Aquaculture 437 (2015) 344–350

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Misuse of multiple comparison tests and underuse of contrast procedures in aquaculture publications

Rodrigue Yossa [a,*], Marc Verdegem [b]

[a] Department of Biology, Gus R. Douglass Institute, WV State University, Institute, WV, USA
[b] Aquaculture and Fisheries Group, Wageningen University, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 19 July 2014
Received in revised form 3 November 2014
Accepted 15 December 2014
Available online 20 December 2014

Keywords:
Aquaculture research
Contrast procedures
Multiple comparison tests
Structured treatments
Biostatistics

ABSTRACT

In aquaculture research, independent variables are qualitative (with or without a structure), quantitative, or factorial combinations. A qualitative independent variable is a variable that has unquantifiable, nominal variants (levels), which represent different categories such as the fish gender. The structure in a qualitative independent variable refers to the existence of a relation between its different variants, in a way that suggests that some variants can be grouped together and then compared to other groups of variants. A quantitative independent variable is a variable with measurable variants that are expressed numerically and are fixed throughout the experiment, such as water temperatures. In a study with one independent variable, each variant of this variable represents a treatment. In a study with two or more independent variables, also called a factorial or multifactorial experiment, the treatments represent all the possible combinations of the two or more independent variables. Following an analysis of variance (ANOVA) (or a multiple factor ANOVA) showing that there is a significant difference among the three or more treatment means, a multiple comparison test, an orthogonal contrast procedure, or a polynomial contrast procedure is applied to separate or present the relationship among the treatment means, in accordance with the nature and structure of the independent variable. The use of multiple comparison tests such as Least Significant Difference, Duncan's Multiple Range, Tukey's Honest Significant Difference, Bonferroni and Scheffé's tests, is more relevant when there is no structure in the qualitative independent variable; otherwise the use of the orthogonal contrast procedure, which allows the comparison of related treatment means or groups of means to other treatment means, is more appropriate. The orthogonal contrast procedure is also appropriate for factorial experiments. With quantitative independent variables, the use of polynomial procedure, which detects the trend of the relationship or regression that exists between the independent and response variables, is appropriate.

The present paper critically analyzed the statistical methods used in articles published in ten selected international peer-reviewed aquaculture journals in the year 2013. This analysis showed that in none of the studies in which the independent variable was qualitative with a structure, the data have been analyzed using orthogonal contrast procedure. Also, the data of only 34% of the studies in which the independent variable was quantitative have been analyzed using polynomial contrast (regression), whereas the data of only 13% of studies with a factorial design have been analyzed using contrast procedure. More attention should be paid on publishing only studies that used appropriate statistical procedures, which conform to the nature of the independent variables of interest.

1. Introduction

Aquaculture is the fastest growing sector in agricultural production across the world, and it currently contributes over 42% in global fish production (FAO, 2014). The growth in aquaculture production during the last four decades coincided with increasing global efforts in aquaculture research and development. For every finfish and shellfish belonging to the 600 aquatic species currently raised in captivity worldwide, aquaculture research essentially focuses on investigating the range of water physicochemical parameters (temperature, pH, hardness, ammonia, dissolved oxygen, etc.), nutrient requirement and substitution levels (macronutrients such as proteins, lipid and carbohydrates, and micronutrients such as vitamins and minerals), feeding (feeding rate, feeding frequency, etc.), use of therapeutants, and production system and technology (light intensity levels, photoperiod, animal density, etc.). In other words, in aquaculture like in other sectors of agricultural sciences, most of the independent variables

* Corresponding author at: Department of Biology, Gus R. Douglass Institute, West Virginia State University, P.O. Box 1000, Institute, WV 25112, USA. Tel.: +1 304 766 4165x4165; fax: +1 304 7665244.
E-mail addresses: rodyossa@yahoo.fr, ryossa@wvstateu.edu (R. Yossa).

http://dx.doi.org/10.1016/j.aquaculture.2014.12.023

(experimental variables) are qualitative (with a structure), quantitative, or factorial combinations. The use of qualitative variable with a structure and quantitative experimental variables, alone or in combination, allows making "planned comparisons" (Steel et al., 1997).

A qualitative independent variable is a variable that has unquantifiable, nominal variants (levels), which represent the different categories included in this variable such as the presence or absence of a character or the fish gender (male or female). The structure in a qualitative independent variable refers to the existence of a relation between the different variants of this variable, in a way that suggests that some variants can be grouped together and then compared to other groups of variants (Steel et al., 1997). For example, assuming that the objective of an aquaculture experiment is to determine the dietary protein optimizing fish growth, there might be four protein types (treatments) tested: soybean meal, corn gluten meal, fish meal, and beef blood meal. There is a structure among these protein types as the two plant proteins (soybean meal and corn gluten meal) can be grouped and compared to a group including the animal proteins (fish meal and beef blood meal). In addition, soybean meal can be compared to corn gluten meal and fish meal can be compared to beef blood meal. Another example is an aquaculture experiment aiming to identify the plant protein that when substituting 10% of fish meal in a salmon diet results in the best growth. Four experimental plant proteins (treatments) might be tested: corn gluten meal, soybean meal, black eye pea meal and canola meal. There is no relevant relationship among the experimental plant proteins that can be considered in order to classify them into definable treatment groups; therefore there is no structure in the independent variable of this experiment.

A quantitative independent variable is a variable with levels that are measurable quantities that are expressed numerically such as the water temperatures, dietary protein levels or fish densities per tank. The levels of a quantitative independent variable are maintained unchanged throughout the experimental period. An example is an experiment in which the independent variable is the water temperature, with five levels, 20, 22, 26, 28, and 30 °C.

In a study with one independent variable (unifactorial experiment), each variant of this variable represents a treatment, and the total number of treatments (k) equals the total number of variants of the independent variable. In a study with two or more independent variables, also called a factorial or multifactorial experiment, the total number of treatments (k) represents all the possible combinations of the two or more independent variables (Petersen, 1977; Steel et al., 1997). For instance, a study with the independent variables (factors) water temperature with two levels (20 °C and 30 °C) and dietary protein source with three levels (soybean meal, gluten meal, and black eye pea meal) is a 2×3 factorial experiment, with $2 \times 3 = 6$ treatments (Table 1).

Several studies have pointed out the extensive misuse of pairwise multiple comparison tests in agricultural sciences (Chew, 1976; Gates, 1991; Gill, 1973; Little, 1981; Madden et al., 1982; Petersen, 1977). However, the examination of the use of statistics in aquaculture science has received little attention. The present paper therefore critically analyzes the statistical methods used in manuscripts published in international aquaculture journals, to draw the attention of researchers on the importance of good statistical planning and proper data analysis. Nevertheless prior to performing this critical analysis, it is worth reviewing some basic and applied concepts in relation to the planning and execution of data analysis (and interpretation).

Table 1
Example of the different treatments obtained by combining the level of the two independent variables (protein sources and water temperatures) in a factorial experiment.

		Protein sources		
		Soybean meal (SB)	Corn gluten meal (CG)	Black eye pea meal (BEP)
Water temperatures (°C)	20	SB20*	CG20*	BEP20*
	30	SB30*	CG30*	BEP30*

*SB20 refers to soybean meal fed to fish reared at 20 °C, SB30 refers to soybean meal fed to fish reared at 30 °C, CG20 refers to corn gluten meal fed to fish reared at 20 °C, CG30 refers to corn gluten meal fed to fish reared at 30 °C, BEP20 refers to black eye pea meal fed to fish reared at 20 °C, and BEP30 refers to black eye pea meal fed to fish reared at 30 °C.

2. Materials and methods

We start with a comparative description of multiple comparison tests and contrast procedures, presenting the circumstances in which it is more appropriate to use these statistics. Then we present the methodological approach that was used to collect and evaluate statistical methods applied in aquaculture experiments published in peer-reviewed journals.

2.1. Multiple comparison tests versus contrast procedures

In an experiment with one independent variable that has more than three levels, and assuming that the independence, normality and homogeneity of the residuals are satisfied, analysis of variance (ANOVA) is used to test the null hypothesis: "there is no significant difference between the treatment means". In an experiment with a combination of two or more independent variables (factorial experiment) the null hypothesis is tested using (multi)factorial ANOVA. When there is a significant difference among the treatment means, the null hypothesis is rejected and a multiple comparison test, an orthogonal contrast procedure or a polynomial contrast procedure is applied to separate treatment means or to analyze the relationships that might exist among the treatment means. The choice of one of these statistical procedures depends on the nature and structure of independent variables (qualitative with or without structure, quantitative, or factorial combinations).

Multiple comparison tests are statistical methods that are intended to compare each factor level mean with every other factor level mean assuming that there is no definable structure among the factor levels (Gates, 1991; Montgomery, 1997; Petersen, 1977; Saville, 1990; Steel et al., 1997). In this manner, a pairwise multiple comparison compares factor level effects on the response variable. For instance, in the above experiment with the four experimental plant proteins, the use of a multiple comparison test is relevant, as no definable structure exists among the experimental plant proteins (treatments). Examples of multiple comparison tests include Least Significant Difference (LSD), Duncan's Multiple Range, Waller and Duncan's, Tukey's Honest Significant Difference (Tukey-HSD), Bonferroni and Scheffé's procedures. The merit of each of these tests over the others has extensively been discussed (Montgomery, 1997; Steel et al., 1997), and is beyond the scope of this paper. However, with qualitative independent factors showing a structure, statistical procedures exist which lead to more meaningful conclusions on treatment effects; furthermore, pairwise multiple comparison tests should not be used on quantitative main factors and factorial interaction terms as this leads to misinterpretation of research results and flawed conclusions (Gates, 1991; Montgomery, 1997; Olsen, 2003; Petersen, 1977; Rafter et al., 2002; Saville, 1990; Steel et al., 1997).

In an experiment with a qualitative variable showing a structure, an adequate statistical procedure for comparing treatment means is the orthogonal contrast procedure (Davis, 2010; Montgomery, 1997; Steel et al., 1997). With this procedure related treatment means or groups of treatment means are specifically compared to other treatment means, for a total of comparisons equal to the degrees of freedom. Each comparison is planned prior to the execution of the experiment and can represent an objective in the research protocol. For instance, in a fish nutrition experiment aiming at i) selecting the best protein source for fish growth, ii) determining the best plant-based protein for fish growth, and iii) determining the best animal-based protein source for fish growth, the independent variable is the fish diet formulation (qualitative variable). In this example, there is only one source of variation, the diet, and the four experimental treatments (k) that can be