A descriptive analysis of the replication applied in aquaponic experimental studies

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***Abstract*— *A literature search was performed via SCOPUS for publications between January 2000 and April 2020 that contained the keywords aquaponic and hydroponic. Sixty-one articles were identified that stated a comparison and a form of comparative statistical analysis was performed. These articles were identified via the principle author, year of publication, the system type tested (coupled or decoupled aquaponic; irrigated nutrient solution from a separated RAS), the number of treatments tested, the number of replicates applied to each treatment and the region or country within which the experiment was performed. An experimental comparison context was assigned to each study to identify the requirement for replication. Sixty-one percent (61 %) of all the studies were deemed to have applied no or incorrect replication (no or incorrect replication: 56 % of fully recirculating system studies, 100 % of decoupled system studies, 86 % of irrigated RAS water studies). In terms of the comparison context, 54 % of system comparison studies, 100 % of solution comparison studies and 63 % of plant component associated comparison studies, applied no or incorrect replication. The association between study location and the incidence of no or incorrect replication was also determined (Europe – 71 %, USA – 80 %, South America – 63 %, Australia – 33 %, West Asia – 5 %, South East Asia; including China – 20 % and South Asia – 14 %). An experimental replication decision matrix was developed to assist future aquaponic researchers in determining the application of correct replication and several example research articles were discussed to demonstrate and explain the correct and incorrect application of replication in experimental designs for aquaponic associated research studies.*

Keywords*— Aquaponic, Hydroponic, Aquaculture, RAS, Replication, Statistics, Experimental design.

I. INTRODUCTION

Aquaponics is a technology that produces both animal (usually fish) and plant products and confers advantages such as water savings, nutrient input savings, lowered environmental impact and an ability for universal location (Lennard, 2017; Ayipio et al., 2019). A proportion of broader aquaculture production is done using Recirculating Aquaculture Systems (RAS), which are analogous to the fish culturing component in an aquaponic context (Lennard, 2017). Vegetable production is partly being done using hydroponic or substrate culture technologies, which are analogous to the plant culturing component in an aquaponic context (Lennard, 2017). Therefore, both fish and vegetables are being produced with existing water-culturing technologies that are analogous to aquaponics. This, along with the intrinsic goal of improving all efficiencies associated with aquaponic production technologies and methodologies, allows a comparative pathway for aquaponics in terms of fish and vegetable production (i.e. fish and plant growth rates may be compared between RAS and aquaponics and between hydroponics and aquaponics to establish the relative efficiencies of the aquaponic technique).

Much aquaponic research is associated with establishing and/or improving the productive potential of the technique, especially via comparison to conventional hydroponic crop production (Ayipio et al., 2019). To measure and establish any perceived improvement, researchers compare the outputs of aquaponics (e.g. plant growth or yield) with either internal aquaponic variables (e.g. fish species cultured, fish stocking density, solution pH, presence/absence of additional nutrients, presence/absence of solution sterilisation, hydraulic parameters – flow rate or hydraulic retention time, planting media, etc.) or externally appropriate comparative technologies with known high productive rates for the associated organism (e.g. hydroponics and substrate culture - plants) (Ayipio et al., 2019). Therefore, especially in terms of plant growth and production, a proportion of aquaponic research is associated with experimentally comparing a proposed aquaponic technology variation with a known hydroponic culturing technique which possesses expected plant production outcomes that are generally considered to be industry best practice (Resh, 2013).

A pivotal requirement of comparative research is the correct application of experimental design, which allows the researcher(s) to determine that the comparison they envisage and desire to test may be done in a context where the measured outcomes may be clarified and verified via statistical analysis (Hurlbert, 1984; Searcy-Bernal, 1994; Poorter & Garnier, 1996; Quinn & Keough, 2002; Ling & Cotter, 2003; Thorarensen et al., 2015). For comparisons with measured animal (fish) or plant (vegetable) productive outcomes, one of the most important factors that determines statistical rigour is the application of appropriate replication (Hurlbert, 1984; Searcy-Bernal, 1994; Quinn & Keough, 2002). Replication means having two or more observations at a spatial or temporal scale that matches and repeats the application of the experimental treatment and are essential because biological systems are inherently variable (Hurlbert, 1984; Quinn & Keough, 2002). Also, and importantly, replication assists to avoid confounding treatment differences with other systematic differences between experimental units (Hurlbert, 1984; Quinn & Keough, 2002).

An important factor in ensuring that replication is adequate and correct in aquaculture or hydroponic studies is determining what constitutes an independent experimental unit (Tlusty, 2010; Thorarensen et al., 2015)? In a RAS fish culture context, an understanding of, and respect for, what is being tested is very important because it dictates the experimental design. For example, the experimental design to test a particular overall RAS technical design (Design A) against another overall RAS technical design (Design B), when fish growth is the measured comparative parameter, is different from what is required when fish feed variations (e.g. Feed A vs Feed B) are being compared within a RAS context and again, fish growth is the measured comparative parameter. In both instances, fish growth is the measured outcome used for comparison. However, in the first case (RAS technical design), the test variable is the RAS itself; in the second case (the feed being fed to the fish within the RAS) the test variable is the fish feed.

Replication is adequate when the variable is repeated in an environment where other variables are completely negated or minimised (Hurlbert, 1984). Replication is inadequate (or "pseudo") if samples come from a single experimental unit (Hurlbert, 1984). In the first example case, both entire RAS's (A and B) must be repeated (or replicated). This is because the test is to determine if one RAS performs better than the other and what is therefore, being compared or tested, are the RAS designs themselves. This means a minimum of three RAS's of Design A and three RAS's of Design B are required. This replication of the entire RAS design allows the determination of acceptable absence of variability within that RAS design (e.g. if the fish growth results from all three RAS A designs are statistically similar, the within RAS A inherent variability is low and therefore, it is acceptable that any of the three replicates of RAS A represents the RAS A design truly, and that the results from all three replicates of RAS A may be pooled to make a common data set). In the second case, because the effect of the fish feed on fish growth is being tested, it is adequate to apply individual fish tanks as the repeatable experimental unit and therefore, one RAS is suitable (as long as it contains three or more individual fish tanks for each treatment – fish feed, being tested). In fact, a centralised filtration system that treats all the water from all experimental units (fish tanks) is a better design, because this removes the filtration unit as a potential variable in the comparison (i.e. if each fish tank has its own filter, or if each treatment of fish feed variation, of three fish tanks, have their own filter, how can it be determined if it is the fish feed or the filter that is causing any fish growth differences detected?) (Tlusty, 2010).

Determining the replication requirement in a hydroponic context is similar and replication is inadequate, again, if all samples arise from one experimental unit (Hurlbert, 1984). If one wishes to compare hydroponic system Design A to hydroponic system Design B, in terms of plant growth outcomes, three replications of each system is required (three hydroponic systems of Design A and three hydroponic systems of Design B). If one wishes to compare the effect of different planting media (e.g. coir, perlite, vermiculite) on a plant growth outcome, a single hydroponic system is adequate, with replicated planting units (e.g. at least three NFT channels or three DWC beds, etc.) (Gomez & Gomez, 1984; Poorter et al., 2012; Gupta et al., 2015).

Many contexts exist within RAS, hydroponics and aquaponics that may be compared, and it is apparent that because aquaponics includes two technologies (RAS and hydroponics) and two productive organisms (fish and plants), the contexts become more complex. For example, one may determine to compare within a system context (e.g. aquaponic system vs hydroponic system), a delivered nutrient solution context (e.g. aquaponic nutrient solution vs hydroponic nutrient solution), an aquaponic system plant component context (e.g. deep water culture vs NFT vs media culture), an aquaponic system plant growing media context (e.g. no media, coco fibre, gravel, sand, perlite, vermiculite, mixtures of all of these media, etc.), an aquaponic system fish species context (e.g. Tilapia vs Trout vs Catfish, etc), mechanical or hydraulic contexts (e.g. applied filtration, hydraulic retention times, flow rates), chemical contexts (nutrient solution strength, nutrient solution mixture, presence/absence of plant growth promoters, etc.) and on it goes! Similarly, different parameters may be used as the measure of performance or comparison (e.g. fish growth, SGR, feed conversion ratio, etc., plant growth, solution nutrient removal or accumulation, etc.). These myriad contexts have different and unique requirements in terms of the replication applied within the overall experimental design. Therefore, a complex matrix exists for the researcher to navigate to determine what replication is appropriate to confer statistical confidence in the results produced and to ensure that the inferences made from those results are valid and ultimately, applicable to broader situations (e.g. commercial applications of the technology).

RAS fish and hydroponic plant production are existing technologies with decades of applied research (Timmons et al., 2002; Resh, 2013). Therefore, techniques in experimental design, replication and statistical analysis have been well-developed and proven within these fields (Tlusty, 2010; Poorter et al., 2012; Gupta et al., 2015). Both RAS and hydroponics research demonstrate that replication determination is highly dependent upon the variable being compared (Tlusty, 2010; Poorter et al., 2012; Gupta et al., 2015). Determination of the context of the comparison within both RAS and hydroponics is well established and should be used as a key driver for similar determinations applied to aquaponic research. In this way, within aquaponics experimentation, context determination enables the identification of which factor requires replication within the experimental design and is pivotal to a statistically valid outcome, whilst enabling the conservation of valuable resources (funds, space, apparatus, consumables, etc.) within the often constrained, experimental environment (Thorarensen et al., 2015).

The aim of this study was to analyse the context of comparison and the associated replication applied to that context, in a randomly selected cohort of peer reviewed, published, scientific articles about aquaponic technology which applied scientific comparisons. The studies were examined, and the context of comparison determined. The replication applied was then examined to determine if it was appropriate for the context of the comparison. The outcomes of the analysis were then used to determine the frequency of inappropriate replication applications within several sub-sets of aquaponic research. The analytical outcomes were then used to develop a decision matrix for researchers in an attempt to improve the application of appropriate replication in experimental designs for aquaponics experimental research that could produce valid, comparative statistical outcomes.

II. MATERIALS AND METHODS

A literature search was performed between the dates 12thMay 2020 and 19th May 2020, via SCOPUS. Sixty-one articles (excluding reviews and editorials) published in peer reviewed journals were identified between January 2000 and April 2020 that contained the keywords *aquaponic* and *hydroponic*, stated a comparison for some form of plant growth or production parameter, stated the inclusion of replication and stated that a form of comparative statistical analysis was performed.

The identified studies were tabulated to include the name of the first author, the year of publication, the system type(s) applied (see below), the number of treatments applied, the number of replications applied per treatment, the region or country within which the experiments were performed and the applied context of comparative analysis.

In terms of the system type, a recirculating aquaponic system was identified as a system that contained fish (in a fish tank), a form of filtration (mechanical, biological or both) and a hydroponic component where the system water recirculated between the fish and plant components perpetually. A hydroponics system irrigated with RAS water was identified as a system not containing fish, whereby a hydroponic component was irrigated with water that originated from a separate RAS that was not part of the experimental design (i.e. the RAS was not connected to the hydroponic component of the experimental design in any hydraulic form or sense, but RAS water was used to fill the sump or nutrient tank of the experimental hydroponic component). A decoupled system was identified as a system that contained fish (in a fish tank or fish component with associated filtration) and a hydroponic component whereby the water flowed from the fish component to the hydroponic component but did not return to the fish component from the hydroponic component.

The number of treatments applied was identified from the article text with a primary relation to a plant production outcome via an associated parameter of identified testable difference. For example, hydroponic vs aquaponic, a plant nutrient solution difference (pH, nutrient concentration, nutrient mixture, hydraulic retention time), type of plant growing component, type of plant substrate applied, etc.

The number of replications applied per treatment was identified from the article text, the written description of the applied experimental set-up and design and any associated diagrammatic or pictorial description of the experimental set-up and design. The comparison parameter applied was identified and assigned to one of three categories; a system comparison (i.e. where different system types are compared to each other – e.g. aquaponic v hydroponic, variations in fish component configuration, variations in fish species cultured, variations in flow rates applied, differences in fish management, etc.), a nutrient solution comparison (i.e. where different nutrient solutions are compared, variations in solution chemical parameters, variations in nutrient solution concentrations, variations in nutrient solutions mixture, etc.), or a plant component comparison (i.e. where different plant component configurations, designs, substrates, etc. were applied).

A series of descriptive statistics were identified and tabulated to include the total number of studies identified, the number of fully recirculating system designs, the number of decoupled system designs and the number of irrigated RAS water designs, the number of system comparisons, the number of solution comparisons and the number of plant component comparisons, the number and percentage of correctly replicated studies, the number and percentage of incorrectly replicated studies and a break-down of the same descriptive statistics based on the region or country of location of the research.

The above outlined information was then used to develop an experimental design decision matrix so that future researchers have an available primer for correct experimental design in an aquaponic research context. In addition, several studies were isolated, examined and discussed in terms of the applied experimental design and replication to provide examples of valid and invalid application of replication, to support and illustrate the developed decision matrix.

III. RESULTS

Study Analysis and Descriptive Statistics:

Table 1 outlines all sixty-one identified studies included in the analysis (by primary author), and includes the year of publication, the system type tested (Recirculating or Coupled, Hydroponics Irrigated with RAS Water and Decoupled), the number of treatments tested, the number of replications applied to each treatment, the region or country where the experiments were conducted and the comparison context.

A series of descriptive statistics for the identified studies are presented in Table 2. Of the sixty-one identified studies, fifty-two were identified as testing recirculating (coupled) aquaponic systems, two were identified as testing decoupled aquaponic systems(where fish were present in a connected, hydraulic context) and seven were identified as testing a variation of irrigating water to a hydroponic component that was sourced from a separate, operating RAS.

Forty-six studies were identified as comparing the whole or complete aquaponic systems (i.e. system comparisons), seven studies were identified as comparing nutrient solution(s) (i.e. solution comparisons) and eight were identified as comparing a variation associated with a plant component (i.e. plant component comparisons). Of the forty-six system comparisons, twenty-one (46 %) were identified to have applied correct replication of the treatments tested and twenty-five (54 %) were identified to have applied incorrect replication of the treatments tested. Of the seven solution comparisons, zero (0 %) were identified to have applied correct replication of the treatments tested and seven (100 %) were identified to have applied incorrect replication of the treatments tested. Of the eight plant component comparisons, three (37 %) were identified to have applied correct replication of the treatments tested and five (63 %) were identified to have applied incorrect replication of the treatments tested.

Of the fifty-two recirculating (coupled) aquaponic systems studied, twenty-three (44 %) were identified to have applied correct replication of the treatments tested and twenty-nine (56 %) were identified to have applied incorrect replication of the treatments tested. Of the seven hydroponic components irrigated with RAS water and/or hydroponic nutrient solution controls, one (14 %) was identified to have applied correct replication of the treatments tested and six (86 %) were identified to have applied incorrect replication of the treatments tested. Of the two decoupled aquaponic systems studied, zero (0 %) were identified to have applied correct replication of the treatments tested and two (100 %) were identified to have applied incorrect replication of the treatments tested.

Overall, of the sixty-one identified studies, twenty-four (39 %) were identified to have applied correct replication of the treatments tested and thirty-seven (61 %) were identified to have applied incorrect, or no, replication of the treatments tested.

Table 2 also contains a regional breakdown of descriptive statistics which includes all of those outlined above, within a regional context. Importantly, this outlines the percentage of studies performed per region that applied incorrect or no

Table 1: Author, year of publication, type of system comparison performed, treatment number, replicate number, region and comparison parameter for the identified aquaponic studies, 2000 – 2020.

Author	Year	System Type	Treatments	Replicates	Region	Comparison Context
Alcarraz et al.	2018	Recirculating	2	3	Chile	System (AP v HP)
Alvarez-Garcia, et al.	2019	RAS irrigated	5	1	Europe	Solution - nutrients
Anderson, et al.	2017	Recirculating	3	1 AP; 2 HP	USA	System (pH)
Blanchard, et al.	2020	RAS irrigated	4	1	USA	Solution (pH)
Blidariu, et al.	2013	Recirculating	2	1	Europe	System (AP v Soil)
Calone, et al.	2019	RAS irrigated	4	1 AP; 3 HP	Europe	Solution (Nutrients)
Castillo-Castellanos, et al.	2016	Recirculating	4	3	Mexico	System (AP v HP)
Dediu et al	2012	Recirculating	2	1	Europe	System (HRT)
Delaide, et al.	2017	Recirculating	2	1	Europe	Plant Comp (Ebb & flow v DWC)
Delaide, et al.	2016	RAS irrigated	3	1	Europe	Solution (HP v AP)
Delaide, et al.	2019	RAS irrigated	2	1	Europe	Solution (HP v AP)
Effendi et al	2017	Recirculating	3	3	Indonesia	System (suppl bact)
Estrada-Perez, et al.	2018	Recirculating	3	3	Mexico	System (Fish density)
Goddek & Vermeulen	2018	RAS irrigated	2	1	Europe	Solution (Nutrients)
Graber & Junge	2009	Recirculating	3	1	Europe	System - (AP v HP v Soil)
Hundley et al	2018	Recirculating	4	1	Brazil	System (Fish density)
Husain, et al.	2014	Recirculating	5	3	India	System (Fish density)
Jordan, et al.	2018	Recirculating	4	1	Brazil	System (AP v HP)
Knaus & Palm (a)	2017	Recirculating	2	1	Europe	System (Carp v Tilapia)
Knaus & Palm (b)	2017	Recirculating	2	1	Europe	System (Catfish v Tilapia)
Lennard & Ward	2019	Recirculating	2	1	Australia	System (AP v HP)
Lennard & Leonard	2006	Recirculating	4	3	Australia	System (Plant component)
Lennard & Leonard	2004	Recirculating	2	3	Australia	System (Recip v constant)
Maucieri, et al.	2019	Recirculating	3	3	Europe	System (Fish density)
Medina, et al.	2016	Recirculating	2	3	USA	System (Feed: fishmeal v veg)
Monsees, et al.	2017	Reci vs Decoup	3	1	Europe	System (Coupled v decoupled)
Monsees, et al.	2019	RAS irrigated	3	3	Europe	System (Steril v unsteril)
Moya et al	2016	Recirculating	3	1	Mexico	System (3 diff plants)
Ngo, et al.	2017	Recirculating	3	1	Vietnam	System (1 - flow; 2 - fish dens)
Nicoletto, et al.	2018	Recirculating	3	3	Europe	Solution (AP v HP)
Nozzi, et al.	2018	Recirculating	4	1	Europe	System (AP v HP)
Nuwansi, et al.	2017	Recirculating	4	3	India	System (Fish ratios)
Palm, et al.	2014	Recirculating	2	1	Europe	System (Catfish v Tilapia)
Pérez-Urrestarazu, et al.	2019	Recirculating	4	1	Europe	System (raft v DWC media)
Pinho, et al.	2018	Recirculating	2	1	Brazil	System (Fish: Pacu v Tilapia)
Rafiee, et al.	2018	Recirculating	2	3	Malaysia	System (AP v AP complim)
Rayhan, et al.	2018	Recirculating	4	3	Bangladesh	System (Fish density)
Roosta	2014	Recirculating	2	3	Iran	Plant (K v no-K)
Roosta & Afsharipoor	2012	RAS irrigated	2	1	Iran	System (HP v AP)
Roosta & Hamidpour	2011	Recirculating	8	3	Iran	System (HP v AP)
Roosta & Mohsenian	2012	Recirculating	4	3	Iran	System (HP v AP)
Saha, et al.	2016	Recirculating	2	4	USA	System (HP v AP)
Sace, & Fitzsimmons	2013	Recirculating	2	1	USA	System (Prawn: present v absent)
Salam, et al.	2014	Recirculating	3	1	Bangladesh	Plant component (Substrates)
Saufie, et al.	2015	Recirculating	2	3	Malaysia	System (AP v HP)
Schmautz, et al.	2016	Recirculating	3	3	Europe	Plant Compon. (NFT v DWC v Drip)
Shete, et al.	2017	Recirculating	3	3	India	Plant Component (Rock v Raft)
Shete, et al.	2016	Recirculating	3	3	India	System (HRT)
Shete, et al.	2013	Recirculating	4	3	India	System (Fish density)
Silva, et al.	2018	Recirculating	2	1	Mexico	Plant Compon. (Raft v Dyn. RAFT)
Simeonidou, et al.	2012	Recirculating	3	1	Europe	System (Fish densities)
Sirakov	2020	Recirculating	2	1	Europe	Plant component (RAFT v Media)
Suhl, et al.	2018	Decoupled	2	1	Europe	System (AP v HP)
Suhl, et al.	2016	Decoupled	2	1	Europe	System (AP v HP)
Vandam, et al	2017	Recirculating	3	1	USA	System (Hydro - 2 v AP)
Velichkova, et al.	2019	Recirculating	2	1	Europe	Plant component (Raft v Media)
Weilgosz, et al.	2017	Recirculating	4	1	USA	System (HP v AP; Ster v Non-steril)
Wilson, et al	2017	Recirculating	2	1	USA	System (HP v AP)
Yang & Kim	2019	Recirculating	3	2	USA	System (HP v AP)
Yang, et al.	2020	Recirculating	3	2	USA	System (Flow rates)
Zou, et al.	2016	Recirculating	3	3	China	System/Solution (pH)

replication to their experimental designs; Europe – 71 %, USA – 80 %, South America, 63 %, Australia – 33 %, West Asia – 25 %, South East Asia (including China) – 20 % and South Asia – 14 %.

Table 2 (A&B): (A) Descriptive statistics (Total studies, total studies with Correct Replication, % Correct Replication, total studies with Incorrect Replication, % Incorrect replication) and (B) breakdown per region (Europe, USA, South America – includes Central America, Australia, West Asia, South East Asia – includes China, South Asia) for the identified aquaponic studies 2000 – 2020.

Parameter	Total	Correct Replication.	% Correct	Incorrect Replication.	% Incorrect
Number of studies identified	61	24	**39**	37	**61**
Fully Recirculating	52	23	**44**	29	**56**
Proper decoupled (fish present)	2	0	**0**	2	**100**
Irrigated RAS water (no fish)	7	1	**14**	6	**86**
System Comparisons	46	21	**46**	25	**54**
Solution Comparisons	7	0	**0**	7	**100**
Plant Component Comparisons	8	3	**37**	5	**63**
No. Incorrect Replications				37	
% Incorrect Replications				61	

Parameter	Europe	USA	Sth America	Australia	West Asia	SE Asia/China	South Asia
Number of studies identified	24	10	8	3	4	5	7
Fully Recirculating	17	9	8	3	3	5	7
Proper decoupled (fish present)	2	0	0	0	0	0	0
Irrigated RAS water (no fish)	5	1	0	0	1	0	0
System Comparisons	13	9	7	3	3	5	5
Solution Comparisons	7	1	0	0	0	0	0
Plant Component Comparisons	4	0	1	0	1	0	2
No. Incorrect Replications	17	8	5	1	1	1	1
% Incorrect Replications	**71**	**80**	**63**	**33**	**25**	**20**	**14**

Experimental Replication Decision Matrix:

Figure 1 outlines a Decision Matrix to enable the determination of valid replication for the devised experimental comparisons in an aquaponic context associated with a plant growth or production measured outcome being statistically compared. A difference in a plant production or growth parameter is the most often applied comparison to determine an assumed difference within an aquaponic system context (e.g. comparison of one aquaponic system to another – coupled v decoupled aquaponics, etc.) or via a comparison to an external, differentiated context (e.g. comparison of aquaponics to hydroponics).

The outlined Decision Matrix (Figure 1) will not be applicable to each and every experiment performed using aquaponic systems. However, in the context of using a plant measure outcome as the determinant of establishing a difference in treatments where aquaponic systems are being tested, it is expected it should account for most situations.

IV. DISCUSSION

Study Analysis and Descriptive Statistics

It must be noted that a specific search technique was applied to identify studies suitable for this analysis (see Materials and Methods section). While the author believes a relatively high proportion of aquaponic studies using a plant growth measure as the comparative determinant (or one of the comparative determinants) were identified and included, it is acknowledged there will be studies that were not identified or included here. Ayipio et al. (2019) performed a recent meta-analysis of crop

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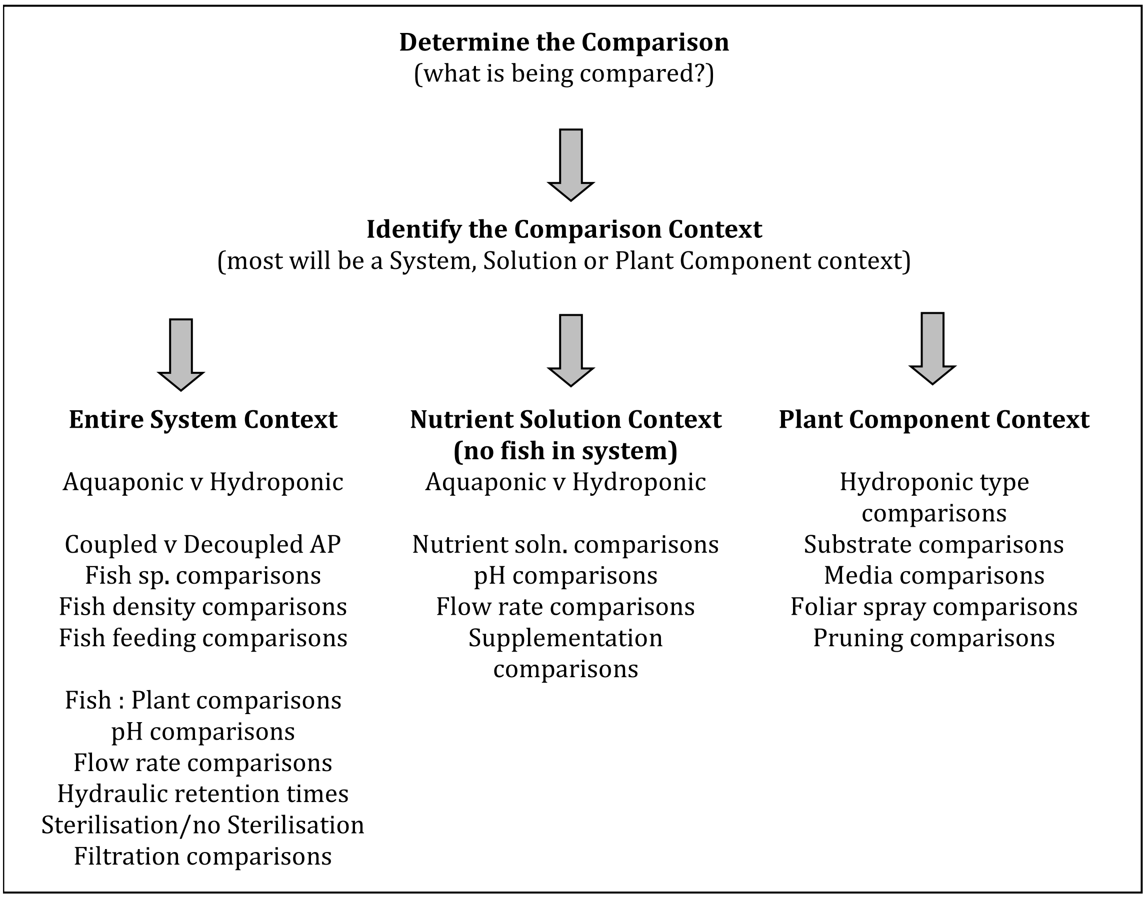

Fig.1: Decision matrix for determining required replication in an aquaponic experimental context.

yields in studies comparing conventional hydroponics to aquaponics and identified twenty-seven studies within a year range of 2009 to 2018, even though no publication date limitation was placed on the search range. However, the current study identified two studies that compared aquaponics to hydroponics (Lennard & Leonard, 2004; Lennard & Leonard, 2006) that were not identified and included by Ayipio et al. (2019); demonstrating that searches, no matter how directed the applied criteria, can miss relevant studies. Therefore, the sixty-one studies identified were considered representative of the studies using the stated search criteria for the time range (2000 to early 2019).

There have been a number of authors who have conducted comparative aquaponic experiments or trials in a number of countries across most continents (Table 1). While some authors claim that decoupled aquaponic system designs are important and the most appropriate to future commercial adoption of aquaponics (Delaide et al., 2016; Goddek et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Delaide et al., 2019; Goddek et al., 2019; Blanchard et al., 2020), only two out of the sixty-one (3 %) studies identified, applied true decoupled designs (i.e. a system with a direct hydraulic linkage from a fish component containing fish to the plant component), and only one study (2 %) directly compared a true decoupled aquaponic system with a coupled (or fully recirculating) aquaponic system (Monsees et al., 2017). Seven studies compared a plant growth measure of some form using nutrient solutions only (i.e. there was no direct hydraulic linkage from a fish component containing fish to the plant component), with several arguing this approach was a valid analogue for true aquaponics (Delaide et al., 2016; Goddek & Vermeulen, 2018; Nicoletto, et al., 2018). This demonstrates the decoupled approach only represented 15 % of the total identified studies. In addition, seven of the nine (78 %) decoupled or nutrient solution studies were located within Europe; suggesting that Europe is the epicentre of decoupled aquaponic design research.

The descriptive statistics (Table 2) illustrate several interesting points associated with aquaponic system research and experimentation. The vast majority of studies (fifty-two of sixty-one studies – 85 %) used a form of fully recirculating (coupled) aquaponic system and these studies applied replication correctly to the highest proportion (44 %). Only two studies used true decoupled aquaponic systems and none (0 %) of these studies applied correct replication. Of the seven studies that irrigated collected RAS water as a nutrient solution (i.e. no fish present in a direct hydraulic link to the plant component), only one (14 %) applied correct replication.

Overall, 61 % of all studies applied replication incorrectly or not at all. Hulbert (1984) points out that 48% of the ecological field studies he examined which applied inferential statistics (e.g. identification of significant differences) contained pseudo replication. Thorarensen et al. (2015) points out how little attention is given to experimental design in aquaculture fish growth experiments, especially in terms of the number of required rearing units for adequate treatment replication and Araujo (2008) argued there is a lack of appreciation of basic statistics in aquaculture experiments. Ruohonen (1998) also points out that a lack of tank replication in fish aquaculture experiments is common, leading to a lack of independent statistical outcomes or pseudoreplication. Raudonius (2017) found that almost 55% of the articles associated with crop research he examined applied experimental design and statistical analysis incorrectly and Kramer et al. (2016) found a similar result of almost 50% for crop studies. Therefore, the outcome of the current study is not unexpected, and while researchers of aquaponics apply statistical analysis to their work, it appears it is more likely than not that it is applied incorrectly.

The result of the current study for incorrect replication application (61 %) in aquaponic studies should be a major concern to the aquaponic research community as inferences are being made based on invalid experimental designs and statistical outcomes that do not support those inferences. To this end, determinant arguments are then being made based on these incorrect designs and analyses. Finally, these interpretations are being used to inform commercial applications of aquaponic technology.

Table 2 also outlines the relative frequency of the descriptive statistics calculated for aquaponics research by region. Europe performed the majority of research into comparative aquaponics with plant measure outcomes, accounting for twenty-four (39 %) of all the identified studies (Table 1). The USA (ten – 16 %) and South America (eight – 13 %) were next, with South Asia (Indian sub-continent), South East Asia (including China), West Asia (Iran, Iraq, Middle East) and Australia following. The most frequent experimenters (Europe, USA & South America) also demonstrated the highest incidence of incorrect or no application of replication in their studies (71, 80 & 63 % respectively). This demonstrates that the regions performing the most aquaponic-associated, comparative research, show the lowest application rate of correct replication within their experimental designs.

This trend of incorrect application of replication within aquaponic research is similar to what is seen within aquaculture and crop science fields, which also demonstrate trends towards pseudo replication, no replication and inappropriate statistical analysis (Hulbert, 1984; Ruohonen, 1998; Araujo, 2008; Thorarensen et al., 2015; Kramer et al., 2016; Raudonius, 2017). Precedent does exist within aquaponic research for early (Lennard & Leonard, 2004; Lennard & Leonard, 2006) and mid-twenty-first century (Roosta & Hamidpour, 2011; Roosta & Mohsenian, 2012; Shete, et al., 2013) laboratory, comparative aquaponic trials and experiments that applied correct experimental replication and for later work that referenced early articles for correct experimental design direction (Medina, et al., 2016). Therefore, it is difficult to understand why such a high rate of incorrect replication is still being applied in the field of aquaponics?

An example of industry informing inferences is demonstrated within the field of decoupled aquaponic systems (DAS). Arguments from researchers within this field are regularly made in terms of the inferred advantage of the decoupled aquaponic approach to plant production outcomes (Delaide et al., 2016; Goddek et al., 2016; Suhl et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019; Eck et al., 2019; Goddek et al., 2019; Blanchard et al., 2020). However, as the outcomes of the current research demonstrates, there was only one identified study that directly compared fully recirculating with decoupled aquaponics (with fish present in the direct hydraulic link between the fish and plant components) and that one study applied no replication (Monsees et al., 2017). The applied inference was that the decoupled approach performed better than the fully recirculating approach, as based on total fruit yields, but no mean results, no standard deviations or standard errors and no comparative statistical analyses were reported (Monsees et al., 2017). Of the seven articles that applied a RAS water or RAS water variation (e.g. complimented with additional nutrients) to a plant component, five argued that the decoupled design(s) they tested were equal to, or superior to, hydroponic controls or fully recirculating aquaponic analogues.

However, again, no replication was present in any of these studies and therefore, the inferred statistical differences observed were inappropriate and unreliable (Delaide et al., 2016; Suhl et al., 2016; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019). Therefore, are the arguments being made that decoupled aquaponic designs are better for commercial application than fully recirculating aquaponic designs (Delaide et al., 2016; Goddek et al., 2016; Suhl et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019; Eck et al., 2019; Goddek et al., 2019; Blanchard et al., 2020) supported by scientific data generated from correct experimental designs and appropriately applied, comparative statistical analyses, or a perception generated from theoretical arguments?

Experimental Replication Decision Matrix:

An Experimental Replication Decision Matrix was developed to try and direct future researchers towards the application of correct replication within overall experimental design. Determining the comparison that is being performed within the overall experimental design is of paramount importance and assists to provide a broad understanding of what is being tested and assists to identify the comparison context (Hulbert, 1984; Quin & Keough, 2002).

This is generally associated with the basic question: What is being compared?

Asking what is being compared in an overall experimental design context is not associated with identifying what parameters will be measured to determine any hypothesised differences (e.g. water chemistry – pH, D.O., EC, specific nutrient concentrations, etc.; plant growth or production – plant weight, plant length, leaf area, yield, etc.; fish growth – SGR, FCR, yield, etc.). It is about identifying what variable is being compared within the experimental design (e.g. aquaponic v hydroponic, fish species variations, fish density variations, fish feeding variations, fish to plant ratio variations, etc.).

Identifying the comparison context assists in determining the final experimental context which directly leads to what aspect or component of the experimental set-up requires the replication.

The important question is: Can what is being compared be compartmentalised as a sub-set of the entire culture system?

If it is impossible to compartmentalise what is being compared as a sub-set of the entire culture system (aquaponic or hydroponic; coupled or decoupled), then the experiment is occurring within an aquaponic system (or any other culture system) context and therefore, correct experimental design requires entire culture system replication.

Figure 1 shows there are relatively few comparisons where replication of only a sub-set of the entire system is valid, and these appear to be almost all related to comparisons associated with a plant component context (hydroponic component type comparisons – DWC, NFT, Media; plant growing substrate or media comparisons; direct plant foliar spray comparisons – nutrient, pesticides, etc.) or a direct plant mechanical treatment or similar comparison (pruning, leaf removal, growing tip excision, etc.). It is also important to understand that in these plant component context cases, replication of the component that provides the nutrient solution to the plants (either RAS/fish component of an aquaponic system, or sump/nutrient tank component of a irrigated nutrient solution) should not be applied, because this can then confuse which variable is actually effecting the plant growth outcome; the plant associated variable (e.g. media or substrate; hydroponic technology – NFT, DWC, media; foliar spray applied, etc.) or a fish component variable (fish, fish tank, filtration, etc.). Tlusty (2010) provides an analysis of an analogous situation that explains this difference in a RAS fish diet evaluation context where several different diets were fed to fish when replication was applied to the entire culture system (RAS) rather than the feed treatments (Arockiaraj & Applebaum, 2010). This study demonstrates that it was impossible to determine if it was the diet treatments or the individual filtration systems that caused the differences observed (Tlusty, 2010).

In most experimental situations, entire system context replication will be appropriate. This is because most aquaponic studies attempt to differentiate the aquaponic system(s) they are testing; differentiation of one aquaponic system from another (e.g. coupled v decoupled aquaponic designs) (e.g. Monsees et al., 2017) or differentiation of an aquaponic system from a different system type (e.g. aquaponic v hydroponic v soil) (e.g. Graber & Junge, 2009; Blidariu et al., 2013; Wilson et al., 2017; Jordan et al., 2018;). In these cases, at least three replicates of the entire aquaponic system and any control system (e.g. hydroponic system) would be required.

Some situations will involve using nutrient solutions (no fish; solutions arising from separated RAS, nutrient-complimented RAS solutions and controls), which does differentiate them from entire aquaponic system comparisons, but in general, entire system replication will also be appropriate in these cases because the nutrient solution is being compared (e.g. Delaide et al., 2016;

Goddek & Vermeulen, 2018; Delaide et al., 2019; Blanchard et al., 2020). Again, in these cases, at least three replicates of each treatment system would be required (i.e. three nutrient sumps connected to three, independent plant culture devices for each treatment).One of these example studies, that compared nutrient solutions (hydroponic control vs nutrient complimented RAS water), rather than a complete aquaponic system, acknowledged that the experimental design applied (only one replicate system per treatment) did not meet the requirements of valid replication (Goddek & Vermeulen, 2018).

For most aquaponic experiments (true aquaponic or RAS-derived nutrient solution variations), a good default experimental design is to adopt at least three replicates per treatment. A key point is that, avoidance of replicating entire aquaponic culture systems or units, as well as any control culture system (e.g. hydroponic system or unit), is not a good practice in an experimental design, replication or statistical analysis context for the majority of aquaponic comparative studies.

The real difference lies with experiments that isolate specific, plant-associated effects (e.g. hydroponic type comparisons – DWC v NFT v Media, etc.; plant media or substrate comparisons; direct plant foliar spray comparisons, etc.). In these cases, one aquaponic system may be acceptable (and may be required), but replication of the experimental component (usually the plant component in plant growth studies) will be required and in general, it is recommended that at least three replicates of the plant-holding component will be required (e.g. if comparing NFT v DWC, at least three NFT channels and at least three DWC beds will be required; if comparing plant-growing media in NFT aquaponic culture, at least three separate NFT channels will be required). Salam et al. (2014) compared several different plant growing media (gravel, crushed bricks & a mixture of saw dust and gravel) and used a common fish component to feed the plant growing beds containing the different media, suggesting a correct starting experimental design for the type of comparison being performed. However, only two replicates per treatment were applied and while two is the minimum number of replicates required by definition (Gupta et al., 2015), three or more replicates are usually recommended for statistically valid crop comparisons (Koller et al., 2016).

Example Studies Highlighting Replication Applications:

1. *Recirculating (Coupled) Aquaponics v Hydroponics – example **system** context comparisons:*

   Alcarraz et al., (2018) compared a standard, recirculating, deep flow (DWC or raft) hydroponic system to a recirculating (coupled), deep flow (DWC or raft) aquaponic system using rainbow trout (*Oncorhynchus mykiss*) juveniles (40 fish per replicate) that produced wastes used to provide nutrition to the plants. The experiment consisted of two treatments (hydroponic and aquaponic) and each treatment had three replicates. The replicates were complete system repetitions (i.e. three independent hydroponic systems and three independent aquaponic systems). The plants cultured were lettuce (*Lactuca sativa* L.) and each replicate contained thirty plants. The two culture systems were statistically compared to each other using ANOVA. This study was a good example of applying correct replication when comparing aquaponics to hydroponics. The culture system (hydroponic or aquaponic) was the variable being compared and therefore, replication of the entire culture system (or unit) was the valid approach. This meant that the statistical analysis applied to identify any difference between the two systems via the parameter compared (lettuce yield – $gfwm^{-2}$) was valid and reliable.

   Lennard & Ward, (2019) compared a number of lettuce varieties (*Lactuca sativa* L.) and herbs (dill - *Anethum graveolens* L., rocket - *Eruca sativa*, coriander - *Coriandrum sativum* L. and parsley – *Petroselinum crispum*) grown in a Nutrient Film Technique (NFT) hydroponic system and a NFT aquaponic system using Grass Carp (*Ctenopharyngodon idella*) to produce wastes used to provide nutrition to the plants. This study compared one semi-commercial-scale hydroponic system (1,800 plant spaces) to one semi-commercial-scale aquaponic system (1,800 plant spaces) and therefore, did not apply valid replication that would allow statistical comparison. The authors recognised the study was a crop production trial without replication of the test variable (i.e. the culture systems) and therefore, did not apply any statistical analysis and only reported mean outcomes with standard errors and percentage differences. However, they did argue the calculated percentage differences in plant yields could be used as a measure of difference and argued that in the majority of cases, the aquaponic treatment outperformed the hydroponic treatment. This study was performed in a greenhouse and would be considered a crop or system demonstration trial. In a trial context it is not valid to infer differences based on the parameters measured in the absence of replication, but trends may be highlighted (Koller et al., 2016). This study was an example of correct identification of the lack of replication and therefore, any statistical analysis would not have been valid. However, arguments that identified one culture systems superiority over the other, based on the observed percentage differences in plant yields, are worth scrutiny and not scientifically reliable (Koller et al., 2016).

2. *Aquaponic Solution v Hydroponic Solution (no fish) – example* ***solution*** *context comparisons:*

Nicoletto et al., (2018) compared three different nutrient solutions; water from an operating recirculating aquaponics system, the same aquaponic water complimented with phosphorous, potassium and a micro-nutrient mixture and a hydroponic nutrient solution control. Each treatment was replicated three times; therefore, a total of nine separate, independent culture systems or units were used. Each culture system consisted of a nutrient tank attached to four NFT channels, with a pump pumping the solution from the nutrient tank to the channels with a gravity return. The plants tested for growth parameters (plant height, yield, dry matter) were rocket (*Eruca vesicaria* R) and mizuna (*Brassica rapa* L. *spp. Nipposinica* M). None of the culture systems tested contained fish. This study was a good example of applying correct replication when comparing aquaponics nutrient solutions to a hydroponics nutrient solution. Even though fish were not present in any system, and even though only nutrient solutions were compared, the experiment was still comparing different culture systems (hydroponic and aquaponic) and therefore, replication of the entire system (unit) was the appropriate and valid approach. This meant that the statistical analysis applied to identify any difference between the two systems via the parameters compared was valid and reliable.

Goddek & Vermeulen, (2018) compared aquaculture (RAS) nutrient complimented water with a hydroponic nutrient solution in a similar experimental culture system set-up to Nicoletto et al., (2018) (nutrient solution tanks attached to NFT channel arrays). They used only one nutrient tank per treatment and therefore, did not apply any replication to the two treatments. They identified that replication was not present within the experimental design ("Hydrologically speaking, this approach, however, cannot be considered as a repetition."). However, despite the acknowledgement of the lack of replication, they still applied a statistical analysis (ANOVA) to the identified plant growth parameters measured (plant wet and dry weights) and used these statistical results to argue that one system (complimented RAS water) was better than the other (hydroponic nutrient solution). This study was an example of inappropriate statistics being applied in a situation with no replication (essentially, a trial) and using the identified statistical differences to infer the superiority of one approach over the other, was not a valid approach.

3. *Three Plant Growing Media Compared in One Aquaponic System – example* ***plant component*** *context*

Salam et al., (2014) compared three different plant growing media (gravel, crushed brick and a sawdust/gravel mixture) in an aquaponics system. The experimental design utilised a single fish tank attached to six separate plant grow beds (two beds for each different media), which therefore, realised two replicates per treatment tested. Because the "system" was not being compared or tested, but the plant growing media was, the use of a single fish tank feeding all the plant grow beds was appropriate, because this design removed the possibility of the fish tank or fish culture component being a variable, and concentrated any detected differences onto the media. Two replicates are considered the lowest number for valid replication (Gomez & Gomez, 1984; Gupta et al., 2015) and therefore, the replication applied in this study was theoretically acceptable. This study is an example of good experimental design and appropriate application of replication for aquaponic studies concentrating on plant-associated component variables. However, it is suggested that three replicates would be more robust.

V. CONCLUSION

It is concerning that aquaponic researchers are not applying correct replication to their experimental designs and then making inferences based on incorrectly applied statistical analyses and outcomes. It is more concerning that these studies are being published in peer reviewed journals, which are supposed to be providing a process to expertly review and ensure the validity and appropriateness of the experimental designs and statistics applied of the studies they publish. This suggests, that a lack of understanding of correct experimental design, correct application of replication and correct application of appropriate statistical analysis is far too common within the cohort of journals publishing aquaponic articles and among the associated reviewers assigned by these journals; a situation that is seen at similar levels within the associated disciplines of aquaculture (Hulbert, 1984; Ruohonen, 1998; Araujo, 2008; Thorarensen et al., 2015) and crop science (Kramer et al., 2016; Raudonius, 2017). An experimental replication decision matrix was developed, and several example articles discussed, in an attempt to assist future aquaponic researchers to determine the replication requirements of the aquaponic studies they design.

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