Assessing the Reliability of Water Test Kits for Use in Small-Scale Aquaculture

By

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Abstract

Water analysis kits are useful for practical aquaculture only if they give results as accurate as those obtained by standard water analysis methods. This study used weighted Cohen's Kappa statistic to compare management decisions made by farmers that used water analysis kits (e.g. Seneye slide kit, Tetra EasyStrips, API test strips, Seachem Ammonia Alert, Salifert Profi test kit and Hach DO and alkalinity kit) and those that used standard methods. The decisions made by farmers were similar for water analysis kits and standard methods except for Tetra and API test strips when measuring nitrate concentration. The highest conformity between the two methods (Kappa value = 1.0, $p < 0.0001$) was obtained with the Hach and Salifert Profi test kits (for measuring DO) and the API test strip (for measuring total hardness). The agreement among the different water analysis kits also was measured by the weighted Cohen's Kappa statistic. Tetra EasyStrips and API test strips had a perfect agreement (kappa value of 1.0 and p value of <0.0001) on the decision made when measuring nitrate and nitrite. The Hach and Salifert Profi kits also had a perfect agreement (kappa value of 1.0 and p value of <0.0001) on the decision made when measuring dissolved oxygen. The rapid, simple measurements by the kits appear suitable for use by farmers.

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Introduction

Water quality is one of the main factors controlling aquaculture production, but it is the most difficult factor to understand, predict and manage (Boyd, 2006). Water quality conditions in culture systems can determine the success or failure of an aquaculture operation (Isyagi, 2009). Most instances of fish kills, disease outbreaks, poor growth and poor feed conversion efficiency are directly or indirectly related to poor water quality. The quality of the water results from its physical and chemical characteristics, and the most important water quality variables in aquaculture usually are dissolved oxygen, un-ionized ammonia, nitrite, nitrate, total alkalinity, total hardness and pH.

Aquaculture is the fastest growing food industry in the world, growing at an annual rate of 8.3%, according to OECD. This growth rate has been possible to a large extent because of improvements in water quality management that allow greater production per unit of pond area over culture system volume. Production managers obviously need a means of assessing water quality so that appropriate management decisions can be made. These decisions often must be made quickly, and there is not time to rely on the results from samples sent to a water testing laboratory. As a result, water test kits have become popular for measuring water quality. They are cheap, easy to use, and take little time to obtain a result. The standard laboratory methods of water quality analysis are only appropriate for larger aquaculture production facilities with more financial resources that can afford a laboratory and person knowledgeable about water analysis.

Despite the advantages of water test kits over standard methods, the reliability of data generated by the former is questionable, and especially with respect to the new water quality kits on the market. This is unfortunate because most of the water management decisions for small-scale aquaculture are based currently on such water test kits. Some studies have shown that certain of the older water analysis kits to be largely unreliable. Other studies reported that certain water analysis kits (e.g. Water Ecology Kit, Model Al-36B, Hach Chemical Co., Ames, Iowa) were unreliable for applications requiring a high degree of accuracy, but suitable for aquaculture management decisions (Boyd, 1976; 1977; Boyd and Hollerman, 1984). Several new and easy to use water test kits recently introduced in the market should be evaluated so that farmers can know if these kits are suitable for assessing water quality conditions in culture systems.

The present research will aid efficient decision making in water quality management for small-scale aquaculture producers by recommending the most reliable water quality test kits. The specific objectives were:

- To compare results of the new generation water test kits and the standard methods,
- To compare results of the different water testing kits,
- To compare the cost of the water quality testing kits and the standard methods.

The comparisons between kits with standard methods and among kits will focus on the degree of agreement with respect to the resulting management decision.

Literature Review

In the early days of modern aquaculture, water quality testing was predominantly done only on large farms using standard methods. The standard methods require knowledge of quantitative chemistry, expensive laboratory equipment and take a relatively long time for completion of analyses. Aquaculture has become more intensive, and rapid, multiple measurements are required for efficient water quality monitoring and management intervention. Water test kits are rapid, inexpensive, portable and suitable for field use. The kits vary in complexity from those used for single water quality variable to those that can be used for 10 or more water quality variables. However, the reliability of information generated by water test kits has not been verified.

There has been no work published on the reliability of the data obtained from test strips in water quality monitoring despite their popular use, especially in developing countries practicing small-scale aquaculture. Also, most of the research has focused on the reliability and precision of different water quality testing kits compared to standard methods of analysis, and these studies were done many years ago. Test kits such as La Motte TRL-05 Water Testing Kit (La Motte Chemical Products Company) and Hellige Water Testing Outfits (Hellige Inc.,) (Boyd, 1980), Water Ecology Kit (Model Al-36B, Hach Chemical Co. Ames, Iowa) (Boyd, 1976), Bausch and Lomb Spectrokits, Ecological Test Kits and CHEMetrics Test Kits (Boyd, 1980), and Hach DR-EL/2 Direct Engineer's Labarotory Kit (Hach Chemical Co. Ames, Iowa) (Boyd, 1977) were evaluated. Results showed that the water analysis kits were unsuitable for most variables where a high accuracy and precision was required. But, some of the kits were suitable for determining concentrations of most variables important in aquaculture management decisions. These studies are also outdated (18 years since the last publication), and new water testing kits have been introduced to the market in recent years. Moreover, previous studies did not consider the reliability of the management decisions that resulted when using kits – they simply compared accuracy and precision of measurements as compared to those made by standard methods of analysis. Thus, one cannot discern whether the same management decision would result from using a particular kit or the standard method to determine the concentration of a given water quality variable.

Dissolved oxygen, pH, ammonia, nitrate, nitrite, total alkalinity and total hardness are important water quality parameters that influence fish production, but no studies have been done to assess how different measurement methods influence farmers' decisions for their management. The water testing kits on the market have been reported to be accurate, easy to use, portable and quick by manufacturers. However, the literature review revealed no information about the reliability of the new generation of water quality test kits. A review of websites for water analysis equipment resulted in a number of rapid methods of assessing water quality in aquaculture and fisheries. These kits usually are contained in a box or case and do not require additional reagents, glassware, or instruments. The kits selected for the study are listed below:

- The Salifert Profi test kit measures dissolved oxygen (DO) concentration in both freshwater and marine water. It is reported to measure over the range of 2-14 mg/l, and 50 tests can be conducted with reagents provided.

- The Hach 146900 dissolved oxygen test kit, Model OX-2P includes reagents and test apparatus in a plastic carrying case. There are reagents sufficient for 100 measurements of DO concentration within the range of 0-15 mg/l. Of course, additional reagents may be purchased separately.

- The Hach alkalinity kit, Model AL-AP is simple and involves an economical titration method with all required reagents, solution and glassware in a plastic casing appropriate for field use. It utilizes a drop count titration method to estimate alkalinity concentrations. It can measure alkalinity in two ranges (5-100 mg/l and 20-400 mg/l). The kit comes with reagents for 100 analyses, but additional reagents may be purchased separately.

- The YSI Model IP-67 DO/pH meter features a waterproof, impact resistant case and innovative field-replaceable DO electrode module. It is fast, and easy to use with one-hand operation. This meter has an easy to use backlit display, automatic push button calibration and a weighted, quick-sinking probe. The more traditional DO meter, the YSI

550 A-12 DO meter is also available to measure DO concentration over a range of 0-50 mg/l. This meter also measures pH.

- The Seneye slide kit measures temperature, pH, unionized ammonia (NH_3), and light in water of different salinities. It allows constant monitoring and take readings of these parameters every 30 min. It is equipped with a warning device to let the farmer know when the water parameters are outside the desired range. Each slide can be used for a total of 1,500 analyses of pH and NH_3 over a 30 day period. It stores data on an iCloud which can be assessed conveniently either on a mobile phone or computer. It is built to improve frequency, accuracy, sensitivity and robustness of water sensing. Additional slides may be purchased separately

- The Thermo Scientific Orion STAR A215 pH meter also measures conductivity, salinity, total dissolved solids, and temperature with all results displayed at once. It offers up to five point pH calibration with automatic recognition for USA/NIST and DIN buffers and calibration editing to fix errors without a complete calibration. It comes with a ROSS Solution kit (475 ml each of pH 4, 7 and 10 buffers; storage solution; cleaning solution; and pH electrode storage bottle).

- The Seachem Ammonia Alert has a disc sensor that changes reversibly from yellow to green to blue depending on the level of NH_3 in water. It continuously takes readings of NH_3 and it lasts for 1 year. No chemicals are needed to use this kit.

- The API 5 in 1 aquarium test strips kit measures total hardness, pH, nitrite, nitrate and carbonate hardness. It involves easy procedures: just dip the strip and compare its color to a color chart. The kit contains 25 test strips and comes with a manual for interpretation of the results.

- The Tetra EasyStrips 6 in 1 aquarium test strips can measure nitrate, nitrite, chlorine, total hardness, pH and alkalinity. It contains 100 test strips and involves easy procedures: just dip the strip and compare its color to a color chart.

The only data found related to the validity of results presented by these water testing kits were those provided by manufacturers. The reliability of advertising data is questionable, and the percent study was conducted to provide an objective assessment of the kits. The effort focused on the agreement between the decisions that would be drawn by the farmer when using these new water testing kits as compared to the standard methods.

Materials and Methods

Design

The study was conducted at the E. W. Shell Fisheries Center, Auburn University, Auburn, Alabama. Water samples for the analyses were collected from research ponds at this station. Pond water samples were analyzed for total hardness, total alkalinity, pH, nitrite, nitrate, unionized ammonia and dissolved oxygen. API test strips and Tetra EasyStrips were used to measure nitrite, nitrate and total hardness. The standard method for hardness was versenate titration to the eriochrome black-T endpoint, while that for alkalinity was titration with standard sulfuric acid to the methyl orange endpoint. Nitrate was measured using the Szechrome NAS reagent, while nitrite was determined by the diazotization method (Boyd and Tucker, 1992).

The standard DO meter, Hach DO kit and Salifert Profi kit were used to measure DO concentration. The DO meter was the standard method. The Seneye, Tetra EasyStrips, and API test strips and a pH meter were used for testing pH. Tetra EasyStrips and a Hach alkalinity test kit were used for testing total alkalinity. Seachem Ammonia Alert and Seneye were used to measure NH_3. The salicycate method (Zhou and Boyd, 2015 was the standard method for ammonia. All the water testing kits were used according to the manufacturer's instructions.

The range in water quality variables in pond waters often was not great enough for assessing the reliability of the kits. Thus, laboratory chemicals often were added to pond water samples to provide the desirable concentration ranges. Concentration of variables less than those presented by the pond water were made by adding laboratory chemical to distilled water.

Analyses

A solution of sodium nitrite was made which was 100 mg/l in nitrite (NO_2^-). This was used as the standard solution. Different concentrations of nitrite (0, 0.5, 1, 2, 3, 4, 5, 7.5, 10 and 12.5 mg/l) were prepared and their nitrite concentrations were analyzed by Tetra EasyStrips and API test strips.

A solution of sodium nitrate was made which was 1,000 mg/l in nitrate (NO_3^-). This was used as the standard solution. Different concentrations of nitrite (0, 5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 mg/l) were prepared and their nitrite concentrations were analyzed by Tetra EasyStrips and API test strips and recorded.

A solution of 500 mg/l total hardness was prepared from calcium sulfate dihydrate ($CaSO_4.2H_2O$) and magnesium sulfate heptahydrate ($MgSO_4.7H_2O$). Concentrations of 0-37.5 mg/l were prepared from the standard solution of 500 mg/l and Tetra EasyStrips and API test strips were used to determine total hardness. Pond water of average total hardness of 46.3 mg/l was adjusted to have concentrations of 50-350 mg/l total hardness. Total hardness was determined with the Tetra EasyStrips and API test strips.

Pond water pH was adjusted with 1.0 N hydrochloric acid and 1.0 N sodium hydroxide. Water samples of pH values ranging from of 6-8.75 were prepared by aid of a laboratory pH meter. Then, Seneye, Tetra EasyStrips and API test strips also were used to make pH readings.

A standard solution of 100 mg/l total ammonia nitrogen (TAN) was prepared from ammonia chloride. A working solution of 1 mg/l of TAN was prepared and its pH was adjusted using a pH meter. Ranges of pH of 6.98-9 were prepared with aid of a pH meter and NH_3 concentrations were calculated with an on-line ammonia calculator www.hbuehrer.ch/Rechner/Ammonia.html. Readings for NH_3 were made using Seneye and Seachem Ammonia Alert.

A standard solution of 1,000 mg/l total alkalinity (as $CaCO_3$ equivalent) was prepared from potassium bicarbonate. Pond water of average total alkalinity 50 mg/l was adjusted to have concentrations of 50 – 350 mg/l total alkalinity using a standard solution of 1000 mg/l total alkalinity. Total alkalinity was determined with the Tetra EasyStrips and a Hach alkalinity kit.

Pond water samples were deoxygenated (0 mg/l) using a sodium sulfite addition of 8 mg/l for each mg/l of DO. A colbalt chloride catalyst (0.15 mg/l) was used to accelerate the deoxygenation of water by sodium sulfite. The water samples were stirred for different lengths of time to provide a range in DO concentration. Analyses made with the DO meter were used as the standard method. Dissolved oxygen readings also were taken with a Hach DO kit and Salifert Profi kit at different DO concentrations (0-12.2) mg/l and were recorded.

Statistical tests

The agreement of the decisions that would result when water testing kits and the standard methods of analyses was determined using the weighed Cohen's Kappa Statistic (a chance-adjusted measure of agreement when response is more than two categories). The null hypothesis was: in the population represented by this sample, the level of agreement between the decisions drawn from results of the different methods is no better than chance at the 95% confidence level. All results were divided into three levels of decision making as shown in the Table 1. The degree of agreement when kappa is used was ranked according to Leeds and Koch (1977) as shown in Table 2. Dissolved oxygen concentration of the different water kits used and DO meter was further analyzed on their agreement using the Altman Bland test (Altman and Bland, 1986) as they gave point values. The null hypothesis for this test was: in the population represented by this sample, the level of agreement between the decisions drawn from results of the different DO methods is no better than chance.

Costs of analyses with kits

The costs of conducting analyses with the different water testing kits were calculated from the purchase prices of kits, and where appropriate, the cost of replacement reagents. The costs of reagents were calculated for the standard methods of analyses, but laboratory glassware and instruments were not included in the cost estimates.

Results and Discussion

Comparison with standard method

The suitability of the kits for making decisions on water quality management rather than the precision and accuracy of measurements was the focus of the data analysis. In Table 3, the agreement between management decisions recommended from analytical results by test kits were compared to the decision recommended by results obtained by the standard methods. The probabilities for the Cohen Kappa (k) values were less than 0.05 for all variables other than nitrate. This reveals that the agreement between decisions by the kits and standard methods was better than would be expected by chance alone for all water quality variables besides nitrate. The poor performance by the kits for nitrate analyses was likely because the reagents used for nitrate analysis are not well-suited for incorporation into kits. Earlier comparisons of the accuracy of the older water quality test kits for nitrate analyses also revealed that kits generally gave inaccurate results (Boyd, 1977; 1980).

The levels of agreement suggested for different ranges of the k-value (Table 2) were used to assess the reliability of the kits versus the standard method for water quality management decisions. The Tetra EasyStrips had substantial agreement with the standard method for total alkalinity, total hardness, and pH. These strips had only moderate agreement with the standard method for nitrite, and they did not agree with the standard method for nitrate.

The API test strips had almost perfect agreement with the standard method for total hardness. Agreement between the two methods was substantial for pH and moderate for nitrite. However, there was no agreement for nitrate.

The Seneye slide kit had moderate agreement with the standard method for NH_3, but agreement for pH was only fair. This is a puzzling case, because the concentration of NH_3 is a function of pH. It is surprising that the degree of agreement would differ between the two variables, and no explanation can be provided.

Perfect agreement was achieved between the DO management decision by the standard DO meter and those resulting from analyses by the Hach DO kit and the Salifert Profi test kit. This is a highly desirable outcome, because DO concentration usually is the most important water quality variable in aquaculture. As a rule, if DO concentrations are adequate in feed-based aquaculture – in which water quality problems are most common – other water quality variables tend to be acceptable (Boyd and Tucker, 1998).

The relationships between the determination of DO with the standard DO meter and the Hach and Salifert Profi DO kits (Figs. 1 and 2) revealed that the plots of the kit concentrations versus the standard meter concentrations fell very close to the Altman Bland identity line. There obviously was a good correlation between the DO concentrations measured by the kits and the standard DO meter. There were, however, differences between the estimates as shown in Altman-Bland difference plots (Figs. 3 and 4). The differences were less than 1 mg/l in all but one instance for each kit. Thus, the Altman-Bland plots support the conclusion of the Cohen k analysis that the kits and standard DO meter gave similar results and management decisions.

Another way of assessing the agreement between the kits is to compare the frequency with which the standard method and the test kits give the identical management decision. This can be done by making a management decision matrix showing the number and percentage of samples for which the same management decision would result regardless of whether a water quality variable is measured by the standard method or with a kit. An example of such a matrix for nitrate is provided to illustrate the estimation of percentage agreement (Table 4).

The estimates of percentage agreement between the standard method and the kits were generally related to the degree of agreement based on Cohen's k value. The best percentage agreement with the standard method was for dissolved oxygen by either of the kits, total hardness by API test strips, and NH_3 by the Seachem Ammonia Alert – these percentages of agreement were between 83 and 100%.

The results of the individual matrices for comparisons of the test kits with the standard methods are provided in the Appendix.

Comparison of different kits

The decision for alkalinity made by the Tetra EasyStrips and by the Hach alkalinity kit involved a significantly better-than-chance level of agreement between them (k = 0.5078, p-value = <.0001). A k value of this magnitude is generally considered moderate. There was an identical decision by the two methods for 76.2% of samples (Table 5).

The decision for total hardness made by the API test strips and by the Tetra EasyStrips involved a significantly better-than-chance level of agreement between them (k=0.7941, p-value

= <.0001). A k value of this magnitude generally is considered substantial. In 80.96% of samples, the two methods would provide identical decisions (Table 5).

With a point estimate of 1.0000 and a 95% confidence interval that does not include zero, p-value < 0.0001, the decisions made by the Tetra EasyStrips and by the API test strips for nitrite involved a significantly better-than-chance level of agreement between them. A k value of this magnitude is generally considered perfect. The actual agreement level between decisions by the two methods was 100%.

With a point estimate of 1.0000 and a 95% confidence interval that does not include zero, p-value < 0.0001, the decisions made by the Tetra EasyStrips and by the API test strips for nitrate involved a significantly better-than-chance level of agreement between them. A k value of this magnitude is generally considered perfect, and agreement in the decision by the two methods was 100% (Table 5).

The decision made for pH by the Seneye slide and by the Tetra EasyStrips involved a significantly better-than-chance level of agreement between them (k=0.2000, p-value = 0.0146). A k value of this magnitude is generally considered slight. The decisions by use of results from the two methods had an agreement of only 37.5% (Table 5).

The decision made by the Seneye slide and by the API test strips involved a significantly better-than-chance level of agreement between them (k=0.2558, p-value = 0.0107). A kappa value of this magnitude is generally considered slight. This conclusion is verified by the two methods giving the same decision for only 50% of samples (Table 5).

The decision made by the Tetra EasyStrips and by the API test strips involved a significantly better-than-chance level of agreement between them (k=0.7895, p-value = 0.0001). A k value of this magnitude is generally considered substantial; agreement between the decisions by the two methods was 87.5% (Table 5).

The decision made for unionized ammonia by the Seneye slide and by the Seachem Ammonia Alert involved a significantly better-than-chance level of agreement between them (k=0.5161, p-value = 0.0071). A k value of this magnitude is generally considered moderate. The level of agreement was 58.33% (Table 5).

With a point estimate of 1.0000 and a 95% confidence interval that does not include zero, p-value < 0.0001, the decisions made for dissolved oxygen by the Hach DO kit and by the Salifert Profi test involved a significantly better-than-chance level of agreement between them. A k value of this magnitude is generally considered perfect, and 100% agreement on decisions by the two methods was achieved (Table 5).

Using the Altman-Bland method, the identity plot showed a perfect agreement of values of the Hach DO kit and the Salifert Profi test (Fig. 5). But, agreement between the two kits is better illustrated with the Altman-Bland difference plot. There was no obvious pattern between the difference of decisions of the Hach DO kit and the Salifert Profi test and the average (Fig. 6). With a p value of 0.7412, which is greater than alpha (0.005), a bias of 0.1667 and difference between limits of agreement of 4.5826 which are large, it was concluded that there is no agreement between the Hach DO kit and the Salifert Profi test decisions. This is an important point in that it demonstrates that the results of two methods may be highly correlated as illustrated by the Altman-Bland identity plot, yet the decisions that result from the actual measured concentrations may not agree. Thus, in evaluating test kits, correlation analysis would not be a good indicator for deciding that the two kits will result in the same decision.

Comparison of costs of kits with the standard method

The standard methods and standard equipment are more expensive compared to their alternative water analysis kits (Table 6). This is in line with the literature as water analysis kits are considered cheaper than the standard methods, and has been a major factor leading to their popular use in aquaculture. Tetra EasyStrips were cheapest to use for total alkalinity, API test strips for total hardness, nitrite and nitrate; Seachem Ammonia Alert for unionized ammonia, and Salifert Profi test for DO.

Reliability of the kits

One of the major issues in comparing the results of the kits with those of the standard methods is the level of precision of the analyses. Several of the kits do not provide a specific concentration for a variable. Rather the kits reveal that the concentration of a variable falls within an indicated range. Thus, there is no way of determining the accuracy of these kits. This is the main reason that emphasis was given to the decision that would result from using the kit.

In the case of oxygen, it was possible to obtain an estimate of the actual DO concentration rather than an estimate of the range of the DO concentration. This allowed the actual measurements to be compared. The fact that a kit gave estimates that were closely related to those obtained by the standard method was, however, not proof that the same management decision would result from both kits.

The comparisons suggested that the level of agreement in decision making between kits and the standard methods of analysis were variable ranging from no agreement to 100% agreement. However, in general, most of the kits gave a degree in decision making that was considerably better than would be expected by chance alone. Thus, several of the kits seem useful for decision making in aquaculture water quality management.

The kits certainly are quicker, easier, and less expensive to use than the standard methods. Therefore, by selecting the most reliable kit for each of the variables evaluated here, a farmer could expect to obtain data that generally would allow efficient water quality management.

Conclusions

1. Water analysis kits can be used to make acceptable decisions related to water quality management in aquaculture.

2. Considering agreement with the standard method of analysis and expense of analyses, the following techniques were considered to be the best for individual variables:

- Total alkalinity – Tetra EasyStrips
- Total hardness – API test strips
- Nitrite – API or Tetra EasyStrips
- Nitrate – None of the kits gave reliable results
- pH – API test strips
- Unionized ammonia – Seachem Ammonia Alert
- Dissolved oxygen – Hach DO kit

3. New kits and reagents were used in all tests conducted in this study. The farmer should store the kits in a dry location protected from the sun. The instructions for measurements should be followed exactly, and reagents should be replaced before the shelf-life expiration date to assure reliable results.

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Table 1: Threshold levels of the different water parameters measured that were used in assessing agreement using weighted Cohen's Kappa statistic

Water parameter	**Decision level**		
Total alkalinity	0-60	60-150	150-300
	Liming needed	Best	High
Total hardness	0-60	60-150	150-350
	Liming needed	Best	Ok but more fertilizer
Nitrite	0-1	1-2	>2
	Best	Stress fish	Add salt and exchange water
Nitrate	0-40	40-120	120-240
	Fertilize	Best	Water exchange
pH	6-7	7-8.5	>8.5
	lime needed	Best	High
Unionized ammonia	<0.003	0.005-0.1	0.1-0.2
	Best	Ok	Water exchange
Dissolved oxygen	0-4	4-8	>8
	Aerate	Good	Possible gas trauma

Table 2: Ranking of degree of agreement according to kappa value by Leeds and Koch (1977)

Kappa value	**Degree of agreement**
< = 0	Poor
0 – 0.2	Slight
0.2 – 0.4	Fair
0.4 – 0.6	Moderate
0.6 – 0.8	Substantial
0.8 – 1	Almost perfect
1	Perfect

Table 3: Comparison of agreement of each water test kit with the standard methods of analysis using Cohen's Kappa test

Water parameter	**Water test kit**	**N**	**k value**	**p value**	**Degree of Agreement based on k**	**Same decision by both standard method and kit (% of samples)**
Total alkalinity	Hach alkalinity kit	39	0.6512	<.0001	Substantial	76.19
	Tetra EasyStrips	39	0.6053		Substantial	76.18
Total hardness	Tetra EasyStrips	21	0.7368	<.0001	Substantial	76.18
	API test strip	21	0.9446		Almost perfect	95.24
Nitrite	Tetra EasyStrips	10	0.5455	0.0125	Moderate	76.92
	API test strip	10	0.5455	0.0125	Moderate	
Nitrate	Tetra EasyStrips	17	0.1472	0.1695	No agreement	41.17
	API test strip	17	0.1472	0.1695	No agreement	41.17
pH	Tetra EasyStrips	13	0.4783	0.0053	Moderate	62.5
	API test strip	13	0.6364	0.0007	Substantial	75
	Seneye slide	13	0.3333	0.0088	Fair	62.5
Unionized ammonia (NH_3)	Seneye slide	9	0.4737	0.0117	Moderate	58.33
	Seachem Ammonia Alert	9	0.8000		Substantial	83.33
Dissolved oxygen	Hach DO kit	12	1.0000	<.0001	Perfect	100
	Salifert Profi test	12	1.0000	<.0001	Perfect	100

Table 4: Management decisions (fertilization, best condition, and water exchange) resulting from results of analyses by the standard method and API test strips for nitrate

Decision				
Standard Method	**API Strips**			
	Fertilization	**Best condition**	**Water exchange**	**Sum**
Fertilization	1 5.88	1 5.88	5 29.41	7 41.18
Best condition	0 0.00	0 0.00	4 23.53	4 23.53
Water exchange	0 0.00	0 0.00	6 35.29	6 35.29
Sum	1 5.88	1 5.88	15 88.24	17 100.00

[1]The rows of entries are for the standard method and the columns are for the API strips. The upper entry in each pair is the number of samples and the lower entry is for the percentage of samples for which the same decision resulted from each method. The numbers and percentage in the diagonal box can be summed to assess overall agreement.

Table 5: Comparison of agreement between the different water test kits

Water parameter	Water test kit	N	k value	p value	Degree of Agreement based on k	Same decision by both methods (% of samples)
Total alkalinity	Hach alkalinity kit & Tetra EasyStrips		0.5078	<0.0001	Moderate	76.2
Total hardness	Tetra EasyStrips & API test strip		0.7941	<0.0001	Substantial	80.96
Nitrite	Tetra EasyStrips & API test strip		1.0000	<0.0001	Perfect	100
Nitrate	Tetra EasyStrips & API test strip		1.0000	<0.0001	Perfect	100
pH	Tetra EasyStrips & API test strip		0.7895	<0.0001	Substantial	87.5
	Tetra EasyStrips & Seneye slide		0.2000	0.0146	Slight	37.5
	API test strip & Seneye slide		0.2558	0.0107	Slight	50
Unionized ammonia	Seneye slide & Seachem Ammonia Alert		0.5161	0.0071	Moderate	58.33
Dissolved oxygen	Hach DO kit & Salifert Profi test		1.0000	<0.0001	Perfect	100

Table 6: Cost comparison of standard methods used to measure different water quality parameters and their corresponding water test kits

Parameter measured	Method	Cost per sample (US $)
Total alkalinity	Standard lab procedure*	0.52
	Hach alkalinity kit	0.52
	Tetra EasyStrips	0.20
Total hardness	Standard lab procedure*	0.56
	Tetra EasyStrips	0.20
	API test strip	0.33
Unionized ammonia	Standard lab procedure*	0.45
	Seneye slide	32.99
	Seachem Ammonia Alert	7.68
pH	pH meter	<0.01
	Seneye slide	≈0.22
	API test strip	0.33
	Tetra EasyStrips	0.20
Nitrate	Standard lab procedure*	≈1.00
	API test strip	0.33
	Tetra EasyStrips	0.20
Nitrite	Standard lab procedure*	0.03
	API test strip	0.33
	Tetra EasyStrips	0.20
Dissolved oxygen	DO meter	<0.01
	Hach Do kit	0.83
	Salifert Profi test	0.49

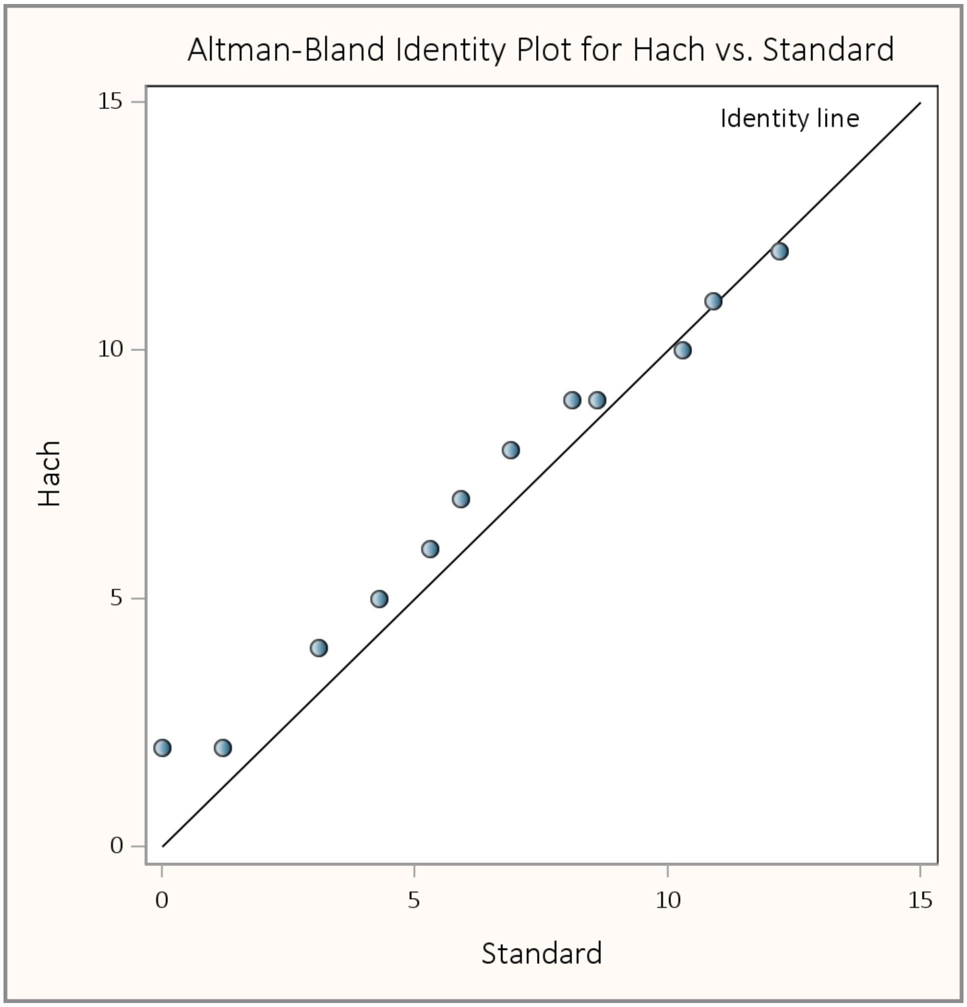

Figure 1: Altman-Bland identity plot for Hach DO kit Vs. standard, DO meter

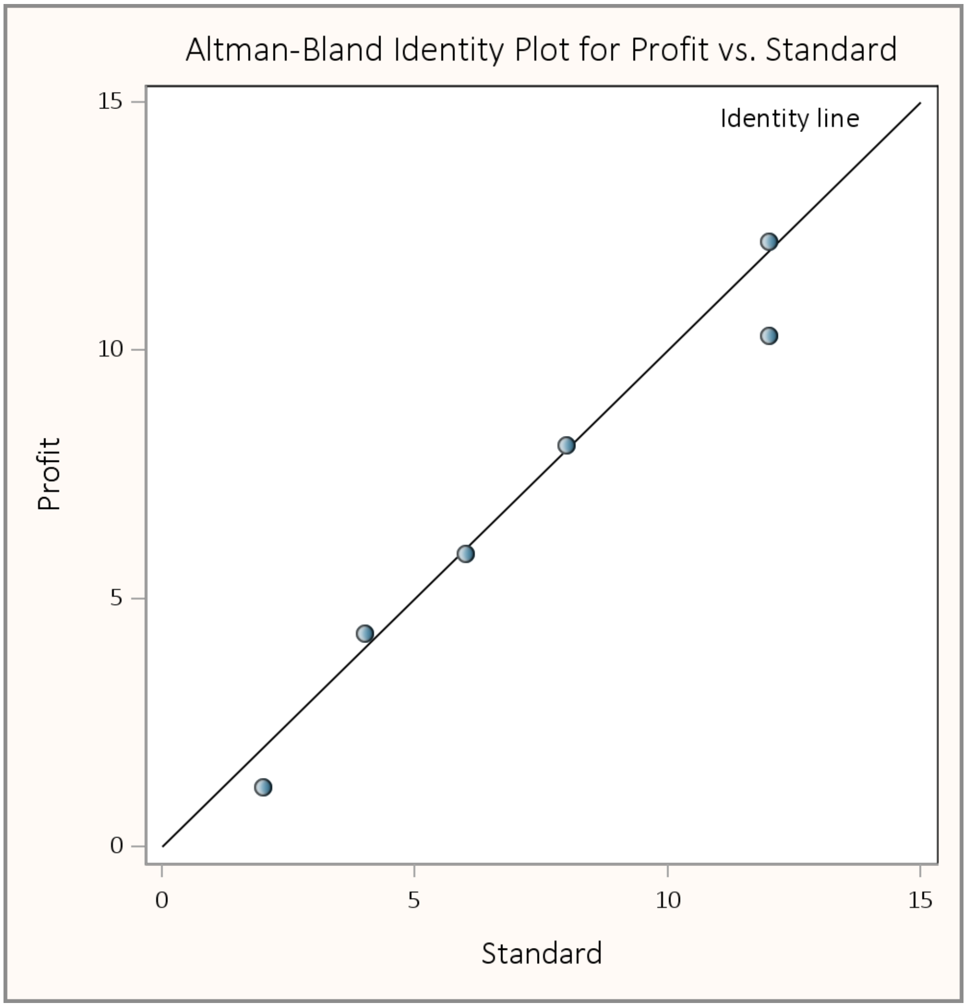

Figure 2: Altman-Bland identity plot for Salifert Profi test Vs. standard, DO meter

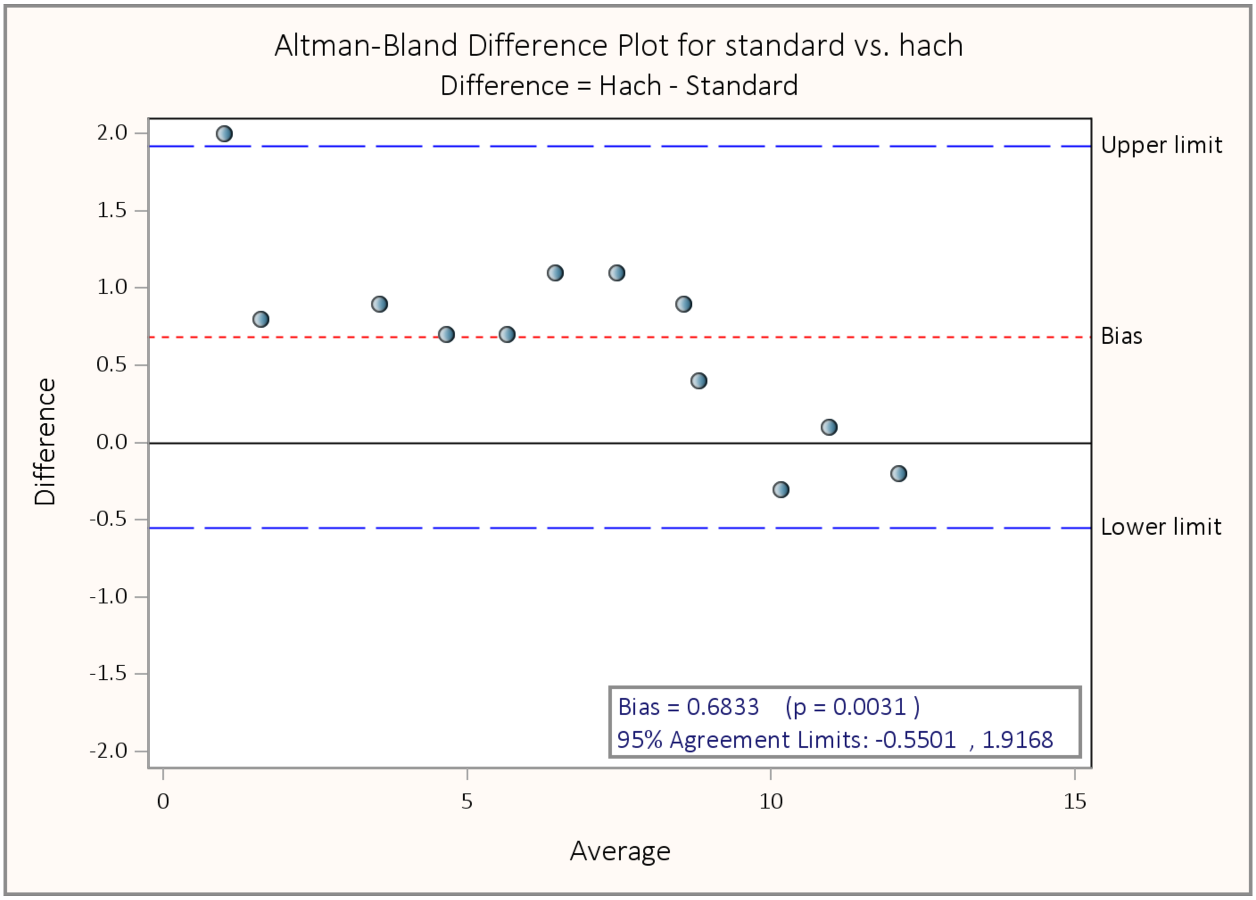

Figure 3: Altman-Bland difference plot for Hach DO kit standard, DO meter Vs. average

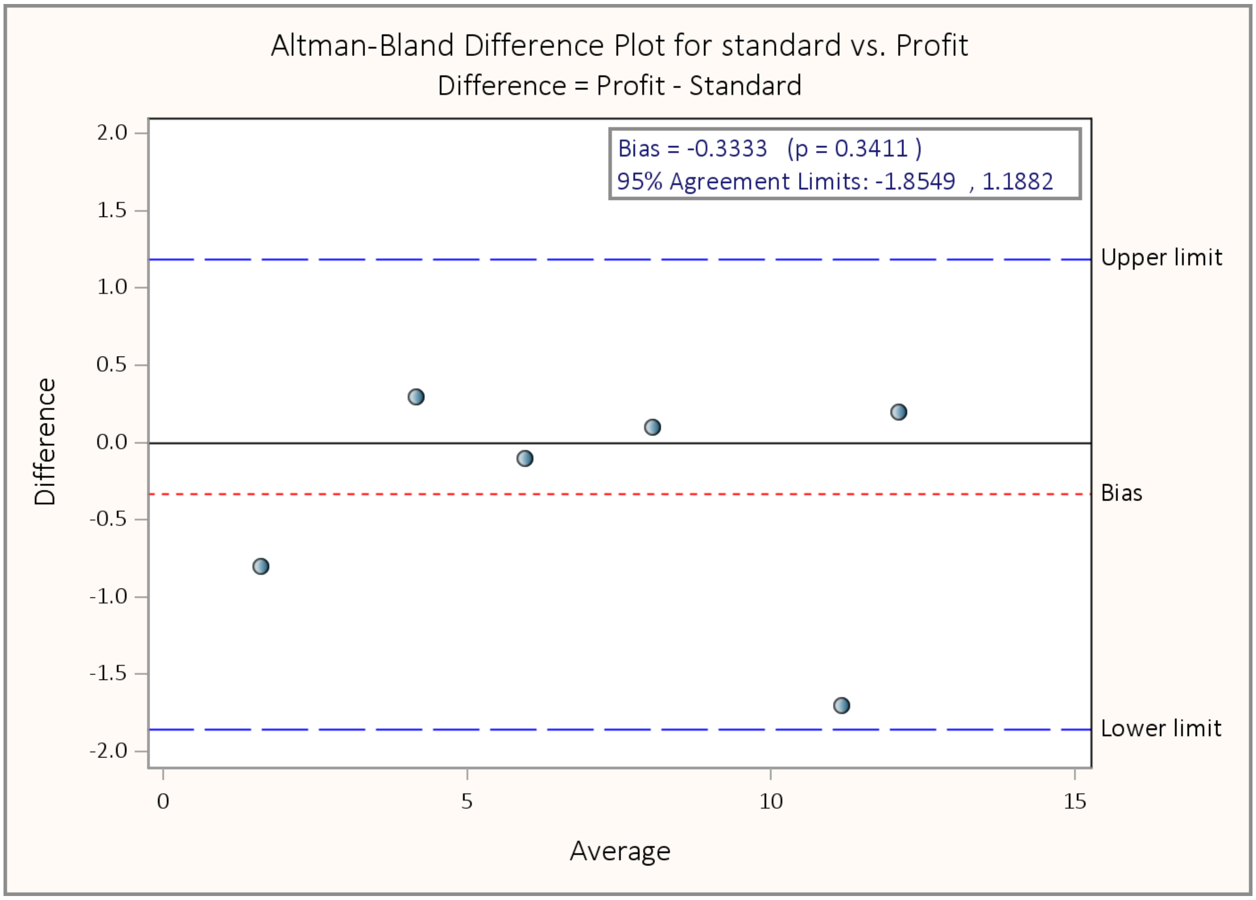

Figure 4: Altman-Bland difference plot for standard, DO meter - Salifert Profi test Vs. average

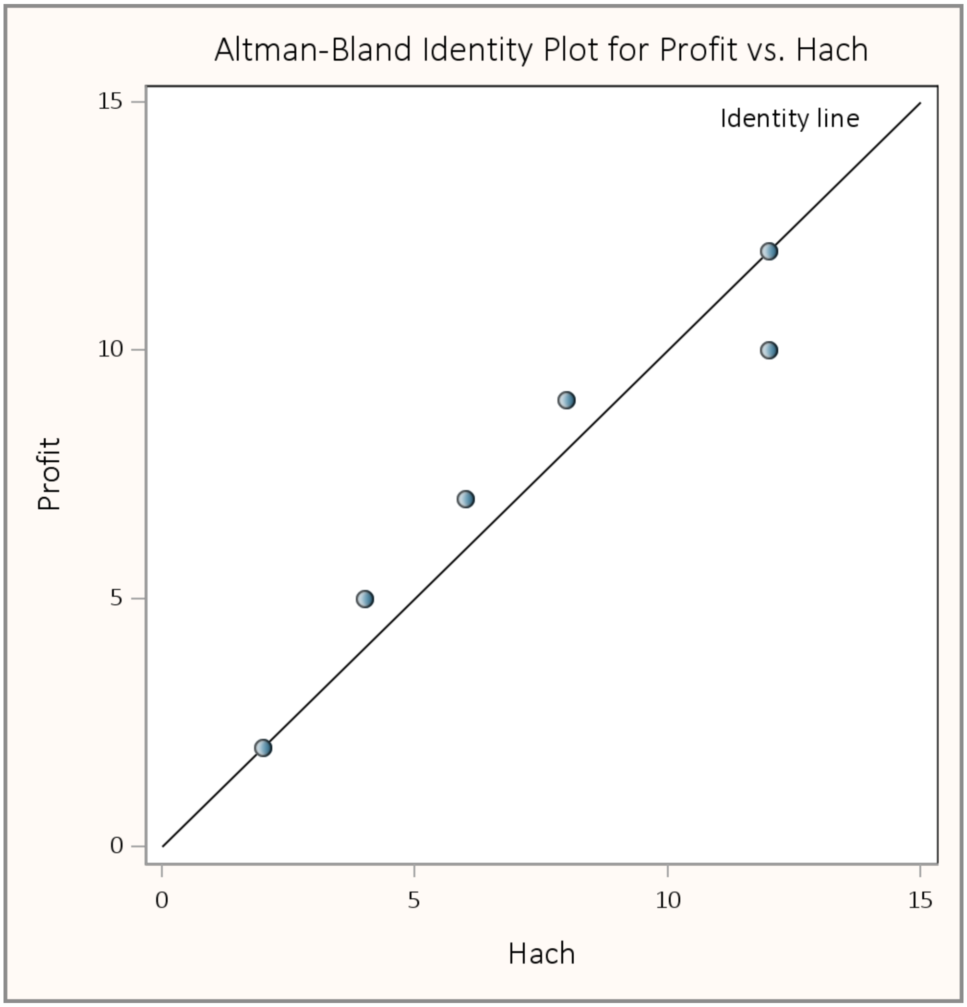

Figure 5: Altman-Bland identity plot for Salifert Profi test Vs Hach DO kit

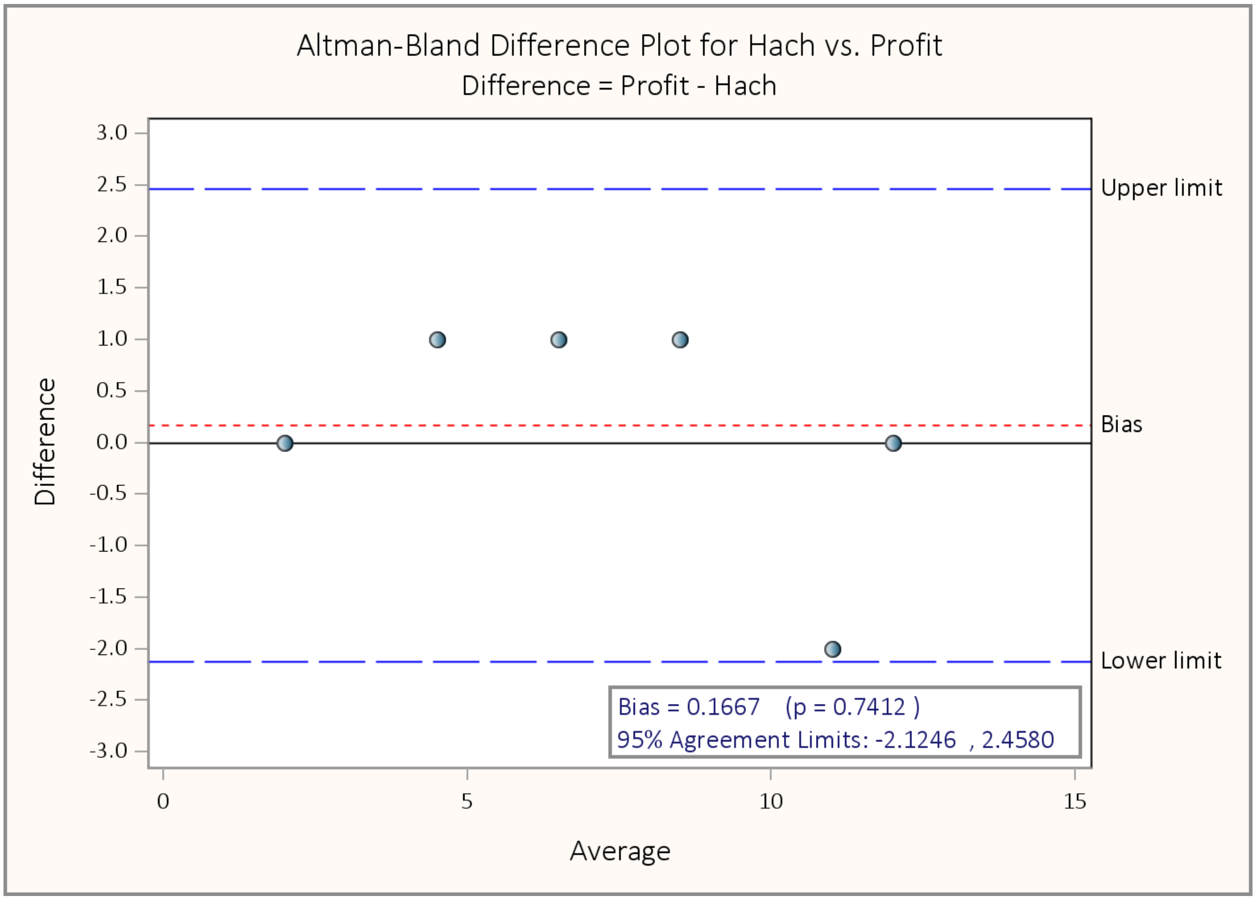

Figure 6: Altman-Bland difference plot for Salifert Profi test - Hach DO kit Vs. average

Appendix

Standard alkalinity method decision by Hach alkalinity decision

standard_decision	Hach_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	2 4.76	4 9.52	0 0.00	6 14.29
Best	0 0.00	10 23.81	0 0.00	10 23.81
High	0 0.00	6 14.29	20 47.62	26 61.90
Total	2 4.76	20 47.62	20 47.62	42 100.00

Standard alkalinity method decision by Tetra Easy test alkalinity decision

standard_decision	Tetra_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	1 2.38	5 11.90	0 0.00	6 14.29
Best	0 0.00	5 11.90	5 11.90	10 23.81
High	0 0.00	0 0.00	26 61.90	26 61.90
Total	1 2.38	10 23.81	31 73.81	42 100.00

Hach alkalinity decision by Tetra Easy test alkalinity decision

Hach_decision	Tetra_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	1 2.38	1 2.38	0 0.00	2 4.76
Best	0 0.00	9 21.43	11 26.19	20 47.62
High	0 0.00	0 0.00	20 47.62	20 47.62
Total	1 2.38	10 23.81	31 73.81	42 100.00

Standard hardness method decision by Tetra Easy test total hardness decision

Standard_decision	Tetra_Easy_decision			
Frequency Percent	Liming needed	Best	OK	Total
Liming needed	5 23.81	0 0.00	0 0.00	5 23.81
Best	1 4.76	3 14.29	4 19.05	8 38.10
OK	0 0.00	0 0.00	8 38.10	8 38.10
Total	6 28.57	3 14.29	12 57.14	21 100.00

Standard hardness method decision by API test strip hardness decision

Standard_decision	API_decision			
Frequency Percent	Liming needed	Best	OK	Total
Liming needed	5 23.81	0 0.00	0 0.00	5 23.81
Best	1 4.76	7 33.33	0 0.00	8 38.10
OK	0 0.00	0 0.00	8 38.10	8 38.10
Total	6 28.57	7 33.33	8 38.10	21 100.00

Tetra Easy test hardness decision by API test strip hardness decision

Tetra_Easy_decision	API_decision			
Frequency Percent	Liming needed	Best	Ok	Total
Liming needed	6 28.57	0 0.00	0 0.00	6 28.57
Best	0 0.00	3 14.29	0 0.00	3 14.29
OK	0 0.00	4 19.05	8 38.10	12 57.14
Total	6 28.57	7 33.33	8 38.10	21 100.00

Standard nitrite method decision by Tetra Easy nitrite decision

Standard_decision	Tetra_Easy_decision			
Frequency Percent	Best	Stress fish	add salt and water exchange	Total
Best	2 15.38	0 0.00	2 15.38	4 30.77
Stress fish	0 0.00	1 7.69	1 7.69	2 15.38
add salt and water exchange	0 0.00	0 0.00	7 53.85	7 53.85
Total	2 15.38	1 7.69	10 76.92	13 100.00

Standard nitrite method decision by API test strip nitrite decision

Standard_decision	API_decision			
Frequency Percent	Best	Stress fish	add salt and water exchange	Total
Best	2 15.38	0 0.00	2 15.38	4 30.77
Stress fish	0 0.00	1 7.69	1 7.69	2 15.38
add salt and water exchange	0 0.00	0 0.00	7 53.85	7 53.85
Total	2 15.38	1 7.69	10 76.92	13 100.00

Tetra Easy nitrite decision by API test strip nitrite decision

Tetra_Easy_decision	API_decision			
Frequency Percent	Best	Stress fish	add salt and water exchange	Total
Best	2 15.38	0 0.00	0 0.00	2 15.38
Stress fish	0 0.00	1 7.69	0 0.00	1 7.69
add salt and water exchange	0 0.00	0 0.00	10 76.92	10 76.92
Total	2 15.38	1 7.69	10 76.92	13 100.00

Standard nitrate method by Tetra Easy nitrate decision

Standard_decision	Tetra_Easy_decision			
Frequency Percent	Fertilizer	Best	Water exchange	Total
Fertilizer	1 5.88	1 5.88	5 29.41	7 41.18
Best	0 0.00	0 0.00	4 23.53	4 23.53
Water exchange	0 0.00	0 0.00	6 35.29	6 35.29
Total	1 5.88	1 5.88	15 88.24	17 100.00

Standard nitrate method decision by API test strip nitrate decision

Standard_decision	API_decision			
Frequency Percent	Fertilizer	Best	Water exchange	Total
Fertilizer	1 5.88	1 5.88	5 29.41	7 41.18
Best	0 0.00	0 0.00	4 23.53	4 23.53
Water exchange	0 0.00	0 0.00	6 35.29	6 35.29
Total	1 5.88	1 5.88	15 88.24	17 100.00

Tetra Easy nitrate decision by API test strip nitrate decision

Tetra_Easy_decision	API_decision			
Frequency Percent	Fertilizer	Best	Water exchange	Total
Fertilizer	1 5.88	0 0.00	0 0.00	1 5.88
Best	0 0.00	1 5.88	0 0.00	1 5.88
Water exchange	0 0.00	0 0.00	15 88.24	15 88.24
Total	1 5.88	1 5.88	15 88.24	17 100.00

The pH meter decision by Seneye pH decision

pH meter_decision	Seneye_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	1 6.25	5 31.25	0 0.00	6 37.50
Best	0 0.00	8 50.00	0 0.00	8 50.00
High	0 0.00	1 6.25	1 6.25	2 12.50
Total	1 6.25	14 87.50	1 6.25	16 100.00

Table 7: The pH meter decision by Tetra Easy test pH decision

pH meter_decision	Tetra_Easy_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	6 37.50	0 0.00	0 0.00	6 37.50
Best	5 31.25	3 18.75	0 0.00	8 50.00
High	0 0.00	1 6.25	1 6.25	2 12.50
Total	11 68.75	4 25.00	1 6.25	16 100.00

The pH meter decision by API test strip pH decision

pH meter_decision	API_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	6 37.50	0 0.00	0 0.00	6 37.50
Best	3 18.75	5 31.25	0 0.00	8 50.00
High	0 0.00	1 6.25	1 6.25	2 12.50
Total	9 56.25	6 37.50	1 6.25	16 100.00

Seneye pH decision by Tetra Easy test pH decision

Seneye_decision	Tetra_Easy_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	1 6.25	0 0.00	0 0.00	1 6.25
Best	10 62.50	4 25.00	0 0.00	14 87.50
High	0 0.00	0 0.00	1 6.25	1 6.25
Total	11 68.75	4 25.00	1 6.25	16 100.00

Seneye pH decision by API test strip pH decision

Seneye_decision	API_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	1 6.25	0 0.00	0 0.00	1 6.25
Best	8 50.00	6 37.50	0 0.00	14 87.50
High	0 0.00	0 0.00	1 6.25	1 6.25
Total	9 56.25	6 37.50	1 6.25	16 100.00

Tetra Easy test pH decision by API test strip pH decision

Tetra_Easy_decision	API_decision			
Frequency Percent	Lime needed	Best	High	Total
Lime needed	9 56.25	2 12.50	0 0.00	11 68.75
Best	0 0.00	4 25.00	0 0.00	4 25.00
High	0 0.00	0 0.00	1 6.25	1 6.25
Total	9 56.25	6 37.50	1 6.25	16 100.00

The standard calculation unionised ammonia decision by Seneye unionised ammonia decision

Calculation_decision	Seneye_decision			
Frequency Percent	Best	OK	Water exchange	Total
Best	1 8.33	2 16.67	0 0.00	3 25.00
OK	0 0.00	3 25.00	3 25.00	6 50.00
Water exchange	0 0.00	0 0.00	3 25.00	3 25.00
Total	1 8.33	5 41.67	6 50.00	12 100.00

The standard calculation unionized ammonia decision by Ammonia alert disc decision

Calculation_decision	Alert_decision			
Frequency Percent	Best	OK	Water exchange	Total
Best	3 25.00	0 0.00	0 0.00	3 25.00
OK	1 8.33	4 33.33	1 8.33	6 50.00
Water exchange	0 0.00	0 0.00	3 25.00	3 25.00
Total	4 33.33	4 33.33	4 33.33	12 100.00

The pH meter decision by Seneye unionized ammonia decision by Ammonia alert disc decision

Seneye_decision	Alert_decision			
Frequency Percent	Best	OK	Water exchange	Total
Best	1 8.33	0 0.00	0 0.00	1 8.33
OK	3 25.00	2 16.67	0 0.00	5 41.67
Water exchange	0 0.00	2 16.67	4 33.33	6 50.00
Total	4 33.33	4 33.33	4 33.33	12 100.00

The DO meter decision by Salifert Profi O_2 test decision

DO meter_decision	Profit_decision			
Frequency Percent	Aerate	Good	Possible gas trauma	Total
Aerate	3 25.00	0 0.00	0 0.00	3 25.00
Good	0 0.00	4 33.33	0 0.00	4 33.33
Possible gas trauma	0 0.00	0 0.00	5 41.67	5 41.67
Total	3 25.00	4 33.33	5 41.67	12 100.00

The DO meter decision by Hach DO kit decision

DO meter_decision	Hach_decision			
Frequency Percent	Aerate	Good	Possible gas trauma	Total
Aerate	3 25.00	0 0.00	0 0.00	3 25.00
Good	0 0.00	4 33.33	0 0.00	4 33.33
Possible gas trauma	0 0.00	0 0.00	5 41.67	5 41.67
Total	3 25.00	4 33.33	5 41.67	12 100.00

Salifert Profi O_2 test decision by Hach DO kit decision

Profit_decision	Hach_decision			
Frequency Percent	Aerate	Good	Possible gas trauma	Total
Aerate	3 25.00	0 0.00	0 0.00	3 25.00
Good	0 0.00	4 33.33	0 0.00	4 33.33
Possible gas trauma	0 0.00	0 0.00	5 41.67	5 41.67
Total	3 25.00	4 33.33	5 41.67	12 100.00