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# Study identifies maximum holding times for water samples

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## Samples should always be analyzed promptly

Information on water quality is increasingly used in making management decisions at aquaculture farms. Some farms establish water quality-monitoring programs as a voluntary means of demonstrating environmental stewardship. Other farms must monitor water quality to confirm compliance with effluent guidelines imposed by governments, lending agencies, and certification programs.

Some farms have laboratories where water quality analyses are done by employees, while others send water samples to commercial laboratories for analysis. Either way, samples can be held up to two days before analyses are initiated.

Samples usually are held on ice in insulated chests or refrigerated. The authors recently conducted a study to determine the maximum time that water samples can be stored at near-freezing temperatures without influencing the concentrations of common water quality variables. Their results are being considered for adoption within GAA's Best Aquaculture Practices standards.



Values for water quality variables in refrigerated samples can shift significantly over time. Prompt analysis is always suggested.

## Sample study

Surface water samples for the study were collected in plastic bottles from ponds at the Auburn University Fisheries Research Unit in Auburn, Alabama, USA, and held on ice in insulated chests. The samples were analyzed in the laboratory within 30 minutes after collection and again after six, 12, 24, 48 and 72 hours. Three samples of different concentration were analyzed for each variable. Triplicate determinations were performed for each variable and holding time.

A dozen common water quality variables were measured in the study. Dissolved oxygen was not included, for it is known to change rapidly during storage and should be measured in situ.

## Results

Results for several variables are summarized in Figs. 1-4. Variation among the sample replicates of each variable was small, so only mean values are plotted.

Differences in the mean concentrations of variables did not always occur after the same holding time for all samples, as illustrated for total ammonia nitrogen in Figure 1. Samples with the lowest and highest concentrations remained unchanged for 24 hours, but the sample of intermediate concentration did not change until after 48 hours.

The concentrations of variables also exhibited different trends in change. Some decreased over time, some increased, and some increased and then decreased. In the case of nitrate nitrogen, the mean concentrations did not differ between the initial and six-hour samples, but after 12 and 24 hours were higher than those of the initial samples (Fig. 2). Concentration declined after 24 hours, but in one sample, the 48- and 72-hour values did not differ from the initial and six-hour ones.

## Recommended holding times

When conducting water quality monitoring, investigators do not know the concentrations of water quality variables at the time of sampling. Therefore, for each variable, the time the first difference occurred in any of the samples was noted. The dotted vertical lines in Figs. 1-4, which mark the time after which changes in concentration of the variables initially occurred, indicate the recommended maximum holding times for the variables.

For example, in two samples, the first difference in total ammonia nitrogen concentration was observed after a holding time of about 24 hours (Fig. 1). In the other sample, the total ammonia nitrogen concentration was stable for 48 hours. Nevertheless, 24 hours was taken as the maximum recommended holding time.

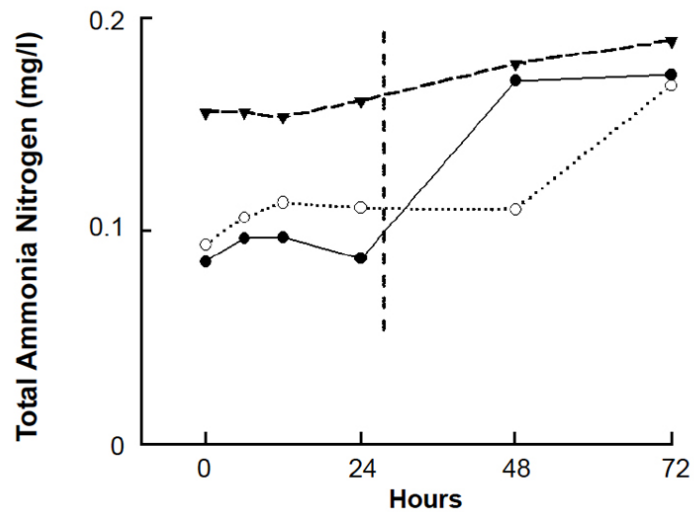


Fig. 1: Mean concentrations of total ammonia nitrogen during sample storage on ice. The vertical line indicates the time of initial change in concentration ( $P < 0.05$ ).

Differences in nitrate nitrogen concentration first occurred in samples held longer than six hours (Fig. 2). The fact that one sample increased in nitrate concentration and then declined to the initial level after 48 hours does not justify holding samples for 48 hours before doing nitrate analyses. The nitrate concentration was declining because of uptake by bacteria and algae in the sample, and there is no assurance that all samples would decline to the initial concentration after 48 hours.

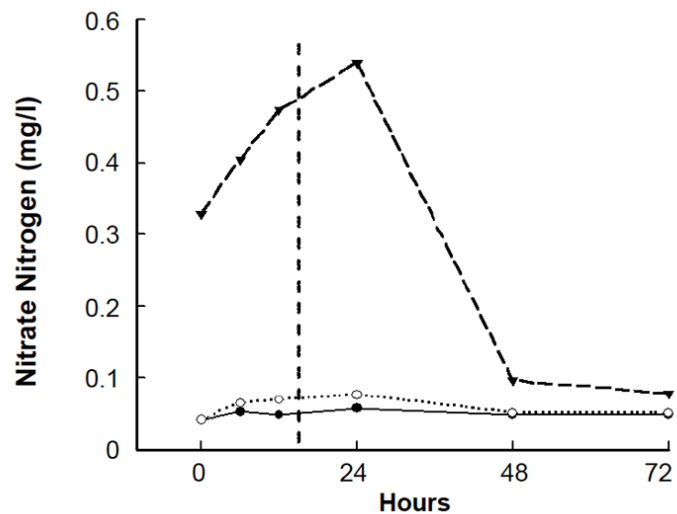


Fig. 2: Mean concentrations of nitrate nitrogen during sample storage on ice. The vertical line indicates the time of initial change in concentration ( $P < 0.05$ ).

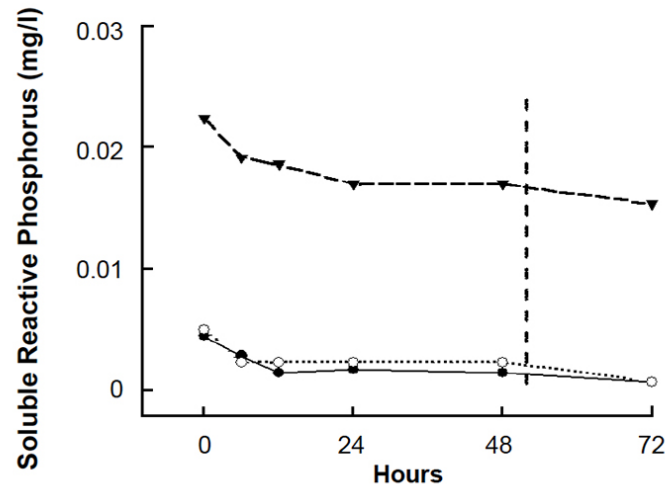


Fig. 3: Mean concentrations of soluble reactive phosphorus during sample storage on ice. The vertical line indicates the time of initial change in concentration ( $P < 0.05$ ).

Fig. 4: Mean concentrations of five-day biochemical oxygen demand during sample storage on ice. The vertical line indicates the time of initial change in concentration ( $P < 0.05$ ).

Recommended holding times for these and other variables are summarized in Table 1. Sample storage was limited to 72 hours because this was considered adequate time for transporting samples to a laboratory and initiating analyses. Variables such as chloride concentration, total suspended solids, turbidity, total alkalinity, and total hardness, which were stable for 72 hours, likely could be held longer, but samples should always be analyzed promptly.

## Boyd, Recommended holding times, Table 1

Variable	Time (hours)
pH	Analyze on site
Dissolved oxygen site	Analyze on site
Total phosphorus	12
Total nitrogen	12
Nitrate nitrogen	12
5-day biochemical oxygen demand	12
Total ammonia nitrogen	24
Soluble reactive phosphorus	48
Chloride	72
Total suspended solids	72

Table 1. Recommended holding times for water samples.

Study data revealed that pH readings taken after six hours can be different than initial readings. Without further study in which pH is measured at more frequent intervals during storage, it is suggested that pH should be measured in situ and samples not held on ice.

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