The Fine Art
of BOD testing
Aarrrrrggh!!
My blank failed!

Our Mission Today

This workshop is intended to focus on understanding the critical parts of the BOD test.

This will not be a step by step review of procedure, but rather a focus on the most important elements of BOD.

WHAT Matters…and WHY it matters

BOD is not like other analyses.

It's not chemistry and not subject to laws of chemistry.

It's keeping a bunch of bacteria happy enough to eat poop

I'm not gonna try it…YOU try it

Yeah…um…NO!
I'm good.

What every analyst, auditor, plant mgr should know

- **Generally speaking, NO ONE likes failure**
- **NO ONE wants to fail a blank or GGA**
- **NO ONE likes to add QC Comments to the DMR**
- **Accept it!...BOD is not a test that follows rules**
- **You can do everything right…and still fail a blank or GGA.**
- **Because there are things that affect the BOD test that are absolutely beyond your control.**
- **What you CAN do is be aware of events that may affect your results and anticipate what could happen.**
- **You CAN also learn about the test and come to understand what you can and cannot control.**

Where does all that leave us?

- The method sucks and is contradictory.
- It’s difficult to control.
- But it’s what we have.
- Still, by understanding how things work, piece by piece, we can control those things that are within our power and document the rest.
- We’ll discuss calibration issues in detail, sample pre-treatment, blanks, GGA, and factors that affect them as well as things like nitrification and toxicity

In the immortal words of Dr. McCoy…
Dammit, Jim! It’s a bioassay!

BOD...where it all began

...about 68 miles from London to the sea

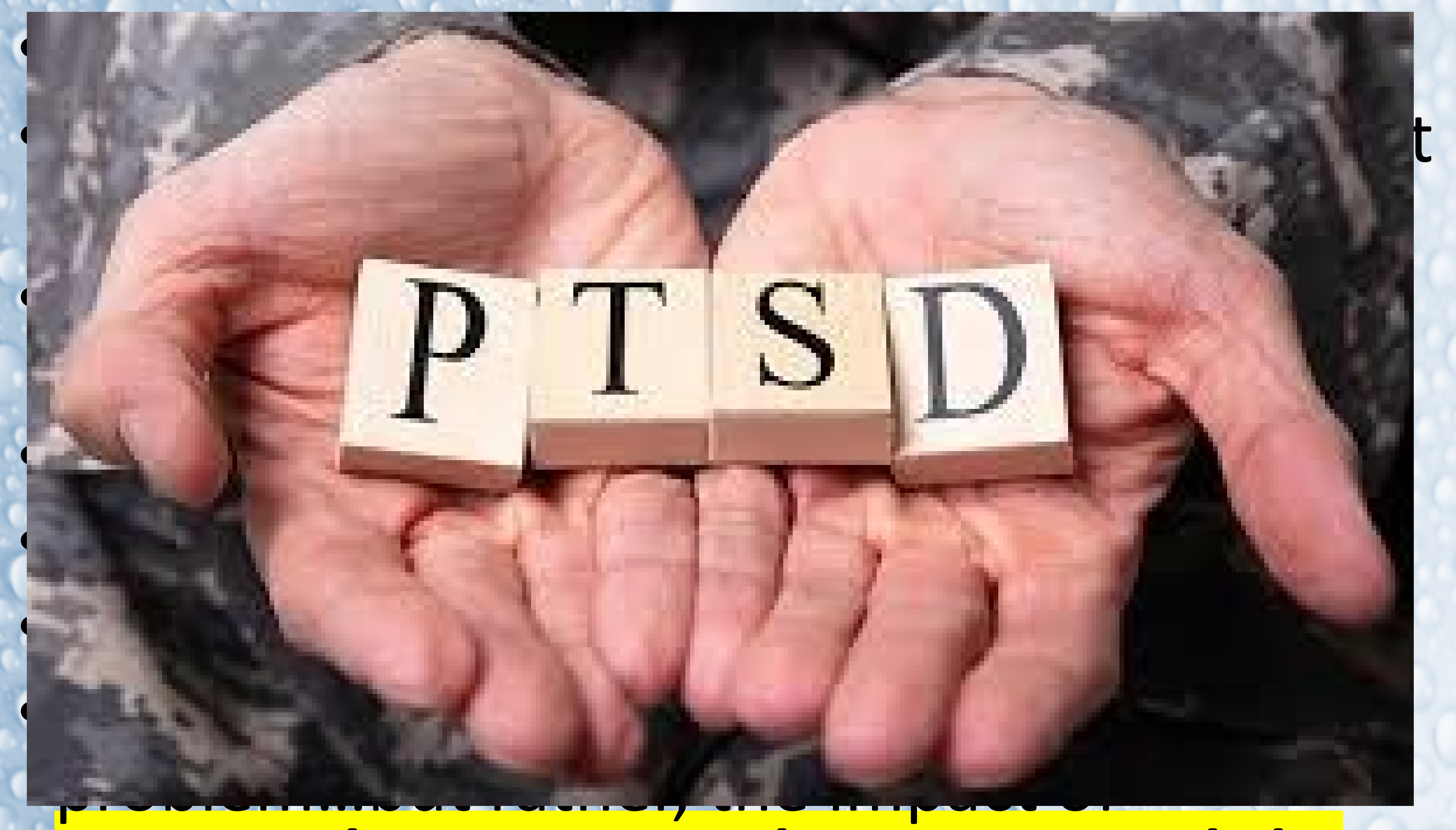
1908

Tower Bridge over the Thames, London

And it still happens today!
Venice,
Italy
Scale (m)
0
100
200
300
400
500

Down the rabbit hole we go!

Calibration and BOD



pressure/temperature/saturation (P/T/S)

Will spend a lot of time on this because it is so critical!

Is that ***really*** "calibrating"?

It's only ONE point (stick probe in the bottle)

You aren't really using a standard!

AND you're measuring AIR when samples are water! How does the even work?

It's all automated!

You merely "ACCEPT" what it offers.

Isn't it really just performing a measurement and then manually verifying that the measurement makes sense?

Barometers…What You Need To Know

- You're **not REQUIRED** to have a barometer!
- Could simply rely on local airport or meterological station data for pressure data.
- …but then, MUST **un**correct pressure data.
- Most onboard barometers are going to be more stable than an aneroid barometer.
- Onboard barometers will fluctuate as much as 2-4 mm.
- **Required:** annual verification of barometers
- Recommend: monthly verification
- To verify: use a local meterological station, airport, or the internet (and **un**correct data).

Verifying Barometers

Top 10 BOD Tip #8.

- **Don’t adjust the DO meter barometer unless it’s off by more than 5 mm**

The facts:

- each 5 mm (0.2” Hg) pressure changes DO saturation by only 0.05-0.07 mg/L.
- Blank depletion can be up to 0.24 mg/L and not fail.

So let’s be careful not to be too prescriptive about pressure adjustment.

A 5 mm pressure difference is insignificant

Fact Checking:

5 mm Hg = only 0.05-0.07 mg/L DO?

Oxygen Saturation Chart

°C											
in. Hg	29.92	29.72	29.53	29.33	29.13	28.94	28.74	28.54	28.35	28.15	27.95
mm Hg	**760**	755	750	745							
19.5 'C	**9.15**	9.09	9.03	8.97	8.91						
19.6 'C	**9.13**	9.07	9.01								
19.7 'C	**9.11**	9.05	8.99								
19.8 'C	**9.1**	9.04	8.98								
19.9 'C	**9.08**	9.02	8.96								
20 'C	**9.06**	9.00	8.94								
20.1 'C	**9.04**	8.98	8.92								
20.2 'C	**9.02**	8.96	8.90	8.84	8.78						
20.3 'C	**9.01**	8.95	8.89	8.83	8.77						
20.4 'C	**8.99**	8.93	8.87	8.81	8.75	8.69	8.64	8.58	8.52	8.46	8.40
20.5 'C	**8.97**	8.91	8.85	8.79	8.73	8.67	8.62	8.56	8.50	8.44	8.38

← Drop of 15 mm Hg →

in. Hg	28.94	28.74	28.54	28.35
mm Hg	735	730	725	720
20 'C	**8.76**	8.70	8.64	8.58

Loss of only 0.18 mg/L O_2

With an average of 0.06 mg/L change per 5 mm, it's not until >15 mm of pressure difference that we risk blank exceedances.

So…I shouldn't worry about pressure?

NOAA: The average daily total fluctuation of barometric pressure in Wisconsin is **0.21 inches of Hg (5 mm)**

But… a 7-yr study of major US cities in 2013 determined that major WI cities were found to change more than 0.2" Hg over 24 hours about 25% of the time

Bear in mind that pressure change is just one part. We also have to factor in temperature, super-saturation.

Mood swings can be quite serious…

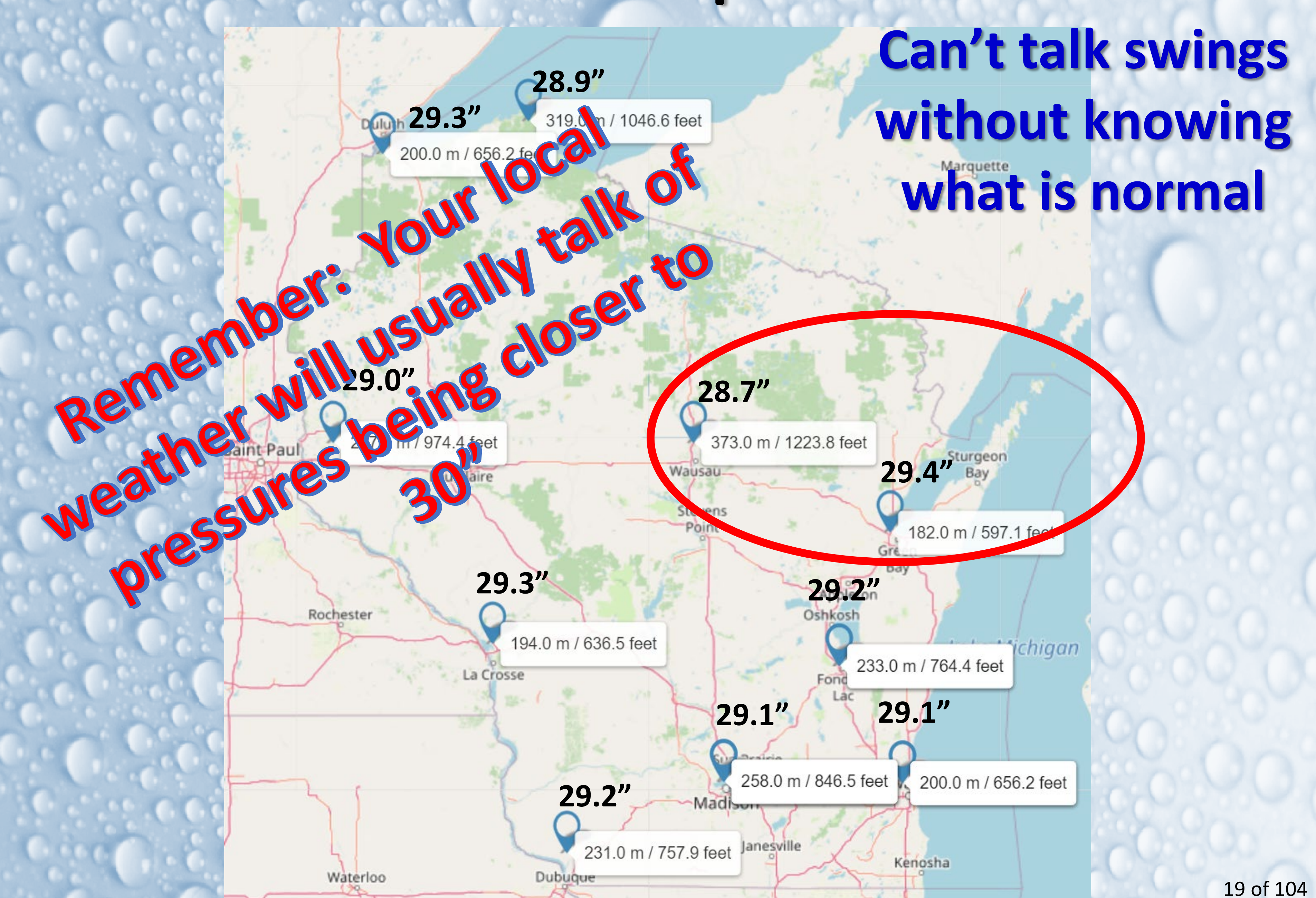
And so can pressure swings

If a picture is worth 1000 words, what does this picture tell you?

Let’s look at how weather patterns in Wisconsin impact calibration and DO measurements associated

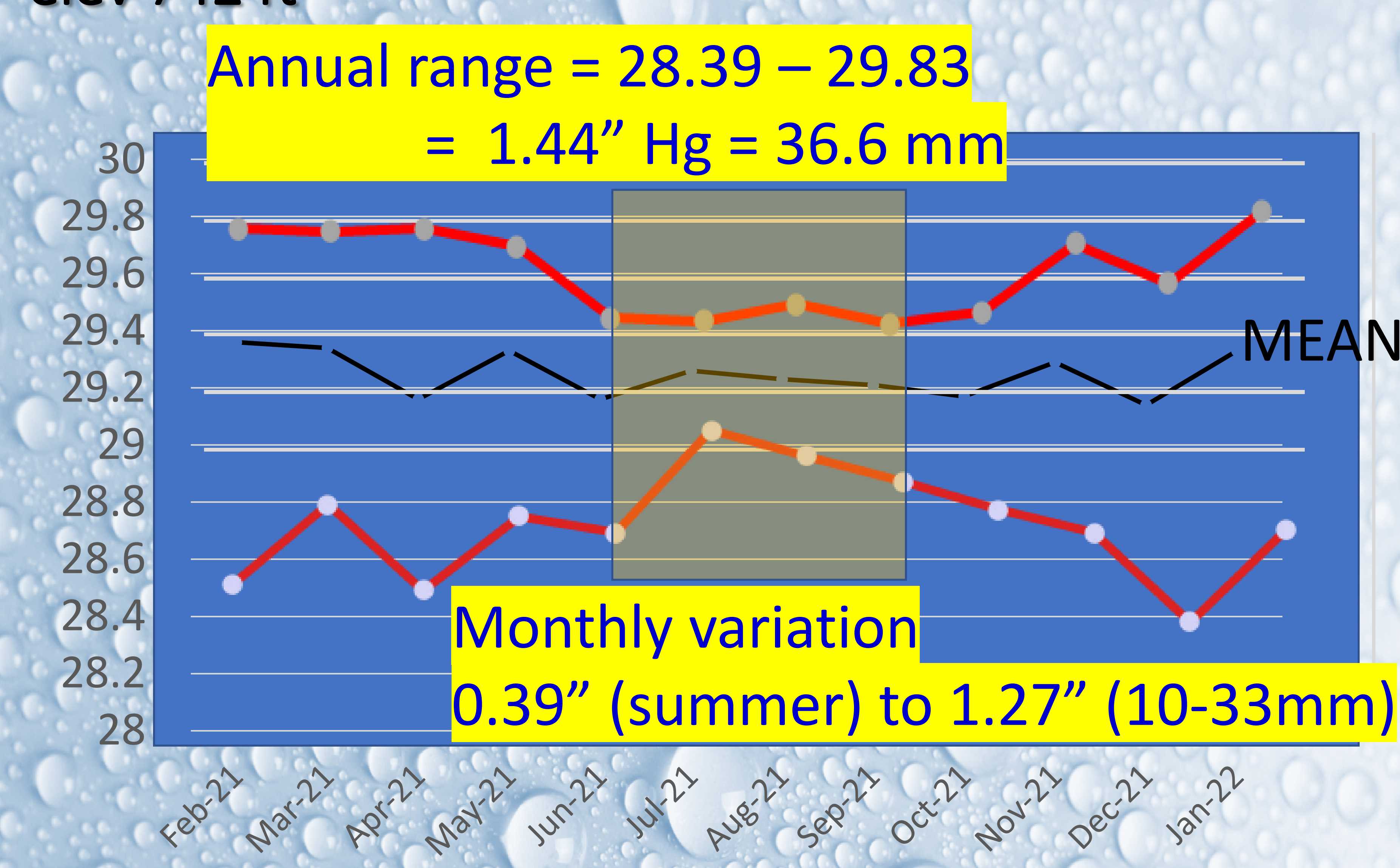
5 to 8 months of the year you can exceed blank requirements just from pressure differences

What is "Normal" pressure for WI



Rockford Muni Airport (airport code FIB)

elev 742 ft



Monthly Barometric pressure (Mean, Min, Max)

Barometric Pressure Correction

- **Need to know:** that nearly every airport would be expected to provide barometric pressure corrected to sea level. Therefore…

- …**you need to** "uncorrect" this for your local elevation

- **Need to know:** what a "normal" pressure is.

- **Need to know:** that everyone has a different normal, depending on the elevation of their facility

TV and airport reports of pressure

- The local TV weather person talks about pressure being around 30 (inches of mercury)
- Standard pressure at sea level (Altitude 0 ASL) is 760mm or 29.96" of Hg.
  Your weatherman is NOT at sea level!
- Pressure drops 1 inch (25.4 mm) for every 1000 feet above sea level *[0.0254 mm per foot]*
- WI Mean Elevation: of WI is 1,050 ft ASL. (*but it varies significantly*). *1000 ft = 1" drop from standard*

So YOUR <u>average</u> pressure should be about 29.0"
about 735 mm

- *Source: http://www.netstate.com/states/geography/wi_geography.htm*

Uncorrecting pressure readings (Green Bay, WI)

https://whatismyelevation.com/

Your facility's altitude is **590** ft ASL

760 - (**590** x 0.0254) = 760 -15.0 = 745

745/760 =0.9803 = press. corr. factor

TV weather says pressure is

30.21 in. x 0.9803

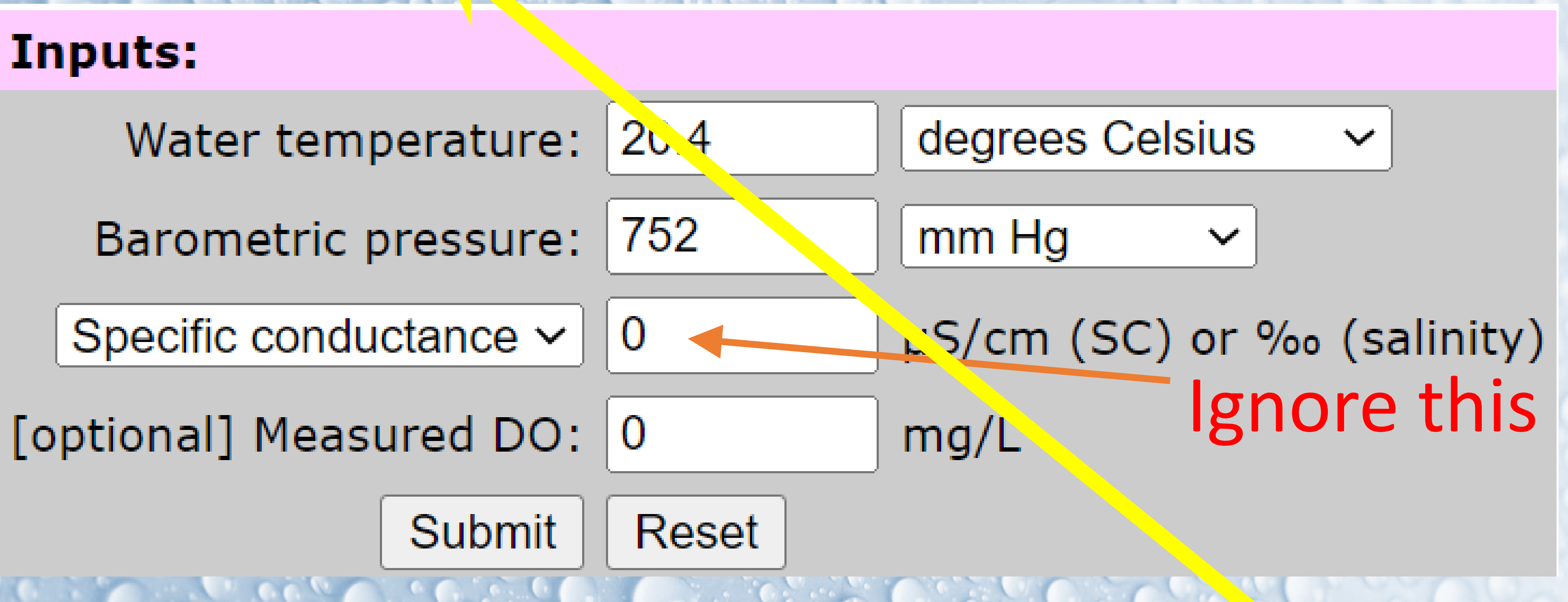
°C Oxygen Saturation

in. Hg	29.92	29.72	29.53	29.33	29.13	28.94	28.74	28.54
mm Hg	**760**	755	750	745	740	735	730	725
20.4 'C	**8.99**	8.93	8.87	8.81	8.75	8.69	8.64	8.58

On the DO Saturation chart, that's halfway between the 750(8.87) and 755 (8.93) columns. **So saturation is 8.90 mg/L**

OR USE ON-LINE DO SATURATION TOOLS

https://water.usgs.gov/water-resources/software/DOTABLES/



Results:

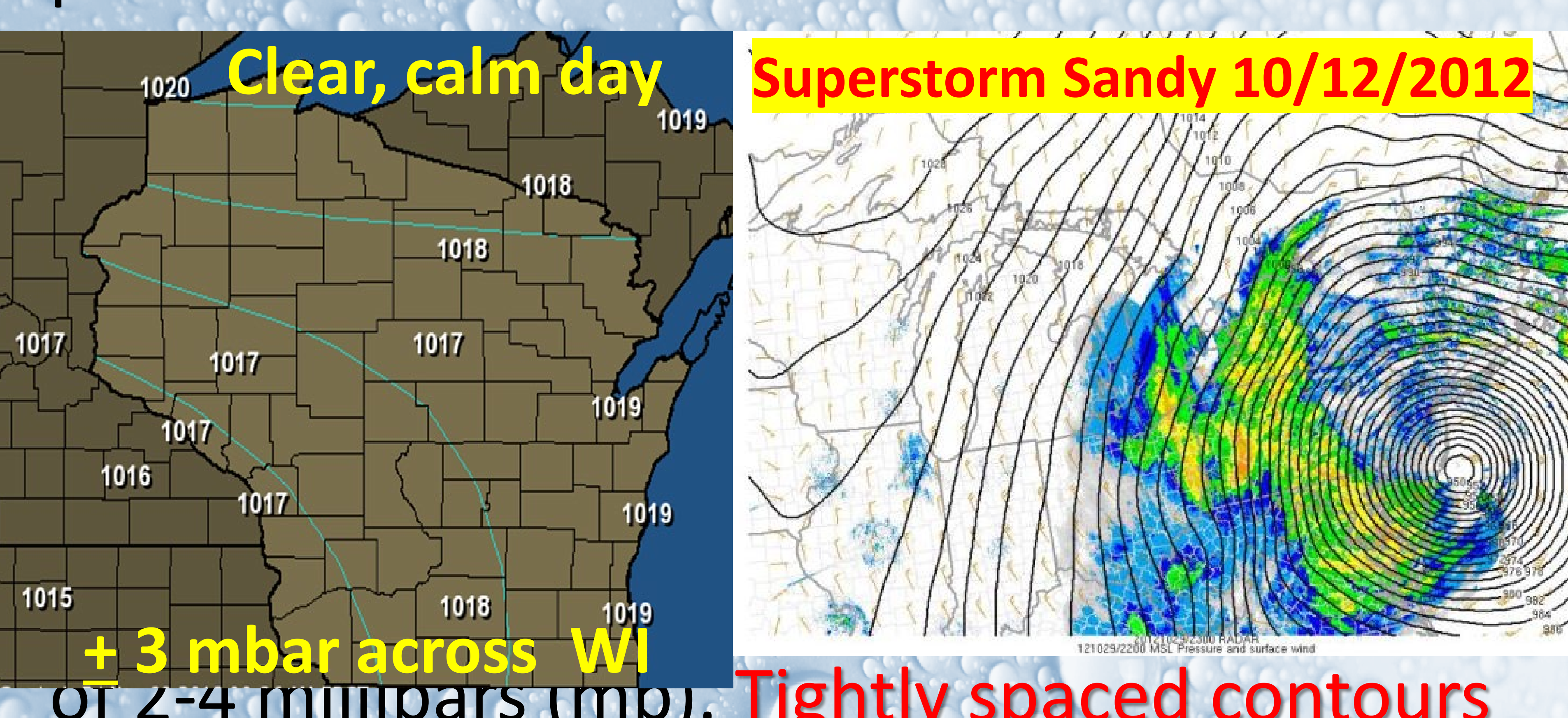
Oxygen solubility: 8.92 mg/L

Percent saturation: 0.00 percent

Close enough for govt work!

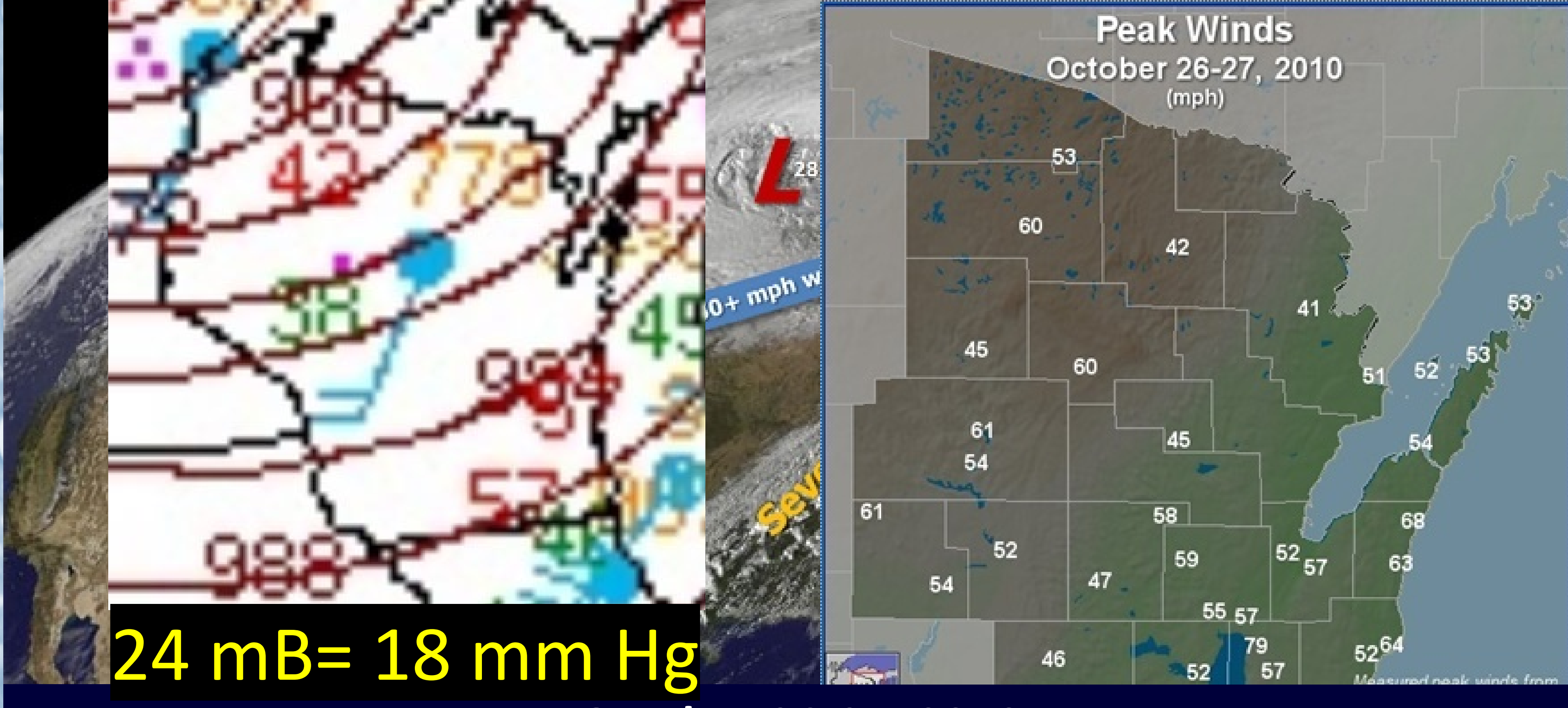
The impact of major pressure changes

- A **pressure gradient** is a change in barometric pressure over a distance.



of 2-4 millibars (mb). Tightly spaced contours indicate high winds.

Severe storms = Big pressure swings



October 26-27, 2010

New low pressure record for WI

Superior, WI, recorded a pressure of 28.39" (961.3 mb, 721 mm) on October 26. Previous state record was set in Green Bay in April 1982.

Superior's elevation is 642 ft ASL

True uncorrected pressure was 705 mm (27.8") Hg

How did 10/26/2010 storm affect DO?

in. Hg	29.33	29.13	28.94	28.74	28.54	28.35	28.15	27.95	27.76	27.56	27.36	27.17
mm Hg	745	740	735	730	725	720	715	710	705	700	695	690
19 'C	9.06	9.00	8.94	8.88	8.81	8.75	8.69	8.63	8.57	8.51	8.45	8.39
19.1 'C	9.04	8.98	8.92	8.86	8.80	8.73	8.67	8.61	8.55	8.49	8.43	8.37
19.2 'C	9.02	8.96	8.90	8.84	8.78	8.72	8.66	8.59	8.53	8.47	8.41	8.35
19.3 'C	9.01	8.95	8.89	8.83	8.77	8.71	8.65	8.59	8.52	8.46	8.40	8.34
19.4 'C	8.99	8.93	8.87	8.81	8.75	8.69	8.63	8.57	8.51	8.45	8.39	8.33
19.5 'C	8.97	8.91	8.85	8.79	8.73	8.67	8.61	8.55	8.49	8.43	8.37	8.31
19.6 'C	8.95	8.89	8.83	8.77	8.71	8.65	8.59	8.53	8.47	8.41	8.35	8.29
19.7 'C	8.93	8.87	8.81	8.75	8.69	8.63	8.57	8.51	8.45	8.39	8.33	8.27
19.8 'C	8.92	8.86	8.80	8.74	8.68	8.62	8.56	8.50	8.44	8.38	8.32	8.26
19.9 'C	8.90	8.84	8.78	8.72	8.66	8.60	8.54	8.48	8.42	8.36	8.30	8.24
20 'C	8.88	8.82	**8.76**	8.70	8.64	8.58	8.52	8.46	8.40	8.34	8.29	8.23

8.88 normal
8.40 storm
0.48 mg/L!

"Normal" pressure

Historical low pressure

Wh ... ight
see ... f the
BO

Oxyg ... P)

5 mm

0 inch

+ 3°C

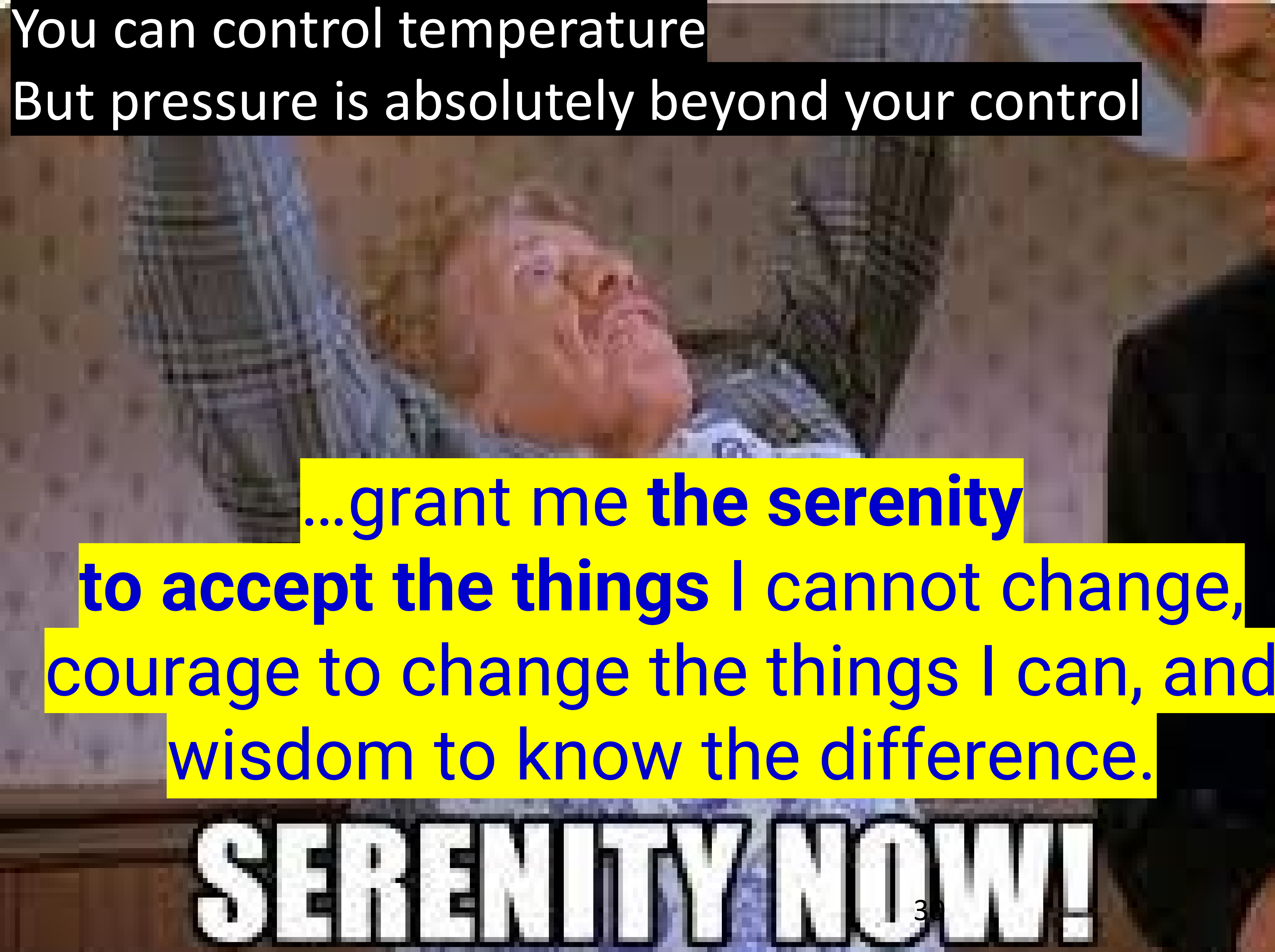
Pres ... h

pres

If a storm is brewing, or an arctic high occurs you cannot stop it. But you CAN take note of the event to identify impact later

Takeaways

- Pressure can change dramatically over 5 days.
- Big swings between Day 0 and Day 5 can impact results.
- Oxygen saturation is greatest under high pressure and cold temps
- Pressure changes can cause those annoying minor failures of blanks (0.25-0.4 mg/L depletion/gain).
- You can’t control the weather, but you can certainly document it.
- Make notes of severe swings to help you explain sudden blank failures (either depleting too much or appearing to gain oxygen.


You can control temperature
But pressure is absolutely beyond your control
…grant me **the serenity**
to accept the things I cannot change,
courage to change the things I can, and
wisdom to know the difference.
SERENITY NOW!

DO Meter
Calibration &
verification
R 1 3 5
2 4 6

How do you VERIFY that DO measurements are accurate?

- **You need a "known" standard**
  - Air saturated water (YOUR daily blank iDO!)
- **You need some basic physical data**
  - Temperature
  - (uncorrected) barometric pressure
- **Physical data → standard "true" value**
  - Determine theoretical TRUE value for oxygen in mg/L (i.e. saturation point)
  - **If measured value = True value ± 0.2 mg/L; calibration is accurate**

Not in SM! This is why blank criteria are a challenge.

Wait…calibration only has to be ± 0.2 mg/L of "true"??? Won't that cause me to fail blanks?

Actually, No. **Measuring the change in DO** from Day 0 to Day 5, not absolute DO.

Similar to using a burette. To measure out 5mLs, you CAN start at the "0" line and allow the fluid to drop to the 5 mL mark. But you can start at any mark and still deliver exactly 5 mLs.

Kinda like when you travel to a different time zone. Your watch may not show the actual time, but you can still tell when an hour or any number of minutes has passed. While there (unless you re-set it) you're consistently off by that hour (or 7 if you're in Italy).

Calibration & consistency

Top 10 BOD Tip #1. Calibrate the same way each day; every analyst. Consistency is the key!

- **How long till it returns to 100% saturation?**

How's this going to work?

"A" removes the probe, dabs any drops, ensures water is 1" deep, places it back in the bottle, sets a timer for 30 minutes and calibrates immediately after.

"B" shakes the probe, fills the bottle up anywhere from 1" deep to just below the probe and calibrates whenever **they** are able to.

Day 0, Analyst A

Day 5, Analyst B

Areas for Consistency Improvement

- Meter warm up before calibrate (≥30 mins) non-LDO
- How drops removed from probe tip (Shake? Dab?)
- Amount of water in the BOD bottle (~1 inch…mark it)
- How long does the probe sit in the BOD bottle before calibration (≥ 30 mins.) Set a time!
- Consistent temperature conditions in Lab
- MUST be consistent on day 0 and day 5
- Get into a routine and STICK WITH IT!

- **Benefits of consistency?**
  - Your calibrations will work even if you don't wait for the air to be 100% saturated….as long as you do your calibration the **SAME WAY EVERY DAY!**

… and if that isn't enough to convince you…consider this

Every time you calibrate a DO probe inconsistently, a puppy dies.

R 1 3 5
2 4 6
Sample
Pre-treatment

4 key elements

1. Sample pH requirements

   pH extremes …kill bugs

2. Presence of residual chlorine

   Residual chlorine …kills bugs

3. Sample/ **[room??]** temperature

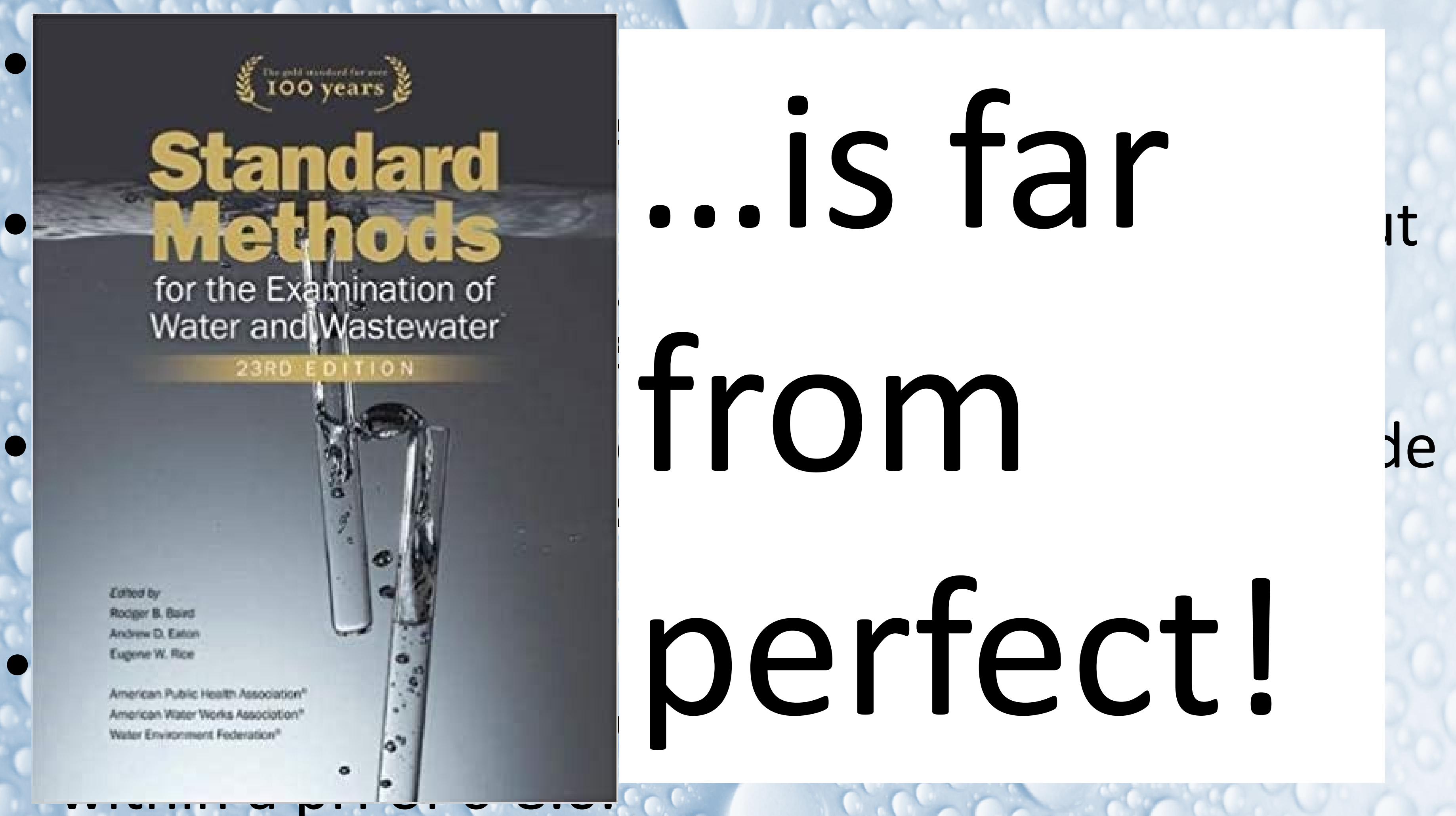
   Blame Bowman…kind of

4. Oxygen saturation (& super-saturation)

   Blank problems

Sample PreTreatment - pH

- pH extremes defined as < pH 6 or > pH 8.0



- IF pH is adjusted, MUST seed samples. Why? *Because you've just added a toxin to the sample!*

Sample PreTreatment – residual chlorine

- ONLY required if chlorine is used
- Work with your engineers (if possible) to sample effluent U/S of chlorination.
- If residual chlorine is present, must de-chlorinate w/ sodium sulfite
- Cannot be overstated, an excess of sodium sulfite WILL scavenge oxygen. *This is the stuff we used to use to create a "zero DO" check for probes/meters*

Test strips offer a rapid test for residual chlorine, but **you must use strips that are sensitive enough**!

In 1996, the State Lab of Hygiene received its first audit from the LabCert Program and Bowman got busted for his lab not maintaining “lab temps” at 20 +/- 1 C.

He received a deficiency and was unable to convince the auditor of their error.

Bowman was not amused.

Being a long-time member of the Standard Methods Task Group that wrote the BOD method, he fought back.

you even need an incubator?

Arvada,CO 1985

5,423

7.50

Steamboat Springs

6,732

6.95

Dammit, Jim!
It's a bioassay!

Real-life super-saturation examples

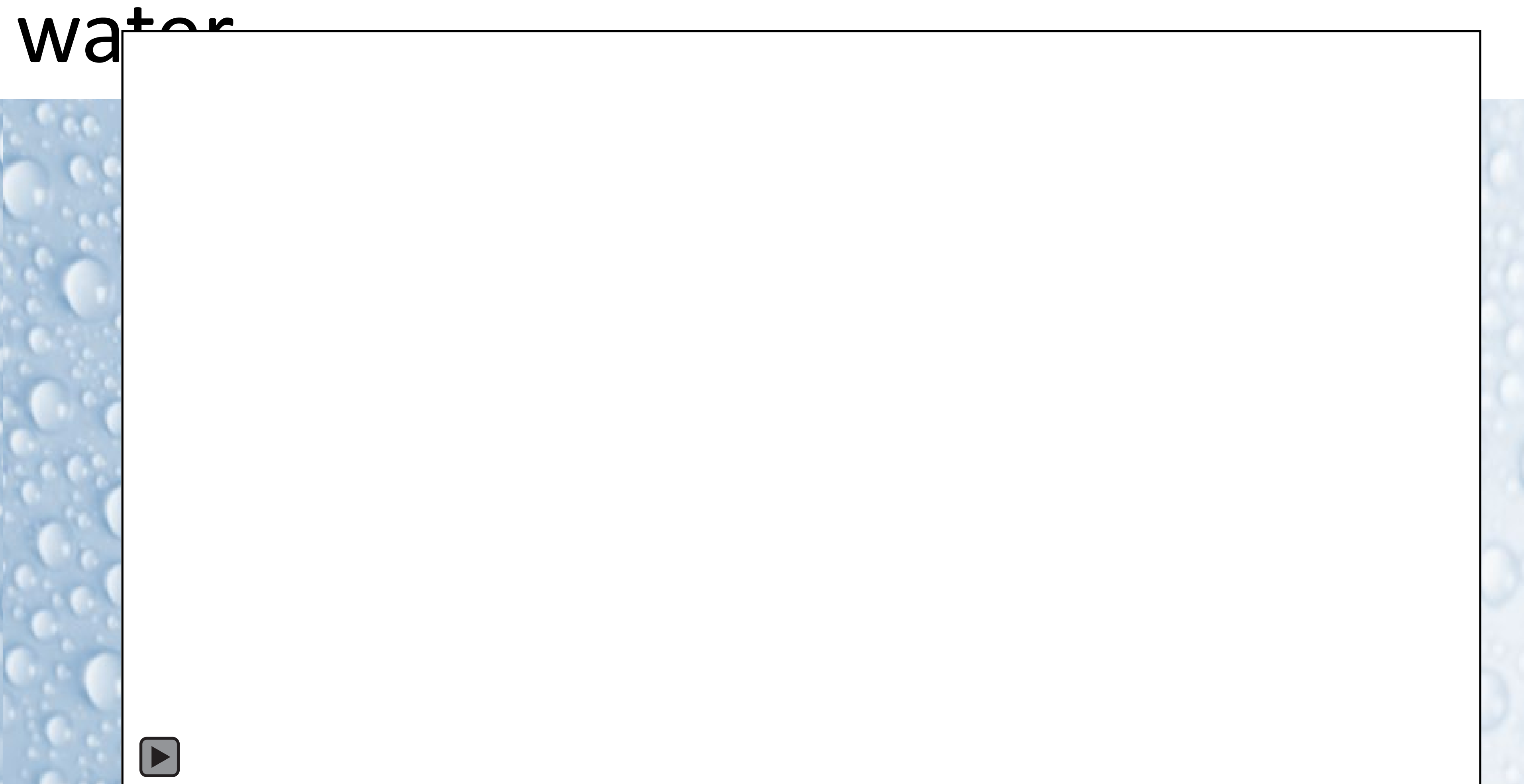
- Carbon dioxide is supersaturated in soft drinks. Pressure forces more CO2 into the water/soda than ordinarily d ... ses the pressure a ... 2 escape as gas bubl

- Sugars in clear honey are supersaturated. Crystals tend to form slowly at room temperature, although you can knock sugar out of solution quickly if you refrigerate honey.

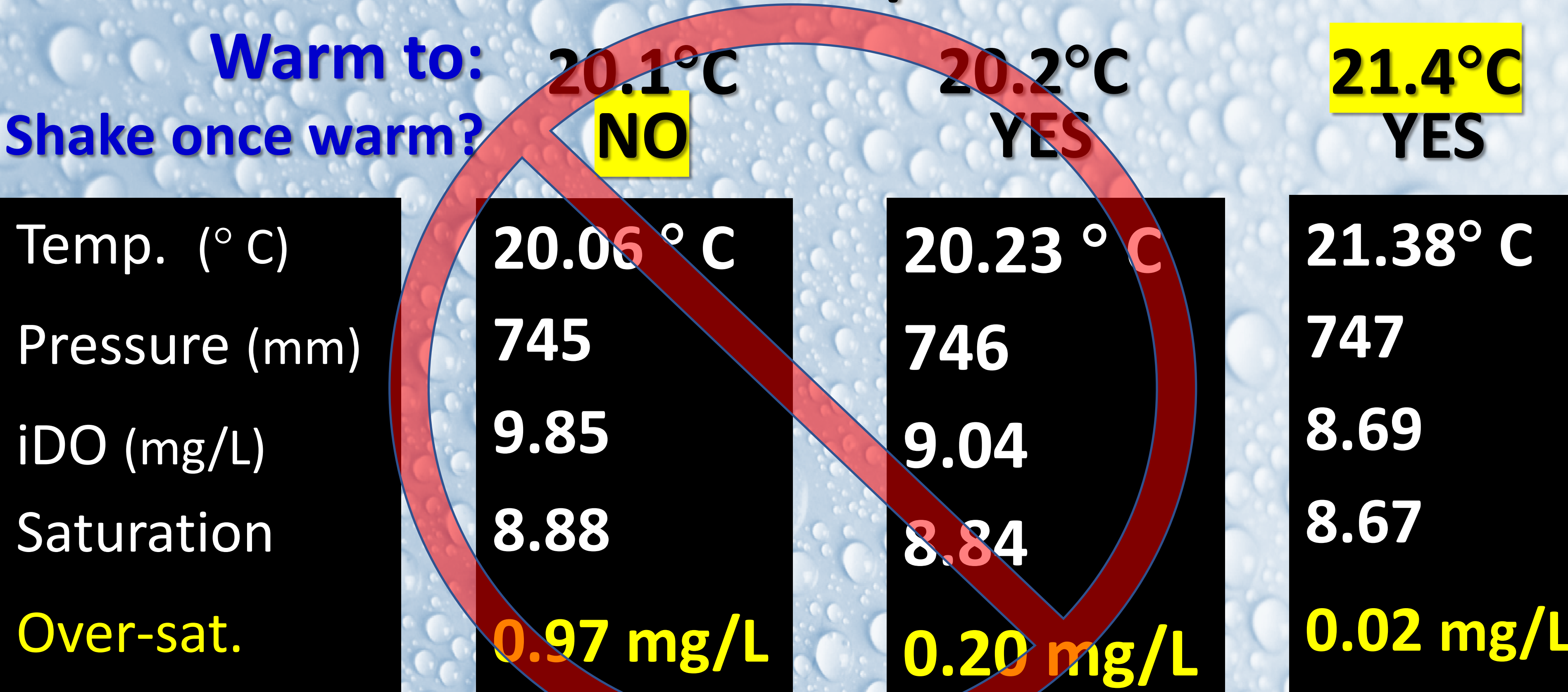
- Cloudbursts result from supersaturation of water in air. A change in temperature or pressure triggers the conversion from water vapor into liquid water.

How do you get rid of super-saturation?

George Bowman is infamous for telling people that the solution is to use holy water



We experimented and here's what we learned about supersaturation…



Warm to:	20.1°C	20.2°C	21.4°C
Shake once warm?	NO	YES	YES
Temp. (° C)	20.06 ° C	20.23 ° C	21.38° C
Pressure (mm)	745	746	747
iDO (mg/L)	9.85	9.04	8.69
Saturation	8.88	8.84	8.67
Over-sat.	0.97 mg/L	0.20 mg/L	0.02 mg/L

It's an interesting physical phenomenon, but warming sample temp closer to 22° C initially is much more efficient in dealing with super-saturation.

Supersaturation Wall of Shame

Chart: 733 mm & 23.6C……Saturation= 8.20mg/L

BOD SAMPLE DATE Tue 2-14

SETUP DATE Wed 2-15 TIME 0808 ANALYST Barometer 732.5 mmHg

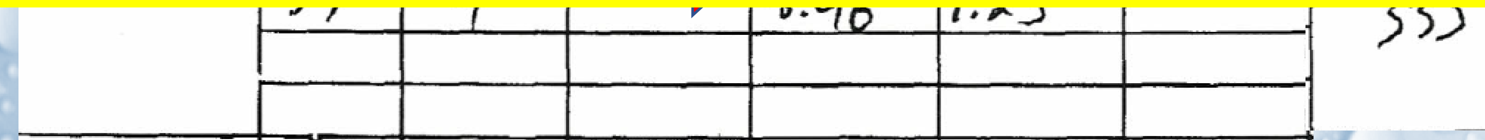
Temperature 23.6

In this case,

There was an extra 1.5 to 2 mg/L DO

The effluent BOD reported of 6 mg/L was about 50% higher than actual BOD (4.3 mg/L)

This doesn't even consider the fact that this lab's temp was outside the acceptable range!



R 1 3 5
2 4 6
Seed &
Seeding

When is seeding required?

Samples must be seeded if they:

…are collected after ANY type disinfection,

…have been pH adjusted,

… are untreated industrial wastes, or

…have been inhibited for cBOD.

Each of these situations poses significant risk for a sample to be toxic to micro-organisms.

Seed Sources

- We encourage labs to try using either their raw, primary or mixed liquor as a seed source.
- **Mixed liquor may be the best choice** since it tends to be more uniform than raw, and it is less likely to be affected by I & I.
- Synthetic seeds certainly can be used, but be aware that each is different and reports of reliability vary greatly.
- Experience shows that synthetic seeds tend to be weaker requiring more volume of seed.

How to use Mixed Liquor as a seed

1. Perform a mixed liquor (ML) settleability test.

2. DO NOT allow ML to settle overnight. Must be used fresh so organisms remain viable.

Pour about 250 mL of clear supernatant into a 500 mL beaker.

3. Transfer 2 to 5 mL (wide tip!) of settled floc to the beaker containing the 250 mL of supernatant. Fortifies with extra suspended solids (*i.e., more bugs*).

4. Stir mixture at a moderate speed to ensure the solids in the supernatant stay suspended. Use this mix to prepare the seed controls and to seed GGA.

Note: *Important to keep the beaker mixing while withdrawing portions for the seed controls and when seeding the individual BOD bottles.*

Figuring out seed controls & GGA

- For most labs, seed controls of 10, 15 and 20 mL work,
- Goal: at least 2 seed controls meet depletion criteria.
- Prepare 3 GGA samples w/different seed volume. Try seeding 1, 2, and 3 mL of the seed mixture. One of these will likely yield GGA in range.
- Use the seed volume that produces the best GGA results for routine analysis.
- DO NOT be overly concerned if the seed correction factor is not in the 0.6 to 1.0 mg/L range.
- Once optimal volumes are determined, document the seeding process in the lab BOD SOP and post instructions.

If you change seeds (or your seed changes), all of this changes!

Seed & Seeding – **Unknown samples**

If analyzing samples from other facilities how do you know that they haven't been disinfected and therefore require seeding and/or de-chlorination?

- **Mealy's Law of Inverse Documentation**: If you have NO documentation that a sample has NOT been disinfected, then you have NO documentation to support a decision NOT to seed.
- **Keep it simple, Stanley**….when in doubt, seed. As long as your seed is consistent, the seed correction factor will adjust for anything you add.
- **Seeding unnecessarily does no harm.** In the worst case, it reduces the working range for a dilution.

Seed controls….Evaluate the DPMS

DPMS: mg/L DO Depletion Per mL of Seed

Seed correction factor (SCF) is the average DPMS.

Averages can be misleading. Look at the data that goes into the SCF!

Lab analyzes 3 GGAs in a set of samples:

165,232,200.

Average is 199…pass! (do you agree?)

Consistency of seed controls: Most likely cause of inconsistency is drawing up settled seed.

Consistency is critical.

Does the average reflect reality?

If not, when multiplied by DF of 50, you could fail GGA

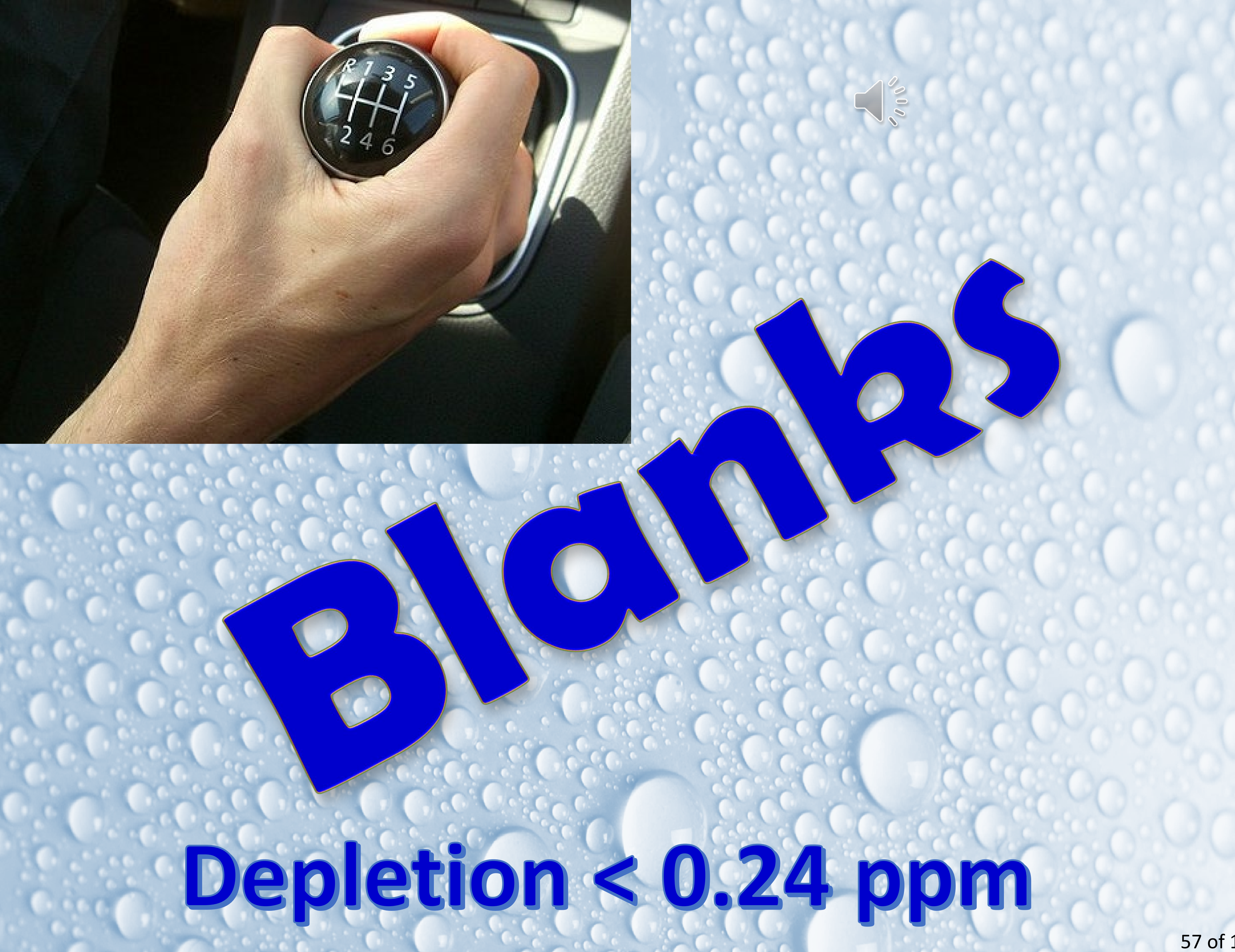
Examples of poor seeds

Good quality seed DPMS is 0.35-0.5 (BOD of 130-180) and is consistent.

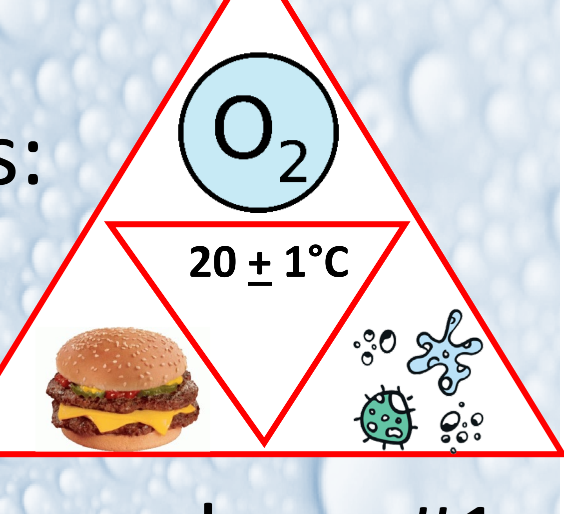
Inconsistent

		A	B	C	D	E	F		
		Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	DO_I	DO_F	B-C	DPMS	300/A	F x (D-E)	SCF
Seed Control	A	6	8.5	6.5	2	0.33	50	100	
	B	10	8.5	1.1	7.4	0.74	30	222	0.54
	C	12	8.5	2.1	6.4	0.53	25	160	

Inconsistent (poor precision) seed affects result accuracy. Are you comfortable averaging 100,160, and 222 to arrive at a BOD of 161 for a sample?


R 1 3 5
2 4 6
Blanks
Depletion < 0.24 ppm

Why/how do blanks "fail"



- O_2 Depletion after 5 days requires:
  1. Oxygen
  2. Microbes…."bugs"
  3. Food→sustainability
- Blanks should be O_2 saturated, so we have #1.
- Without #2 **and** #3 … depletion can't occur.
- **And you shouldn't have either in a blank!**
- MOST blank failures occur due to inconsistency or weather/calibration factors **(P/T/S)**.
- Blank "depletion" should fluctuate between ± 0.2 mg/L. (*error of the method*).

IF Blank depletion exceeds 0.24 mg/L

- Cannot underscore the importance of pressure/temperature/supersaturation (P/T/S) on blanks.
- Most assume blank problems stem from contamination vs. (more likely) P/T/S.
- For contamination, it MUST be both bugs and dirty glassware
- Blank **contamination** typically involves significant depletion (> 0.5 mg/L).
- **P/T/S issues** usually means depletions ≅ 0.3-0.4 mg/L
- If a blank is super-saturated going IN, that extra DO **will** appear as depletion.

R 1 3 5
2 4 6
GGA
Our LCS..or
Lab Control Standard

GGA seems so simple…

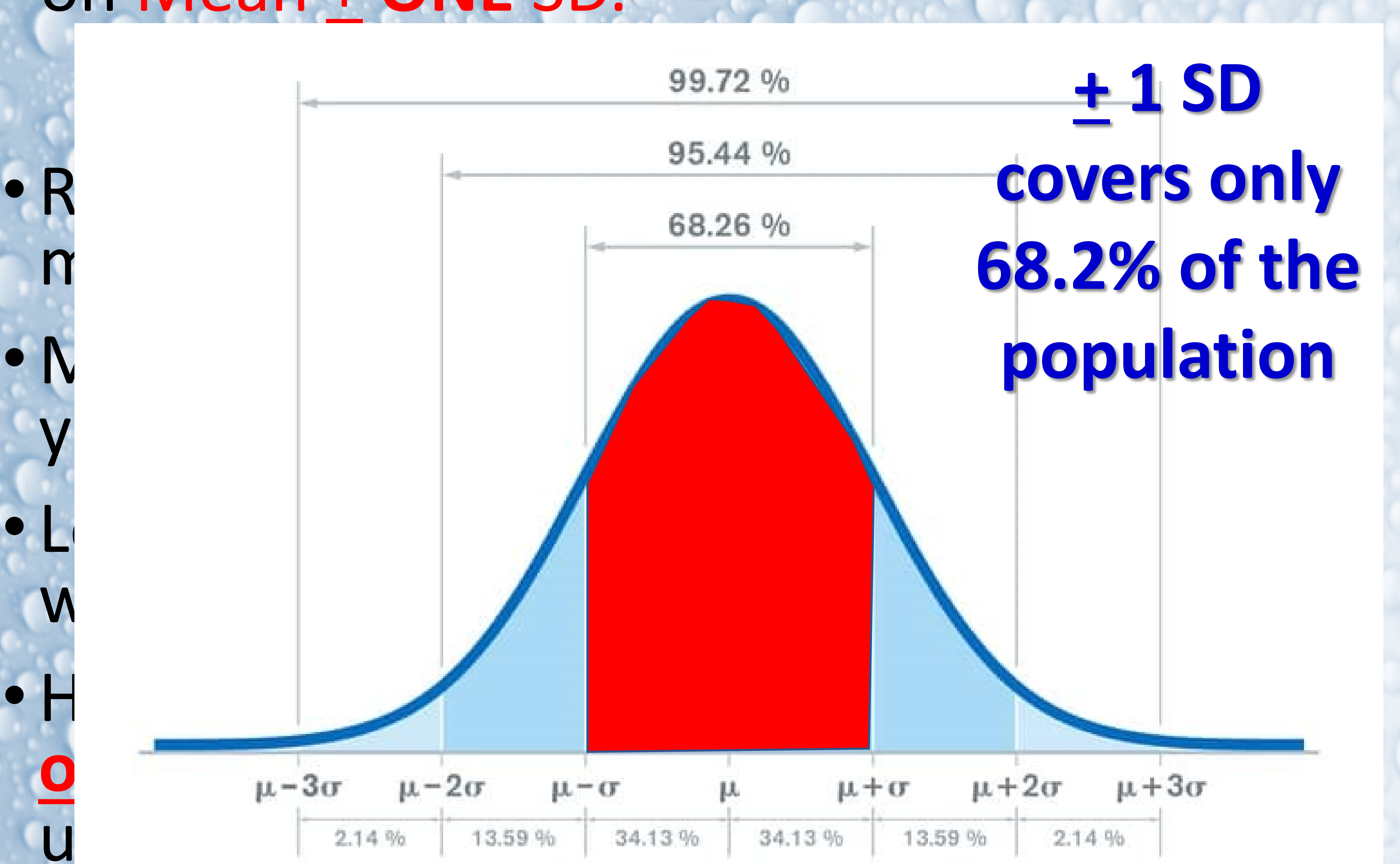
- 1 per 20 samples or 1 per week
  …whichever is more frequent
  …which means 1/week for most labs
- 6 mLs of method mandated GGA solution in a 300 mL bottle (*50 x dilution*)
- Acceptance criteria of 198 ± 30.5 mg/L
  - = 167.5 - 228.5 mg/L
  - = 85 to 115% (of 198)
- **NEVER** add inhibitor!

0.2 mg/L OVER depletion means +10 mg/L GGA

…yet it remains a challenge

GGA tidbits

- Remember that the "control limits" are based on Mean + **ONE** SD.
- R
  m
- M
  y
- L
  w
- H
  o
  u



Contamination? Calibration? Or Seed?

Contamination?

- Can affect BOTH blanks (rarely) and GGA
- Tends to be LARGE effect
- Will be HIGH bias (GGA high)

Calibration (P/T/S)?

- Mainly affects blanks
- Tends to be SMALL effect
- Can be LOW or HIGH bias
- (blanks deplete > 0.2 mg/L **OR** GAIN > 0.2 mg/L)

Or Seed (weak/inconsistent)?

- Affects GGA only
- Tends to be LARGE effect (enough to fail GGA)
- Tends to be LOW bias (GGA low)

If it's Contamination: Bugs? or Dirty Dishes?

Unlike blanks, where contamination must mean **BOTH** bugs/crud, with GGA (a food source), contamination can be **EITHER** bugs or crud (because GGA is a food source).

Scenario 1: Microbial contamination only

- possibly from a bad filter in the DI system.
- GGA fails high but blanks are fine
- Blanks likely fine because glassware is clean and there is no "food source" to keep bugs going and expending oxygen.
- GGA fails high due to the extra oxygen consumed by extra bugs attacking the GGA.

Water purification systems <u>can</u> affect BOD?

- Inadequate maintenance ⇨ problems.
- Over-engineeered systems can create "dead" zones (allowing stagnation…good for bugs).
- DI systems can harbor bacteria and mold and can leach organics.
- Activated charcoal in deionizer systems can become contaminated with bacteria and mold, and can slough-off BOD.
- Chlorinated water feeding ion exchange systems can breakdown and leach BOD.
- Use quality virgin or nuclear-grade resins. Poorer grade resins WILL leach BOD.

Don't sacrifice quality for a few bucks.

If it’s Contamination: Bugs? or Dirty Dishes?

Scenario 2: Dirty glassware only

- GGA fails high but blanks are fine.
- Likely just “dirty glassware”, providing a food source.
- Blanks are likely fine because –despite availability of a food source (the “crud”)--- there is no source of bugs and therefore no oxygen can be used.
- GGA fails high due to the extra oxygen consumed by the bugs (seed) as they attack GGA and the “crud”

Scenario 3: Source is microbial AND dirty glassware

- Both GGA and blanks fail high (blanks deplete >1 mg/L)
- Likely a combination of “dirty glassware”, dilution water, and “bugs”.
- Not only is there a food source (“crud”) but also unaccounted for (seed correction) “bugs” using O_2.

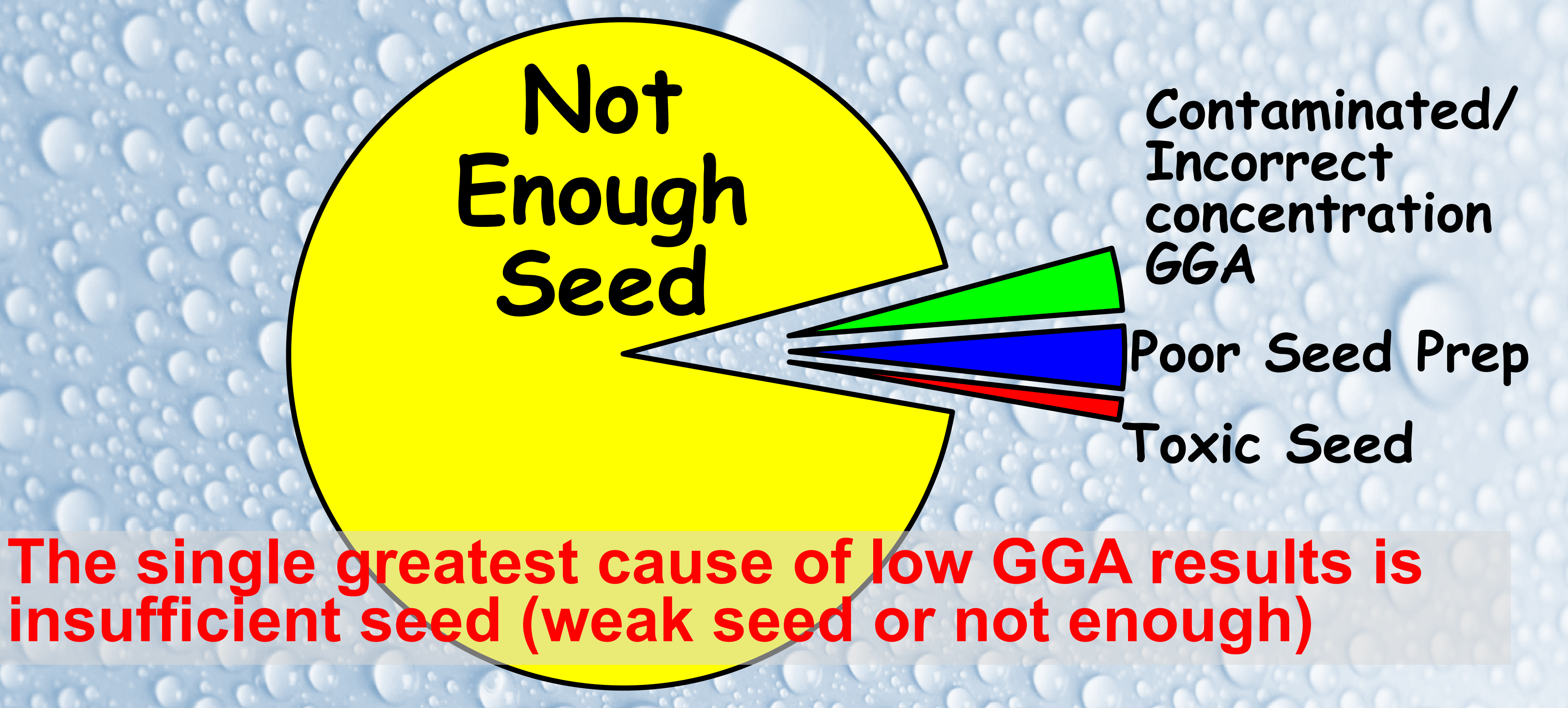
Seed & Seeding - GGA

Top 10 BOD Tip #3. Low GGA results with synthetic seed? Consider using primary WW; mixed liquor or raw WW for seed. Low GGA is nearly always caused by weak or inadequate seed.

- Synthetic seeds have haunted labs for years. Sometimes they work quite well, but other times, they appear to **be plain voodoo.**
- There is simply no substitute for a real seed which has been acclimated to your waste.
- Seed just needs to settle and be maintained at a good temperature.
- **Seed is best used fresh. Too many problems with people stressing the critters by putting them in the incubator overnight…or even longer!**

GGA Killers
99% ISOPROPYL
Alcohol
ALCOHOL FOR TOPICAL ANTIMICROBIAL USE
FIRST AID ANTISEPTIC
16 FL OZ (1 PT) 473 mL
febreze
FABRIC
refresher
68 of 104

REASONS FOR GGA FAILING LOW (< 180)



The single greatest cause of low GGA results is insufficient seed (weak seed or not enough)

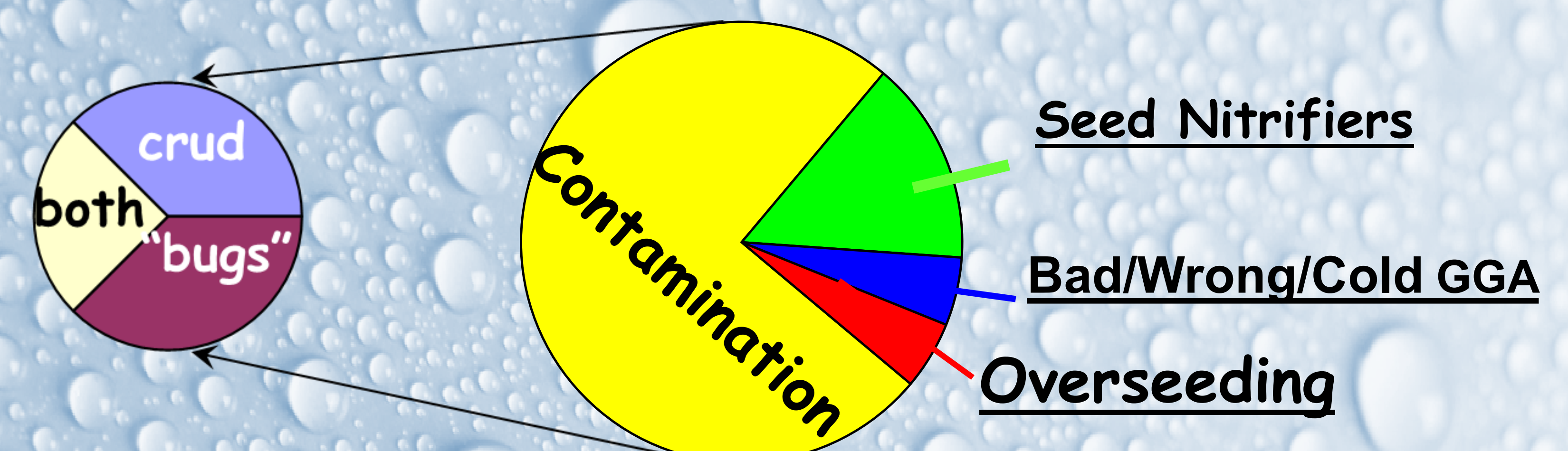
Make sure you have enough seed (and it's not weak)

Use whatever seed volume it takes to pass GGA

Make sure your seed controls are consistent

Monitor DPMS in seed controls

REASONS FOR GGA FAILING HIGH (> 228)



Contamination ("Bugs"): Sources could be from the lab itself, or possibly from buildup in a lab reagent water system.

Nitrifiers in Seed: Recall that there is ammonia in dilution water (294 mLs!) and GGA contains significant (7.5%) nitrogen.

GGA prep: Contaminated GGA, incorrectly prepared GGA, and use of GGA while cold can all cause high GGA bias.

OverSeeding: Remotely possible, but as long as your SCF (**DPMS**!) is accurate, GGA should be fine.

Toxicity
R 1 3 5
2 4 6

Sample Toxicity

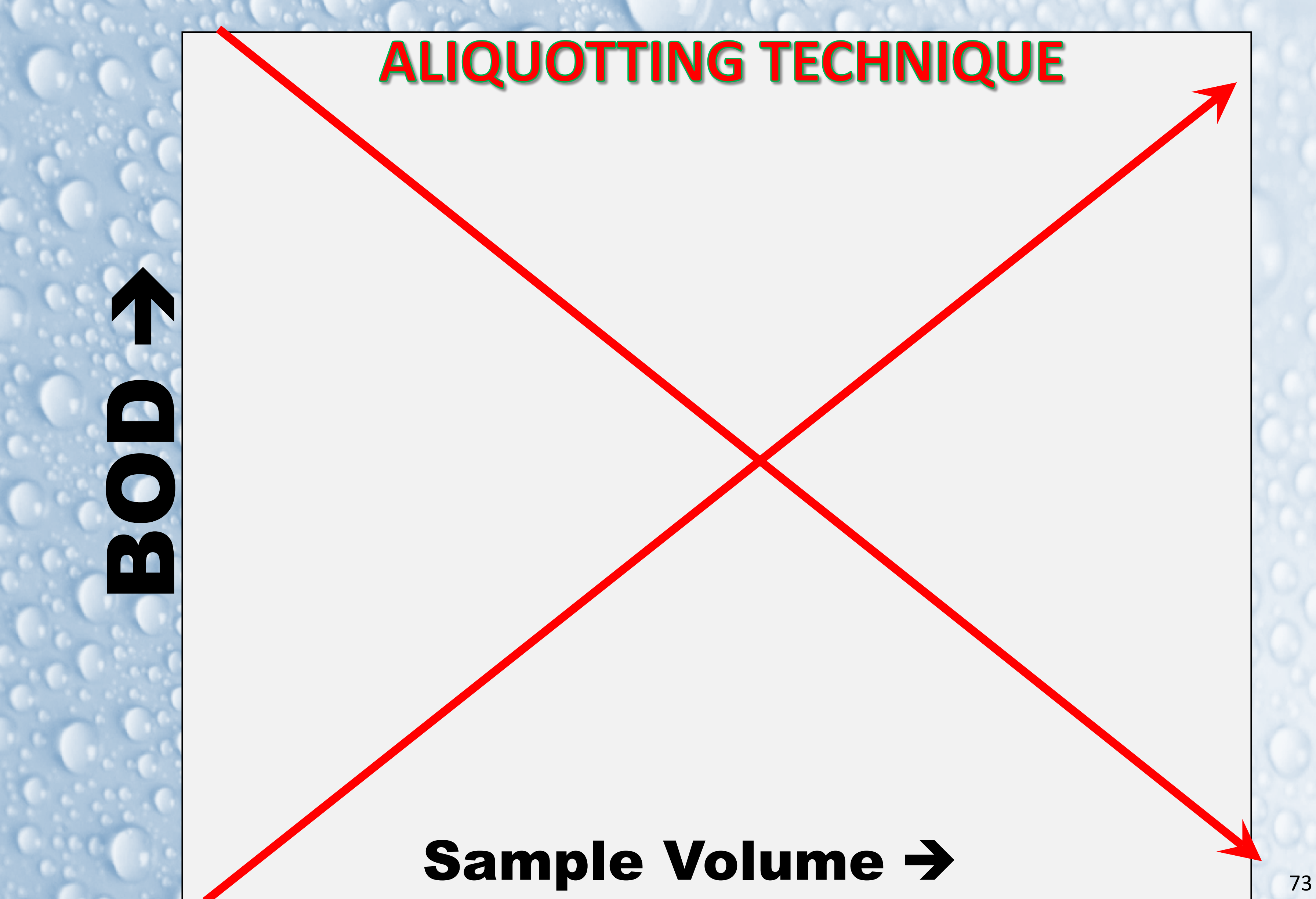
- Often referred to as "sliding" BODs
- BOD drops as dilution volume increases (less dilute)
- Often occurs in systems receiving industrial waste
- Amounts to killing off (*or severe shock to*) "the bugs"
- Results in UNDER-reporting the BOD of a waste
- Failure to mix sample b/w dilutions can APPEAR as toxicity. (*the opposite can happen as well!)*
- Even pH adjustments can result in this effect
- Poor technique (pipetting, pouring samples)

If nitrification IS occurring

(*remember : dilution water contains* ***NH_3***)

…as dilution ↑ , available NH_3 ↑ ==> final BOD ↑

Sliding BODs - aliquoting technique

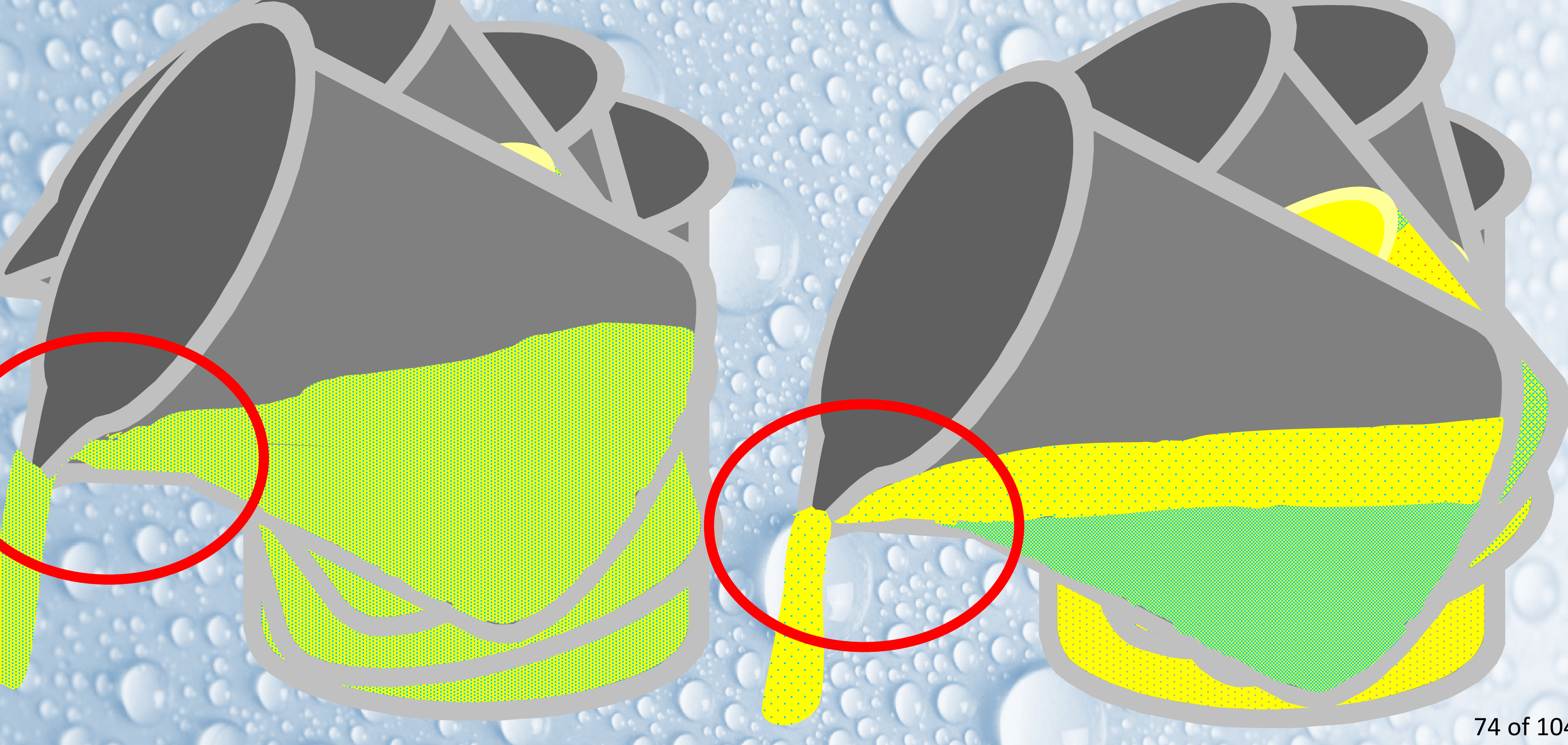


Aliquotting: How you pour

When pouring, solids are also actively settling during the entire transfer process

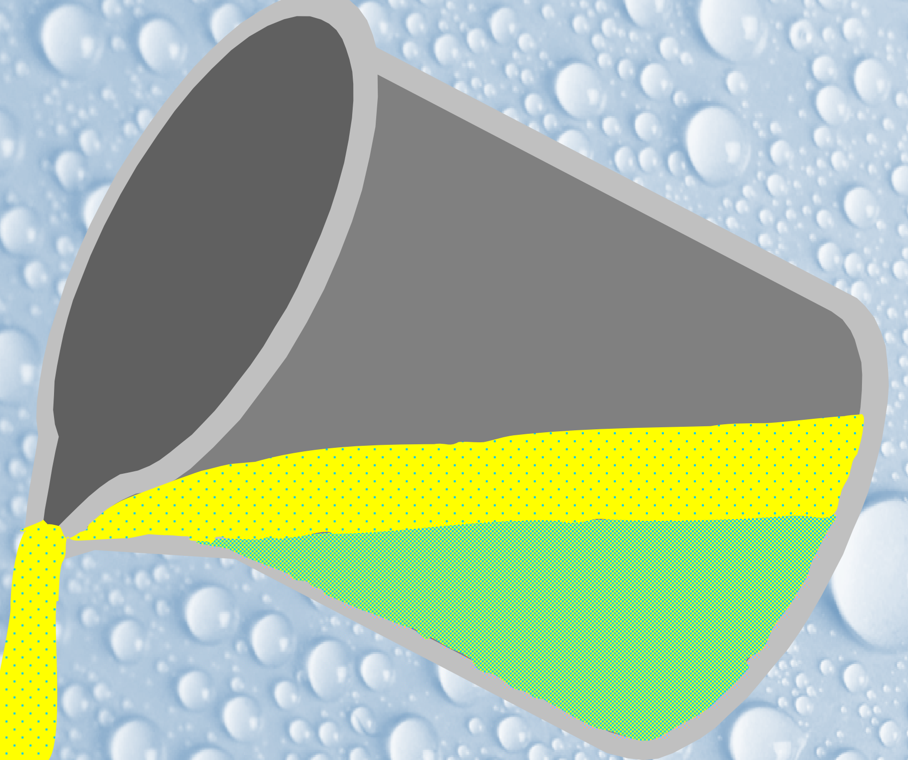
Pouring **quickly** favors an aliquot containing an even mix of sample.

Pouring **slowly** favors an aliquot containing a significant portion of diluted supernatant. Solids settle.



How could “aliquoting” go both ways?

Let’s say you are a “slower, methodical pourer”

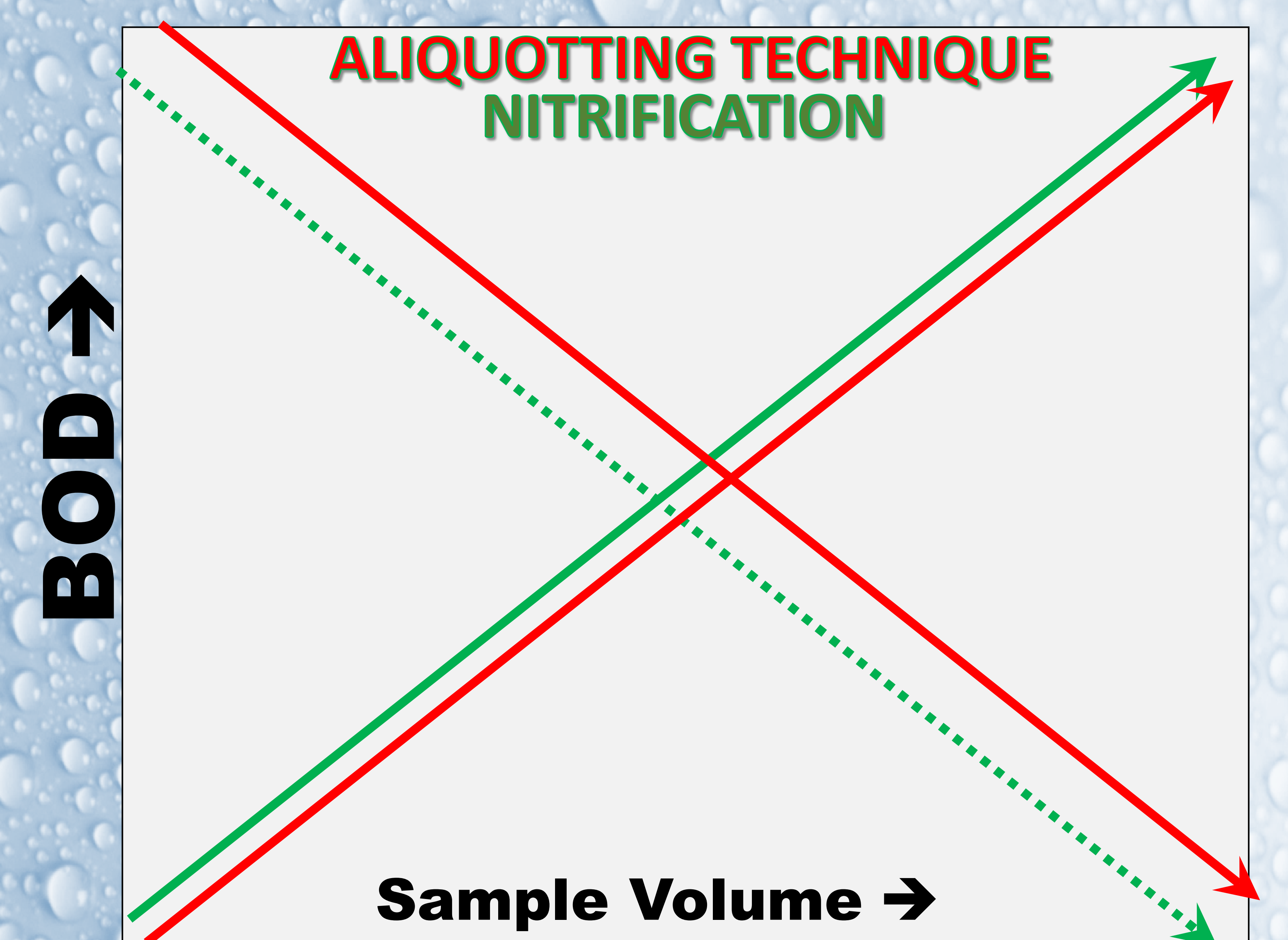


What happens if you pour out 100 mLs, then 200 mLs, and then finally 300 mLs?

Now ask yourself what if you did it in reverse order and poured 300 mLs, then 200 mLs, and then finally 100 mLs?

Remember: BOD and TSS are typically 1:1 ***(the BOD is in the TSS)***

Sliding BODs - Nitrification



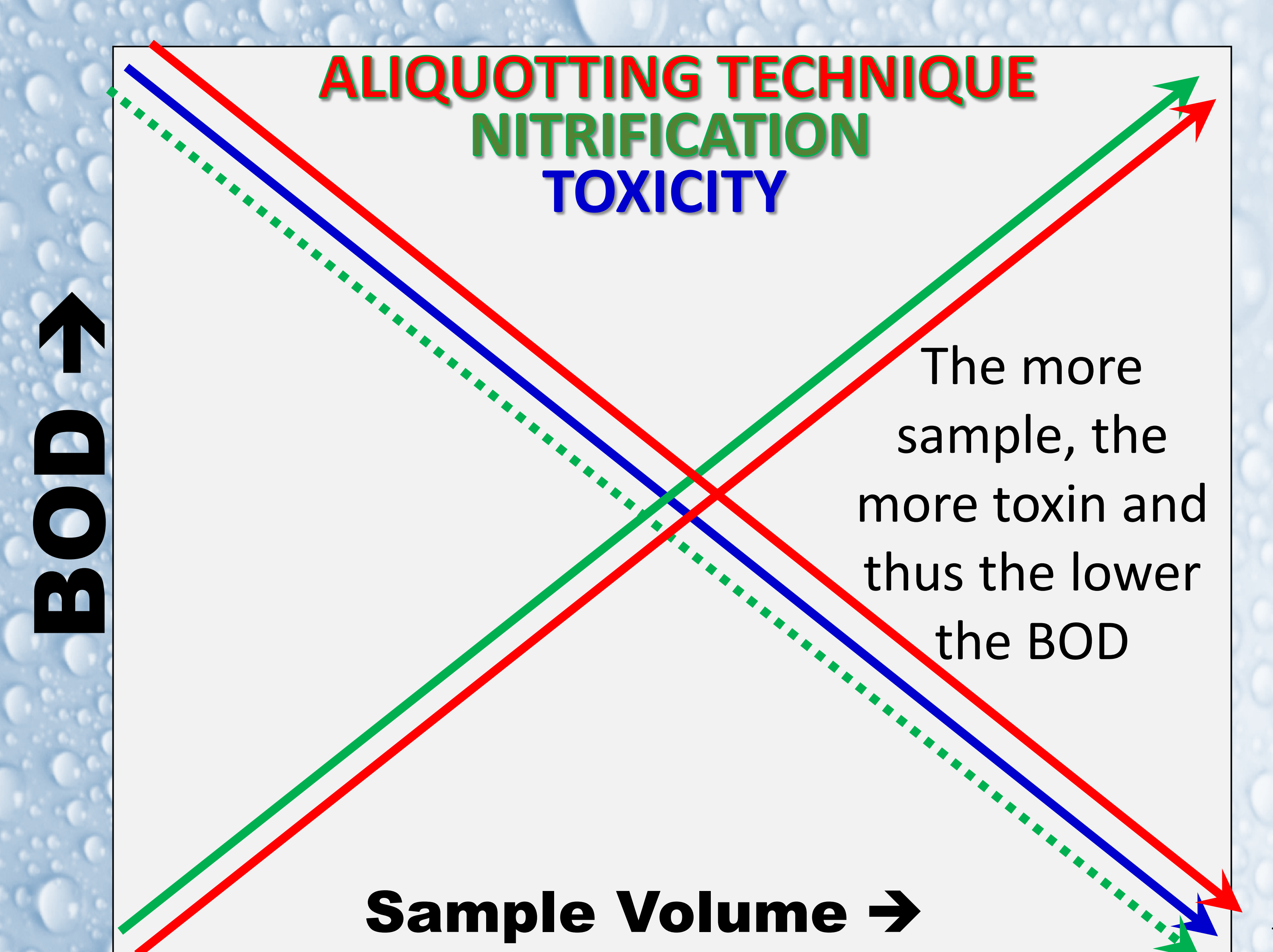
Wait!
Nitrification does that dual thing too?

Yes… given proper conditions (temperature, right bacteria, etc.) for nitrification to occur

Lots of NH3 in sample. NOD increases as sample volume increases. Thus BOD increases with increasing sample volume. *NOTE: The contribution from dilution water (below) is negligible in this case*

NO/minimal NH3 in sample. There is still NH3 in dilution water! Dilution water has an "NOD" of 2.04 mg/L.
Therefore, as the amount of dilution water in a sample (dilution) increases… i.e., sample volume DECREASES….BOD will increase.

Sliding BODs: add Toxicity to mix



What is NOT a “sliding” BOD

300 mLs → 2 mg/L BOD

200 mLs → 3 mg/L BOD

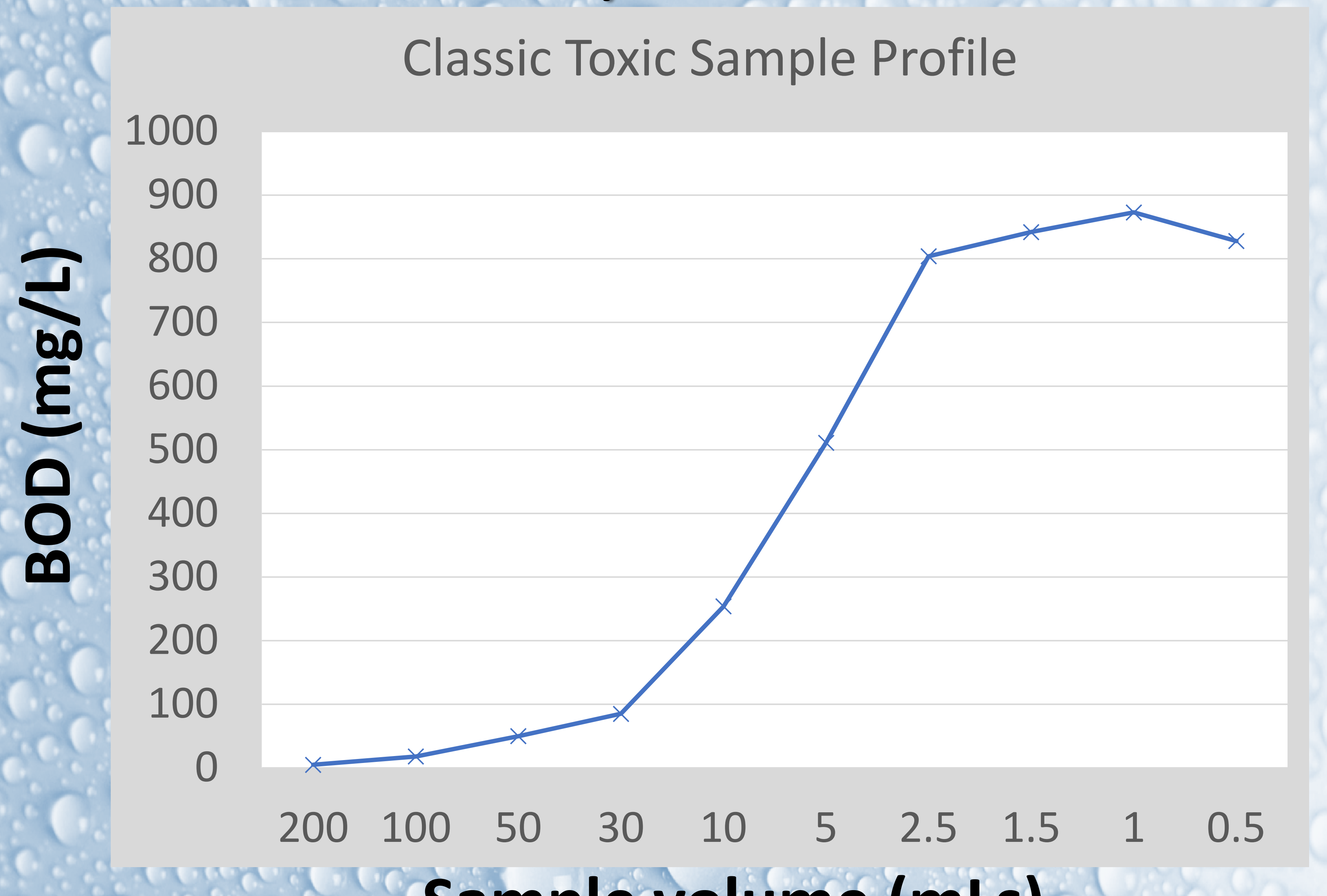
Difference of 2 and 4	= 4 - 2 = 2
Average of 2 and 4	= (2 + 4)/2 = 6/2 = 3
% difference	= 2/3 × 100 = **66.7%**

SM now says, “Identify samples in test reports when serial dilutions show more than 30% difference between high and low values.”

Sadly, SM does not understand that the scenario above, by their criteria, would require flagging.

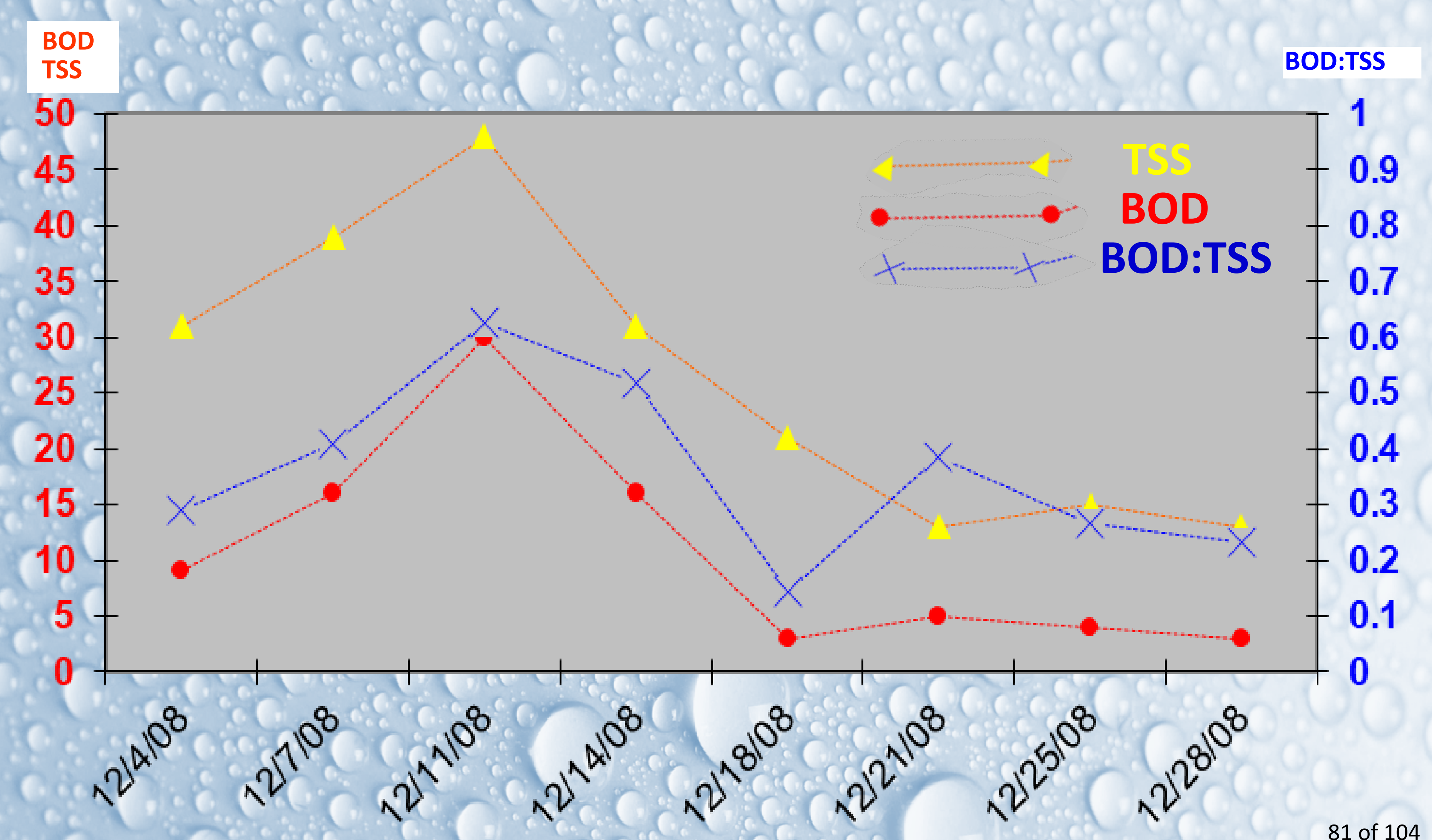
Hello! Std Methods! You just cant make up numbers!

What Does Toxicity Look Like?



Example of a WWTP Toxic plant upset

TSS consistently 2-4 times BOD (BOD:TSS ratio < 0.5).



Dealing with (and proving) Toxicity

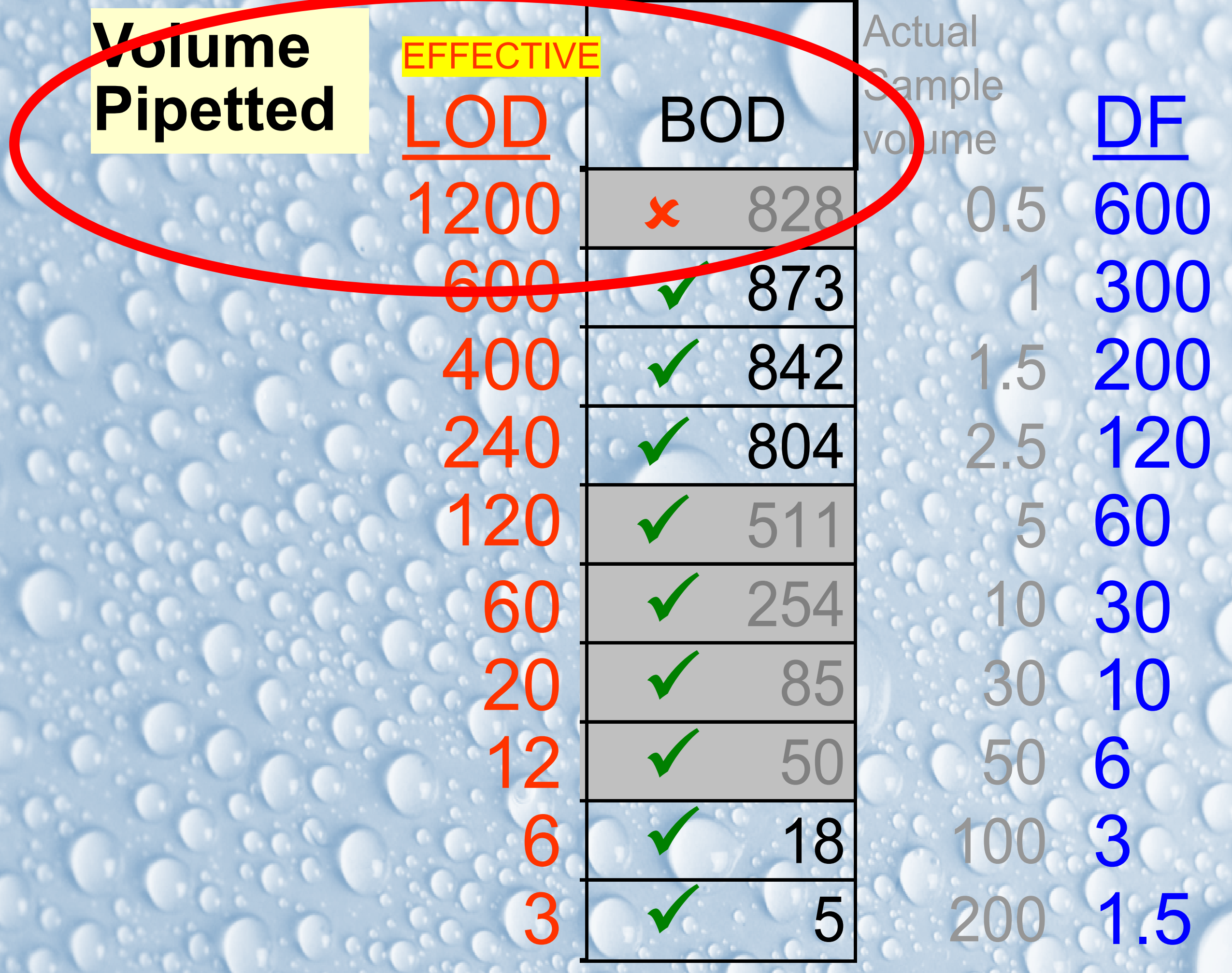
At least **LOOK** at dilutions that do not meet depletion criteria.
It will require MANY dilutions to prove toxicity.
You need enough dilutions to dilute out the toxic effect

The only way to PROVE toxicity is to analyze 10 or more dilutions and try to find the plateau where BOD levels off

You wouldn't "see" the trending unless you "calculated BOD (assuming DO_f=0) for each of the dilutions that over-depleted

Sample Volume	Initial Dilution	DO_I	DO_F	DO Depletion	BOD	Actual Sample volume
100	1	8.40	2.40	6.00	18	100
200	1	8.31	5.19	3.12	5	200

Applying a radical concept to the toxicity example



Volume Pipetted EFFECTIVE LOD	BOD	Actual Sample volume	DF
1200	✗ 828	0.5	600
600	✓ 873	1	300
400	✓ 842	1.5	200
240	✓ 804	2.5	120
120	✓ 511	5	60
60	✓ 254	10	30
20	✓ 85	30	10
12	✓ 50	50	6
6	✓ 18	100	3
3	✓ 5	200	1.5

If you consider an “LOD” to be unique for each dilution, we get even better information proving that we have diluted out the toxin and determined a valid result.

Just so we're perfectly clear…

- NOT suggesting you do something differently than what Standard Methods says.
- Just suggesting that this is an area where SM needs some outside-the-box thinking.
- The BOD calculated from each dilution **should** be considered relative to the LOD for that dilution.
- This is a RADICAL concept that, in some cases, represents a

- SM 5210B (22nd ed.) 7.b. *Reporting:*
- Average the test results for all qualified bottles within each dilution series. Report the result as BOD5 if nitrification is not inhibited.
- Samples showing large differences between the computed BOD for different dilutions, for example, greater than 30%, may indicate the presence of a toxic substance or analytical problems.
- Identify results in the test reports when any of the following quality control parameters is not met:
  - *[dilutions] …*more than 30% difference between high and low values

R 1 3 5
2 4 6
Nitrification

Nitrification is….

- The oxidation (using up oxygen) of reduced ammonia ($NH3/NH4^+$).
- Which means the conversion of ammonia (NH_3) to nitrite (NO_2) and then to nitrate (NO_3).
- The use of oxygen during nitrification translates to an oxygen depletion that is measured as BOD.
- It's called "nitrogenous demand"…or "NOD"
- It requires the presence of specific bacteria: *Nitrosomonas* and *Nitrobacter*
- *…and usually only occurs at temps > 10C (50F)*

Nitrogenous Oxygen Demand (NOD)

Reduced Nitrogen + Oxygen → Nitrite (NO_2) → Nitrate (NO_3)

$$NH_3 + 1.5\ O_2 \rightarrow HNO_2 + H_2O + \text{cells}$$

$$HNO_2 + 0.5\ O_2 \rightarrow HNO_3 + \text{cells}$$

$$NH_3 + 2\ O_2 \rightarrow HNO_3 + \text{cells}$$

Theoretically 1 mg/L of NH_3-N requires 4.57 mg/L O_2 to oxidize NH3 to NO3-N

NOTE that the equation generates acid (HNO_2, HNO_3), which neutralizes substantial sample alkalinity in order to maintain pH

In case you need to know…

1 "N" yields 2 "O_2"

1 "N"= 14 g/mol

2 "O_2"= 64 g/mole

∴ 64/14= 4.57

Dilution Water & GGA NOD

NH_3-N in dilution water alone can contribute up to 2 mg/L NOD without accounting for any sample dilution factor.

"one bottle" nutrients packages add the equivalent of 0.45 mg/L as N. *(2 mg/L NOD).*

A 200 mL sample dilution, to which additional nutrients are added contains as much as 4.4 mg/L of BOD in the form of NOD.

GGA also contains NH3 and thus has a nitrogenous demand.

Remember, this doesn't occur w/o specific bugs

Nitrification Benchmarks

- If TSS ≤ 50% of BOD result (BOD: TSS ratio > 2:1) then consider…don't assume…the possibility of nitrification.
- Look at NH3 levels. If no measurable NH3, it can't be NOD!
- NH3 mg/L x 4.57 = potential "BOD" due to NOD.
- Nitrification occurs most often in warmer months. Nitrification doesn't occur below 10ºC.
- Could BOD:TSS ratio high due to soluble BOD?
- Run side-by-side: (A)an effluent BOD as is and (B)also after passing effluent thru a TSS filter.
- Finally, run side-by-side cBOD/BOD determinations. cBOD should be considerably less than BOD (*depends on available NH3, of course!*). cBOD is about 80-90% of BOD if no nitrification is occurring.

cBOD < BOD Isn't Enough

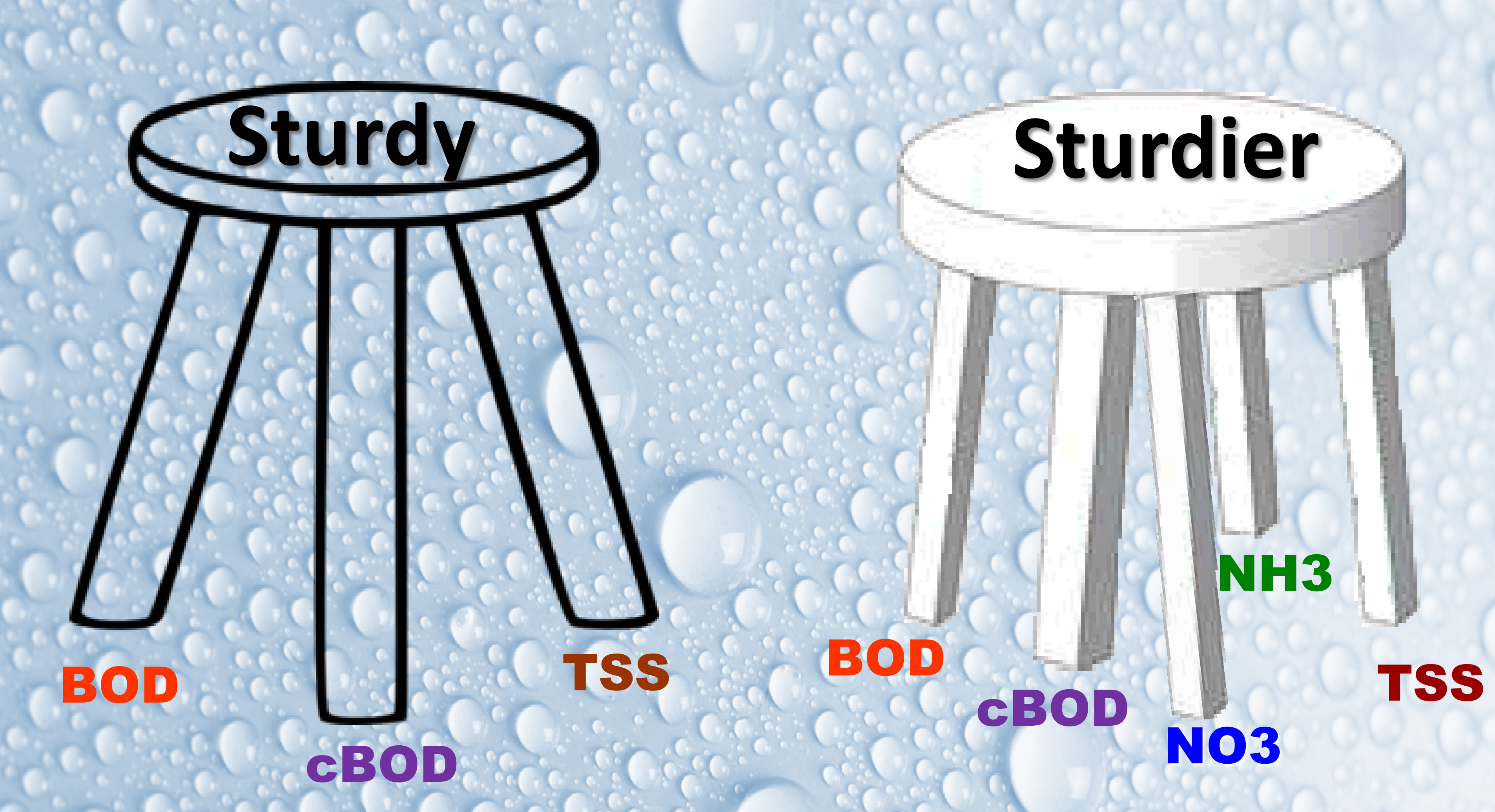
Just having a cBOD that is less than BOD is not enough to make a claim that nitrification is occurring

- Addition of cBOD nitrification inhibitor reagent will ALWAYS lower BOD.

HELLO MCFLY

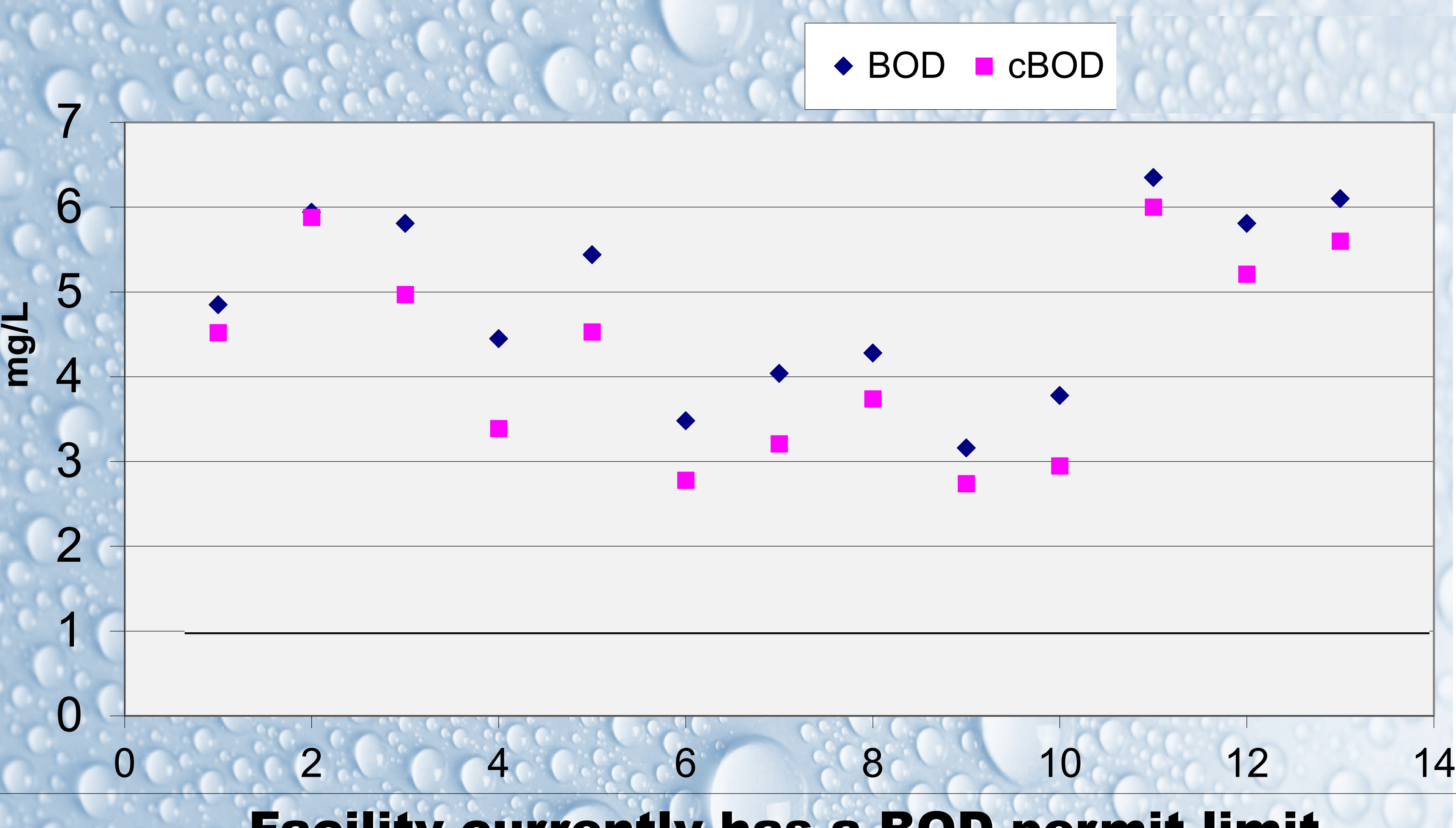
- **Hello!**…**McFly!** It's a toxin!
- It's the EXTENT of the reduction (BOD:cBOD ratio) that tells the tale.
- How MUCH less cBOD is-- relative to BOD-- matters!

Nitrification: 4 Legged Stool



BOD, TSS and cBOD data are a start.
But to prove nitrification, you will need to run NH3.
Showing that NO3 is increasing is even better!

Pelican WWTP: Is this Nitrification?

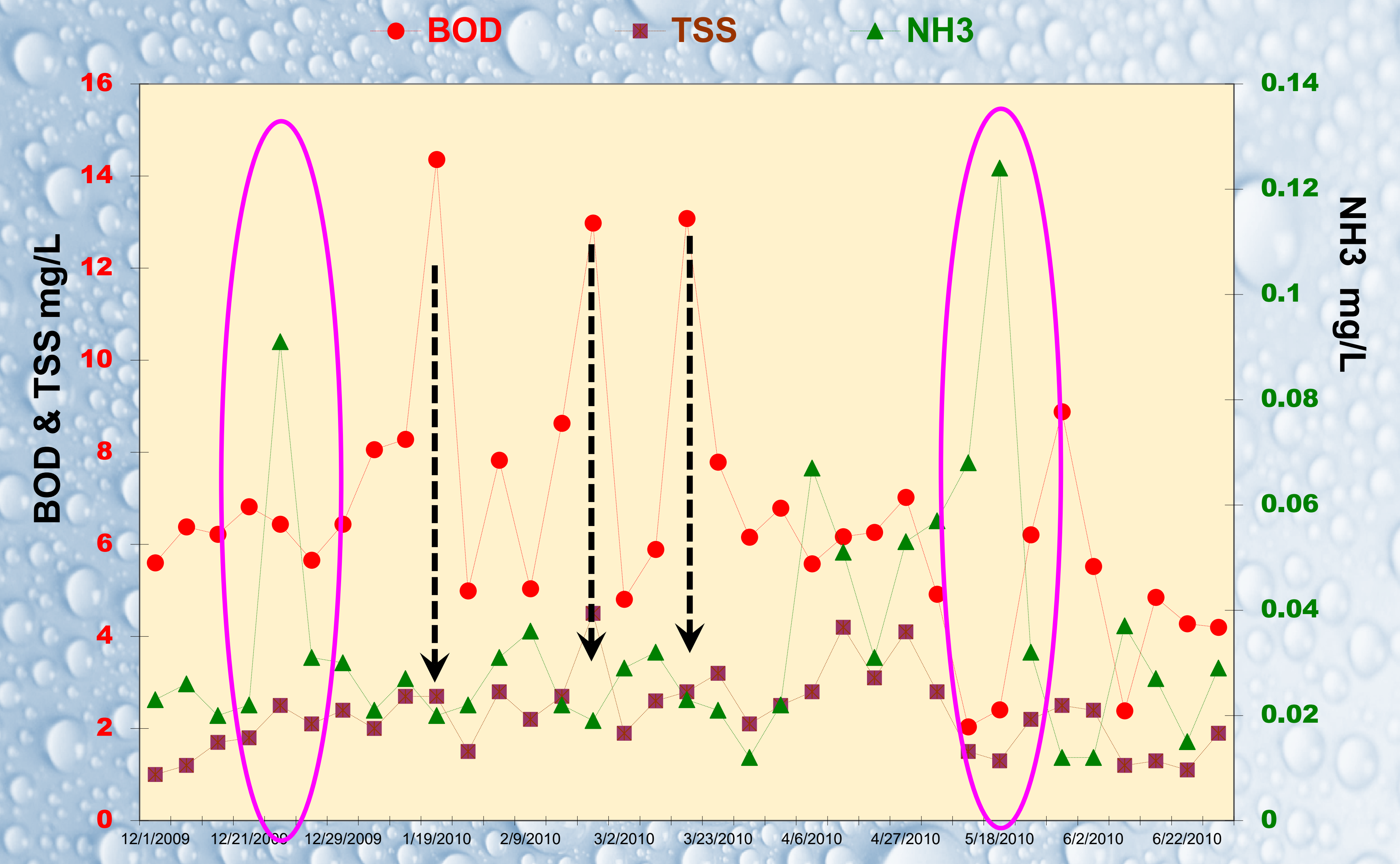


Facility currently has a BOD permit limit

With nitrification, should see cBOD <<< BOD

Pelican WWTP: Is this Nitrification?

Complete picture. How does BOD match with NH3?



Judging Nitrification overreactions

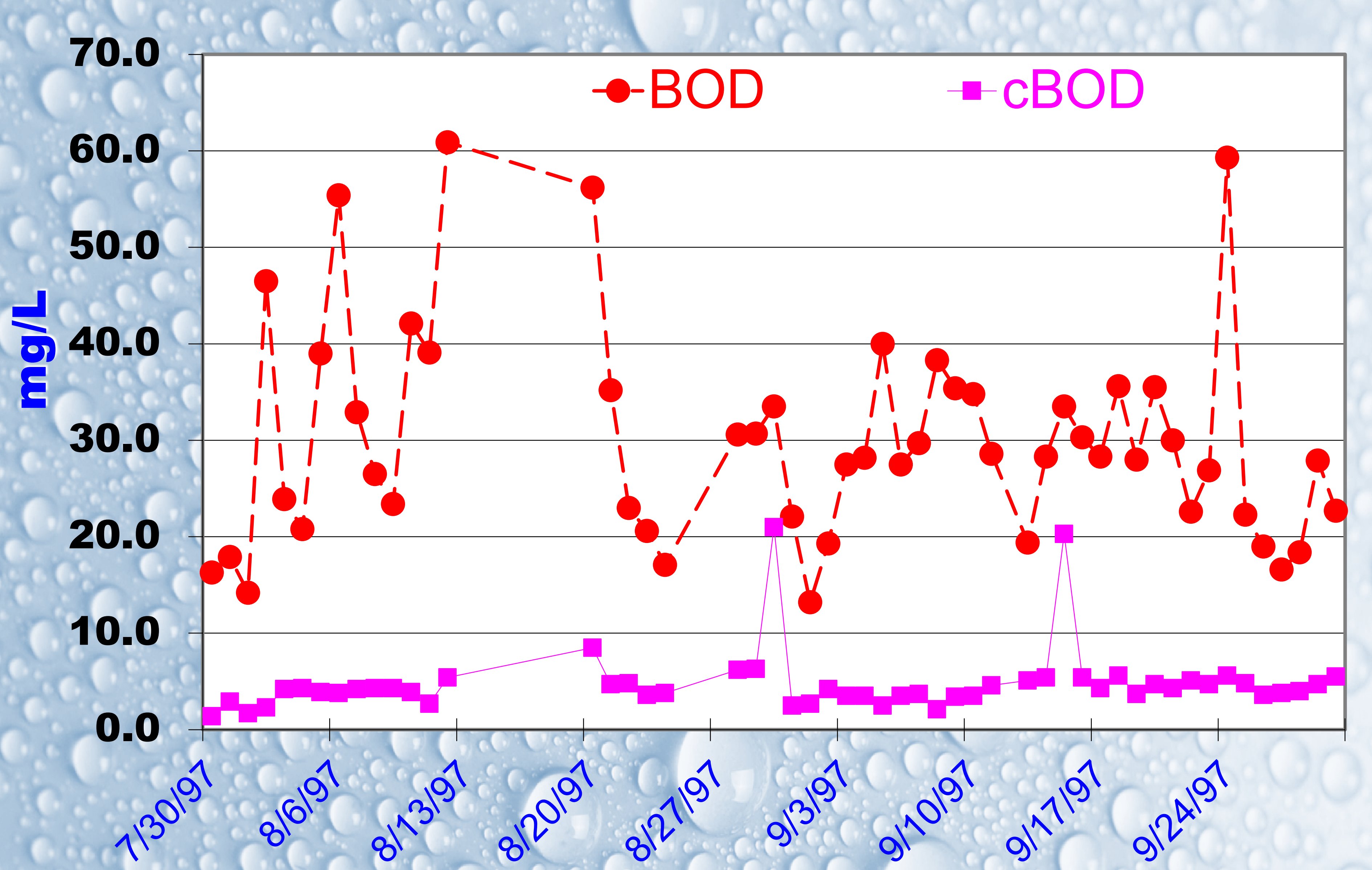
Pelican WWTP is nitrifying and needs a cBOD permit limit

- If the average NH3 level is about 0.03 ppm, that only explains about 2.4 ppm of "BOD" (vs. 8-14ppm).
- The difference between BOD and cBOD is less than the Nitrogenous demand of dilution water and sample NH3
- NH3 levels increase on several occasions while BOD actually drops.

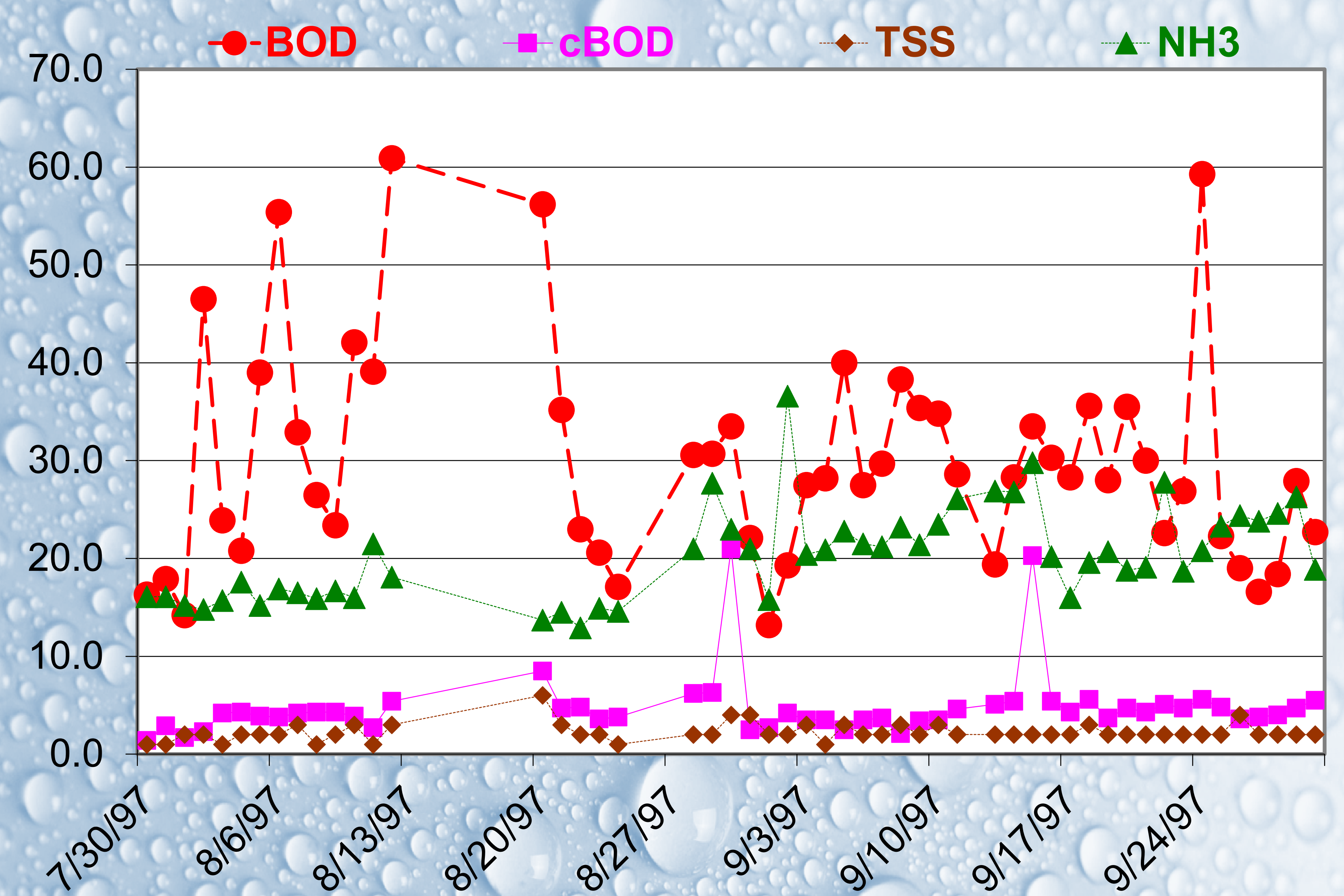
The verdict:

OVERREACTION

“Hoover” WWTP is this Nitrification?



“Hoover” WWTP Nitrification (+ TSS & NH3)



Judging Nitrification overreactions

Hoover WWTP is a classic case of nitrification

- BOD:cBOD ratios as much as 15:1, consistently 5-6:1
- cBOD tracks almost perfectly with TSS
- THIS is a fairly classic nitrification pattern
- NO3 data would be icing on the cake
- Not perfect…nitrification is limited: NOD of at least 70 ppm; BOD averages about 40 ppm

The verdict:

NOT an OVERREACTION

Main Takeaways from today

- Much of blank issues are related to PTS(D)
- Be aware of weather and its impact on BOD
- Be consistent in every analyst in every detail.
- Apply your knowledge to situations
- Document everything.
- Remember that Standard Methods is NOT perfect.
- Apply common sense when methods seem illogical (because they often are)

It's nice to have goals, but…

If you expect to meet criteria for every GGA and blank *(or even 90% of),* you are setting yourself up for disappointment.

YOU CAN DO IT!
memegenerator.net

THANK
YOU!

Aaaahhhh…Venice. Just DO NOT think about what goes into the canals!

Nitrification & Other Parameters

- Ammonia N (NH_3-N) – [Inf and eff] - If much nitrification is occurring, would see a significant decrease in ammonia levels as it is used up.
- Nitrite (NO_2) – [Eff] -generally <0.5 mg/L, anything greater would be considered high.
- Nitrate (NO_3) – [Eff] - Expect undetected without nitrification. If nitrification is occurring, expect nitrates anywhere from 3-15 mg/L or greater ***depending on NH3 levels***.
- Alkalinity (as $CaCO_3$) [Inf and Eff] - Expect a significant decrease if nitrification is taking place. Effluent alkalinity levels <50 mg/L indicate potential for pH problems.