



Application of Biostatistics in Aquaculture Experiments

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Aquaculture has become one of the fastest-growing food-production sectors in the world, and the scientific evaluation of farming practices, feeds, water quality, genetics, and disease management increasingly depends on sound quantitative analysis. Biostatistics provides the tools needed to design valid experiments, summarize biological variation, and draw defensible conclusions from data collected on fish, shrimp, and other cultured organisms. This article reviews the role of biostatistics in aquaculture research, with emphasis on experimental design, replication, sample size determination, and the statistical methods most frequently applied to growth trials, water-quality monitoring, and disease studies. Analysis of variance, regression, mixed models, and multivariate techniques such as principal component analysis are discussed alongside recurring methodological problems, particularly pseudo replication and inadequate statistical power. The review draws on established literature in aquaculture science and biometry to highlight both the value and the current limitations of statistical practice in the field, and it concludes with recommendations for improving the rigor of future aquaculture experiments.

Keywords: Biostatistics; aquaculture; experimental design; analysis of variance; pseudo replication; statistical power; multivariate analysis

Introduction

Aquaculture, the farming of fish, crustaceans, mollusks, and aquatic plants, now supplies close to half of all aquatic animal products consumed globally. As production systems intensify and diversify, researchers routinely conduct experiments to evaluate feed formulations, stocking densities, water-treatment methods, genetic lines, and disease-control strategies. Because living organisms display substantial natural variability in growth, survival, and physiological response, conclusions about the effect of any treatment cannot be drawn reliably from raw observation alone. Biostatistics, the application of statistical methods to biological data, supplies the framework needed to plan such experiments, quantify uncertainty, and separate genuine treatment effects from random variation. Its use spans the entire research cycle, from the design of a trial and the calculation of an adequate sample size to the choice of analytical model and the interpretation of results.

The Role of Biostatistics in Aquaculture Research

Biostatistics enters the field of aquaculture research in several distinct phases. It informs decisions at the planning stage about the number of experimental units (tanks, ponds or cages), how animals should be allocated to treatments and the level of replication needed to detect a biologically meaningful difference. During data collection, statistical thinking informs the taking of measurements so that they are independent and representative of the population under study. In the analysis, appropriate models are selected to fit the structure of the data whether that structure is a simple comparison of two feeds, a factorial design testing

multiple factors at once or a longitudinal record of growth over time. Finally, interpretation depends on statistical inference, which lets researchers say, with a known

Experimental Design in Aquaculture Studies

Replication and the Problem of Pseudoreplication

A central design decision in any aquaculture trial is the level of replication, that is, how many independent tanks, ponds, or cages receive each treatment. Replication increases the reliability of an estimate and allows the natural variability among experimental units to be quantified. A well-known and long-standing problem in the field is pseudoreplication, in which measurements from many individual animals housed together in a single tank are mistakenly treated as independent replicates, when in fact the tank, not the individual fish, is the true experimental unit. Because animals sharing a tank also share the same water quality, feeding regime, and social environment, their responses are correlated rather than independent. Treating such correlated observations as if they were independent inflates the apparent sample size, understates the true error variance, and greatly increases the risk of falsely concluding that a treatment effect exists. This issue was first formally described in ecological research and has since been shown to remain common in aquaculture and aquaponic studies, underscoring the continuing need for researchers to correctly identify the experimental unit before applying inferential statistics.

Sample Size and Statistical Power

Closely related to replication is the question of statistical power, the probability that an experiment will detect a real treatment effect if one exists. Reviews of published growth trials have found that many aquaculture studies use only a small number of replicate tanks, commonly around three per treatment, together with a modest number of fish per tank, and that the number of replicates and sample size are rarely chosen with reference to a formal power calculation. Because the biological variance in body mass tends to be broadly similar across many cultured fish species, general guidelines can be developed for the level of replication and sample size needed to detect a given percentage difference between treatment means at an acceptable level of statistical power. Increasing the number of replicate units, increasing the number of animals measured within each unit, and reducing unwanted sources of variability are all recognized ways to improve the sensitivity of an aquaculture experiment, although each comes at a cost in terms of space, labor, and expense that must be balanced against the value of a more reliable result.

Commonly Used Statistical Methods

Analysis of Variance and Mixed Models

Analysis of variance (ANOVA) remains the most widely applied statistical technique in aquaculture growth and nutrition trials, used to compare mean responses such as weight gain, feed conversion ratio, or survival among two or more treatment groups. When data are hierarchical, for example when many fish are measured within each tank and tanks are nested within treatments, a nested or mixed-model ANOVA is generally more appropriate than a simple one-way ANOVA, because it correctly separates the variation occurring between tanks from the variation occurring between individual fish within a tank. Mixed models are particularly valuable for dose-response feeding trials, where a graded series of dietary inclusion levels is tested, and for repeated-measures growth data collected from the same animals over successive sampling occasions.

Regression and Correlation Analysis

Regression techniques are widely used to describe relationships between continuous variables, such as the relationship between fish age and body weight, between dietary protein level and growth rate, or between water temperature and oxygen consumption. Multiple regression techniques, such as growth models based on the von Bertalanffy growth function, make it possible to analyze several environmental or nutritional variables simultaneously and to determine the factors that most strongly influence production results. Correlation analysis

is also used to study the relationships among physiological or water-quality variables before an elaborated causal model is developed.

Multivariate Statistical Techniques

Multivariate statistical methods have become increasingly important because aquaculture systems generate data on many interrelated variables simultaneously, including temperature, dissolved oxygen, ammonia, pH and salinity. Principal component analysis and factor analysis are methods of reducing a large number of correlated water-quality parameters to a smaller number of underlying components, which helps to identify the dominant sources of variation in a pond or recirculating system. Cluster analysis groups together sampling sites or time periods with similar water-quality profiles. Discriminant analysis has been used to classify different production systems, such as recirculating, pond-based or integrated systems, by their characteristic water-quality patterns. These techniques address the joint behavior of multiple variables that might individually exhibit only weak or inconsistent patterns, and complement univariate methods.

Applications in Specific Areas of Aquaculture

Growth and Feeding Trials

Feeding trials designed to compare diets, feed additives, or feeding frequencies rely on biostatistics to determine whether observed differences in growth or feed efficiency are genuine. Careful attention to the appropriate error term, replication level, and sample size is essential, since feeding trials are among the aquaculture studies most frequently criticized for inadequate replication and unreported statistical power.

Water Quality Assessment

Statistical monitoring of water quality is central to sustainable aquaculture management. Multivariate statistical analysis of physicochemical parameters has been used to assess pond and river water intended for aquaculture, revealing seasonal and spatial patterns in variables such as biological oxygen demand, suspended solids, and coliform counts, and to distinguish the environmental performance of different pond-based production systems. Time-series and regression-based forecasting models are also being combined with statistical and machine-learning approaches to anticipate water-quality changes before they affect stock health.

Disease and Health Studies

Biostatistics supports the design and analysis of challenge trials and epidemiological surveys used to evaluate disease resistance, vaccine efficacy, and the physiological or immune response of cultured animals to stressors. Survival analysis and proportion-based tests are commonly used to compare mortality rates among treatment groups, while hematological and biochemical indicators are analyzed using ANOVA or multivariate methods to characterize the health status of fish under different management regimes.

Challenges in Applying Biostatistics to Aquaculture

Despite its importance, statistical practice in aquaculture research continues to face recurring challenges. Pseudoreplication remains common, particularly when logistical or financial constraints limit the number of independent tanks or ponds available for an experiment. Statistical power is frequently not calculated in advance, and retrospective analyses suggest that many published studies operate below the conventional eighty percent power threshold, increasing the risk of failing to detect real treatment effects. Peer review does not always catch these problems, meaning that flawed experimental designs and inappropriate error terms sometimes appear in the published literature. Addressing these challenges requires closer collaboration between aquaculture scientists and biostatisticians at the design stage, rather than only at the point of data analysis, along with wider adoption of general guidelines on minimum replication and sample size for common types of aquaculture experiments.

Conclusion

Biostatistics is indispensable to modern aquaculture research, shaping how experiments are designed, how data are collected, and how conclusions are drawn from inherently variable

biological systems. Analysis of variance, regression, mixed models, and multivariate techniques each address different aspects of aquaculture data, from simple treatment comparisons to the joint behavior of many interacting water-quality variables. At the same time, longstanding methodological problems, especially pseudoreplication and insufficient statistical power, continue to compromise the reliability of some published work. Strengthening statistical literacy among aquaculture researchers, planning experiments with an explicit power calculation, and correctly identifying the true experimental unit are practical steps that would substantially improve the quality and reproducibility of future aquaculture experiments.

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