Survival and Growth Performance of *Crassostrea virginica* in the NYC Harbor

Cézanne Bies and Zain Bin Khalid

Advisor: Mauricio González, M.Sc.

Mentor: Matthew P. Hare, Associate Professor, Natural Resources (NTRES/DNR), College of Agriculture and Life Sciences (CALS)

Source: C.Bies

Marine Biology Research Program

New York Harbor School

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Abstract

Bacterial contamination, over harvesting, pollution, and sewage overflows have depleted the Eastern Oyster population. (Munoz, *et al*. 2010). Many restoration projects are using wild oysters to replenish the decreasing supply; however, these projects lack enough scientific data to allow for comparison of the growth performance and phenotype for the different spats and their spawning methods. In this study, oyster growth performance was compared between mass and Individually spawned Eastern Oysters, (*Crassostrea virginica*). The process was performed by measuring the size and mortality of both spats over the span of 4 weeks, and physical– chemistry data was collected and analyzed at the site to determine if there were any environmental disturbances present in the water. If two wild spats of *Crassostrea virginica* are produced with different spawning methods and compared using growth performance and mortality as measures, then the individual spawned oyster group will exhibit faster growth rate and have less mortality. After conducting three statistical analyses, the performance between the mass and Individual spawning methods were found to have a significant difference. Physical chemistry did not appear to significantly alter the growth and mortality patterns of the oysters. From this research, mass spawned Eastern Oysters, (*Crassostrea virginica*), were found to have better growth and mortality patterns.

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Introduction

New York City's ground and surface waters are being contaminated with heavy metals, persistent organic pollutants, and nutrients that have an adverse effect on human health (Bharali, 2007). However, eastern oysters (Crassostrea virginica) act as nature's biofiltration system, each providing ~50 gallons of purified water per day (Merrill, 2014). New York harbor once had up to 350 square miles of oysters, producing roughly about 300,000 bushels a year (Nigro, 2011). Today, that population has substantially decreased due to the effect of bacterial contamination, over harvesting, pollution and sewage (Munoz, et al. 2010). Only a small number of oysters remain in the NYC harbor, and that damage cannot be fixed without proactive conservation efforts. By tracking the growth and mortality each week, a better comparison between spawnings can be found.

Background Information

The Eastern Oyster (Crassostrea Virginica) also called the Atlantic Oyster, American Oyster, Common Oyster and Virginia Oyster inhabits the North Atlantic shores from the rocky coastlines of Maine to the southern shores of the Gulf coast. The Eastern Oyster shell (valve) length can grow in to approximately 8 inches (20cm). The covering shells are known as bivalve connect by a natural joint and single large muscle (abductor). The coloration may range from alabaster white to dark gray. The shell texture also varies from those patterned with smooth but ridged surfaces to others with coarse, ridge– like surfaces. They mainly ingest algae and plankton, and their biggest predators are humans who harvest them. Other predators are crabs, snails, sea stars,

sea nettles, sea stars, sea anemones and parasites threatening them while also sharing their marine habitat. The spawning season for oysters is approximately seven months from late spring to early fall.

During this season a female can produce and release over 100 million eggs. Oyster larvae are about the size of pepper grains. They will dive down several feet below water, and excrete a gorilla– like glue substance that serves to attach it to rock or dead shell, thereby becoming a permanent, lifelong resident. Eastern Oysters can live in a variety of water conditions, ranging from shallow near– fresh waters to the saltier ocean water bays and estuaries. They are found in depths of water from 8 to 25 feet deep and with water temperatures that vary from 28 and 90 degrees. They are found attached to rocks, boats, bottles, crabs, and ideally other oysters (also known as an oyster reef). In a conductive environment, an oyster can grow in a short period of time – with spat growing one inch in three months, two inches in seven months, and three inches in 15 months. Approximately one percent of fertilized eggs reach maturity. Lifespans of oysters vary based on freshwater and the number of predators. Oysters are protandric in the first year (having male sexual organs while young and female organs later in life). Oysters survive using their gills to filter plankton and algae for nutrition. Due to bacterial contamination, over harvesting, pollution and sewage overflows, the number of wild Eastern oysters *(Crassostrea virginica)* have decreased dramatically over the last few decades. There is not much scientific data available to compare phenotypes of mass and individual cohorts of eastern oysters. A city– wide effort has been started with the goal of restoring a self– sustaining population of Eastern oysters in the Hudson River and other regional waters. The *C. virginica*, or eastern oysters, play a major role in the proper functioning of estuary ecosystems (Dame 1972) especially within New York harbor.

Project Design Chart

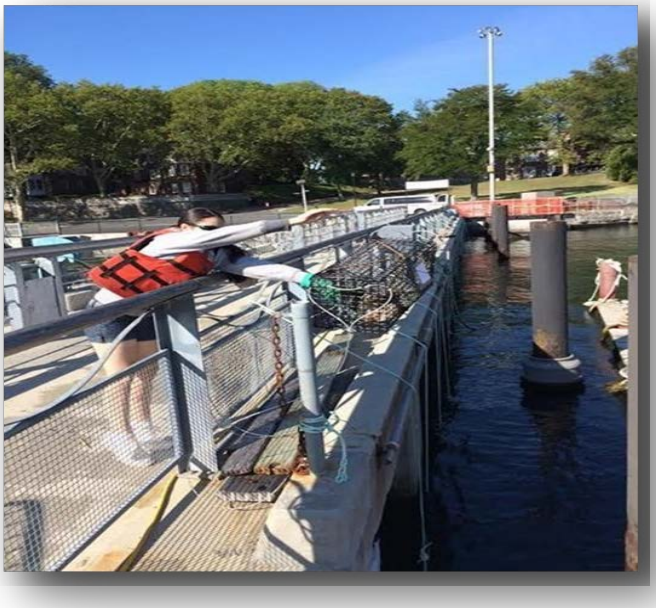
Table 01. Project Design Chart	
Category	Entry
Scientific Problem:	Which method oyster spawning is better adapted to the conditions of the Hudson River Estuary, mass or Individually spawned oysters?
Hypothesis 01:	Individually spawned oyster groups will exhibit faster growth rates and experience less mortality
Hypothesis 02:	Mass spawned oyster groups will exhibit less mortality rates and faster growth rates
Null Hypothesis:	Both groups mass and individual spawned will show similar mortality and growth rates
Objective 01:	Determine which spawning method (mass, individually) will exhibit a faster growth and/or lower mortality rate
Objective 02:	Understand how water stressors and changes in water quality affect oyster performance

Table 02. Experimental Design			
Proposed Variables		Proposed Controls	Proposed Constants
Independent	**Dependent**	N/A observational project	• Location of the cage containing the oysters • Depth placement of the cage • Size and Shape of cage • Number of spat counted
Spawning Method Type (Mass or Individual)	Growth & Fitness of both oyster groups		

Locality

Fig.1: The Oyster crates were tested on Pier 101 on Governors Island, located at 40.691453, 74.012461)

Fig.2: Testing cage with mass and Individually spawned oysters being released at Pier 101



Materials

Item	Function	Quantity
Waste beaker	To remove possible toxic chemicals from surfaces	1
Moshnet cage with screen– boxed bottom. 26x16x14(in) / 6.04x40.64x35.56 (cm)	To contain Oysters	1
Temperature monitors	To measure temperature of oysters	1
Rope	To secure moshnets	2
Nursery tanks (Individual, Mass)	To hold larvae strains and breeding oysters	2
Water heater	To incubate Nursery tanks	2
Dead Oyster Shells	Surface for the larvae to set and cement properly	2 Bushels full ~300 shells
Winkler Method test kits	To test the Dissolved Oxygen	1
Plankton, algae particles	Food for the oysters	~ 30 ml bottle
YSI ProPlus Sans	To measure Dissolved Oxygen (ppm), PH, Salinity (ppt) and Temperature (C)	2
Test Strips	To test Ammonia, Nitrite, Nitrate, and PH	5 bottles
Caliper	To Measure oysters (cm)	2
Refractometer	For measuring Salinity (ppt)	1
Secchi Disk	To measure water transparency	2
Sample Vials	To hold water samples	4

Procedures

A selected collection of 50 spat were provided by a first generation of mature oysters, *C. virginica* cultivated in Martha's Vineyard. The mature oysters, *C. virginica* were originally collected from the shores of Martha's Vineyard, placed into two tanks of standard dimensions, and then acclimated in hatcheries (41.395614, – 70.521689). The oysters were divided into two groups to be bred: Mass spawning, and Individually spawned. The oyster strain bred through Mass spawning was successfully created by removing the mature oysters from their tank, keeping them together in the same bucket, and subsequently bred collectively. The Individual spawning cohort was established by redistributing oyster semen equally, coming from each mature male sexed to each female via micropipette. This ensured that each male's gametes had an equal chance of becoming a zygote. Both groups were placed in identically salinized and heated tanks water temperatures between 20°C to 30°C (74°F to 86°F) and salinity was at set at a level above 10ppt (Meritt, 2012) simulating an environment for breeding. After breeding, sedimentation was facilitated by placing larvae onto dead shell. In the shared habitat, once in juvenile state (when the oyster spat has successfully settled itself), both spawnings were packed into two different nets with 50 cohorts (1– 2 spat per shell). These two different nettings were identified by the amount of zip ties attached to each – 2 zip ties for Mass, and 1 zip tie for Individual Spawning. The nets were packaged into a cage with the dimensions of 26x16x14(in) / 6.04x40.64x35.56 (cm), which was then set in the New York (Upper Bay) waters of Pier 101 off Governors Island, located at (40.691453, – 74.012461). Fitness (mortality and growth) and Physical Chemistry (salinity, temperature, pH, dissolved oxygen, ammonia, phosphate, nitrate, nitrite, and secchi depth) were measured every week, in the span of 4 weeks, from the release of

the cage. Mortality is assessed by how many shells are emitting an “ooze” (releasing water when pressure is added), half open, or have only half of a shell. For analyzing the data collected from each spawning, 3 t– tests were used to evaluate the significant difference between Mass and Individual spawning:

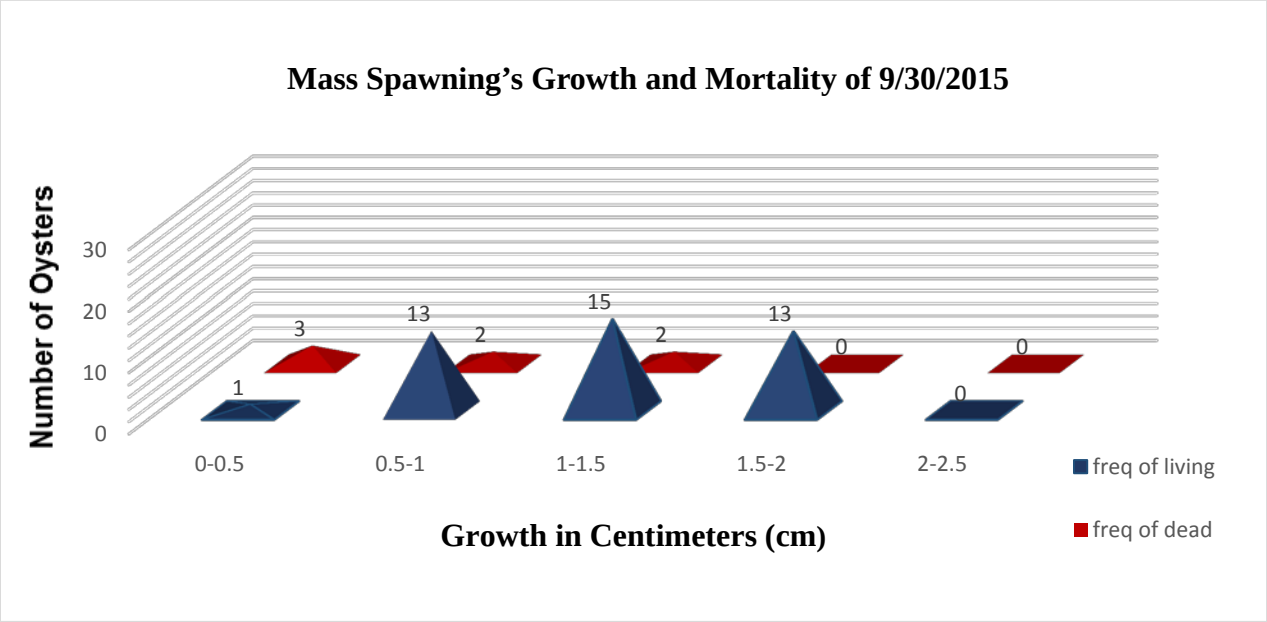
1.) An evaluation of the total data collected over the course of four sample weeks.
2.) A comparison of this new data with the last oyster measurement data collected, performed on 11/18/15; and
3.) Analysis of dead oyster measurements.

Each t–test was created using the following equation:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\left(\frac{(N_1 - 1)s_1^2 + (N_2 - 1)s_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

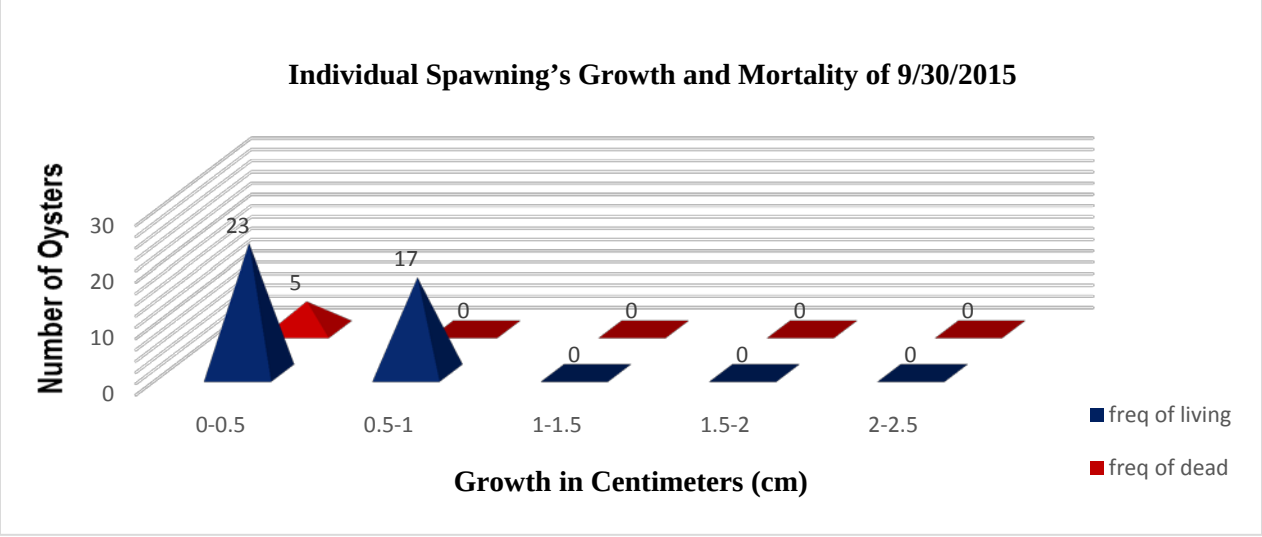
Observations and Results

Fig. 3: Mass spawning on 9/30/15



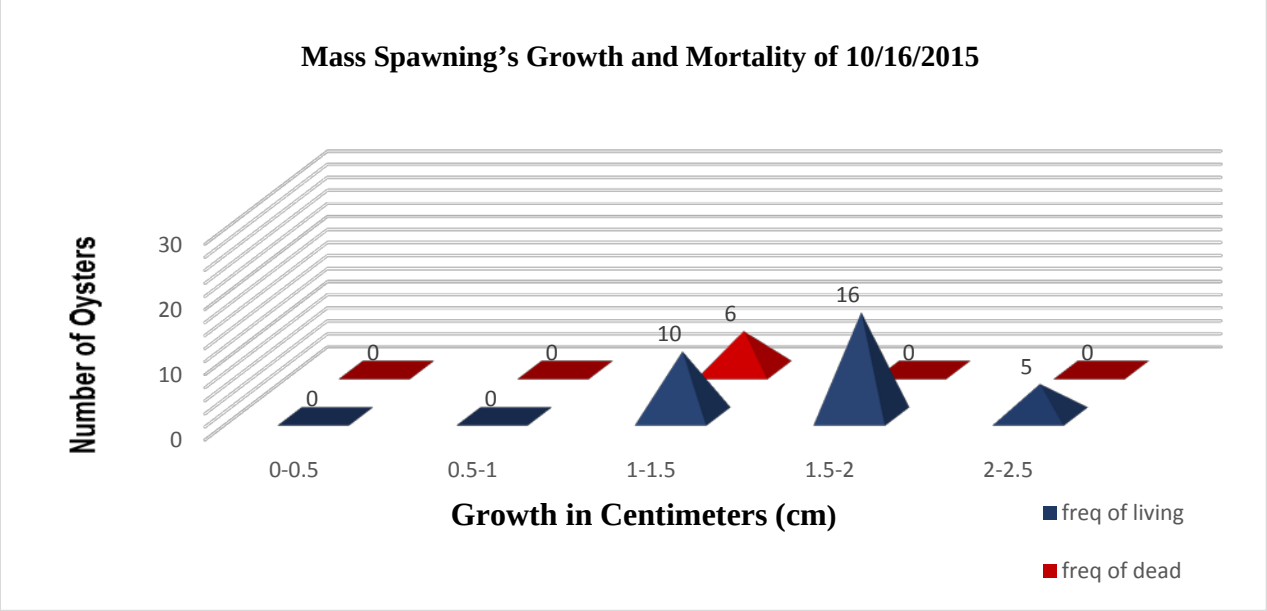
The first measurement of the Mass spawning shows a high frequency of living oysters between the measurements of 0.5–1.0, 1–1.5 cm, and 1.5–2.0 cm and the highest mortality level of 3 oysters in the 0– 0.5 cm frequency bin.

Fig. 4: Individual spawning on 9/30/15



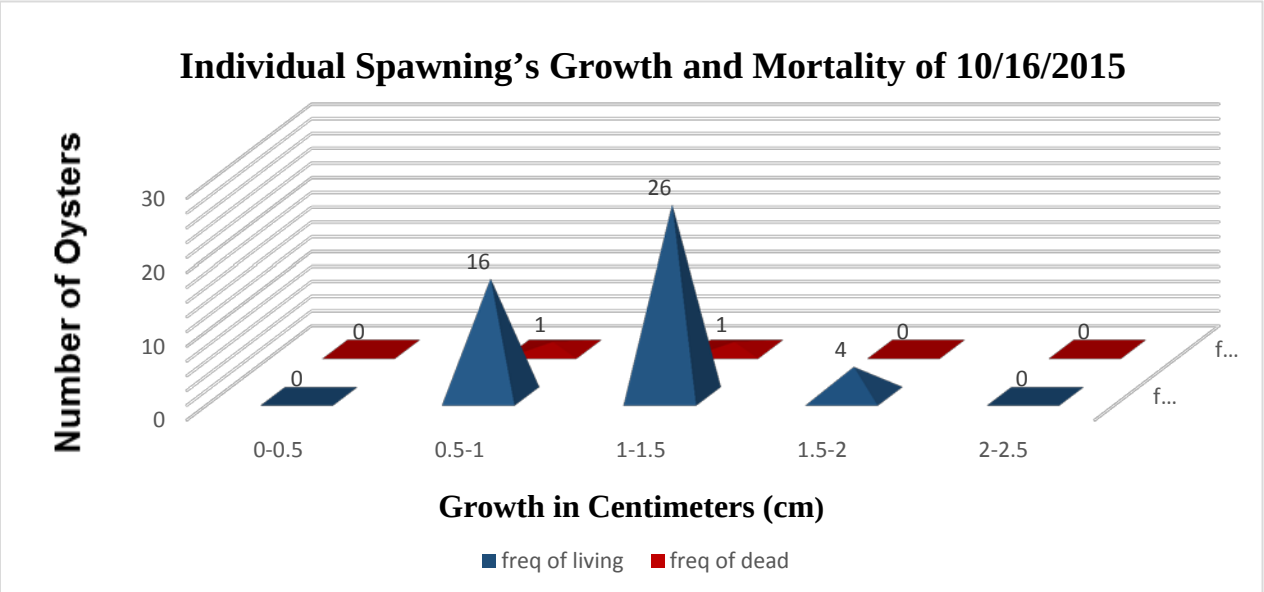
The first measurement of the Individual spawning displays a high frequency of living oysters between the measurements of 0–0.5cm and .05-1cm. There is also a beginning mortality of 5 in the 0-0.5 bin.

Fig. 5: Mass spawning on 10/16/15



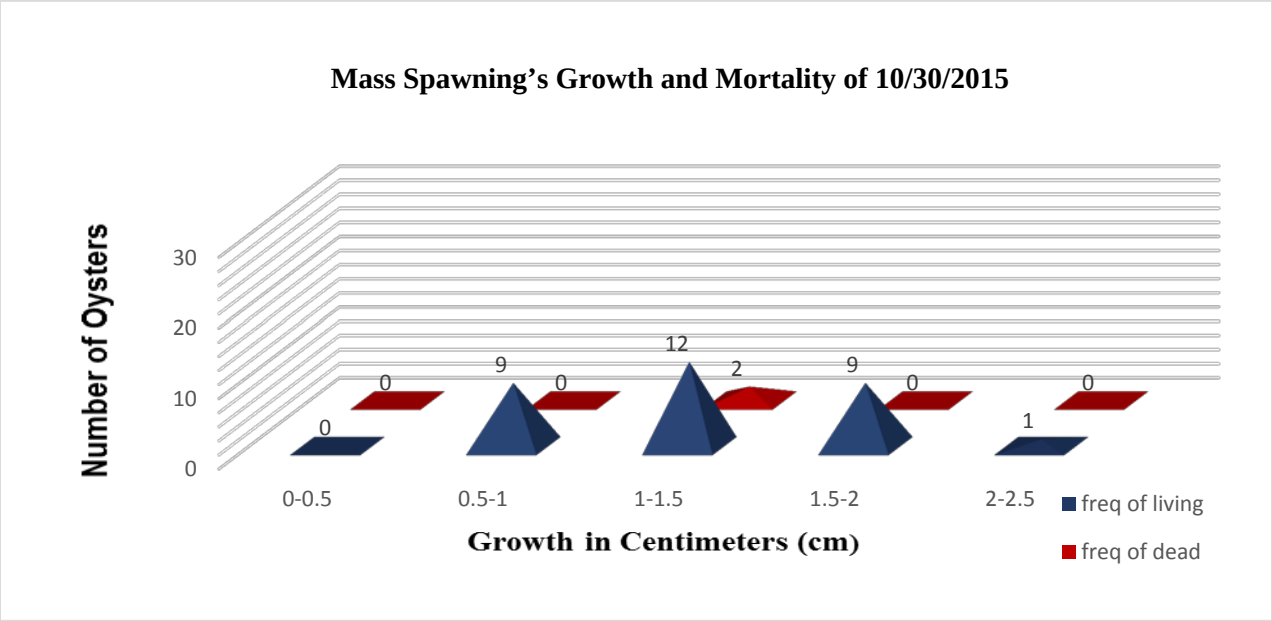
The second measurement of the Mass spawning showed a decrease in the frequencies between the measurements of 0–0.5 cm, and 1-1.5 cm. However, the measurements of .5-1 cm have increased by 10. No dead oysters in the 0–0.5 cm bin were counted in the random selection process. There is a new peak of oysters in the 2-2.5 bin, meaning those in the 1.5- bin must have grown.

Fig. 6: Individual spawning on 10/16/15



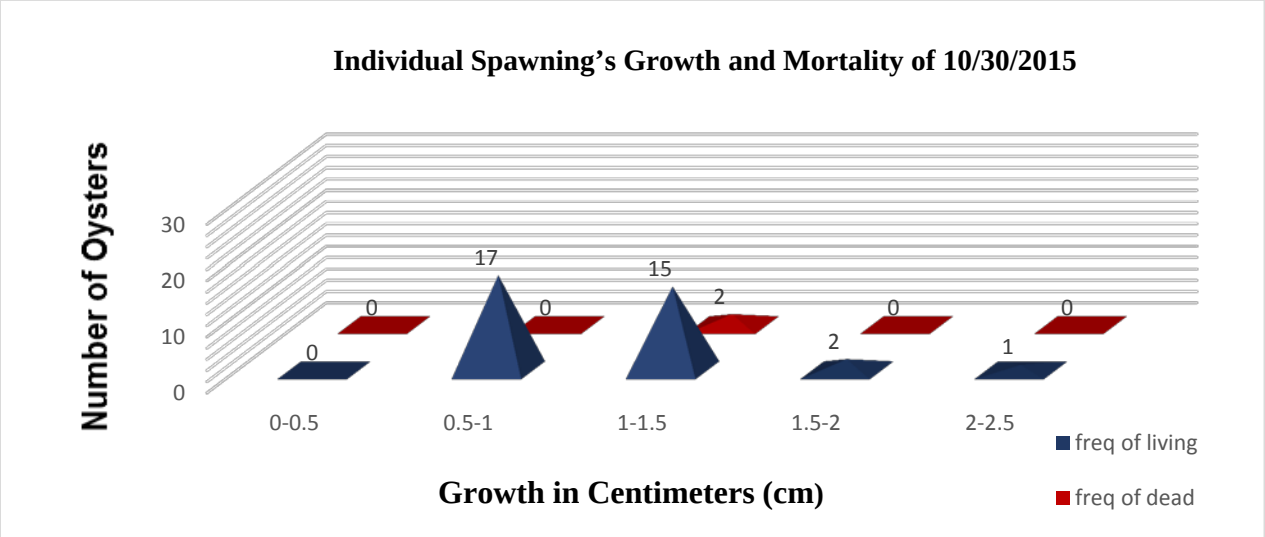
The second measurement of Individual spawning shows a significant growth of the oysters, spiking at 1.0–1.5 cm, and starting to make a peak at 1.5–2.0 cm. This means that the oysters that once had a frequency of 0–0.5 cm, have now began to grow without significant mortality.

Fig. 7 Mass spawning of 10/30/15



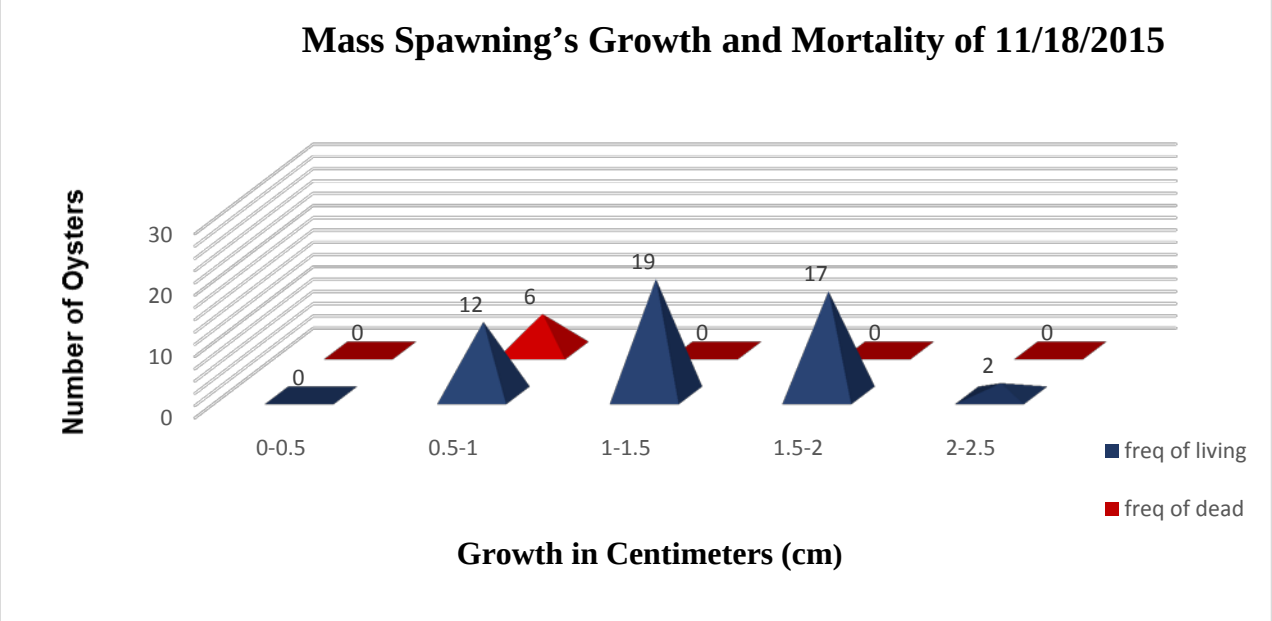
The third measurement of Mass spawning, similarly to the third Individual spawning, displays a dramatic decrease of bins 1.0–1.5 cm, 1.5–2.0cm, and 2.0 cm compared to the last sampling,

Fig. 8 Individual spawning of 10/30/15



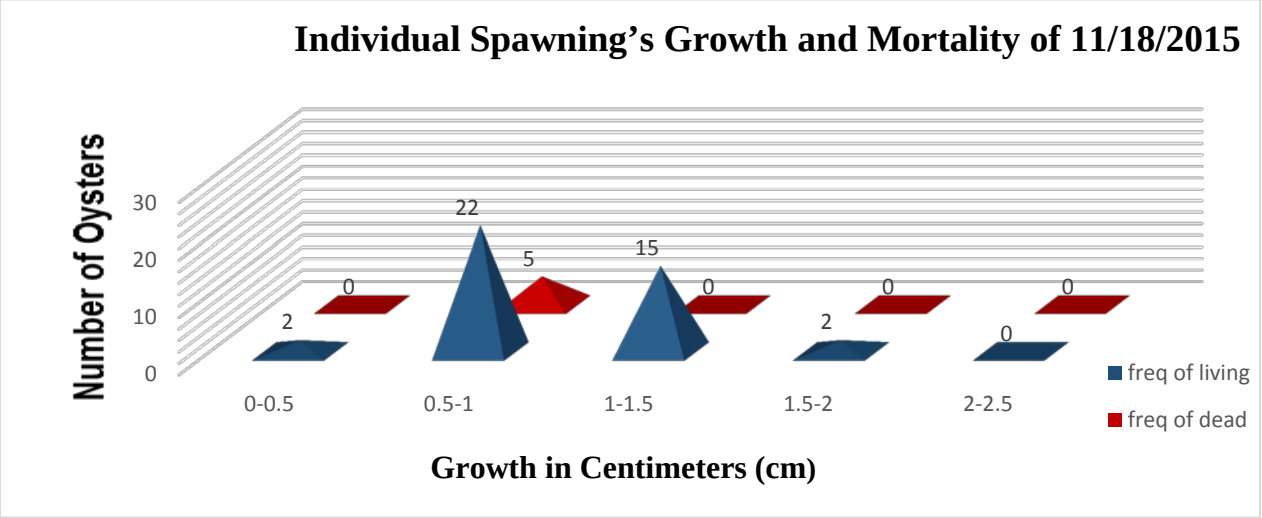
The third measurement of Individual spawning shows a dramatic decrease in the frequency in the bins 0.5– 1.0 cm, 1.0– 1.5 cm and 1.5– 2.0 cm. This extreme change could have been the product of three variables–the predatory species, Microciona prolifera, began appearing on some of the shells and could be a cause of the decrease of large oysters. Next, spat could have been overlooked during the process of collecting 50 spat, due to their poor visibility.

Fig. 9. Sizes of Mass spawning of 11/18/15



The last measurement of Mass spawning, there was a dramatic shift in measurements, bin 1.5-2 cm holding up to 17 oysters in addition to 1.5-2 cm bin frequency showing growth of 17. This displays that the oysters measured on the previous sampling maintained their performance and grew in size.

Fig.10 Individual spawning of 11/18/15



The last Individual spawning had an increase in frequency 0.5– 1.0 cm by 5 oysters and an increase in mortality of the 0.5-1 cm measurement. This increase in mortality of the 0.5-1 cm bin could have been an effect of the sudden increase of 0.5-1 cm frequencies measured.

Observations and Results (Cont'd)

A Range and an average of Physical– chemistry parameters (9/29/15– 11/18/15) Average (Range)

Temp (C)	D. Oxygen (ppm)	pH	Salinity (ppt)	Nitrite (ppm)	Nitrate (ppm)	Secchi Depth (m)	Alkalinity (ppm)	Ammonia (ppm)	Phosphate (ppm)
17.6 (13.26– 24.4)	6.22 (4.5– 9.5)	7.9 (6– 9.5)	27 (25– 35)	0 (0– 0)	0 (0– 0)	1m	84 (80– 120)	1 (0.5– 1)	8 (0.5– 15)

Analysis of Results

Test 1 revealed the differences between Mass and Individually spawned groups from the total growth measurements. The absolute value of the derived t = 0.668 does not exceed the critical value of t = 1.96 at p = 0.5 with d*f* = 334. Based on the statistical test, we accepted H_0 which stated that there are no significant differences in the total growth rate between the two groups.

Test 2 The next two-tailed t-test preformed to compare the variation between the last oyster measurements collected, performed on 11/18/15. Specifically, this compared the last live oyster growth sampling, comparing Mass and Individual spawning. When tested, the derived t = 2.00 exceeds the critical value of t = 2.00 at p = .05 with d*f* = 37. Therefore H_a is accepted and it is concluded that the mean growth of Mass spawning (1.53) was significantly higher than the mean of the Individual spawning (0.9), t (37) = 2.00 p< .05.

Test 3 The last t– test preformed analyzed the total dead oyster measurements collected. When tested, the derived t = 1.99 exceeds the critical value of t = 1.98 at p = .05 with d*f* = 87. Therefore H_a is accepted and it is concluded that the mean mortality of Mass spawning ~(1.00) was significantly lower than the mean of the Individual spawning ~(0.8), t (87) = 1.99 p< .05.

CONCLUSION

The goal of this project was to find significant differences, if any, in the growth rate and mortality of Crassostrea virginica, and to see if that had any correlation to the water parameters present. After thoroughly investigating the correlation of the mortality samples and their matching water physical chemistry, there was no obvious correlation between physical, chemical data readings and mortality rates to draw any conclusions that the physical chemistry of the water significantly affected the oysters. From our findings, we concluded that the Mass spawning had a faster growth rate and a lower mortality rate. Therefore, from this research, conservationists should favor Mass over Individual methods of spawning.

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Annex

Measuring Salinity

VITALSINE General Use

1. Open the daylight plate and apply one or two drops of the sample solution to the surface of the prism. Hold the prism at an angle close to parallel with the floor so the sample will not run off of the prism.
2. Gently close the daylight plate over the prism. The sample solution should spread as a thin, even layer in between the daylight plate and the prism. By looking through the daylight plate, ensure that the sample solution covers the ENTIRE surface of the prism. If there are bubbles and gaps or if the sample is only on one portion of the prism, the sample solution must be reapplied (Figure 1). Inaccurate readings will result if the prism is not covered correctly.
3. Looking through the eyepiece, hold the refractometer and direct the daylight plate upwards towards light. If the scale is not in focus, adjust it by gently turning the eyepiece (rubber hood) either clockwise or counterclockwise. Be careful not to overturn the focusing mechanism.
4. When the refractometer scale is viewed through the eyepiece, the upper field of view will be seen as blue and the lower field will be seen as white (Figure 4). The reading is taken at the point where the boundary line of the blue and white fields crosses the scale (Figure 5). The value (either permillage or specific gravity) is the salinity level of water.
5. When each measurement is complete, the sample must be cleaned from the prism using tissue paper and water.

Figure 4

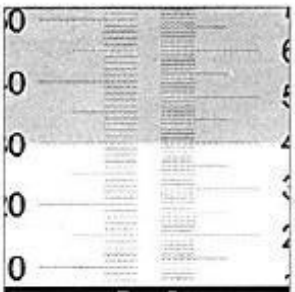
Figure 5

VITALSINE Conversion Table

Salinity (‰)	NaCl (w/w)	$MgCl_2$ (w/w)	$MgSO_4$ (w/w)	K_2SO_4 (w/w)	$CaCl_2$ (w/w)	Brix%
0	0.0	0.0	0.0	0.0	0.0	0.0
10	1.0	0.7	0.9	1.4	0.8	1.3
20	2.1	1.4	1.8	2.9	1.5	2.5
30	3.1	2.1	2.7	4.3	2.3	3.7
40	4.1	2.8	3.6	5.8	3.0	4.9
50	5.1	3.5	4.5	7.3	3.8	6.2
60	6.2	4.2	5.4	8.8	4.5	7.4
70	7.2	5.0	6.3	10.3	5.3	8.6
80	8.3	5.7	7.2	11.8	6.0	9.8
90	9.4	6.4	8.2	13.4	6.8	11.0
100	10.5	7.2	9.1	15.0	7.6	12.3

VITALSINE Specifications

Range:	0-100‰ / 1.000-1.070 Specific Gravity
Resolution:	1.0‰ / 0.001 Specific Gravity
Accuracy:	±1.0‰ / ±0.001 Specific Gravity
ATC Range:	10-30°C
Dimensions:	40 x 40 x 185mm (1.6 x 1.6 x 7.3")
Weight:	285g (10.0 oz.)
Supplied With:	Vinyl Carrying Case (1), Plastic Transfer Pipet (1), Calibration Screwdriver (1)

Annex Con't

VITALSINE Calibration

1. Calibration of ATC refractometers should only be conducted when the previous calibration setting has shifted and is noticeably affecting measurements. **DO NOT PERFORM CALIBRATIONS IN THE FIELD!** Calibration must take place in a controlled environment of 20°C (68°F) using distilled water of the same temperature. It's recommended to allow the refractometer and the distilled water to reach temperature equilibrium with the controlled environment before calibration takes place.
2. Open the daylight plate and apply one or two drops of distilled water on to the surface of the prism. Hold the prism at an angle close to parallel with the floor so the distilled water will not run off of the prism.
3. Gently close the daylight plate over the prism. The distilled water should spread as a thin, even layer in between the daylight plate and the prism. By looking through the daylight plate, ensure that the distilled water covers the ENTIRE surface of the prism. If there are bubbles and gaps or if the distilled water is only on one portion of the prism, the distilled water must be reapplied (Figure 1). Inaccurate calibrations will result if the prism is not covered correctly.
4. Looking through the eyepiece, hold the refractometer and direct the daylight plate upwards towards light. If the scale is not in focus, adjust it by gently turning the eyepiece (rubber hood) either clockwise or counterclockwise. Be careful not to overturn the focusing mechanism.
5. When the refractometer scale is viewed through the eyepiece, the upper field of view will be seen as blue and the lower field will be seen as white (Figure 2). Confirm that the boundary line crosses the scale at "0" (Figure 3).
6. If the boundary line falls above or below zero, gently loosen the set screw on the calibration ring with the supplied screwdriver. While looking through the eyepiece, gently turn the calibration ring clockwise or counterclockwise until the boundary line is at zero. Once this is achieved gently tighten down the set screw with the supplied screwdriver. (NOTE: Do not over-tighten. If the set screw is over-tightened, the boundary line may shift slightly).
7. When calibration is complete, gently wipe the prism using tissue paper.

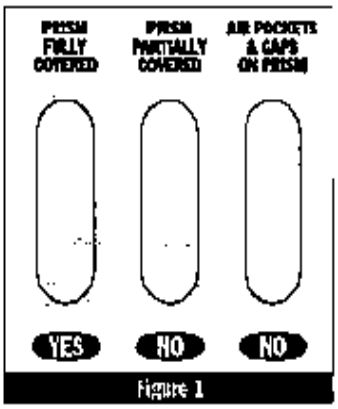

Figure 1

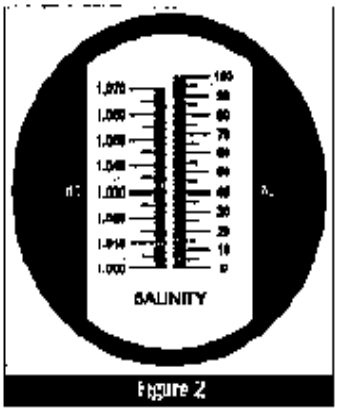

Figure 2

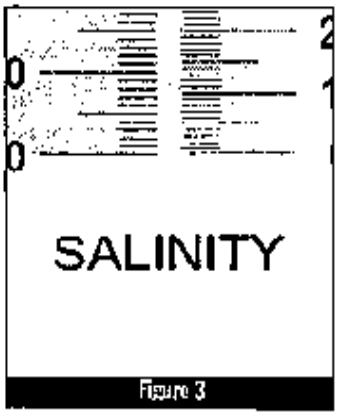

Figure 3

3

Annex Con't

Dissolved Oxygen (ppm) with the Modified Winkler Method

✓	Acquire Water Sample by lowering either a 7 gallon (26.4979 Liter) Beta Bottle or a 5 gallon (18.9271 Liter) bucket with an attached rope.
✓	Fill Sampling Bottle by submerging bottle fully in water
✓	Empty and refill bottle for accuracy
✓	Once filled, cap while underwater and set aside on a flat surface
✓	Uncap and add 8 drops of Maganous Sulfate Solution (Note: all chemical dropper bottles should be held at a 90°angle directly facing the Sample bottle)
✓	Add 8 drops of Alkaline Iodide Azide
✓	Cap and invert bottle vigorously 3 times
✓	Set aside and let precipitate settle to the neck of the sample bottle
✓	Add 8 drops of Sulfuric Acid to Sample Bottle
✓	Cap and invert bottle vigorously 3 times
✓	Set aside and let precipitate settle to the bottom of the sample bottle
✓	Fill Test Tube with the mixture from the Sample Bottle to the 20 mL line. Cap and set aside
✓	Depress the plunger of the Titrator
✓	Insert the Titrator into the opening of the Sodium Thiosulfate
✓	Invert the bottle of Sodium Thiosulfate and slowly withdraw the plunger until slightly over zero (for next step)
✓	If any air bubbles have occurred, flick Titrator lightly with finger and push plunger up to the zero line
✓	With the Titrator, add Sodium Thiosulfate to the Test Tube until the mixture turns a pale yellow (Note: Slowly swirling Test Tube in clockwise directions while adding Sodium Thiosulfate is recommended) Set aside Titrator
✓	Add 8 drops of Starch indicator to Test Tube; shake slightly until mixture turns black or purple
✓	With the Titrator, add Sodium Thiosulfate drops until clear
✓	Read result by measuring how much Sodium Thiosulfate is remaining in Titrator in ppm
✓	For accuracy, perform sampling test twice

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Dissolved Oxygen (ppm), pH, Salinity (ppt), Temperature (C) with the YSI ProPlus Galvanic Probe Method

BP reading has been corrected to sea level, use the following equation to determine the true BP in mmHg for your altitude:

True BP = (Corrected BP in mmHG) – {2.5 * (Local Altitude in feet/100)}

2. Press the Cal key.
3. Highlight **Barometer** and press Enter. Use the arrow keys to highlight the desired units and press Enter to confirm.
4. Highlight **Calibration Value** and press enter to adjust.
5. Use the Alpha/Numeric screen to enter your True BP, then highlight <<<ENTER>>> and press enter.
6. Highlight **Accept Calibration** and press enter to finish the calibration.

CONDUCTIVITY, PH, AND ORP CALIBRATION

1. Press the Cal key.
2. Highlight the parameter you wish to calibrate and press enter. For Conductivity, a second menu will offer the option of calibrating Specific **Conductance, Conductivity,** or **Salinity**. Calibrating one automatically calibrates the other two. An additional sub-menu will require you to select the calibration units. For pH, auto-buffer recognition will determine which buffer the sensor is in and it will allow you to calibrate up to 6 points.
3. Place the correct amount of calibration standard into a clean, dry or pre-rinsed container.
4. Immerse the probe into the solution, making sure the sensor and thermistor are adequately immersed. Allow at least one minute for temperature to stabilize.
5. For any of parameters, enter the calibration solution value by highlighting **Calibration Value**, pressing enter, and then using the alpha/numeric keypad to enter the known value. Once you have entered the value of the calibration standard, highlight <<<ENTER>>> and press enter.
6. Wait for the readings to stabilize, highlight **Accept Calibration** and press enter to calibrate.
7. For pH, continue with the next point by placing the probe in a second buffer and following the on-screen instructions or press Cal to complete the calibration.

DO CALIBRATION

The Pro Plus offers four options for calibrating dissolved oxygen. The first is an air calibration method in % saturation. The second and third calibrates in mg/L or ppm to a solution with a known DO concentration (usually determined by a Winkler Titration). Calibration of any option (% or mg/L and ppm) will automatically calibrate the other. The fourth option is a zero calibration. If performing a zero calibration, you must perform a % or mg/L calibration following the zero calibration. For both ease of use and accuracy, YSI recommends performing the following 1-point DO % calibration:

1. Moisten the sponge in the cal/transport sleeve with a small amount of water and install it on the probe. The cal/transport sleeve ensures venting to the atmosphere. For dual port and Quatro cables, place a small amount of water (1/8 inch) in the calibration/transport cup and screw it on the probe. Disengage a thread or two to ensure atmospheric venting. **Make sure the DO and temperature sensors are not immersed in the water.**
2. Turn the instrument on. If using a polarographic sensor, wait 10 minutes for the DO sensor to stabilize. Galvanic sensors do not require a warm up time.
3. Press the Cal key, highlight DO and press enter.
4. Highlight DO%, then press Enter.
5. Verify the barometric pressure and salinity displayed are accurate. Once DO and temperature are stable, highlight **Accept Calibration** and press enter.

TAKING MEASUREMENTS AND STORING DATA

1. The instrument will be in Run mode when powered on.
2. To take readings, insert the probe into the sample. Move the probe in the sample until the readings stabilize. This releases any air bubbles and provides movement if measuring DO.
3. **Log One Sample** is already highlighted in Run mode. Press enter to open a submenu. Highlight **Sites** or **Folders** and press enter to select the site or folder to log the sample to.
4. If necessary, use the keypad to create a new Site or Folder name. If Site List and Folder List are disabled in the System menu, you will not see these options when logging a sample.
5. Once the Site and/or Folder name is selected, highlight **Log Now** and press enter. The instrument will confirm that the data point was logged successfully.
6. If you would like to log at a specific interval vs. logging one sample at a time, press the System key. Use the arrow keys to highlight **Logging** and press enter. Enable **Continuous Mode** and adjust the time **Interval** if necessary. On the Run screen, the option to log will change from **Log One Sample** to **Start Logging** based on the time interval entered.
7. During a continuous log, the **Start Logging** dialog box on the Run screen will change to **Stop Logging**.

UPLOADING DATA TO A PC WITH DATA MANAGER

1. Make sure Data Manager and the USB drivers are installed on the PC. The USB drivers will be installed during the Data Manager installation.
2. Connect the Communications Saddle to the back of the Pro Plus instrument and use the USB cable to connect the saddle to the USB port on the PC.
3. If connecting for the first time, Windows' may prompt you through two 'New Hardware Found' Wizard in order to complete the USB driver installation.
4. Open Data Manager on the PC and turn on the Pro Plus.
5. Click on the correct instrument in Data Manager under the **Select Instrument** heading. Once you've highlighted the correct instrument, click the **Retrieve Instrument Data** tab and check **Data, GLP, Site List, Configuration** or **Select All** options to retrieve data. Click **Start**.
6. After the file transfer is complete, the data is available for viewing, printing, and exporting from Data Manger and the data can be deleted from the Pro Plus if desired.
7. Press the File key and choose **Delete Data** if you no longer need the data on the Pro Plus.

CONTACT INFORMATION

YSI
1700/1725 Brannum Lane
Yellow Springs, OH 45387
Tel: 800-765-4974 (937-767-7241)
Fax: 937-767-1058
E-Mail: proseries@ysi.com
Website: www.ysi.com

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Revision B
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Professional Plus Quick-Start Guide

This Quick-Start Guide is meant to serve as a quick reference in operating the Professional Plus. It is not intended to replace the information found in the Operations Manual. For your convenience, this quick start guide will enable you to unpack your instrument and get to the field quickly.

GETTING STARTED

Unpack the instrument and install (2) C size batteries in the back of the instrument. Tighten the four screws of the battery plate on to the back of the instrument.

If necessary, install the sensors into the cable assembly by inserting the sensors into the ports and then hand tightening them. Do not use a tool and do not over tighten.

If using a 1010 cable, a sensor must be installed in port 1 for correct operation. If installing a pH/ORP combo sensor into a 1010 cable, ORP will not be measured. If using a 1020 cable, install a pH, ORP, pH/ORP, or an ISE sensor in port 1 and a DO sensor in port 2.

If using a Quatro cable, install a pH, ORP, or ISE sensor in ports label 1 and 2. A sensor must be installed in port 1 for port 2 to operate correctly. If you install a pH/ORP combo sensor into port 1 or port 2, ORP will not be measure. Install the Dissolved Oxygen sensor in the port labeled DO. Install the Conductivity/Temperature sensor in the port labeled CT following the instructions included with the sensor. For ease of installation, YSI recommends that you install a sensor into port 1 first; followed by DO installation, then port 2, and lastly C/T.

Please refer to the Getting Started Setup section of the Manual for a complete list of sensor/cable port configurations.

Install a port plug into any port that does not have an installed sensor. Attach the cable assembly to your instrument.

INSTALLING THE DO MEMBRANE

Note: The DO sensor is shipped with a red protective cap to protect the electrode. A new membrane cap must be installed before the first use.

1. Prepare the O_2 probe solution according to the instructions on the bottle. After mixing, allow the solution to sit for 1 hour. This will help prevent air bubbles from later developing under the membrane.
2. Remove, and discard or save the red protective cap.
3. Thoroughly rinse the sensor tip with distilled or deionized water.
4. Fill a new membrane cap with probe solution. Avoid touching the membrane portion of the cap.
5. Thread the membrane cap onto the sensor, moderately tight. A small amount of electrolyte will overflow.
6. Screw the probe sensor guard on moderately tight.

MENU FUNCTIONS

The Professional Plus has a menu-based interface. Press the "hot keys" to access the System, Sensor, Calibration, and File menus (from left to right at the top of the keypad). To navigate through the menus, use the up and down arrow keys to highlight a desired menu option with a highlight bar, and press the Enter key to activate the selection. Use the left arrow key to go back one screen. Press the Esc key to return to the run screen or to exit an alpha/numeric entry screen. The Pro Plus will automatically power on to the Run screen.

SETTING THE DATE AND TIME

1. Press the System key .
2. Highlight **Date/Time** and press Enter.
3. Highlight **Date Format** and press Enter. Highlight the correct format and press Enter.
4. Highlight **Date** and press Enter. Use the keypad to enter the correct date, then highlight ↵on the display keypad, and press Enter.
5. Highlight **Time Format** and press Enter. Highlight the correct format and press Enter.
6. Highlight **Time** and press Enter. Use the keypad to enter the correct time, then highlight ↵on the display keypad, and press Enter.
7. Press Esc to return to the Run screen.

SETTING UP SENSORS & REPORTING UNITS

A sensor must be enabled in the **Sensor** menu for it to operate. Once a sensor is enabled, the desired units for that sensor must be selected in the **Display** menu to determine what will be displayed.

1. Press the Sensor key.
2. Highlight **Setup** and press enter. Highlight the parameter of interest and press enter. Highlight **Enabled** and press enter to ensure a checkmark in the box. When enabling the ISE1 and ISE2 ports, you must select the correct sensor after enabling the port.
3. When Dissolved Oxygen is enabled, a submenu allows the user to select the sensor type (Polarographic or Galvanic) and membrane type being used. Highlight **Sensor Type** or **Membrane** and press **Enter** to modify these settings.
4. Press the left arrow key to return to the previous screen or press Esc to return to the Run screen.

Once changes to the Sensor menu have been completed, you must determine which units will be reported (i.e. %, mg/L, °C, °F, etc.).

1. Select the Sensor hot key on the keypad, highlight **Display**, and press enter.
2. Highlight the parameter you want to access and press the Enter.
3. A submenu will open allowing you to select the reporting units. Some parameters can be reported in multiple units. For example, DO can be reported in DO%, DO mg/L, and DO ppm. Other parameters, for example temperature, can only be reported in one unit. Make selections from the submenu, and then press the left arrow key to return to the Display menu or press Esc to return to the Run screen.

BAROMETER CALIBRATION

1. Determine your local barometric pressure (BP) in mmHg from a mercury barometer, an independent laboratory, or from a local weather service. If the

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Taking a Secchi Disk Reading

✓ The Secchi disk into the water in the spot of testing. Keep lowering the disk slowly until it disappears. Note the depth on the cord.
✓ Record the average Secchi depth on the data sheet along with the date and time of the reading.
✓ pH, Nitrite, and Nitrate with Aquacheck Colorimetry

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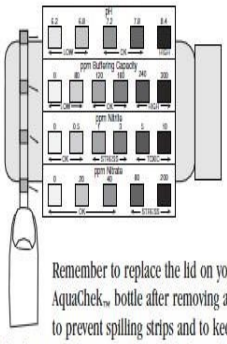
AquaChek™
POND TEST STRIPS

AquaChek™ is a test for pH, Buffering Capacity, Nitrite and Nitrate levels in your pond. The test pads on the strip will change color to indicate the levels in your pond.



Remember to replace the lid on your AquaChek™ bottle after removing a strip to prevent spilling strips and to keep them fresh. **Keep the strips in a cool, dry place,** and leave the packet of drying agent in the bottle — it will help keep the test strips at their best.

Follow these easy, step-by-step instructions

Step 1

Remove an AquaChek™ Pond Test Strip from the bottle and replace the cap tightly. Dip test strip into your pond water for 1 second and remove. Do not shake excess water from strip.

Step 2

Hold strip level for 30 seconds.

Step 3

pH

Compare the end pad of the strip to the pH color chart on the label. The pH pad should turn a shade of red-orange, between 7.2 and 7.8.

Buffering Capacity

Compare the second pad from the end of the strip to the Buffering Capacity color chart on the label. The Buffering Capacity pad should turn a shade of green. The correct range is 120 ppm (parts per million) to 180 ppm.

Nitrite

Compare the third pad from the end of the strip to the Nitrite color chart on the label. The Nitrite pad should remain white or turn a shade of pink. The safe range is between 0 ppm and 0.5 ppm.

Step 4

Nitrate

At 60 seconds after dipping strip, compare the pad nearest the handle to the Nitrate color chart on the label. The pad should remain tan or turn a shade of pink. The safe range is between 0 ppm and 40 ppm.

For recommendations on the importance of maintaining proper water conditions, see the reverse side of this instruction sheet.

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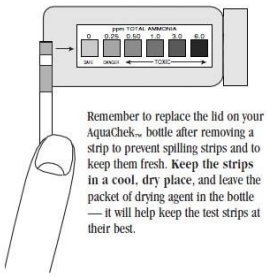
Ammonia with Aquacheck Colorimetry

AquaChek™ POND TEST STRIPS

AquaChek™ Ammonia tests for Total Ammonia in your pond. The test pad on the strip will change color to indicate the Total Ammonia level in your pond.



Remember to replace the lid on your AquaChek™ bottle after removing a strip to prevent spilling strips and to keep them fresh. **Keep the strips in a cool, dry place**, and leave the packet of drying agent in the bottle — it will help keep the test strips at their best.

Follow these easy, step-by-step instructions

Step 1

Remove an AquaChek™ Ammonia Pond Test Strip from the bottle and replace the cap tightly. Fill the sample vial to top line with pond water. Dip the test strip into water sample. Move strip vigorously up and down in the water sample for 30 seconds. Make sure both pads are always submerged.

Step 2

Remove test strip and shake off excess water. Hold strip level (pad side up) for 30 seconds.

Step 3

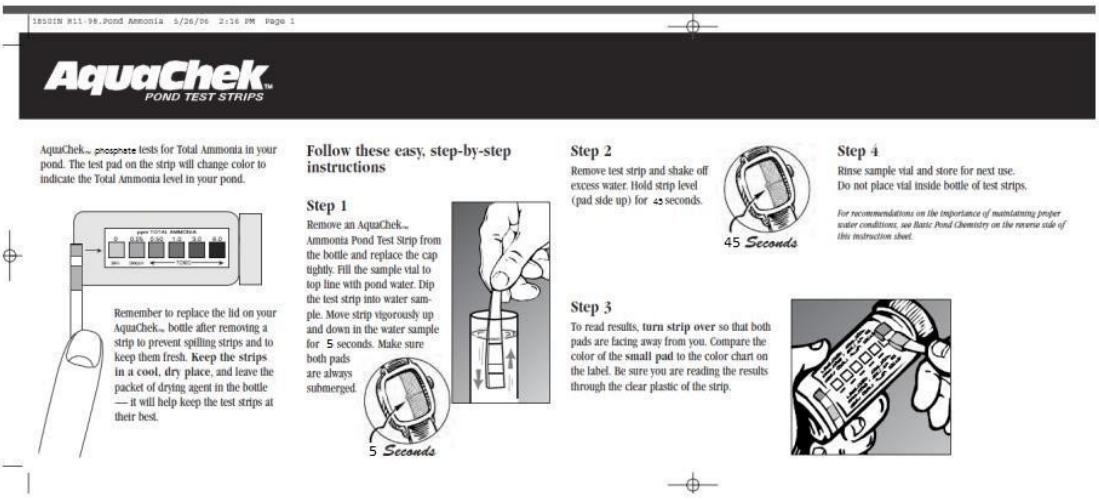
To read results, **turn strip over** so that both pads are facing away from you. Compare the color of the **small pad** to the color chart on the label. Be sure you are reading the results through the clear plastic of the strip.

Step 4

Rinse sample vial and store for next use. Do not place vial inside bottle of test strips.

For recommendations on the importance of maintaining proper water conditions, see Basic Pond Chemistry on the reverse side of this instruction sheet.

Phosphate with Aquacheck Colorimetry



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