

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Escola de Veterinária
Pós-graduação em Zootecnia

Vinícius Monteiro Bezerra

**CARACTERIZAÇÃO E IMPACTO REPRODUTIVO DA DISMORFIA DA
PAPILA UROGENITAL DE TILÁPIAS DO NILO**

Belo Horizonte
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**CARACTERIZAÇÃO E IMPACTO REPRODUTIVO DA DISMORFIA DA
PAPILA UROGENITAL DE TILÁPIAS DO NILO**

Tese apresentada ao Programa de Pós-Graduação em
Zootecnia da Escola de Veterinária da Universidade
Federal de Minas Gerais como requisito parcial para
obtenção do grau de Doutor em Zootecnia.

Área de Concentração: Produção Animal

Orientador: Prof. Dr. Edgar de Alencar Teixeira

Coorientadores: Dra. Érika Ramos de Alvarenga

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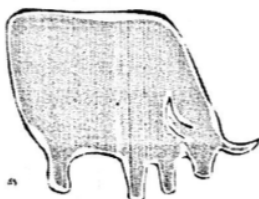
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ATA DE DEFESA DE TESE DO ALUNO VINICIUS MONTEIRO BEZERRA

Às 09:00 horas do dia 22 de março de 2024, reuniu-se, a Comissão Examinadora de Tese, aprovada em reunião ordinária no dia 12/02/2024, para julgar, em exame final, a defesa da tese intitulada:
Caracterização e impacto reprodutivo da distorção da
papila uregênital de tilápias do Nilo

_____, como requisito final para a obtenção do Grau de Doutor em Zootecnia, área de concentração Produção animal.

Abrindo a sessão, o Presidente da Comissão, Prof. Edgar de Alencar Teixeira, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Prof.(a)/Dr.(a) <u>Edgar de Alencar Teixeira</u>	☒	☐
Prof.(a)/Dr.(a) <u>Érika Romar de Oliveira</u>	☒	☐
Prof.(a)/Dr.(a) <u>Yves Moreira Ribeiro</u>	☒	☐
Prof.(a)/Dr.(a) <u>FRANCISCO REZENDE WZ</u>	☒	☐
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Pelas indicações, o (a) candidato (a) foi considerado (a): ☒ Aprovado (a)

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Para concluir o Doutorado, o(a) candidato(a) deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 22 de março de 2024.

Assinatura dos membros da banca:

Yves Moreira Ribeiro
Érika Romar de Oliveira
FRANCISCO REZENDE WZ

Edgar de Alencar Teixeira

Dedico este trabalho à infinita curiosidade
que todo pesquisador possui. Que ela
sempre nos acompanhe.

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“It has long been an axiom of mine that the little things are infinitely the most important.” — Sir Arthur Conan Doyle, As
memórias de Sherlock Holmes

RESUMO

A tilápia do Nilo é amplamente produzida em sistemas convencionais devido à sua rusticidade e adaptabilidade a diversas condições de qualidade da água, tornando-se a terceira maior espécie produzida pela aquacultura mundial. A escolha de reprodutores eficientes e a determinação de fatores que impactam negativamente no desempenho reprodutivo devem ser mais investigadas para garantir alta produtividade na tilapicultura. Além disso, problemas reprodutivos em tilápias do Nilo são pouco estudados e seu impacto na cadeia produtiva não é explorado. Este trabalho investigou detalhadamente uma má formação reprodutiva identificada em papilas urogenitais de fêmeas de tilápia do Nilo. Primeiramente, a alteração anato-morfológica foi classificada e foram descritos os diferentes graus de acometimento, sua progressão, associação com distúrbios ovarianos e impacto na eficiência reprodutiva em fêmeas portadoras da anomalia comparadas as não portadoras. Em um segundo momento, foram explorados os possíveis fatores causais, a prevalência da má formação em diferentes condições de temperatura e de linhagens genéticas, impactos dessas diferentes condições nos índices biológicos, nos perfis hormonais, e na eficiência reprodutiva. No primeiro experimento, 96 fêmeas normais e 96 fêmeas anormais foram divididas em três tanques/grupo. As fêmeas anormais foram usadas para criar um padrão de classificação da dismorfia na papila urogenital. Para determinar o impacto da dismorfia na eficiência reprodutiva, foi delineado um ensaio reprodutivo dividido em três ciclos de três semanas, com novos grupos de reprodutoras normais e anormais a cada semana. Os machos usados no ensaio tinham papilas urogenitais normais. Todas as fêmeas e machos foram chipados. Durante os ensaios, foram monitoradas a qualidade da água e variáveis reprodutivas. Após os ensaios, os animais foram eutanasiados e os pesos do corpo, vísceras, gônadas e fígado foram obtidos para cálculo dos índices biológicos. Fragmentos de cérebro, rim, baço e gônadas de fêmeas anormais foram inoculados em ágar sangue e Macconkey para avaliação bacteriológica. Os resultados indicaram que fêmeas com anormalidade na papila urogenital apresentaram menor desempenho reprodutivo. A má formação na papila urogenital foi associada a alterações ovarianas consideráveis e nos índices biológicos. Portanto, a morfologia dessa estrutura reprodutiva externa pode ser usada como critério de seleção para reprodutoras de tilápia do Nilo. Esse desempenho reprodutivo reduzido não foi associado a uma doença bacteriana, então outras causas foram investigadas. No segundo experimento, foram exploradas as possíveis causas, como os efeitos genéticos e

ambientais (temperatura e agente bacteriano) na prevalência da dismorfia da papila urogenital. Foram definidas três amplitudes de temperatura para investigação: 25°C-28°C (Controle), 27°C-30°C (TM) e 30°C-33°C (TA). Os efeitos genéticos foram avaliados com duas linhagens de tilápias: uma melhorada para peso corporal (G8) durante 7 gerações de seleção e outra não melhorada geneticamente (G0). O grupo controle foi realizado apenas com a linhagem melhorada devido à limitação de tanques. Os animais foram submetidos às temperaturas por 60 dias após a absorção do saco vitelínico, depois cultivados em tanques comuns e chipados para acompanhamento individual. Após atingirem a idade adulta, determinou-se a proporção de fêmeas com anomalia da papila urogenital em cada tratamento e sua capacidade reprodutiva. Temperaturas mais elevadas durante os 60 dias após a absorção do saco vitelínico resultaram em uma maior prevalência de fêmeas de tilápias do Nilo portadoras da anomalia, com consequente decréscimo na eficiência reprodutiva e produção de larvas desses grupos. Fêmeas com as alterações na papila urogenital também apresentaram alterações consideráveis em seus ovários, nos índices biológicos e nos perfis hormonais. Animais G8 apresentaram uma relação linear entre o aumento da temperatura e a prevalência da dismorfia da papila urogenital. Entre 30°C e 33°C, 90% das fêmeas G8 possuíam anomalia, contra 54% de prevalência na linhagem G0. Em conclusão, a inspeção da papila urogenital pode contribuir para a seleção de reprodutoras na tilapicultura e a condução da larvicultura nos primeiros 60 dias iniciais em temperaturas próximas a 25°C previne a ocorrência de anomalia da papila urogenital. Por fim, como as faixas de temperatura investigadas não foram extremas, os resultados ilustram como o aquecimento global e as mudanças climáticas podem afetar negativamente a produção de tilápia.

Palavras-chave: *Oreochromis niloticus*; disfunção reprodutiva; reprodutores eficientes; ovário; produção de alevinos; aquecimento global; dismorfia da papila urogenital

ABSTRACT

Nile tilapia is widely produced in conventional systems due to its rusticity and adaptability to various water quality conditions, making it the third largest species produced by aquaculture worldwide. Selecting efficient breeders and identifying factors that negatively impact reproductive performance should be further investigated to ensure high productivity in tilapia farming. Additionally, reproductive problems in Nile tilapia are poorly studied, and their impact on the production chain is not explored. This study investigated in detail a reproductive dysmorphia identified in the urogenital papillae of female Nile tilapia. First, the anatomical-morphological alteration was classified, and the different degrees of involvement, its progression, association with ovarian disorders, and impact on reproductive efficiency in females with the dysmorphia were compared to those without it. In the second step, possible causal factors were explored, as well as the prevalence of the anatomical dysmorphia under different temperature conditions and genetic lineages, and the impact of these conditions on biological indices, hormonal profiles, and reproductive efficiency. In the first experiment, 96 normal females and 96 abnormal females were divided into three tanks per group. The abnormal females were used to create a classification standard for urogenital papilla dysmorphia. To determine the impact of dysmorphia on reproductive efficiency, a reproductive trial was designed and divided into three three-week cycles, with new groups of normal and abnormal females each week. The males used in the trial had normal urogenital papillae. All females and males were chipped. During the trials, water quality and reproductive variables were monitored. After the trials, the animals were euthanized, and the weights of the body, viscera, gonads, and liver were obtained to calculate the biological indices. Fragments of brain, kidney, spleen, and gonads from abnormal females were inoculated on blood and MacConkey agar for bacteriological evaluation. The results indicated that females with abnormal urogenital papilla showed lower reproductive performance. Dysmorphia of the urogenital papilla was associated with significant ovarian alterations and biological indices. Therefore, the morphology of this external reproductive structure can be used as a selection criterion for Nile tilapia broodstock. This reduced reproductive performance was not associated with bacterial disease, so other causes were investigated. In the second experiment, possible causes, such as genetic and environmental effects (temperature and bacterial agents) on the prevalence of urogenital papilla dysmorphia, were explored. Three temperature ranges were defined for investigation: 25°C-28°C (Control), 27°C-

30°C (MT), and 30°C-33°C (HT). Genetic effects were evaluated with two tilapia strains: one improved body weight (G8) over seven generations of selection and another genetically unimproved (G0). The control group was created only with improved strain due to tank limitations. The animals were subjected to the same temperatures for 60 days after absorption of the yolk sac, then maintained in common tanks and chipped for individual monitoring. After reaching adulthood, the proportion of females with a urogenital papilla anomaly in each treatment and their reproductive capacity were determined. Higher temperatures during the 60 days after absorption of the yolk sac resulted in a higher prevalence of female Nile tilapia with the anomaly, with a consequent decrease in reproductive efficiency and larval production in these groups. Females with alterations in the urogenital papilla also presented significant changes in their ovaries, biological indices, and hormonal profiles. G8 animals showed a linear relationship between increasing temperature and the prevalence of urogenital papilla dysmorphia. Between 30°C and 33°C, 90% of G8 females had the anomaly, compared to 54% prevalence in the G0 lineage. In conclusion, inspection of the urogenital papilla can contribute to the selection of breeders in tilapia farming, and conducting larviculture in the first 60 days at temperatures close to 25°C prevents the occurrence of urogenital papilla dysmorphia. Finally, since the temperature ranges investigated were not extreme, the results illustrate how global warming and climate change can negatively affect tilapia production.

Keywords: *Oreochromis niloticus*, reproductive dysfunction, efficient broodstock, ovary, seed production, global warming, urogenital papilla dysmorphia

LISTA DE FIGURAS

CAPÍTULO 2

Figura 1: Esquema do processo neuroendócrino regulatório do eixo pineal-hipotálamo-hipófise-gônadas em peixes.....	31
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CAPÍTULO 3

Figure 1: Experimental design to compare the reproductive performance of Nile tilapia (<i>Oreochromis niloticus</i>) females with normal or abnormal urogenital papilla.....	49
Figure 2: Comparison of Nile tilapias (<i>Oreochromis niloticus</i>) females with urogenital papilla considered normal (A, A1) and abnormal (B, B1 and C, C1).....	56
Figure 3: Macroscopic classification of the ovaries.....	57
Figure 4: The distribution of normal ovary and ovary with low or high severity lesions between Nile tilapia (<i>Oreochromis niloticus</i>) with normal and abnormal urogenital papilla.....	59
Figure 5: Proportion of effective reproduction (females that effectively produced larvae divided by the total number of females in reproduction tanks) (top) and number of larvae produced per tank (bottom) of Nile tilapia (<i>Oreochromis niloticus</i>) females with normal and abnormal urogenital during three different reproduction cycles	60
Figure 6: Larvae number produced per female of Nile tilapia (<i>Oreochromis niloticus</i>), considering only females that produced larvae.....	61
Figure S1: Viscerosomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI) and viscerosomatic fat index (VFI) of Nile tilapia (<i>Oreochromis niloticus</i>) females with normal and abnormal urogenital papilla.....	65

CAPÍTULO 4

Figure 1: The complete experiment design was divided in five phases.....	80
Figure 2: Distribution of mean temperature from the first to the 60th day of Nile tilapia after yolk sac absorption.....	87
Figure 3: Urogenital papilla of Nile tilapia classified as normal. Anus (green arrow), oviduct exit (red arrow) and urinary pore (black arrow) are easily identified.....	90
Figure 4: Urogenital papilla of Nile tilapia classified as mild (abnormal grade 1) involvement.....	91
Figure 5: Description of Nile tilapia with urogenital papilla classified as severe involvement (abnormal grade 2).....	93

Figure 6: New alterations in urogenital papilla observed in anormal female Nile tilapia.....	94
Figure 7: Differentiation of ovaries classified as normal, mild (abnormal grade 1) and severe (abnormal grade 2) involvement caused by urogenital papilla dysmorphia.....	94
Figure 8: Prevalence of urogenital papilla dysmorphia of two distinct populations, the base generation (G0) and generation 8 (G8) from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption)	95
Figure 9: Biological indexes of female Nile tilapia of two distinct generations, the base generation (G0) and generation 8 (G8) of from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).....	100
Figure 10: Hormonal dosage of plasma estradiol and 11-ketotestosterone of female Nile tilapia of two distinct populations, the base generation (G0) and generation 8 (G8) of from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).....	101

LISTA DE TABELAS

CAPÍTULO 3

Table S1: Mean, standard deviation (SD), median, minimum (Min) and maximum (Max) of water quality variables in stocking tanks* of Nile tilapia (<i>Oreochromis niloticus</i>) during three reproductive cycles.....	54
Table S2: Mean, standard deviation (SD), median, minimum (Min) and maximum (Max) of water quality variables in reproduction tanks of Nile tilapia (<i>Oreochromis niloticus</i>) with normal and abnormal urogenital papilla during three reproductive cycles.....	55
Table 1: Results of fitting the logistic regression model to effective reproduction (proportion of females that effectively generated larvae) in reproduction tanks of Nile tilapia (<i>Oreochromis niloticus</i>) with normal and abnormal urogenital papilla during three reproductive cycles.....	63
Table 2: Results of fitting quasipoisson generalized linear model for number of larvae produced per tank of Nile tilapia (<i>Oreochromis niloticus</i>) with normal and abnormal urogenital papilla during three reproductive cycles.....	64

CAPÍTULO 4

Table S1: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in culture tanks of Nile tilapia (<i>Oreochromis niloticus</i>) from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).....	88
Table S2: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in BFT system stocking tanks of females and male of Nile tilapia (<i>Oreochromis niloticus</i>) reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).....	89
Table S3: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in the clear water reproduction tanks of Nile tilapia (<i>Oreochromis niloticus</i>).....	89
Table 1: Results of fitting the logistic regression model to effective reproduction (proportion of females that effectively generated larvae) of Nile tilapia from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared on different temperature ranges (25°C – 28°C: control, 27°C – 30°C: moderate temperature, and 30°C – 33°C: high temperature) during the first 60 days after yolk sac absorption.....	97
Table 2: Results of fitting quasipoisson generalized linear model for number of larvae produced per female of Nile tilapia from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared on different temperature ranges (25°C – 28°C: control, 27°C – 30°C: moderate temperature, and 30°C – 33°C: high temperature) during the first 60 days after yolk sac absorption.....	98

LISTA DE ABREVIATURAS

11-KT - *11- ketotestosterone*

ANOVA - *Analysis of Variance*

APHA - *American Public Health Association*

AQUACEN - Laboratório Central Oficial

AQUA-VET - Laboratório de Doenças de Animais Aquáticos da Escola de Veterinária

BFT - *Biofloc Technology*

BW - *Body Weight*

C:N ratio - *Carbon:Nitrogen ratio*

CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CGPs - Células Germinativas Primordiais

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

DHP - *Dihydroprogesterone*

DNA - *Deoxyribonucleic Acid*

DO - *Dissolved Oxygen*

E2 - *Estradiol*

FAO - Organização das Nações Unidas para a Alimentação e a Agricultura

FAPEMIG - Fundação de Amparo à Pesquisa do Estado de Minas Gerais

FSH - Hormônio folículo-estimulante

GnRH - Hormônio liberador de gonadotrofinas

GSI - *Gonadosomatic Index*

GW - *Gonad Weight*

HHG - Hipotálamo-Hipófise-Gônadas

HHT - Hipotálamo-Hipófise-Tireoide

HSI - *Hepatosomatic Index*

IC - *Confidence Interval*

ICB - Instituto de Ciências Biológicas

LAQUA - Laboratório de Aquacultura

LH - Hormônio luteinizante

LW - *Liver Weight*

Max - *Maximum*

Min - *Minimum*

N - *Number*

NGT-Aqua - *Nutrition, Genetics and Technology for Aquaculture research group*
OR - *Odds Ratio*
PEIXE BR - *Anuário Brasileiro da Piscicultura*
PIT - *Passive Integrated Transponder tags*
SD - *Standart Deviation*
SNK - *Student–Newman–Keuls test*
SS - *Settleable Solids*
T - *Testosterone*
T3 - *Hormônio triiodotironina*
T4 - *Hormônio tiroxina*
TAN - *Total Ammonia Nitrogen*
TRH - *Hormônio liberador de tireotropina*
TSAB - *Tryptic Soy Blood Agar*
TSH - *Hormônio tireotrofina*
UFMG - *Universidade Federal de Minas Gerais*
UNESCO - *United Nations Educational, Scientific and Cultural Organization*
VFI - *Viscerosomatic Fat Index*
VFW - *Viscerosomatic Fat Weight*
VSI - *Viscerosomatic Index*
Vtg - *Vitellogenin*
WOAH - *World Organisation for Animal Health*

Sumário

1 APRESENTAÇÃO DA TESE	20
1.1 Introdução geral.....	20
1.2 Objetivos da tese.....	23
1.2.1 Objetivo Geral	23
1.2.2 Objetivos Específicos.....	23
Referências bibliográficas.....	24
2 REVISÃO DE LITERATURA: DA EMBRIOLOGIA À FISIOLOGIA REPRODUTIVA E DISFUNÇÕES REPRODUTIVAS EM PEIXES	25
2.1 Introdução.....	25
2.2 Tilapicultura	25
2.3 Embriologia e importância na tilapicultura	27
2.4 Diferenciação sexual de tilápia	28
2.5 Fisiologia da reprodução	30
2.6 Disfunções reprodutivas	33
Referências bibliográficas.....	37
3 DECREASE OF REPRODUCTIVE PERFORMANCE OF NILE TILAPIA (<i>OREOCHROMIS NILOTICUS</i>) FEMALES WITH DYSMORPHIA IN UROGENITAL PAPILLA	43
3.1 Abstract.....	45
3.2 Introduction	46
3.3 Material and Methods.....	47
3.3.1 Location.....	47
3.3.2 Breeder stocking and fish conditions.....	47
3.3.3 Reproductive assay.....	48
3.3.4 Water quality.....	50
3.3.5 Biological indexes	51
3.3.6 Bacteriological analysis.....	51
3.3.7 Statistical analysis	51
3.4 Results	53
3.4.1 Water quality.....	53
3.4.2 Anatomical description of Nile tilapia with abnormal urogenital papilla.....	56
3.4.3 Reproductive performance.....	59
3.4.4 Biological Indexes.....	65
3.4.5 Bacteriological analysis.....	65
3.5 Discussion.....	66
3.6 Conclusion.....	69
3.7 Acknowledgements.....	69

3.8 Data Availability Statement	69
3.9 Conflict of interest.....	69
References	70
4 A LONG-TERM HIGH TEMPERATURE ON YOUNG NILE TILAPIA FEMALES AFFECTS ITS UROGENITAL PAPILLA MORPHOLOGY AND FUTURE REPRODUCTIVE PERFORMANCE.....	74
4.1 Abstract.....	75
4.2 Introduction	76
4.3 Material and Methods.....	77
4.3.1 Experimental design and fish management during gonadal differentiation	77
4.3.2 Fish management after experimental treatments.....	81
4.3.3 Sexing and evaluation of urogenital papilla.....	81
4.3.4 Impact of temperature treatments on reproductive performance.....	82
4.3.5 Bacteriological analysis.....	84
4.3.6 Hormonal dosages of 17-beta-estradiol and 11-ketotestosterone.....	84
4.3.7 Statistical analysis	85
4.4 Results	86
4.4.1 Heat treatments application and water quality parameters.....	86
4.4.2 Urogenital papilla dysmorphia classification and anatomic ovarian changes associated	90
4.4.3 Prevalence of urogenital papilla dysmorphia and ovaries commitment in females of Nile tilapia	95
4.4.4 Bacteriological analysis.....	96
4.4.5 Reproductive performance.....	96
4.4.6 Biological indexes	99
4.4.7 Hormonal dosages of 17-beta-estradiol and 11-ketotestosterone.....	101
4.5 Discussion.....	102
4.6 Conclusion.....	108
4.7 Acknowledgements.....	108
4.8 Data Availability Statement	108
4.9 Conflict of interest.....	108
References	109
5 CONCLUSÃO GERAL	114
APÊNDICE – PRODUÇÃO RESULTANTE DA TESE	116
Anomalia na estrutura reprodutiva externa de tilápias do Nilo: causas e impactos na produção de alevinos.....	116
Referências bibliográficas.....	127

1 APRESENTAÇÃO DA TESE

1.1 Introdução geral

A tilápia do Nilo (*Oreochromis niloticus*) é a terceira espécie de peixe de água doce mais produzida pela piscicultura mundial, alcançando um total de 4,41 milhões de toneladas de peso vivo de tilápias produzidas em 2020, segundo último relatório publicado pela Organização das Nações Unidas para a Alimentação e a Agricultura (FAO, 2022), correspondendo a 9% da produção mundial naquele ano. O Brasil foi o quarto maior país produtor da espécie em 2023, e foram produzidas 579.080 toneladas de peso vivo de tilápias, correspondendo a 65,3% da produção nacional de piscicultura, segundo a Associação Brasileira da Piscicultura em seu Anuário Brasileiro da Piscicultura (PEIXE BR, 2024).

Apesar da produção mundial expressiva em 2020, a tilapicultura mundial ainda não alcançou a produção das espécies que encabeçam o setor, como a Carpa capim (*Ctenopharyngodon idellus*) (5,80 milhões de toneladas de peso vivo) e Carpa prateada (*Hypophthalmichthys molitrix*) (4,90 milhões de toneladas de peso vivo) (FAO, 2022), mostrando o potencial de mercado que a tilapicultura pode conquistar. A dificuldade de atingir este potencial se deve principalmente pela produção inconstante de alevinos, decorrentes de características reprodutivas da espécie, como reprodução em ciclos assíncronos e recorrentes, desova também assíncrona, baixa fecundidade relativa e incubação oral dos embriões pela fêmea (Bhujel, 2000; Little et al., 1993; Ng e Romano, 2013; Azevedo et al., 2021). O cuidado parental pós eclosão das larvas, também aumenta o intervalo entre ciclos reprodutivos e diminui a produção total de alevinos por reprodutora na criação (Bhujel, 2000). O manejo dos reprodutores também influencia na eficiência reprodutiva e a manutenção de parâmetros de qualidade de água ideais é imprescindível para uma reprodução adequada, principalmente a temperatura, cujos níveis acima ou abaixo da faixa ideal podem influenciar diretamente a reprodução e a produção de larvas (Alvarenga e França, 2009; Bhujel, 2000; El-Sayed, 2019). Além de manter condições ideais de qualidade de água para os reprodutores, a eficiência do plantel pode ser otimizada pela determinação de indicadores que permitam a predição da capacidade reprodutiva dos animais. Tais indicadores poderão permitir a seleção de reprodutores adequados e mais eficientes.

Existem poucos relatos a respeito de patologias reprodutivas em peixes, sendo as mais comuns neoplasias e alterações secundárias à reações inflamatórias (Roberts, 2012). A identificação de alterações patológicas ou anatômicas, que sejam de origem infecciosa ou ambiental, mas que a influenciem a reprodução é necessária para que o mercado continue a garantir segurança sanitária, possa implementar práticas de manejo preventivo adequado e atinja um crescimento competitivo na produção nacional e mundial. A escassez de informações sobre alterações patológicas ou anatômicas pode ser atribuída mais a ausência de regulamentação normativa para acompanhamento e controle sanitário de organismos aquáticos (Santos et al., 2023), do que a sua não ocorrência. As alterações ou anormalidades anatômicas reprodutivas são ainda menos exploradas, com dificuldade de especificar suas causas e possíveis impactos produtivos na tilapicultura, especialmente se afetarem órgãos importantes para a reprodução, como a papila urogenital dos peixes (Jensen e Shelton, 1979; Alcántar-Vázquez, et al., 2014; Alcántar-Vázquez, et al., 2015; Khoo, 2019; Nozu e Nakamura, 2020).

A papila urogenital é um órgão gênito-urinário que participa da liberação de urina e gametas no ambiente para fecundação. Nas tilápias, existe dismorfia sexual na papila urogenital, permitindo a diferenciação dos animais em machos e fêmeas (Baroiller e D'Cotta, 2018). Nos machos de tilápias há apenas um orifício em sua papila urogenital, com ducto comum para a liberação de urina e sêmen. Nas tilápias fêmeas, a papila urogenital possui dois orifícios: o poro urinário, com formato de ponto e o poro genital, com formato de vírgula. Anomalias anatômicas na papila urogenital de fêmeas de tilápias no Brasil já foram observadas em criações comerciais e laboratórios de diagnóstico (pelo prof. Dr. Eduardo Maldonado Turra, LAQUA/UFMG e prof. Dr. Carlos Augusto Gomes Leal, AQUACEN/UFMG em comunicação pessoal), entretanto, não encontramos na literatura estudos científicos a respeito dessa alteração, seus impactos na eficiência reprodutiva e suas possíveis causas em tilápias do Nilo.

O diagnóstico e determinação do impacto reprodutivo deste tipo de anomalia é necessário para que reprodutores sejam corretamente selecionados e manejados, pois são os indivíduos que permanecem por mais tempo nas fazendas aquícolas e responsáveis pela produção dos alevinos (Bhujel, 2000; El-Sayed, 2019). Animais com subfertilidade, problemas reprodutivos ou doenças que afetam a reprodução devem ser identificados o quanto antes e as causas de problemas reprodutivos devem ser investigadas. Diante disso, é crucial identificar fatores que influenciam a eficiência reprodutiva na tilapicultura (El-Sayed, 2019).

Esta tese foi idealizada após a identificação de fêmeas de tilápias do Nilo portadoras de anomalias (má-formações anatômicas) em suas papilas urogenitais com causas desconhecidas, até então. Porém com efeitos negativos para a reprodução. A observação de anormalidades ocorreu em alguns animais nascidos dentro do programa de melhoramento genético do Núcleo NGTAqua, alocado no biotério LAQUA da Escola de Veterinária da Universidade Federal de Minas Gerais. Porém, relatos da ocorrência de anomalia semelhante em produções comerciais de tilápias, sem parentesco com o material genético do grupo NGTAqua, instigaram a realização deste estudo com a expectativa de identificar, classificar e investigar esta anomalia da papila urogenital dessa espécie, compreendendo sua origem e seus impactos anátomo-fisiológicos e reprodutivos.

Assim, a pesquisa se dividiu em duas investigações científicas, em um primeiro momento, a pesquisa teve como objetivo identificar macroscopicamente e classificar as malformações na papila urogenital de fêmeas de tilápias do Nilo, avaliar suas implicações na eficiência reprodutiva e investigar possíveis causas infecciosas associadas. Ao final da primeira fase do projeto foi publicado, em caráter inédito, o primeiro artigo com a descrição desta anomalia anatômica da papila urogenital em fêmeas de tilápia do Nilo e suas consequências reprodutivas, não sendo identificados organismos patogênicos associados. Em um segundo momento, foram avaliados fatores possivelmente envolvidos com a prevalência da anomalia da papila urogenital. Para tal, investigou-se uma possível associação entre diferentes faixas de temperatura na água de cultivo durante a larvicultura e dois materiais genéticos diferentes (um submetido ao processo de melhoramento genético para peso corporal e outro não submetido a esse processo). Foram determinados então, a prevalência da anomalia da papila urogenital, dosagens hormonais e índices biológicos para avaliar as fêmeas e desempenho reprodutivo dos animais dos diferentes tratamentos de temperatura e grupos genéticos.

1.2 Objetivos da tese

1.2.1 Objetivo Geral

Estabelecer parâmetros de identificação da dismorfia da papila urogenital de fêmeas de tilápias do Nilo, avaliar suas apresentações macroscópicas na papila e em ovários, e comparar seus impactos reprodutivos em relação a fêmeas normais.

Explorar a possível influência da temperatura de cultivo na prevalência de animais portadores da dismorfia, avaliando em um estudo ao longo do tempo as consequências na reprodução, em seu perfil hormonal, índices biológicos, investigando ainda se houve envolvimento patogênico no desenvolvimento da doença.

1.2.2 Objetivos Específicos

- (1) identificar, dentre as fêmeas selecionadas como matrizes do Programa de Melhoramento de tilápias do Nilo da Escola de Veterinária/UFMG, aquelas que apresentam papila urogenital diferente do padrão da espécie;
- (2) caracterizar as anomalias da papila urogenital de fêmeas de tilápias;
- (3) comparar a eficiência reprodutiva de animais com alteração da estrutura reprodutiva externa com aqueles considerados normais;
- (4) comparar a estrutura dos ovários de fêmeas com estrutura reprodutiva externa normal e anormal;
- (5) avaliar se a ocorrência de anomalias da estrutura reprodutiva externa está correlacionada com alterações dos ovários;
- (6) investigar se há evidências de que as anomalias na estrutura reprodutiva externa possuem componente genético envolvido;
- (7) explorar a influência da temperatura da água durante o desenvolvimento gonadal nas linhagens melhorada e não melhorada geneticamente na prevalência da má-formação;
- (8) avaliar a possível presença de patógenos bacterianos na etiopatogenia da anomalia;
- (9) avaliar se fêmeas anormais e normais apresentam perfis hormonais diferentes;
- (10) avaliar diferenças nos índices biológicos de fêmeas mantidas nos tratamentos térmicos.

Referências bibliográficas

- Alcántar-Vázquez, J.P., la Torre, R.M., Calzada-Ruíz, D., Antonio-Estrada, C. 2014. Production of YY-male of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) from atypical fish. *Lat. Am. J. Aquat. Res.* 42(3): 644-648.
- Alcántar-Vázquez, J.P., Rueda-Curiel, P., Calzada-Ruíz, D., Antonio-Estrada, C., la Torre, R.M. 2015. Feminization of the Nile tilapia *Oreochromis niloticus* by estradiol-17 β Effects on growth, gonadal development and body composition. *Hidrobiologica*, 25(2), 275-283.
- Alvarenga, E.R., França L.R. 2009. Effects of different temperatures on testis structure and function, with emphasis on somatic cells, in sexually mature Nile tilapias (*Oreochromis niloticus*). *Biol Reprod.* 2009;80(3):537-544.
- Azevedo, R.O., Alvarenga, E.R., Fernandes, A.F.A., Silva, M.A., Alves, G.F.O., Menezes, W.F., Turra, E.M. 2021. Use of hCG hormone in the natural and artificial reproduction of Nile tilapia (*Oreochromis niloticus*). *Aquac. Res.*, 52(12), 6380-6388. <https://doi.org/10.1111/are.15502>
- Baroiller, J.F., D'Cotta, H.D. 2018. Sex control in tilapias. In: Wang, H.P., Piferrer, F., Chen, S.L. (Eds.). *Sex Control in Aquaculture*. John Wiley & Sons Ltd, 191–234.
- Bhujel, R.C. 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. *Aquaculture*, 181(1-2), 37-59. [https://doi.org/10.1016/S0044-8486\(99\)00217-3](https://doi.org/10.1016/S0044-8486(99)00217-3)
- El-Sayed, A.-F. M. 2019. *Tilapia culture*, 2nd ed. Elsevier Inc, Academic Press, London.
- FAO (Food and Agriculture Organization). 2022. *The State of World Fisheries and Aquaculture (SOFIA) – Sustainability in action*. FAO/WHO, Rome, Italy.
- Jensen, G.L., Shelton, W.L. 1979. Effects of estrogens on Tilapia aurea: implications for production of monosex genetic male tilapia. *Aquaculture*, 16(3), 233-242. [https://doi.org/10.1016/0044-8486\(79\)90111-X](https://doi.org/10.1016/0044-8486(79)90111-X)
- Khoo, L. 2019. Reproductive disorders. In: Smith, S. A. (Ed.), *Fish diseases and medicine*. CRC Press, 244-256.
- Little, D.C., Macintosh, D.J., Edwards, P. 1993. Improving spawning synchrony in the Nile tilapia, *Oreochromis niloticus* (L.). *Aquac. Res.*, 24, 399–405.
- Ng, W., Romano, N. 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Rev.Aquac.*, 5, 220–254.
- Nozu, R., Nakamura, M. 2020. Influence of prolonged cultivation on sexual characteristics of sterilized female tilapia, *Oreochromis mossambicus*, induced by high-temperature treatment. *Aquaculture*, 524, 735245. <https://doi.org/10.1016/j.aquaculture.2020.735245>
- Peixe BR (Associação Brasileira da Piscicultura). 2024. *Anuário Brasileiro da Piscicultura*. Peixe BR, São Paulo, Brasil.
- Roberts R.J. 2012. *Fish Pathology*. John Wiley & Sons. Scherer, Hoboken, NJ, 592p.
- Santos, S.B., Fernandez-Alarcon M., Ballaben, A.S., Harakava, R., Galetti, R., Guimarães, M.C., Natori, M.M., Takahashi, L.S., Ildefonso, R., Rozas-Serri, M. 2023. First report of *Aeromonas veronii* as an emerging bacterial pathogen of farmed Nile tilapia (*Oreochromis niloticus*) in Brazil. *Pathogens*, 12, 1020. <https://doi.org/10.3390/pathogens12081020>

2 REVISÃO DE LITERATURA: DA EMBRIOLOGIA À FISIOLOGIA REPRODUTIVA E DISFUNÇÕES REPRODUTIVAS EM PEIXES

2.1 Introdução

A seleção de indivíduos superiores para fins de reprodução na tilapicultura facilita a produção mais eficiente de alevinos viáveis e saudáveis, favorecendo a sustentabilidade da criação de tilápias do Nilo (*Oreochromis niloticus*) (Turra, 2012). Alterações hormonais, má formação e anomalias na estrutura reprodutiva interna (gônadas) e externa (papila urogenital), causadas por diversos fatores, têm sido observadas mundialmente em pisciculturas (Sumon et al., 2022). No entanto, há poucas informações na literatura se tais anormalidades podem causar perdas significativas no número de alevinos produzidos.

A tilápia do Nilo é uma das espécies tropicais de água doce mais cultivadas e sua produção está se expandindo nos últimos anos. Segundo relatório sobre a situação mundial da pesca e da aquicultura, a produção de espécies aquícolas totalizou 49,12 mil toneladas em 2020 (FAO, 2022). Destas, 4,41 mil toneladas foram provenientes da tilapicultura, representando 9% do total. A tilapicultura cresceu 10% de 2015 a 2020, embora esse crescimento pudesse ser maior (FAO, 2022). E uma das possibilidades de aumento na produção é maximizar a eficiência reprodutiva desta espécie.

Uma compreensão da anatomia e fisiologia reprodutiva de peixes teleósteos é particularmente importante em espécies cultivadas, entre elas a tilápia do Nilo, pois a produção de ovos e os estágios larvais são normalmente os pontos mais críticos em termos de eficiência econômica e biológica do sistema (Hong et al., 2021).

Assim, este capítulo tem como objetivo fornecer uma compreensão abrangente e detalhada dos principais aspectos da tilapicultura, incluindo a embriologia e sua importância no cultivo de tilápias, os processos de diferenciação sexual, a fisiologia da reprodução, e as disfunções reprodutivas que podem afetar essa e outras espécies.

2.2 Tilapicultura

A tilapicultura é uma atividade mundial, e seu crescimento é impulsionado pela crescente demanda no consumo de peixes e pela capacidade adaptativa da tilápia a uma variedade de ambientes (Castilho-Barros et al., 2020). Os países de maior destaque na

produção de tilápia são China (2 milhões de tonelada), Indonésia (1,4 milhão de tonelada), Egito (1 milhão de tonelada) e Brasil (550 mil toneladas). Estes países se beneficiam de condições climáticas favoráveis a criação desta espécie, infraestrutura adequada e tecnologias para aumento da produtividade interna (Peixe BR, 2023). A tilapicultura então é uma atividade valorizada pela eficiência da espécie quanto aos índices produtivos, tornando-a uma opção econômica e sustentável.

No Brasil a produção de tilápia tem se destacado como uma importante atividade dentro do setor aquícola. Com condições climáticas favoráveis em várias regiões do seu território, e uma vasta extensão de água doce disponível (Silva et al., 2023). Lideram a produção de tilápia os estados do Paraná (187.800 mil toneladas), São Paulo (77.300 mil toneladas), Minas Gerais (51.700 mil toneladas), Santa Catarina (42.500 toneladas) e Mato Grosso do Sul (32.200 toneladas) (Peixe BR, 2023). A tilapicultura brasileira tem sido impulsionada pela demanda crescente no mercado interno e externo, com foco na adoção de práticas sustentáveis de produção (Castilho-Barros et al., 2020; Tavares et al., 2021).

Os entraves produtivos da atividade são multifacetados e abrangem diversos aspectos que impactam diretamente o desempenho produtivo e a sustentabilidade da atividade (Carneiro et al., 2022; Silva et al., 2023). Um dos principais desafios enfrentados é a manutenção da qualidade da água de cultivo, pois a poluição, o acúmulo de sedimentos e o excesso de nutrientes podem comprometer a saúde e o crescimento dos peixes (Mercanti et al., 2018; Cavalcanti et al., 2020; Dilmi et al., 2021). Além disso, pode ser veículo de doenças que representam uma preocupação significativa, como infecções bacterianas (Amal et al., 2011; Haenem et al., 2023), fúngicas e parasitárias (Dong et al., 2015; Mahboub e Adel 2021) que podem causar sérias perdas na produção (Tavares-Dias e Martins 2017; MacKinnon et al., 2023).

A nutrição adequada é fundamental para otimizar o crescimento e melhores índices produtivos, exigindo formulações precisas de rações que atendam às necessidades específicas dos peixes em seus diferentes estágios de desenvolvimento (Rodrigues et al., 2020; Oliveira et al., 2021; Gule et al., 2022). O manejo adequado, incluindo a seleção de linhagens geneticamente melhoradas e práticas de reprodução, é essencial para maximizar a produção e a qualidade dos peixes (Sgnaulin et al., 2020). Além disso, estratégias de controle de doenças, como vacinação e monitoramento da saúde dos peixes, são indispensáveis para prevenção e uma forma de minimizar possíveis perdas (Tavares-Dias e Martins 2017). No entanto, desafios regulatórios e ambientais, como licenciamento

ambiental e preocupações com impactos ambientais, também precisam ser enfrentados para garantir a sustentabilidade da atividade a longo prazo (Marques et al., 2020; Silva et al., 2023).

Os desafios na reprodução de tilápias são diversos e têm um impacto significativo na tilapicultura (Valenti et al., 2021). Um dos principais desafios é o controle eficaz da reprodução em cativeiro (Azevedo et al., 2021), uma vez que as tilápias têm um comportamento reprodutivo complexo e são sensíveis a fatores ambientais, como temperatura e qualidade da água (Silva et al., 2020). Além disso, garantir uma taxa de reprodução consistente ao longo do ano pode ser difícil, especialmente em regiões com variações sazonais extremas (Mahmoud et al., 2020).

Outro desafio é a seleção e o manejo adequados dos reprodutores para otimizar características desejáveis, como crescimento rápido, resistência a doenças e qualidade da carne (Yoshida et al., 2022). A consanguinidade e a perda de diversidade genética também são preocupações relevantes, pois podem levar à diminuição do vigor e da produtividade (Silva et al., 2020; Gunadi et al., 2021). Questões relacionadas à qualidade dos ovócitos e ao sucesso na incubação também representam desafios significativos (Quiang et al., 2021). Problemas como baixa taxa de fertilização, desenvolvimento anormal dos embriões e alta taxa de mortalidade durante o período de incubação, afetam a produção de alevinos viáveis (Fernandes et al., 2013; Sarmiento et al., 2018).

O impacto desses desafios na reprodução de tilápias se reflete diretamente na produtividade e na rentabilidade da atividade (Yoshida et al., 2022). Dificuldades como obtenção de alevinos de qualidade e na manutenção de estoques de reprodutores saudáveis podem resultar em uma produção inconsistente e custos mais elevados para os produtores (Marques et al., 2020; Valenti et al., 2021). Portanto, superar os desafios na reprodução de tilápias pode impulsionar o crescimento sustentável e aprimorar a eficiência da tilapicultura.

2.3 Embriologia e importância na tilapicultura

A reprodução das tilápias ocorre naturalmente em cativeiro e a maioria das produções de formas jovens em unidades de produção de alevinos usam a reprodução em hapas dentro de viveiros (Bhujel, 2001). Uma vez efetivada a reprodução, o processo de embriogênese é iniciado com a fertilização dos ovos, que ocorre no ambiente externo. Após a fertilização, os ovos são incubados em condições cuidadosamente controladas de

temperatura e qualidade da água, essenciais para o desenvolvimento embrionário saudável (Valenti et al., 2021; Quiang et al., 2022).

Durante a embriogênese ocorre o desenvolvimento gonadal das larvas. As células germinativas primordiais (CGPs) desempenham um papel principal nesse processo, migrando para os locais onde se formarão os órgãos reprodutivos (Kobayashi et al., 2000; Robles et al., 2017). O desenvolvimento gonadal ocorre logo no início no estágio embrionário e continua durante as fases larvais. As larvas de tilápia passam por uma série de transformações morfológicas enquanto seu sistema reprodutivo se desenvolve, preparando-se para o crescimento, desenvolvimento e a maturidade sexual (Morrison et al., 2001; Zhao et al., 2020).

É essencial notar que a qualidade da água, temperatura e nutrição adequada desempenham um papel crítico no desenvolvimento gonadal das larvas de tilápia (Mercanti et al., 2018; Cavalcanti et al., 2020; Gule et al., 2022). Condições ambientais inadequadas podem afetar negativamente o desenvolvimento dos órgãos reprodutivos, comprometendo a saúde e a viabilidade reprodutiva futura dos peixes (Yoshida et al., 2015; Santos e Coimbra, 2017). Além disso, fatores genéticos também influenciam significativamente o desenvolvimento gonadal das larvas (Piferrer et al., 2012).

2.4 Diferenciação sexual de tilápia

A diferenciação sexual em tilápias inicia-se no estágio larval, e é um processo mediado por uma interação de fatores genéticos, hormonais e ambientais, que coordenam a formação dos órgãos reprodutivos específicos de cada sexo (Piferrer et al., 2012).

Durante a fase embrionária, a diferenciação sexual é iniciada por meio de um delicado balanço entre genes determinantes do sexo, que são expressos em resposta a sinais endócrinos (Strüssmann et al., 2002; Robles et al., 2017) e ambientais (Yoshida et al., 2015).

Em peixes, a determinação sexual pode ser desencadeada por fatores genéticos e ambientais. O sistema genético de determinação sexual pode envolver o controle poligênico, genes dominantes de determinação sexual (como DMY), estes genes dominantes associados com controle de genes autossômicos e por ação de cromossomos sexuais (Devlin e Nagahama, 2002; Guerrero-Estévez e Moreno-Mendoza, 2010). Não se sabe ao certo o que engatilha a determinação sexual em tilápias. Baroiller e D'Cotta (2018), em uma extensa revisão sobre controle sexual de tilápias, apontam o LG1 como

o principal locus genético de determinação sexual em tilápias. De acordo com esses autores, esse locus age conjuntamente com vários outros loci (LG3 e LG23) para determinar o sexo. Apesar da determinação genética, a temperatura pode sobrepor e alterar o mecanismo de diferenciação quando a gônada está indiferenciada. Uma vez que a determinação sexual é iniciada e o processo de diferenciação entre os sexos ocorre, esse processo não pode ser alterado, e a gônada fica comprometida com o desenvolvimento de um dos sexos. O período crítico de diferenciação sexual em tilápias cultivadas a 26°C está entre 9 e 15 dias pós fertilização (Ijiri et al., 2008). É nesse período que a temperatura ou tratamento hormonal precisam ser aplicados para ser eficiente na masculinização dos animais, quando o objetivo é produzir lotes monosexo (Ijiri et al., 2008; Baroiller e D'Cotta, 2018).

No início da formação das gônadas em peixes, as células germinativas primordiais (CGPs), que se originam do mesoderma extraembrionário no início da gastrulação, migram para a crista genital (Nakamura et al., 1998; Guerrero-Estévez e Moreno-Mendoza, 2010). Em tilápias do Nilo, as células germinativas primordiais foram localizadas no mesoderma próximo ao intestino dorsal no dia da eclosão das larvas e na crista gonadal 3 dias pós-eclosão (Ijiri et al., 2008). Ao colonizar a gônada primitiva, as PGCs se associam às células somáticas e passam a ser denominadas de gonócitos, os quais, potencialmente, podem se diferenciar em ovogônias ou espermatogônias (Foyle, 1993; Yoshizaki et al., 2002; Guerrero-Estévez e Moreno-Mendoza, 2010). Diferentemente das fêmeas, os gonócitos nos peixes machos normalmente permanecem quiescentes até o início do processo espermatogênico, que ocorre mais tardiamente (Strüssmann & Nakamura, 2002). A meiose das células germinativas inicia-se nas gônadas femininas de 25 a 30 pós-eclosão e nas gônadas XY só após a diferenciação sexual (Kobayashi et al., 2000; Ijiri et al., 2008). Sob microscopia de luz, os primeiros sinais de diferenciação sexual aparecem em tilápias aos 23-26 dias pós-eclosão, com a formação da cavidade ovariana nas gônadas XX e dos ductos eferentes nas gônadas XY (Nakamura et al., 1998).

Externamente, a diferenciação sexual pode ser observada por meio de características morfológicas distintas. Nos machos, as larvas desenvolvem papilas genitais próximas a região anal, de aspecto pontudo apresentando apenas um orifício na papila: a saída do ducto geniturinário, enquanto as fêmeas geralmente exibem uma papila na região anal de formato mais arredondado e sem protuberâncias, e nas papilas das fêmeas existem dois orifícios, a saída do oviduto e o poro urinário, estruturas separadas.

Além disso, as fêmeas tendem a apresentar uma coloração corporal mais escura em comparação aos machos, que muitas vezes exibem cores mais vivas e padrões avermelhados distintos durante a época de reprodução (De Alba et al., 2021; Li et al., 2024).

A diferenciação sexual é influenciada por uma série de fatores, incluindo temperatura da água, composição da ração e densidade populacional. Condições ideais de criação, como temperatura estável e nutrição adequada, podem promover uma diferenciação sexual mais eficiente e um desenvolvimento saudável dos órgãos reprodutivos, facilitando o controle da reprodução e o gerenciamento da população (Piferrer et al., 2012; Santos e Coimbra, 2017; Li et al., 2019). Dessa forma, alterações de variáveis ambientais no período de diferenciação sexual podem afetar o desenvolvimento reprodutivo normal dos animais (Baroiller e D'Cotta, 2018; Nozu e Nakamura, 2020). Nozu e Nakamura (2020), por exemplo, induziram esterilidade funcional em tilápias (*Oreochromis mossambicus*) por manter altas temperaturas (37 °C) na água de cultivo de larvas/juvenis (de 9 a 56 dias de idade) dessa espécie. Esses autores descreveram que a papila urogenital das fêmeas submetidas ao tratamento com altas temperaturas foi subdesenvolvida, e indicaram essa característica como um marcador de esterilidade funcional.

2.5 Fisiologia da reprodução

Diversos fatores abióticos, fisiológicos e endocrinológicos estão envolvidos na reprodução dos peixes, os sinais obtidos por mudanças ambientais são captados e traduzidos por órgãos e tecidos específicos, que transmitem para o eixo hipotálamo-hipófise-gônadas (Da Silva Ribeiro e Moreira, 2012), ativando o sistema neuroendócrino, estimulando os hormônios reprodutivos e ocasionando mudanças fisiológicas e morfológicas que preparam tanto os machos quanto as fêmeas para o processo reprodutivo (Salazar et al., 2018). A reprodução é regulada por uma rede de hormônios, sendo os principais eixos hormonais envolvidos na reprodução da tilápia o eixo hipotálamo-hipófise-gônadas (HHG) e o eixo hipotálamo-hipófise-tireoide (HHT) (De Alba et al., 2021).

No eixo Hipotálamo-Hipófise-Gônadas (HHG) (Figura 1), o hipotálamo secreta o hormônio liberador de gonadotrofinas (GnRH), que estimula a glândula pituitária (hipófise) a sintetizar e secretar os hormônios gonadotróficos, o hormônio luteinizante (LH) e o hormônio folículo-estimulante (FSH) (De Alba et al., 2019; Blanco, 2020). O LH e o FSH atuam nos ovários das fêmeas e nos testículos dos machos para estimular o desenvolvimento e a maturação dos gametas (oócitos nos ovários e espermatozoides nos testículos) (Gabilondo et al., 2022). Nos machos, o LH também estimula as células de Leydig nos testículos a produzirem testosterona, essencial para o desenvolvimento e manutenção das características sexuais secundárias e a regulação da espermatogênese. Nas fêmeas, o FSH desempenha um papel importante na estimulação do crescimento dos folículos ovarianos e na produção de estrógenos pelos ovários (Li et al., 2019; Li et al., 2021).

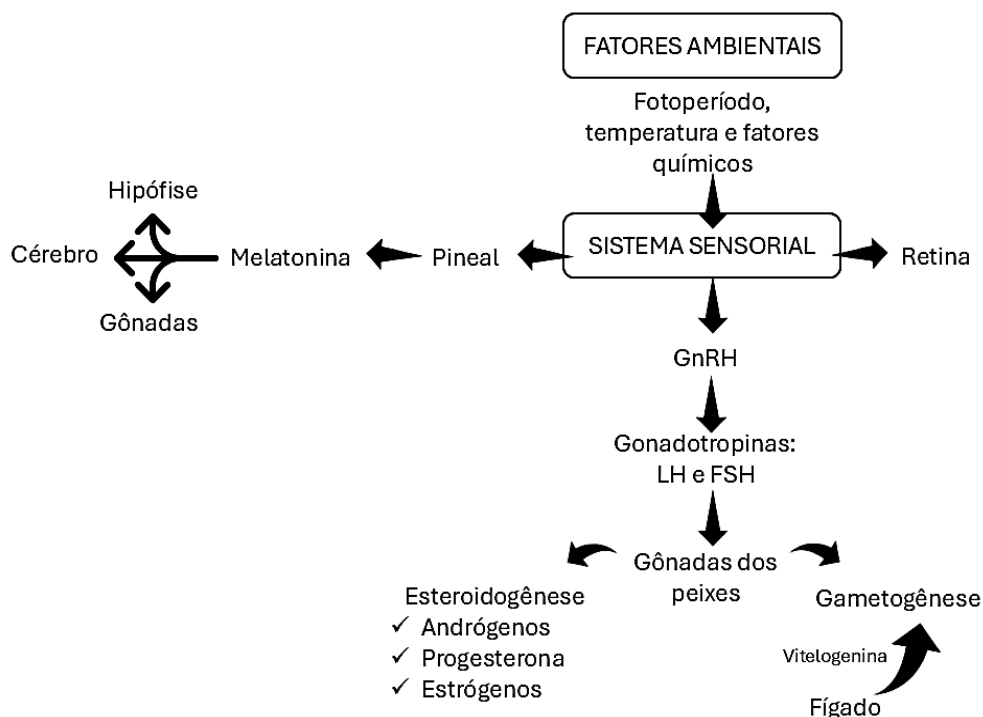


Figura 1: Esquema do processo neuroendócrino regulatório do eixo pineal-hipotálamo-hipófise-gônadas em peixes. GnRH: hormônio liberador de gonadotrofina; LH: hormônio luteinizante; FSH: hormônio folículo-estimulante. A secreção de GnRH age sobre células gonadotróficas na glândula pituitária, liberando os hormônios LH e FSH que agirão nas gônadas de peixes machos e fêmeas. Adaptado de Estevez (2009).

No eixo Hipotálamo-Hipófise-Tireoide (HHT), o hipotálamo secreta o hormônio liberador de tireotropina (TRH), que estimula a hipófise a secretar o hormônio tireotrofina (TSH) (Rodrigues et al., 2020; Zhang et al., 2022). O TSH atua na tireoide para estimular a síntese e secreção dos hormônios tireoidianos, triiodotironina (T3) e tiroxina (T4). Os

hormônios tireoidianos desempenham um papel na regulação do metabolismo energético e do crescimento, que são importantes para suportar os altos requerimentos metabólicos durante o período reprodutivo (Deal e Volkoff, 2020).

Além desses eixos hormonais principais, outros hormônios também desempenham papéis importantes na reprodução da tilápia, como os esteroides sexuais (estrógenos e testosterona), que influenciam diretamente a maturação sexual e a expressão de comportamentos reprodutivos, e os hormônios do estresse, como o cortisol, que podem afetar a reprodução em resposta a condições adversas do ambiente (Ma et al., 2020). Em conjunto, esses eixos hormonais trabalham para regular todos os aspectos do ciclo reprodutivo da tilápia, desde a maturação dos gametas até o comportamento reprodutivo e uma reprodução bem-sucedida (De Alba et al., 2021; Gabilondo et al., 2022).

Durante o período reprodutivo no ambiente natural, os animais apresentam mudanças comportamentais e morfológicas distintas (Li et al., 2024). Os machos frequentemente exibem comportamentos territoriais, como escavação de ninhos e defesa de território, para atrair as fêmeas. Além disso, eles podem desenvolver cores mais vibrantes e padrões distintos, como uma forma de cortejo. As fêmeas, por sua vez, exibem comportamentos relacionados à busca por abrigo e proteção para seus ovos (De Alba et al., 2021; Mashaii et al., 2022).

Morfologicamente, tanto os machos quanto as fêmeas de tilápia (*Oreochromis* spp.) passam por mudanças significativas nas papilas genitais, estruturas especializadas que desempenham um papel importante no comportamento reprodutivo e na reprodução. Estas mudanças nas papilas estão diretamente relacionadas com a busca de parceiros e o processo de cortejo (De Alba et al., 2021).

Nos machos, as papilas genitais se tornam mais desenvolvidas e proeminentes durante o período reprodutivo. Estas estruturas são visíveis na região anal e são utilizadas durante o cortejo para estimular as fêmeas e facilitar a cópula. Os machos podem exibir comportamentos específicos, como movimentos vibratórios das papilas, para atrair as fêmeas e demonstrar sua aptidão reprodutiva. Além disso, as papilas genitais liberam e direcionam o sêmen durante o processo de fertilização (Li et al., 2024).

Nas fêmeas, as mudanças nas papilas genitais são evidentes, e desempenham um papel importante durante a reprodução (Baroiller e Cotta, 2018). Durante o período reprodutivo, as fêmeas podem exibir comportamentos específicos para permitir que os machos depositem o sêmen próximo aos ovos, onde ocorrerá a fertilização (Mashaii et al., 2022; Li et al., 2024). Após a fertilização, conforme as larvas crescem e se

desenvolvem, passam por uma série de estágios de desenvolvimento, incluindo metamorfose, quando mudam de forma e adquirem características de peixes adultos.

2.6 Disfunções reprodutivas

O manejo dos reprodutores abrange processos reprodutivos críticos, como: maturação, produção de ovócitos e espermatozoides e desova, e continua sendo um grande obstáculo para o avanço da indústria da aquicultura. Em condições de criações comerciais desfavoráveis como em condições de qualidade da água inadequada, os reprodutores podem se tornar mais vulneráveis à interrupção das atividades reprodutivas por desequilíbrio hormonal, resultando em disfunção reprodutiva (Gioacchini et al., 2014). Tais deficiências reprodutivas incluem produção de ovócitos ou espermatozoides de baixa qualidade e/ou de quantidade, problemas na desova, menor crescimento e alta mortalidade de larvas e alevinos (Selvaraj et al., 2021). Um problema comum enfrentado por peixes machos cultivados é a baixa quantidade e/ou qualidade de liberação de sêmen durante a espermição (Mylonas et al., 2010).

O eixo hipotálamo-hipófise-gônadas regula a função reprodutiva na maioria dos peixes. O hipotálamo e a glândula pituitária modulam a produção de gonadotrofinas, LH e FSH, e os esteroides sexuais gonadais regulam a vitelogênese e a maturação ovocitária. FSH e LH foram confirmados como importantes no crescimento e maturação do oócito (Zhang et al., 2015). Em geral, a literatura considera que a tilápia do Nilo se adapta bem as condições em cativeiro e se reproduz de maneira satisfatória. Entretanto, há problemas de sincronização da reprodução de fêmeas desta espécie, o que reduz sua eficiência reprodutiva (Bhujel, 2000). Assim, identificar características externas nos animais, especialmente em fêmeas, que indiquem quais indivíduos estão prontos para a reprodução e apresentam maior capacidade reprodutiva seria de grande relevância na produção de alevinos de tilápias. A criação de reprodutores e suas estratégias de manejo têm sido consideradas vitais para o aumento da produção aquícola nas últimas três décadas. Proteínas, ácidos graxos, lipídios, vitaminas, especialmente E e C, e carotenóides na alimentação influenciam a fecundidade, taxa de fecundação, taxa de eclosão e o desenvolvimento larval dos peixes (Izquierdo, 2001).

Gioacchini et al. (2014) categorizaram três tipos de disfunções reprodutivas das fêmeas com base no padrão afetado do ciclo reprodutivo, enquanto Selvaraj et al. (2021) documentaram dois modos de disfunção em peixes teleósteos. Ambos os grupos de

pesquisadores associaram as disfunções reprodutivas mais disruptivas na fase vitelogênica, como exemplificado pela falha na vitelogênese em enguias (*Anguilla* spp.), peixe lírio (*Seriola* spp.) (Abellan e Basurco, 1999) e tainha cinzenta (*Mugil cephalus*) (Monbrison et al., 1997). O segundo modo é categorizado como a incapacidade de atingir a maturação final do oócito até a ovulação, como visto em robalos brancos (*Centropomus undecimalis*) (Selvaraj et al., 2021). O terceiro conjunto de disfunções leva a falhas na desova na época de reprodução; algumas fêmeas de garoupas brancas (*Epinephelus aeneus*), robalo muge (*Morone saxatilis*) e capatão-legítimo (*Dentex dentex*) podem liberar ovos após a ovulação sem exibir comportamento reprodutivo característico (Gioacchini et al., 2014). Estes são exemplos de falhas entre as fêmeas em reprodução que não parecem estar acompanhados de problemas reprodutivos que podem ser atribuídos aos machos.

No geral, disfunções reprodutivas podem ser desencadeadas por variações diretas ou indiretas dos gametas e sistema endócrino, estresse induzido em cativeiro, inadequação do ambiente de desova e deficiências nos perfis nutricionais da dieta (Akhtar et al., 2017). Estes problemas em peixes normalmente estão associados a exposição de substâncias diversas que alteram a fisiologia reprodutiva e a condições inadequadas de manejo, como temperatura e fotoperíodo, principalmente (Mylonas et al., 2010; Hong et al., 2021). Antes da ação direta na reprodução de organismos aquáticos, a presença de fertilizantes químicos, antibióticos, hormônios, efluentes industriais, degradação ambiental e frequentes flutuações de temperatura suprimem a imunidade e a atividade microbiana benéfica nos reprodutores, tornando-os mais suscetíveis a doenças infecciosas (Arslan et al., 2021). Os autores também relatam que os efeitos tóxicos dos desreguladores endócrinos nos peixes dependem do período e duração da exposição, das propriedades químicas das substâncias, se a substância exposta é única ou mista.

Produtos químicos no ambiente, como fungicidas, herbicidas, inseticidas e compostos desreguladores endócrinos, podem inibir a reprodução e são altamente tóxicos para peixes e outros organismos na cadeia alimentar (Sabra e Mehana, 2015). Além disso, em sistemas aquáticos, os pesticidas têm impactos adversos nas atividades reprodutivas dos peixes, como fecundidade, taxa de fecundação, índice gonadossomático, maturação do oócito, ovulação, desova e espermição (Murty, 2018). As atividades reprodutivas dos peixes são afetadas por inúmeros pesticidas, como organoclorados e organofosforados (El-Gawad et al., 2012) que prejudicam o estado morfológico dos testículos e ovários dos peixes, e causam atrasos no desenvolvimento do oócito e inibição da síntese de hormônios

esteroides (Delbes et al., 2022). Já se demonstrou que a absorção de pesticidas (lindano, pentaclorofenol e propoxur) teve efeitos adversos no índice gonadossomático, bem como no crescimento geral e desempenho reprodutivo de tilápias do Nilo e bagres africanos (*Clarias gariepinus*) (Hanson et al., 2007). Ainda de acordo com esses autores, a contaminação por inseticidas comumente usados como os carbamatos, organoclorados, organofosforados e piretróides, pode levar à redução do crescimento e, a disfunções reprodutivas nos peixes.

Os efeitos de desregulação endócrina podem ocorrer durante os estágios iniciais de desenvolvimento dos peixes, por exemplo, baixas concentrações de pesticidas podem atuar como imitadores ou bloqueadores de hormônios sexuais, causando desenvolvimento sexual anormal, feminização de machos, proporções anormais de sexo e padrões comportamentais incomuns associados ao acasalamento (Tazeen e Kulkarni, 2018). A presença de células germinativas masculinas e femininas no tecido testicular é referida como um estado intersexual, que pode ser causado por desregulação endócrina através da exposição química e desequilíbrio hormonal (Hutchinson et al., 2006), e ocorre nos primeiros momentos do desenvolvimento gonadal de peixes. A avaliação histopatológica do tecido gonadal durante a fase de desenvolvimento permite a detecção de disfunção reprodutiva intersexual, porém o ideal é que os fatores associados ao aparecimento desta condição sejam investigados e evitados rigorosamente (Grim et al., 2007).

A interrupção do desempenho reprodutivo dos peixes também pode ocorrer associada as condições do ambiente de cultivo e manejo. O estresse, quando presente demasiado durante a fase reprodutiva causa impacto prejudicial no processo de vitelogênese (por exemplo: atresia folicular), tendo sido registrado em salmonídeos como truta arco-íris (*Oncorhynchus mykiss*) e truta marrom (*Salmo trutta*) décadas atrás (Carragher et al., 1989). Além disso, mudanças repentinas na temperatura da água podem causar estresse agudo, resultando em extensa atresia folicular em ovários de peixes sexualmente maduros (Schreck, 2010). A temperatura da água e o fotoperíodo são os principais fatores ambientais que desencadeiam a maturação e a desova do ovócito em peixes (Hong et al., 2021; Polat et al., 2021). O fotoperíodo desempenha um papel significativo na aceleração da maturação e desova de pregados (*Scophthalmus maximus*) (Polat et al., 2021). Muitos peixes comercialmente importantes, como truta arco-íris (Selvaraj et al., 2021), tilápia do Nilo (Onumah et al., 2010) e paulistinha (*Danio rerio*) (Abdollahpour et al., 2020), respondem às mudanças de fotoperíodo, levando ao início e conclusão da maturação gonadal e desova.

Apesar da diversidade de relatos de disfunções do sistema reprodutivo em teleósteos, o mesmo não acontece quanto a alterações histopatológicas. Além das alterações neoplásicas e inflamatórias, como as que podem ser encontradas em qualquer tecido, notavelmente poucas alterações histopatológicas foram descritas no sistema reprodutivo de peixes (Sumon et al., 2022). Aparentemente, tumores ovarianos são menos comuns em peixes, se comparados a outros grupos de vertebrados, embora haja um alto nível de tumores testiculares relatados (Akhtar et al., 2017).

Peixes normalmente apresentam alta prolificidade, o que significa que poucos reprodutores podem gerar um número grande de prole (Turra, 2012). A ausência de relatos de alterações patológicas em peixes pode estar relacionada com a menor ocorrência dessas alterações ou a baixa percepção dos produtores e pesquisadores de disfunções reprodutivas deste tipo, dada a alta produção de formas jovens por poucos indivíduos. Entretanto, quando se considera unidades produtoras de alevinos, a identificação de afecções ou distúrbios reprodutivos pode auxiliar consideravelmente a eficiência produtiva do plantel de reprodutores (Turra, 2012).

Referências bibliográficas

- Abdollahpour, H., Falahatkar, B., Lawrence, C. 2020. The effect of photoperiod on growth and spawning performance of zebrafish, *Danio Rerio*. Aquac. Rep. 17: 100295. doi: 10.1016/j.aqrep.2020.100295.
- Abellan, E., Basurco, B. 1999. Marine Finfish Species Diversification: Current Situation and Prospects in Mediterranean Aquaculture. Ciheamiamz: Zaragoza, Spain; FAO: Rome, Italy. 4: 1-139.
- Akhtar, M., Ciji, A., Sarma, D., Rajesh, M., Kamalam, B., Sharma, P., Singh, A. 2017. Reproductive dysfunction in females of endangered golden mahseer (*Tor putitora*) in captivity. Anim. Reprod. Sci. 182: 95-103. doi: 10.1016/j.anireprosci.2017.05.004.
- Amal, M.N.A., & Zamri-Saad, M. (2011). Streptococcosis in tilapia (*Oreochromis niloticus*): a review. Pertanika J. Trop. Agric. Sci, 34(2), 195-206.
- Arslan, P., Özeren, S.C.C., Dikmen, B.Y. 2021. The effects of endocrine disruptors on fish. Environ. Res. Technol. 4: 145-151. doi: 10.35208/ert.860440.
- Azevedo, R.O., Alvarenga, E.R., Fernandes, A.F.A., Silva, M.A., Alves, G.F.O., Menezes, W. F., Turra, E. M. (2021). Use of hCG hormone in the natural and artificial reproduction of Nile tilapia (*Oreochromis niloticus*). Aquac. Res. 52(12), 6380-6388. <https://doi.org/10.1111/are.15502>
- Baroiller, J.F., Cotta, H.D. 2018. Sex control in tilapias. In: Wang, H.-P., Piferrer, F., Chen, S.-L. (Eds.). Sex Control in Aquaculture. John Wiley & Sons Ltd., pp. 191–234
- Bhujel, R.C. 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. Aquaculture, 181: 37-59
- Blanco, A.M. 2020. Hypothalamic-and pituitary-derived growth and reproductive hormones and the control of energy balance in fish. Gen. Comp. Endocrinol. 287, 113322. <https://doi.org/10.1016/j.ygcen.2019.113322>
- Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G., Teletchea, F., Tomasso, J.R., Tucker, C.S., Valenti, W.C. 2020. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J. World Aquac. Soc. 51: 578-633. doi: 10.1111/jwas.
- Cavalcanti, L.D., Gouveia, E.J., Leal, F.C., Figueiro, C.S.M., Rojas, S. S., & Russo, M.R. 2020. Responses of monogenean species to variations in abiotic parameters in tilapiculture. J. Helminthol. 94, e186. <https://doi.org/10.1017/S0022149X20000711>
- Carneiro, C.J., Brum, A.L., Thesing, N.J., & Prochnow, D.A. 2022. Cadeia produtiva da piscicultura: um olhar para a evolução da tilapicultura no Brasil. Revista Perspectiva. 46 (175), 25-34. <https://doi.org/10.31512/persp.v.46.n.175.2022.223.p.25-34>
- Carragher, J., Sumpter, J., Pottinger, T., Pickering, A. 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta L.* and *Salmo gairdneri Richardson*. Gen. Comp. Endocrinol. 76: 310-321. doi: 10.1016/0016-6480(89)90163-9.
- Castilho-Barros, L., Owatari, M. S., Mouriño, J. L. P., Silva, B. C., Seiffert, W. Q. 2020. Economic feasibility of tilapia culture in southern Brazil: A small-scale farm model. Aquaculture. 515, 734551. <https://doi.org/10.1016/j.aquaculture.2019.734551>
- Da Silva Ribeiro, C., & Moreira, R.G. 2012. Fatores ambientais e reprodução dos peixes. R. bras. Biol. 8: 58-61.
- Deal, C. K., Volkoff, H. 2020. The role of the thyroid axis in fish. Front. Endocrinol. 11, 596585. <https://doi.org/10.3389/fendo.2020.596585>

- Delbes, G., Blázquez, M., Fernandino, J., Grigorova, P., Hales, B., Metcalfe, C., Navarro-Martín, L., Pai, L., Robaire, B., Rwigemera, A., Van Der Kraak, G., Wade, M., Marlatt, V. 2022. Effects of endocrine disrupting chemicals on gonad development: Mechanistic insights from fish and mammals. *Environ. Res.* 204: 112040. doi: 10.1016/j.envres.2021.112040.
- de Alba, G., Mourad, N.M.N., Paredes, J.F., Sánchez-Vázquez, F.J., López-Olmeda, J.F. 2019. Daily rhythms in the reproductive axis of Nile tilapia (*Oreochromis niloticus*): Plasma steroids and gene expression in brain, pituitary, gonad, and egg. *Aquaculture*. 507, 313-321. <https://doi.org/10.1016/j.aquaculture.2019.04.047>
- de Alba, G., Sánchez-Vázquez, F.J., López-Olmeda, J.F. 2021. Sex Determination and Differentiation of Tilapia. *Biology and Aquaculture of Tilapia*. CRC Press. pp. 137-156.
- Dilmi, A., Refes, W., Meknachi, A. 2021. Effects of C/N ratio on water quality, growth performance, digestive enzyme activity and antioxidant status of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in biofloc based culture system. *TrJFAS*. 22(1).
- Dong, H.T., Nguyen, V.V., Le, H.D., Sangsuriya, P., Jitrakorn, S., Saksmerprom, V., Senapin, S., Rodkhum, C. 2015. Naturally, concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) farms. *Aquaculture*, 448, 427-435. <https://doi.org/10.1016/j.aquaculture.2015.06.027>
- El-Gawad, E., Abbass, A., Shaheen, A. 2012. Risks Induced by Pesticides on Fish Reproduction. *Massive Conferences and Trade Fairs: Cairo, Egypt*. p.329-338.
- Fernandes, A.F.A., Alvarenga, E.R., Oliveira, D.A.A., Aleixo, C.G., Prado, S.A., Luz, R.K., Sarmiento, N.L.A.F., Teixeira, E.A., Turra, E.M. 2013. Production of oocytes of Nile tilapia (*Oreochromis niloticus*) for in vitro fertilization via hormonal treatments. *Reprod. Domest. Anim.* 48(6), 1049-1055. <https://doi.org/10.1111/rda.12212>
- FAO, F. 2022. The state of world fisheries and aquaculture. Opportunities and challenges. Food and Agriculture Organization of the United Nations. FAO/WHO, Rome, Italy.
- Gabilondo, A.R., Pérez, L.H., Rodríguez, R.M. 2022. Hormonal and neuroendocrine control of reproductive function in teleost fish. *Rev. Bionatura*, 6, 2122.
- Gioacchini, G., Giorgini, E., Vaccari, L., Carnevali, O. 2014. Can Probiotics Affect Reproductive Processes of Aquatic Animals? In: D. Merrifield, E. Ringø, editors, *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*. John Wiley & Sons, Ltd, Chichester, UK. 328-348. doi: 10.1002/9781118897263.ch12.
- Grier, H.J., Uribe-Aranzábal, M.C. 2009. The testis and spermatogenesis in teleosts. *Reproductive biology and phylogeny of fishes (agnathans and bony fishes)*, 8, 119-142.
- Guerrero-Estévez, S., Moreno-Mendoza, N. 2010. Sexual determination and differentiation in teleost fish. *Rev Fish Biol Fisheries* 20, 101–121. <https://doi.org/10.1007/s11160-009-9123-4>
- Guiguen, Y., Fostier, A., Piferrer, F., Chang, C.F. 2010. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. *Gen. Comp. Endocrinol.* 165(3), 352-366. <https://doi.org/10.1016/j.ygcen.2009.03.002>
- Gule, T.T., Geremew, A. 2022. Dietary strategies for better utilization of aquafeeds in Tilapia farming. *Aquac. Nutr.* <https://doi.org/10.1155/2022/9463307>
- Gunadi, B., Robisalmi, A. 2021. Improving genetic quality of cultivated aquatic species under a breeding program: Case study of first generation (G1) population of red tilapia (*Oreochromis spp*). In *IOP Conference Series: Earth and Environmental Science* IOP Publishing. 744, No. 1, p. 012020).

- Grim, K.C., Wolfe, M., Hawkins, W., Johnson, R., Wolf, J. 2007. Intersex in Japanese medaka (*Oryzias latipes*) used as negative controls in toxicologic bioassays: A review of 54 cases from 41 studies. *Environ. Toxicol. Chem.* 26: 1636-1643.
- Haenen, O.L.M., Dong, H.T., Hoai, T.D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S.L., Zadoks, R., Kiermeier, A., Wang, B. Gamaro, E.G., Takeuchi, M., Azmai, M.N.A.; Fouz, B., Pakingking Jr. R., Wei Wei, Z., Bondad-Reantaso, M.G. 2023. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. *Rev. Aquac.* 15, 154-185. <https://doi.org/10.1111/raq.12743>
- Hanson, R., Dodoo, D., Essumang, D., Blay, J., Yankson, K. 2007. The effect of some selected pesticides on the growth and reproduction of fresh water *Oreochromis niloticus*, *Chrysichthys nigrodigitatus* and *Clarias gariepinus*. *Bull. Environ. Contam. Toxicol.* 279: 544-547. <https://doi.org/10.1007/s00128-007-9279-3>.
- Hong, B.S., Lee, H.B., Park, J.Y., Yoon, J.H., Lee, I.Y., Lim, H.K. 2021. Effects of Photoperiod, water temperature, and exogenous hormones on spawning and plasma gonadal steroid in starry flounder, *Platichthys stellatus*. *Isr. J. Aquac.* 73: 1-12. <https://doi.org/10.46989/001c.28425>
- Hutchinson, T.H., Ankley, G.T., Segner, H., Tyler, C.R. 2006. Screening and testing for endocrine disruption in fish - Biomarkers as “signposts,” not “traffic lights,” in risk assessment. *Environ. Health Perspect.* 114: 106-114. <https://doi.org/10.1289/ehp.8062>
- Ijiri S., Kaneko H., Kobayashi T., Wang, D.S., Sakai, F., Paul-Prasanth, B., Nakamura, M., Nagahama, Y. 2008. Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. *Biol Reprod* 78(2):333–341
- Izquierdo, M., Fernandez-Palacios, H., Tacon, A. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture.* 197: 25-42. [https://doi.org/10.1016/S0044-8486\(01\)00581-6](https://doi.org/10.1016/S0044-8486(01)00581-6)
- Kobayashi, T., Kajiura-Kobayashi, H., & Nagahama, Y. 2000. Differential expression of vasa homologue gene in the germ cells during oogenesis and spermatogenesis in a teleost fish, tilapia, *Oreochromis niloticus*. *Mech. Dev.* 99 (1-2), 139-142.
- Li, M., Sun, L., & Wang, D. 2019. Roles of estrogens in fish sexual plasticity and sex differentiation. *Gen. Comp. Endocrinol.* 277, 9-16. <https://doi.org/10.1016/j.ygcen.2018.11.015>
- Li, J., Liu, Z., Kang, T., Li, M., Wang, D., Cheng, C. H. 2021. Igf3: a novel player in fish reproduction. *Biol. Reprod.* 104(6), 1194-1204. <https://doi.org/10.1093/biolre/ioab042>
- Li, M., Sun, L., Zhou, L., Wang, D. 2024. Tilapia, a good model for studying reproductive endocrinology. *Gen. Comp. Endocrinol.*, 345, 114395. <https://doi.org/10.1016/j.ygcen.2023.114395>
- Ma, Y., Ladisa, C., Chang, J. P., Habibi, H.R. 2020. Multifactorial control of reproductive and growth axis in male goldfish: Influences of GnRH, GnIH and thyroid hormone. *Mol. Cell. Endocrinol.* 500, 110629. <https://doi.org/10.1016/j.mce.2019.110629>
- MacKinnon, B., Debnath, P.P., Bondad-Reantaso, M.G., Fridman, S., Bin, H., Nekouei, O. 2023. Improving tilapia biosecurity through a value chain approach. *Rev. Aquac.* 15, 57-91. <https://doi.org/10.1111/raq.12776>
- Mahboub, H. H. 2021. Mycological and histopathological identification of potential fish pathogens in Nile tilapia. *Aquaculture.* 530, 735849. <https://doi.org/10.1016/j.aquaculture.2020.735849>
- Mahmoud, S., Sabry, A., Abdelaziz, A., Shukry, M. 2020. Deleterious impacts of heat stress on steroidogenesis markers, immunity status and ovarian tissue of Nile tilapia

- (*Oreochromis niloticus*). J. Therm. Biol. 91, 102578. <https://doi.org/10.1016/j.jtherbio.2020.102578>
- Marques, F.B., Watterson, A., da Rocha, A.F., Cavalli, L. S. 2020. Overview of Brazilian aquaculture production. Aquac. Res. 51(12), 4838-4845. <https://doi.org/10.1111/are.14828>
- Mercante, C.T., David, G.S., Rodrigues, C.J., do Carmo, C.F., Silva, R.J. 2018. Potential toxic effect of ammonia in reservoirs with Tilapia culture in cages. Int. J. Fish. Aquat. Stud. 6(5), 256-261.
- Monbrison, D.D., Tzchori, I., Holland, M.C., Zohar, Y., Yaron, Z., Elizur, A. 1997. Acceleration of gonadal development and spawning induction in the Mediterranean grey mullet, *Mugil cephalus*: Preliminary studies. Isr. J. Aquac. 49- 214-221.
- Morrison, C.M., Miyake, T., Wright Jr, J.R. 2001. Histological study of the development of the embryo and early larva of *Oreochromis niloticus* (Pisces: Cichlidae). J. Morphol. 247(2), 172-195. [https://doi.org/10.1002/1097-4687\(200102\)247:2<172::AID-JMOR1011>3.0.CO;2-H](https://doi.org/10.1002/1097-4687(200102)247:2<172::AID-JMOR1011>3.0.CO;2-H)
- Murty, A.S. 2018. Toxicity of Pesticides to Fish, CRC Press: Boca Raton, FL, USA.
- Mylonas, C.C., Fostier, A., Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. Gen. Comp. Endocrinol. 165: 516-534. doi: 10.1016/j.ygcen.2009.03.007
- Nakamura, M.; Kobayashi, T., Chang., X. Gonadal sex differentiation in teleost fish. J. Exp. Zool. 281: 362-372, 1998.
- Oliveira, L.K., Pilz, L., Furtado, P.S., Ballester, E.L.C., & de Almeida Bicudo, A.J. 2021. Growth, nutritional efficiency, and profitability of juvenile GIFT strain of Nile tilapia (*Oreochromis niloticus*) reared in biofloc system on graded feeding rates. Aquaculture, 541, 736830. <https://doi.org/10.1016/j.aquaculture.2021.736830>
- Onumah, E.E., Wessels, S., Wildenhayn, N., Brümmer, B., Hörstgen-Schwark, G. 2010. Stocking density and photoperiod manipulation in relation to estradiol profile to enhance spawning activity in female Nile tilapia. Turk. J. Fish. Aquat. Sci. 10: 463-470. <https://doi.org/10.4194/trjfas.2010.0404>
- Nakamura, M., Nagahama, Y. 1989. Differentiation and development of Leydig cells, and changes of testosterone levels during testicular differentiation in tilapia *Oreochromis niloticus*. Fish Physiol. Biochem. 7, 211-219. <https://doi.org/10.1007/BF00004709>
- Nakamura, M., Bhandari, R.K., Higa, M. 2003. The role estrogens play in sex differentiation and sex changes of fish. Fish Physiol. Biochem, 28, 113-117. <https://doi.org/10.1023/B:FISH.0000030495.99487.17>
- Nozu, R., Nakamura, M., 2020. Influence of prolonged cultivation on sexual characteristics of sterilized female tilapia, *Oreochromis mossambicus*, induced by high-temperature treatment. Aquaculture 524, 735245.
- Parenti, L.R., Grier, H.J. 2004. Evolution and phylogeny of gonad morphology in bony fishes. Integr Comp Biol. 44(5), 333-348. <https://doi.org/10.1093/icb/44.5.333>
- Piferrer, F., Ribas, L., Díaz, N. 2012. Genomic approaches to study genetic and environmental influences on fish sex determination and differentiation. Mar. Biotechnol. 14, 591-604. <https://doi.org/10.1007/s10126-012-9445-4>
- Polat, H., Ozturk, R.C., Terzi, Y., Aydin, I., Kucuk, E. 2021. Effect of photoperiod manipulation on spawning time and performance of turbot (*Scophthalmus maximus*). Aquac. Stud. 21: 109-115. https://doi.org/10.4194/2618-6381-v21_3_03
- Qiang, J., Duan, X. J., Zhu, H. J., He, J., Tao, Y. F., Bao, J. W., Zhu, X.-W., Xu, P. 2021. Some “white” oocytes undergo atresia and fail to mature during the reproductive cycle in female genetically improved farmed tilapia (*Oreochromis*

- niloticus*). Aquaculture, 534, 736278.
<https://doi.org/10.1016/j.aquaculture.2020.736278>
- Robles, V., Riesco, M.F., Psenicka, M., Saito, T., Valcarce, D.G., Cabrita, E., Herraez, P. 2017. Biology of teleost primordial germ cells (PGCs) and spermatogonia: Biotechnological applications. Aquaculture. 472, 4-20.
<https://doi.org/10.1016/j.aquaculture.2016.03.0044>
- Rodrigues, M.S., Tovo-Neto, A., Rosa, I.F., Doretto, L.B., Fallah, H.P., Habibi, H.R., Nóbrega, R. H. 2022. Thyroid Hormones Deficiency Impairs Male Germ Cell Development: A Cross Talk Between Hypothalamic-Pituitary-Thyroid, and—Gonadal Axes in Zebrafish. Front. Cell Dev. Biol. 10, 865948.
<https://doi.org/10.3389/fcell.2022.865948>
- Rodrigues, A.T., Mansano, C.F., Khan, K.U., Nascimento, T.M., Boaratti, A.Z., Sakomura, N.K., Fernandes, J.B. 2020. Ideal profile of essential amino acids for Nile tilapia (*Oreochromis niloticus*) in the finishing growth phase. Aquac. Res. 51(11), 4724-4735. <https://doi.org/10.1111/are.14819>
- Sabra, F.S., Mehana, E.-S.E.-D. 2015. Pesticides toxicity in fish with particular reference to insecticides. Asian J. Agric. Food Sci. 3: 68.
- Salazar, J.E.L., Jim, C.E.D., Caro, N.E.L., Dar, D., Marin, I.R. 2018. La reproducción del *Arapaima gigas*: revisión sistemática. Rev Sinergia, 1(2), 96-109.
- Guerrero-Estévez, S.M. Moreno-Mendoza, N.J. 2010. Sexual determination and differentiation in teleost fish. Rev. Fish Biol. Fish. 20, 101-121.
<https://doi.org/10.1007/s11160-009-9123-4>
- Santos, D., Luzio, A., Coimbra, A.M. 2017. Zebrafish sex differentiation and gonad development: a review on the impact of environmental factors. Aquat. Toxicol. 191, 141-163. <https://doi.org/10.1016/j.aquatox.2017.08.005>
- Sarmento, N.L., Martins, E.F., Costa, D.C., Mattioli, C.C., da Costa Julio, G.S., Figueiredo, L.G., Luz, M.R., Luz, R.K. 2018. Reproductive efficiency and egg and larvae quality of Nile tilapia fed different levels of vitamin C. Aquaculture, 482, 96-102. <https://doi.org/10.1016/j.aquaculture.2017.08.035>
- Schreck, C.B. 2010. Stress and fish reproduction: The roles of allostasis and hormesis. Gen. Comp. Endocrinol. 165; 549-556. <https://doi.org/10.1016/j.ygcen.2009.07.004>
- Selvaraj, S., Chidambaram, P., Ezhilarasi, V., Kumar, P.P., Moses, T., Antony, C., Ahilan, B.A. 2021. Review on the Reproductive Dysfunction in Farmed Finfish. Annu. Res. Rev. Biol. 36: 65-81.
- Silva, A.C.F., Corrêa Filho, R.A.C., Ventura, A.S., Nunes, A.L., Laice, L.M., Ribeiro, R.P., Oliveira, C.A.L., Almeida, L.C., Barbosa, P.T.L., Povh, J.A. 2020. Reproductive traits in different Nile tilapia genetic groups. Arq. Bras. Med. Vet. Zootec. 72, 1797-1804. <https://doi.org/10.1590/1678-4162-11764>
- Silva, Á.F.S., de Lima, M.A.B., de Campos Takaki, G.M. 2023. Main advances in tilapia production for the sustainable development of aquaculture in Brazil and its economic benefits. Caderno de ANAIS HOME.
- Sgnaulin, T., Durigon, E.G., Pinho, S.M., Jeronimo, G.T., de Alcantara Lopes, D.L., Emerenciano, M.G.C. 2020. Nutrition of Genetically Improved Farmed Tilapia (GIFT) in biofloc technology system: Optimization of digestible protein and digestible energy levels during nursery phase. Aquaculture. 521, 734998.
<https://doi.org/10.1023/A:1023343023556>
- Strüssmann, C.A., Nakamura, M. 2002. Morphology, endocrinology, and environmental modulation of gonadal sex differentiation in teleost fishes. Fish Physiol. Biochem. 26, 13-29.

- Sumon, M.A.A., Molla, M.H.R., Hakeem, I.J., Ahammad, F., Amran, R.H., Jamal, M.T., Hasan, M.T. 2022. Epigenetics and Probiotics Application toward the Modulation of Fish Reproductive Performance. *Fishes* 7: 189. <https://doi.org/10.3390/fishes7040189>
- Tavares-Dias, M., Martins, M. L. 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. *J. Parasit. Dis.* 41, 913-918. <https://doi.org/10.1007/s12639-017-0938-y>
- Tavares, S.G., de Queiroz, S.S., Bertolini, G.R.F., de Grandi, A.M., Rodrigues, M.L., Signor, A. 2021. A produção científica mundial sobre a tecnologia de bioflocos na tilapicultura como propulsora do desenvolvimento sustentável. *Res., Soc. Dev.* 10 (5), e56910515200-e56910515200. <https://doi.org/10.33448/rsd-v10i5.15200>
- Tazeen, S., Kulkarni, R. 2018. Histopathological impact of profenofos on ovary of the freshwater fish *Notopterus notopterus*. *Asian J. Res. Zool.* 1: 1-7. doi: 10.9734/AJRIZ/2018/v1i229682
- Turra, E.M. 2012. Melhoramento genético na Aquacultura. In: J.C.C. Pereira, editor, Melhoramento genético aplicado à produção animal. FEPMVZ. Belo Horizonte, MG. 577-600.
- Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O. 2021. Aquaculture in Brazil: past, present, and future. *Aquac. Rep.* 19, 1006. <https://doi.org/10.1016/j.aqrep.2021.1006111>
- Yoshida, G.M., Oliveira, C.A.L.D., Kunita, N.M., Rizzato, G.S., Ribeiro, R.P. 2015. Reproduction performance of female Nile tilapia under different environments and age classes. *Acta Sci. Anim. Sci.* 37, 221-226. <https://doi.org/10.4025/actascianimsci.v37i3.25907>
- Yoshida, G.M., de Oliveira, C.A.L., Campos, E.C., Todesco, H., Araújo, F.C., Karin, H.M., Zardin, A.M.S.O. Bezerra Jr, J.S. Alexandre Filho, L. Vargas, L. Ribeiro, R.P. 2022. A breeding program for Nile tilapia in Brazil: Results from nine generations of selection to increase the growth rate in cages. *J Anim Breed Genet Journal*.139(2), 127-135. <https://doi.org/10.1111/jbg.12650>
- Zhang, Z., Zhu, B., Ge, W. 2015. Genetic analysis of zebrafish gonadotropin (FSH and LH) functions by TALEN-mediated gene disruption. *Mol. Endocrinol.* 29: 76-98. doi: 10.1210/me.2014-1256
- Zhang, S., Guo, X., Lu, S., He, J., Wu, Q., Liu, X., Han, Z., Xie, P. 2022. Perfluorohexanoic acid caused disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. *Ecotoxicol Environ Saf.* 232, 113283.
- Zhao, Y., Chen, H. J., Wang, Y. Y., Mei, Y., Huang, L. B., Wang, H., Ji, X. S. 2020. Gonad development examination of high-temperature-treated genetically female Nile tilapia. *Aquaculture.* 515, 734535. <https://doi.org/10.1016/j.aquaculture.2019.734535>

3 DECREASE OF REPRODUCTIVE PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FEMALES WITH DYSMORPHIA IN UROGENITAL PAPILLA

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Decrease of reproductive performance of Nile tilapia (*Oreochromis niloticus*) females with dysmorphia in urogenital papilla

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ABSTRACT

The seed production of Nile tilapia (*Oreochromis niloticus*) can be considerably reduced when inefficient breeders are present in the broodstock. Females with dysmorphia in urogenital papilla were identified and characterized in the present study and their reproductive performance were compared to normal tilapias in three different temperatures (28 °C, 26 °C and 24 °C). We also evaluated the effects of dysmorphia in urogenital papilla on biological indexes and its possible association with pathological bacteria. From a broodstock of Nile tilapia (600 females and 150 males), 96 normal males, 96 females with normal urogenital papilla and 96 females with abnormal urogenital papilla were selected. All tilapias were individually identified by tags and reared in a biofloc system during the stocking. We performed three reproductive cycles in clear water, each one of three weeks, totaling 9 weeks of reproduction. Different temperatures were used in each cycle, being the first at 28 °C, the second at 26 °C and the third at 24 °C. In each week, we used eight 3.6 m³ tanks with 8 females and 6 males, half of the tanks (4 tanks) had normal females and the other half with abnormal females. For each female group, there was a resting period of two weeks between each reproductive cycle. During the assays, we monitored the water quality. After reproductive assays, the animals were photographed, euthanized and the body weights, viscera, gonads, and liver were obtained for biological indexes calculation. We performed bacteriological examination, where fragments of brain, kidney, spleen, and gonads from 8 abnormal females were inoculated onto blood and MacConkey agar. The macroscopic alteration in the urogenital papilla was also associated with lesions in ovaries. The odds of females with normal urogenital papilla effectively reproduce was approximately 3, 5 and 12 times than females with abnormal papilla, in the first, second, and third cycles, respectively. The larvae number per tank produced by normal females was approximately 3, 5 and 5 times than females with abnormal urogenital papilla in the first, second and third reproductive cycle, respectively. The gonadosomatic, viscerosomatic, hepatosomatic and viscerosomatic fat indexes were not different between the normal and abnormal females. The bacteriological examination was negative for all samples. In conclusion, the morphology of urogenital papilla is a good predictor of reproductive performance of Nile tilapia and should be used for breeder selection.

1. Introduction

Nile tilapia easily reproduces under captivity conditions (Pullin

et al., 1991; Bhujel, 2000; Piamsomboon et al., 2019; Azevedo et al., 2021), reaches sexual maturity precociously (4 to 5 months after hatching) and has short spawning cycles (Longalongo et al., 1999; Sun

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3.1 Abstract

The seed production of Nile tilapia (*Oreochromis niloticus*) can be considerably reduced when inefficient breeders are present in the broodstock. Females with dysmorphia in urogenital papilla were identified and characterized in the present study and their reproductive performance were compared to normal tilapias in three different temperatures (28°C, 26 °C and 24°C). We also evaluated the effects of dysmorphia in urogenital papilla on biological indexes and its possible association with pathological bacteria. From a broodstock of Nile tilapia (600 females and 150 males), 96 normal males, 96 females with normal urogenital papilla and 96 females with abnormal urogenital papilla were selected. All tilapias were individually identified by tags and reared in a biofloc system during the stocking. We performed three reproductive cycles in clear water, each one of three weeks, totalizing 9 weeks of reproduction. Different temperatures were used in each cycle, being the first at 28°C, the second at 26°C and the third at 24°C. In each week, we used eight 3.6 m³ tanks with 8 females and 6 males, half of the tanks (4 tanks) had normal females and the other half with abnormal females. For each female group, there was a resting period of two weeks between each reproductive cycle. During the assays, we monitored the water quality. After reproductive assays, the animals were photographed, euthanized and the body weights, viscera, gonads, and liver were obtained for biological indexes calculation. We performed bacteriological examination, where fragments of brain, kidney, spleen, and gonads from 8 abnormal females were inoculated onto blood and Macconkey agar. The macroscopic alteration in the urogenital papilla was also associated with lesions in ovaries. The odds of females with normal urogenital papilla effectively reproduce was approximately 3, 5 and 12 times than females with abnormal papilla, in the first, second, and third cycles, respectively. The larvae number per tank produced by normal females was approximately 3, 5 and 5 times than females with abnormal urogenital papilla in the first, second and third reproductive cycle, respectively. The gonadosomatic, viscerosomatic, hepatosomatic and viscerosomatic fat indexes were not different between the normal and abnormal females. The bacteriological examination was negative for all samples. In conclusion, the morphology of urogenital papilla is a good predictor of reproductive performance of Nile tilapia and should be used in breeder selection.

Keywords: *Oreochromis niloticus*, ovary, seed production, reproductive dysfunction.

3.2 Introduction

Nile tilapia easily reproduces under captivity conditions (Pullin et al., 1991; Bhujel, 2000; Piamsomboon et al., 2019; Azevedo et al., 2021), reach sexual maturity precociously (4 to 5 months after hatching) and have short spawning cycles (Longalong et al., 1999; Sun et al., 2014). Despite these advantageous reproductive characteristics, the seed production of Nile tilapias faces some limitations. Nile tilapia is a multiple spawner and maternal mouthbrooder and has low fecundity and asynchronous spawning (Bhujel, 2000; Little et al., 1993; Ng and Romano, 2013; Azevedo et al., 2021). These limitations imply the need of considerable number of females required to meet the demand for seed, high labor cost or a long time to reach the expected quantity of offspring (Bhujel, 2000; Azevedo et al., 2021). Therefore, optimizing hatchery management becomes necessary for sustainability of seed production (El-Sayed, 2019), especially in countries where the Nile tilapia production has been expanding at a high rate (Barroso et al., 2018).

Optimizing the reproduction of Nile tilapia can contribute to a more efficient seed production, which favors the sustainability of tilapia farming due the rational use of natural resources (inputs, water, and land). To achieve such optimizing, a variety of management strategies involving abiotic factors manipulation (Campos-Mendoza et al., 2004; Alvarenga and França, 2009; Yoshida et al., 2015; El-Sayed, 2019), nutritional management (El-Sayed and Kawanna, 2008; Lima et al., 2020, Nascimento et al., 2023), use of a specific production system (Ekasari et al., 2015; Alvarenga et al., 2017; Young et al., 2021; Khanjani et al., 2022) and hormonal treatments (Fernandes et al., 2013; Piamsomboon et al., 2019; Azevedo et al., 2021) have been tested and recommended. Another strategy that can be adopted is the identification and selection of animals with high reproductive capacity or the exclusion of those with reduced reproduction efficiency. Such identification may allow the use of animals with high reproductive performance, resulting in a seed production more economically and environmentally sustainable (Trong et al., 2013).

Variations in the urogenital papilla have been described in tilapia breeders under experimental conditions (Jensen and Shelton, 1979; Alcántar-Vázquez, et al., 2014; Alcántar-Vázquez, et al., 2015; Nozu and Nakamura, 2020). Recently, we identified females with external reproductive structure different of the typical female urogenital papilla in the broodstock our laboratory (a description of typical urogenital papilla of Nile tilapia can be accessed in Barroiler and D'Cotta, 2018). These female tilapias with atypical urogenital

papilla have also been observed in fish farms in Brazil since 2005 (Eduardo Maldonado Turra, personal communication). Recently, the Laboratory of Aquatic Animal Diseases of Department of Preventive Veterinary Medicine, Veterinary School/ UFMG analyzed two batches of Nile tilapia breeders (120 animals per batch) from farms in the States of Minas Gerais and São Paulo (Brazil) and identified 8-10% of animals with this reproductive anomaly (Carlos Augusto Gomes Leal, personal communication). However, according to our knowledge, there is no information in the literature whether such abnormalities can impair the reproductive capacity or the fingerlings production. Thus, the objectives of this study were: (1) to perform an anatomical characterization of urogenital papilla and ovary of female tilapia with the dysmorphia; (2) to verify whether urogenital papilla dysmorphia is related to changes in ovarian morphology; (3) to compare the reproductive performance (reproductive capacity and larvae production) of females with abnormal external reproductive structure with those considered normal in three different temperatures (28 °C, 26 °C and 24 °C); (4) to assess whether pathological bacteria are related with the dysmorphia of urogenital papilla; and (5) to evaluate the effects of dysmorphia in urogenital papilla on biological indexes.

3.3 Material and Methods

3.3.1 Location

The experiment was conducted in a greenhouse at the Aquaculture Laboratory of the Veterinary School of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais - UFMG), Brazil. All procedures were previously approved by the Ethics Committee on Animal Use of UFMG under protocol number 44/2019.

3.3.2 Breeder stocking and fish conditions

The animals for the experiment were obtained from a broodstock of Nile tilapia (*O. niloticus*, Chitralada line, with seven to twelve months of age) with 600 females and 150 males. From this broodstock, we identified 102 females with dysmorphia in the urogenital papilla (17% of the females) and randomly selected 96 of them (abnormal group) for the reproductive assay. We also randomly selected 96 females with normal urogenital papilla

(normal group), and 96 normal males. The description of the abnormal group is detailed in the Results section. Male and female Nile tilapia were individually identified by Passive Integrated Transponder (PIT) tags. The broodstock was reared in a biofloc technology (BFT). The tanks were completely filled with mature biofloc previously development in our laboratory. We used sugar cane (50% carbon) as carbon source and a C:N ratio of 6:1 based on the total ammonia nitrogen (TAN) according to Ebeling et al. (2006).

3.3.3 Reproductive assay

The 96 females of each experimental group (females with normal or abnormal urogenital papillae) were divided into three groups of 32 fish, and each group was allocated to a different tank (totaling three 3.6 m³ tanks). These tanks were divided in half using hapas and each tanks received a group of 32 animals from each experimental group (64 females per tank). That is, the animals of the different groups shared the same tank to ensure that they were under the same conditions during the stocking period (when the females were not in tanks for reproduction). The 96 males, all with normal urogenital papilla, were reared in a 3.6 m³ tank divided into two hapas with 48 males each.

Each stocking tank of females was used in one of three consecutive weeks of reproduction (Figure 1). For males, one of these two groups with 48 fish was used in tanks for reproduction each week. For each week of natural breeding, 32 females of each type (normal or abnormal) were randomly distributed in 4 tanks for reproduction (3.6 m³), totaling 8 tanks in each week. The 48 males also were randomly distributed in these 8 tanks for reproduction. Thus, in three weeks of reproduction, all females of each group (32 females per week, 96 females in total) were in the tank for reproduction once. In each tank, 8 females and 6 males were allocated in 1 mm mesh hapa and they remained together for 7 days. Tanks for reproduction were maintained with clear water and constant aeration through compressors and diffusers. The experimental unit in this experiment was the tank for reproduction.

The entire procedure described above was called a reproductive cycle, corresponding to three weeks of reproduction (32 different females per week and per group, 96 different females per group in total), in which each female was in a tank for reproduction in one week and was in a tank for stocking for one (whether the female reproduced) or two consecutive weeks (whether the female reproduced or not) for the gonadal recrudescence. This cycle was

repeated three times with the same animals (that is, each animal was in a tank for reproduction at three separate periods). The average temperature in each reproductive cycle was different, being 28°C, 26°C and 24°C in the first, second and third cycles, respectively. In total, we performed nine weeks of reproduction. During the third reproductive cycle, an hapa ruptured in a tank for reproduction with normal females and the tilapias escaped, resulting in one tank less in this reproductive cycle.

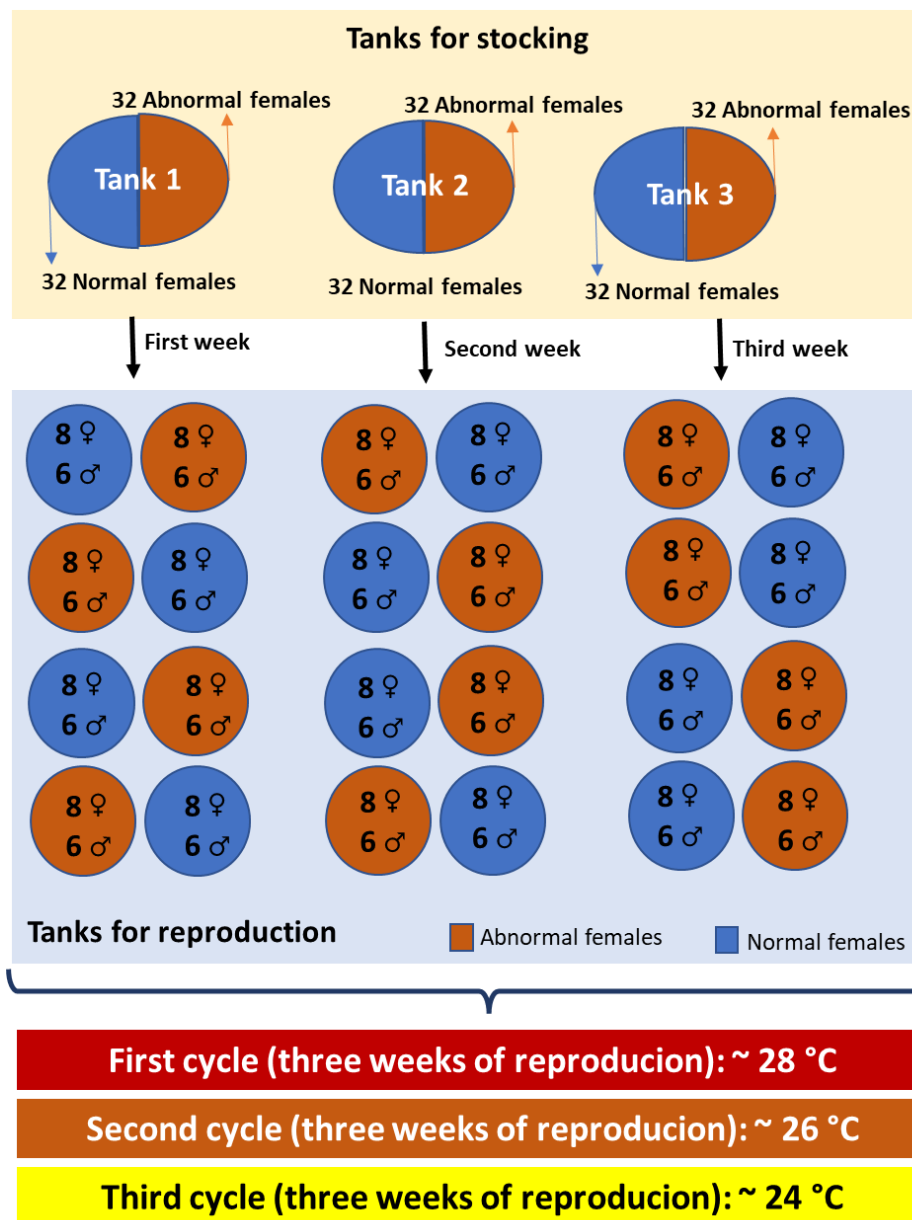


Figure 1: Experimental design to compare the reproductive performance of Nile tilapia (*Oreochromis niloticus*) females with normal or abnormal urogenital papilla. All males used were normal. The schematic summary (in light blue and orange) represents the experimental procedure of a reproduction cycle, corresponding to three weeks of reproduction, in which each female was in a tank for reproduction in one week and was in a tank for stocking for two consecutive weeks for the gonadal recrudescence until the next reproductive cycle. In

each week, 32 different females were evaluated per group (normal and abnormal), totaling 96 females evaluated per group in each temperature (192 females in total). Each cycle (corresponding three weeks of reproduction) was performed three times, one at each different temperature (28°C, 26°C and 24°C) with the same 192 females (96 females per group), totaling nine weeks of reproduction.

At the end of the 7 days, the females with eggs in their mouths in tanks for reproduction were transferred to individual hapas (1 m³ volume) allocated in other 3.6 m³ tanks for mouthbrooding. Close to 1 week of incubation and after absorption of the yolk sac, the larvae from each female were counted. Thus, females that reproduced were one week in the tanks for mouthbrooding and one week in the stocking tank for gonadal recrudescence, while females that did not reproduce were two weeks in the stocking tank until the next reproductive cycle. Alvarenga et al. (2017) studied the gonadal recrudescence of Nile tilapia in BFT and described that the ovaries were completely recovered two weeks after spawning. Proportion of females that effectively produced larvae per tank and the number of larvae per gram of female and per female were evaluated to compare the reproductive performance of females with normal or abnormal urogenital papilla.

3.3.4 Water quality

In tanks for stocking (BFT) and reproduction (clear water system), the water temperature, dissolved oxygen (DO) and pH were measured twice a day (at 8:00 am and 4:00 pm) using the multiparameter Probe AKSO. The TAN and nitric nitrogen (N-NO₂⁻) concentrations were measured twice a week by spectrophotometry according to the methodology generated by UNESCO (1983) and Bendschneider and Robinson (1952), respectively. Total alkalinity was assessed twice in the first reproductive cycle, and once in the second and third reproductive cycles according to the methodologies proposed by APHA (2012). The DO was maintained above 7.0 mg·L⁻¹ through side-channel blowers and air diffusers in tanks for reproduction and above 4.5 mg·L⁻¹ in stocking tanks.

In tanks for stocking (BFT), when TAN concentrations reached values above 1 mg·L⁻¹, cane sugar was added to stimulate heterotrophic bacteria growth and to reduce TAN, using a C:N ratio of 6:1 (Ebeling et al., 2006). To avoid an excessive presence of suspended solids, water renewal rates of 17.5% were adopted twice a week (about 5% daily), as recommended by Manduca et al. (2021). The volume of settleable solids (SS) was measured once in each reproductive cycle through 1,000 mL Imhoff cones after sedimentation for 15 min.

In tanks for reproduction (clear-water system), a water renewal rate of 30% was performed before each week of reproduction to ensure low concentration of TAN and nitrite.

3.3.5 Biological indexes

At the end of experiment, the females with abnormal urogenital papilla (n=28) and females with normal urogenital papilla (n=40) were euthanized, the urogenital papillae were photographed, and the body weight (BW) was obtained. The animals were dissected to obtain the weights of the gonad (GW), liver (LW) and viscerosomatic fat (VFW). The ovaries were also photographed. The following biological indexes were calculated for each female: gonadosomatic index ($GSI = GW \times 100 / BW$), hepatosomatic index ($HSI = LW \times 100 / BW$), and viscerosomatic fat index ($VFI = VFW \times 100 / BW$).

3.3.6 Bacteriological analysis

To evaluate if the abnormality in urogenital papilla could be associated with an infectious disease, bacteriological examination was performed to address potential systemic or gonadal infections. Fragments of brain, kidney, spleen, and gonads from eight abnormal females were aseptically sampled, streaked onto tryptone soybean agar (Kasvi, Italy) supplemented with 5% defibrinated sheep blood (TSAB), and MacConkey agar. The plates were incubated at 28 °C for 48 h to bacterial isolation.

3.3.7 Statistical analysis

For water quality and biological indexes, when the assumptions of ANOVA (normality and homogeneity) were not met, the variables were analyzed by Mann-Whitney non-parametric tests. Chi-square test was used to analyze the distribution of ovaries in three classes: normal, or with lower or with high severity of lesions in the different female groups.

For the analysis of reproductive performance (proportion of effective reproduction and number of larvae produced, non-Normal variables) in each cycle (first cycle = 28 °C, second cycle = 26 °C and third cycle = 24 °C), tanks of females with normal papilla were compared to tanks of females with abnormal papilla using Mann-Whitney test. In addition, a logistic

regression (Model 1) and quasipoisson regression (Model 2) (Agresti, 2015) were fitted for proportion of effective reproduction and number of larvae produced per tank, respectively.

In logistic regression, we considered the probability of the female effectively reproduce (π) as a binary response variable (if female produced larvae: $\pi = 1$, otherwise $\pi = 0$), the intercept, the effect of the female group (normal = 1 or abnormal = 0), and the effect of the week of reproduction (effects of second (2) and third (3) week of reproduction compared to first week of reproduction), and the effects of water temperature of tank for reproduction. The effect of week of reproduction includes the effects of females and water quality (other than temperature) in each week. The temperature and week of reproduction were control variables, i.e., they are not of direct interest to our study of each cycle (temperature was the direct interest when the analyses were between the cycles), but they were controlled to prevent possible biases caused by their influences in the analysis of the reproductive performance of the different groups (normal and abnormal females).

In quasipoisson regression, tanks of females with normal papilla were compared to tanks of females with abnormal papilla for the number of larvae produced per tank. The model included the intercept, the effect of the female group (normal = 1 or abnormal = 0), the effect of the week of reproduction (effects of second (2) and third (3) week of reproduction compared to first week of reproduction), and the effects of tank water temperature. Again, temperature and week of reproduction were control variables. The effect of week of reproduction includes the effects of females and water quality (other than temperature) in each week.

The goodness of fit of logistic and quasipoisson regression models was evaluated by the Hosmer-Lemeshow test (used only for the Model 1) and Pearson chi-square goodness-of-fit test (used in Model 1 and 2).

The results were analyzed using the 'R' software (R Core Team, 2022) and Tidyverse (Wickham et al., 2019), Car (Fox and Weisberg, 2019), gridExtra (Auguie, 2017) and ResourceSelection packages (Lele et al., 2019).

3.4 Results

3.4.1 Water quality

In the tanks for stocking, where females with normal and abnormal urogenital papilla shared the same tank, the water quality variables (Supplementary material: Table S1) were within the recommended range for the tilapia culture (dissolved oxygen $> 4 \text{ mg}\cdot\text{L}^{-1}$, 6-9 pH (Wedemeyer, 1996), alkalinity $> 50 \text{ mg of CaCO}_3\cdot\text{L}^{-1}$ (Avnimelech, 2012), TAN $< 1 \text{ mg}\cdot\text{L}^{-1}$ and nitrite $< 8 \text{ mg}\cdot\text{L}^{-1}$ (El-Sayed, 2019). Water quality parameters (temperature, pH, TAN, nitrite, and alkalinity) did not differ between tanks for reproduction of Nile tilapias with normal and abnormal urogenital papilla (Supplementary material: Table S2). The three reproduction cycles were performed with different average temperatures, being about 28°C , 26°C and 24°C for the first, second and third cycle, respectively. TAN presented values $< 1 \text{ mg}\cdot\text{L}^{-1}$ and nitrite $< 8 \text{ mg}\cdot\text{L}^{-1}$, as recommended by El-Sayed (2019). Alkalinity had values above $60 \text{ mg de CaCO}_3\cdot\text{L}^{-1}$ (recommended by Sá (2012) for freshwater fish production) and values of pH were near to neutrality in both experimental groups and during all reproductive cycles.

Table S1: Mean, standard deviation (SD), median, minimum (Min) and maximum (Max) of water quality variables in stocking tanks* of Nile tilapia (*Oreochromis niloticus*) during three reproductive cycles.

Water quality parameters		Stocking tanks		
First reproduction	n	Mean $\pm$ SD	Median	(Min -Max.)
Temperature (°C) (daily mean)	3	28.59 $\pm$ 0.22	28.62	(28.36 - 28.80)
Temperature (°C) - 8 am	3	27.35 $\pm$ 0.27	27.40	(27.06 - 27.60)
Temperature (°C) - 4 pm	3	28.83 $\pm$ 0.32	29.67	(29.63 - 30.20)
pH (daily mean)	3	6.45 $\pm$ 0.70	6.11	(5.97 - 7.25)
pH - 8 am	3	6.72 $\pm$ 0.64	6.65	(6.12 - 7.40)
pH - 4 pm	3	6.16 $\pm$ 0.90	6.10	(5.3 - 7.10)
TAN (mg/L)	3	0.54 $\pm$ 0.32	0.63	(0.13 - 0.87)
Nitrite (mg/L)	3	0.41 $\pm$ 0.24	0.45	(0.16 - 0.64)
Second reproduction	n	Mean $\pm$ SD	Median	(Min -Max.)
Temperature (°C) (daily mean)	3	27.05 $\pm$ 1.01	26.65	(26.30 - 28.20)
Temperature (°C) - 8 am	3	26.46 $\pm$ 1.01	26.60	(25.40 - 27.40)
Temperature (°C) - 4 pm	3	27.63 $\pm$ 1.52	27.90	(26.00 - 29.00)
pH (daily mean)	3	6.67 $\pm$ 0.37	6.78	(6.25 - 6.98)
pH - 8 am	3	6.68 $\pm$ 0.43	6.85	(6.20 - 7.00)
pH - 4 pm	3	6.65 $\pm$ 0.33	6.70	(6.30 - 6.95)
TAN (mg/L)	3	0.23 $\pm$ 0.09	0.26	(0.13 - 0.31)
Nitrite (mg/L)	3	0.66 $\pm$ 0.65	0.30	(0.27 - 1.40)
Third reproduction	n	Mean $\pm$ SD	Median	(Min -Max.)
Temperature (°C) (daily mean)	3	24.91 $\pm$ 1.58	24.15	(23.85 - 26.71)
Temperature (°C) - 8 am	3	24.14 $\pm$ 2.39	24.50	(21.60 - 26.33)
Temperature (°C) - 4 pm	3	25.67 $\pm$ 1.69	26.10	(23.80 - 27.10)
pH (daily mean)	3	6.71 $\pm$ 0.68	6.55	(6.13 - 7.45)
pH - 8 am	3	6.66 $\pm$ 0.51	6.57	(6.20 - 7.20)
pH - 4 pm	3	6.77 $\pm$ 0.85	6.55	(6.05 - 7.70)
TAN (mg/L)	3	0.50 $\pm$ 0.30	0.33	(0.33 - 0.84)
Nitrite (mg/L)	3	1.72 $\pm$ 2.50	0.31	(0.25 - 4.60)

* In the tanks for stocking, females with normal and abnormal urogenital papilla shared the same tank. Thus, the water quality values were the same for both groups of animals (females with normal and abnormal urogenital papilla). Alkalinity and volume of settleable solids were not measured in all weeks and their means in the stocking tanks were 92.70 ± 35.69 mg of $\text{CaCO}_3 \cdot \text{L}^{-1}$ and 5.47 ± 2.63 $\text{ml} \cdot \text{L}^{-1}$, respectively.

Table S2: Mean, standard deviation (SD), median, minimum (Min) and maximum (Max) of water quality variables in reproduction tanks of Nile tilapia (*Oreochromis niloticus*) with normal and abnormal urogenital papilla during three reproductive cycles.

Water quality parameters		Normal Females			Abnormal Females			
First reproduction	N	Mean $\pm$ SD	Median	(Min -Max.)	N	Mean $\pm$ SD	Median	(Min -Max.)
Temperature ($^{\circ}$ C) (daily mean) *	12	28.12 $\pm$ 0.86	28.09	(26.60 -29.62)	12	28.15 $\pm$ 0.75	28.00	(27.05 - 29.28)
Temperature ($^{\circ}$ C) - 8 am *	12	27.29 $\pm$ 0.62	27.45	(26.00 -28.25)	12	27.40 $\pm$ 0.44	27.40	(26.55 - 28.20)
Temperature ($^{\circ}$ C) - 4 pm *	12	28.95 $\pm$ 1.13	28.80	(27.20 - 31.00)	12	28.89 $\pm$ 1.13	28.61	(26.85 - 30.37)
pH (daily mean) *	12	7.17 $\pm$ 0.14	7.18	(6.92 -7.38)	12	7.12 $\pm$ 0.09	7.11	(6.94 - 7.25)
pH - 8 am *	12	7.32 $\pm$ 0.16	7.37	(7.05 -7.55)	12	7.26 $\pm$ 0.16	7.24	(6.93 - 7.50)
pH - 4 pm **	12	7.02 $\pm$ 0.19	7.00	(6.70 -7.30)	12	6.98 $\pm$ 0.11	7.00	(6.80 - 7.15)
TAN (mg·L ⁻¹) **	12	0.28 $\pm$ 0.10	0.25	(0.13 - 0.46)	12	0.31 $\pm$ 0.18	0.27	(0.11 -0.76)
Nitrite (mg·L ⁻¹) **	12	1.40 $\pm$ 1.23	0.79	(0.07 -3.32)	12	1.16 $\pm$ 0.83	0.91	(0.27 - 2.78)
Alkalinity (mg of CaCO ₃ ·L ⁻¹) **	8	105.62 $\pm$ 21.29	102.50	(85.00 -150.00)	8	96.25 $\pm$ 20.66	90.00	(80.00 – 145.00)
Second reproduction	N	Mean $\pm$ SD	Median	(Min -Max.)	N	Mean $\pm$ SD	Median	(Min -Max.)
Temperature ($^{\circ}$ C) (daily mean) *	12	26.15 $\pm$ 0.88	26.34	(24.68 - 27.52)	12	26.21 $\pm$ 0.86	26.04	(24.73 - 27.52)
Temperature ($^{\circ}$ C) - 8 am *	12	25.52 $\pm$ 0.63	25.67	(24.40 - 26.50)	12	25.59 $\pm$ 0.73	25.83	(23.80 - 26.40)
Temperature ($^{\circ}$ C) - 4 pm *	12	26.77 $\pm$ 1.35	26.95	(24.60 - 28.55)	12	26.83 $\pm$ 1.35	26.80	(25.00 - 28.65)
pH (daily mean) **	12	7.15 $\pm$ 0.15	7.16	(6.94 - 7.35)	12	7.22 $\pm$ 0.21	7.16	(6.97 - 7.71)
pH - 8 am **	12	7.32 $\pm$ 0.14	7.35	(7.10 - 7.50)	12	7.37 $\pm$ 0.21	7.32	(7.15 - 7.95)
pH - 4 pm **	12	6.98 $\pm$ 0.19	7.00	(6.60 - 7.25)	12	7.07 $\pm$ 0.25	7.00	(6.70 - 7.50)
TAN (mg·L ⁻¹) **	12	0.13 $\pm$ 0.08	0.12	(0.03 - 0.29)	12	0.22 $\pm$ 0.17	0.15	(0.04 - 0.52)
Nitrite (mg·L ⁻¹) **	12	0.27 $\pm$ 0.15	0.27	(0.07 - 0.66)	12	0.38 $\pm$ 0.28	0.33	(0.07 - 1.01)
Alkalinity (mg of CaCO ₃ ·L ⁻¹) **	4	86.25 $\pm$ 17.5	87.50	(65.00 – 105.00)	4	100.00 $\pm$ 21.21	92.50	(85.00 – 130.00)
Third reproduction	N	Mean $\pm$ SD	Median	(Min -Max.)	N	Mean $\pm$ SD	Median	(Min -Max.)
Temperature ($^{\circ}$ C) (daily mean) *	11	23.95 $\pm$ 1.30	23.55	(22.40 - 26.05)	12	23.66 $\pm$ 1.45	23.58	(21.00 - 26.25)
Temperature ($^{\circ}$ C) - 8 am *	11	22.94 $\pm$ 1.85	22.40	(20.00 - 25.70)	12	22.79 $\pm$ 1.68	22.15	(20.80 – 26.00)
Temperature ($^{\circ}$ C) - 4 pm *	11	24.95 $\pm$ 1.75	25.40	(22.40 - 27.10)	12	24.52 $\pm$ 1.99	25.05	(21.20 - 27.50)
pH (daily mean) *	11	7.27 $\pm$ 0.26	7.27	(6.85 - 7.70)	12	7.27 $\pm$ 0.15	7.29	(7.05 - 7.50)
pH - 8 am *	11	7.48 $\pm$ 0.26	7.50	(7.00 - 7.90)	12	7.47 $\pm$ 0.27	7.50	(7.00 - 7.90)
pH - 4 pm **	11	7.06 $\pm$ 0.29	7.03	(6.50 - 7.50)	12	7.06 $\pm$ 0.12	7.10	(6.80 - 7.20)
TAN (mg·L ⁻¹) **	11	0.16 $\pm$ 0.07	0.17	(0.02 -0.26)	12	0.17 $\pm$ 0.12	0.18	(0.02 - 0.34)
Nitrite (mg·L ⁻¹) **	11	0.56 $\pm$ 0.64	0.21	(0.06 - 1.96)	12	0.29 $\pm$ 0.23	0.20	(0.09 - 0.93)
Alkalinity (mg of CaCO ₃ ·L ⁻¹) **	3	108.33 $\pm$ 17.56	110.00	(90.00 – 125.00)	4	121.25 $\pm$ 13.15	117.50	(110.00 – 140.00)

* Means in the same row did not differ according to ANOVA ($p > 0.05$); ** Values in the same row did not differ by Mann-Whitney test ($p > 0.05$)

3.4.2 Anatomical description of Nile tilapia with abnormal urogenital papilla

The female tilapias were classified into normal and abnormal urogenital papillae, and when anomalies were present, they were categorized into groups of animals with mild or severe involvement (Figure 2). Normal females had prominent urogenital papillae, rosy or reddish, with three well-formed and delimited orifices, corresponding to anus, oviduct, and urinary pore. In addition, females with normal papillae had a slightly distended abdomen, soft, consistent with females in breeding period (Vazzoler, 1996).

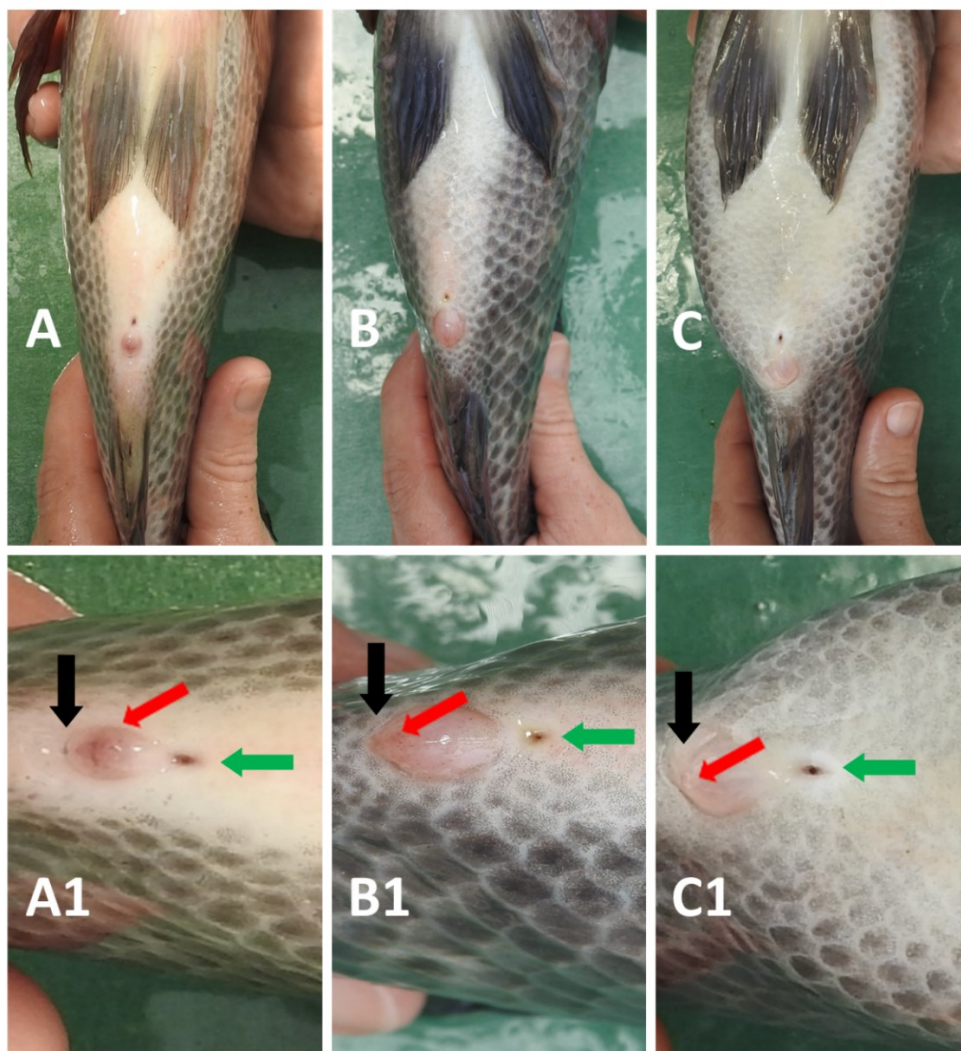


Figure 2: Comparison of Nile tilapias (*Oreochromis niloticus*) females with urogenital papilla considered normal (A, A1) and abnormal (B, B1 and C, C1). A and A1) image and enlarged image, respectively, of a female papilla classified as normal, well-formed, rosy, and/or reddish papillae with anus (green arrow), oviduct (red arrow) and urinary pore (black pore) well defined. B and B1) female classified with mild malformation, with misshapen, swollen and slightly reddened papilla, slightly distended abdomen. C and C1) papilla with severe involvement was remarkably similar to male papilla, with marked dysmorphia and abdominal severe swelling.

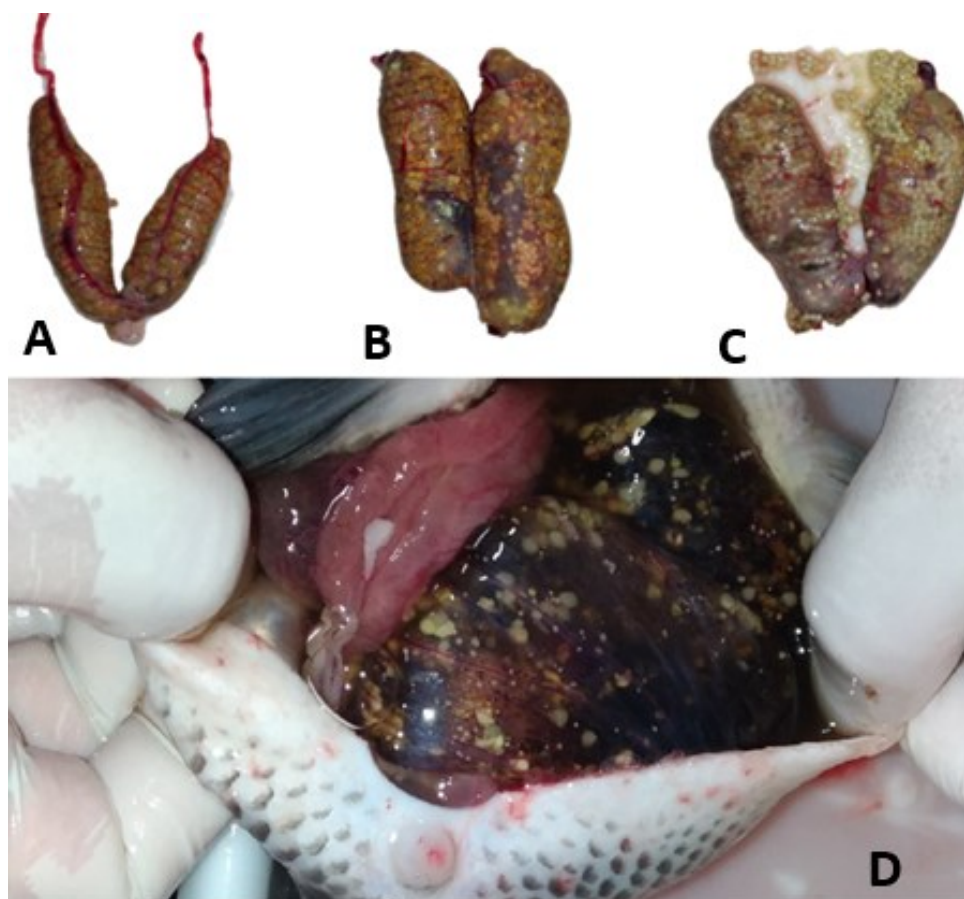


Figure 3: Macroscopic classification of the ovaries. A) Normal ovaries: bilateral, with similar sizes, saccular and fusiform shape, with yellowish orange mature eggs with normal vascularization and normal thickness capsule, B) ovaries with low severity lesions: non-uniform, increase in volume and shape loss, due to excess retained eggs and difficulties in spawning, appearance of atretic and necrotic eggs in greater quantity, hemorrhagic foci, with fluid collection, altered vascularization with decrease in thickness C) ovaries with high severity lesions: non-uniform, normal shape loss with excessive size increased, full of atretic eggs and hemorrhagic foci, presence of firm, blackened masses with variable shapes and sizes. Friable organ that is easy to rupture during removal and D) open coelomic cavity with ovaries occupying practically the entire cavity, high severity ovarian lesions, with extensive internal hemorrhage, atretic and necrotic eggs, in addition to diffusely distributed blackened masses.

The abnormal female papillae showed malformation signs, and according to the degree of involvement, they were classified as mild (grade 1: 79.5% of abnormal females) or severe (grade 2: 20.5% of abnormal females) involvement. The females with grade 1 abnormal papillae had the oviduct outlet poorly delimited or with mild stenosis, in addition to a slight abdominal bulge in some cases. Females classified as grade 2 had conical papillae with an appearance like that of males, severely stenosed oviducts exit, which probably impaired spawning, causing congestion and petechial hemorrhages in the

papilla. Discrete and severe alterations of urogenital papilla were often accompanied by distended abdomens, with considerable to severe involvement.

The Nile tilapia females in this study were from a fish group of same age. However, the mean female's weight with abnormal urogenital papilla (mean weight = 717.0 ± 149.6) was significantly higher than the female's weight with normal urogenital papilla (mean weight = 544.8 ± 134.7) at Mann-Whitney test ($p < .05$).

Figure 3 shows the ovaries of females with urogenital papilla considered normal and abnormal. The ovaries morphology was classified into normal, low-severity lesions and high-severity lesions. The normal ovaries were bilateral organs, with similar length between them, located in the upper part of the coelomic cavity, ventral to the swim bladder, sacculiform, tubular and elongated, with diffuse vascularization. In adult females kept in conditions suitable for reproduction, normal tilapia ovaries develop, occupying approximately one third of the coelomic cavity, being easily identified among the other organs. Macroscopically, a greater amount of large yellowish orange mature eggs was observed, and in distinct stages of maturation, abundantly distributed throughout the organ.

The ovaries distribution considered normal and with different degrees of involvement was significantly different between female tilapia with normal and abnormal urogenital papilla (Chi-square test – $p < .05$) (Figure 4). More than 65% of the females with altered urogenital papilla presented some injury degree in the ovaries, and 29% of the animals in this group presented high severity lesions. In contrast, 64% of the females with a normal urogenital papilla had completely normal ovaries and only 4% of them had gonads with high severity injuries.

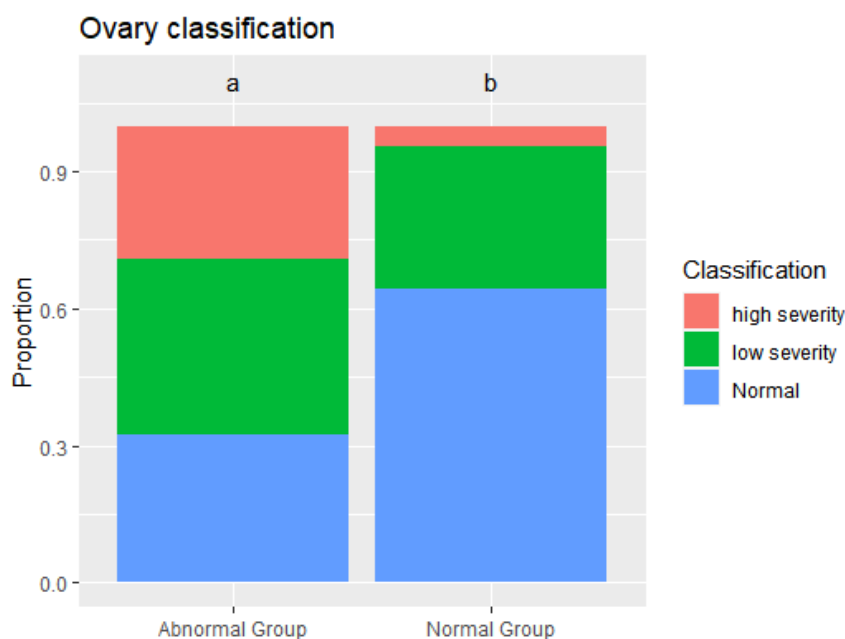


Figure 4: The distribution of normal ovary and ovary with low or high severity lesions between Nile tilapia (*Oreochromis niloticus*) with normal and abnormal urogenital papilla. Ovaries classified as low severity lesions had slightly increased volume due to retained eggs and difficulties in spawning, appearance of atretic and necrotic eggs in greater quantity compared to normal ovaries. Ovaries with high severity lesions were non-uniform and had excessive size increased, full of atretic eggs and hemorrhagic foci, presence of firm, blackened masses with variable shapes and sizes. The distribution of ovary types was significantly different by Chi-square test ($p < .05$).

3.4.3 Reproductive performance

The proportion of animals that effectively reproduced and the larvae number produced per tank during the three reproductive cycles evaluated were considerably higher in females with normal urogenital papilla in comparison to females with abnormal urogenital papilla (Figure 5, Mann-Whitney test: $p < .05$). On average, 33.63%, 40.77% and 21.59% of normal females reproduced in the first, second and third cycle of reproduction. In contrast, the mean proportion of abnormal females that reproduced per tank was 14.29%, 13.69% and 2.08% in the first ($\sim 28^\circ\text{C}$), second ($\sim 26^\circ\text{C}$) and third ($\sim 24^\circ\text{C}$) reproduction cycle, respectively. Only one female categorized with abnormal papilla with severe involvement (grade 2) reproduced in the first reproduction cycle. In the second and third reproduction cycles, no abnormal female with papilla classified as grade 2 reproduced. When only females that effectively reproduced were considered, the number of produced larvae per female was similar between the groups (Figure 6, top, Mann-Whitney test: $p > .05$). This suggests that females with abnormal urogenital papillae

have a considerable reduction in reproductive capacity, however, those animals that reproduce even with this dysmorphia, generate a number of offspring similar to that of females with normal urogenital papilla. The number of larvae produced per female was similar between the groups, but the larvae production per kilogram of female was significantly higher in the group of animals with normal urogenital papilla (Figure 6 (bottom), Mann-Whitney test: $p < .05$), since these females had lower body weight than those with abnormal urogenital papilla.

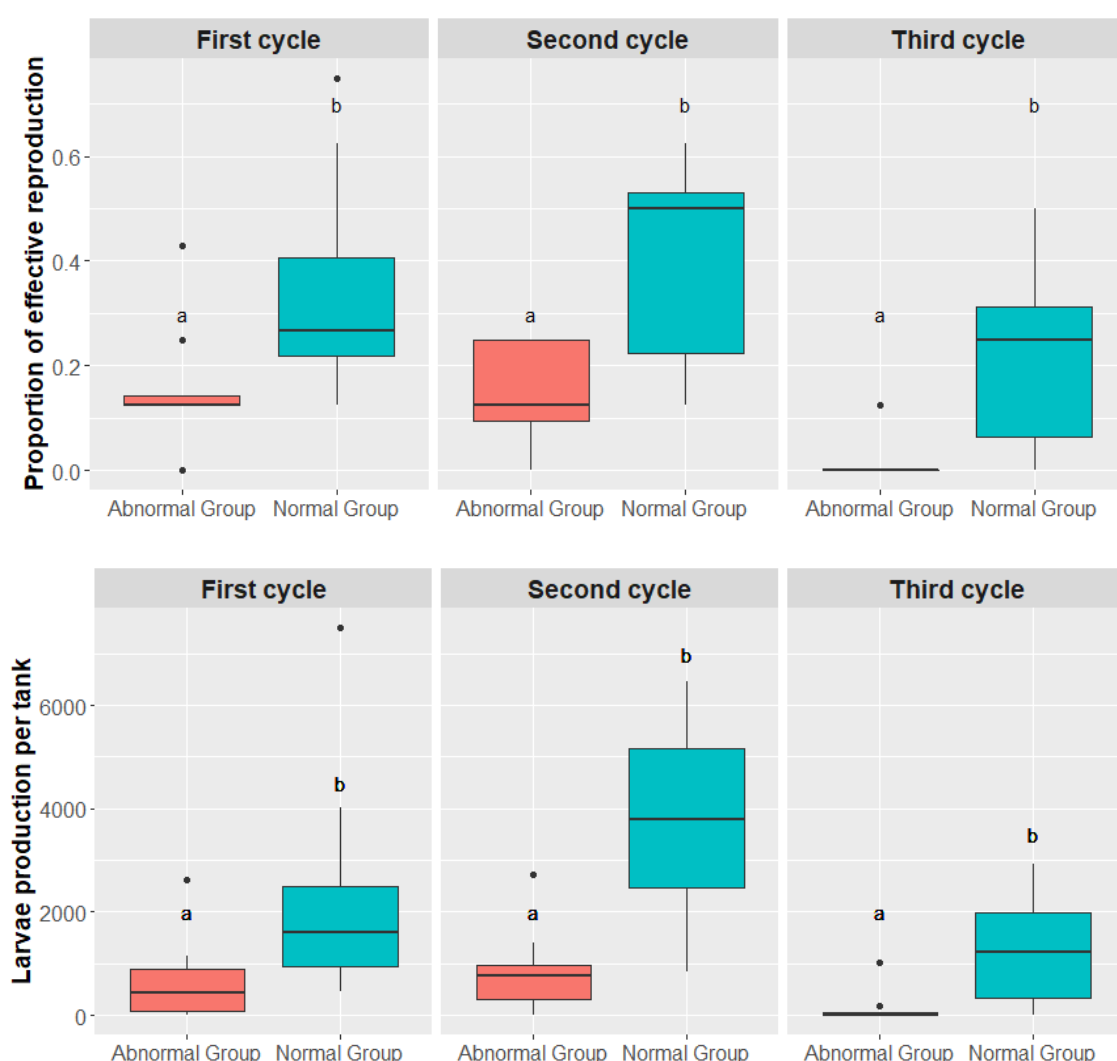


Figure 5: Proportion of effective reproduction (females that effectively produced larvae divided by the total number of females in reproduction tanks) (top) and number of larvae produced per tank (bottom) of Nile tilapia (*Oreochromis niloticus*) females with normal and abnormal urogenital papilla during three different reproduction cycles. In the same reproductive cycle, different letters indicate significant differences by Mann-Whitney test ($p < .05$).

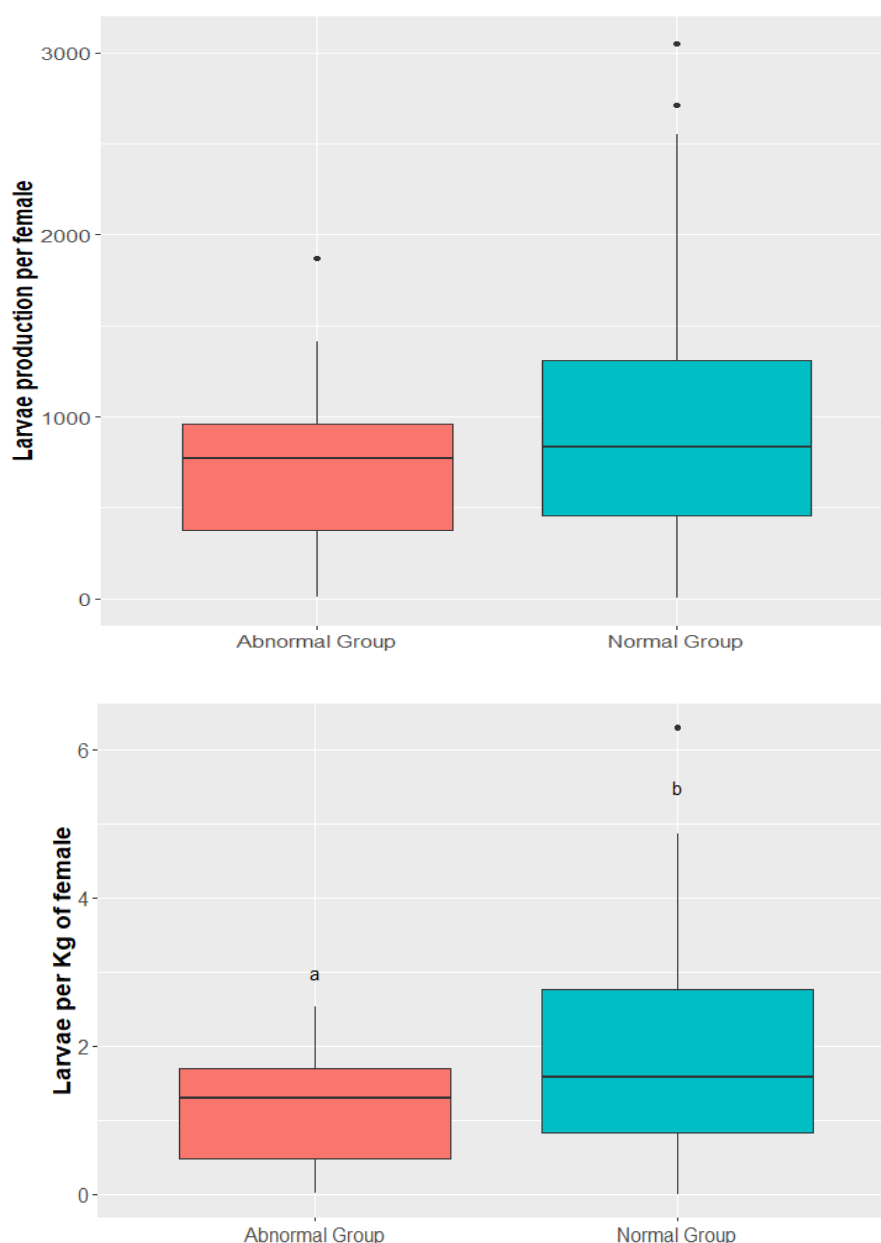


Figure 6: Larvae number produced per female of Nile tilapia (*Oreochromis niloticus*), considering only females that produced larvae. The larvae production did not differ between females with normal and abnormal urogenital papilla by Mann-Whitney test ($p > .05$) (top); The production of larvae per female kilogram (bottom) in Nile tilapia females with normal urogenital papilla was significantly higher than those of females with abnormal urogenital papilla by Mann-Whitney test ($p < .05$).

The results of fitted logistic regression models to effective reproduction during three consecutive cycles are in Table 1. In all reproductive cycles, the group effect (normal or abnormal) was significant ($p < .05$). The odds of females with normal urogenital papilla effectively reproduced was approximately 3, 5 and 12 times that of females with abnormal papilla in the first (at 28 °C), second (at 26 °C) and third (at 24 °C) cycles,

respectively. Temperature and reproduction weeks were control variables in the models since subtle variations in temperature or other water quality variables may occur in tanks for reproduction throughout the three-weeks period of each cycle. The effects of temperature of tanks for reproduction and the week of reproduction was not significant in the first cycle of reproduction. The third week of reproduction in the second cycle favored the effective reproduction compared to the first one. In the third reproduction cycle (temperature $\sim 24^{\circ}\text{C}$), a positive effect of temperature on reproduction was identified, and an increase of one degree Celsius in tanks for reproduction was associated with an increase of 448 % in the odds of effective reproduction. It is noteworthy that the temperature of the tanks for reproduction did not differ between the experimental groups, but temperature fluctuations between the tanks occur naturally in a greenhouse. The logistic model indicated that slightly higher temperatures in the third cycle favored reproduction.

The quasipoisson generalized linear model was used to evaluate the urogenital papilla type effect on the production of larvae per tanks (Table 2). The number of larvae per tank is approximately 3.4, 4.7 and 5.2 times that females with normal urogenital papilla compared to females with abnormal urogenital papilla in the first, second and third reproductive cycle, respectively. Again, temperature and reproduction weeks were control variables in the models. The effect of temperature was significant in third cycle, and an increase of one degree Celsius in tanks for reproduction was associated with an increase of 263 % in the number of larvae produced per tank.

Table 1: Results of fitting the logistic regression model to effective reproduction (proportion of females that effectively generated larvae) in reproduction tanks of Nile tilapia (*Oreochromis niloticus*) with normal and abnormal urogenital papilla during three reproductive cycles.

First cycle (~ 28 °C)					
	Coefficient	OR**	IC - 95% - OR	Interpretation (%) ***	p-value
Intercept	-5.50	-	-	-	0.490
Normal Group	1.17	3.23	1.55 - 6.72	223.33	0.002
Temperature (°C)	0.13	1.14	0.65 - 1.97	-	0.646
Reproduction week (2) *	-0.44	0.64	0.26 - 1.60	-	0.343
Reproduction week (3) *	0.46	1.58	0.63 - 3.95	-	0.326
Second cycle (~ 26 °C)					
	Coefficient	OR**	IC - 95% - OR	Interpretation (%)	p-value
Intercept	-2.20	-	-	-	0.72
Normal Group	1.53	4.61	2.22 - 9.59	361.00	<0.001
Temperature (°C)	-0.01	0.99	0.62 - 1.58	-	0.964
Reproduction week (2) *	0.56	1.76	0.66 - 4.66	-	0.258
Reproduction week (3) *	1.13	3.11	1.16 - 8.33	210.87	0.024
Third cycle (~ 24 °C)					
First cycle	Coefficient	OR**	IC - 95% - OR	Interpretation (%)	p-value
Intercept	-46.91	-	-	-	0.019
Normal Group	2.503	12.2 2	2.57 - 58.04	1121.91	0.002
Temperature (°C)	1.701	5.48	1.22 - 24.64	447.94	0.027
Reproduction week (2) *	2.226	9.26	0.39 - 217.36	-	0.167
Reproduction week (3) *	3.901	49.5	0.43 - 5654.9	-	0.107

*The first week of reproduction (reference) was compared to the second and third weeks of reproduction. **OR = Odds ratio. *** Interpretation (%) indicates the percentage increase in proportion of females effectively reproduced of the group with normal urogenital papilla compared to the group with abnormal urogenital papilla.

Table 2: Results of fitting quasipoisson generalized linear model for number of larvae produced per tank of Nile tilapia (*Oreochromis niloticus*) with normal and abnormal urogenital papilla during three reproductive cycles.

First cycle (~ 28 °C)					
	Coefficient	Impact on the rate	IC - 95%	Interpretation (%) **	p-value
Intercept	11.19	-	-	-	0.180
Normal Group	1.23	3.42	[1.44 - 8.10]	242.12	0.011
Temperature (°C)	-0.24	0.79	[0.45 - 1.37]	-	0.408
Reproduction week (2) *	-0.57	0.57	[0.19 - 1.65]	-	0.310
Reproduction week (3) *	0.24	1.27	[0.51 - 3.14]	-	0.6115
Second cycle (~ 26 °C)					
	Coefficient	Impact on the rate	IC - 95%	Interpretation (%)	p-value
Intercept	0.20	-	-	-	0.959
Normal Group	1.54	4.65	[2.71 - 8.04]	365.48	<0.001
Temperature (°C)	0.15	1.17	[0.87 - 1.55]	-	0.186
Reproduction week (2) *	0.45	1.57	[0.82 - 2.98]	-	0.076
Reproduction week (3) *	0.60	1.83	[0.97-3.43]	-	0.311
Third cycle (~ 24 °C)					
First cycle	Coefficient	Impact on the rate	IC - 95%	Interpretation (%)	p-value
Intercept	-29.23	-	-	-	0.045
Normal Group	1.65	5.18	[1.88 - 14.45]	418.15	0.005
Temperature (°C)	1.29	3.63	[1.30 - 10.14]	263.00	0.025
Reproduction week (2) *	1.32	3.76	[0.14 - 34.70]	-	0.258
Reproduction week (3) *	3.22	25.07	[0.93 - 672.34]	-	0.071

*The first week of reproduction (reference) was compared to the second and third weeks of reproduction. ** Interpretation (%) indicates the percentage increase in number of larvae produced of the group with normal urogenital papilla compared to the group with abnormal urogenital papilla.

3.4.4 Biological Indexes

The viscerosomatic, hepatosomatic, gonadosomatic and viscerosomatic fat indexes (Supplementary material: Figure S1) were not different between the normal and abnormal group (Mann-Whitney test, $p > .05$). As described in Figure 3D, some animals from abnormal groups presented enlarged ovaries with many inviable oocytes and fluid accumulation, which explain the higher value of GSI (outliers in Figure S1).

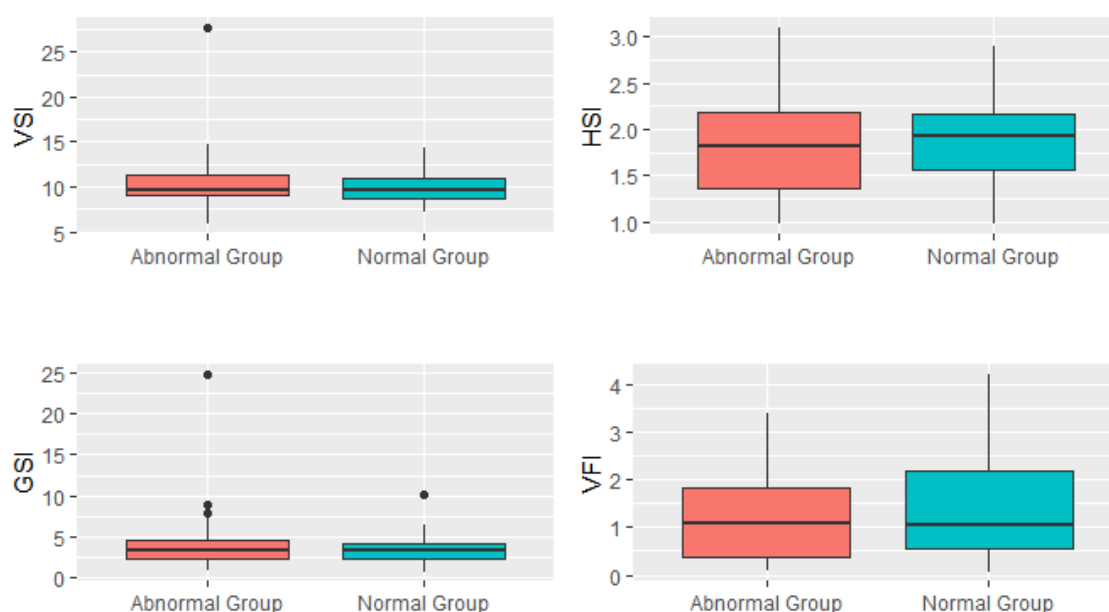


Figure S1: Viscerosomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI) and viscerosomatic fat index (VFI) of Nile tilapia (*Oreochromis niloticus*) females with normal and abnormal urogenital papilla. Values did not differ by Mann-Whitney test ($p > .05$).

3.4.5 Bacteriological analysis

Negative results were obtained in the bacteriological analysis of the eight evaluated fish. Therefore, the inflammatory and necrotic alterations verified in the gonads could not be associated with bacterial agents.

3.5 Discussion

The major problems described in literature for seed production of Nile tilapias are the low egg production during spawning and a lack of spawning synchrony (Bhujel, 2000, Alvarenga et al., 2017; Azevedo et al., 2021). According to our knowledge, a description of dysmorphia in urogenital papilla of Nile tilapia females and its impact on reproductive capacity and fingerlings production has not been reported in the literature. Atypical papilla of tilapia female has only been described in experiments of hormonal feminization (Jensen and Shelton, 1979; Alcántar-Vázquez et al., 2015). In the present study, we described a macroscopic alteration in the urogenital papilla of Nile tilapia females, also associated with lesions in ovaries, which considerably reduce the reproductive performance and the seed production. Pathologies of reproductive systems are poorly described in the literature of teleost fish, and the reports of their impact on seed production are scarce (Khoo, 2019). The most common fish pathologies reported are neoplastic and inflammatory responses (Roberts, 2012). Neoplasms are less reported in fish compared to other vertebrates, but testicular and ovarian tumors are the most frequently reported pathologies in fish reproductive systems (Akhtar et al., 2017). The dysmorphia of the urogenital papilla identified in this work is characterized by loss of the normal conformation of this organ and narrowing of oviduct exit, with different degrees of involvement, which reduces spawning capacity. The reduction of reproductive performance is even more pronounced in females whose urogenital papilla was categorized as grade 2 involvement. In abnormal females, non-spawned oocytes accumulate in ovaries inducing the inflammatory response, fluid accumulation, and finally resulting in changes in morphology of ovary. The hypothesis that the cause of the pathological alterations is due to the narrowing of the oviduct is supported by the fact that the females with abnormal urogenital papillae that reproduced present a total production of larvae similar to the production of normal females and to values obtained in the literature (Ekasari et al., 2015, Azevedo et al., 2021). In this case, it is possible that the degree of obstruction of the oviduct exit is lower, allowing spawning to occur. Interestingly, the efferent duct obstruction was associated to sterility of *cyp19a1b* mutant tilapia males (Zhang et al., 2019). These reproductive alterations in mutant tilapia males were related to decrease in the concentration of 17 β -estradiol in serum of the fish.

The pathology described in this study did not have its etiopathogenesis defined. In fish, reproductive dysfunctions have already been associated with bacterial infection (Khoo, 2019), inbreeding depression (Crnokrak and Roff, 1999; Gjerdem and Baranski, 2009; Fessehaye et al., 2009), stress (Pickering, et al., 1987; Carragher et al., 1989, Schreck, 2010), nutritional deficiencies (Tsadik and Bart, 2007; Lima et al., 2020, Nascimento et al., 2023), exposure to various substances that impair reproductive physiology (Hanson et al., 2007; Murty, 2018; Tazeen and Kulkarni, 2018), and inadequate management conditions such as temperature, photoperiod or other water quality parameters that do not meet the needs of fish breeders (Bhujel, 2000; Mylonas et al., 2010). For several years, technicians and field veterinarians associated the inflammatory and necrotic processes of tilapia gonads with a possible ascending bacterial infection of the oviduct (Khoo, 2019), that resulted in the reproductive dysfunctions described in the present study. Based on the absence of systemic and gonadal bacterial infections in affected fish verified in the present study, the hypothesis of an infectious etiology could not be proved. An effect of inbreeding depression is unlikely in this population since the pedigree of our breeders is recorded in each generation and the estimated coefficients of inbreeding (F) is around 2% (Manduca, 2022). Effects of exposure to substances that alter reproductive physiology, or nutritional deficiencies are also not plausible causes of this pathology since the water quality and the nutrition of breeders are monitored and the conditions for abnormal females were identical to females with normal urogenital papilla.

We consider some plausible hypotheses for the cause of urogenital papilla dysmorphia: (1) the dysmorphia of urogenital papilla might be initially triggered by traumatic events followed by local secondary and transitory infections (not detected in well-established affected fish) which could explain the narrowing of the oviduct. However, the relatively high prevalence of dysmorphia (17 %) in this batch of tilapia females weakens this hypothesis; (2) considering that females with abnormal urogenital papillae were larger than those with normal papillae (even though they were the same age) and had an external reproductive structure similar to that of males, our hypothesis for the dysmorphia described in this work is related to the temperature during the gonadal development phase. The breeders used in this study were produced in a twelve-week period in the summer. About 100 families were produced and they were distributed in hapas placed in different tanks inside a greenhouse. During development, these animals

may have been subjected to different temperatures due to the week they were produced (at the beginning or end of summer), or due to the position of their tanks inside the greenhouse (some tanks have lower temperatures due to the proximity of windows, for example). We think some animals were exposed to slightly higher temperatures, which were not enough to masculinize the animal, however, the normal development of the reproductive ducts and urogenital papilla was compromised. Corroborating this hypothesis, Nozu and Nakamura (2020) induced sterility in tilapias (*Oreochromis mossambicus*) by implementing only a high-temperature water treatment (37 °C) in fry stages. These authors described that the urogenital papillae in treated females were noticeably undeveloped and suggested their possible use as an indicator of sterility. The females evaluated in the present study were not reared in high temperatures (37 °C) during the initial development. However, eventually higher temperatures (>30 °C) may have occurred since the animals were reared in greenhouses during Brazilian summer in early development. These temperatures would not be sufficient to induce sterility, but they could impair the normal development of the reproductive system, probably by affecting the hormonal balance. In fact, the urogenital papilla development in blue tilapia (*Oreochromis aureus*) females is commonly thought to be estrogen dependent (Jensen and Shelton, 1979) and sterilized Mozambique tilapia females evaluated by Nozu and Nakamura (2020) also exhibited low estrogen levels.

Considering the effects of temperature on Nile tilapia reproduction (Brummett, 1995; Bhujel, 2000; Alvarenga and França, 2009; El-Sayed, 2019; Qiang et al., 2022), three reproductive cycles were performed with the same animals at 28 °C, 26 °C and 24 °C (average of temperatures). As expected, the reproductive performance was higher in the cycles of reproductive at 26 °C and 28 °C (El-Sayed, 2019). In all temperatures evaluated, both, the proportion of reproduced females and the number of larvae produced by tank were consistently reduced in females with abnormal urogenital papillae, which provides robust evidence of the negative effects of the dysmorphia of urogenital papilla. Apparently, the reduction of reproductive performance of abnormal females is more pronounced at lower temperatures (the odds ratio of reproduction in normal/abnormal females increases as the temperature decreases). However, the experimental design carried out did not allow inferences about the interaction between temperature and papilla shape, as the three different temperatures were applied at different periods. To confirm this hypothesis, it is necessary to perform an assay in which different temperatures would

be applied in the same period to independent samples of females with normal and abnormal urogenital papillae. The other water quality variables were monitored, and their values were kept in a suitable range of the species.

3.6 Conclusion

The results described in this study can have substantial impact on both seed production and grow-out phase of Nile tilapia. Since evaluation of the urogenital papillae can be easily performed and the morphology of this structure is a good predictor of reproductive capacity and ovary status, it is recommended that tilapia fry producers use this evaluation when selecting females that will be breeders. On the other hand, since the tilapia females with this dysmorphia are characterized by a drastic reduction in reproductive success, the investigation of the causes of this anomaly opens the possibility of developing a methodology to induce sterility, which can be an alternative to the existing methods of sexual control during the grow-out phase. The investigation of potential causes of this dysmorphia by monitoring the breeders from the larval stage is in progress.

3.7 Acknowledgements

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3.8 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3.9 Conflict of interest

The authors have no conflict of interest to declare.

References

- Agresti, A. Foundations of linear and generalized linear models. John Wiley & Sons, 2015.
- Akhtar, M.S., Ciji, A., Sarma, D., Rajesh, M., Kamalam, B.S., Sharma, P., Singh, A.K. 2017. Reproductive dysfunction in females of endangered golden mahseer (*Tor putitora*) in captivity. Anim. Reprod. Sci. 182: 95–103.
- Alcántar-Vázquez, J.P., Rueda-Curiel, P., Calzada-Ruíz, D., Antonio-Estrada, C., la Torre, R.M. 2015. Feminization of the Nile tilapia *Oreochromis niloticus* by estradiol-17 β Effects on growth, gonadal development and body composition. Hidrobiologica, 25(2), 275-283.
- Alcántar-Vázquez, J.P., la Torre, R.M., Calzada-Ruíz, D., Antonio-Estrada, C. 2014. Production of YY-male of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) from atypical fish. Lat. Am. J. Aquat. Res. 42(3): 644-648.
- Alvarenga, E.R., França L.R. 2009. Effects of different temperatures on testis structure and function, with emphasis on somatic cells, in sexually mature Nile tilapias (*Oreochromis niloticus*). Biol Reprod. 2009;80(3):537-544.
- Alvarenga, E. R., Sales, S. C. M., Brito, T. S., Santos, C. R., Corrêa, R. D. S., Alves, G. F. O., ... Turra, E. M. 2017. Effects of biofloc technology on reproduction and ovarian recrudescence in Nile tilapia. Aquac. Res., 48, 5965– 5972.
- APHA - American Public Health Association. Standard methods for the examination of water and wastewater. Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesceri, L.S. 2012. Washington, D.C.: American Public Health Association, American Water Works Association, Water Environment Federation, 1496p.
- Auguie, B. 2017. `_gridExtra`: Miscellaneous Functions for "Grid" Graphics. R package version 2.3, <<https://CRAN.R-project.org/package=gridExtra>>.
- Avnimelech, Y. 2012. Biofloc Technology - A Practical Guidebook, 2d Edition. The World Aquaculture Society, Baton Rouge, Louisiana, United States. doi: 10.13140/2.1.4575.0402.
- Azevedo, R.O., Alvarenga, E.R., Fernandes, A.F.A., da Silva, M.A., Alves, G.F.O.A., Menezes, W.F., Turra, E. M. 2021. Use of hCG hormone in the natural and artificial reproduction of Nile tilapia (*Oreochromis niloticus*). Aquac. Res., 52(12), 6380-6388.
- Baroiller, J.F., Cotta, H.D., 2018. Sex control in tilapias. In: Wang, H.-P., Piferrer, F., Chen, S.-L. (Eds.), Sex Control in Aquaculture. John Wiley & Sons Ltd., pp. 191–234.
- Barroso, R.M., Munoz, A.E.P., Tahim, E.F., Tenório, R.A., Muehlmann, L.D., Silva, F.M., Barreto, L.E.G.S., Hein, G. 2018. Dimensão Socioeconômica da Tilapicultura no Brasil. Embrapa, Brasília, 110p.
- Bendschneider, K., Robinson, R.J. 1952. A new spectrophotometric method for the determination of nitrite in seawater. J. Mar. Res. 11, 87–96.
- Bhujel, R.C. 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. Aquaculture, 181(1-2): 37-59.
- Brummett, R.E. 1995: Environmental regulation of sexual maturation and reproduction in tilapia, Rev.Fish. Sci., 3:3, 231-248
- Campos-Mendoza, A., McAndrew, B.J., Coward, K., Bromage, N. 2004. Reproductive response of Nile tilapia (*Oreochromis niloticus*) to photoperiodic manipulation: Effects on spawning periodicity, fecundity, and egg size. Aquaculture, 231, 299–314.

- Carragher, J.F., Sumpter, J., Pottinger, T. J., Pickering, A.D. 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. Gen. Comp. Endocrinol. 76:310–321.
- Crnokrak, P., Roff, D.A., 1999. Inbreeding depression in the wild. Heredity (Edinb). 83, 260–270.
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture, 257(1-4), 346-358.
- Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawidjaja, E. H., Zairin, M., Bossier, P., De Schryver, P. 2015. Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. Aquaculture, 441, 72– 77.
- El-Sayed, A.-F.M., Kawanna, M. 2008. Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. Aquaculture, 280, 179–184.
- El-Sayed, A.-F. M. 2019. Tilapia culture. 2nd ed. Elsevier Inc, Academic Press, London 348 pp.
- Fessehaye, Y., Bovenhuis, H., Rezk, M.A., Crooijmans, R., Van Arendonk, J.A.M., Komen, H., 2009. Effects of relatedness and inbreeding on reproductive success of Nile tilapia (*Oreochromis niloticus*). Aquaculture 294, 180-186.
- Fernandes, A., Alvarenga, É., Oliveira, D., Aleixo, C., Prado, S., Luz, R., Turra, E., 2013. Production of oocytes of Nile tilapia (*Oreochromis niloticus*) for in vitro fertilization via hormonal treatments. Reprod. Domest. Anim. 48, 1049–1055. <https://doi.org/10.1111/rda.12212>.
- Fox, J., Weisberg, S. 2019. An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Gjedrem, T., Baranski, M. 2010. Selective breeding in aquaculture: an introduction, Volume 10, SSBM, 221p.
- Hanson, R., Dodoo, D.K., Essumang. D.K., Blay Jr.J., Yankson, K. 2007. The effect of some selected pesticides on the growth and reproduction of fresh water *Oreochromis niloticus*, *Chrysichthys nigrodigitatus* and *Clarias gariepinus*. Bull. Environ. Contam. Toxicol. 279: 544–547.
- Jensen, G.L., Shelton, W.L. 1979. Effects of estrogens on Tilapia aurea: implications for production of monosex genetic male tilapia. Aquaculture, 16(3), 233-242.
- Khanjani, M.H., Sharifinia, M., Hajirezaee, S. 2022. Recent progress towards the application of biofloc technology for tilapia farming. Aquaculture, 552, 738021.
- Khoo, L. 2019. Reproductive disorders. In: Smith, S. A. (Ed.), Fish diseases and medicine. CRC Press, 244-256.
- Lele S.R., Keim J.L., Solymos P. 2019. ResourceSelection: Resource Selection (Probability) Functions for Use-Availability Data. R package version 0.3-5, <https://CRAN.R-project.org/package=ResourceSelection>.
- de Lima, S.A., Pedreira, A.C. de O., de Freitas, J.M.A., Dalmaso, A.C.S., Chiella, R.J., Meurer, F., Romão, S., Bombardelli, R.A. 2020. Diets containing purified nucleotides reduce oxidative stress, interfere with reproduction, and promote growth in Nile tilapia females. Aquaculture 528, 735509. <https://doi.org/10.1016/j.aquaculture.2020.735509>.
- Little, D.C., Macintosh, D.J., Edwards, P. 1993. Improving spawning synchrony in the Nile tilapia, *Oreochromis niloticus* (L.). Aquac. Res., 24, 399–405.
- Longalong, F.M., Eknath, A.E., Bentsen, H.B., 1999. Response to bidirectional selection

- for frequency of early maturing females in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 178, 13–25.
- Manduca, L. G. Seleção para peso corporal em tilápia do Nilo em três sistemas de produção: Efeitos sobre características reprodutivas. 2022. Tesis. Universidade Federal de Minas Gerais. Belo Horizonte. 55p.
- Murty, A.S. 2018. Toxicity of Pesticides to Fish, CRC Press: Boca Raton, FL, USA.
- Mylonas, C.C., Fostier, A., Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocrinol.* 165, 516–534.
- Nascimento C.Z., Meurer F., Romão S., Cazarolli L.H., Marcon S., Chagas, T.V., Bombardelli, R.A. 2023. Feed for Nile tilapia broodstock and offspring supplemented with purified nucleotides boosts the juvenile's health, growth, and the resistance face to transport and *Aeromonas hydrophila* challenges. *Anim. Feed Sci. and Technol.* 297, 115568. <https://doi.org/10.1016/j.anifeedsci.2023.115568>
- Ng, W., Romano, N. 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Rev. Aquac.*, 5, 220–254.
- Nozu, R., Nakamura, M. 2020. Influence of prolonged cultivation on sexual characteristics of sterilized female tilapia, *Oreochromis mossambicus*, induced by high-temperature treatment. *Aquaculture*, 524, 735245.
- Piamsomboon, P., Mehl, N.S., Sirivaidyapong, S., Wongtavatchai, J. 2019. Assisted reproduction in Nile tilapia *Oreochromis niloticus*: Milt preservation, spawning induction and artificial fertilization. *Aquaculture*, 507, 139–143.
- Pickering, A.D., Pottinger, T.G., Carragher, J., Sumpter, J.P. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta L.* *Gen. Comp. Endocrinol.*, 68(2), 249-259.
- Pullin, R.S., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M., Abella, T.A. 1991. The genetic improvement of farmed tilapias (GIFT) project: the story so far. *Naga: the ICLARM quarterly*, 14, 3-6.
- Qiang, J., Tao, Y.F., Zhu, J.H., Lu, S.Q., Cao, Z.M., Ma, J.L., ... Xu, P. 2022. Effects of heat stress on follicular development and atresia in Nile tilapia (*Oreochromis niloticus*) during one reproductive cycle and its potential regulation by autophagy and apoptosis. *Aquaculture*, 555, 738171.
- Roberts R.J. 2012. *Fish Pathology*. JohnWiley & Sons.scherer, Hoboken, NJ, 592p.
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Sá, M.V.C. 2012. *Limnocultura: Limnologia para Aquicultura*. Fortaleza, Edições UFC, 219p
- Schreck, C.B. 2010. Stress and fish reproduction: The roles of allostasis and hormesis. *Gen. Comp. Endocrinol.* 165, 549–556.
- Sun, Y., Jiang, D., Zeng, S., Hu, C., Ye, K., Li, M., Wang, D. 2014. Screening and characterization of sex-linked DNA markers and marker-assisted selection in the Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 433(20), 19–27.
- Tazeen, S., Kulkarni, R. 2018. Histopathological impact of profenofos on ovary of the freshwater fish *Notopterus notopterus*. *Asian J. Res. Zool.* 1–7.
- Trong, T.Q.; van Arendonk, J.A.M., Komen, H. 2013. Genetic parameters for reproductive traits in female Nile tilapia (*Oreochromis niloticus*): I. Spawning success and time to spawn. *Aquaculture*, 416–417, 57–64. doi: 10.1016/j.aquaculture.2013.08.032

- Tsadik, G.G., Bart, A.N. 2007. Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 272, 380–388.
- UNESCO. 1983. Chemical methods for use in marine environmental monitoring. Em: *Manual and Guides* 12. IOC, Paris, France.
- Vazzoler, A. E. A. M. 1996. *Biologia da reprodução de peixes teleósteos. Teoria e Prática*. Maringa, EDUEM, 169p.
- Wedemeyer, G. 1996. *Physiology of fish in intensive culture systems*. Springer Science & Business Media, New York, 232p.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., ..., Yutani, H. 2019. “Welcome to the Tidyverse.” *J. Open Source Softw.*, 4, 1686.
- Young, B.C., Alfaggeh, R.H., AlMoutiri, I. 2021. Growth, Fry Production, and Cost Analysis for Sabaki Tilapia Production Systems. *North Am J Aquaculture*, 83, 290-293.
- Yoshida, G.M., Oliveira, C.A.L. De, Kunita, N.M., Rizzato, G.S., Ribeiro, R.P. 2015. Reproduction performance of female Nile tilapia under different environments and age classes. *Acta Scientiarum. Anim. Sci.*, 37, 221–226.
- Zhang, X., Min, Q., Li, M., Liu, X., Li, M., Wang, D. 2019. Mutation of *cyp19a1b* results in sterile males due to efferent duct obstruction in Nile tilapia. *Mol. Reprod. Dev.*, 86(9), 1224-1235.

4 A LONG-TERM HIGH TEMPERATURE ON YOUNG NILE TILAPIA FEMALES AFFECTS ITS UROGENITAL PAPILLA MORPHOLOGY AND FUTURE REPRODUCTIVE PERFORMANCE

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4.1 Abstract

Dysmorphia in urogenital papilla of Nile tilapia drastically reduces the reproductive performance of this species. However, the study of factors that affect the prevalence of this anomaly has not yet been performed. This study evaluated genetic and environmental (temperature and bacterial agent) effects on the prevalence of urogenital papilla dysmorphia and their impact on reproductive performance, hormonal profile, and biological indices. For such, 2,400 larvae from the base generation of a breeding program (without selection or G0) and 3,600 larvae from generation 8 (generation obtained after seven generations of selection for body weight - G8) were distributed in 20 tanks of 150L and exposed to temperature treatments for 60 days after yolk sac absorption (daysa). The G0 and G8 fish were under two and three different ranges of temperature, respectively, as following: control temperature from 25-28°C (CT-G8), moderate temperature from 27-30°C (MT-G8 and MT-G0), and high temperature from 30-33°C (HT-G8 and HT- G0), totaling 5 treatments with 4 replicates. After 60 days, tilapias from the different treatments were reared in the same tank and identified by PIT tags when they achieved 50 g. The animals at six months of age were sexed, and females have the urogenital papillae classified as normal or abnormal (mild or severe), and ten abnormal females were sampled for bacteriological evaluation. The tilapia females were reevaluated at 8 months of age to determine the prevalence of the dysmorphia. To evaluate the impact of treatments on reproductive performance, 70 females per treatment were used in a reproductive assay for 8 weeks. At the end of reproductive assay, the blood plasma was collected to determine the levels of 17-beta-estradiol and 11-ketotestosterone. All samples evaluated were negative in bacteriological analysis. The prevalence of urogenital papilla dysmorphia in CT-G8, MT-G8, HT-G8, MT-G0 and HT-G0 were 20.49%, 61.59%, 89.48%, 68.39%, and 43.70%, respectively. The dysmorphia prevalence was similar only between MT-G0 and MT-G8 ($p>.05$). Except by the HT-G0 group, all treatments presented significant reduction close to 70% in the reproductive performance in comparison to the control ($p<.05$). Animals with severe dysmorphia exhibited the highest values of gonadosomatic and viscerosomatic indexes. The HT-G8 group presented a higher concentration of estradiol in plasma, but like MT-G0 group. The CT-G8 showed the highest value of 11-ketotestosterone among all treatments. In conclusion, the rearing of Nile tilapia in temperature close to 25°C in the first 60 daysa is recommended to produce an efficient broodstock.

Keywords: efficient broodstock, environmental and genetic effects, *Oreochromis niloticus*, urogenital papilla dysmorphia, global warming.

4.2 Introduction

Tilapia culture is expanding at a high rate (FAO, 2022) and the more it is expanded, the more crucial it becomes to produce enough seeds to meet the increasing demand of tilapia farmers (El-Sayed, 2019). Nile tilapias easily reproduce under captivity conditions when they meet suitable water quality and food supply (Pullin et al., 1991; Bhujel, 2000; El-Sayed, 2019; Piamsomboon et al., 2019; Azevedo et al., 2021). However, the presence of inefficient breeders in broodstock and poor hatchery management can result in insufficient production of seeds and reduced growth rates of them (Bhujel, 2000; El-Sayed, 2019; Bezerra et al., 2023). Therefore, identification of predictable factors that impact reproductive capacity is necessary for optimizing hatchery operation and maximizing tilapia culture outputs (El-Sayed, 2019).

Bezerra et al. (2023) described a dysmorphia in urogenital papilla of Nile tilapia that drastically reduces its reproductive performance. These authors did not find association between this dysmorphia and bacterial agents and hypothesized that higher temperatures during the gonadal development phase would promote an increase in the prevalence of dysmorphia. A Chitralada strain of Nile tilapia was used in the previous study of Bezerra et al. (2023). The investigation of this dysmorphia in different genetic groups could indicate the role of genetic effects on etiopathogenesis of alterations of urogenital papilla. Identifying the causes of reproductive dysfunctions can help fish farms prevent them, avoiding economic losses in commercial hatcheries (Bhujel, 2000; Mylonas et al., 2010). Checking the possible effects of higher temperature on the genesis of the urogenital dysmorphia is essential for tilapia culture in face of the possible effects of global warming. Several authors have reported various physiological effects in Nile tilapia when exposed to high temperatures. These effects include morphological changes in gonads during high-temperature-induced masculinization (Sun et al., 2016; Wang et al., 2019; Zhao et al., 2020; Lu et al., 2021; Jiang et al., 2023; De Alba et al., 2024), an increase in follicular atresia (Qiang et al., 2022), and different hormonal changes (Qiang et al., 2022; Naziat et al., 2024). Some authors considered the warmer periods (within the limits tolerated by the species) caused by climate change may promote longer growing seasons, especially in temperate regions, and favor the production of warmer water species, such as, tilapia (Troell et al., 2017; Collins et al., 2020; Maulu et al., 2021). However, if higher temperatures increase the prevalence of urogenital papilla

dysmorphia, climate changes may have negative impacts in seed production of Nile tilapia, indicating that fish farmers, especially in tropical countries, may face new challenges in seed production in near future.

Thus, the main objective of this study was investigating the etiopathogenesis of the urogenital papilla dysmorphia described by Bezerra et al. (2023) in Nile tilapia. The specific objectives were, as follows: (1) to evaluate genetic and environmental effects on the prevalence of urogenital papilla dysmorphia, reproductive performance and hormonal profile through the application of different temperature ranges in the first 60 days right after yolk sac absorption in two different populations of Nile tilapia, the base generation of a breeding program (without selection) and generation 8 (generation obtained after seven generations of selection for body weight); (2) to determinate if the presence of bacterial agents are associated to the dysmorphia of urogenital papilla; (3) to describe the progression of the urogenital papilla dysmorphia through a long-term follow-up (approximately 15 months) of Nile tilapias and evaluation of biological indices after this period.

4.3 Material and Methods

The experiment was conducted in a greenhouse of Aquaculture Laboratory of the Veterinary School of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais – UFMG, Brazil). All procedures were previously approved by the Ethics Committee on Animal Use of UFMG under protocol number 06/2017.

4.3.1 Experimental design and fish management during gonadal differentiation

The animals used in this experiment were produced using two distinct populations, the base generation (G0) and generation 8 (G8), from the genetic improvement program of Nutrition, Genetics and Technology for Aquaculture research group (NGT-Aqua). This genetic improvement program is based on genetic selection for body weight in BFT and recirculating aquaculture system (for further reference on two distinct generations, see Cavatti Neto et al., 2023). Spawning of eleven couples of each generation were utilized, and all breeders had normal urogenital papilla. From these breeding, larvae of G0 and G8 were equivalently selected and distributed in the treatments.

To evaluate the influence of temperature during the gonadal differentiation on the prevalence of dysmorphia of the urogenital papilla in tilapia, three cultivation temperature ranges were used on this phase (first 60 days right after yolk sac absorption): (1) Control: mean temperature of 26.5°C, range from 25°C to 28°C; (2) Moderate temperature: mean temperature of 27.5°C, range from 27°C to 30°C; (3) High temperature: and mean temperature of 31°C, range from 30°C to 33°C. The temperature means of the treatments were significantly different from each other (Supplementary material: Table S1 – shown on item 4.4 Results). To evaluate whether the two generations have different sensitivity to temperature, fish from G0 and G8 were submitted the moderate and elevated temperature. Due to space limitations (number of tanks available), it was necessary to choose one of the generations for the control group. The G8 generation was chosen because fish farms are more interested in using genetically improved breeders. Therefore, the trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0), with 4 replicates in each group.

Right after yolk sac absorption, the larvae produced were randomly distributed in 20 tanks, 300 larvae per tank, totaling 2,400 G0 larvae ($2 \text{ mg} \pm 1$), in 8 tanks (4 experimental units per treatment), and 3,600 G8 larvae ($6 \text{ mg} \pm 3$), in 12 tanks (the complete experimental design was shown in Fig. 1). The tanks were made of polypropylene and had 150 L of useful volume, with constant aeration and individualized thermostat.

During the first 42 days post yolk sac absorption, the Nile tilapia fry was fed with a commercial mashed feed (Propescado-Nutriave Foods) containing 55% crude protein. From 42 to 60 days post yolk sac absorption, fish were fed with 0.8 mm feed containing 45% crude protein. The feed was offered 5 times a day during this period (first 60 days of experiment). The feeding rate applied was 30% of the fish weight for the first week, 25% for the second, 20% for the third, and 15% for the fourth week and 10% for the second month of experiment.

Temperature, pH, salinity was measured using a multiparameter probe (HANNA HI98130), and dissolved oxygen (DO) parameters were measured using oximeter (Alfakit® AT 155) three times a week. Temperature was measured at 8 a.m., 12 p.m. and 4 p.m., the other parameters at 8 a.m. and 4 p.m. The DO was maintained above 5.0 mg · L⁻¹ through side-channel blowers. Salinity was initially maintained at 1 ppt, but from the

45th day to 60th day of experiment it was increased due to the accumulation of salts derived from eventual leftover feed and lower water exchange in BFT systems (as described by Alvarenga et al., 2018). Total alkalinity was assessed once a week according to the methodologies proposed by American Public Health Association (APHA, 2012).

The concentrations of total ammonia nitrogen (TAN) and nitrite (NO_2^-) were measured twice a week by spectrophotometry according to the methodology recommended by UNESCO (1983) and Bendschneider and Robinson (1952), respectively. Nitrate concentration was measured once a month according to Monteiro et al. (2003). The partial water exchange (75% per day) was necessary to maintain the lower temperature of water in CT-G8 and it was also used to control nitrogenous components. The biofloc technology (BFT) was used to control of nitrogenous components in MT-G0, MT-G8, HT-G0 and HT-G8. The tanks from these treatments were completely filled with biofloc previously developed in our laboratory. When TAN concentrations reached values above $1 \text{ mg} \cdot \text{L}^{-1}$ in BFT, cane sugar was added to stimulate the growth of heterotrophic bacteria and reduce TAN, using a C:N ratio of 6:1 (Ebeling et al., 2006). Settable solids were measured weekly through 1,000 mL Imhoff cones after sedimentation for 15 min and total solids were measured monthly according to Strickland and Parsons (1972).

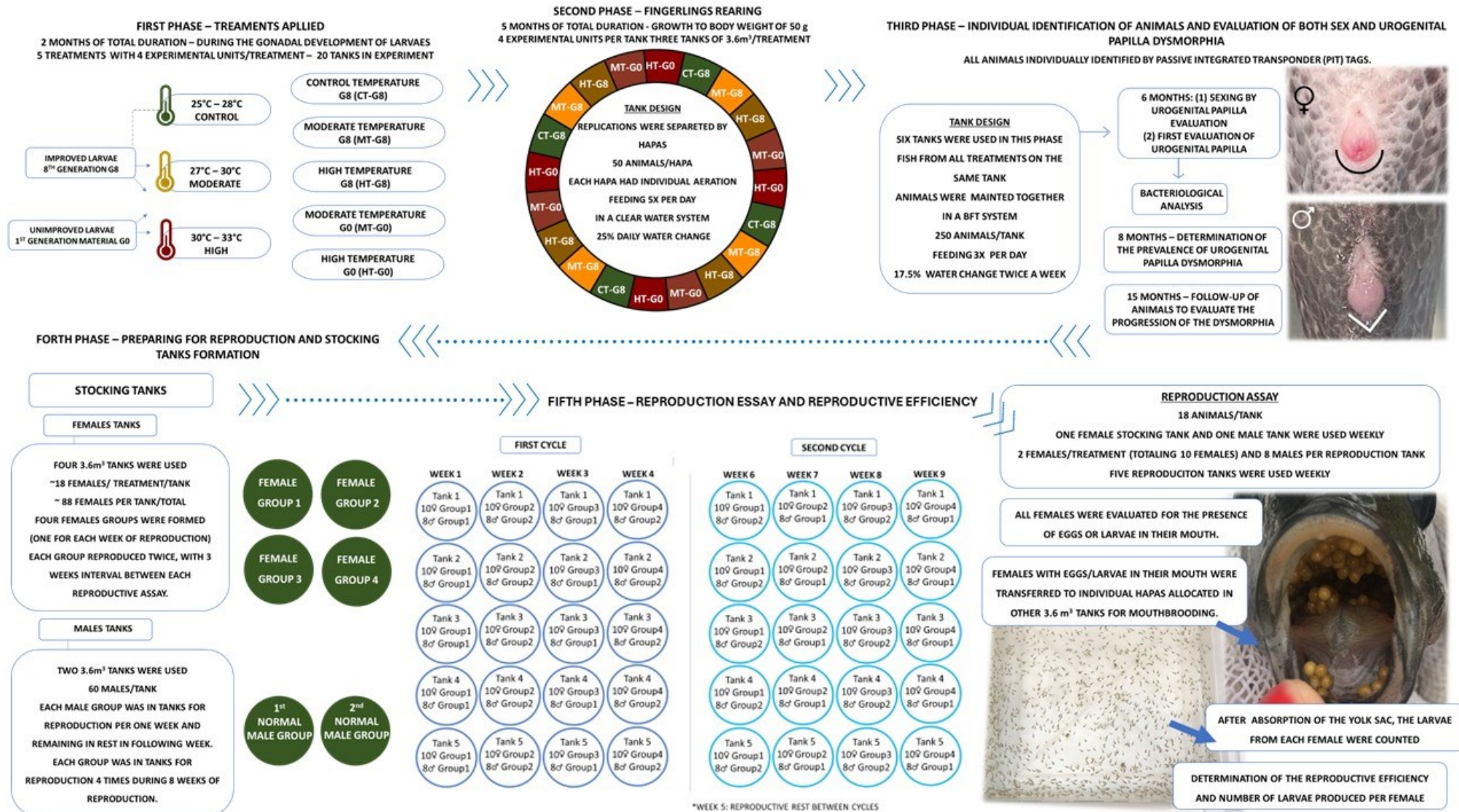


Figure 1: The complete experiment design was divided in five phases. **1st phase**, 300 larvae of each population (G0 or G8) was placed to 150L tanks, with individual aeration and thermostat. The G0 and G8 tilapias were randomly distributed into 8 tanks and 12 tanks, respectively. The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0), with 4 replications in each group. **2nd phase**, the animals from each original tank grew separated by hapas in the same tank until the identification by PIT tags. **3rd phase**, after chipping, fish from different treatments were mixed in the same tank, and the sex and urogenital papilla evaluated to determine the prevalence of dysmorphia per treatment. Ten females were collected for bacteriological analysis. **4th phase**, 70 females of each treatment (totalizing 350 females) were chosen, maintaining the proportion of normal and abnormal females according to the prevalence of urogenital papilla dysmorphia found previously. These females were placed in 4 stocking tanks of 3.6 m³. Normal males were kept in other 2 tanks. **5th phase**, reproduction essay was performed for 8 weeks. Females with eggs in their mouths in tanks for reproduction were transferred to individual hapas, and the larvae produced were counted 7 days after.

4.3.2 Fish management after experimental treatments

On 60 days post yolk sac absorption, fish from each 150L tank were transferred to identified hapas in two tanks of 3.6 m³ of useful volume. Each tank had 20 hapas of 90L and each hapa received 50 animals randomly collected from one experimental unit. So, the initial 20 experimental units (20 tanks of 150L each) were relocated to 40 hapas (20 hapas in each 3.6 m³ tank). The aim was to maintain the identification of the original experimental unit. This transfer of animals to hapas within the same tank was done to guarantee that, post-treatment, all tilapia shared a common environment, irrespective of the treatment. In this experimental phase, the water quality was maintained by daily water exchange of 20% of the total water volume. Nile tilapias were fed with pelleted commercial feed 1.7 mm containing 40% of crude protein (60-90 days of experiment) and 2-4 mm containing 36% of crude protein (90-120 days of experiment) (Propescado-Nutriave Foods), 3 times a day, at a rate of 10% of the biomass of each hapa until the animals reached approximately 50 g.

After reaching approximately 50g, the animals were identified by Passive Integrated Transponder (PIT) tags and a communal rearing of individuals from different treatments was established. In communal tanks of 3.6 m³ of useful volume, 50 individuals from each treatment were allocated, totaling 250 animals per tank. Six 3.6 m³ tanks of useful volume were used in this phase (300 animals per treatment). Fish were fed with pelleted commercial feed (2-4 mm and 4-6 mm) (Propescado-Nutriave Foods) containing 32% crude protein, 3 times a day, at a rate of 5% of the biomass until the animals were 6 months of age. An inoculum of 50% of bioflocs was used to fill the tanks, and the water quality was monitored as described above. The BFT was used to control of nitrogenous compounds in this experimental stage.

4.3.3 Sexing and evaluation of urogenital papilla

Specimens of *O. niloticus* were sexed by visual inspection about six months after yolk sac absorption. At this age, males were identified by two ventral orifices: the anus, located more cranially, and the genitourinary pore within the urogenital papilla. On the other hand, females presented three ventral orifices: the anus cranially, the oviduct exit and the urinary pore caudally on the urogenital papilla. Additionally, the shape of the

papilla differs between sexes; in males, it is funnel-shaped, whereas in females, it is rounded (Baroiller and D'Cotta, 2018) (Fig. 1). It is important to note that although females with dysmorphia presented urogenital papillae similar to those of males, the observation of oocytes in oviduct exit was possible by a subtle massage in ventral region of them.

After sexing, males and females were placed in six different tanks, three for males and three for females (stocking density of males and females were 175 and 195 fish per 3.6m³ tank of useful volume, respectively). The urogenital papillae were also examined and classified as normal or abnormal. The dysmorphia was identified only in female tilapias. The abnormal females were divided into two degrees of involvement (mild: grade 1 and severe: grade 2) following the description of Bezerra et. al (2023). In this first evaluation, there were a considerable proportion of animals with small urogenital papillae, which impaired the determination of prevalence of urogenital papillae dysmorphia in this moment. Due this, at eight months of age, all animals were evaluated for the second time to ensure the correct sexing and the determination of prevalence of urogenital papillae dysmorphia.

It is important to note, the animals were identified with microchips and fish from different treatments were in the same tank in both evaluations. During the diagnostic of urogenital papilla dysmorphia, fish were randomly caught, and the researcher had only the number of microchips, i.e., the information of treatment group of each animal was not available in this moment to avoid bias in diagnostics. After all evaluations, the microchip number were associated with the treatments and the prevalence of urogenital papilla dysmorphia was calculated as the proportion of the total females evaluated who had abnormal urogenital papillae in each experimental group.

To describe the progression of the urogenital papilla dysmorphia, the females were evaluated and photographed at six, eight and fifteen months after yolk sac absorption.

4.3.4 Impact of temperature treatments on reproductive performance

To evaluate the impact of temperature treatments during gonadal differentiation in reproductive performance of adult females, 70 females of each treatment (totalizing 350 females) were chosen, maintaining the proportion of normal and abnormal females according to the prevalence of urogenital papillae dysmorphia found previously. These

females were placed in 4 stocking tanks of 3.6 m³ of useful volume (2 tanks with 87 animals and 2 tanks with 88 animals). Each stocking tank had 17-18 females of each treatment. That is, the animals of the different experimental groups continued to share the same tank to ensure that they were under the same conditions after the initial period of temperature treatment.

Each stocking tank of females was used twice (tank 1: first and fifth weeks; tank 2: second and sixth weeks, tank 3: third and seventh weeks; and tank 4: fourth and eighth weeks) (Fig. 1) during eight weeks of reproductive assay. One hundred and twenty normal males were used in reproductive assay. They were placed in two tanks, each one with sixty fish. Males from one of these two tanks were used for reproductive assay in each week. For each week of natural breeding, 10 females of each experimental group (CT-G8, MT-G0, MT-G8, HT-G0 and HT-G8) were distributed in 5 tanks for reproduction (3.6 m³ of useful volume), i.e., two females from each treatment per tank, totaling 10 females · tank⁻¹ and 50 females · week⁻¹ in reproductive assay. Forty males also were randomly distributed in these 5 tanks for reproduction (8 males · tank⁻¹). In each tank, 10 females and 8 males (ratio male: female of 1:1.25) were allocated in 1 mm mesh hapa and they remained together for 7 days. Tanks for reproduction were maintained with clear water and constant aeration through compressors and diffusers. The experimental unit was each female.

At the end of the 7 days, the females incubating eggs in their mouths in tanks for reproduction were transferred to individual hapas (1 m³ volume) allocated in other 3.6 m³ tanks of useful volume for mouthbrooding. After close to 1 week of incubation and absorption of the yolk sac, the larvae from each female were counted. Thus, females that reproduced were one week in the tanks for mouthbrooding and two weeks in the stocking tank for gonadal recrudescence (as described by Alvarenga et al., 2017), while females that did not reproduce were three weeks in the stocking tank until the next reproductive week. Proportion of females that effectively produced larvae per treatment and the number of larvae per female were evaluated to compare the reproductive performance of females from different experimental groups.

The BFT was used in stocking tanks and the water quality (pH, oxygen, temperature, alkalinity, TAN, nitrite, and settleable solids) of them was evaluated and monitored as described above for BFT tanks. Tanks of reproduction were maintained with clear water and the values of oxygen, pH, temperature, TAN, and nitrite were also

monitored as already described. The breeders were fed with commercial feed 4-6 mm (Propescado-Nutriave Foods) containing 32% crude protein, enriched with 500 mg of Vitamin C · kg⁻¹ of feed (as recommended by Mataveli, 2018), three times a day, at a rate of 1.5% of the biomass.

4.3.5 Bacteriological analysis

As previously described (Bezerra et al., 2023), the gonadal alterations caused by the stenosis of urogenital papilla promoted the enlargement of gonads, colors alterations and necrosis of the oocytes. Macroscopically at necropsy, these alterations could be associated with abnormal ascending bacterial infections. To ascertain the potential association of infectious etiologies of gonadal pathology instead of the stenosis of urogenital papilla, a bacteriological examination was conducted in tilapias as soon as the dysmorphism was identified. To investigate the presence of systemic and gonadal infections, aseptic sampling of brain, kidney, spleen, and gonadal fragments from two females from each treatment (10 females in total) were collected. These specimens were aseptically streaked onto tryptone soybean agar (Kasvi, Italy) supplemented with 5% defibrinated sheep blood (TSAB) and MacConkey agar. Incubation of the plates occurred at 28 °C for 48 hours to facilitate bacterial isolation. The TSAB medium was chosen to support the growth of fastidious bacteria and to enable the selection of different microbial groups, particularly *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*. MacConkey agar was used for the isolation and differentiation of non-fastidious gram-negative rods, especially members of the family Enterobacteriaceae and the genus *Pseudomonas*.

4.3.6 Hormonal dosages of 17-beta-estradiol and 11-ketotestosterone

For hormonal measurements of 17-beta-estradiol and 11-ketotestosterone, 8 females per treatment (40 females in total) were chosen for blood collection after the reproductive assay. Three milliliters of blood were collected from each female and centrifuged at 675 G-force for 5 minutes. Plasma samples were collected and stored at -80 °C freezer. The samples were submitted in duplicate to ELISA assay using the Estradiol-17β Enzyme Immunoassay kit (Cayman, Michigan, USA) and 11-

ketotestosterone Enzyme Immunoassay kit (BT Lab, Shanghai, China) according to the manufacturer's instructions.

4.3.7 Statistical analysis

When the assumptions of normality and homogeneity were met, data of water quality, 17-beta-estradiol and 11-ketotestosterone levels, and biological indexes were analyzed by ANOVA followed by Tukey's test ($CV < 10\%$), Student–Newman–Keuls (SNK) test (CV from 10% to 15%) or Duncan test ($CV > 15\%$). When the assumptions of ANOVA were not met for these variables, they were analyzed by Kruskal-Wallis non-parametric test.

Both the prevalence of urogenital papilla dysmorphia and the distribution of ovaries in three classes (normal, or with lower or with high severity of lesions) into the different treatments were analyzed by the Chi-square test of independence to determine whether there is an association between categorical variables (i.e., whether the prevalence of urogenital papilla dysmorphia and the distribution of ovaries in three classes are independent or related to experimental groups) (Conover, 1999; Quinn and Keough, 2002; Box et al., 2005). Then, the association test of the prevalence of urogenital papilla dysmorphia (presence or absence) between pairs of treatment were performed with Fisher Exact test with Bonferroni correction and the association test of proportion of the three classes of ovary between pairs of experimental groups were performed with chi-square test with Bonferroni correction. Since significance values have been adjusted by Bonferroni correction for multiple tests, the p-value was 0.005 ($0.05/10$) for ten pairwise comparisons in these statistical analyses.

For the analysis of reproductive performance (proportion of effective reproduction and number of larvae produced, non-Normal variables), females from control group (CT-G8) were compared to females from the other treatments by a logistic regression (Model 1) and quasipoisson regression (Model 2) (Agresti, 2015). These models were fitted for effective reproduction and number of larvae produced per female, respectively.

In logistic regression, we considered the probability of the female effectively reproduce (π) as a binary response variable (if female produced larvae: $\pi = 1$, otherwise $\pi = 0$), the intercept, the effect of the treatments (MT-G8, HT-G8, MT-G0, HT-G0) in comparison to the reference group (CT-G8), and the effect of the temperature of tanks for

reproduction and the effect of the body weight of female. The effects of temperature and body weight of females were control variables, i.e., they are not of direct interest to our study, but they were controlled to prevent biases caused by their influences in the analysis of the reproductive performance of the different groups.

In quasipoisson regression, only females that effectively reproduced were included. The females from CT-G8 (control group) were compared to females from the other treatments for the number of larvae produced. The model included the intercept, the effect of the treatment group compared to control group, the effect of body weight of female, and the effect of tank water temperature. Again, body weight of female and water temperature of tank were control variables.

The goodness of fit of logistic and quasipoisson regression models was evaluated by the Hosmer-Lemeshow test (used only for the Model 1) and Pearson chi-square goodness-of-fit test (used in Model 1 and 2). During the 8-week reproductive assessment period, 3 from 40 tanks exhibited errors in the distribution of females and all animals (30 from 400 Nile tilapia female) from these tanks were subsequently excluded during statistical analysis.

In all statistical analysis, differences were considered significant at $p < .05$. The results were analyzed using the Infostat (Di Rienzo et al., 2015) and R software (R Core Team, 2022). Tidyverse (Wickham et al., 2019), Car (Fox and Weisberg, 2019), gridExtra (Auguie, 2017), and ResourceSelection packages (Lele et al., 2019) were used in R software.

4.4 Results

4.4.1 Heat treatments application and water quality parameters

The heat treatments implemented during the first 60 days after yolk sac absorption (gonadal development) maintained the temperatures into the range expected for each treatment (Fig. 2). Average and standard deviation of temperature for CT-G8, MT-G8, MT-G0, HT-G8, and HT-G0 were $26.41^{\circ}\text{C} \pm 0.68$, $27.46^{\circ}\text{C} \pm 0.57$, $27.24^{\circ}\text{C} \pm 0.83$, $31.21^{\circ}\text{C} \pm 0.82$, and $31.05^{\circ}\text{C} \pm 0.84$, respectively (Supplementary material: Table S1). There was a significant difference between control, moderate, and high-temperature treatments ($p < .05$), while no difference was found between MT-G8 and MT-G0 ($p > .05$).

nor between HT-G8 and HT-G0 ($p>.05$), indicating equal temperature conditions for both populations of Nile tilapia.

Dissolved oxygen, pH, TAN, nitrite, salinity, and alkalinity values of tanks during the first 60 days after yolk sac absorption are detailed in Supplementary material: Table S1. These values remained within the recommended range for the species (dissolved oxygen $>4 \text{ mg} \cdot \text{L}^{-1}$, pH 6–9, TAN $<1 \text{ mg} \cdot \text{L}^{-1}$, nitrite $<8 \text{ mg} \cdot \text{L}^{-1}$, salinity $<15 \text{ ppt}$, alkalinity $>50 \text{ mg of CaCO}_3 \cdot \text{L}^{-1}$, and settleable solids $<50 \text{ ml} \cdot \text{L}^{-1}$). Temperature, dissolved oxygen, pH, TAN, nitrite, salinity, alkalinity, and settleable solids values of stocking tanks and tanks for reproduction also met the recommendations for Nile tilapia (Supplementary material: Table S2 and Table S3). After the initial period of temperature application (first 60 days), the fish from different treatments shared the same tanks, thereat they were exposed to the same cultivation conditions during stocking and reproduction.

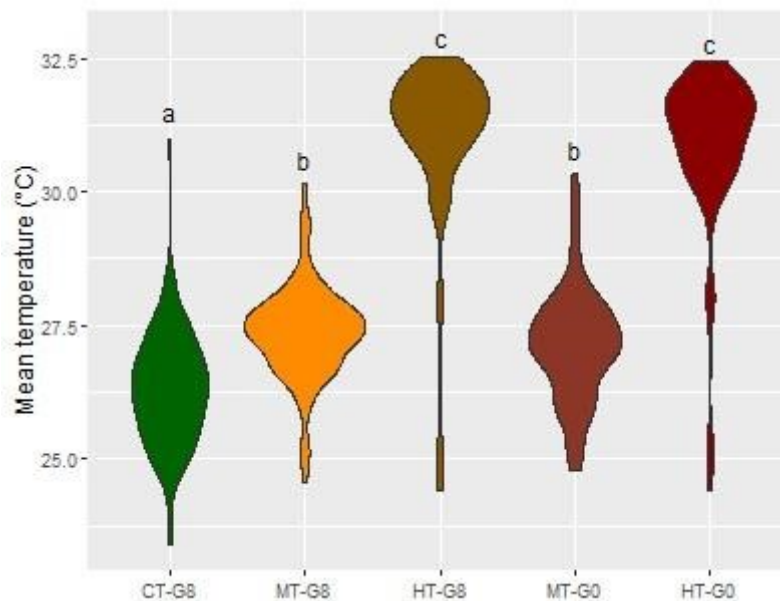


Figure 2: Distribution of mean temperature from the first to the 60th day of Nile tilapia after yolk sac absorption. The larvae from two distinct populations, the base generation (G0) and generation 8 (G8) of from a genetic breeding program based on genetic selection for body weight, submitted to three temperature ranges (CT: 25°C to 28°C, MT: 27°C to 30°C and, HT: 30°C to 33°C) during the larvae gonadal differentiation phase. The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). Different letters indicate significant differences by Kruskal-Wallis test ($p<.05$).

Table S1: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in culture tanks of Nile tilapia (*Oreochromis niloticus*) from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).

Water quality parameters	CT-G8*			MT-G8			HT-G8			MT-G0			HT-G0		
First Phase	n	Mean±SD	(Min -Max.)	n	Mean±SD	(Min -Max.)	n	Mean±SD	(Min -Max.)	n	Mean±SD	(Min -Max.)	n	Mean±SD	(Min -Max.)
Temperature (°C) (daily mean)	52	26.41±0.68 ^c	(24.86-27.9)	52	27.46±0.57 ^b	(26.43-28.85)	52	31.21±0.82 ^a	(27.59-31.99)	52	27.24±0.83 ^b	(26.04-29.92)	52	31.05±0.84 ^a	(27.83-31.93)
Temperature (°C) (8 a.m.)	52	24.66±0.75 ^c	(23.15-25.8)	52	25.80±0.87 ^b	(24.55-28.10)	52	29.54±1.13 ^a	(24.68-30.60)	52	25.65±1.24 ^b	(24.25-30.08)	52	29.37±1.15 ^a	(24.75-30.33)
Temperature (°C) (12 a.m.)	52	26.42±0.74 ^c	(25.03-27.7)	52	27.45±0.66 ^b	(26.25-28.65)	52	31.42±1.19 ^a	(26.13-32.13)	52	27.29±1.07 ^b	(25.88-31.23)	52	31.29±1.20 ^a	(26.30-32.10)
Temperature (°C) (4 p.m.)	52	28.14±0.94 ^c	(26.20-30.4)	52	29.13±0.74 ^b	(27.90-31.08)	52	32.66±0.97 ^a	(29.05-33.73)	52	28.79±0.76 ^b	(27.55-30.75)	52	32.48±0.88 ^a	(29.30-33.50)
Dissolved oxygen (mg.L ⁻¹)	52	6.49±0.56 ^a	(5.41-7.6)	52	6.38±0.65 ^a	(5.24-7.89)	52	5.98±0.87 ^b	(4.62-7.32)	52	6.23±0.74 ^{ab}	(4.85-7.39)	52	5.75±1.11 ^b	(3.20-7.65)
pH (daily mean)	52	7.44±0.22 ^a	(7.04-7.8)	52	7.13±0.47 ^b	(5.88-7.81)	52	6.92±0.74 ^c	(5.51-7.95)	52	7.03±0.58 ^{ab}	(5.87-7.67)	52	7.07±0.54 ^{ab}	(6.01-7.94)
TAN (mg.L ⁻¹)	30	0.45±0.54	(0.01-1.9)	30	0.83±0.82	(0.02-2.39)	30	0.52±0.73	(0.02-2.71)	30	0.69±1.15	(0.03-4.38)	30	0.46±0.47	(0.01-1.57)
Nitrite (mg.L ⁻¹)	30	0.60±1.43	(0.01-5.1)	30	1.71±3.92	(0.02-11.52)	30	1.68±2.68	(0.01-7.40)	30	1.21±2.37	(0.01-7.93)	30	1.67±3.06	(0.01-9.12)
Salinity (g.L ⁻¹)	52	0.13±0.02 ^b	(0.11-0.2)	52	1.61±0.83 ^a	(0.63-2.92)	52	1.67±0.81 ^a	(0.63-3.05)	52	1.59±0.80 ^a	(0.63-2.83)	52	1.68±0.82 ^a	(0.63-3.03)
Alkalinity (mg of CaCO ₃ .L ⁻¹)	15	74.00±7.15 ^a	(65.0-82.5)	15	71.88±29.47 ^{ab}	(27.50-102.50)	15	59.88±19.91 ^b	(12.50-63.70)	15	58.63±27.44 ^b	(27.50-92.50)	15	54.00±19.61 ^b	(26.25-73.75)

*The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). Values with different letters are significantly different by Kruskal-Wallis test ($p < .05$).

Table S2: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in BFT system stocking tanks of females and male of Nile tilapia (*Oreochromis niloticus*) reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption).

Water quality parameters	Females			Males		
	n	Mean $\pm$ SD	(Min -Max.)	n	Mean $\pm$ SD	(Min -Max.)
Temperature ($^{\circ}$ C) (daily mean)	96	26.60 $\pm$ 2.31	(23.70 - 33.9)	48	28.19 $\pm$ 2.41	(23.90 - 33.30)
Dissolved oxygen (mg.L ⁻¹)	96	6.29 $\pm$ 0.83	(3.64 - 8.05)	48	5.41 $\pm$ 0.91	(3.71 - 7.03)
pH (daily mean)	96	6.60 $\pm$ 0.61	(5.17 - 7.97)	48	6.55 $\pm$ 0.44	(5.58 - 7.61)
TAN (mg.L ⁻¹)	64	0.47 $\pm$ 0.72	(0.00 - 4.00)	32	1.04 $\pm$ 1.43	(0.00 - 5.08)
Nitrite (mg.L ⁻¹)	64	0.88 $\pm$ 1.07	(0.00 - 4.67)	32	0.20 $\pm$ 0.07	(0.00 - 2.80)
Salinity (g.L ⁻¹)	96	0.21 $\pm$ 0.09	(0.10 - 0.69)	48	0.18 $\pm$ 0.05	(0.10 - 0.35)
Alkalinity (mg of CaCO ₃ .L ⁻¹)	32	65.52 $\pm$ 47.07	(15.00 - 175.00)	16	91.35 $\pm$ 61.32	(21.25 - 200.00)

The female group tank was compound of the mean of the four tanks used in this phase. All the tanks of females received animals of the five treatments in the same proportion. The all males used were normal tilapia.

Table S3: Mean, standard deviation (SD), minimum (Min) and maximum (Max) of water quality variables in the clear water reproduction tanks of Nile tilapia (*Oreochromis niloticus*)

Water quality parameters	Tanks for reproduction*		
	n	Mean $\pm$ SD	(Min -Max.)
Temperature ($^{\circ}$ C) (daily mean)	40	27.86 $\pm$ 0.91	(24.91 - 29.68)
Temperature ($^{\circ}$ C) (8 a.m.)	40	27.29 $\pm$ 0.79	(25.53 - 28.70)
Temperature ($^{\circ}$ C) (4 p.m.)	40	28.43 $\pm$ 1.37	(21.84 - 30.67)
Dissolved oxygen (mg.L ⁻¹)	40	7.24 $\pm$ 0.42	(5.83 - 8.12)
pH (daily mean)	40	7.07 $\pm$ 0.17	(6.74 - 7.42)
TAN (mg.L ⁻¹)	40	0.25 $\pm$ 0.20	(0.00 - 0.87)
Salinity (g.L ⁻¹)	20	0.09 $\pm$ 0.04	(0.04 - 0.13)

*All the reproduction tanks received Nile tilapias females from the five treatments in the same proportion.

4.4.2 Urogenital papilla dysmorphia classification and anatomic ovarian changes associated

Females with a consistent urogenital papilla conformation with the species were classified as normal (Fig. 3). The normal papillae had rounded caudal region with the cranial presence of the anal orifice, and two well-defined orifices located in the papilla itself - the oviduct outlet (cranial) and the urinary pore (caudal).

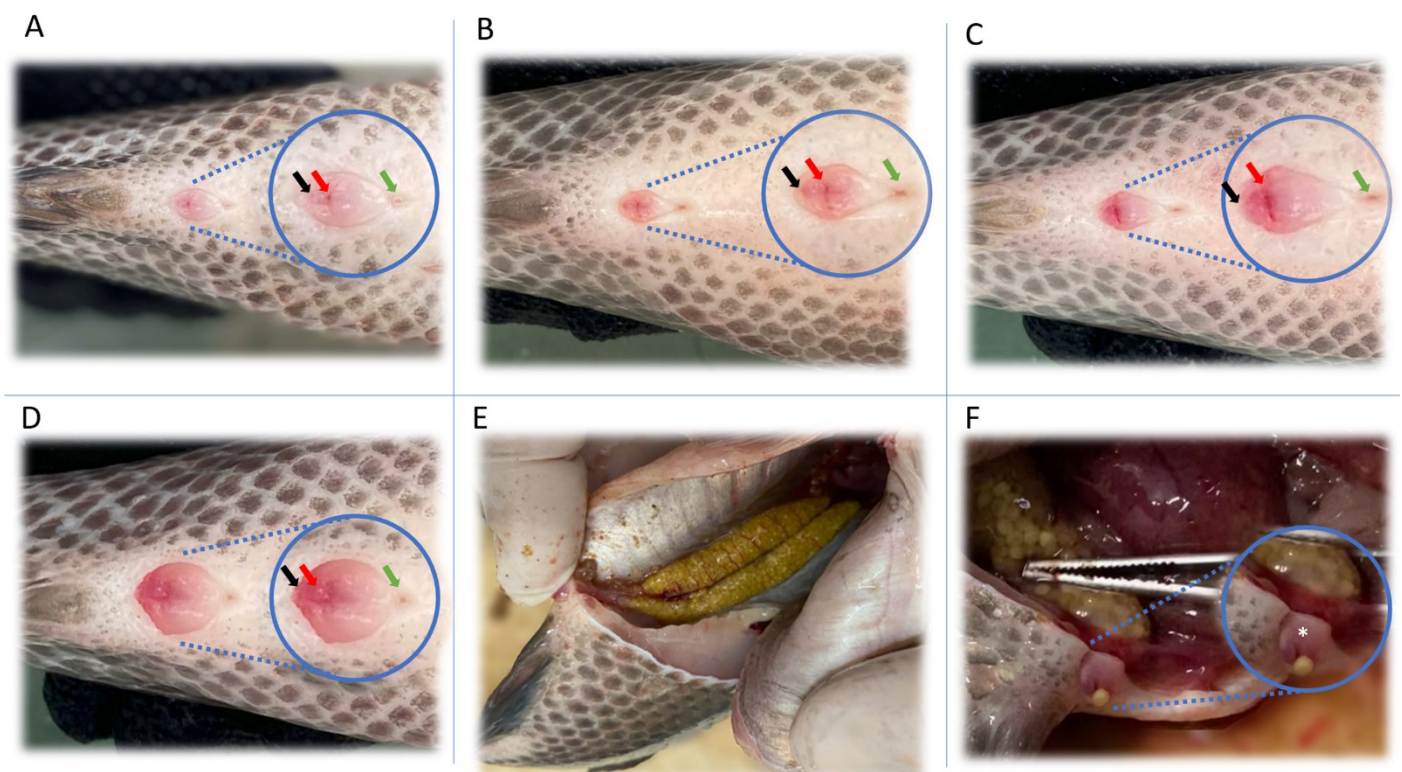


Figure 3: Urogenital papilla of Nile tilapia classified as normal. Anus (green arrow), oviduct exit (red arrow) and urinary pore (black arrow) are easily identified. Normal females exhibited a clear "comma shape" in the oviduct outlet (red arrows). Enlarged images shown into blue circles. The normal urogenital papilla underwent anatomical changes during the reproductive cycle (Figure A to D). As the female progressed in oocyte maturation, vascularization increased, and the papilla changed from a pale pink (A) to a reddish color (B), with swelling (C and D). Most females with normal papilla had normal ovaries (E), located dorsally in the coelomic cavity, occupying a maximum of one-third of the coelomic cavity, with normal color and mature oocytes for spawning. Females with normal papilla and ovaries experienced easy spawning, as observed during massage in ventral region for oocytes extrusion (F).

Abnormal females with urogenital papilla dysmorphia were identified in all treatments, with the highest prevalence in moderate and high-temperature treatments (Fig. 4). These females exhibited alterations in structure and shape, classified as mild (abnormal grade 1) affected by dysmorphia. These abnormalities included a less defined oviduct exit, deviating from the typical shape, resembling "one dot," leading to difficulties in distinguishing the urinary pore from the oviduct exit.

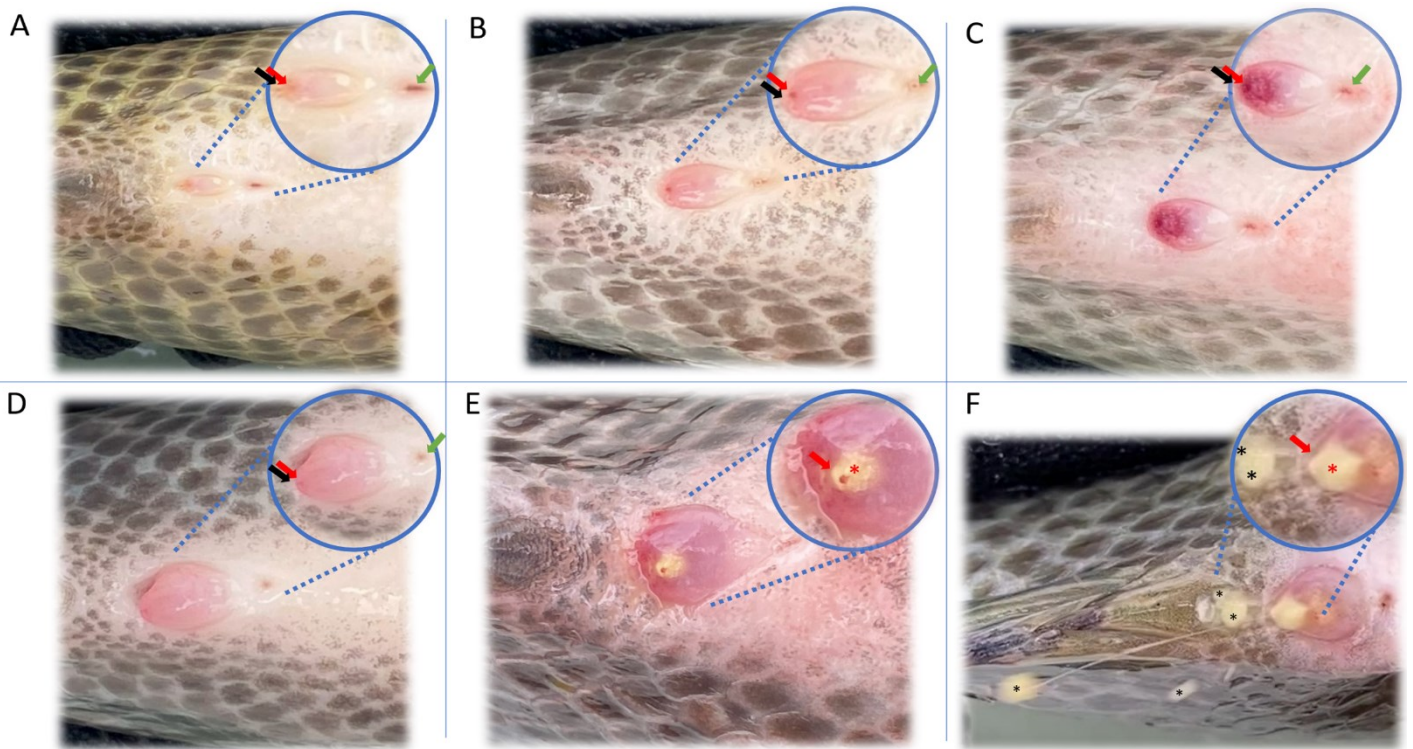


Figure 4: Urogenital papilla of Nile tilapia classified as mild (abnormal grade 1) involvement. The alterations in papilla, classified as grade 1, throughout the reproductive process depicted in images A, B, C, D, and E. Females with dysmorphia of urogenital papilla exhibited change in anatomic papilla shape. Initial consequences were shown in D, resulting in a conic conformation (similar a male urogenital papilla in Nile tilapias). Despite dysmorphic characteristics, these animals maintained a normal production of oocytes, but with increased difficulty in spawning. The remaining oocytes trapped at the oviduct exit (E), hindering a normal release of them. Massage in ventral region led to abrupt release, causing deformation of the oocytes and, in several times, their rupture, explaining the presence of light-yellow liquid accompanying expelled oocytes in these females (F). Anus (green arrow), oviduct exit (red arrow) and urinary pore (black arrow) are shown in the images.

In severe cases, females were unable to spawn normally, exhibiting an excessively distended ventral region compared to normal females, due to altered urogenital papillae formation (Fig. 5).

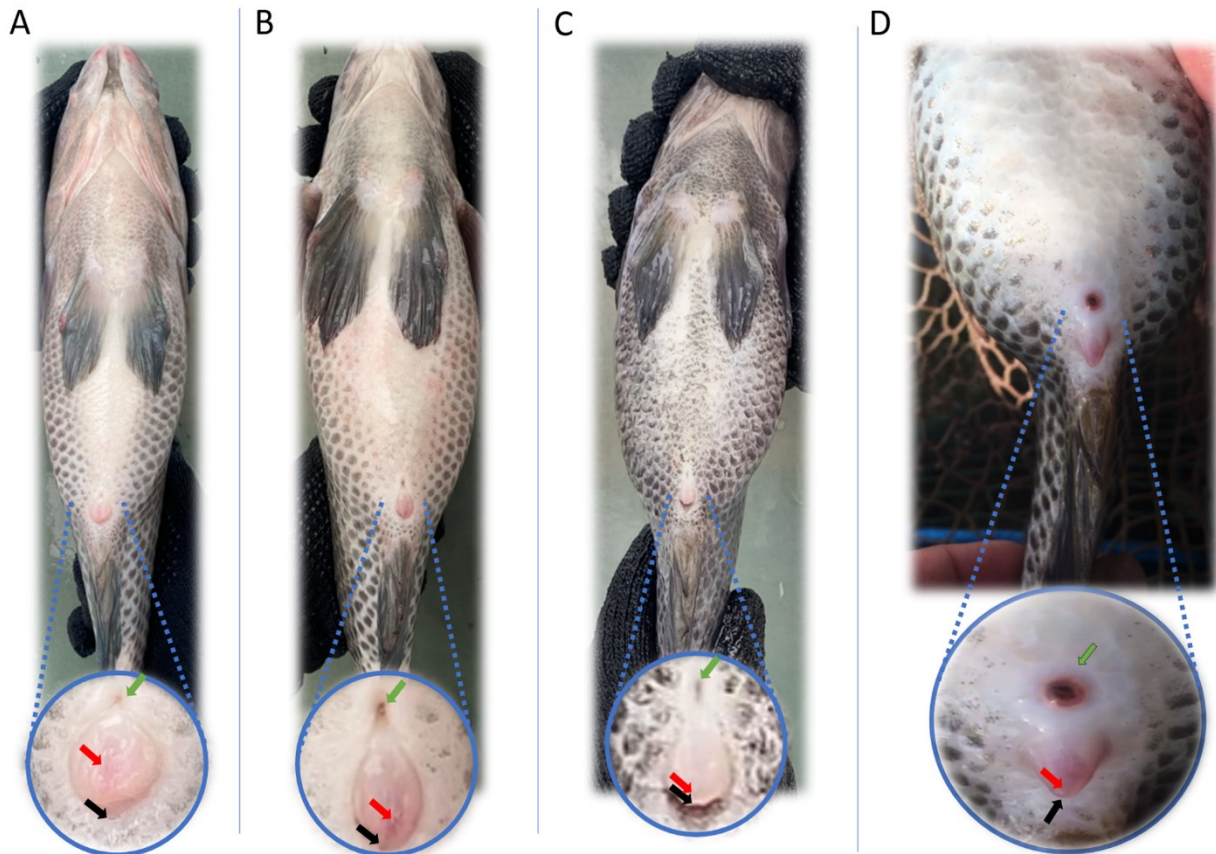


Figure 5: Description of Nile tilapia with urogenital papilla classified as severe involvement (abnormal grade 2). Images A to D depicts the progression of distension in ventral region, with initially distended ventral region, causing a possible discomfort during brief massage. In more severe conditions, the oviduct exit (red arrows) is very reduced, and the ventral region became excessively distended with increased flaccidity, causing signs of discomfort upon touch or brief massage in ventral region. The papilla commonly assumed a conical shape, likely due to the pressure exerted by ovaries filled with retained oocytes (D). Black and green arrows indicate urinary pore and anus, respectively.

The study monitored changes resulting from the persistent inability to spawn in atypical females (Fig. 6). Some of these females developed a new opening on the side of the papilla. In these cases, the oocytes were released intact by the new opening, and the females have regained the ability to spawn.

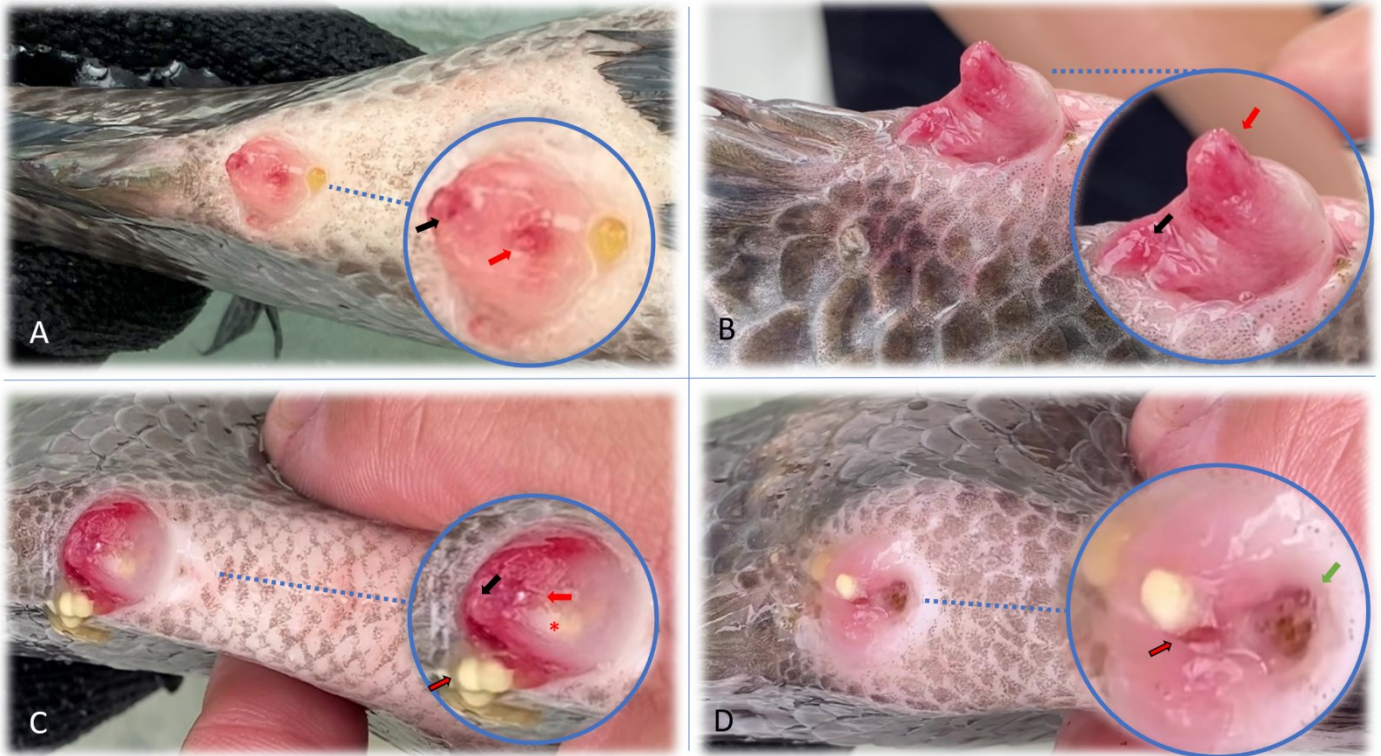


Figure 6: New alterations in urogenital papilla observed in anormal female Nile tilapia. In the papilla of these atypical females, the oviduct exit, and urinary pore pronounced, and the papilla appeared more swollen (A and B) than in females with urogenital papilla classified as mild or severe involvement. In image C, one oocyte retained at the exit of the oviduct (red arrow), while others released through the newly formed opening on the side of the papilla (red arrow). In severe cases (D), some females lacked a distinct urogenital papilla. Urinary pore (black arrow) and anal orifices (green arrow).

The ovaries were also assessed and categorized (Fig. 7). Normal ovaries in Nile tilapias have a symmetrical bilateral wedge-shaped structure, central vasculature branching throughout, and mostly mature oocytes with few atretic counterparts. Moderately impaired ovaries exhibit an altered, occasionally asymmetrical shape, with changed vascularization compared to normal organs and a higher prevalence of atretic oocytes. Severely compromised ovaries (grade 2) show symptoms of excessive egg retention, atretic oocytes exceeding the absorptive capacity, leading to the accumulation of translucent greenish fluid and firm, brownish masses - features absent in normal ovaries.

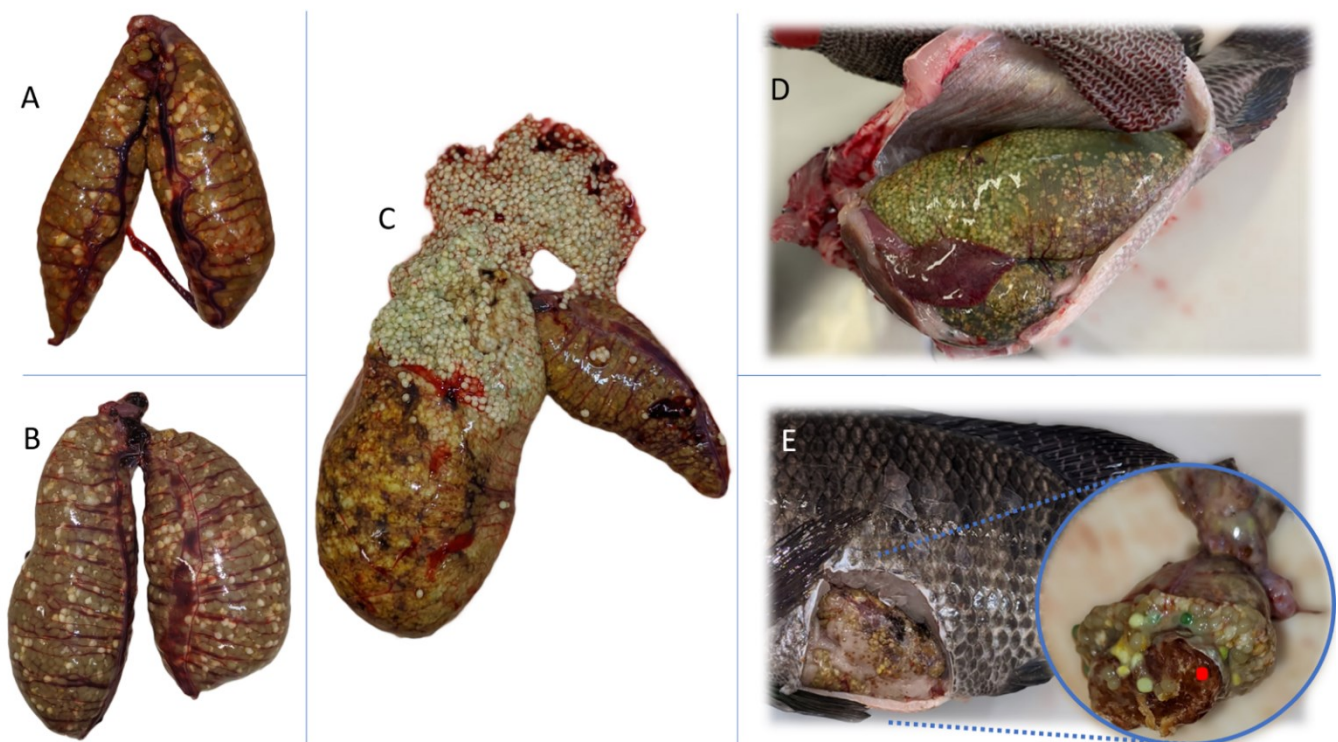


Figure 7: Differentiation of ovaries classified as normal, mild (abnormal grade 1) and severe (abnormal grade 2) involvement caused by urogenital papilla dysmorphia. Normal ovaries exhibited a symmetrical bilateral wedge-shaped structure, central vasculature branching throughout the organ, and majority of mature oocytes with few atretic counterparts (A). Moderated impairment ovaries displayed an altered, occasionally asymmetrical shape, leading to changed vascularization compared to normal organs and a higher prevalence of atretic oocytes (B). Severely compromised ovaries (grade 2) exhibited symptoms of excessive egg retention, with a predominant presence of atretic oocytes surpassing the absorptive capacity of tilapia ovaries (C). This retention led to the accumulation of translucent greenish fluid (D) and, occasionally, firm, brownish masses (red point), detached in blue circle (E), characteristics not observed in normal Nile tilapias ovaries. Due to the excessive distension of these organs, severe involvement ovaries occupied over two-thirds of the coelomic cavity in these females, presenting two possible outcomes: the pressure-induced opening of a passage for spawning or led the death of the female.

4.4.3 Prevalence of urogenital papilla dysmorphia and ovaries commitment in females of Nile tilapia

The heat treatments applied during gonadal development (first 60 days after yolk sac absorption) affected the prevalence of urogenital papilla dysmorphia in females ($p < .05$) (Fig. 8). The chi-square test revealed significant differences between the proportions of females classified as normal, moderate (abnormal grade 1), and severe (abnormal grade 2) involvement of urogenital papilla dysmorphia among the treatments. HT-G8 exhibited the highest prevalence of urogenital papilla dysmorphia, being 88.16% of females classified as abnormal grade 1, 1.32% as abnormal grade 2, and only 10.53% of females classified as normal. MT-G8 and MT-G0 did not differ significantly, both showing the second-highest proportion of abnormal females. For MT-G8, 61.59% were abnormal grade 1, none were classified as abnormal grade 2, and 38.41% were classified as normal. MT-G0 had 64.47% classified as abnormal grade 1 and 3.92% as abnormal grade 2, and 31.58% classified as normal, respectively. HT-G0 had 43.70% of females considered abnormal grade 1 and 56.30% as normal. CT-G8 showed the lowest proportion of abnormal females (18.85% abnormal grade 1 and 1.64% abnormal grade 2) with a higher proportion of normal females (79.51%).

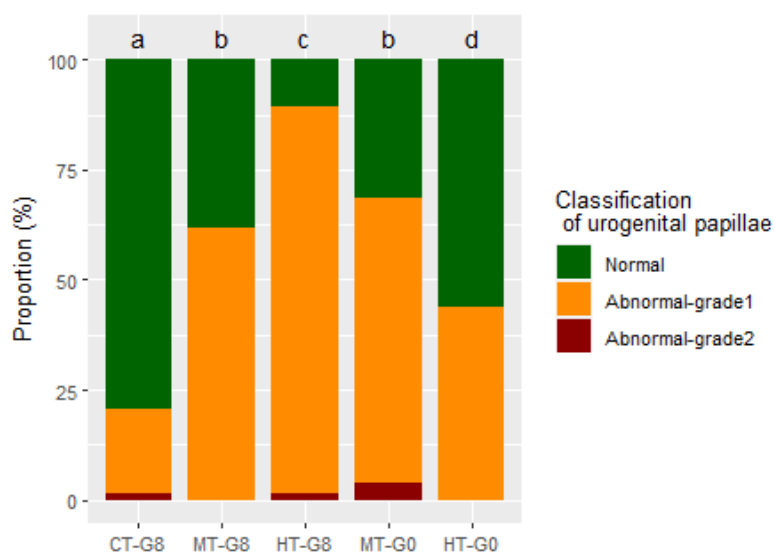


Figure 8: Prevalence of urogenital papilla dysmorphia of two distinct populations, the base generation (G0) and generation 8 (G8) from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption). The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). Urogenital papilla of females classified as normal, mild (abnormal grade 1) or severe (abnormal grade 2) involvement. Since the p-value of Chi-

square test of independence was significant ($p < .05$), the association test of the prevalence of urogenital papilla dysmorphia (presence or absence) between pairs of treatment were performed with Fisher Exact test with Bonferroni correction ($p < .005$).

4.4.4 Bacteriological analysis

All samples evaluated from the ten females sent for bacteriological analysis had negative results for bacterial growth involved in pathogens. The inflammatory and necrotic alterations identified in the gonads were not correlated with infection.

4.4.5 Reproductive performance

The results of logistic regression models for reproductive performance are summarized in Table 1. The control group (CT-G8) was the reference group in the model and the other treatments were compared to it. Except by the HT-G0 group, all treatments presented a reduction close to 70% in the reproductive performance in comparison to the control ($p > .05$). Female body weight and temperature were control variable in the model and demonstrated significant effects on reproductive performance. In this study, the female used had body weights ranging from 130 g to 610 g. For each additional gram of body weight, there was a decrease of 0.6% in the likelihood of reproduction of female, i.e., heavier females were less likely to reproduce. The temperature of the water in tanks for reproduction was at 24.91°C to 29.68°C during 8 weeks of reproduction. In this range of temperature, the increase of each degree Celsius resulted in the reduction of 37.71% in the chance of the female reproduces.

The quasipoisson generalized linear model was employed to examine the effect of treatments on larvae production of females (Table 2). It is important to highlight that this analysis focused exclusively on females that successfully reproduced, aiming to identify factors influencing larval production. Mean temperature and female body weight were included as control variables in the models. The results showed no significant differences between treatments, indicating that when reproduction occurred, the number of larvae was similar among females from different genetic origin and temperatures treatment in first 60 days after yolk sac absorption.

Table 1: Results of fitting the logistic regression model to effective reproduction (proportion of females that effectively generated larvae) of Nile tilapia from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared on different temperature ranges (25°C – 28°C: control, 27°C – 30°C: moderate temperature, and 30°C – 33°C: high temperature) during the first 60 days after yolk sac absorption.

	Coefficient	OR**	IC - 95% - OR	Interpretation (%) ***	p-value
Intercept	14,43	-	-	-	0,0070
MT-G8*	-1,33	0,27	0,11 - 0,63	-73,49	0,0072
HT-G8*	-1,17	0,31	0,14 - 0,70	-69,03	0,0049
MT- G0*	-1,11	0,33	0,15 - 0,74	-67,14	0,0049
HT-G0*	-0,43	0,65	0,31 - 1,37	-	0,2582
Female weight (g)*	-0,01	0,99	0,99 - 1,00	-0,60	0,0013
Mean temperature of tank during reproduction (°C)	-0,47	0,62	0,44 - 0,89	-37,71	0,0024

*

* The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). The CT-G8 was the reference group and compared to other treatments. The factors mean temperature of tank during reproduction and female body weight were control variables and had a significant effect on effective reproduction **OR = Odds ratio. *** Interpretation (%): negative values indicate the percentage decrease in females that effectively reproduced. Therefore, there was a reduction in reproductive performance of treated group (except for HT-G0) compared to the control group. In addition, the increase of both female weight and temperature of tank during reproduction (from ~25°C to 30°) reduced the effective reproduction in Nile tilapia.

Table 2: Results of fitting quasipoisson generalized linear model for number of larvae produced per female of Nile tilapia from two distinct populations (the base generation (G0) and generation 8 (G8) of from a genetic improvement program based on genetic selection for body weight) reared on different temperature ranges (25°C – 28°C: control, 27°C – 30°C: moderate temperature, and 30°C – 33°C: high temperature) during the first 60 days after yolk sac absorption.

	Coefficient	Impact on the rate	IC - 95%	Interpretation (%)	p-value
Intercept	12,11				0,0003
MT-G8*	0,01	1,52	0,99 - 2,31	-	0,0573
HT-G8*	-0,23	0,80	0,44 - 1,43	-	0,4455
MT-G0*	0,09	1,10	0,65 - 1,85	-	0,7298
HT-G0*	0,42	1,01	0,54 -1,88	-	0,9865
Female weight (g)	0,00	1,00	1,00 - 1,00	-	0,8051
Mean temperature of tank during reproduction (°C)	-0,20	0,82	0,66 - 1,01	-	0,0716

* The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). The CT-G8 was the reference group and compared to other treatments. For the number of larvae produced per female, treated groups did not differ from the control group. The factors mean temperature of tank during reproduction and female body weight were control variables and did not significantly affect the effective reproduction ($p > .05$).

4.4.6 Biological indexes

The gonadosomatic, hepatosomatic, and viscerosomatic index per treatments and per classifications of the urogenital papilla were shown in Fig. 9. Among the applied heat treatments, no distinctions were observed in the gonadosomatic index and viscerosomatic index ($p > .05$), but a notable difference emerged in the hepatosomatic index values ($p < .05$). Females from the control group presented higher values for hepatosomatic index and were statistically different from MT-G0. However, these groups did not differ from the MT-G8, HT-G0 and HT-G8 treatments.

Considering biological indexes through the urogenital papillae classification, the animals displaying severe dysmorphia (grade 2 abnormal) exhibited highest values for gonadosomatic index and viscerosomatic index ($p < .05$) compared to animals classified as normal and abnormal-grade1.

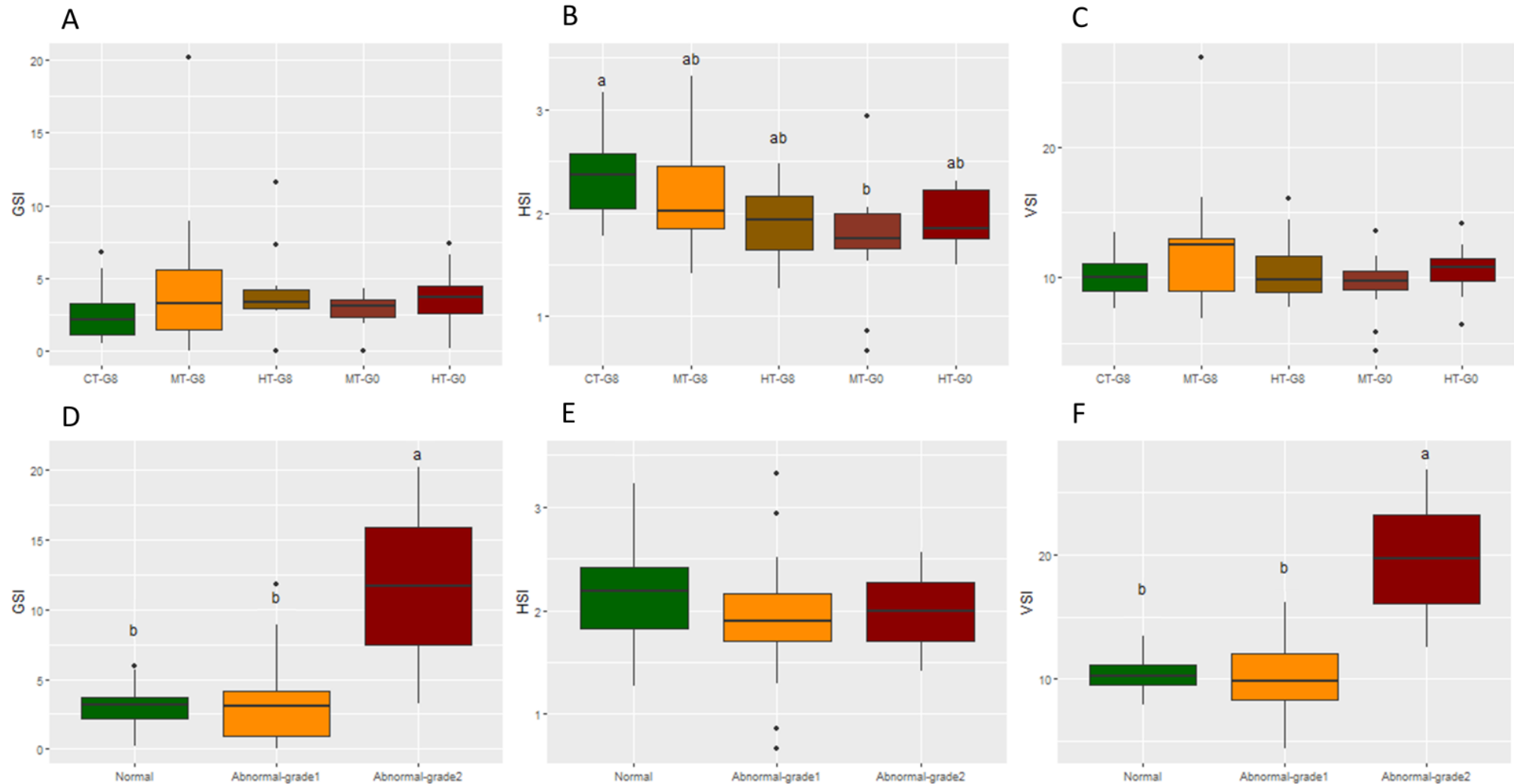


Figure 9: Biological indexes of female Nile tilapia of two distinct generations, the base generation (G0) and generation 8 (G8) of from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption). The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). The gonadosomatic (GSI), hepatosomatic (HSI) and viscerosomatic (VSI) indices compared among heat treatments (A, B and C) and regarding the classification of the urogenital papilla as normal, moderately (abnormal grade 1) or severely affected (abnormal grade 2). The HSI means differ among the experimental groups, GSI and VSI differed among fish with different urogenital papilla classes by SNK post-test. Significant differences indicated by different letters ($p < .05$).

4.4.7 Hormonal dosages of 17-beta-estradiol and 11-ketotestosterone

A significant difference was observed in the hormonal profiles of females exposed to the heat treatments (Fig. 10). Specifically, the HT-G8 group exhibited a higher plasma estradiol concentration ($1.68 \text{ pg} \cdot \text{ml}^{-1} \pm 0.04$) ($p < .05$), but it was like the MT-G0 group ($1.63 \text{ pg} \cdot \text{ml}^{-1} \pm 0.08$). Difference was also not significant when MT-G0 was compared to the other treatments (HT-G0, MT-G8, and CT-G8) ($p > .05$). Regarding plasma 11-ketotestosterone levels, the CT-G8 control group showed the highest average value among all treatments ($1,067.36 \text{ ng} \cdot \text{L}^{-1} \pm 217.54$).

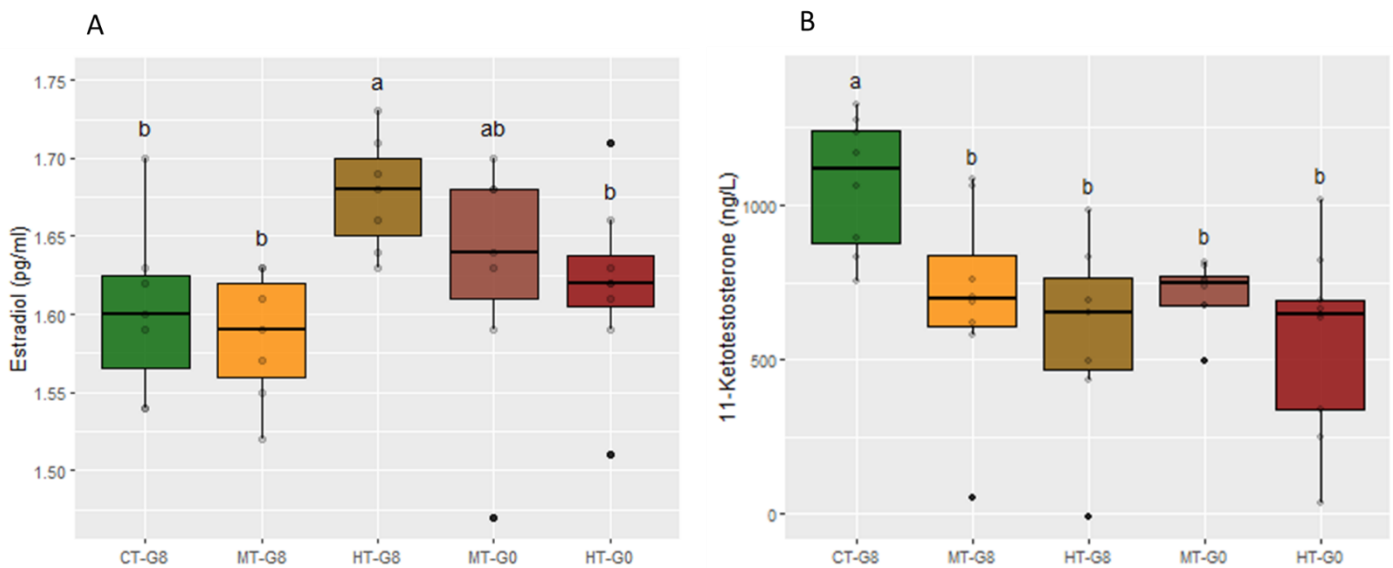


Figure 10: Hormonal dosage of plasma estradiol and 11-ketotestosterone of female Nile tilapia of two distinct populations, the base generation (G0) and generation 8 (G8) of from a genetic breeding program based on genetic selection for body weight, reared in different temperature ranges during gonadal development (first 60 days after yolk sac absorption). The trial presented 5 experimental groups: control temperature G8 (CT-G8), moderate temperature G8 (MT-G8), high temperature G8 (HT-G8), moderate temperature G0 (MT-G0), and high temperature G0 (HT-G0). Different letters indicated significant hormonal dosages differences among treatments by Kruskal-Wallis test ($p < .05$).

4.5 Discussion

According to our knowledge, this is the first prospective investigation exploring the etiopathogenesis of the urogenital papilla dysmorphia described by Bezerra et al. (2023) in Nile tilapia. The identification of predictable factors that impact reproductive capacity is crucial to tilapia culture expansion, and these factors may include both infectious and non-infectious elements (Jalabert e Zohar, 1982; Coward and Bromage, 2000; Bhujel et al., 2001; Yoshida et al., 2017; Qiang et al., 2021). Bezerra et al. (2023) performed bacteriological analysis in adult Nile tilapia with dysmorphia of urogenital papilla and did not find bacterial agents associated. In the present study, the bacteriological analysis was performed as soon as female Nile tilapia with urogenital papilla dysmorphia were identified. Again, negative results were obtained in the bacteriological analysis. Most severe infectious agents in aquaculture are bacteria, and infections by *Aeromonas* spp., *Pseudomonas fluorescens*, *Flavobacterium columnare*, *Streptococcus* spp. (*S. agalactiae* and *S. iniae*), *Edwardsiella tarda*, *Francisella noatunensis*, *Vibrio* spp., and *Mycobacterium* are listed notifiable diseases in tilapia culture (Bondad-Reantaso, 2020). Despite many studies describing the symptoms, diagnosis, treatment and prevention of the diseases caused by bacteria, a symptomatic representation similar to those reported in this study for females with dysmorphia in the urogenital papilla has not yet been identified (El-Sayed, 2019; Haenen et al., 2022; WOA, 2024). It is important to note the NGTAqua vivarium have a biosecurity plan, which includes physical biosecurity measures (barriers to prevent the entry of unauthorized people and aquatic animals other than those of breeding program, use of closed system, use of secure water source), constant control of water quality and health monitoring (including regular sampling and testing of animals) (Aly and Fathi, 2024). Therefore, together the results indicated that an infectious cause for this dysmorphia is unlikely. On the other hand, our results strongly indicate that genetic and temperature effects are associated with the etiopathogenesis of the urogenital papilla dysmorphia.

The occurrence of urogenital papilla dysmorphia increased significantly in females reared in high-temperature water during the early 60 days post yolk sac absorption, and it developed to a considerable reduction in reproductive efficiency of these breeders. Bezerra et al. (2023) suggested that high temperature during gonadal development could be related to the malformation in the urogenital papilla of female Nile

tilapia. Due to the phenotypic plasticity of initial stages of Nile tilapia and its response to water temperature, this species is considered one of the best models to evaluate the thermosensitivity and its effects on development pattern (Ijiri et al., 2008; Baroiller et al., 2009; Yúfera, 2021). Water temperature is one of the most influent environmental factors on sex determination in teleost fishes, and it is used in tilapia larviculture as a non-hormonal masculinization method, an ecologically viable alternative to the use male steroidogenic hormones to generate batches of males (Baroiller et al., 2009). Nile tilapias have a critical phase of sex determination, first 9-15 days after fertilization, and exposure to high temperatures around 36°C at this moment induces genotypic XX females to undergo masculinization, transforming in XX pseudomales (Ijiri et al., 2008; Baroiller et al., 2009). Nevertheless, it is important to note that exposure to temperatures lower or higher than this critical phase has no impact on the sex determination, as demonstrated by studies conducted by Sun et al. (2018) and Zhao et al. (2019). Despite the absence of sexual reversal, recent studies described changes in anatomy of the reproductive system of adult female tilapia, which could be caused by high temperatures (Nozu and Nakamura, 2020; Bezerra et al., 2023). The high temperatures (30°C-33°C) used in this study were not enough to produce a monosexual group of males but generated high prevalence of abnormal females (close to 90% and 44% of abnormal females in G8 and G0, respectively) carrying a dysmorphia in the external reproductive system, resulting in plausible ovarian involvement in these females.

Comparatively, tilapias from G8 responded more clearly to the effects of temperature than tilapias from G0. The lowest prevalence of dysmorphia was found in CT-G8 group reared in the treatment with lower water temperature (25°C-28°C). While the prevalence of the dysmorphia of urogenital papilla was similar between G0 (close to 68%) and G8 (close to 62%) in moderate temperature (27°C-30°C), the Nile tilapias from G8 were more susceptible to high temperature treatment (HT-G8: close to 90% of abnormal females) than those from G0 (close to 44%). Two hypotheses related to the process of genetic selection for body weight could explain these results: (1) This outcome may be related to higher uniformity of animals of G8, a result of the selection pressure applied in the breeding program (Cavatti Neto et al., 2023). Our hypothesis is that animals of G0 group are more genetically and phenotypically diverse, potentially resulting in varied responses to temperature treatments. While the matings in tilapias from G0 are random, the G8 population is the result of seven consecutive process of genetic selection,

which reduced the variability and increase the probability of mating between relatives. Therefore, a possible explanation of elevated prevalence of urogenital papilla dysmorphia of G8 in higher temperature could be the inbreeding depression. In fact, inbreeding depression is expected to be more severe in stressful environments (Bickley et al., 2013). However, the inbreeding is controlled in the NGTAqua breeding program (Cavatti Neto et al., 2023), and it is estimated be lower than 5% in G8 (specifically 4.3%, according to Eduardo Maldonado Turra, personal communication), which partly weakens this hypothesis. Evidence of inbreeding depression on survival and growth of rainbow trout were reported by Gjerde et al. (1983) with high levels of inbreeding ($F=0.25$, 0.375 , and 0.5). Gallardo et al. (2004) found significant inbreeding depression only for the gonadosomatic index (5.3% per each 10% increase in inbreeding) in population of Coho salmon. According to these authors the inbreeding did not significantly reduce other traits such as body weight at spawning, weight of the gonad, or relative fecundity. Therefore, an evaluation of effects of temperature on young tilapia females from genetic groups with different levels of inbreeding is necessary to elucidate the hypothesis of inbreeding depression; (2) Alternatively, genetic correlation between growth and temperature sensibility caused by linkage disequilibrium and/or pleiotropy could explain these results (Stearns, 2010; Etterson, 2016; Chebib and Guillaume, 2021). The genetic selection of tilapias with higher estimated breeding value for body weight may have resulted in simultaneous selection of autosomal alleles for thermosensitivity, due the close physical proximity of these set of loci in on a chromosome (linkage disequilibrium). In fact, since temperature-influence on sex is inherited genetically and minor genetic factors can affect the sex ratios (Baroiller and D'Cotta, 2018), the association between growth and thermosensitivity genes is possible. The occurrence of pleiotropy may also be a plausible explanation of the results. The families of the NGTAqua breeding program are produced in a greenhouse in the summer, when temperatures can reach values above 30°C. Eventually, the breeding program may have selected females with higher estimated breeding values for body weight that had this phenotype because presented some degree of urogenital papilla dysmorphia that contributed to their higher body weight but did not prevent their reproduction. Corroboration this hypothesis, Bezerra et al. (2023) described higher mean of body weight in females with abnormal urogenital papilla, as compared to female with normal urogenital papilla. A clearer view of genetic effects on etiopathogenesis of urogenital papilla dysmorphia will be provided by a genome-wide

association study, and fin collection of both genetic groups was performed to allow such analysis.

During the follow-up period, we observed the greater degree of severity of the dysmorphia is due to the inability of the tilapia females to spawn due to the possible stenosed oviduct. We also were able to identify newfound changes. In some abnormal females in this study, the urogenital papilla exposes the exit of the oviduct and urinary pore in such a way that they resemble two distinct papillae, each one like the urogenital papilla of males. In the most severe cases, females presented conical and pointed papilla associated with a pronounced distension in ventral region. Females with severe dysmorphia progressed to severe distension in ventral region, presented muscle tremors while palpated indicating discomfort and possibly pain. In these females, a possible fistula at the base of the urogenital papillae occurred, through which the oocytes were released intact in some individuals. In addition of the presence of a new exit from the oviduct, that restored the ability of some females to spawn, the progression of severe dysmorphia may involve: (1) the distension of genital pore as the animal grows, which may eventually restore reproductive capacity in rare cases, or (2) the death of fish, probably due the rupture of enlarged ovaries caused by the large number of retained oocytes and fluid accumulation. The ovaries of females with severe dysmorphia of urogenital papilla often exhibited firm material - often dark-brown color, associated with adhesions between the ovary and adjacent structures, including the body wall, intestine, bladder, and liver. Reproductive anomalies are not widely reported in tilapia (Akhtar et al., 2017), and this study is the first report of the long-term follow-up of Nile tilapia with dysmorphia of urogenital papilla according to our knowledge.

The reproductive performance of the experimental groups revealed that the treatments most affected by urogenital papilla dysmorphia also demonstrated lower reproductive capacity when compared to the control group. The lower reproductive performance was caused by the reduced spawning capacity of females affected by urogenital papilla dysmorphia. In a study conducted by Bezerra et al. (2023), it was observed that some females with abnormal urogenital papillae were able to reproduce and produce larvae comparable to normal females. However, the presence of these abnormal females had a direct impact on the overall reproductive capacity of broodstock. Specifically in this study, the tilapia females from the G8 group reared in high-temperature conditions had a reduction of up to 73.49% in larval production. Our results

reinforce that suitable management strategies for broodstock are crucial in tilapia culture (Coward and Bromage, 2000; Bhujel et al., 2001; Campos Mendoza et al., 2004), and indicated that the maintenance of future tilapia breeders at temperatures close to 25°C during the first two months after yolk sac absorption is essential for their future reproductive efficiency.

Global methylation studies performed in tilapia revealed that females had higher levels of DNA methylation in several chromosomes, compared with males (Sun et al 2016, Wan et al., 2016). The masculinization in Nile tilapia induced by high-water temperatures involves the higher methylation in genes, especially those involved in the aromatase/estrogen levels (Wan et al., 2016). Thus, the mechanism that induces the dysmorphia in urogenital papilla may be related to downregulation of genes induced by higher temperatures in females. In fact, the hormonal levels were different among experimental groups, indicating the hormonal pathway may be affected by temperature. Different studies induced sterility in tilapia through a high-temperature water treatment of fry for a period of greater than 45 days (Nakamura et al., 2015; Pandit et al., 2015; Nozu and Nakamura, 2020). Nozu and Nakamura (2020) applied temperature of 37°C in fry of *Oreochromis mossambicus* from 9 to 56 days of age and observed that the genital papillae were not developed in the treated females. Like our study, these authors also described permanent effect of temperature, since the plasma sex steroid hormone levels in treated tilapia were significantly different than those of control group in adult animals (19 months of age). Previous studies have already indicated that the development of the genital papilla in *Oreochromis aureus* is strongly linked to estrogen levels (Jensen and Shelton, 1979), and the effects of temperature on gene expression involved in the aromatase/estrogen levels may be a cause of urogenital papilla dysmorphia.

On the other hand, the different hormonal profile among tilapia experimental groups also may be a consequence of urogenital papilla dysmorphia. Reproductive cycles are successive in sexually mature tilapia, with intervals of 4-6 weeks. The estradiol (E2) and dihydroprogesterone (DHP) are recognized as the main regulators of cell divisions (mitotic and meiotic) in oögonia (Alba et al., 2021). Androgens, such as, 11-ketotestosterone (11-KT) and its precursor testosterone (T) also play an additional role during oocyte development (Wang et al., 2020; Alba et al., 2021). A rapid increase in E2 and T levels in the first days of the cycle is associated with an accelerated process of vitellogenesis. Furthermore, testosterone also plays an important role as a precursor of E2

(Tacon et al., 2000; Ortiz et al., 2017) and as a gonadotropin stimulator (Melamed et al., 1997) on ovarian development. Serum 11-KT concentrations were high during oocyte maturation, especially during vitellogenesis in some fish species (Barannikova et al., 2004; Wang et al., 2020). After spawning, plasma levels of E2, vitellogenin (Vtg), and gonadotropins dramatically drop and return to baseline levels (Tacon et al., 2000; Poleo et al., 2005; Fujimura and Okada, 2007). The plasma collection of tilapia female in this study was performed two weeks after reproductive assay, when females were in stocking tanks. The lower E2 level and the highest concentration of 11-KT in control group (CT-G8) may be related to the higher proportion of spawned female in this group, since the E2 level decrease (Alba et al., 2021) and 11-KT increase after spawning (Barannikova et al., 2004; Wang et al., 2020). Therefore, the results pave the way toward investigating if the differences in steroid profile among the fish from different experimental groups are a cause or consequence of the urogenital papilla dysmorphia.

The effects of treatments or the presence of urogenital papilla dysmorphia on biological indices were evaluated. The liver plays a crucial role in metabolism, including vitellogenin and protein synthesis, and lipid metabolism (Melamed et al., 2000). The biological indexes of females from different treatments were not statistically different, probably because normal and abnormal females were present in all experimental groups. Considering the classifications of female urogenital papilla, both viscerosomatic (VSI) and gonadosomatic indices (GSI) were elevated in abnormal females. The retention of oocytes by these females contributed to the increased GSI and VSI. Values between 4.6% and 10.2% indicate the presence of mature gonads and suggest that the females are ready to spawn (Vazzoler, 1996). However, in severely affected abnormal females, the GSI exceeds 15%. Such higher values in these indices indicate the difficulty in spawning of females with urogenital papilla dysmorphia, which will have a considerably reduced reproductive efficiency, and their use as breeders is not recommended.

4.6 Conclusion

Our results suggest the genetic factors and temperature play important role in etiopathogenesis of the urogenital papilla dysmorphia, while an infectious cause seems unlikely. The increase in prevalence of dysmorphia of the urogenital papilla results in a decrease of the reproductive performance. It still needs to be investigated whether differences in the hormonal profile are causes or consequences of the urogenital papilla dysmorphia. Our results indicated that the rearing of Nile tilapia in temperature close to 25°C in the first 60 days after yolk sac absorption is recommended to produce an efficient broodstock. Lastly, since the temperature ranges investigated were not extreme, our results illustrate how global warming and climate change can negatively affect tilapia production.

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4.8 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

4.9 Conflict of interest

The authors have no conflict of interest to declare.

References

- Agresti, A. 2015. Foundations of linear and generalized linear models. John Wiley & Sons.
- Akhtar, M.S., Ciji, A., Sarma, D., Rajesh, M., Kamalam, B.S., Sharma, P., Singh, A.K. 2017. Reproductive dysfunction in females of endangered golden mahseer (*Tor putitora*) in captivity. Anim. Reprod. Sci, 182, 95-103. <https://doi.org/10.1016/j.anireprosci.2017.05.004>
- Alvarenga, E.R., Alves, G.F.O., Fernandes, A.F.A., Costa, G.R., Da Silva, M.A., Teixeira, E.D.A., Turra, E.M. 2018. Moderate salinities enhance growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in the biofloc system. Aquac. Res, 49(9), 2919-2926. <https://doi.org/10.1111/are.13728>
- Alvarenga, E.R., Sales, S.C.M., Brito, S.T., Santos, C.R., Corrêa, R.D.S., Alves, G.F.O., Manduca, L.G., Turra, E.M. 2017. Effects of biofloc technology on reproduction and ovarian recrudescence in Nile tilapia. Aquac. Res., 48, 5965-5972. <https://doi.org/10.1111/are.13420>
- Aly, S.M., Fathi, M. 2024. Advancing aquaculture biosecurity: a scientometric analysis and future outlook for disease prevention and environmental sustainability. Aquacult. Int. <https://doi.org/10.1007/s10499-024-01589-y>
- APHA. 2012. Standard methods for the examination of water and wastewater, 22nd edition. In: Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. 2012. American Public Health Association, AWWA. Washington, D.C.
- Auguie, B. 2017. _gridExtra: Miscellaneous Functions for "Grid"Graphics. R package version 2.3. Available at: <https://CRAN.R-project.org/package=gridExtra>.
- Azevedo, R.O., Alvarenga, E.R., Fernandes, A.F.A., Silva, M.A., Alves, G.F.O., Menezes, W.F., Turra, E.M. 2021. Use of hCG hormone in the natural and artificial reproduction of Nile tilapia (*Oreochromis niloticus*). Aquac. Res., 52(12), 6380-6388. <https://doi.org/10.1111/are.15502>
- Barannikova, I.A., Bayunova, L.V., Semenkova, T.B. 2004. Serum levels of testosterone, 11-ketotestosterone and estradiol-17 β in three species of sturgeon during gonadal development and final maturation induced by hormonal treatment. J. Fish Biol., 64, 1330-1338. <https://doi.org/10.1111/j.0022-1112.2004.00395.x>
- Baroiller, J.F., D'Cotta, H.D. 2018. Sex control in tilapias. In: Wang, H.P., Piferrer, F., Chen, S.L. (Eds.). Sex Control in Aquaculture. John Wiley & Sons Ltd, 191–234.
- Baroiller, J.F., D'Cotta, H.D., Bezault, E., Wessels, S., Hoerstgen-Schwark, G. 2009. Tilapia sex determination: Where temperature and genetics meet. CBPA: Part A, 153, 30-38. <https://doi.org/10.1016/j.cbpa.2008.11.018>
- Bendschneider, K., Robinson, R.J. 1952. A new spectrophotometric method for the determination of nitrite in seawater. J. Mar. Res, 11, 87–96. Available at: <http://hdl.handle.net/1773/15938>
- Bezerra, V.M., Teixeira, E.A., Silva, F.B., Silva, S.C.D.C., Teixeira, J.P., Leal, C.A.G., Silva, M.A., Turra, E.M., Colosimo, E.A., Alvarenga, E.R. 2023. Decrease of reproductive performance of Nile tilapia (*Oreochromis niloticus*) females with dysmorphia in urogenital papilla. Aquaculture, 571, 739495. <https://doi.org/10.1016/j.aquaculture.2023.739495>
- Bhujel, R.C., Turner, W.A., Yakupitiyage, A., Little, D.C. 2001. Impacts of environmental manipulation on the reproductive performance of Nile tilapia (*Oreochromis niloticus*). J. Aquacult. Trop, 16(3), 179-209.

- Bhujel, R.C. 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. *Aquaculture*, 181(1-2), 37-59. [https://doi.org/10.1016/S0044-8486\(99\)00217-3](https://doi.org/10.1016/S0044-8486(99)00217-3)
- Bickley L.K., Brown, A.R., Hosken, D.J., Hamilton, P.B., Le Page, G., Paull, G.C., Owen, S.F., Tyler, C.R. 2013. Interactive effects of inbreeding and endocrine disruption on reproduction in a model laboratory fish. *Evol Appl.*, 6(2):279-89. <https://doi.org/10.1111/j.1752-4571.2012.00288.x>.
- Bondad-Reantaso, M.G., C. R. Lavilla-Pitogo, Karunasagar, I., Arthur, J.R., Hao, B., Garrido-Gamarro, E. and Penarubia, O.R. 2020. Outputs and activities of FAO Project FMM/RAS/298/MUL on antimicrobial resistance in fisheries and summary of FAO's recent work on antimicrobial resistance in aquaculture. *FAO Fisheries and Aquaculture Circular*, 1215, 83-84. Available at: <https://openknowledge.fao.org/server/api/core/bitstreams/05d689c2-3b16-4331-906b-e4e490f6461f/content>
- Box, G.E.P., Hunter, S., Hunter, W.G. 2005. *Statistics for Experimenters: Design, Innovation, and Discovery*, Wiley, 2nd Edition. 633. Hoboken, New Jersey.
- Campos-Mendoza, A., McAndrew, B.J., Coward, K., Bromage, N. 2004. Reproductive response of Nile tilapia (*Oreochromis niloticus*) to photoperiodic manipulation: Effects on spawning periodicity, fecundity, and egg size. *Aquaculture*, 231, 299–314. <https://doi.org/10.1016/j.aquaculture.2003.10.023>
- Cavatti Neto, A.C., Alvarenga, E.R., Toral, F.L.B., Leite, N.R., da Costa, F.F.B., Goulart, L.Q., Corrêa, R. D. S., Silva, M. A., Santos, B. D., Fernandes, A. F. A., Turra, E.M. 2023. Impact of selection for growth and stocking density on Nile tilapia production in the biofloc system. *Aquaculture*, 577, 739908. <https://doi.org/10.1016/j.aquaculture.2023.739908>
- Chebib J., Guillaume, F. 2021, Pleiotropy or linkage? Their relative contributions to the genetic correlation of quantitative traits and detection by multitrait GWA studies. *Genetics*, 219(4), 159. <https://doi.org/10.1093/genetics/iyab159>
- Collins, C., Bresnan, E., Brown, L., Falconer, L., Guildler, J., Jones, L., Kennerley, A., Malham, S., Murray, A., Stanley, M. 2020. Impacts of climate change on aquaculture. *MCCIP Sci. Rev*, 482-520. <https://doi.org/10.14465/2020.arc21.aqu>
- Conover, W. J. 1999. *Practical Nonparametric Statistics*, Wiley, 3rd Edition., New York, New York.
- Coward, K., Bromage, N.R. 2000. Reproductive physiology of female tilapia broodstock. *Rev. Fish Biol. Fish.*, 10(1), 1-25. <https://doi.org/10.1023/A:1008942318272>
- De Alba, G.D., Michele, N., Mourad, N., Paredes, J.F., Sánchez-Vázquez, F.J., López-Olmeda, J.F. 2019. Daily rhythms in the reproductive axis of Nile tilapia (*Oreochromis niloticus*): Plasma steroids and gene expression in brain, pituitary, gonad, and egg. *Aquaculture*, 507, 313-321. <https://doi.org/10.1016/j.aquaculture.2019.04.047>
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. 2015. *InfoStat Version 2015*. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available at: <http://www.infostat.com.ar>
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*, 257(1-4), 346-358. <https://doi.org/10.1016/j.aquaculture.2006.03.019>
- El-Sayed, A.-F. M. 2019. *Tilapia culture*, 2nd ed. Elsevier Inc, Academic Press, London.

- Etterson, J.R. 2016. Climate Change, Quantitative Genetics and, Editor(s): Richard M. Kliman, Encyclopedia of Evolutionary Biology, Academic Press, 277-286. <https://doi.org/10.1016/B978-0-12-800049-6.00057-3>.
- FAO (Food and Agriculture Organization). 2022. The State of World Fisheries and Aquaculture (SOFIA) – Sustainability in action. FAO/WHO, Rome, Italy.
- Fox, J., Weisberg, S. 2019. An R Companion to Applied Regression, 3rd ed. Thousand Oaks CA: Sage.
- Fujimura, K., Okada, N. 2007. Development of the embryo, larva and early juvenile of Nile tilapia *Oreochromis niloticus* (Pisces: Cichlidae). Developmental staging system. Dev Growth Differ, 49, 301-324. <https://doi.org/10.1111/j.1440-169X.2007.00926.x>
- Gallardo, J. A., García, X., Lhorente, J. P., Neira, R. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of Coho salmon selected using BLUP predictors of breeding values. Aquaculture, 234(1-4), 111–122. <https://doi.org/10.1016/j.aquaculture.2004.01.009>
- Gjerde, B., Gunnes, K., Gjedrem, T., 1983. Effects of inbreeding on survival and growth in rainbow trout. Aquaculture 34, 327– 332. [https://doi.org/10.1016/0044-8486\(83\)90212-0](https://doi.org/10.1016/0044-8486(83)90212-0)
- Haenen, O. L. M., Dong, H. T., Hoai, T. D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S. L., Zadoks, R., Kiermeier, A., Wang, B., Gamarro, E. G., Takeuchi, M., Azmai, M. N. A., Fouz, B., Pakingking, R., Wei, Z. W., and Bondad-Reantaso, M. G. 2023. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev Aqua. 15(S1), 154–185. <https://doi.org/10.1111/raq.12743>
- Ijiri, S., Kaneko, H., Kobayashi, T., Wang, D.S., Sakai, F., Paul-Prasanth, B., Nakamura, M. Nagahama, Y. 2008. Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol. Reprod. 78, 333-341. <https://doi.org/10.1095/biolreprod.107.064246>
- Jalabert, B., Zohar, Y. 1982. Reproductive physiology in cichlid fishes, with particular reference to Tilapia and Sarotherodon. In: Pullin, R.S.V., Lowe-McConnell, R.H. The Biology and Culture of Tilapias (ICLARM conference proceedings). ICLARM, 7, 129-140.
- Jensen, G.L., Shelton, W.L. 1979. Effects of estrogens on *Tilapia aurea*: implications for production of monosex genetic male tilapia. Aquaculture, 16(3), 233-242. [https://doi.org/10.1016/0044-8486\(79\)90111-X](https://doi.org/10.1016/0044-8486(79)90111-X)
- Lele, S.R., Keim, J.L., Solymos, P. 2019. ResourceSelection: Resource Selection (Probability) Functions for Use-Availability Data. R package version 0.3-5. Available at: <https://CRAN.R-project.org/package=ResourceSelection>.
- Mataveli, M., Moraes, G.V., Streit Junior, D.P., Ribeiro, R.P., Gasparino, E. 2010. Qualidade do sêmen em tilápia do Nilo (*Oreochromis niloticus*), alimentadas com dietas contendo diferentes níveis de vitamina C. Acta Sci., Anim. Sci. 32(3), 345-349. <https://doi.org/10.4025/actascianimsci.v32i3.7836>
- Maulu, S., Hasimuna, O.J., Haambiya, L.H., Monde, C., Musuka, C.G., Makorwa, T.H., Munganga, B.P., Phiri, K.J., Nsekanabo, J.D. 2021. Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. Front. Sustain. Food Syst., 5. <https://doi.org/10.3389/fsufs.2021.609097>
- Melamed, P., Gur, G., Rosenfeld, H., Elizur, A., Schulz, R.W., Yaron, Z. 2000. Reproductive development of male and female tilapia hybrids (*Oreochromis niloticus* × *O. aureus*) and changes in mRNA levels of gonadotropin (GtH) Iβ and IIβ subunits. J. Exp. Zool., 286, 64-75. [https://doi.org/10.1002/\(SICI\)1097-010X\(20000101\)286:1<64::AID-JEZ6>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1097-010X(20000101)286:1<64::AID-JEZ6>3.0.CO;2-D)

- Melamed, P., Gur, G., Rosenfeld, H., Elizur, A., Yaron, Z. 1997. The mRNA levels of GtH I β , GtH II β and GH in relation to testicular development and testosterone treatment in pituitary cells of male tilapia. *Fish Physiol. Biochem.*, 17, 93-98. <https://doi.org/10.1023/A:1007737414565>
- Monteiro, M.I.C., Ferreira, F.N., Oliveira, N.M.M., Avila, A.K. 2003. Simplified version of the sodium salicylate method for analysis of nitrate in drinking waters. *Anal. Chim. Acta*, 477, 125-129. [https://doi.org/10.1016/S0003-2670\(02\)01395-8](https://doi.org/10.1016/S0003-2670(02)01395-8)
- Mylonas, C.C., Fostier, A., Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocrinol.*, 165, 516-534. <https://doi.org/10.1016/j.ygcen.2009.03.007>
- Nakamura, R., Nozu, R., Ijiri, S., Kobayashi, T., Hirai, T., Yamaguchi, Y., Seale, A., Lerner, D.T., Grau, G.E. 2015. Sexual characteristics of high-temperature sterilized male Mozambique tilapia, *Oreochromis mossambicus*. *Zool. Lett.*, 1, 21. <https://doi.org/10.1186/s40851-015-0021-4>
- Nozu, R., Nakamura, M. 2020. Influence of prolonged cultivation on sexual characteristics of sterilized female tilapia, *Oreochromis mossambicus*, induced by high-temperature treatment. *Aquaculture*, 524, 735245. <https://doi.org/10.1016/j.aquaculture.2020.735245>
- Ortiz, J., Valladares, L., Muñoz, D., Caza, J., Manjunatha, B., Kundapur, R.R. 2017. Levels of 17 β -estradiol, vitellogenin, and prostaglandins during the reproductive cycle of *Oreochromis niloticus*. *Lat. Am. J. Aquat. Res.*, 45, 930-936. <https://doi.org/10.3856/vol45-issue5-fulltext-8>
- Pandit, N.P., Bhandari, R.K., Kobayashi, Y., Nakamura, M. 2015. High temperature induced sterility in the female Nile tilapia, *Oreochromis niloticus*. *Gen. Comp. Endocrinol.*, 213, 110-117. <https://doi.org/10.1016/j.ygcen.2015.01.028>
- Piamsomboon, P., Mehl, N.S., Sirivaidyapong, S., Wongtavatchai, J. 2019. Assisted reproduction in Nile tilapia *Oreochromis niloticus*: Milt preservation, spawning induction and artificial fertilization. *Aquaculture*, 507, 139-143. <https://doi.org/10.1016/j.aquaculture.2019.04.019>
- Poleo, G.A., Lutz, C.G., Cheuk, G., Tiersch, T.R. 2005. Fertilization by intracytoplasmic sperm injection in Nile tilapia (*Oreochromis niloticus*) eggs. *Aquaculture*, 250, 82-94. <https://doi.org/10.1016/j.aquaculture.2005.03.025>
- Pullin, R.S., Eknath, A.E., Gjerdem, T., Tayamen, M.M., Macaranas, J.M., Abella, T.A. 1991. The genetic improvement of farmed tilapias (GIFT) project: The story so far. *Naga: The ICLARM Quarterly*, 14(2), 3-6.
- Qiang, J., Duan, X.J., Zhu, H.J., He, J., Tao, Y.F., Bao, J.W., Zhu, X.W., Xu, P. 2021. Some 'white' oocytes undergo atresia and fail to mature during the reproductive cycle in female genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, 534, 736278. <https://doi.org/10.1016/j.aquaculture.2020.736278>
- Quinn, G. P., Keough, M. J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>
- Stearns, F.W. 2010. One hundred years of pleiotropy: a retrospective. *Genetics*, 186(3), 767-73. <https://doi.org/10.1534/genetics.110.122549>. Erratum in: *Genetics*. 2011, 187(1), 355. PMID: 21062962; PMCID: PMC2975297.
- Strickland, J.D.H., Parsons, T.R. 1972. *A Practical Handbook of Seawater Analysis*, 2nd ed. *J. Fish. Res. Board Can.*, 167. <https://doi.org/10.25607/OBP-1791>

- Sun, L.N., Wang, Y., Zhao, Y., Wang, H., Li, N., Ji, X.S. 2016. Global DNA methylation changes in Nile tilapia gonads during high temperature- induced masculinization. *PLOS one*, 11, 158483. <https://doi.org/10.1371/journal.pone.0158483>
- Sun, L.X., Teng, J., Zhao, Y., Li, N., Wang, H., Ji, X.S. 2018. Gonad transcriptome analysis of high-temperature-treated females and high-temperature-induced sex-reversed neomales in Nile tilapia. *Int. J. Mol. Sci*, 19, 689. <https://doi.org/10.3390/ijms19030689>
- Tacon, P., Baroiller, J.F., Le Bail, P.Y., Prunet, P., Jalabert, B. 2000. Effect of egg deprivation on sex steroids, gonadotropin, prolactin, and growth hormone profiles during the reproductive cycle of the mouthbrooding cichlid fish *Oreochromis niloticus*. *Gen. Comp. Endocrinol.*, 117, 54-65. <https://doi.org/10.1006/gcen.1999.7388>
- Troell, M., Eide, A., Isaksen, J., Hermansen, Ø., Crépin, A.S. 2017. Seafood from a changing Arctic. *Ambio*, 46(Suppl 1), S368-S386. <https://doi.org/10.1007/s13280-017-0954-2>
- UNESCO. 1983. Chemical methods for use in marine environmental monitoring. Manual and Guides12. IOC, 53.
- Vazzoler, A. E. A. M. 1996. Biologia da reprodução de peixes teleósteos. Teoria e Prática. Maringá, EDUEM, 169.
- Wan, Z.Y., Xia, J.H., Lin, G., Wang, L., Lin, V.C.L., Yue, G.H. 2016. Genome-wide methylation analysis identified sexually dimorphic methylated regions in hybrid tilapia. *Sci. Rep*, 6, 35903. <https://doi.org/10.1038/srep35903>
- Wang, W., Zhu, H., Tian, Z., Sun, A., Dong, Y., Dong, T., Hu, H. 2020. Effects of 11-Ketotestosterone on Development of the Previtellogenic Ovary in the Sterlet, *Acipenser ruthenus*. *Front. Endocrinol*, 11, 115. <https://doi.org/10.3389/fendo.2020.00115>
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemond, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Lin Pedersen, T., Miller, E., Milton Bache, S., Müller, K., Ooms, J., Robinson, D., Paige Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H. 2019. Welcome to the tidyverse. *J. Open Source Softw*, 4, 1686. Available at: <https://tidyverse.tidyverse.org/articles/paper.html>.
- World Organisation for Animal Health (WOAH). 2024. Notification of diseases and provision of epidemiological information. Aquatic Animal Health Code: Chapter 1.3. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmlfile=chapitre_diseases_listed.htm.
- Yoshida, G.M., Lhorente, J.P., Carneiro, R., Yáñez, J.M. 2017. Bayesian genome-wide association analysis for body weight in farmed Atlantic salmon (*Salmo salar* L.). *Anim. Genet.*, 48, 698-703. <https://doi.org/10.1111/age.12621>
- Yúfera, M. 2021. Embryonic and Larval Development of Tilapias. In: *Biology and Aquaculture of Tilapia*. CRC Press, 178-195.
- Zhao, Y., Mei, Y., Chen, H.J., Zhang, L.T., Wang, H., Ji, X.S. 2019. Profiling expression changes of genes associated with temperature and sex during high temperature-induced masculinization in the Nile tilapia brain. *Physiol. Genomics*, 51, 159-168. <https://doi.org/10.1152/physiolgenomics.00117.2018>

5 CONCLUSÃO GERAL

A identificação desta anomalia na papila urogenital em fêmeas de tilápias do Nilo tende a auxiliar melhorias nas técnicas de seleção de reprodutores e manejo na larvicultura nesta espécie, já que sua presença reduziu consideravelmente a eficiência reprodutiva de portadoras em ambos os experimentos que compõem esta tese. A possibilidade do diagnóstico visual (realizado através de características físicas macroscópicas da papila urogenital das fêmeas) contribui para que seja implementada uma seleção de reprodutoras de tilápia do Nilo que exclua animais com a anomalia descrita nesse estudo, fomentando ganhos reprodutivos na tilapicultura.

Houve uma associação alta entre a presença da papila anômala com alterações consideráveis nos ovários destas fêmeas, com o aumento considerável do volume do órgão, pela retenção de ovócitos e lesões ovarianas que deverão ser investigadas histologicamente e quantificadas para melhor entendimento da progressão para casos graves e eventuais óbitos. Os índices gonadossomático e viscerossomático das fêmeas acometidas pela anomalia foram significativamente maiores quando comparados com fêmeas normais, e influenciados pelo aumento considerável do volume ovariano.

Foi possível compreender com mais detalhes os mecanismos responsáveis pela origem da anomalia na papila urogenital em tilápias, sendo fatores genéticos e altas temperaturas da água de cultivo durante os primeiros 60 dias após a absorção do saco vitelínico das larvas identificados como importantes causas na etiopatogênese desta anomalia. Quanto ao caráter genético da anomalia, a maior prevalência de fêmeas portadoras dentro do grupo de animais melhorados (G8) quando comparados ao grupo de fêmeas nos quais não se aplicou quaisquer métodos de seleção ou controle na reprodução (G0), há duas possíveis vertentes de explicação para os resultados observados. 1) houve seleção simultânea de genes envolvidos na característica de ganho de peso corporal associados a genes responsáveis pela anomalia; 2) em grupos de animais selecionados espera-se maior homogeneidade entre os indivíduos, o que resultaria em animais mais homogêneos neste grupo e uma prevalência mais perceptível, quando comparado a animais não selecionados, que por sua vez teriam maior heterogeneidade e por isso, respostas variáveis aos fatores causadores da anomalia. Estas hipóteses fazem parte dos objetivos de futuros projetos oriundos desta tese, para os quais coletaram-se nadadeiras de todos os animais do experimento para avaliação genética.

Uma hipótese que surge a partir dos resultados é que a temperatura ambiental influenciou no surgimento da anomalia através de um possível aumento nos níveis de metilação de DNA em vários cromossomos, gerando alteração na regulação de genes durante a diferenciação gonadal. Esse efeito é observado para a masculinização de tilápias por temperatura. A hipótese é que as altas temperaturas usadas nesse estudo não foram o bastante para que causassem a masculinização, mas foram o suficiente para gerar as alterações morfológicas nas papilas de fêmeas portadoras da anomalia, e mudanças significativas em seus perfis hormonais. Em animais normais espera-se que, após a desova, haja aumento nas concentrações séricas de 11-ketotestosterona e diminuição de estrógeno, porém nas fêmeas anormais estes níveis se alteraram. O efeito das altas temperaturas na fase do desenvolvimento gonadal sobre a metilação de DNA e o surgimento de papilas urogenitais anormais por esse mecanismo poderão ser investigados futuramente.

O impacto de altas temperaturas da água de cultivo sobre a prevalência de alterações reprodutivas gera a dúvida se as mudanças climáticas, em especial o aquecimento global, enfrentadas atualmente por nosso planeta poderão propiciar aumentos na prevalência geral desta anomalia da papila urogenital em diferentes populações de tilápias. Com o aumento constante das médias da temperatura mundial, as faixas de temperaturas mais altas durante a larvicultura de tilápias poderão causar prejuízos consideráveis, devido às alterações anatômicas e fisiológicas permanentes em animais portadores, e até então irreversíveis.

APÊNDICE – PRODUÇÃO RESULTANTE DA TESE**Anomalia na estrutura reprodutiva externa de tilápias do Nilo: causas e impactos na produção de alevinos**

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Eficiência na produção de formas jovens de tilápias

Embora a tilápia reproduza facilmente em fazendas comerciais, a eficiência de produção de alevinos pode ser reduzida caso animais com baixa capacidade reprodutiva estejam presentes no plantel de reprodutores. Estudos recentes realizados pelo Grupo de pesquisa NGTAqua (Nutrição, Genética e Tecnologia na Aquicultura) em colaboração com o laboratório Aquavet (Laboratório de Doenças de Animais Aquáticos), ambos da Escola de Veterinária da Universidade Federal de Minas Gerais, identificaram alterações anatômicas na estrutura reprodutiva externa (papila urogenital) de fêmeas de tilápias do Nilo, descreveram possíveis causas dessas alterações (efeitos de temperatura, agentes patogênicos e genéticos) e calcularam o impacto dessa anomalia no desempenho reprodutivo de tilápias nilóticas (Bezerra et al., 2023 e Bezerra et al., 2025).

Estrutura reprodutiva externa de tilápias: a papila urogenital

A tilápia nilótica apresenta dimorfismo sexual externo, ou seja, é possível separar machos de fêmeas apenas avaliando sua estrutura reprodutiva externa, chamada de papila urogenital (El-Sayed, 2019). Em machos adultos é possível distinguir um único orifício na papila urogenital, que é o poro geniturinário, enquanto em fêmeas se observa dois orifícios, que são a saída do oviduto, cranialmente, e o poro urinário na porção caudal da papila urogenital (Figura 1). Além disso, o formato da papila urogenital difere entre os sexos sendo mais arredondada em fêmeas e mais alongada em machos (Baroiller e D'Cotta, 2018). A distinção dos sexos dessa forma contribui muito para a atividade dos produtores, pois permite separar machos de fêmeas e evitar reproduções em momento indesejável. Além disso, possibilita manter em tanques de reprodução a proporção ideal de machos e fêmeas e facilita o manejo do plantel de reprodutores. Dessa forma, o formato adequado da papila urogenital é essencial para o manejo correto de reprodutores de tilápias.

Variações no formato da papila urogenital em tilápias do Nilo já foram descritas em experimentos nos quais tratamentos com aplicações de hormônios sexuais foram empregados (como nos trabalhos de Alcántar-Vázquez et al., 2014 e Alcántar-Vázquez et al., 2015). Desde 2005, fêmeas de tilápias com papila urogenital com formato alterado foram observadas em uma fazenda produtora de alevinos de tilápias em Minas Gerais (Prof. Eduardo Maldonado Turra, UFMG, Comunicação pessoal). Mais recentemente, o laboratório Aquavet recebeu dois lotes de reprodutores (aproximadamente 120 animais por lote) de fazendas dos Estados de Minas Gerais e São Paulo e identificaram cerca de 8-10% de fêmeas reprodutoras com alteração na estrutura reprodutiva externa (Prof. Carlos Augusto Gomes Leal, UFMG, Comunicação pessoal). Ainda, o

grupo NGTAqua observou em seu plantel de reprodutores cultivado dentro de estufa agrícola a ocorrência de fêmeas com papila urogenital alterada, similar a papila urogenital de machos. Diante dessas observações, iniciou-se uma pesquisa para investigar por que essas alterações ocorrem e quais seus impactos na eficiência reprodutiva de tilápias do Nilo.

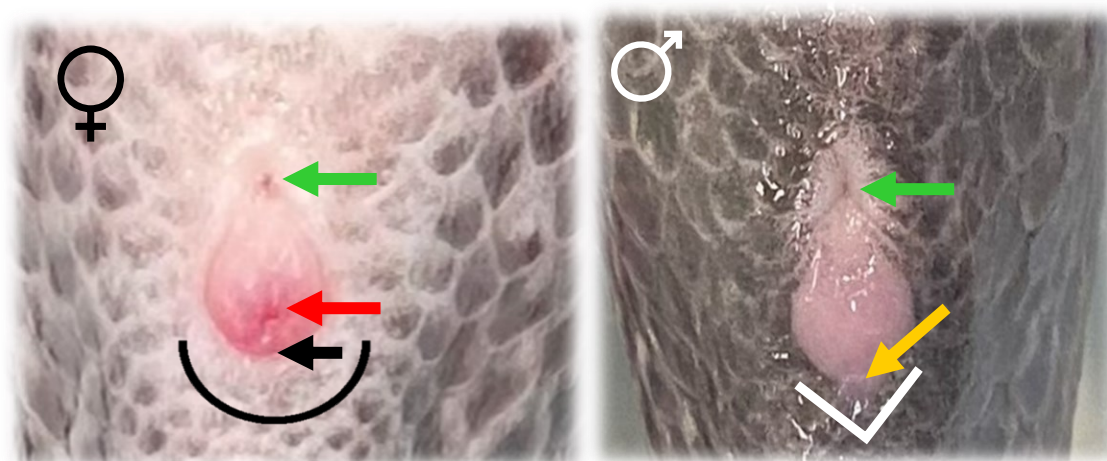


Figura 1: Papilas urogenitais normais de tilápias do Nilo. À esquerda, uma papila urogenital de fêmea, mostrando a saída do oviduto (seta vermelha) e poro urinário (seta preta). O ânus é identificado pela seta verde. Evidencia-se o formato mais arredondado da papila urogenital de fêmeas. À direita é apresentado a papila urogenital de machos, mostrando o poro geniturinário (seta amarela), o ânus é mostrado na ponta da seta verde. Observa-se estrutura mais alongada da papila urogenital de machos.

Anomalia da papila urogenital

Fêmeas com papila urogenital alterada possuem uma papila urogenital alongada, similar a papila urogenital de machos, e sem a saída do oviduto evidente (Figura 2). Nesses animais, sob leve pressão na cavidade celomática, é possível observar ovócitos aprisionados na saída do oviduto (Figura 3). Em casos mais graves, o acúmulo de ovócitos não desovados gera abaulamento do corpo do animal (especificamente da cavidade celomática). Quando se avaliou os ovários de fêmeas anormais, mais de 65% delas apresentaram algum grau de comprometimento do ovário, como volume aumentado, acúmulo de líquido esverdeado, grande volume de ovócitos atresícos e presença de massas amarronzadas e endurecidas (Figura 4), indicando que fêmeas com papilas urogenitais anormais podem também apresentar comprometimento dos ovários.

Impactos da anomalia da papila urogenital no desempenho reprodutivo

Para avaliar o impacto da anomalia da papila urogenital no desempenho reprodutivo de tilápias, os pesquisadores separaram 96 fêmeas de tilápias normais e 96 fêmeas de tilápias com papila urogenital anormais e realizaram 9 semanas de reprodução desses dois grupos de fêmeas. Em cada semana, 32 fêmeas de cada um desses grupos (normais e anormais) foram alocadas junto com machos normais e avaliadas quanto a produção de larvas. Nessas 9 semanas de reprodução, em 3 delas os animais foram mantidos a 28°C, em 3 semanas eles estiveram em água na temperatura de 26°C e nas demais 3 semanas na temperatura de 24°C. Nesse experimento, em média, 33,6%, 40,8% e 21,6% das fêmeas normais reproduziram à 28°C, 26°C e 24°C, respectivamente. Por outro lado, a proporção de fêmeas anormais que reproduziu foi 14,3% a 28°C, 13,7% a 26°C e 2,1% a 24°C (Figura 5). Modelo estatístico dos dados mostrou que a proporção de fêmeas que reproduzem no grupo normal é 3,2 vezes a proporção dos animais que reproduzem no grupo de fêmeas com papila urogenital anormal na temperatura de 28°C, 4,6 vezes na temperatura de 26°C e de 5,5 vezes na temperatura de 24°C. Assim, o desempenho reprodutivo dos animais com a papila urogenital anormal é drasticamente reduzido comparado com os animais com a estrutura reprodutiva externa normal (mais detalhes em Bezerra et al. (2023)).

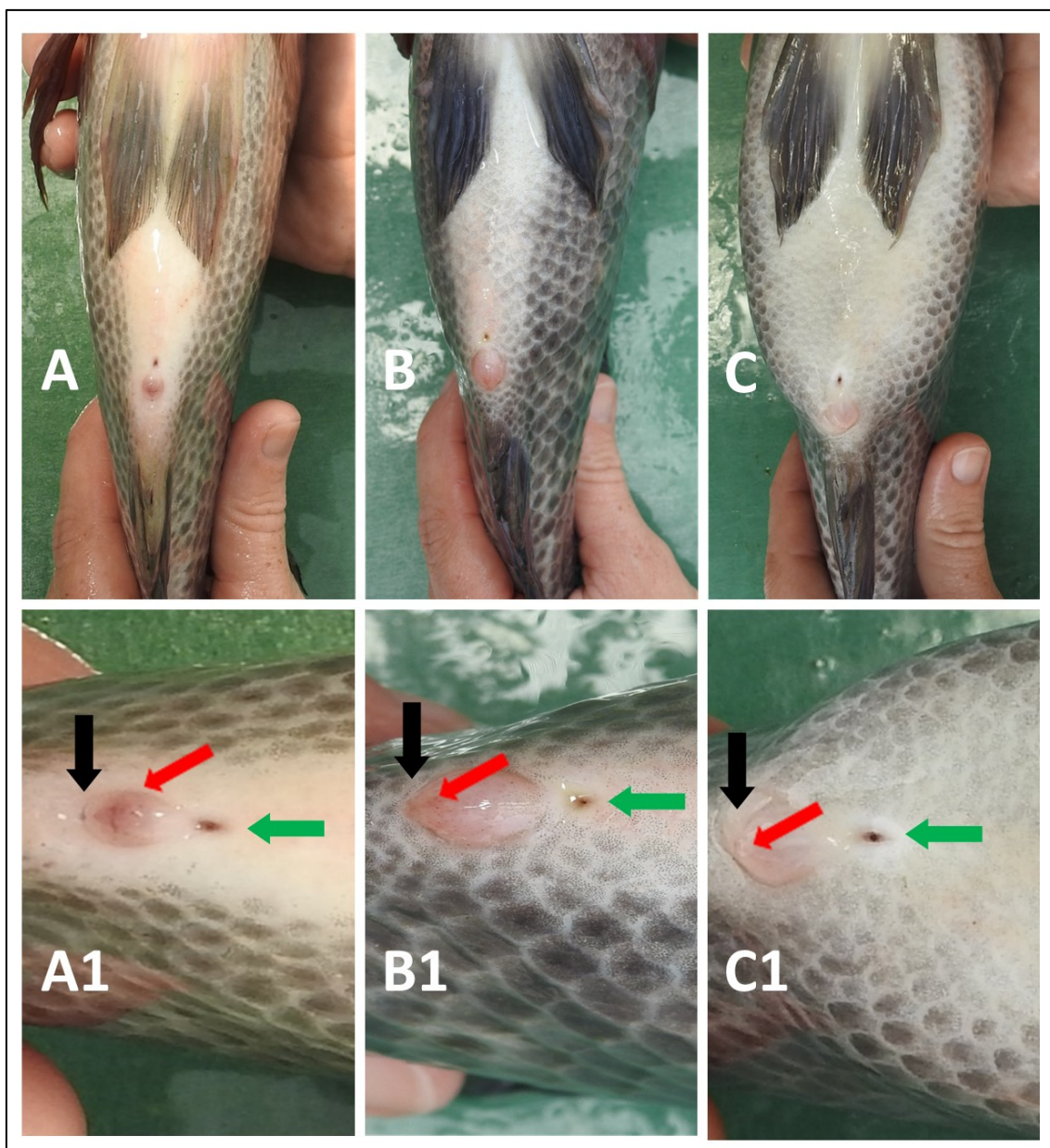


Figura 2: Comparação de papila urogenital de fêmeas de tilápias nilóticas consideradas normais (A, A1) e anormais (B, B1 e C, C1). A e A1 mostram a fotografia e a fotografia aumentada de uma papila urogenital considerada normal mostrando o oviduto (seta vermelha) e poro urinário (seta preta) muito bem definidos. O ânus é identificado pela seta verde. B e B1 mostram fêmeas classificadas com malformação considerada moderada, com formato mais alongada similar a uma papila urogenital de macho e com uma cavidade celomática levemente distendida. C e C1 mostram a papila urogenital classificada como anormal com comprometimento severo, levando a um grande acúmulo de ovócitos e severo abaulamento da cavidade celomática. Adaptado de Bezerra et al. (2023).

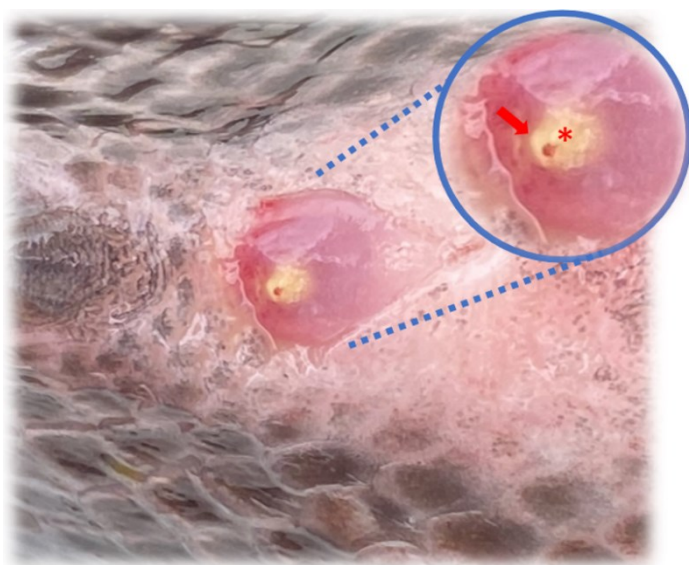


Figura 3: Papila urogenital de fêmea de tilápia do Nilo com anomalia. A papila urogenital da fêmea é similar a uma papila urogenital de macho. Uma leve pressão da cavidade celomática deixa ovócito evidente, mas aprisionados na saída do oviduto. Adaptado de Bezerra et al. (2025).

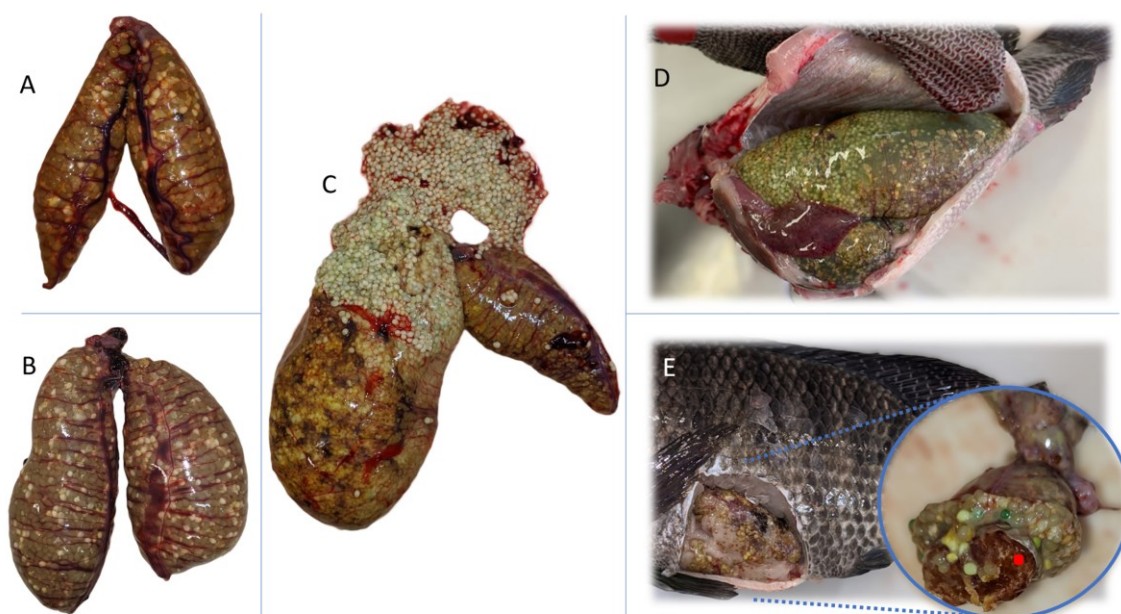


Figura 4: Ovários com aspecto normal (A), levemente alterado (B) e severamente comprometido (C). Ovários severamente comprometidos apresentam grande volume de ovócitos atresícos (ovócitos esbranquiçados), acúmulo de líquido esverdeado (D) e ocasionalmente massas amarronzadas com material endurecido (ponto vermelho destacado no círculo azul) (E).

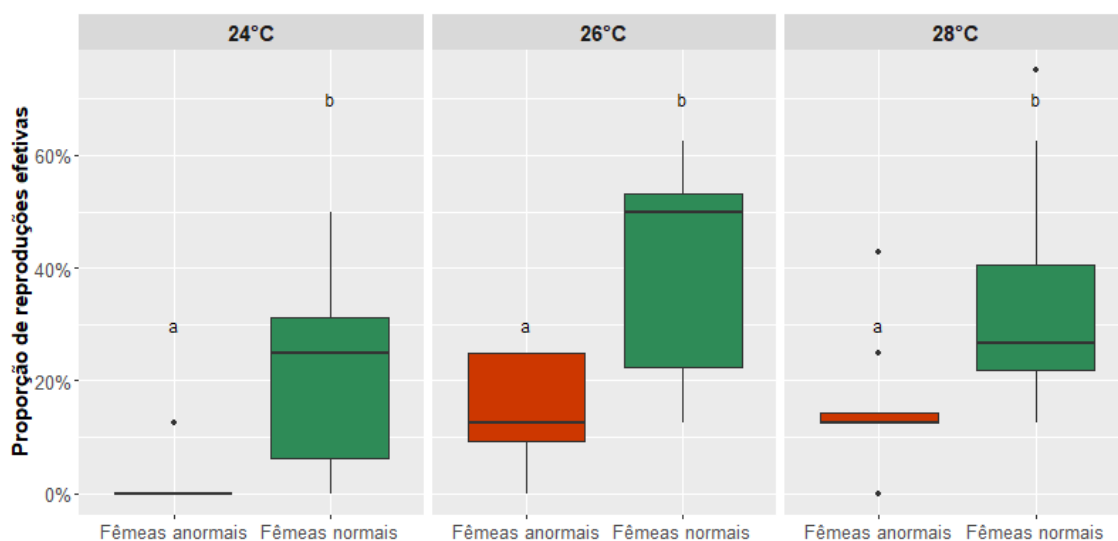


Figura 5: Proporção de reprodução efetiva de fêmeas de tilápias do Nilo com papila urogenital normal (em verde) e anormal (em vermelho). Letras diferentes em cada uma das temperaturas que os animais foram expostos durante a reprodução indicam diferença significativa na proporção de reproduções efetivas pelo teste estatístico de Mann-Whitney ($p < 0,05$).

Fatores que afetam a ocorrência da anomalia da papila urogenital

O baixo desempenho reprodutivo dos animais com papilas urogenitais anormais levou aos pesquisadores a investigarem possíveis causas dessa condição. Uma hipótese inicial seria que o baixo desempenho reprodutivo fosse causado por uma infecção bacteriana ascendente do oviduto, resultando no processo inflamatório e necrótico nos ovários. Para testar essa hipótese, foi feito estudo buscando isolar bactérias nos animais anormais. Essa análise bacteriana foi feita tanto nos animais imediatamente após a identificação da anomalia na papila (quando as fêmeas estavam com cerca de 6 meses de idade) quanto nos animais adultos após o processo reprodutivo. Entretanto, todos os exames bacteriológicos foram negativos, indicando que uma causa infecciosa é pouco provável (Bezerra et al., 2023 e Bezerra et al., 2025).

Uma segunda hipótese era que altas temperaturas no momento da diferenciação sexual poderiam ter um efeito de masculinizar a papila urogenital de fêmeas, tornando-as similares a papila urogenital de machos, mas essa temperatura não seria alta o suficiente para masculinizar os ovários. A hipótese do efeito de altas temperaturas foi baseada no fato de que a frequência de fêmeas com papila urogenital anormal observada no Laboratório de Aquacultura da UFMG aumentou após o cultivo das formas jovens em

estufa agrícola no verão, quando a temperatura da água pode alcançar valores entre 30°C e 33°C. Para testar essa hipótese, foi definido 3 amplitudes de temperatura a serem investigadas: 25°C-28°C, considerada temperatura normal de cultivo (Controle), 27°C-30°C considerada uma temperatura moderadamente alta (TM) e 30°C a 33°C, uma faixa de temperatura alta (TA). Além do efeito de temperatura, efeitos genéticos poderiam afetar a prevalência da anomalia da papila urogenital. Por isso, duas linhagens genéticas de tilápias, uma geneticamente melhorada para peso corporal durante sete gerações de seleção genética (G8) e uma não submetida a esse processo de seleção genética (G0) foram avaliadas. Por limitação de tanques disponíveis para o experimento, o grupo controle foi feito apenas com a linhagem melhorada. Essa escolha foi feita considerando que a linhagem melhorada seria mais utilizada em tilapiculturas. Os animais foram submetidos a essas 3 amplitudes de temperatura durante os 60 dias iniciais pós absorção do saco vitelínico, posteriormente foram cultivados em tanques comuns e chipados para que todos os indivíduos, de todos os tratamentos de temperatura, pudessem ser acompanhados individualmente para se determinar a proporção de fêmeas de tilápias com a anomalia da papila urogenital (prevalência da anomalia) em cada um dos tratamentos e a capacidade reprodutiva delas.

Os resultados mostraram que o aumento da temperatura na fase de larvicultura (nos primeiros 60 dias, logo após a absorção do saco vitelínico) resultou em elevação da prevalência de fêmeas com papila urogenital anormal (Figura 6). O grupo submetido a altas temperaturas da linhagem G8 (TA-G8) apresentou a maior prevalência de anomalia da papila urogenital, quase 90% de fêmeas anormais (essa porcentagem foi de 44% das fêmeas G0). Alevinos mantidos em temperatura moderadamente alta geraram fêmeas adultas que apresentaram 62% de anomalia na papila urogenital de fêmeas em G8 (TM-G8) e 68% de anomalia em fêmeas com papila urogenital em G0 (TM-G0). O grupo com menor prevalência de anomalia da papila urogenital foi o grupo controle (20% das fêmeas). Portanto, a temperatura tem efeito primordial na prevalência das papilas urogenitais anormais, sendo possível identificar também efeitos de origem genética, uma vez que as duas linhagens apresentaram diferentes resultados quanto a ocorrência dessa anomalia. Os animais melhorados apresentaram um claro aumento da anomalia com o aumento da temperatura, o que pode estar relacionado ao fato desse grupo ser mais homogêneo, devido ao processo de seleção genética. Por outro lado, esse claro padrão

não foi tão evidente nos animais não melhorados, provavelmente devido a maior variabilidade genética desse grupo.

Ao atingirem maturidade sexual, os animais provenientes dos diferentes grupos experimentais foram submetidos a ensaios de desempenho reprodutivo. A análise estatística dos dados mostra que o grupo de fêmeas submetidas a temperatura moderada e alta nos primeiros 60 dias pós absorção do saco vitelínico apresentaram uma redução de cerca de 70% do desempenho reprodutivo comparado com o grupo controle. Essa queda de desempenho está relacionada com a maior proporção de animais com papila urogenital anormal nos grupos submetidos a temperaturas mais elevadas no início do desenvolvimento (mais detalhes em Bezerra et al, 2025).

Na pesquisa desenvolvida na UFMG, os animais foram todos marcados com microchips e acompanhados durante 15 meses. Esse acompanhamento permitiu observar que alguns animais com papila urogenital anormal desenvolveram novas aberturas em regiões não comuns (Figura 7). Em alguns desses casos, as fêmeas readquirem a capacidade de liberar os ovócitos mas ainda não se sabe se esta capacidade as permite reproduzir adequadamente, já que este novo orifício não se assemelha a estrutura da papila urogenital normal.

Recomendações aos produtores de alevinos

Os resultados das pesquisas científicas mostraram que a temperatura e fatores genéticos desempenham um papel importante na causa da anomalia da papila urogenital de fêmeas de tilápias, enquanto uma causa infecciosa é pouco provável. Temperaturas elevadas durante os dois primeiros meses de vida da tilápia resultaram em um aumento significativo de papilas urogenitais anormais, que impactam consideravelmente no desempenho reprodutivo do plantel de reprodutores. Sendo assim, o cultivo de futuros reprodutores de tilápias em água na temperatura próxima a 25°C nos primeiros 60 dias após a absorção do saco vitelínico é altamente recomendado para evitar a ocorrência de fêmeas com papilas urogenitais anormais. Vale ressaltar que as amplitudes de temperaturas usadas nos estudos ilustram como o aquecimento global e mudanças climáticas podem afetar negativamente a produção de tilápias.

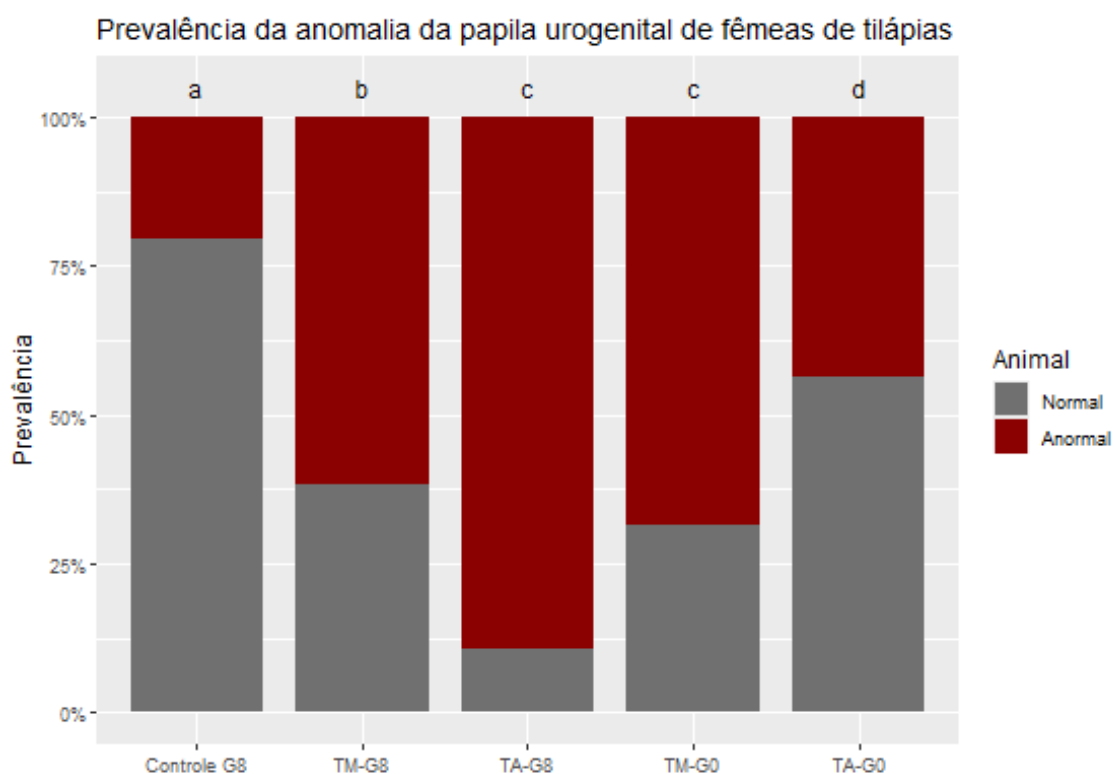


Figura 6: Prevalência da anomalia da papila urogenital de tilápias do Nilo em uma população de tilápias submetidas a sete gerações de seleção genética para peso corporal (G8) e uma população não submetida a esse processo (G0) em três faixas de temperatura durante os primeiros 60 dias pós-absorção do saco vitelínico: temperatura considerada normal entre 25°C-28°C (Controle G8), moderadamente alta entre 27°C-30°C em G8 (TM-G8) e em G0 (TM-G0), e temperatura alta entre 30°C-33°C em G8 (TM-G8) e em G0 (TM-G0). Diferentes letras indicam diferença significativa pelo teste de qui-quadrado com correção do Bonferroni ($p < 0,05$).

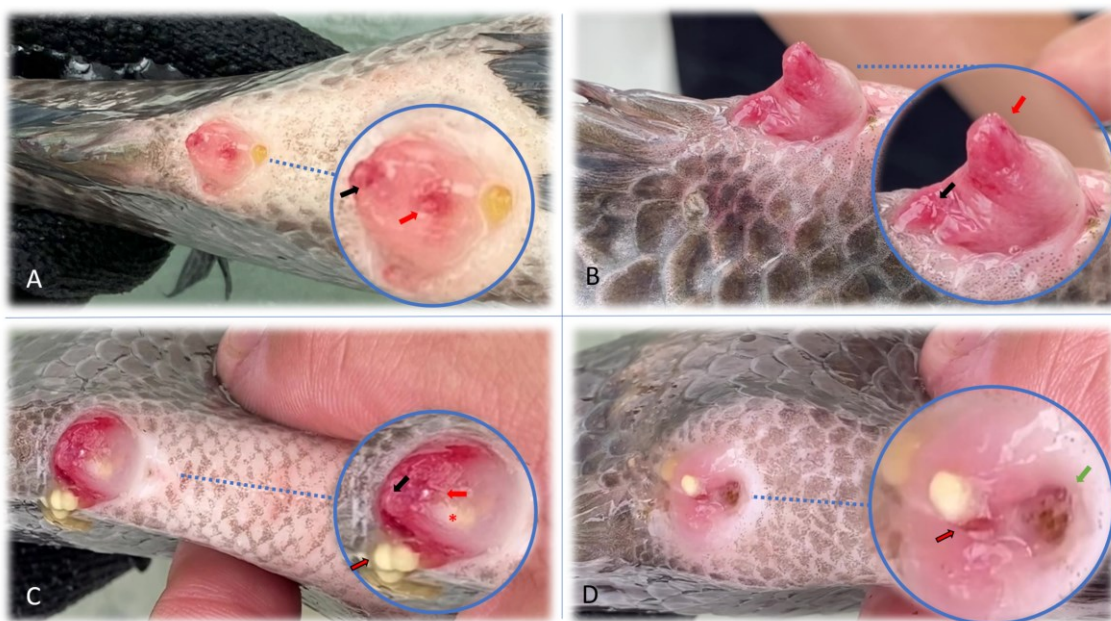


Figura 7: Fêmeas com papilas urogenitais anormais que apresentaram novas saídas do oviduto. Em A e B, uma nova estrutura de papila urogenital é formada e o animal passa a apresentar uma papila urogenital com aspecto duplicado. Em C e D, novas aberturas da papila urogenital são formadas em posições não convencionais. Seta vermelha representa a saída do oviduto, seta verde mostra o ânus, seta preta o poro urinário, e o ponto vermelho em C mostra um ovócito aprisionado na saída do oviduto original.

Referências bibliográficas

- Alcántar-Vázquez, J.P., la Torre, R.M., Calzada-Ruíz, D., Antonio-Estrada, C., 2014. Production of YY-male of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) from atypical fish. *Lat. Am. J. Aquat. Res.* 42 (3), 644–648.
- Alcántar-Vázquez, J.P., Rueda-Curiel, P., Calzada-Ruíz, D., Antonio-Estrada, C., la Torre, R.M., 2015. Feminization of the Nile tilapia *Oreochromis niloticus* by estradiol-17 β effects on growth, gonadal development and body composition. *Hidrobiologica* 25 (2), 275–283.
- Baroiller, J.F., D’Cotta, H.D., 2018. Sex control in tilapias. In: Wang, H.P., Piferrer, F., Chen, S.L. (Eds.), *Sex Control in Aquaculture*. John Wiley & Sons Ltd, pp. 191–234.
- Bezerra, V.M., Teixeira, E.A., Silva, F.B., Silva, S.C.D.C., Teixeira, J.P., Leal, C.A.G., Silva, M.A., Turra, E.M., Colosimo, E.A., Alvarenga, E.R., 2023. Decrease of reproductive performance of Nile tilapia (*Oreochromis niloticus*) females with dysmorphia in urogenital papilla. *Aquaculture* 571, 739495. <https://doi.org/10.1016/j.aquaculture.2023.739495>.
- Bezerra, V.M., Reis, G.P.A., Melo, C. L., Menezes, W. F. Santos, B.D., Ferreira, M. P., Pires, D. C., da Costa, F. B., Ramírez, J. F. B., Teixeira, J.P., Leal, C.A.G., Ribeiro, Y. M., Turra, E.M., Teixeira, E.A., Alvarenga, E.R., 2025. A long-term high temperature on young Nile tilapia females affects its urogenital papilla morphology and future reproductive performance. *Aquaculture* 595 (2025) 741666. <https://doi.org/10.1016/j.aquaculture.2024.741666>
- El-Sayed, A.-F.M., 2019. *Tilapia culture*, 2nd ed. Elsevier Inc, Academic Press, London.