UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ESTIMAÇÃO DE PARÂMETROS GENÉTICOS E RESPOSTA À SELEÇÃO DE CARACTERÍSTICAS MORFOMÉTRICAS E DESEMPENHO EM TAMBAQUI (*Colossoma macropomum*)

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Dissertação apresentada, como parte das exigências para obtenção do título de MESTRE EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de concentração Produção Animal.

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TITULAÇÃO: Mestre em Zootecnia - Área de Concentração Produção Animal

APROVADO em 26 de fevereiro de 2019.

Prof. Dr. Ricardo Pereira Ribeiro

Prof. Dr. Elias Nunes Martins

Prof. Dr. Carlos Antonio Lopes de Oliveira
Orientador

“The highest reward for a person's toil is not what they get for it, but what they become by it.”

John Ruskin

Aos meus queridos pais, Albertino e Patrícia;
minha avó, dona Maria da Conceição e irmãos,
Yumi, Yann e Yuri, pelo amor, força, compreensão e
que antes de todos, tornaram esse sonho possível.

A minha querida namorada, pelo amor, paciência e por
me acompanhar com alma e corpo nessa jornada.

DEDICO.

AGRADECIMENTOS

A Deus, pela vida e por nunca desistir de mim, obrigado!

A meus queridos pais, Albertino e Patrícia, pelo todo o amor e dedicação, tanto comigo e com meus irmãos, sempre lutando para o nosso futuro, muito obrigado por tudo, amo vocês!

A minha avó, Maria da Conceição, que sempre acreditou e apoio meus sonhos.

A minha querida namorada, Nayara, que me apoia e está comigo em todos os desafios, por toda a paciência, porque reconheço que não é fácil, te amo sonsa!

Ao meu orientador e professor Dr. Carlos Antonio Lopes de Oliveira, vulgo Carlão, um ser humano incrível, obrigado pela dedicação, conselhos, conversas, puxões de orelha e amizade, muito obrigado por todos os ensinamentos em melhoramento genético, Deus e profissionalismo, de coração, muito obrigado!

Ao professor Dr. Ricardo Pereira Ribeiro, pelo suporte para o desenvolvimento deste trabalho e tanto outros desenvolvidos no grupo PeixeGen. Obrigado pela oportunidade de ingressar no grupo, pela confiança na coordenação, agredeço imensamente todo o aprendizado e desenvolvimento profissional conquistado, obrigado!

Aos meus queridos amigos e irmãos de pós-graduação, que levarei para o resto da minha vida, Filipe Chagas, Humberto Todesco, Jaísa Casetta, Karla Tsujii, Rodrigo

Feuerharmel, Laís Mantovani, Vanessa Lewandowski, Luiz Fernando, Pedro Castro, Jonathan Carvalho e Johnny Brito, obrigado galera!

Aos funcionários, Vitão, Cleiton e Zé Geraldo, obrigado pelos ensinamentos, tanto nos manejos e no cotidiano da Codapar.

Aos meus amigos e professores do GMAT/UFR, em especial, as professoras Dr.ª Denise Rocha Ayres e Dr.ª Annaiza Braga Bignardi Santana. Ao meu amigo Diogo Osmar Silva, agradeço as discussões sobre melhoramento genético.

Ao Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá, em especial a Solange Iung.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela bolsa concedida nesse período.

Ao Grupo Bom Futuro, por permitir o desenvolvimento deste estudo. Em especial, Hanner Karim, Renan Cucato e Angelo Bonifácio e seus colaboradores.

Por fim, a todos que contribuiram de forma direta ou indireta, tanto no cotidiano da Codapar, Rio do Corvo ou nas coletas de dados em Mato Grosso.

BIOGRAFIA

Eric Costa Campos nasceu em 19/10/1993 na cidade de Viçosa em Minas Gerais, filho de Albertino de Campos Neto e Patrícia Aparecida Costa.

Em outubro de 2016, concluiu o curso de bacharelado em Zootecnia, pela Universidade Federal de Mato Grosso, Campus Universitário de Rondonópolis.

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Em fevereiro de 2019, submeteu-se à banca para defesa da Dissertação de mestrado.

ÍNDICE

Página

RESUMO

O objetivo da realização deste trabalho foi estimar os parâmetros genéticos para características morfométricas e desempenho em um ciclo de produção de tambaqui. Foram utilizados animais da primeira geração do programa de melhoramento genético do Grupo Bom Futuro no Mato Grosso. O Banco de dados continha informações de 2349 animais, a identificação e seis meses de cultivo e 2115 animais aos doze meses de cultivo. Foi realizado teste de adequacidade e o modelo de melhor ajuste foi definido de acordo com o Critério de Akaike - AIC, que continham as combinações dos efeitos aleatórios genéticos aditivos e de ambiente comum de família, e os efeitos fixos do dia da biometria e das covariáveis idade à biometria e peso à identificação, com efeitos lineares ou quadráticos. As estimações dos parâmetros genéticos ocorreram por meio de procedimentos bayesianos, pelo software GIBBS1F90 (BLUPF90 *family programs*), utilizando o modelo animal. As herdabilidades são consideradas baixas (0,10 e 0,19) para as características medidas à identificação e altas para seis e doze meses de cultivo (0,61 a 0,81). O efeito de ambiente comum de família, apresentou redução de importância com a idade. Observou-se correlação genética favorável entre o desempenho e características morfométricas no período de cultivo (> 0,84) e entre períodos de cultivo (> 0,80), possibilitando que a predição dos valores genéticos seja antecipada em seis meses, resultando em ganhos genéticos equivalentes aos obtidos aos doze meses. Em conclusão, é possível a melhoria genética na espécie para desempenho, sem impactos negativos nas características morfométricas, com possíveis ganhos genéticos de 5% a 31% na próxima geração.

ABSTRACT

The objective of this work was to estimate the genetic parameters for morphometric characteristics and performance in a tambaqui production cycle. Animals from the first generation of the Bom Futuro Group breeding program in Mato Grosso were used. The database contained information about 2349 fish at identification and six months of cultivation and 2115 fish at twelve months of cultivation. A suitability test was performed and the best fit model was defined according to the Akaike Criterion -AIC, which contained combinations of the additive genetic and common family random effects, and the fixed effects of the biometry day and covariables age at biometrics and weight at identification, with linear or quadratic effects. The genetic parameters were estimated by Bayesian procedures, using the GIBBS1F90 software (BLUPF90 family programs), using the animal model. Heritabilities are considered low (0.10 and 0.19) for traits measured at identification and high for six and twelve months of cultivation (0.61 to 0.81). The effect of a common family environment showed a reduction of importance with increasing age. A favorable genetic correlation was observed between performance and morphometric traits in the cultivation period (> 0.84) and between periods of cultivation (> 0.80). This genetic correlation allowing the prediction of breeding values to be anticipated in six months, resulting in gains equivalent to those obtained at 12 months. In conclusion, genetic improvement in this specie is possible for performance, without negative impacts on the morphometric traits, with possible genetic gains of 5% to 31% in the next generation.

I- INTRODUÇÃO

1.1 Panorama da aquicultura internacional e nacional

A produção aquícola mundial registrada no ano de 2016, foi de 110,2 milhões de toneladas e movimentando um total de 243,5 bilhões de dólares. Apesar da recessão econômica em alguns países, a aquicultura continua sendo o setor que mais cresce diante de outros setores de produção de alimentos, a cerca de 5,8% durante o período de 2001 a 2016. Estima-se que em 12 anos, ocorra aumento considerável no consumo de pescado, ocasionando crescimento da atividade em nível mundial, particularmente na América Latina e Caribe, onde crescerá 49% (FAO, 2018).

A produção de peixes cultivados possui grande contribuição na produção de pescados total, totalizando 54,1 milhões de toneladas de pescado (US $ 138,5 bilhões) que representam 49%, e, com projeção de aumento por volta de 11% até 2030. Entre as espécies mais cultivadas, as carpas ocupam as três primeiras posições com 29% (carpa capim (11%), carpa prateada (10%) e carpa comum (8%), respectivamente), em seguida, tilápia do Nilo (8%) e na quinta posição, carpa cabeçuda com 7% (FAO, 2018).

No Brasil, segundo dados da Associação Brasileira de Piscicultura (PeixeBr, 2019) a produção de peixes provindos de cultivo, foi de 722,6 mil toneladas em 2018. O país sofreu uma forte recessão da economia nos últimos três anos, promovendo recuo de quase 8 % do Produtor Interno Bruto e milhões de desempregados, este cenário negativo não foi capaz de impedir o crescimento da piscicultura em 2018, com aumento de 5% em relação ao ano anterior. De forma semelhante ao cenário mundial, é esperado no Brasil, mudanças das políticas públicas na atividade piscícola e aumento do consumo

interno de peixes, que atualmente é 9,5 kg/hab/ano, que impulsionarão o crescimento nos próximos anos (PeixeBr, 2018).

A tilápia do Nilo é atualmente a espécie mais cultivada no Brasil, com 357,6 mil toneladas representa 55,4%, em seguida, o grupo das espécies nativas com 44,6 % da produção total (PeixeBr, 2019). No grupo dos nativos, o tambaqui representa 18,2 %; Tambacu e Tambatinga (8,7%) e carpas (3,9%), portanto, o tambaqui e seus híbridos representam 26,9%, ou seja, metade das espécies nativas cultivadas no país (IBGE, 2017).

1.2 Tambaqui – Colossoma macropomum

Pertencente à família *Characidae*, que agrupa, popularmente os “peixes redondos”, o tambaqui é nativo da bacia dos rios Amazonas e Orinoco, e, atinge cerca de 30 quilogramas e 1 metro de comprimento padrão em sua vida adulta (Araujo-Lima and Goulding, 1998; Araujo-Lima and Gomes, 2005). A carne de tambaqui é caracterizada como alimento de alta qualidade nutricional, de boa relação lipídica e capacidade de modulação do perfil de seus ácidos graxos (Paulino et al., 2018), associada a boa composição proteica, apresenta-se como referência na culinária amazônica e com ascendente destaque internacional (Santos, 2010; Mesquita, 2013).

O tambaqui é de suma importância para o cenário da aquicultura na América Latina e Caribe, no qual observou-se aumento de 85 % na produção entre 2000 a 2014, movimentando cerca de 313,5 milhões de dólares em 2014 (CEPAL et al., 2017). O elevado crescimento da produção nos últimos anos em países da América do Sul está associado as características produtivas da espécie, por exemplo a rusticidade, taxas de crescimento e conversão alimentar favoráveis e principalmente, adaptabilidade às condições de cultivo (Araujo-Lima and Goulding, 1998; Silva and Fujimoto, 2015; Corrêa et al., 2018).

As características reprodutivas da espécie, como a alta fecundidade (78 gramas de ovócitos por quilograma de peso vivo), fertilização externa, que garante a flexibilidade na definição de acasalamentos com a formação de famílias, além de diferentes cruzamentos (tambatinga e tambacu), facilitam o avanço do melhoramento genético (Turra et al., 2013). Porém, a qualidade dos alevinos disponíveis atualmente é questionável, devido ao excesso de

consanguinidade no plantel (Calcagnotto and Toledo-Filho, 2000; Araujo-Lima and Gomes, 2005) , desconhecimento da estrutura de parentesco e os valores genéticos aditivos para as características de interesse econômico dos animais utilizados como reprodutores, impedindo o fornecimento de alevinos com qualidade genética comprovada.

A primeira tentativa de melhoria genética na espécie aconteceu no Brasil, por meio do projeto “Bases tecnológicas para o desenvolvimento sustentável da aquicultura no Brasil – Aquabrasil”, desenvolvido pela Embrapa – Empresa Agropecuária Brasileira, no ano de 2009. Como o objetivo de atender às principais demandas da cadeia aquícola brasileira, especialmente na obtenção de alevinos de boa qualidade genética, o tambaqui foi uma das espécies escolhidas (Resende, 2009). O projeto foi finalizado e gerou o primeiro artigo científico de parâmetros genéticos da espécie, com resultados que indicaram que é possível a realização do melhoramento genético (Mello et al., 2016).

Recentemente, foi iniciado pela Embrapa um novo projeto para o fortalecimento da aquicultura no país, o “BRS Aqua”, considerado o maior investimento já realizado para o desenvolvimento do setor. O destaque do projeto em genética, é o fomento do banco de germoplasma de tambaqui geneticamente superior para características de interesse econômico, assim, disponibilizando informações científicas e tecnológicas para o desenvolvimento do setor produtivo (Embrapa, 2018).

1.3 Melhoramento genético de espécies aquícolas

O melhoramento genético em peixes, iniciou, empiricamente, há mais de 2000 anos, com os chineses observando as variações fenotípicas na coloração, e selecionando as cores de interesse na carpa comum (Alves et al., 2013). Apesar dos grandes avanços nos aspectos nutricionais e produtivos, atualmente o melhoramento genético é pouco difundido em nível mundial na piscicultura, mas, existem exemplos de sucesso, como na ciprinocultura (Dong et al., 2015); tilapicultura (Ponzoni et al., 2011; Oliveira et al., 2015) e salmonicultura (Gjedrem, 2005, 2012), em que estão presentes os principais programas de melhoramento.

A falta de conhecimento sobre genética quantitativa é uma das razões para que a reprodução seletiva na piscicultura esteja muito atrás das plantas e animais terrestres (Gjedrem et al., 2012). Estima-se que em 2010, aproximadamente, 8 % da produção aquícola mundial tenha se originado de estoques geneticamente melhorados (Gjedrem et al., 2012). Cenário oposto ocorre na Europa, onde a participação do mercado combinado das empresas de reprodução e produção, auxiliam para que, 80 – 83 % da produção do continente venha da reprodução seletiva (Janssen et al., 2017).

Estudar as características hereditárias e de variação quantitativa, é de grande importância para a piscicultura moderna, pois, a maior parte das características de importância econômica são controladas por vários genes e são fortemente influenciadas pelo ambiente (Eler, 2017). Portanto, faz-se necessário o delineamento adequado de um programa de melhoramento e avaliação genética a partir de metodologias estatísticas, para que os ganhos genéticos sejam satisfatórios nas gerações (Ribeiro and Legat, 2008; Ribeiro et al., 2012).

Na maioria dos programas de melhoramento genético de peixes a taxa de crescimento é o principal critério utilizado, a seleção para esta característica tem revelado resultados satisfatórios para as condições de cultivo atuais. Para a escolha acurada dos animais geneticamente superiores para uma determinada característica, é necessário estimativas precisas de parâmetros genéticos e associações genéticas, principalmente ao longo do tempo (Gjedrem and Baranski, 2009; Yoshida et al., 2013).

Prever com precisão as estimativas de variações genéticas e fenotípicas, herdabilidades e correlações fenotípicas e genéticas é necessário ter o registro de todas as informações do objetivo de seleção. Estas informações podem ser do próprio indivíduo e também, de irmãos completos ou meio irmãos, pais e progenitores, isto é possível, devido os parentes compartilharem alelos com o candidato a seleção (Gjedrem and Baranski, 2009).

A metodologia mais eficiente para estimar os valores genéticos é a das equações de modelos mistos, denominada "BLUP – *Best Linear Unbiased Prediction*", descrita por Henderson (1975). Esta ferramenta é eficiente em conjunto de informações de irmãos e progenitores completos e meios-irmãos, sendo frequentemente usada para predição de parâmetros genéticos em

espécies aquícolas (Gjedrem and Baranski, 2009). A metodologia que considera todas as relações genéticas entre animais, é conhecido como modelo animal, no qual, é descrito na forma matricial:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{e}$$

em que,

y, é o vetor de observações de características medidas nos indivíduos;

X, a matriz de incidência de efeitos fixos;

β, é o vetor de efeitos fixos;

Z, é a matriz de incidência de valores genéticos;

a, é o vetor de valores genéticos (aleatórios);

e, é o vetor de erros aleatórios;

Estimativas de herdabilidade de características de desempenho e morfométricas são descritas em diversas espécies com interesse comercial na aquicultura. Por exemplo, em tilápias do Nilo, que variam de 0,20 a 0,34 (Nguyen et al., 2010; Porto et al., 2015); carpas, 0,17 a 0,32 (Dong et al., 2015; Nguyen, 2016); salmões, 0,20 a 0,40 (Gjedrem, 2000; Leeds et al., 2016); pangasius, 0,19 a 0,34 (Sang et al., 2012); robalo branco, 0,31 a 0,60 (Saillant et al., 2006); linguado japonês, 0,12 a 0,80 (Liu et al., 2014; Li et al., 2018); bacalhau do Atlântico, 0,31 a 0,34 (Kristjánsson and Arnason, 2016); camarão gigante da Malásia, 0,05 a 0,22 (Luan et al., 2012); camarão branco do Pacífico, 0,24 a 0,46 (Zhang et al., 2017); camarão tigre gigante, 0,27 a 0,56 (Kenway et al., 2006; Krishna et al., 2011); vieira caribenha, 0,18 a 0,76 (Barros et al., 2018) e ostras do Pacífico, 0,15 a 0,33 (Li et al., 2011).

Os programas de melhoramento devem considerar as mudanças tanto nas características sob seleção quanto nas mudanças correlacionadas, principalmente em características de interesse econômico (Lopes, 2005). A correlação genética é devido ao pleiotropismo, ou seja, a capacidade da expressão de genes em duas ou mais características (Falconer and Mackay, 1996). Segundo Oliveira et al. (2016), ao estudar mudanças correlacionadas em cinco gerações em tilápia do Nilo, observou que a seleção para taxa de crescimento influenciou a morfologia dos peixes, deixando-os com aspecto oval.

A resposta à seleção pode ser utilizada para estimar o progresso genético em um programa de melhoramento, já que mede os possíveis ganhos genéticos a serem obtidos na próxima geração com o núcleo de seleção. Ganhos genéticos significativos para desempenho são verificados em tilápia do Nilo, com 10 a 15 % (Ponzoni et al., 2011; Oliveira et al., 2015); salmão do Atlântico, 12 a 15%; salmão prateado, 15 a 60%; truta arco íris, 10 a 13%; carpas, 27% (monocultivo) a 29,6 (policultivo); bangre americano, 13%; Goraz, 22%; camarões, 4,2 a 13%; ostras, 9,5 a 20% e vieiras, 16 % (Gjedrem and Baranski, 2009).

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II. Genetic parameters and response to selection of commercial interest traits in *Colossoma macropomum*

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Short title: Genetic Parameters of *Colossoma macropomum*

Abstract

The genetic information has not been used to reproduce *Colossoma macropomum* in Latin American countries. The objectives of this study were to estimate the (co) variance components of economic interes traits at three moments during the cultivation. The datas were collected in three moments: at the identification, at the six and twelve months of cultivation, in 2349 fish from a

commercial breeding program of *Colossoma macropomum* in Brazil. The components of (co) variance, heritabilities and genetic and phenotypic correlations were estimated using Bayesian inference from appropriate statistical models. Heritability estimates were low in magnitude for traits measured at identification (0.10 and 0.19) and moderate to high for traits of six and twelve months (0.23 to 0.81). The effects of the common family environment were higher in the identification period (higher than 73%) with rapid decrease at six months and nonexistent in the twelve months of cultivation. There was a favorable genetic association between the same period traits (0.84 to 0.99) and between cultivation periods (0.80 to 0.92), turnig possible that selection applied in body weight in the sixth month, could result in an increase in the traits from twelve months of cultivation. Genetic gains expected in the next generation are between 5% and 31% in morphometric traits and performance. The results indicate that is possible to develop genetically superior commercial lines of *C. macropomum*, with genetic gains equivalent to or higher than those observed in other aquatic species.

Keywords: genetic improvement, heritability, genetic correlation, tambaqui, cachama, black pacu

Implications

To know the genetic parameters and the structure of genetic and phenotypic correlations of commercial interes traits could help in breeding programs development as well as in the selection criterion definition of *Colossoma macropomum*, because selective breeding based on genetic values does not

occur in this species. The commercial interest traits are highly heritable and correlated, with sufficient genetic variability to allow the response to selection. The favorable genetic correlation between the cultivation periods will allow the fish selection using traits that expressed earlier, thus anticipating the genetic values prediction and, reducing the breeding program costs.

Introduction

Most of the advances in world aquaculture in recent decades are the result of genetically superior varieties development and use, associated with developments in other crop-related aspects such as nutrition and sanitation. For example, in *Salmonidae* (Gjedrem, 2005, 2012; Leeds et al., 2016); *Cyprinidae* (Gjerde et al., 2002; Hussain et al., 2002; Ninh et al., 2013; Dong et al., 2015) and *Oreochromis niloticus* (Nguyen et al., 2011; Ponzoni et al., 2011; Hamzah et al., 2014; Oliveira et al., 2015; Nguyen, 2016). However, selective breeding based on genetic values is still not practiced in *Colossoma macropomum*.

The *Colossoma macropomum* comprises the group of the main aquaculture species in Latin America and the Caribbean. Between years 2000 and 2014, production increased 85%, moving more than US $ 1 billion (CEPAL et al., 2017). Characteristics such as growth potential, high productivity and prolificacy (78 oocytes per gram of live weight) favor its cultivation expansion (Araujo-Lima and Goulding, 1998), being considered a promising fish in several Latin American countries (CEPAL et al., 2017).

In Brazil, *C. macropomum* occupies the second position in the ranking of the most cultivated fish, being the first of the native species group (IBGE, 2017). Due to the economic importance and the potential, one of the first efforts to

implement a breeding program occurred in 2009, through the "Aquabrasil" project, which the objective was to meet the main demands of the aquaculture production chain (Resende, 2009). Genetic parameters estimates for performance traits in *Colossoma macropomum* were described by Mello et al. (2016).

Knowledge of genetic parameters and the response to the selection of economic interest traits can provide important information regarding the selection criteria definition. Therefore, the objective of this study was the estimation of (co) variance components and the genetic correlations structure between performance and morphometric traits of *Colossoma macropomum* at three moments during cultivation.

Material and methods

All animal handling procedures were in accordance with ethical standards and were approved by the Animal Ethics Committee of the Maringá State University (protocolo - CEUA7619040418).

Animals and traits

The fish used in this study refer to the first generation from Bom Futuro Group breeding program of *Colossoma macropomum*, based in Cuiabá, Mato Grosso - Brazil. To form 53 families, 38 males and 31 females were used. The spawning occurred through the hormonal induction method (Filho and Weingartner, 2007). After the gamete extrusion management and fertilization, the eggs were transferred to artificial incubation in 200 liters incubators, with circulation and controlled water temperature. Finally, seven days after hatching,

the post-larvae were transferred to nurseries (2 m²) and kept until the individual identification being feed with 55% crude protein ration.

Individual identification occurred by inserting microchip PIT tags (Passive Integrated Transponder) on the left side of the loin after the fingerlings reached a mean of 9 grams. At this moment, the fish individual weight was measured (PEtag) in grams and total length (CTtag) in centimeters. After being identified, the individuals were transferred to nursery in a density of about two fish per square meter, receiving extruded feed according to the phases: juvenile I (45% crude protein, 10 - 250 grams) and juvenile II (25% crude protein, above 250 grams). The water quality parameters were monitored daily and the mean annual value of the temperature was 22 ºC; 6.5, hydrogenation potential and 3 mg/L of dissolved oxygen.

In the sixth month of cultivation, body weight (PE_6) in grams; loin width (LA_6) in millimeters; body height (AL_6) in centimeters; standard length (CP_6) in centimeters were measured. In the twelfth month of cultivation, body weight (PE_{12}) in grams; head length (CA_{12}) in centimeters; loin width (LA_{12}); body height (AL_{12}); standard length (CP_{12}) and trunk size (TR_{12}) in centimeters, as shown in figure 1 were also measured. The days for slaughter weight (DPA) trait was calculated as suggested by (Castro-Pereira et al., 2007), from the equation:

$$DPA = \frac{\mathbf{2000}\ \boldsymbol{grams}}{\left(\frac{\mathbf{PE12}\ -\ \mathbf{PEtag}}{\boldsymbol{Age}\ \mathbf{12} - \boldsymbol{Age\ tag}}\right)}$$

being: 2000 grams, weight at slaughter; PE_{12}, fish weight at 12 months; PE_{tag}, animal weight at the identification; Age_{12}, animal age at 12 cultivation months; Age_{tag}, animal age at identification.

Database

Analysis of consistency and descriptive statistics of the performance and morphometric traits were carried out at the identification, six and twelve months of cultivation (Table 1).

A total of 2349 fish were measured for all traits and 95 pedigree fish information (parents and grandparents known) were added to the relationship matrix. The identifiable environmental effects were, identification weight; cultivation period age; day of the biometry accomplishment, besides the direct additive genetic effects and common family environment.

Suitability of models

The AIREMLF90 program (Misztal et al., 2016) was used for the adequacy test and the best fit model was defined according to the Akaike information criterion - AIC (Akaike, 1974), in which the best model is the lowest value. The combinations of the models containing the random effects of direct additive genetic (GAD) and common family environment (FAM), the fixed effects of biometry day (DB) as classification were tested and covariates, and biometrics age (IDB) and identification weight (PE_{tag}) (except for PE_{tag} and CT_{tag}) with linear and/or quadratic effects. In each analyzes, it was considered a generalized linear mixed model that can be described as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e},$$

where **y** is the phenotype for the evaluated trait; **X**, **Z and W** correspond to the incidence matrices of fixed effects, genetic and common family environment, respectively; **β** is the fixed effects vector; **a** is the fish additive effect vector assuming that **a** ~ N(0,**G**σ_a^2), where **G** is the kinship matrix and σ_a^2 corresponds to the additive genetic variance; **c** is the common family environment effects vector, assuming that **c** ~ N(0,**I**σ_c^2), where **I** is an identity matrix and σ_c^2 is the variance of the common family environment effect; **e** is the residual effect vector, assuming that **e** ~ N(0,**I**σ_e^2), where **I** is an identity matrix and σ_e^2 is the variance of the residual effect.

Estimating the components of (co) variances and genetic parameters

Using the most appropriate model, the (co) variance components and genetic parameters were estimated in unitrait and bitrait analyzes (for genetic and phenotypic correlations estimation). Bayesian procedures were used, using the programs GIBBS1F90 and POSTGIBBSF90 of the BLUPF90 family (Misztal et al., 2016). The convergence diagnosis was verified by the CODA (Plummer et al., 2006) package implemented in the R programming language (R Development Core Team, 2011).

In the unitrait analyzes, Gibbs chains from 500,000 cycles were used, with a 10% of burn-in and thinning interval of 10 cycles. For the bicaracter analyzes, samples were taken every 20 cycles, after discarding 10% of 1,000,000 initial cycles.

Selection response

The response to direct (ΔG_x) and correlated (ΔG_{xy}) bayesian selection was calculated from Gibbs chains generated by the in unitrait and bitrait analyzes, where:

$$\Delta G_x = h^2_x \,.\, i_x \,.\, \sigma_{Px} \quad \textbf{and} \quad \Delta G_{xy} = r_{Gyx} \,.\, h_y \,.\, h_x \,.\, i_x \,.\, \sigma_{Py}$$

being: h^2, trait heritability x; h, heritability square root of the trait y and x; r_{Gyx}, genetic correlation between the traits y and x; σ_{Py}, phenotypic standard deviation of the trait y; i, selection intensity 1.76 (proportion 10% selected) in trait x.

Simulation of the sex effect

The sex effect for PE_{12} was simulated. Firstly, the phenotypes were classified in descending order and separated into two groups (heavier and lighter) using the median weight with boundary between the groups. In the heavier fish group (higher than the median), the female phenotype was randomly defined in proportions of 50%, 60%, 70%, 80%, 90% and 100%, and males, in a complementary manner. The male phenotype distribution in the second group (lighter) was similar to the previous group, being female in a complementary way.

Results

Suitability of models

The covariable age was significant only for PE_{tag} traits (linear and quadratic effect); PE_6 (linear effect); PE_{12} (linear and quadratic effect) and DPA (linear effect), for the morphometric traits the models containing this effect presented higher value of Akaike's information criterion. The covariates biometry day and weight at identification were significant for all traits (Table 2).

The effect of common family environment was significant in the traits measured at the identification, at six months, PE_6; AL_6; CP_6 and twelve months culture, CA_{12} (Table 2).

Genetic parameters

The estimated heritability for identification traits were lower than the six and twelve months of cultivation. Heritability estimates ranged from 0.19 to 0.73 for morphometric traits, while for body weight were observed higher values, ranged from 0.10 to 0.81, for DPA the heritability was 0.67 (Table 3).

The participation of the common family environment effect in the total variance was higher at identification (0.80 on mean), ranged from 0.06 to 0.10 at six months and was 0.05 for CA_{12}, according to table 3.

Estimates of PE_{12} and DPA genetic and phenotypic correlations with the morphometric traits in the identification period were not different from zero. When correlating PE_{12} and DPA traits with the morphometric ones at six and twelve months of cultivation, a strong genetic and phenotypic association was observed, with values higher than 0.86 and 0.64, respectively, and -0.99 and -0.94 for DPA (Table 4).

Selection response

The direct response to selection at twelve months of cultivation varied from 2.74% to 30.9% with absolute values described in table 5.

When selecting for PE_{12}, the expected correlated responses were 10% (TR_{12}); 5.16% (CA_{12}); 10.33% (LA_{12}); 9.11% (AL_{12}); 8.99% (CP_{12}) and -31.25% (DPA), according to the absolute values described in table 5.

The results indicated that the correlated selection responses performed on body weight at six months were not different from the direct responses obtained for each trait at twelve months of cultivation (table 5).

Simulation of the sex effect

The increase in the females percentage in the heavier fish group and a reduction in the lighter fish group, simulating the existing sexual dimorphism in *C. macropomum*, showed a functional relationship between the heritability and the increase of the female sex proportion. The best fit regression model was polynomial of second degree $(\mathrm{y} = 1.3974 - 0.0197\mathrm{x} + 0.0001\mathrm{x}^2)$, with coefficient of determination (R^2) 0.98 (Figure 2).

The superiority of the female phenotype to the male for each simulation was compared by the Tukey test at 5% significance (Tukey, 1949). The superiority verified for the 50% increase is 10%; 60% is 12%; 70% is 14%; 80% is 16%; 90% is 16% and 100% is 17%.

Discussion

Age of animal (linear or quadratic effect) is commonly used as a covariate to estimate genetic parameters in aquatic species (Leeds et al., 2016; Mello et al., 2016; Oliveira et al., 2016; Garcia et al., 2017; Barros et al., 2018). However, in our study, the age was not significant for several traits. The use of artificial reproduction allowed the reduction in the families formation interval, reducing the age differences between the families tested. This fact, associated with lower coefficient of variation traits, may have influenced the non-significance of the age effect for some traits.

The common family environment effect (c^2) in the two-cultivation period is small compared to those observed in *O. niloticus* (Khaw et al., 2012; Yoshida et al., 2013; Porto et al., 2015) and *C. macropomum* (Mello et al., 2016). While the participation of this effect on phenotypic variation was, on mean, 8% in the sixth month of cultivation, in the identification period it was 10 times higher. According to Nguyen et al. (2010), this effect is higher for growth traits during the early stage of development. Therefore, when transferred to the cultivation environment, this effect quickly decrease in importance and lost its significance at twelve months of cultivation.

The performance traits have favorable genetic correlations with the morphometric traits, similar to the other species (Choe and Yamazaki, 1998; Nguyen et al., 2010; Hung et al., 2013; Porto et al., 2015; Fu et al., 2016). According to Falconer and Mackay (1996), high genetic associations evidenced pleiotropism, so there are changes in the fish morphology when selecting for body weight (Trong et al., 2013; Oliveira et al., 2016). Fu et al. (2016) report

that indirect selection by length is more advantageous and precise, due to the smaller trait variation, which is not influenced by gonads or viscera weights.

The high heritabilities magnitude, in addition to the favorable genetic correlations between the traits, results in expected genetic gains of 15% in mean in the last cultivation period. The estimates of genetic gain per generation corroborate with other species of fish reported in the literature (Gjedrem, 2000, 2005; Gall and Bakar, 2002; Ponzoni et al., 2005; Gjedrem and Baranski, 2009; Rezk et al., 2009; Dong et al., 2015; Leeds et al., 2016).

The selection in PE_{tag} does not result in genetic gains in the morphometric characteristics at twelve months of cultivation. For body weight, there was a reduction of 0.31% and an increase of 4% in the growing period in the next generation, if the selection criterion was the weight in the identification period.

The selection applied in PE_6 could result in genetic gains with the same intensity if applied in PE_{12}. The anticipation of period definition to predict genetic values is acceptable since it is possible to reduce the maintenance costs of the selection nucleus in six months without interfering with the genetic prediction.

The heritability estimates found in the cultivation periods are higher than those reported in *Salmonidae*, 0.20-0.40 (Gjedrem, 2000; Leeds et al., 2016); 0.17-0.32 in *Cyprinidae* (Dong et al., 2015, Nguyen, 2016); 0.20-0.34 in *Oreochromis niloticus* (Nguyen et al., 2010; Porto et al., 2015) and for the same species with 12 and 24 months of age (Mello et al., 2016). High magnitude of heritability suggests high participation of inheritable genetic differences in the observed variation, pointing out that individual phenotypic selection could present a high response to selection.

The results obtained by Mello et al. (2016) reported lower absolute heritability values of weight, height and standard length at 12 months than at 24 months of age, indicating a possible decline with the individuas age. In studies with *O. niloticus* (Yoshida et al., 2013) and *Oncorhynchus mykiss* (Kause et al., 2003; Dupont-Nivet et al., 2010), the heritabilities reduction was verified as individuals became sexually mature. However, our results showed a decrease in heritability only for height and width traits.

In the period in which the traits were measured, it was not possible to identify the sex of the individuals. Several researchers have tended to control this effect in the genetic parameters estimation of *O. niloticus* (Garcia et al., 2017; Nguyen et al., 2017, Oliveira et al., 2005, Ponzoni et al., 2005, Porto et al. Rezk et al., 2009). According to Almeida et al. (2016), there is sexual dimorphism in *C. macropomum*, in which, females show slaughter weight (about 16%) bigger than males.

When comparing the effect of sex phenotype simulation on the database, it was observed heritabilityan reduction to the females percentage of 98.5% among the heaviest (critical point of x). The increase of 90% seems to be consistent with reality, because dimorphism (16%) and heritability (0.45) are similar to those described by Almeida et al. (2016) and Mello et al. (2016), respectively.

Our results indicated that the absence of sex effect in the statistical model may lead to overestimation of inheritable genetic differences, consequently, to the large numbers selection of females in the breeding stock for new generation breeding, if the selection is made with based on the fish phenotype.

It is important to emphasize to the researchers of this species, the need to develop a technique that is functional and can identify the individual sex in the first cultivation year, thus enabling the process of genetic improvement to occur in a simplified way.

However, these results and those described by Mello et al. (2016), it was observed that the share of inheritable genetic differences in the total variation is high and the impact of the selection may be even greater than the results presented, either in the selection nucleus or in the genetically superior lines formation.

Due to the high fecundity of the species, in which a female at the first maturation can produce almost 0.5 million oocytes (Araujo-Lima and Goulding, 1998), it is possible to use a small number of breeding herds intensively, increasing the selection intensity. Therefore, the results of the dissemination and use of genetically superior animals in the productive chain of this species may point to results not yet observed in aquatic species with only one generation of selection.

Acknowledgments

The authors are grateful to Bom Futuro Group for providing access to the database used in this study. This work was conducted during a scholarship supported by Program of Academic Excellence - PROEX at the Maringá State University. Financed by CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Brazil Education Ministry.

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Figures

Figure 1. Traits measured during cultivation periods

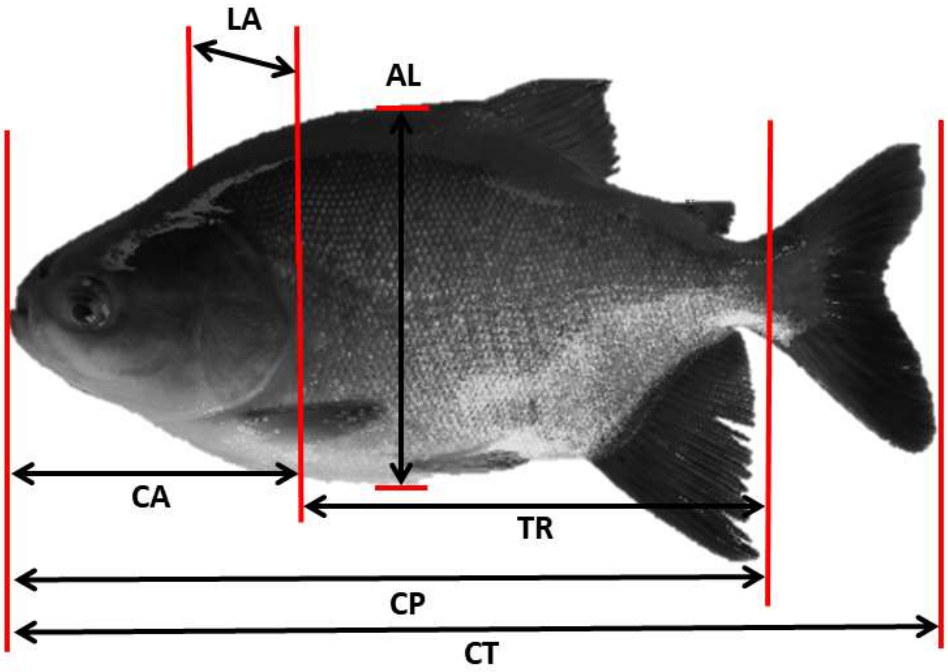
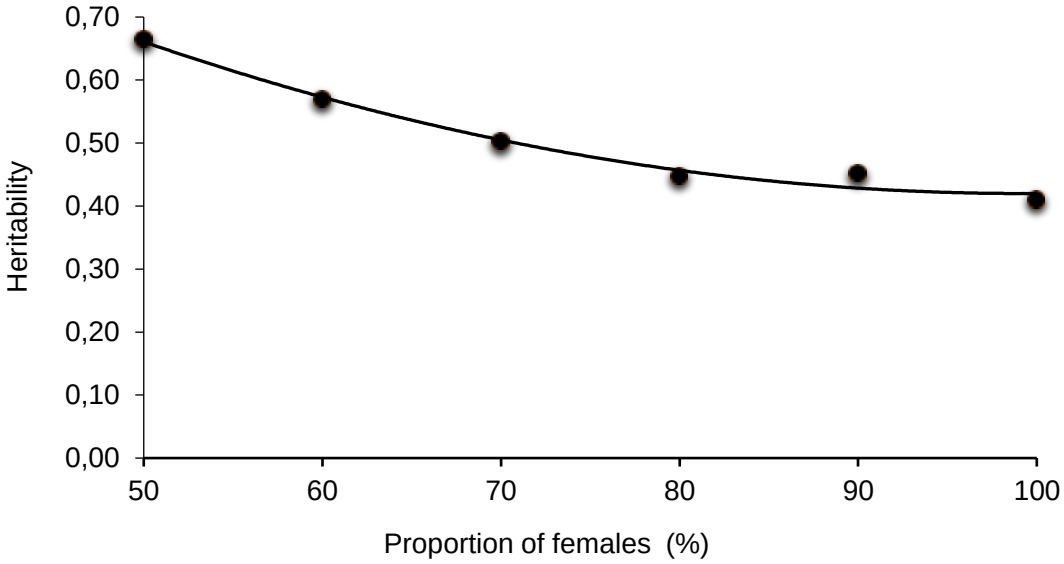


Figure 2. Behavior of the heritability after the females proportion increase inside the heavier fishes



Tables

Table 1. Descriptive statistics of the database used in the analyzes.

Traits	N	MIN	M	MAX	SD	CV (%)
PE_{tag} (g)	2349	2.0	9	61.5	6.95	77.54
CT_{tag} (cm)	2349	4.9	8.1	16.1	1.64	20.33
$Idade_{tag}$ (days)	5	121	159	168	12.89	8.03
PE_6 (g)	2349	74	359	740	89.93	25.04
LA_6 (mm)	2349	20	32.40	43	3.21	9.92
AL_6 (cm)	2349	6.50	10.50	13.80	0.91	8.68
CP_6 (cm)	2349	10.40	21.47	29.90	1.91	8.89
$Idade_6$ (days)	5	329	364	376	12.89	3.54
PE_{12} (g)	2115	404	1204	2324	253.05	21.02
TR_{12} (cm)	2115	12.9	22.7	28.6	1.77	7.79
CA_{12} (cm)	2115	5.3	9.1	11	0.70	7.74
LA_{12} (mm)	2115	36	51.9	94	4.52	8.72
AL_{12} (cm)	2115	11	15.7	20	1.14	7.29
CP_{12} (cm)	2115	21.4	31.8	38.2	2.14	6.72
$Idade_{12}$ (days)	5	567	602	614	12.81	2.13
DPA (days)	2115	384	768	2183	178.86	23.28

N, amount of information; MIN, minimum value; M, mean; MAX, maximum value; SD, standard deviation and CV, coefficient of variation

Table 2. Suitable models for each characteristic of the cultivation periods.

Traits	GAD	FAM	DB	IDB	IDB ²	PE_{tag}	PE_{tag} ²
PE_{tag} (g)	✓	✓	✓	✓	✓	-	-
CT_{tag} (cm)	✓	✓	✓	X	X	-	-
PE_6 (g)	✓	✓	✓	✓	X	✓	✓
LA_6 (mm)	✓	X	✓	X	X	✓	✓
AL_6 (cm)	✓	✓	✓	X	X	✓	✓
CP_6 (cm)	✓	✓	✓	X	X	✓	✓
PE_{12} (g)	✓	X	✓	✓	✓	✓	✓
TR_{12} (cm)	✓	X	✓	X	X	✓	✓
CA_{12} (cm)	✓	✓	✓	X	X	✓	✓
LA_{12} (mm)	✓	X	✓	X	X	✓	✓
AL_{12} (cm)	✓	X	✓	X	X	✓	✓
CP_{12} (cm)	✓	X	✓	X	X	✓	✓
DPA (days)	✓	X	-	✓	X	✓	✓

GAD, effects of direct additive genetic; FAM, common family environment; DB, biometry day of; IDB, age at biometrics linear; IDB², age at biometrics quadratic; PE_{tag}, weight at identification linear e PE_{tag}^2, weight at identification quadratic

Table 3. Estimates of genetic parameters, variances (additive genetics, family and residual) at the identification period, six and twelve months of cultivation.

Traits	h^2	c^2	σ^2_a	σ^2_c	σ^2_e
PE_{tag} (g)	0.10 (0.06) (0.01;0.22)	0.85 (0.04) (0.77;0.92)	6.088 (3.495) (0.004;10.970)	53.419 (12.105) (31.960;77.490)	2.761 (1.747) (0.171;5.741)
CT_{tag} (cm)	0.19 (0.11) (0.01;0.40)	0.73 (0.07) (0.58;0.85)	0.611 (0.323) (0.015;1.095)	2.386 (0.615) (1.283;3.602)	0.255 (0.161) (0.4E-03;0.530)
PE_6 (g)	0.71 (0.12) (0.37;0.96)	0.09 (0.06) (0.01;0.24)	6464 (1826) (3281;9886)	774 (580) (11.66;1921)	1718 (925) (17.19;3239)
LA_6 (mm)	0.73 (0.12) (0.52;0.97)	X	8.186 (2.045) (4.545;12.450)	X	2.804 (1.050) (0.574;4.689)
AL_6 (cm)	0.68 (0.15) (0.34;0.93)	0.06 (0.05) (0.004;0.20)	0.619 (0.181) (0.286;0.983)	0.057 (0.049) (0.003;0.19)	0.227 (0.092) (0.046;0.400)
CP_6 (cm)	0.61 (0.17) (0.21;0.91)	0.10 (0.07) (0.01;0.29)	2.510 (0.895) (0.756;4.242)	0.410 (0.306) (0.012;1.027)	1.121 (0.453) (0.272;2.042)
PE_{12} (g)	0.81 (0.12) (0.60;0.99)	X	56270 (11832) (35750; 79430)	X	12118 (6039) (19.64; 21670)
TR_{12} (cm)	0.68 (0.11) (0.48;0.91)	X	2.194 (0.547) (1.242; 3.324)	X	0.994 (0.283) (0.405; 1.497)
CA_{12} (cm)	0.23 (0.09) (0.07;0.43)	0.05 (0.03) (0.003;0.13)	0.093 (0.041) (0.019; 0.172)	0.018 (0.013) (0.001; 0.05)	0.284 (0.023) (0.238; 0.327)
LA_{12} (cm)	0.58 (0.10) (0.40;0.81)	X	12.071 (3.146) (6.623; 18.330)	X	8.387 (1.652) (5.099; 11.370)
AL_{12} (cm)	0.63 (0.11) (0.44;0.86)	X	0.837 (0.214) (0.463; 1.260)	X	0.477 (0.112) (0.254; 0.676)
CP_{12} (cm)	0.72 (0.11) (0.51;0.94)	X	3.357 (0.822) (1.878; 5.052)	X	1.300 (0.425) (0.413; 2.061)
DPA (days)	0.67 (0.11) (0.48;0.88)	X	22781 (5242) (13020; 33850)	X	10570 (2804) (4765; 15650)

h^2, heritability; c^2, importance of the common family environment effect; σ^2_a, additive genetic variance; σ^2_c, family variance; σ^2_R, residual variance; (SD), Standard deviation and (highest posterior density interval 95 %)

Table 4. Genetic (below diagonal) and phenotypic (above diagonal) correlation at the identification period, six and twelve months of cultivation

	P_{tag}	CT_{tag}	P_6	LA_6	AL_6	CP_6	P_{12}	TR_{12}	CA_{12}	LA_{12}	AL_{12}	CP_{12}	DPA
P_{ch}	1	0.95 (0.01) (0.92;0.97)	0.74 (0.14) (0.40;0.94)	-0.10 (0.28) ns (-0.44;0.28)	-0.99 (0.01) (-0.99;-0.98)	-0.99 (0.01) (-0.99;-0.98)	0.05 (0.07) ns (-0.64;0.18)	0.02 (0.07) ns (-0.08;0.18)	0.05 (0.20) ns (-0.35;0.45)	0.02 (0.07) ns (-0.08;0.16)	0.06 (0.07) ns (-0.05;0.19)	0.03 (0.07) ns (-0.07;0.19)	0.01 (0.06) ns (-0.14;0.10)
CT_{tag}	0.84 (0.25) (0.15;0.99)	1	-0.03 (0.10) ns (-0.29;0.24)	0.09 (0.63) ns (-0.10;0.25)	0.97 (0.02) (-0.92;-0.99)	0.98 (0.01) (0.97;0.99)	-0.33 (0.53) ns (-0.11;0.01)	0.02 (0.07) ns (-0.10;0.19)	0.06 (0.13) ns (-0.21;0.30)	-0.07 (0.06) ns (-0.16;0.10)	0.04 (0.06) ns (-0.12;0.11)	0.02 (0.07) ns (-0.09;0.19)	0.02 (0.06) ns (-0.13;0.12)
P_6	-0.32 (0.64) ns (-0.96;0.99)	-0.73 (0.30) ns (-0.99;0.20)	1	0.91 (0.01) (0.90;0.92)	0.94 (0.01) (0.93;0.95)	0.93 (0.01) (0.91;0.94)	0.78 (0.01) (0.73;0.82)	0.70 (0.01) (0.64;0.75)	0.54 (0.04) (0.47;0.61)	0.67 (0.03) (0.61;0.73)	0.70 (0.03) (0.64;0.75)	0.74 (0.03) (0.68;0.79)	-0.73 (0.02) (-0.77;-0.68)
LA_6	-0.29 (0.74) ns (-0.99;0.93)	0.17 (0.63) ns (-0.99;0.99)	0.99 (0.01) (0.98;0.99)	1	0.88 (0.01) (0.86;0.90)	0.85 (0.02) (0.82;0.88)	0.73 (0.02) (0.68;0.77)	0.64 (0.03) (0.60;0.72)	0.50 (0.04) (0.43;0.57)	0.65 (0.03) (0.59;0.71)	0.66 (0.03) (0.60;0.72)	0.70 (0.03) (0.64;0.91)	-0.70 (0.03) (-0.75;-0.64)
AL_6	-0.98 (0.03) (-0.99;-0.91)	-0.10 (0.53) ns (-0.98;0.87)	0.99 (0.01) (0.97;0.99)	0.99 (0.01) (0.98;0.99)	1	0.89 (0.01) (0.86;0.92)	0.75 (0.03) (0.70;0.79)	0.67 (0.07) (0.60;0.73)	0.54 (0.04) (0.48;0.62)	0.66 (0.03) (0.59;0.71)	0.71 (0.03) (0.66;0.76)	0.71 (0.03) (0.65;0.76)	-0.71 (0.06) (-0.77;-0.48)
CP_6	-0.98 (0.05) (-0.99;-0.90)	0.18 (0.55) ns (-0.97;0.99)	0.99 (0.01) (0.97;0.99)	0.98 (0.02) (0.95;0.99)	0.97 (0.02) (0.90;0.99)	1	0.74 (0.02) (0.70;0.80)	0.71 (0.03) (0.65;0.76)	0.55 (0.04) (0.47;0.63)	0.63 (0.03) (0.56;0.69)	0.65 (0.03) (0.58;0.71)	0.75 (0.02) (0.69;0.79)	-0.71 (0.06) (-0.74;-0.46)
P_{12}	-0.03 (0.62) ns (-0.97;0.95)	-0.57 (0.34) ns (-0.99;0.20)	0.91 (0.01) (0.80;0.99)	0.86 (0.04) (0.76;0.92)	0.88 (0.06) (0.76;0.97)	0.92 (0.05) (0.80;0.99)	1	0.89 (0.01) (0.87;0.91)	0.64 (0.02) (0.60;0.69)	0.85 (0.01) (0.82;0.87)	0.90 (0.01) (0.88;0.91)	0.93 (0.01) (0.92;0.94)	-0.94 (0.01) (-0.96;-0.94)
TR_{12}	-0.11 (0.61) ns (-0.97;0.99)	-0.23 (0.47) ns (-0.99;0.79)	0.84 (0.01) (0.68;0.94)	0.80 (0.06) (0.65;0.90)	0.80 (0.07) (0.63;0.92)	0.89 (0.05) (0.77;0.98)	0.96 (0.01) (0.93;0.98)	1	0.47 (0.04) (0.40;0.54)	0.74 (0.02) (0.69;0.78)	0.78 (0.02) (0.74;0.82)	0.97 (0.01) (0.96;0.97)	-0.95 (0.01) (-0.89;-0.85)
CA_{12}	-0.17 (0.62) ns (-0.98;0.99)	-0.53 (0.46) ns (-0.99;0.68)	0.80 (0.02) (0.40;0.99)	0.90 (0.06) (0.75;0.99)	0.87 (0.10) (0.63;0.99)	0.82 (0.14) (0.47;0.99)	0.97 (0.02) (0.89;0.99)	0.90 (0.06) (0.75;0.99)	1	0.57 (0.03) (0.51;0.63)	0.66 (0.02) (0.61;0.71)	0.68 (0.02) (0.63;0.72)	-0.65 (0.02) (-0.70;-0.61)
LA_{12}	-0.12 (0.61) ns (-0.95;0.99)	-0.68 (0.35) ns (-0.99;0.34)	0.87 (0.08) (0.69;0.99)	0.84 (0.06) (0.70;0.92)	0.89 (0.07) (0.72;0.99)	0.88 (0.10) (0.65;0.99)	0.96 (0.02) (0.92;0.98)	0.85 (0.05) (0.74;0.93)	0.98 (0.02) (0.92;0.99)	1	0.79 (0.02) (0.76;0.83)	0.77 (0.02) (0.74;0.81)	-0.82 (0.02) (-0.85;-0.79)
AL_{12}	0.14 (0.61) ns (-0.90;0.99)	-0.65 (0.35) ns (-0.99;0.33)	0.87 (0.07) (0.69;0.91)	0.83 (0.06) (0.69;0.91)	0.90 (0.06) (0.76;0.99)	0.88 (0.08) (0.69;0.99)	0.97 (0.01) (0.94;0.99)	0.87 (0.04) (0.76;0.93)	0.99 (0.02) (0.94;0.99)	0.96 (0.02) (0.92;0.98)	1	0.84 (0.01) (0.81;0.87)	-0.88 (0.01) (-0.90;-0.85)
CP_{12}	-0.07 (0.62) ns (-0.96;0.99)	-0.24 (0.47) ns (-0.99;0.76)	0.86 (0.06) (0.71;0.99)	0.83 (0.05) (0.70;0.91)	0.84 (0.06) (0.69;0.94)	0.91 (0.05) (0.80;0.99)	0.98 (0.01) (0.95;0.99)	0.99 (0.01) (0.98;0.99)	0.93 (0.04) (0.83;0.99)	0.89 (0.04) (0.80;0.94)	0.91 (0.03) (0.83;0.95)	1	-0.92 (0.01) (-0.93;-0.90)
DPA	0.41 (0.51) ns (-0.91;0.99)	0.44 (0.41) ns (-0.54;0.98)	-0.90 (0.05) (-0.98;-0.78)	-0.86 (0.05) (-0.93;-0.74)	-0.86 (0.06) (-0.95;-0.69)	-0.90 (0.07) (-0.99;-0.69)	-0.99 (0.01) (-0.99;-0.98)	-0.94 (0.02) (-0.97;-0.89)	-0.95 (0.04) (-0.99;-0.86)	-0.92 (0.03) (-0.96;-0.85)	-0.94 (0.01) (-0.97;-0.89)	-0.96 (0.01) (-0.98;-0.93)	1

ns, Non-significant, (SD), Standard deviation e (highest posterior density interval 95 %)

Table 5. Response to direct and correlated distribution

	Selection criteria	PE_{12}	TR_{12}	CA_{12}	LA_2	AL_{12}	CP_{12}	DPA
Direct		$372.45^{(63)}$ (342;404)	$2.13^{(0.09)}$ (1.98;2.34)	$0.25^{(0.01)}$ (0.27; 0.24)	$4.61^{(0.18)}$ (4.31;5.02)	$1.27^{(0.05)}$ (1.18;1.39)	$2.73^{(0.12)}$ (2.52;3.00)	$-215^{(9)}$ (-234; -200)
Correlated	PE_{tag}	$-3.78^{(0.16)}$ (-4.15; -3.51)	$-0.09^{(0.0003)}$ (-0.10; -0.08)	$-0.03^{(0.001)}$ (-0.03; -0.02)	$-0.23^{(0.01)}$ (-0.24; -0.21)	$0.07^{(0.002)}$ (0.06;0.07)	$-0.07^{(0.002)}$ (-0.07; -0.06)	$34^{(1)}$ (32; 36)
	PE_6	$325.80^{(26)}$ (269;369)	$1.86^{(0.19)}$ (1.45;2.18)	$0.37^{(0.08)}$ (0.18;0.46)	$4.52^{(0.49)}$ (3.46;5.33)	$1.19^{(0.12)}$ (0.93;1.40)	$2.37^{(0.22)}$ (1.90;2.75)	$-203^{(16)}$ (-232; -168)
	PE_{12}	$372.45^{(63)}$ (342;404)	$2.27^{(0.11)}$ (2.06;2.48)	$0.47^{(0.02)}$ (0.43;0.51)	$5.36^{(0.24)}$ (4.87;5.80)	$1.43^{(0.06)}$ (1.30;1.54)	$2.86^{(0.13)}$ (2.61;3.09)	$-240^{(9)}$ (-257; -221)

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Authors must write maximum 100 words explaining the implications of their work. Implications explain the expected importance or economic, environmental and/or social impact. This must be in simple English suitable for non science readers. The Implications will be peer-reviewed [Arial 12 regular, style 'ANM main text'].

Introduction [Arial 12 bold, style 'ANM heading 1']

Text [Arial 12 regular, style 'ANM main text'].

Material and methods [Arial 12 bold, style 'ANM heading 1']

Text [Arial 12 regular, style 'ANM main text'].

Subheading [Arial 12 italic, style 'ANM heading 2']

Text [Arial 12 regular, style 'ANM main text'].

Sub-subheading [Arial 12 italic, style 'ANM heading 3']. Text [Arial 12 regular, style 'ANM main text'].

Results [Arial 12 bold, style 'ANM heading 1']

Text [Arial 12 regular, style 'ANM main text'].

Subheading [Arial 12 italic, style 'ANM heading 2']

Text [Arial 12 regular, style 'ANM main text'].

Sub-subheading [Arial 12 italic, style 'ANM heading 3']. Text [Arial 12 regular, style 'ANM main text'].

Discussion [Arial 12 bold, style 'ANM heading 1']

Text [Arial 12 regular, style 'ANM main text']. The discussion section may be combined with the results section, but it is discouraged.

Subheading [Arial 12 italic, style 'ANM heading 2']

Text [Arial 12 regular, style 'ANM main text'].

Sub-subheading [Arial 12 italic, style 'ANM heading 3']. Text [Arial 12 regular, style 'ANM main text'].

Acknowledgements [Arial 12 bold, style 'ANM heading 1']

Text [Arial 12 regular, style 'ANM main text'].

References [Arial 12 bold, style 'ANM heading 1']

Author A and Author D Year. Chapter title. In book title (eds. AB Editor and CD Editor), pp. first page-last page numbers. Publisher name, place of publication (i.e. city, state (if applicable) and country).

Author A, Author B, Author CD and Author E Year. Title of article. Full Name of the Journal Volume number, first page-last page numbers.

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Table 1 [Arial 12 bold, style 'ANM heading 1'] *Effect of x and y on z in w; tables should be sufficiently detailed for being understood without any reference to the text [Arial 12 italic, style 'ANM Table title]*

	Heading [Arial 11 regular, style 'ANM Tab Spanner]						
	Heading[1]		Heading				
Heading	ColH1[2]	ColH2	ColH3	ColH4	ColH5	RMSE	*P*-value
Row heading (units)							
Row subheading							
Row sub-subheading	Value	Value	Value	Value	Value	Value	Value
Row sub-subheading	Value	Value	Value	Value	Value	Value	Value
Row subheading							
Row sub-subheading	Value	Value	Value	Value	Value	Value	Value
Row sub-subheading	Value[a]	Value[ab]	Value[bc]	Value[c]	Value	Value	Value
Row heading[3]	Value	Value	Value	Value	Value	Value	Value
Row heading	Value	Value	Value	Value	Value	Value	Value
Row heading	Value	Value	Value	Value	Value	Value	Value

ColH1 = Column heading 1; ColH2 = Column heading 2; ColH3 = Column heading 3; ColH4 = Column heading 4; ColH5 = Column heading 5

[1] Footnote explaining heading.

[2] Footnote explaining column heading 1.

[3] Footnote explaining row heading.

[a,b] Values within a row with different superscripts differ significantly at $P<0.05$.

Figure captions [Arial 12 bold, style 'ANM heading 1']

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Figure 2 [Arial 12 bold, style 'ANM heading 1'] This is the caption of figure 2 [Arial 12 regular, style 'ANM main text'].

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