# JoVE: Science Education

## Dissolved Oxygen in Surface Water: The Azide-Winkler Titration Method

--Manuscript Draft--

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|---|---|
| **Manuscript Number:** | 10016 |
| **Full Title:** | Dissolved Oxygen in Surface Water: The Azide-Winkler Titration Method |
| **Article Type:** | Manuscript |
| **Section/Category:** | Manuscript Submission |
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**PI: Kimberly Frye and Margaret Workman, DePaul University**

**Environmental Science Education Title:**
Dissolved Oxygen in Surface Water: The Azide-Winkler Titration Method

**Overview:** Dissolved oxygen (DO) measurements calculate the amount of gaseous oxygen dissolved in surface water, of import to all oxygen-breathing life in river ecosystems including fish species preferred for human consumption (e.g. bluegill and bass), as well as decomposer species critical to the recycling of biogeochemical materials in the system.

The Azide-Winkler titration method uses a titration to determine the concentration of an unknown in a sample. Specifically, sodium thiosulfate is used to titrate iodine, which can be stoichiometrically related to the amount of dissolved oxygen in a sample.

**Principles:** The Azide-Winkler method is used to measure DO on site where surface water is collected. Manganese (II) Sulfate and Potassium Hydroxide are added to the sample, and the dissolved oxygen in the sample oxidizes the manganese and forms a brown precipitate.

$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4$

$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2$

Sulfuric acid is then added to acidify the solution and the precipitate dissolves. Under these conditions, the iodide in the solution is converted into iodine.

$2\ MnO(OH)_2 + 4\ H_2SO_4 \rightarrow 2\ Mn(SO_4)_2 + 6\ H_2O$

$2\ Mn(SO_4)_2 + 4\ KI \rightarrow 2\ MnSO_4 + 2\ I_2 + 2\ K_2SO_4$

Thiosulfate is then used to titrate the iodine in the presence of a starch indicator.

$4\ Na_2S_2O_3 + 2\ I_2 \rightarrow 2\ Na_2S_4O_6 + 4\ NaI$

At the endpoint of this titration, the blue solution will turn clear. The amount of dissolved oxygen in the sample is directly proportional to the amount thiosulfate required to reach the endpoint.

1 mole of $O_2 \rightarrow$ 4 moles of $S_2O_3^{2-}$

**Procedure:**

1. **Measure Dissolved Oxygen in sample.**

**1.1.** Using a calibrated pipette, add 2 mL manganous sulfate to a clear 300 mL BOD bottle filled with your sample water. Be careful not to introduce oxygen into the sample by inserting pipette tip under the sample surface and carefully dispensing manganous sulfate to avoid creating bubbles.

**1.2.** Using the same technique, add 2 mL alkaline iodide-azide reagent.

**1.3.** Immediately insert the stopper, tilting the bottle slightly and quickly pushing the stopper in place so no air bubbles are trapped in the bottle.

**1.4.** Carefully invert several times (without creating air bubbles) to mix and a floccule (floc) will form from a precipitated aggregation of material with a cloudy appearance. (Fig 1)

**1.5.** Wait until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled. The sample is now "fixed" and can be stored for up to 8 hours if needed in a cool and dark condition.

**1.6.** If storing, samples should be sealed using a small amount of deionized water squirted around stopper and stopper should be wrapped in aluminum foil secured with a rubber band.

**1.7.** Pipette 2 mL of concentrated sulfuric acid into the sample by holding the pipette tip just above the sample surface. Invert carefully several times to dissolve the floc. (Fig 2)

**1.8.** In a glass flask and using a calibrated pipette, titrate 201 mL of sample water with 0.0025 N standardized sodium thiosulfate, swirling and mixing continuously until a pale straw color forms. (Fig 3)

**1.9.** Add 2 mL droppers of starch indicator solution and swirl to mix. Once you add the Starch Indicator, the solution will turn blue. (Fig 4)

**1.10.** Continue the titration, adding one drop at a time until one drop dissipates the blue causing the colorless endpoint. Be sure to add each drop of titrant carefully and to evenly mix each drop before adding the next. Holding the sample against a white piece of paper can help enhance visualization of the endpoint.

**1.11.** The concentration of DO is equivalent to the volume (mL) of titrant used. Each milliliter of sodium thiosulfate added to the water sample equals 1 mg/L dissolved oxygen.

**2. Representative Results:**

2.1 A dissolved oxygen level of 5 - 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below 3 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 or 1 mg/L will not support fish.

2.2 The maximum amount of oxygen that can be dissolved in water varies by temperature (table 1):

2.3 DO measurements in mg/L are converted to % saturation using water temperature and the conversion chart below.(fig 5)

**DISSOLVED OXYGEN LEVELS (% SATURATION)**

Excellent 91 – 110
Good 71 – 90
Fair 51 – 70
Poor < 50

**Applications:** Slow-moving rivers are particularly vulnerable to low DO levels, and in extreme these DO levels can lead to hypoxic conditions creating “dead zones” where aerobic life is no longer supported by a body of water. Once plants and animals die-off, the build-up of sediment that occurs can also raise the river bed allowing plants to colonize over the water and the loss of the river all together. Surface waters at higher altitudes are also more vulnerable to low DO levels as atmospheric pressure decreases with increasing altitude and less oxygen gas is suspended in the water.

Low DO levels also support life forms considered unappealing or unfit for human use including leeches and aquatic worms (*Oligochaeta)*.

Legend:
Fig 1. A sample after the alkaline iodide-azide reagent has been added and mixed, showing floc formation at the top of the sample before settling.

Fig 2. A sample with dissolved floc after addition of sulfuric acid.

Fig 3. A sample after addition of sodium thiosulfate displaying a plate straw color.

Fig 4. A sample showing the blue color after the starch indicator is added and mixed.

Fig 5: DO measurements are converted to % saturation using the water's temperature. The water's temperature (10 $^{o}$C in this example) on the top horizontal axis and the measured DO value (4.5 mg/L in this example) on the bottom horizontal axis. Use a ruler to draw a line between the two values and record where the line meets the middle diagonal axis for % saturation (40% in this example).

Table 1: Maximum amounts of oxygen that can be dissolved in water by temperature

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| Temp. ($°C$) | DO (mg/L) | Temp. ($°C$) | DO (mg/L) | Temp. ($°C$) | DO (mg/L) | Temp. ($°C$) | DO (mg/L) |
|---|---|---|---|---|---|---|---|
| 0 | 14.60 | 11 | 11.01 | 22 | 8.72 | 33 | 7.16 |
| 1 | 14.19 | 12 | 10.76 | 23 | 8.56 | 34 | 7.16 |
| 2 | 13.81 | 13 | 10.52 | 24 | 8.40 | 35 | 6.93 |
| 3 | 13.44 | 14 | 10.29 | 25 | 8.24 | 36 | 6.82 |
| 4 | 13.09 | 15 | 10.07 | 26 | 8.09 | 37 | 6.71 |
| 5 | 12.75 | 16 | 9.85 | 27 | 7.95 | 38 | 6.61 |
| 6 | 12.43 | 17 | 9.65 | 28 | 7.81 | 39 | 6.51 |
| 7 | 12.12 | 18 | 9.45 | 29 | 7.67 | 40 | 6.41 |
| 8 | 11.83 | 19 | 9.26 | 30 | 7.54 | 41 | 6.41 |
| 9 | 11.55 | 20 | 9.07 | 31 | 7.41 | 42 | 6.22 |
| 10 | 11.27 | 21 | 8.90 | 32 | 7.28 | 43 | 6.13 |

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**Overview:** Dissolved oxygen (DO) measurements calculate the amount of gaseous oxygen dissolved in surface water, which is important to all oxygen-breathing life in river ecosystems, including fish species preferred for human consumption (e.g. bluegill and bass), as well as decomposer species critical to the recycling of biogeochemical materials in the system.

The oxygen dissolved in lakes, rivers, and oceans is crucial for the organisms and creatures living in it. As the amount of dissolved oxygen drops below normal levels in water bodies, the water quality is harmed and creatures begin to die ~~-off~~. In a process called eutrophication, a body of water can become hypoxic and will no longer be able to support living organisms, essentially becoming a "dead zone."

Eutrophication occurs when excess nutrients cause algae populations to grow rapidly in an algal bloom. The algal bloom forms dense mats at the surface of the water blocking out two essential inputs of oxygen for water: gas exchange from the atmosphere and photosynthesis in the water due to the lack of light below the mats. As dissolved oxygen levels decline below the surface, oxygen-breathing organisms die-off in large amounts, creating an increase in organic matter. The excess organic matter causes an increase in the oxygen-breathing decomposer populations in the benthic zone, which further depletes the remaining dissolved oxygen levels during the metabolic decomposition activity. Once the oxygen levels become this low, mobile oxygen-breathing species (e.g. fish) will move away, leaving no aerobic life in the water and creating a dead zone.

The Azide-Winkler titration method uses titration to determine the concentration of an unknown in a sample. Specifically, sodium thiosulfate is used to titrate iodine, which can be stoichiometrically related to the amount of dissolved oxygen in a sample.

**Principles:** The Azide-Winkler method is used to measure DO on site, where surface water is collected. Manganese (II) sulfate and potassium hydroxide are added to the sample, and the dissolved oxygen in the sample oxidizes the manganese and forms a brown precipitate. Azide is added in the form of a purchased alkaline iodide-azide reagent to correct for the presence of nitrites, which are found in wastewater samples and can interfere with the Winkler oxidation procedure.

$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4$

**Comment [1]:** Flesh out more about the importance of dissolved oxygen in ecosystems.

**Comment [2]:** Where does the azide come into the chemistry? Is it just the counter ion for the iodide?

**Comment [3]:** Is this a precipitate, as well?

**Comment [4]:** no

$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2$ (precipitate)

Sulfuric acid is then added to acidify the solution, and the precipitate dissolves. ~~-~~Under these conditions, the iodide from the alkaline iodide-azide reagent in the solution is converted into iodine.

Comment [5]: When is this added to the solution?

Comment [FK6]: In the previous step. Clarification added to the paragraph above.

$2\ MnO(OH)_2 + 4\ H_2SO_4 \rightarrow 2\ Mn(SO_4)_2 + 6\ H_2O$

$2\ Mn(SO_4)_2 + 4\ KI \rightarrow 2\ MnSO_4 + 2\ I_2 + 2\ K_2SO_4$

Thiosulfate is then used to titrate the iodine in the presence of an added starch indicator.

Comment [7]: This is added as well?

$4\ Na_2S_2O_3 + 2\ I_2 \rightarrow 2\ Na_2S_4O_6 + 4\ NaI$

4 moles of $S_2O_3^{2-}$ → 1 mole of $O_2$

At the endpoint of this titration, the blue solution will turn clear. The amount of dissolved oxygen in the sample is quantified in direct proportion to the amount of thiosulfate required to reach the endpoint.

1ml $S_2O_S$ → 1mg/l O:

$$\frac{1\ \text{ml S2O3}}{200\text{ml}} \times \frac{1\text{L}}{1000\ \text{mL}} \times \frac{0.025\ \text{moles}}{\text{L}} \times \frac{1\ \text{moles}}{4\ \text{moles}} \times \frac{32\ \text{g}\ O_2}{1\ \text{mole}} \times \frac{1000\text{mg}}{1\text{g}} \times \frac{1000\text{ml}}{1\text{L}} = 1\text{mg/l}\ O_2$$

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**Procedure:**

1. **Measure Dissolved Oxygen in sample.**

1.1. At water collection site, u~~U~~se~~ing~~ a calibrated pipette to~~,~~ add 2 mL manganous sulfate to a clear 300 mL BOD bottle filled with the sample water. Be careful not to introduce oxygen into the sample by inserting the pipette tip under the sample surface and carefully dispensing manganous sulfate. This will avoid creating bubbles until the sample is “fixed”~~” to~~ and prevent changes to the dissolved oxygen concentration.~~.~~

Comment [8]: How do you limit change in dissolved oxygen content when transporting the sample?

1.2. Using the same technique, add 2 mL alkaline iodide-azide reagent.

1.3. Immediately insert the stopper, tilting the bottle slightly and quickly pushing the stopper in place so no air bubbles are trapped in the bottle.

1.4. Carefully invert several times (without creating air bubbles) to mix. A floccule (floc) will form from a precipitated aggregation of material with a cloudy appearance (Fig. 1).

1.5. Wait until the floc in the solution has settled. Again, invert the bottle several times and wait until the floc has settled. The sample is now “fixed” to prevent change in dissolved oxygen content and can be transported back to the lab and stored for up to 8 hours, ~~-~~if needed, ~~if needed~~ in a cool and dark condition.

1.6. If storing, samples should be sealed using a small amount of deionized water squirted around the stopper, and the stopper should be wrapped in aluminum foil, secured with a rubber band.

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1.11. The concentration of DO is equivalent to the volume (mL) of titrant used. Each milliliter of sodium thiosulfate added to the water sample equals 1 mg/L dissolved oxygen.

2. Representative Results:

**Comment [9]:** Are steps 1.1-1.5 done in the field, or in the lab?

**Comment [10]:** Provide the stoichiometry of going from ml thiosulfate to moles of O2 to mg/L. Also, I did the calculation, and it’s an order of magnitude off. Is the thiosulfate actually 0.025 N?

**Comment [11]:** Add stoichiometry of going from ml thiosulfate to moles of O2 to mg/L to Principles section

2.1 A dissolved oxygen level of ~~-of 5 -~~ 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below ~~3~~ 4 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 mg/L~~or 1 mg/L~~ will not support ~~fish~~aerobic aquatic life (Fig. 5).

2.2 The maximum amount of oxygen that can be dissolved in water varies by temperature (Table 1).~~DO measurements in mg/L are converted to % saturation using water temperature and the conversion chart below.(fig 5)~~

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~~Good 71 – 90~~
~~Fair 51 – 70~~
~~Poor < 50~~

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Fig. 4: A sample showing the blue color after the starch indicator is added and mixed.

Fig. 5: A dissolved oxygen level of 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below 4 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 mg/L will not support fish and below 1 mg/L will not support most species.

**Comment [12]:** Can you provide this image without the red markings? Also, do you own the rights to this image?

**Comment [13]:** I do not own the rights. It's just the standard chart used but we've changed it to skip this last conversion to % saturation and stayed in mg/L instead. Added 2 figures to apply mg/L result to real world application of species oxygen requirements.

**Comment [14]:** What visuals/data can you provide for these applications? With reference images, we might animate the process of dead zones leading to the loss of a river.

**Comment [15]:** I don't have (and could not find any images of a river loss) but was able to fins a Caspian sea image from NASA that shows the loss of water on the north end and included it as Fig 7.

Fig. 6: Map of dissolved oxygen concentrations across the Louisiana shelf showing the dead zone region.

Fig. 7: Photograph of the Caspian Sea showing severe eutrophication in the north end.

Table 1: Maximum amounts of oxygen that can be dissolved in water by temperature.

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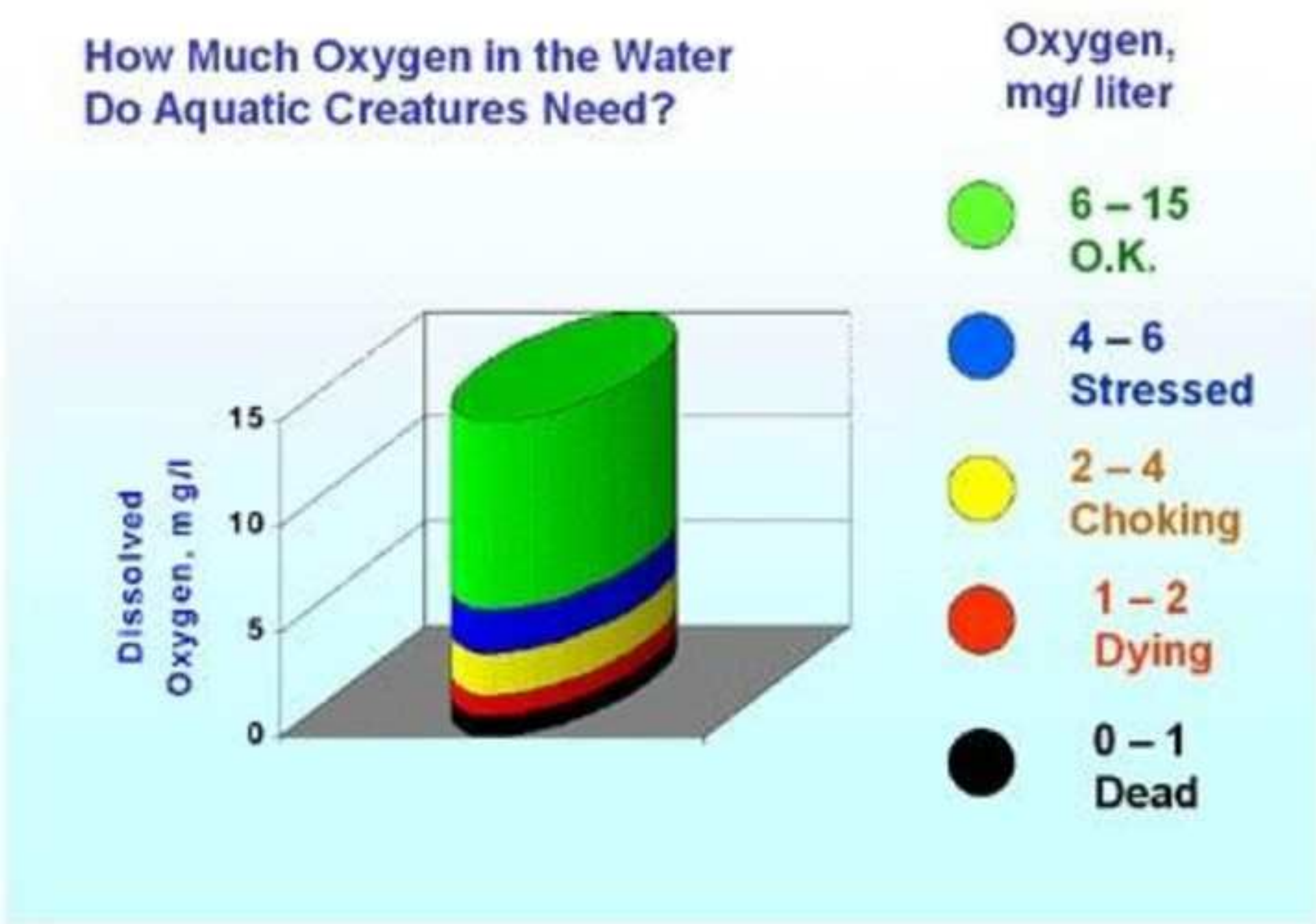

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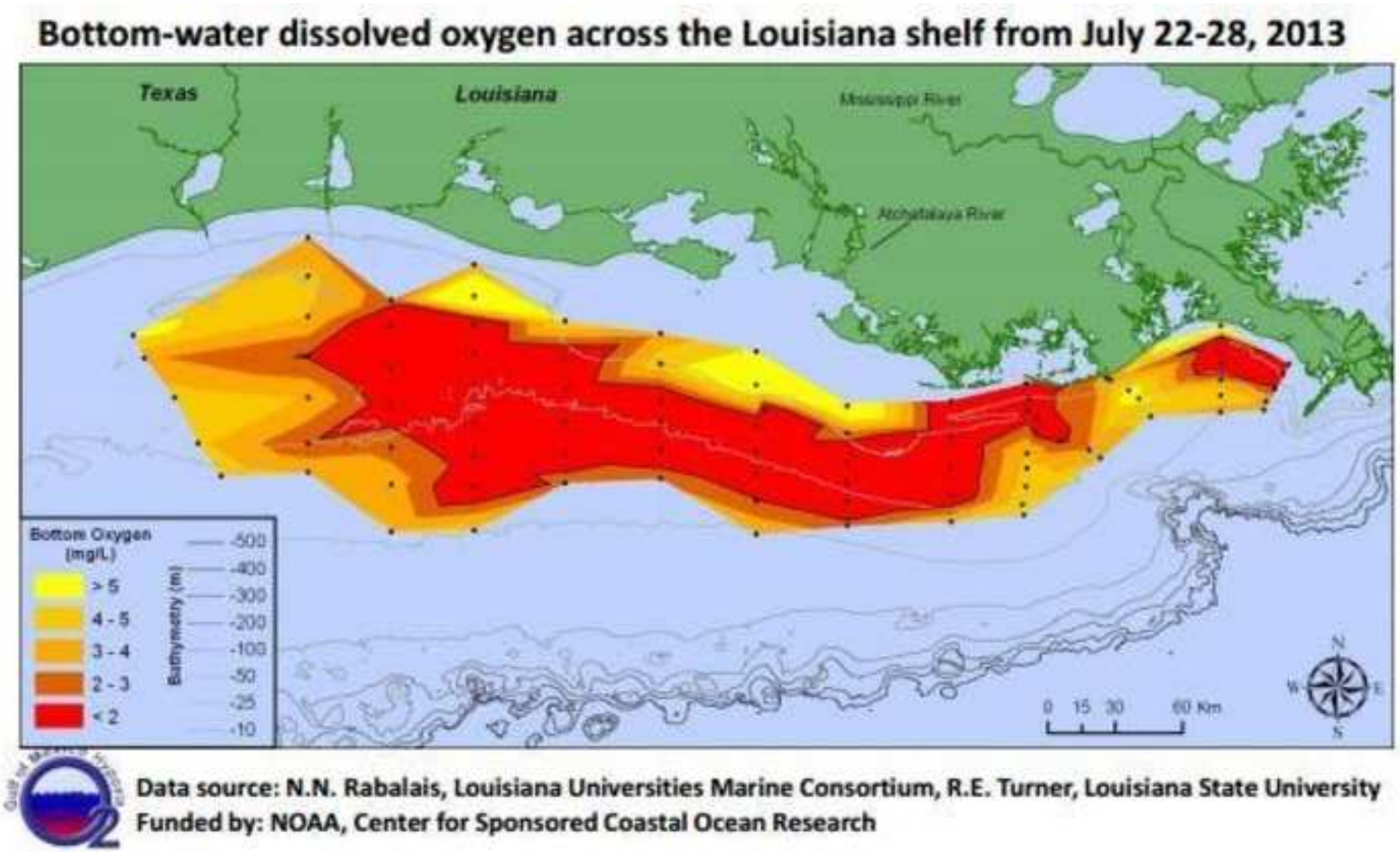

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