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# Sampling Strategies for Epidemiological Studies of Aquatic Animal Diseases: Principles, Methods and Applications

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Aquatic animal diseases are major constraints to sustainable aquaculture and fisheries production. Effective disease surveillance requires collection of representative samples from the target population. Since examination of every animal is usually impractical, appropriate sampling techniques are essential for obtaining reliable information while reducing time and cost. Simple random, systematic, stratified, cluster & multistage sampling are important approaches used in aquatic animal health studies. Sample size is influenced by expected prevalence, desired confidence, precision, diagnostic test characteristics and population structure. This article briefly discusses the major sampling strategies, sample size determination and their applications in fish and shrimp disease surveillance.

**Keywords:** aquatic animal health, disease surveillance, epidemiology, sampling, prevalence, aquaculture

## Introduction

Aquaculture is an important source of food, employment and income, but infectious diseases caused by viruses, bacteria, parasites and fungi can cause considerable production losses. Disease surveillance is therefore essential for early detection, monitoring disease occurrence and evaluating disease-control measures. Sampling is a fundamental component of disease surveillance. Examining every animal in a large fish or shrimp population is generally impractical. Instead, a representative sample is selected and examined, and the results are used to make conclusions about the larger population. The quality of these conclusions depends greatly on the sampling method used. Aquaculture populations have special characteristics that must be considered during sampling. Animals are commonly grouped in ponds, tanks, cages or farms, and individuals within the same production unit may have similar exposure to pathogens. Disease occurrence may also vary according to species, age, stocking density, geographical location, season and management practices. Therefore, sampling design should be selected according to the objective of the study and structure of the population.

## Objectives of Sampling

Sampling in aquatic animal disease epidemiology is mainly conducted to:

- Estimate disease or infection prevalence.
- Detect the presence of a particular pathogen.
- Demonstrate freedom from disease.
- Identify populations or areas at increased risk.
- Monitor disease trends.
- Evaluate disease-control and biosecurity programmes.

The objective should be clearly defined before selecting the sampling method. Random sampling is generally suitable for prevalence estimation, whereas targeted or risk-based sampling can be useful for early detection of diseases in high-risk populations.

## Major Sampling Techniques

### Simple Random Sampling

In simple random sampling, every individual has an equal probability of being selected. For example, a number of fish can be randomly selected from a known population using random numbers or computer-generated selection. It is simple and reduces selection bias, but obtaining a complete list of individual animals may be difficult in large aquaculture systems.

### Systematic Sampling

In systematic sampling, individuals are selected at regular intervals after choosing a random starting point. For example, every tenth shrimp can be selected from a population after randomly choosing the starting position. This method is simple and convenient for field surveys. However, periodic patterns in the population should be considered because they may introduce bias.

### Stratified Sampling

Stratified sampling divides the population into important subgroups called strata, followed by sampling from each group. In aquatic animal health studies, strata may include species, age groups, geographical regions, production systems or farm sizes. This method ensures that important subgroups are represented and is particularly useful when disease prevalence differs between groups.

### Cluster Sampling

In cluster sampling, groups such as farms, ponds, cages or tanks are selected rather than individual animals at the first stage. Animals are then sampled from the selected clusters.

Cluster sampling is useful for large geographical surveys because it reduces travel and sampling costs. However, animals within the same cluster may have similar exposure to disease, reducing statistical efficiency.

### Multistage Sampling

Multistage sampling involves selection at two or more levels. For example:

**District → Farm → Pond → Individual fish/shrimp**

This method is particularly useful for large-scale disease surveillance programmes. It is practical and economical but requires appropriate statistical planning because clustering can occur at multiple levels.

## Sample Size Determination

Determining an appropriate sample size is essential for obtaining reliable results. An insufficient sample may fail to detect an existing infection, whereas an unnecessarily large sample increases cost and laboratory workload.

For prevalence studies, a commonly used formula is:

$$n=Z2P(1−P)d2n=\frac{Z^2P(1-P)}{d^2}$$

where:

**n** = required sample size

**Z** = value corresponding to the confidence level

**P** = expected prevalence

**d** = desired precision.

For a 95% confidence level, the value of ZZ is approximately 1.96.

For disease detection or demonstration of freedom from infection, sample size also depends on design prevalence, diagnostic test sensitivity and specificity, and the required confidence level. Therefore, a fixed sample number should not be applied to every disease investigation.

## Application in Fish and Shrimp Disease Surveillance

Sampling techniques are widely applicable to aquatic animal disease surveillance. In fish farming, samples may be collected from hatcheries, nursery units, ponds, cages and natural

water bodies. In shrimp farming, farms and ponds may be selected first, followed by sampling of individual shrimp. For example, surveillance for **white spot syndrome virus (WSSV)** may involve selecting farms from different geographical areas, ponds within selected farms and shrimp from selected ponds. Similarly, bacterial diseases such as *Vibrio* infections can be investigated by sampling animals and appropriate environmental or farm-level units. In India, the **National Surveillance Programme for Aquatic Animal Diseases (NSPAAD)** provides a national framework for surveillance of important aquatic animal diseases. Such programmes demonstrate the importance of systematic sampling and standardized diagnostic procedures in aquatic animal health management.

## Factors Affecting Sampling Quality

Several factors can affect the reliability of a disease survey. Selection bias may occur when samples are collected only from easily accessible farms or clinically affected animals. Sampling only diseased animals may overestimate prevalence, whereas sampling only apparently healthy animals may underestimate it. Season, age, species, stocking density and environmental conditions can also influence disease occurrence. Sample collection, preservation, transportation and laboratory diagnosis must be standardized because poor sample handling can result in inaccurate results. The clustered nature of aquaculture populations is another important consideration. Fish or shrimp within the same pond or farm may share similar exposure to pathogens and therefore cannot always be considered completely independent observations.

## Conclusion

Appropriate sampling is essential for reliable aquatic animal disease surveillance. Simple random, systematic, stratified, cluster and multistage sampling methods have different applications depending on the study objective and population structure. Sample size should be determined scientifically by considering prevalence, confidence, precision and diagnostic-test performance. Well-designed sampling improves disease detection, prevalence estimation and evaluation of control measures while reducing unnecessary costs. Integration of sampling methodology with epidemiology, diagnostic testing and risk-based surveillance can strengthen aquatic animal health programmes and contribute to sustainable aquaculture development.

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