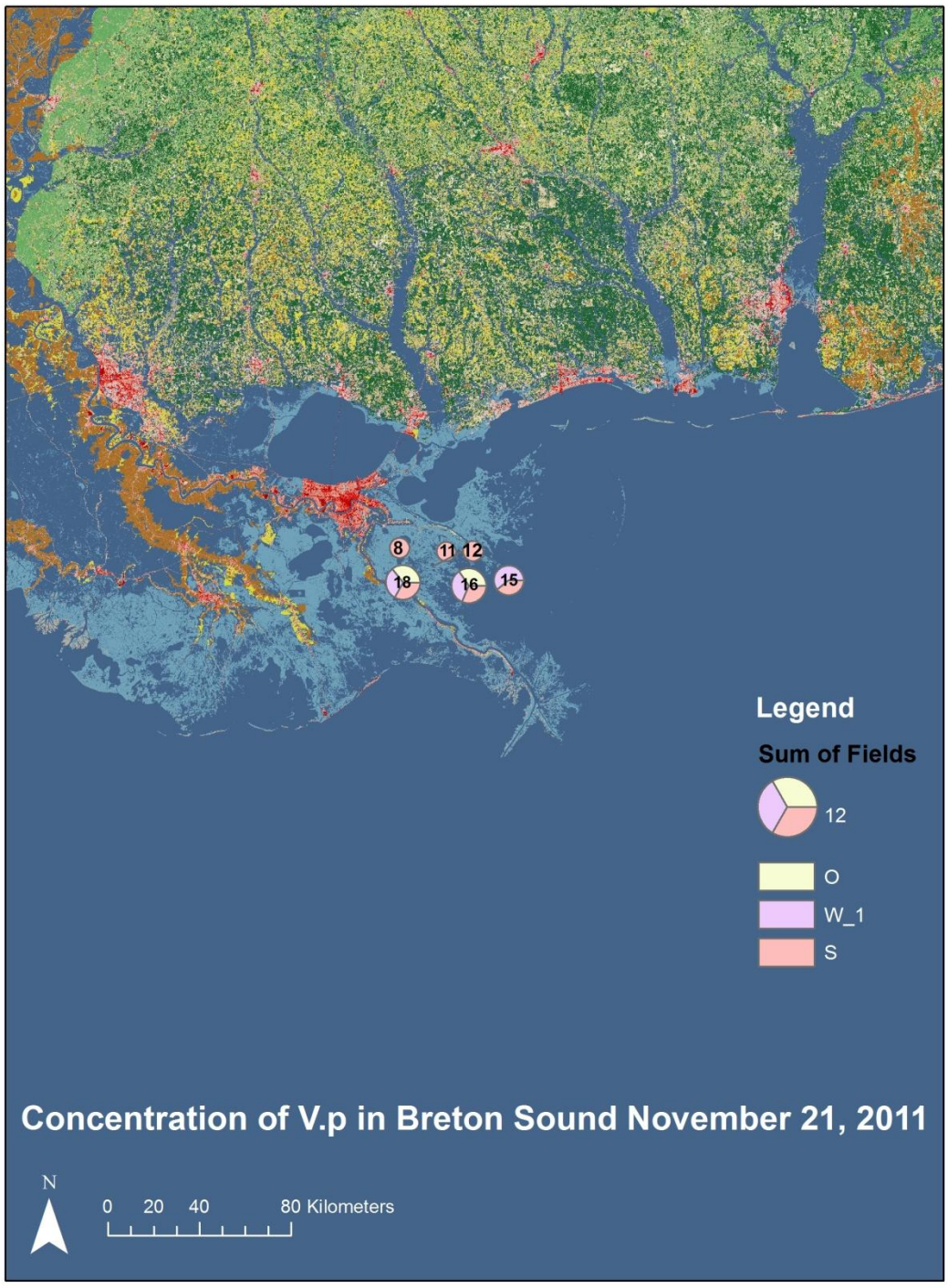
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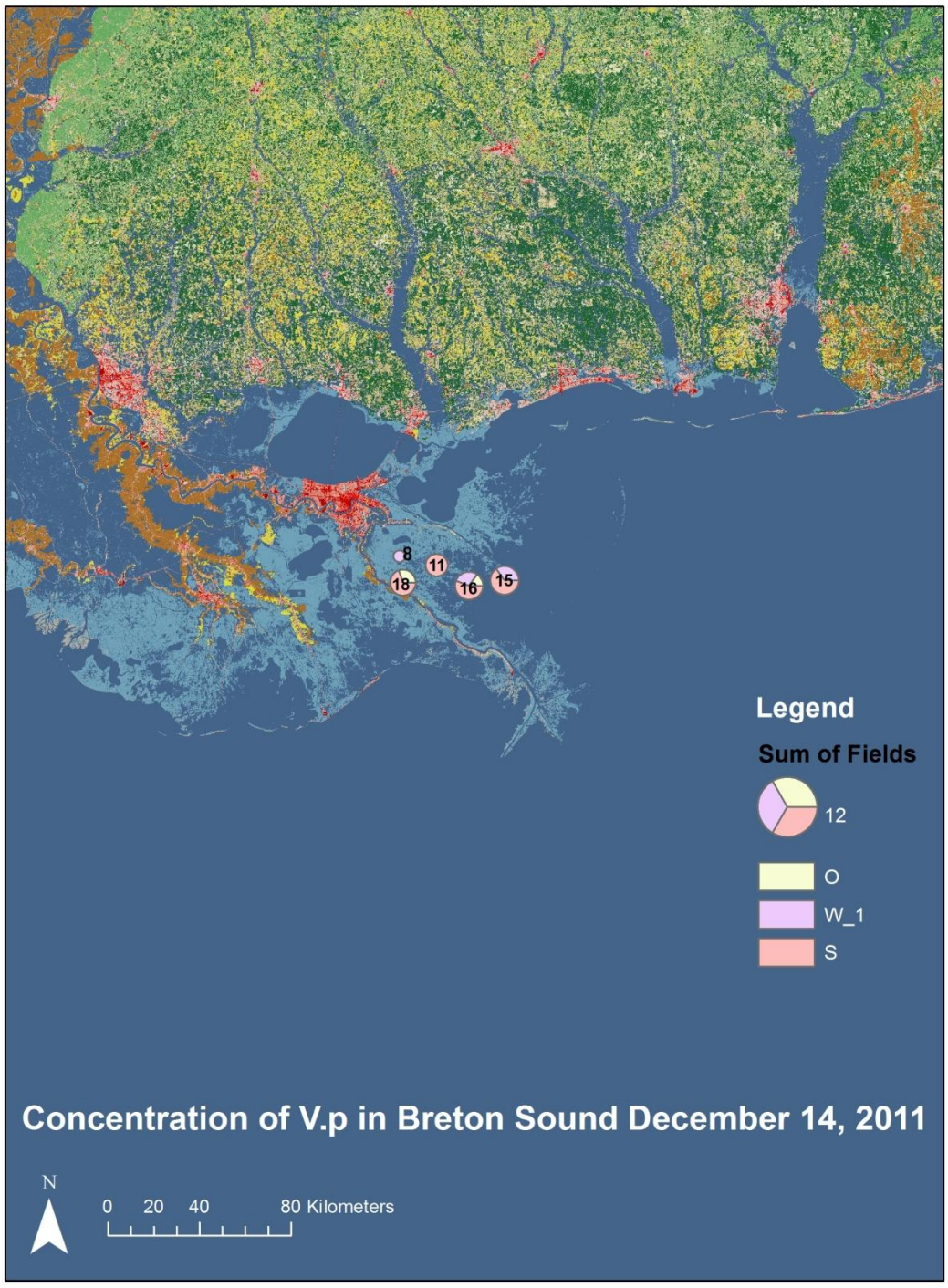
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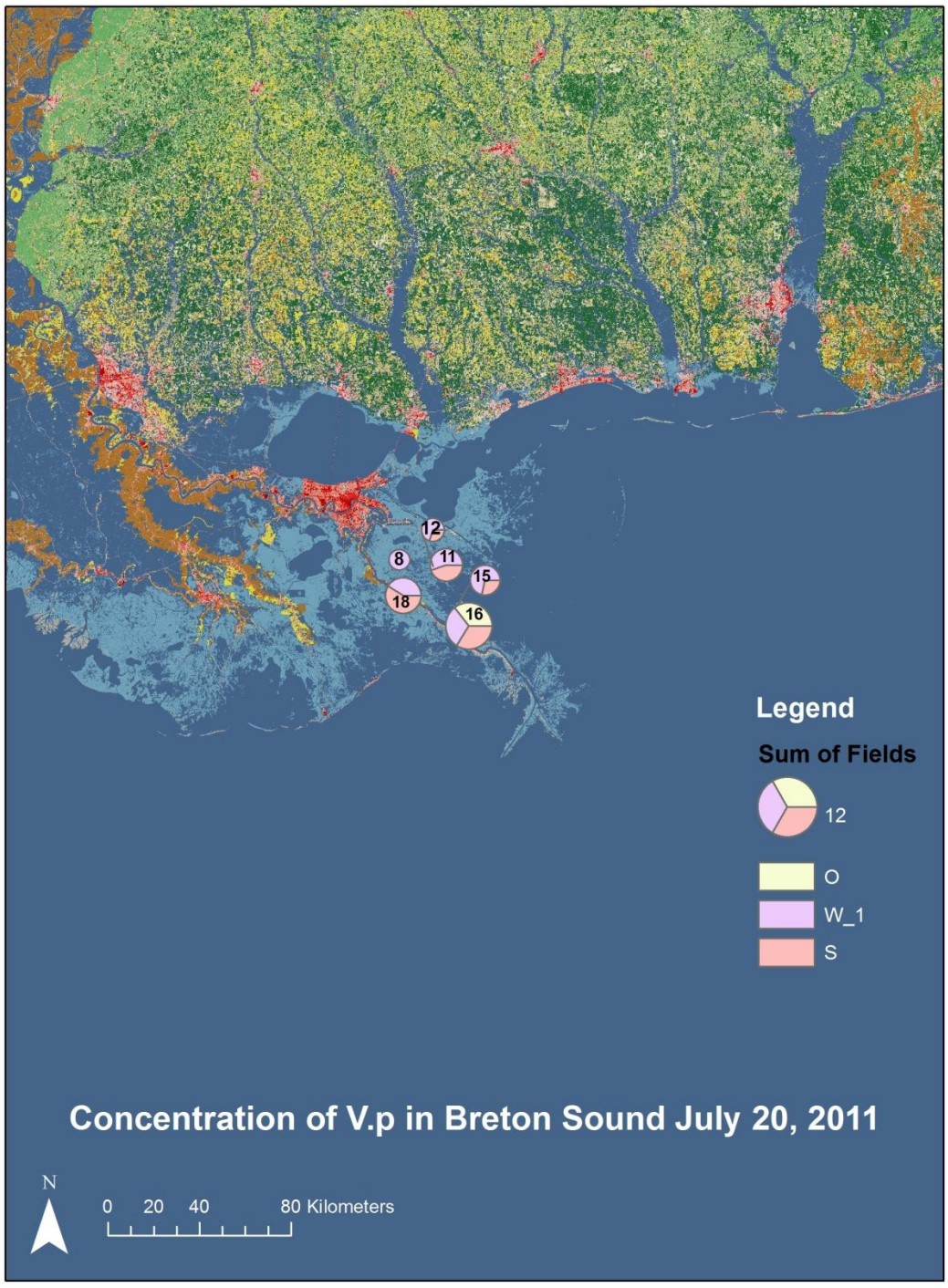
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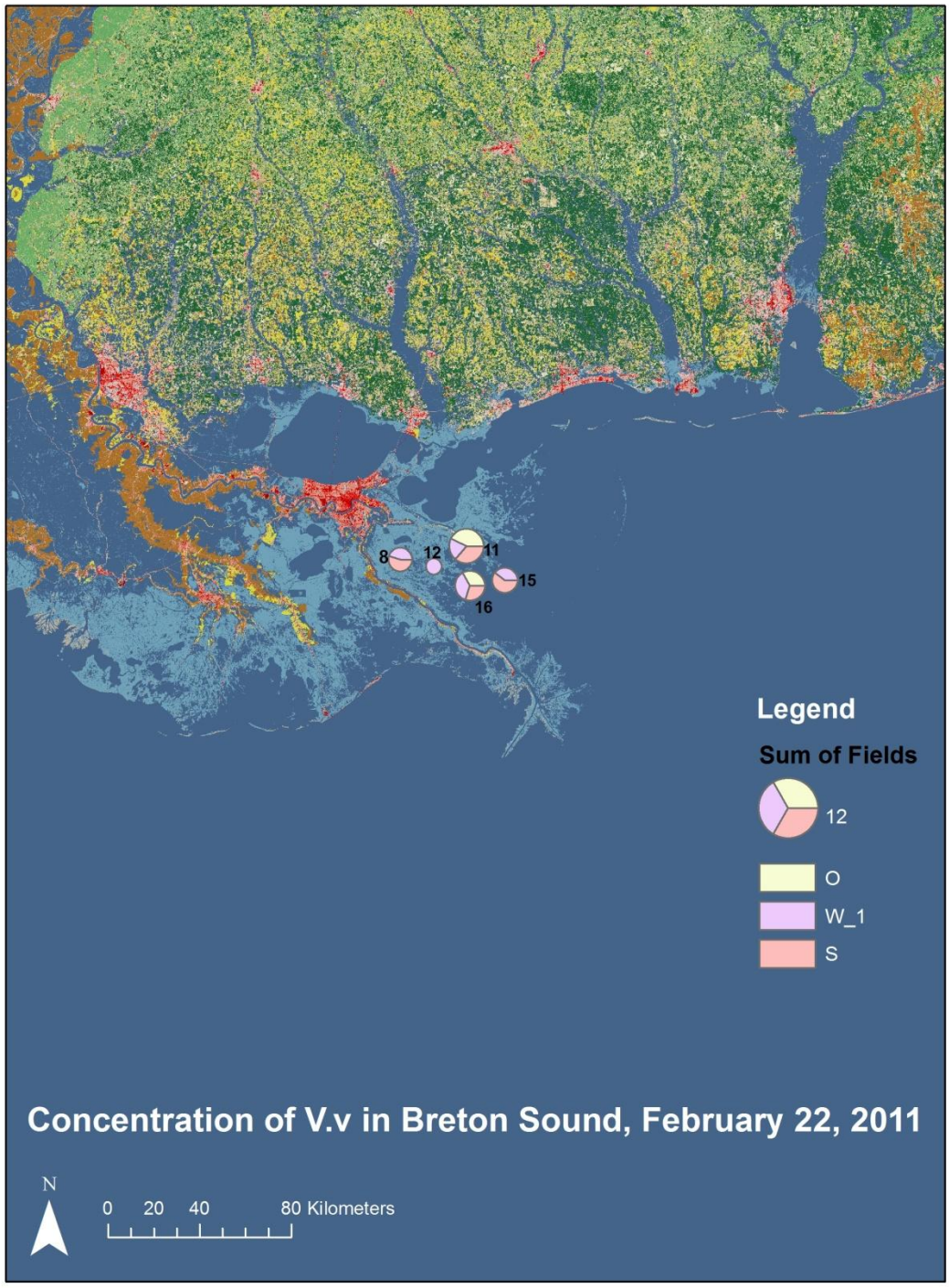
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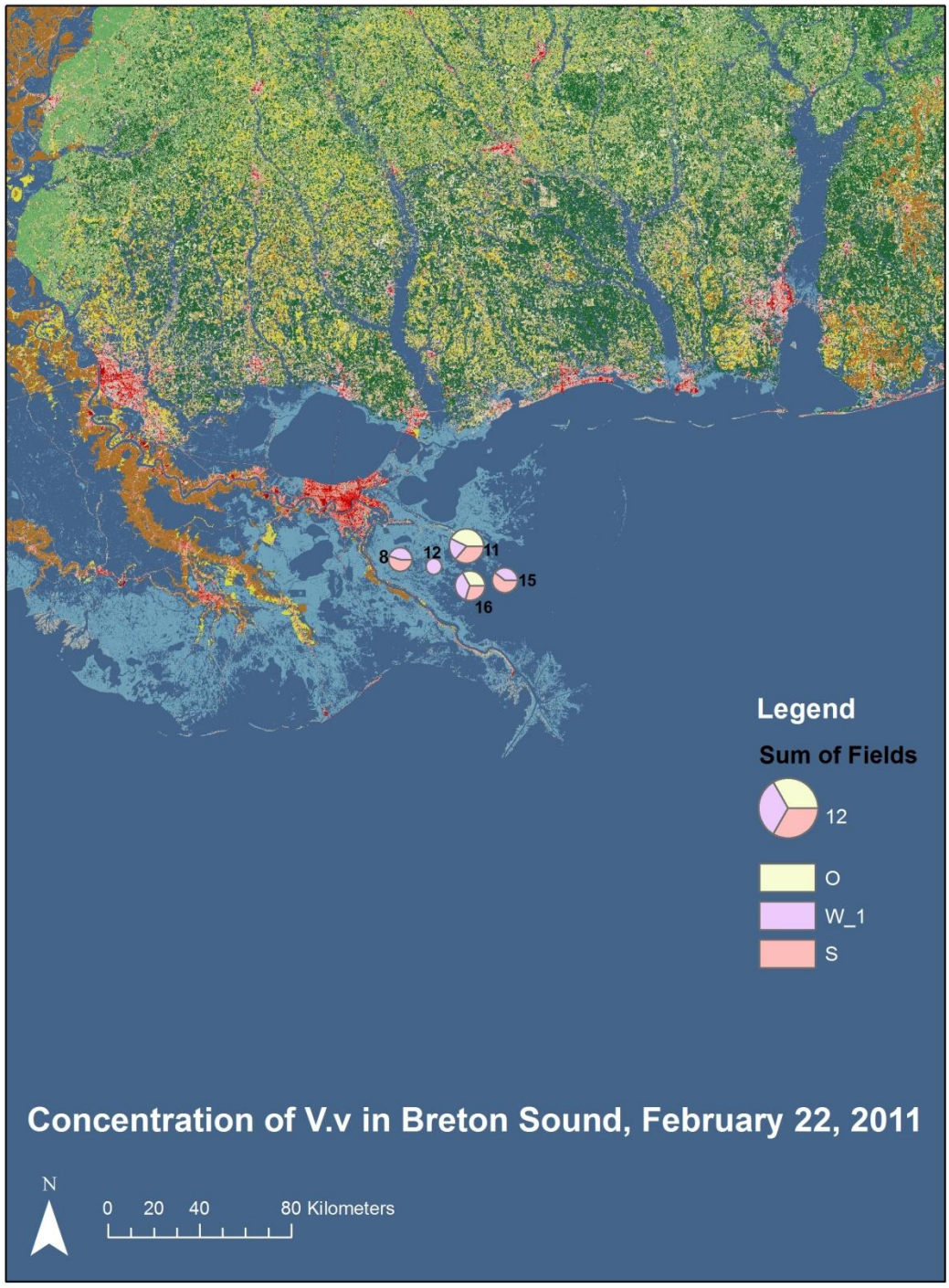
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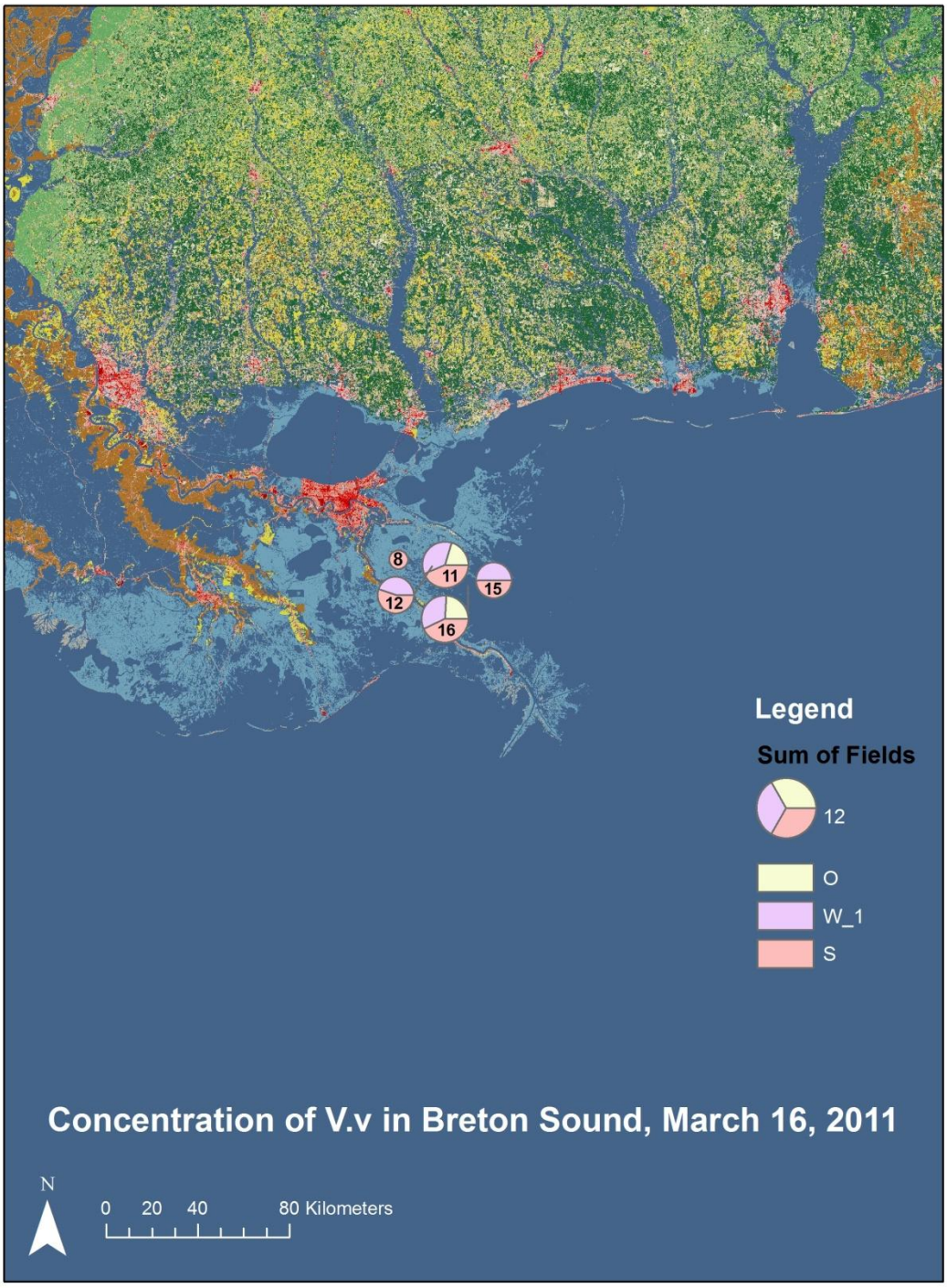
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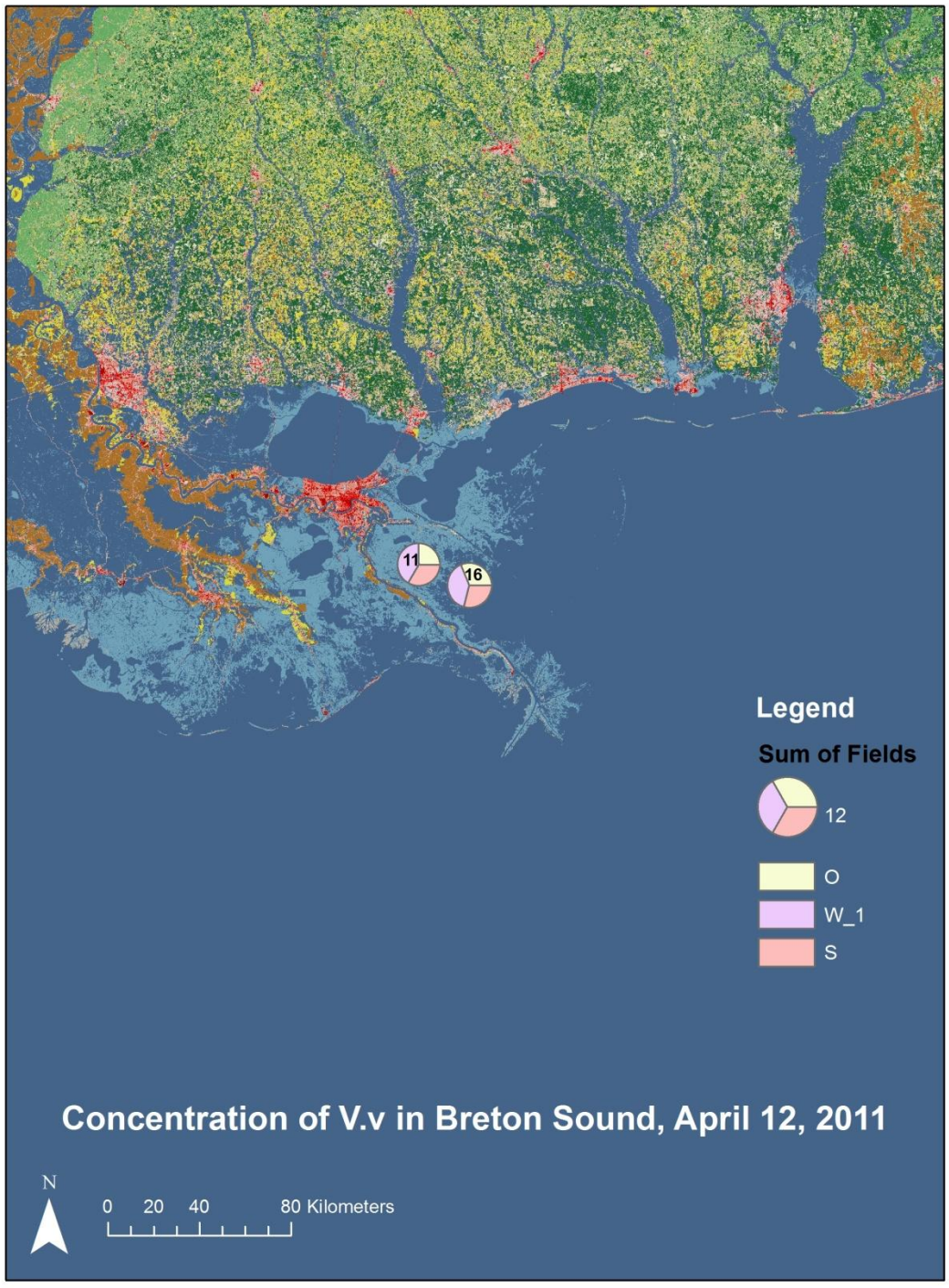
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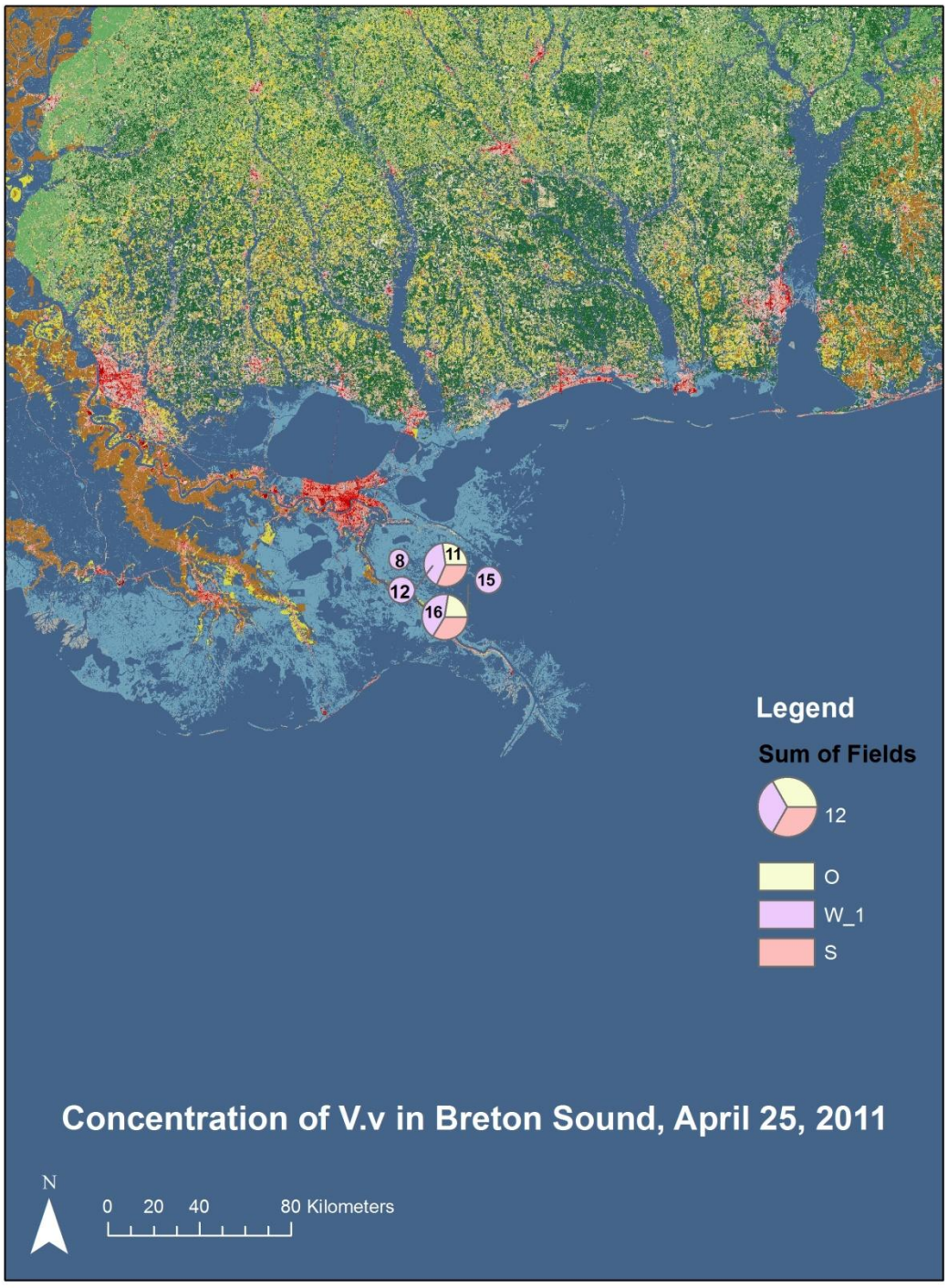
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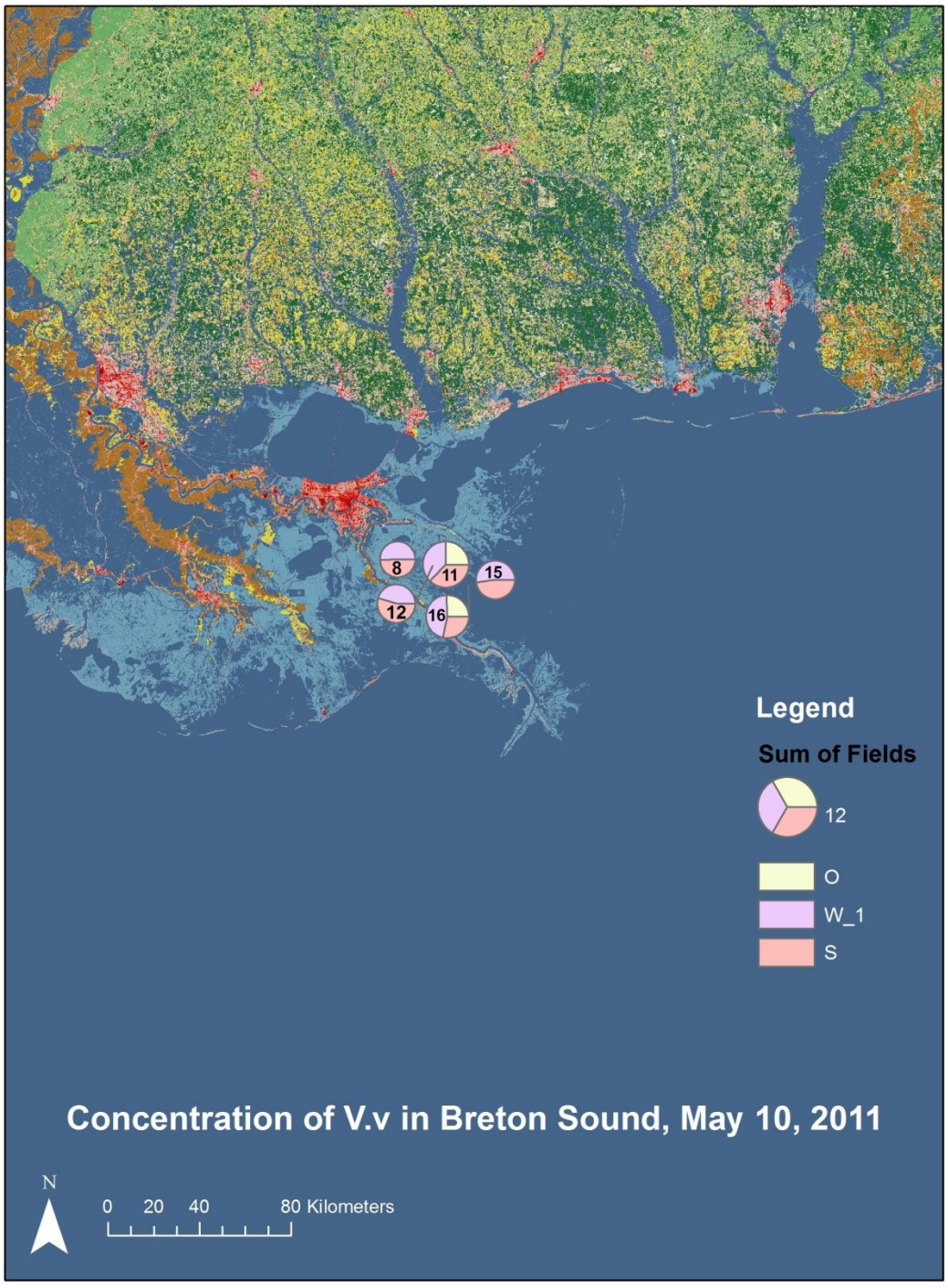
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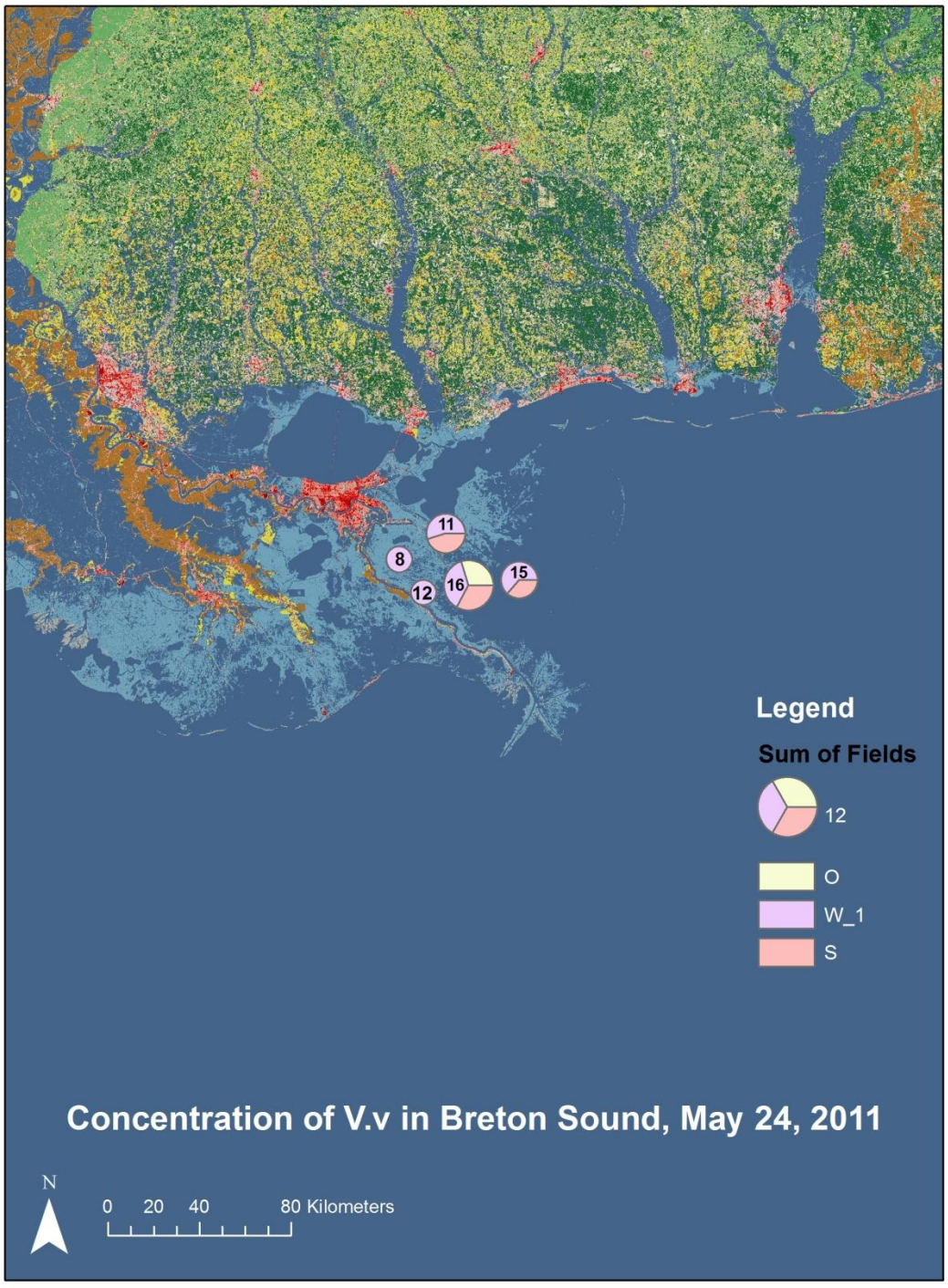
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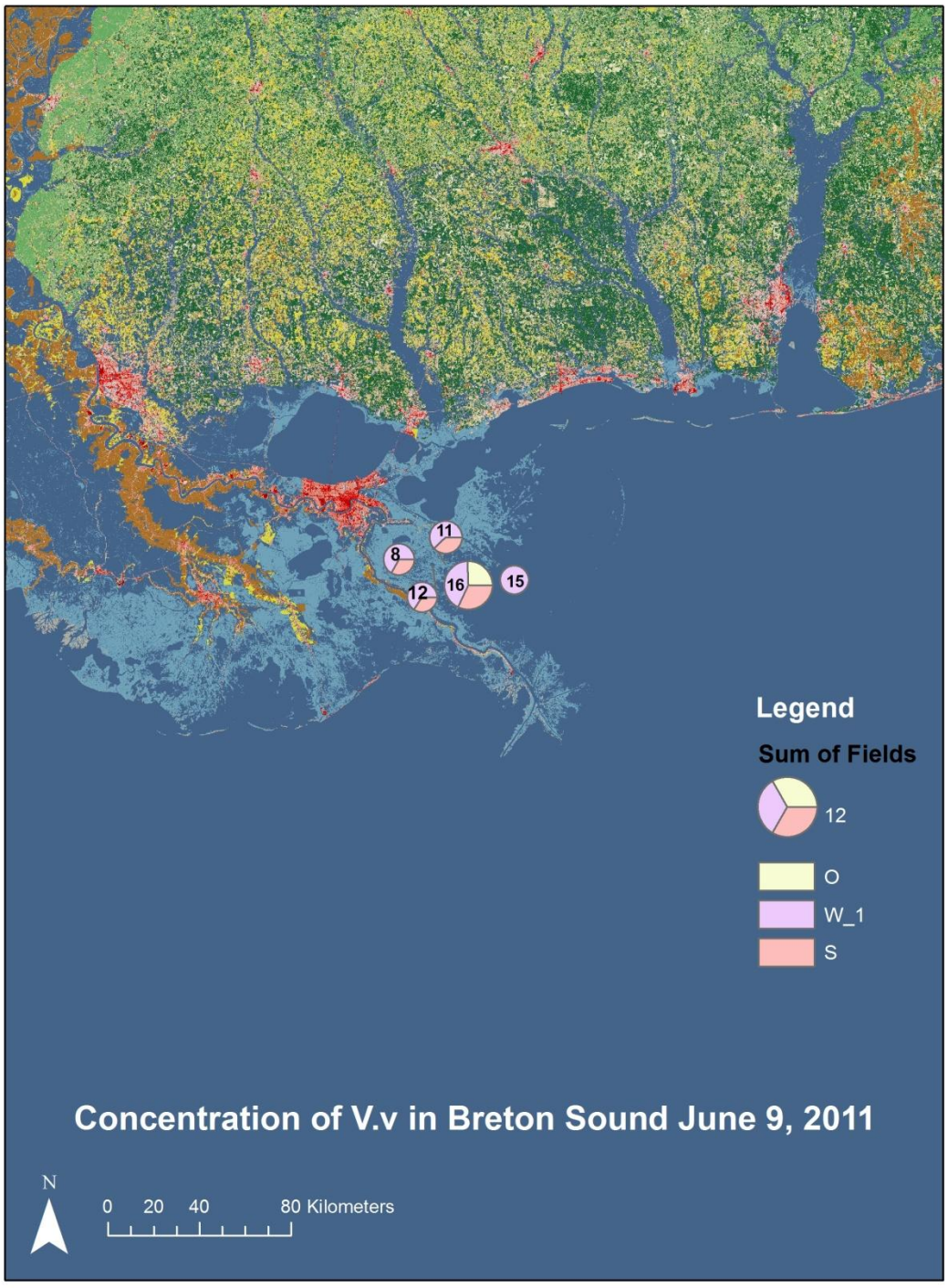
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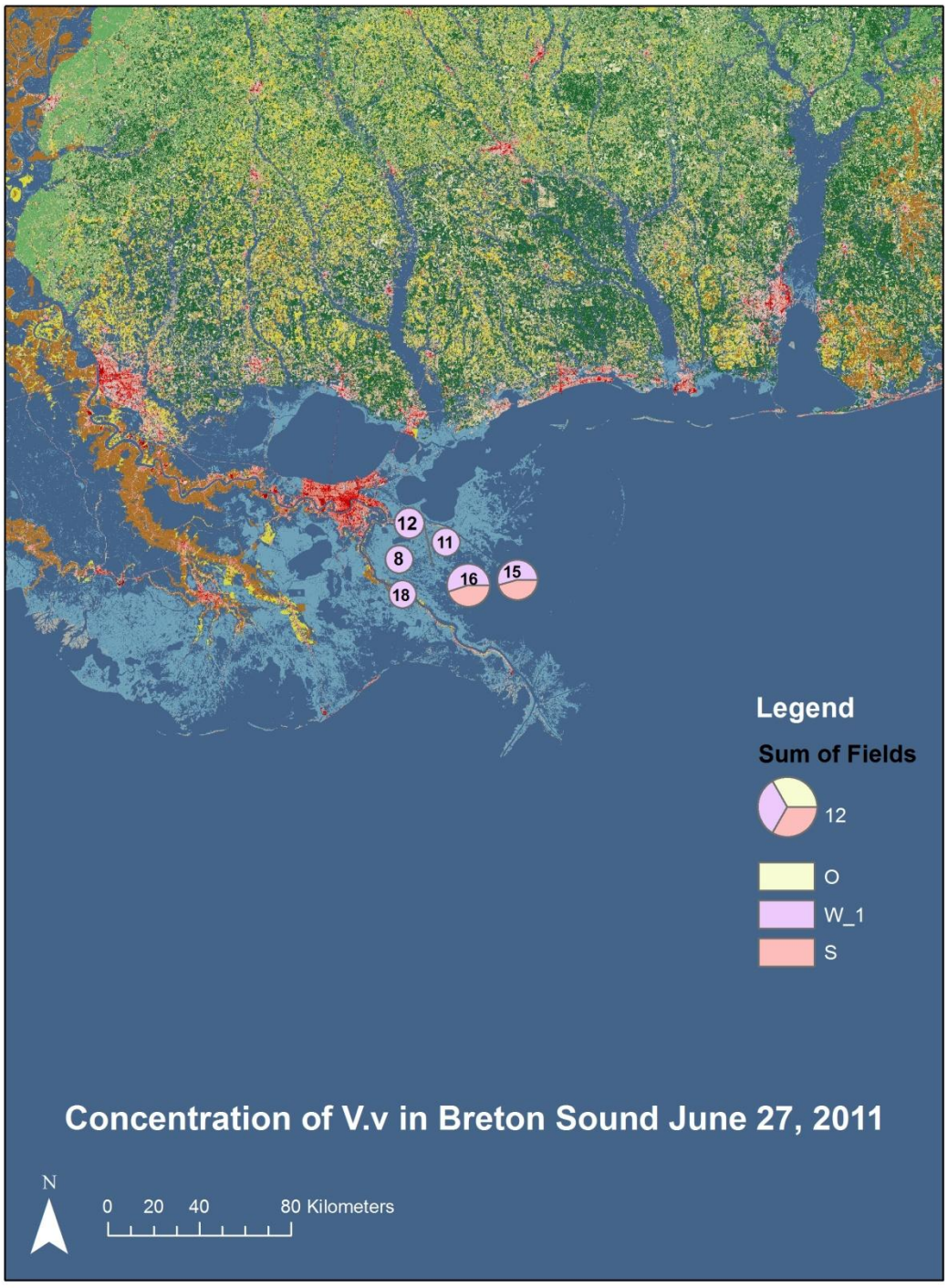
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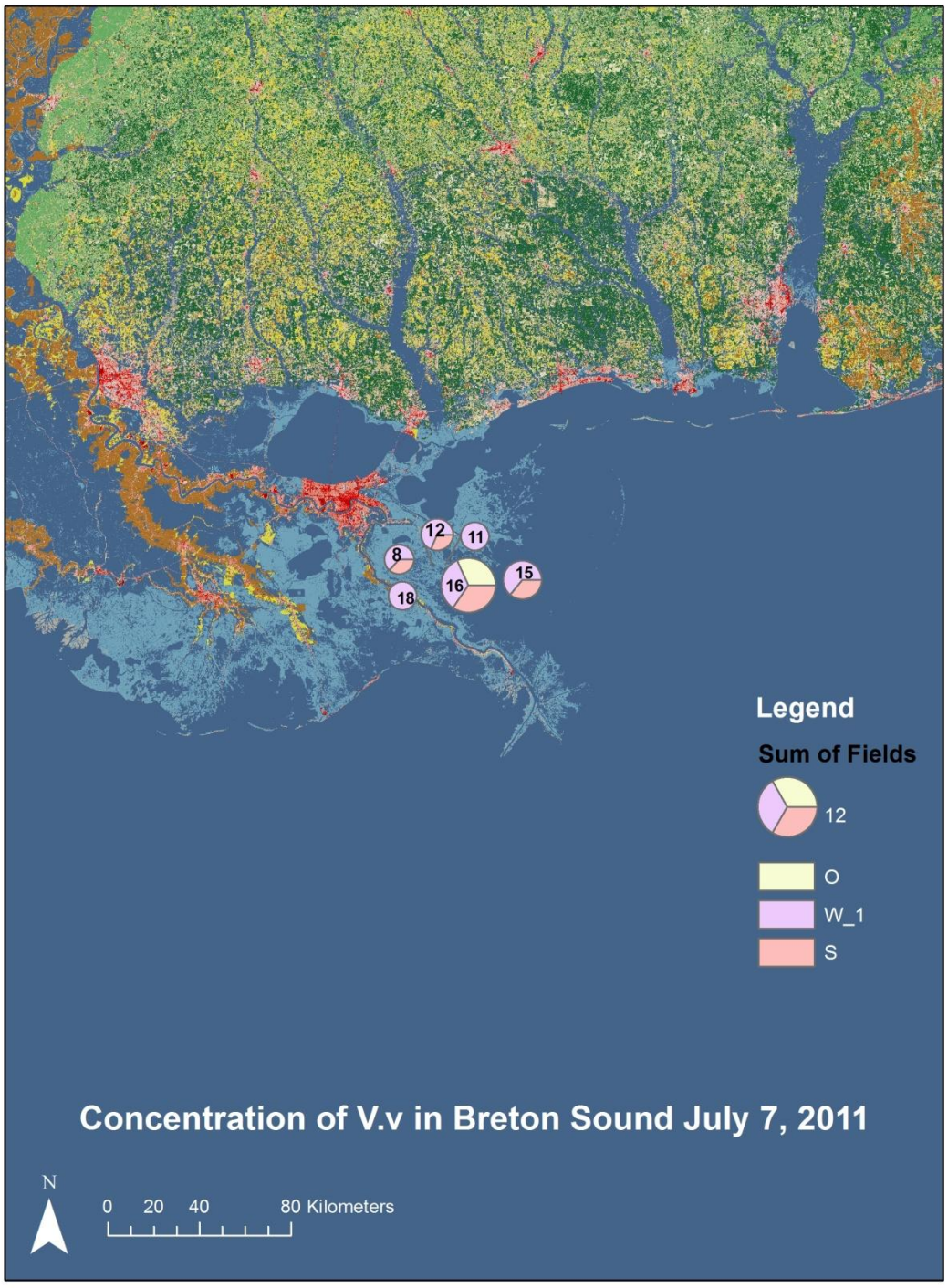
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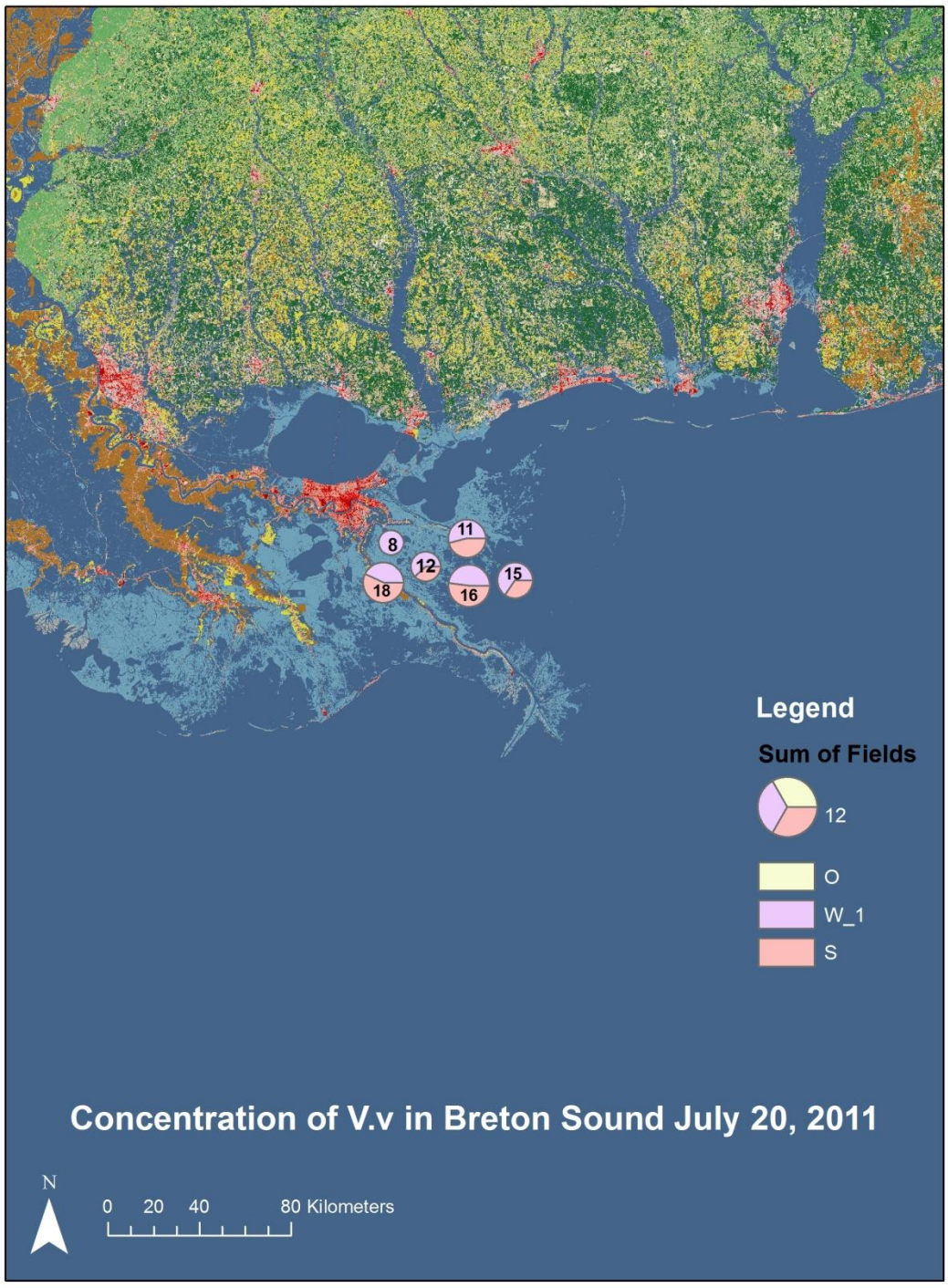
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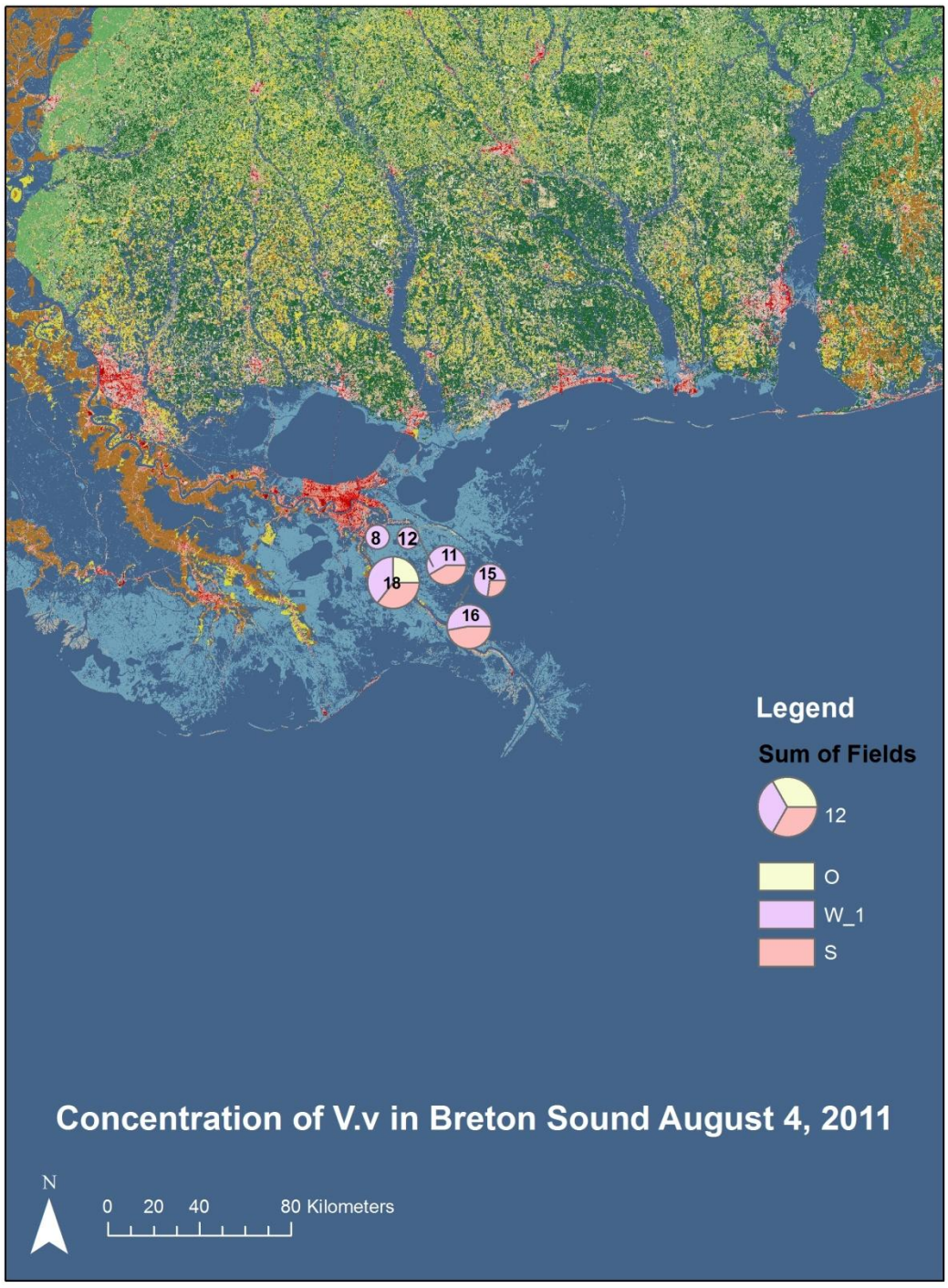
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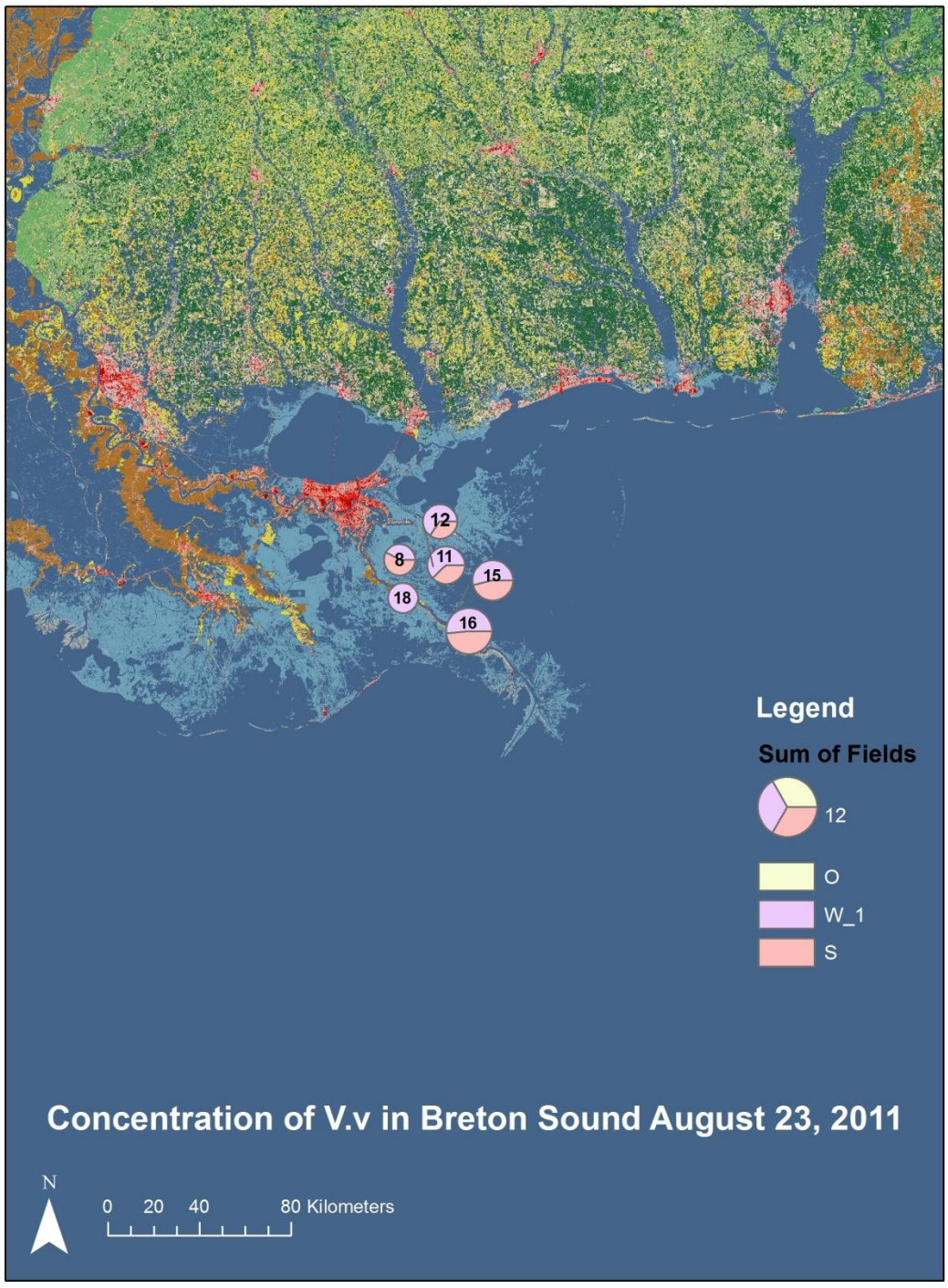
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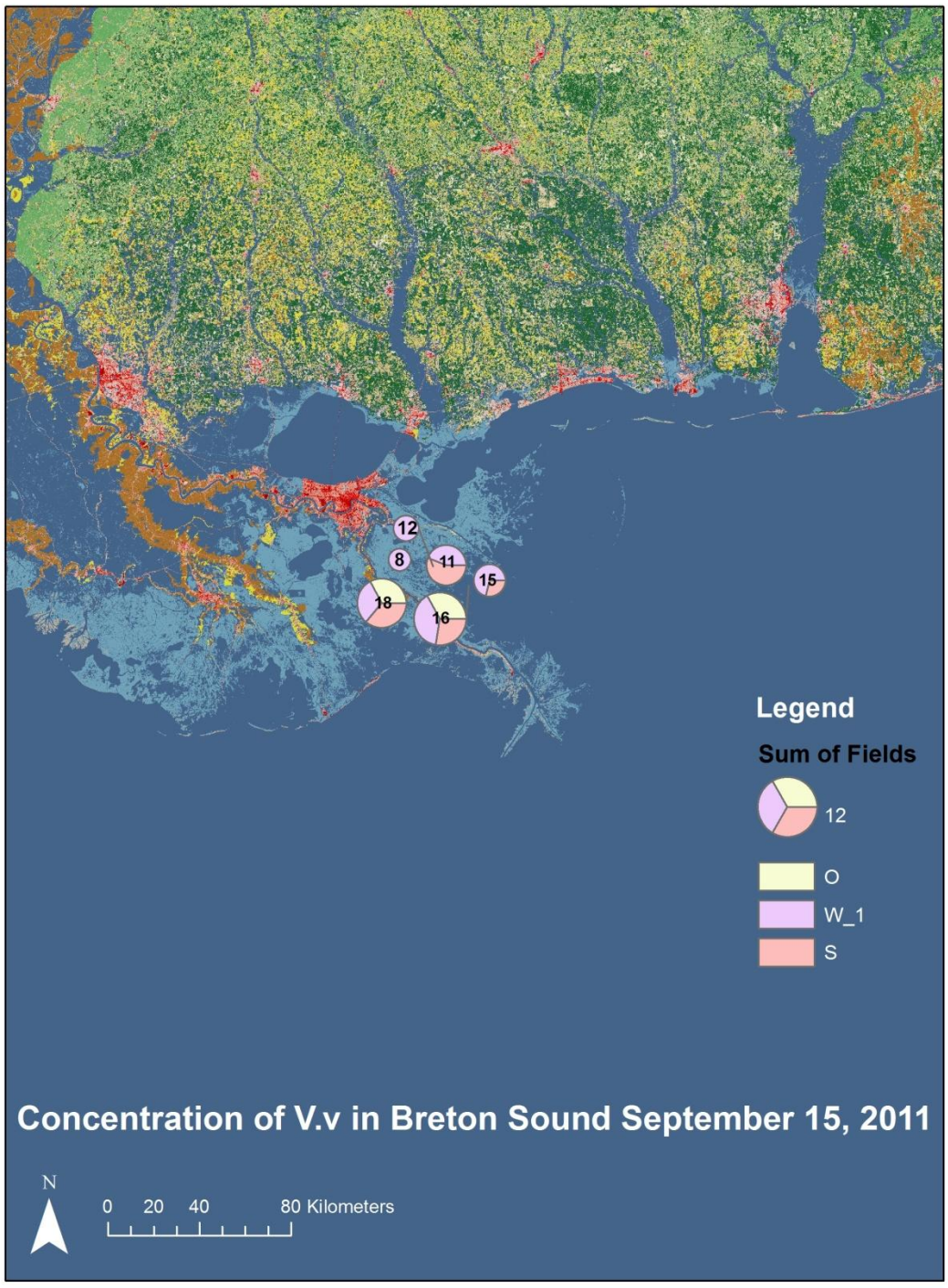
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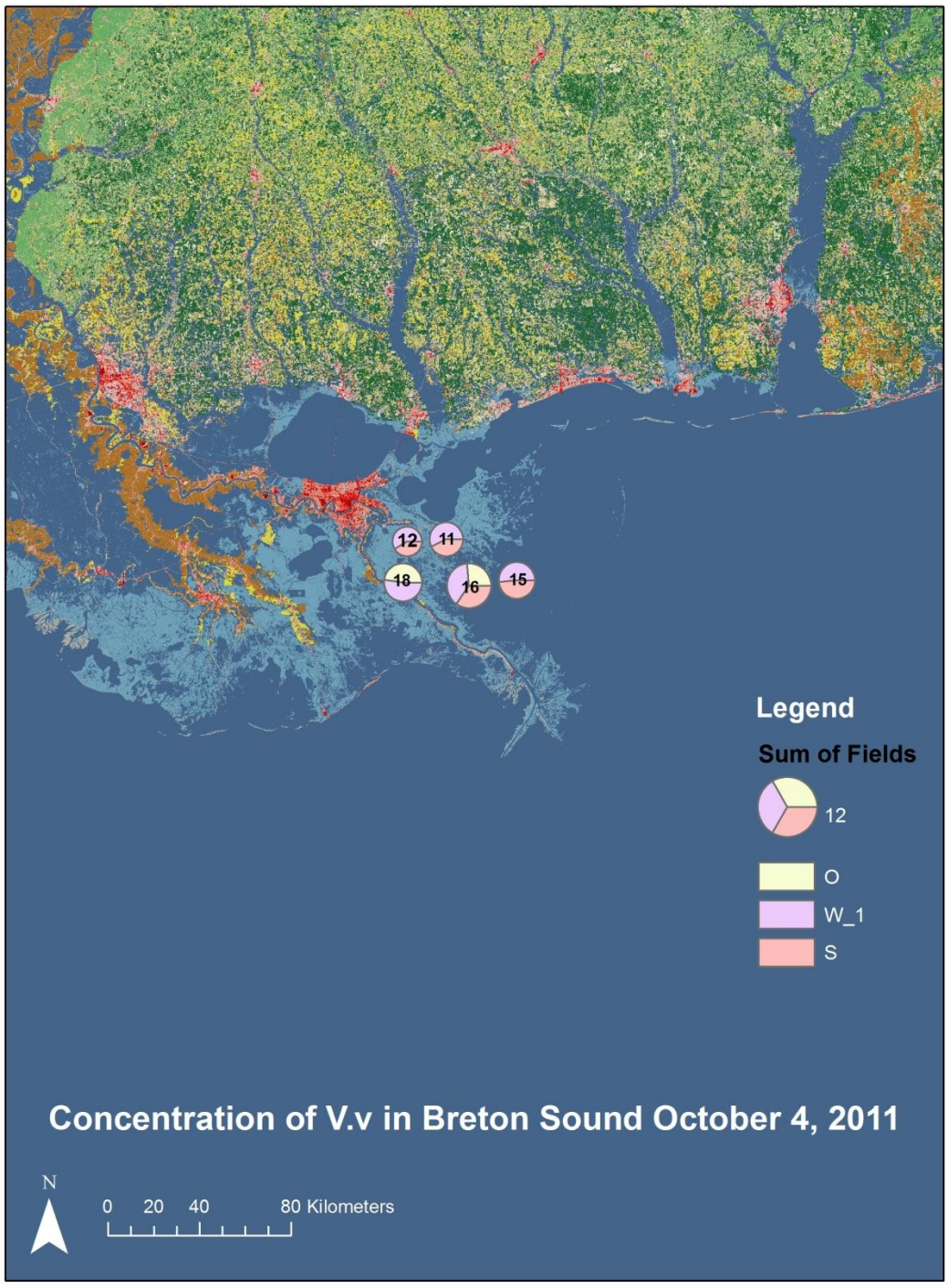
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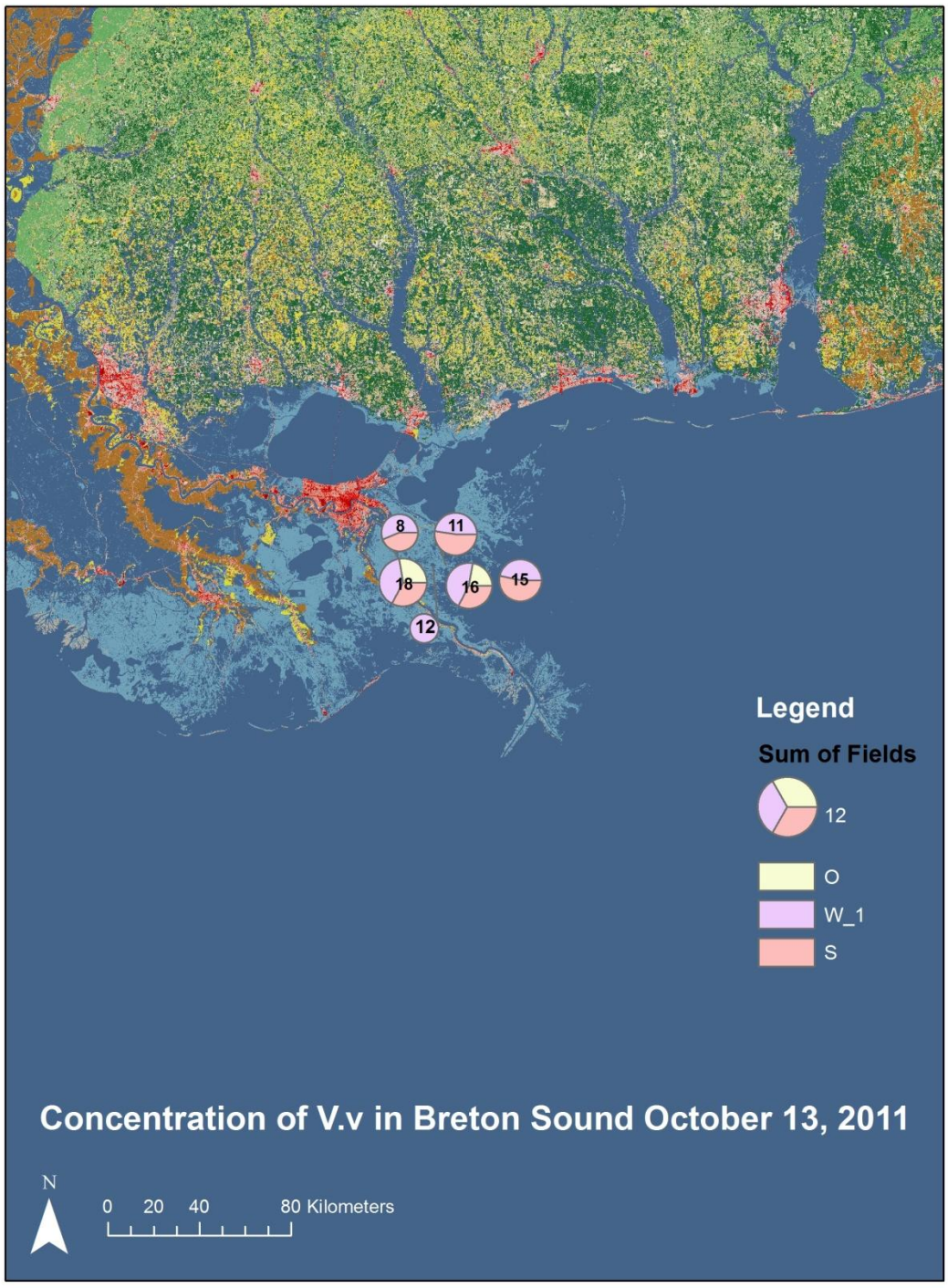
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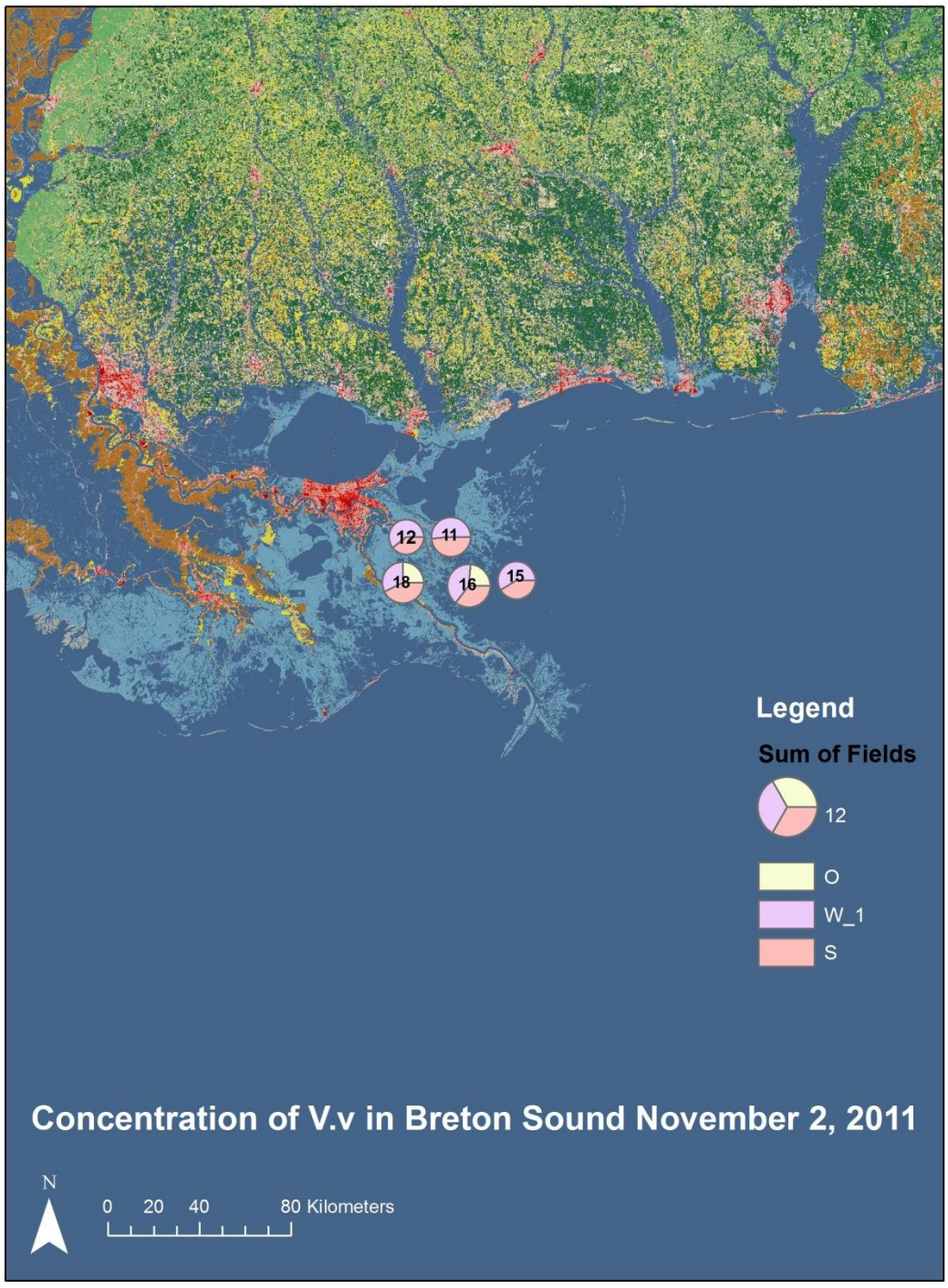
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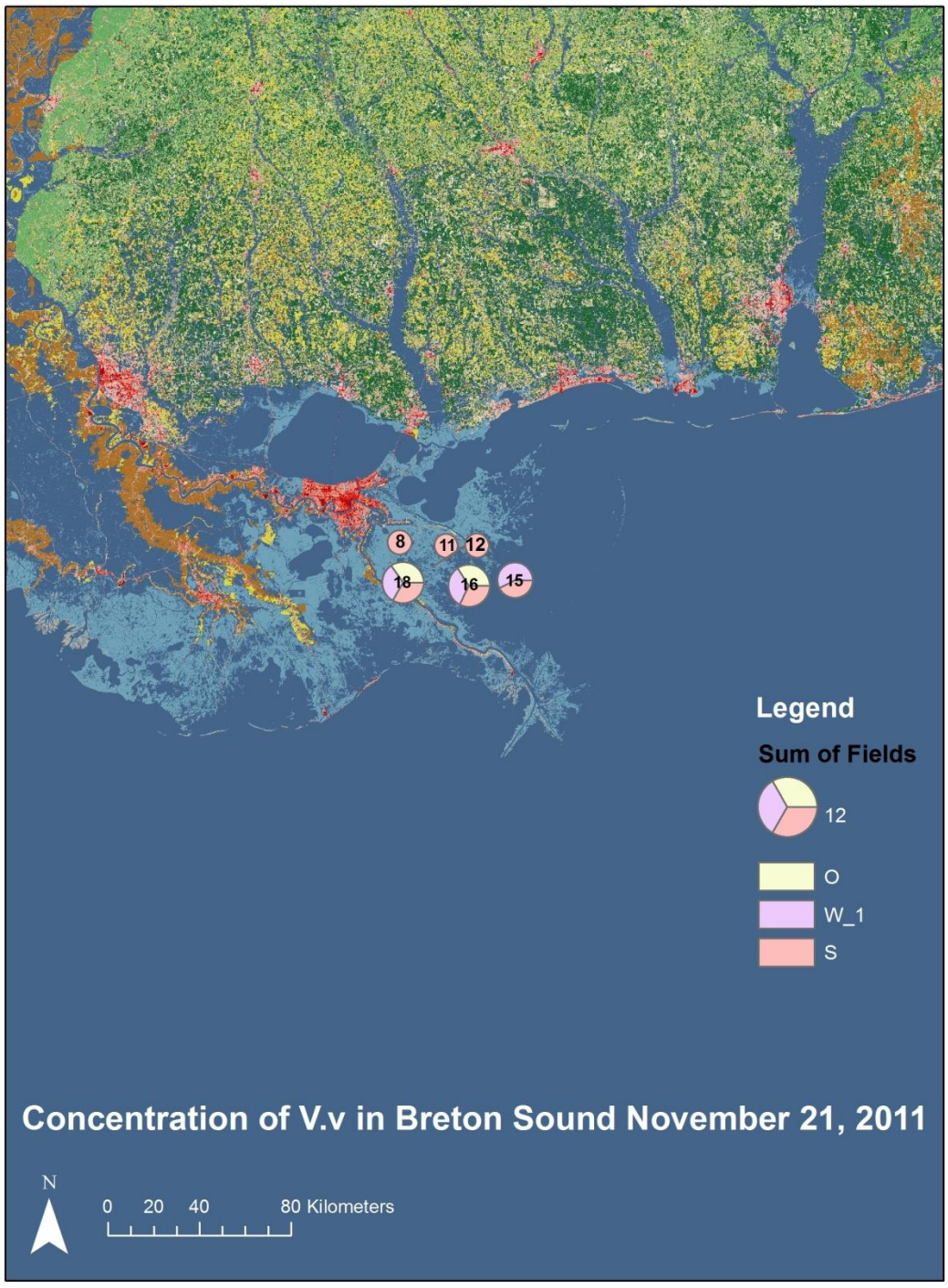
11
18
16
15
Legend
Sum of Fields
12
O
W_1
S
Concentration of V.v in Breton Sound November 2, 2011
N
0 20 40 80 Kilometers


8
11
12
18
16
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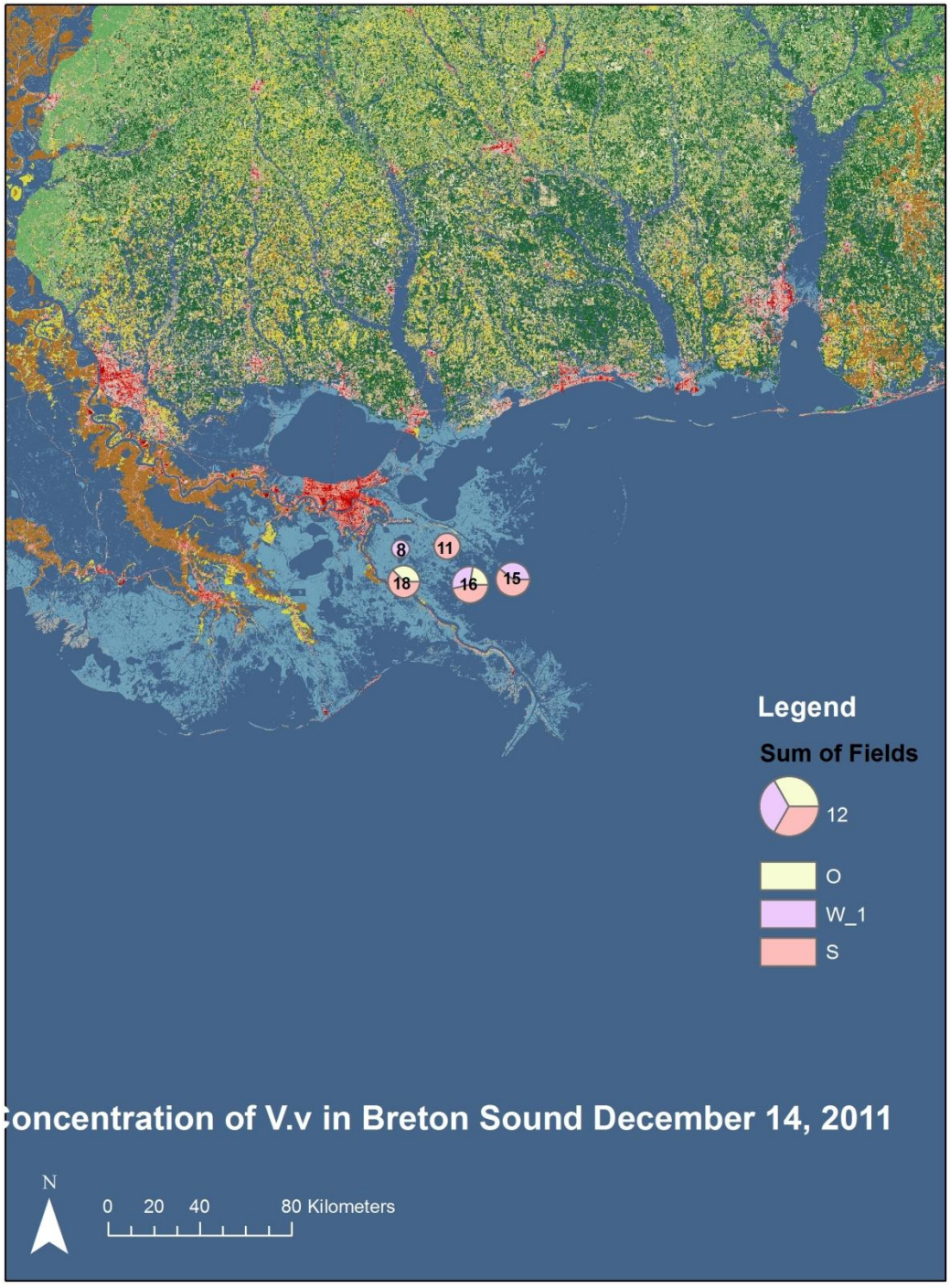
Legend
Sum of Fields
12
O
W_1
S
Concentration of V.v in Breton Sound November 21, 2011
N
0 20 40 80 Kilometers


8
11
18
16
15
Legend
Sum of Fields
12
O
W_1
S
oncentration of V.v in Breton Sound December 14, 2011
N
0 20 40 80 Kilometers