UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

Resposta acústica do camarão *Penaeus vannamei* submetido a diferentes condições ambientais

Fábio Ulisses Ramos Costa Filho

Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Mestre.

Prof. Dr. Sílvio Ricardo Maurano Peixoto
Orientador

Recife,

Fevereiro

2026

Dados Internacionais de Catalogação na Publicação
Sistema Integrado de Bibliotecas da UFRPE
Bibliotecário(a): Auxiliadora Cunha – CRB-4 1134

C838r Costa Filho, Fabio.

Resposta acústica do camarão *Penaeus vannamei* submetido a diferentes condições ambientais / Fabio Costa Filho. – Recife, 2026.

76 f.; il.

Orientador(a): Sílvio Ricardo Maurano Peixoto.

Dissertação (Mestrado) – Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura, Recife, BR-PE, 2026.

Inclui referências.

1. Camarão - Criação. 2. Monitoramento da pesca - Temperatura. 3. Comportamento alimentar. 4. Oxigênio I. Peixoto, Sílvio Ricardo Maurano, orient. II. Título

CDD 639.3

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQÜICULTURA

Resposta acústica do camarão *Penaeus vannamei* submetido a diferentes condições ambientais

Fábio Ulisses Ramos Costa Filho

Dissertação julgada adequada para obtenção do título de mestre em Recursos Pesqueiros e Aquicultura. Defendida e aprovada em 26/02/2026 pela seguinte Banca Examinadora.

Prof. Dr. Sílvio Ricardo Maurano Peixoto
Orientador
Departamento de pesca e
aquicultura /UFRPE

Prof. Dr. Alfredo Olivera Galvez
Membro Interno
Departamento de pesca e
aquicultura /UFRPE

Dr. Otávio Augusto Lacerda Ferreira Pimentel
Membro Externo
Departamento de pesca e
aquicultura /UFRPE

Dedicatória

Dedico este trabalho a todos aqueles que, de maneira direta ou indireta, contribuíram para a realização deste trabalho. Esta conquista também é de vocês.

Agradecimentos

A minha família, meus pais, irmãs e meus avós materno e paternos.

Ao meu orientador, Dr. Silvio Peixoto, pelo ensinamento, orientação, oportunidade de estagiar no laboratório e motivação para continuar na área acadêmica e me dá oportunidades que achei que nunca teria.

Dra. Roberta Borda pela ajuda orientação extra e que junto ao prof. Silvio esteve me ajudadndo nas pesquisas e afins

Aos colegas de laboratório tantos ao que saíram quanto o que estiveram até o fim o meu trabalho presente, ajudarndonos experimentos e no dia a dia.

Dr. Prof. Allen Davis e sua equipe por me ajudar a realizar esse trabalho que só foi possível ser feita em seu laboratório no exterior

À Maria Eduarda, que cruzou meu caminho ainda na graduação. O que começou como uma amizade de faculdade transformou-se em um carinho profundo e, hoje, tenho a alegria de tê-la como minha companheira de vida.

Aos amigos do grupo 'Carai vei', pela amizade que atravessa décadas. Obrigado por serem a minha válvula de escape nos momentos de estresse e por permanecerem ao meu lado desde a adolescência até a conclusão deste ciclo.

Resumo

A intensificação da carcinicultura moderna exige o aprimoramento de estratégias alimentares e o controle rigoroso de variáveis ambientais, uma vez que métodos convencionais de monitoramento visual são frequentemente inviáveis em sistemas intensivos e turvos. Diante da necessidade de soluções tecnológicas que otimizem a produtividade e a sustentabilidade, o uso do Monitoramento Acústico Passivo (PAM) surge como uma alternativa inovadora para avaliar o bem-estar e o comportamento dos animais em tempo real. A presente dissertação investigou a resposta acústica e o consumo de ração do camarão-branco-do-pacífico, *Penaeus vannamei*, sob diferentes condições de temperatura e oxigênio dissolvido (OD), utilizando o PAM como ferramenta central. A atividade acústica e o consumo de ração foram monitorados por meio de hidrofones (PAM) em exemplares submetidos a gradientes térmicos variando de 22,1; 24,1; 27,5 e 31,3 °C, bem como a diferentes niveis de oxigênio dissolvido (OD) 5, 3, 2 e 1 mg/L, incluindo hipóxia gradual e abrupta, para identificar os níveis críticos de supressão alimentar e os padrões de emissão de 'cliques' mandibulares sob estresse ambiental. O estudo validou que a emissão de "cliques" mandibulares possui uma forte correlação positiva com o consumo real de alimento, permitindo o acompanhamento do comportamento alimentar de forma não invasiva e em tempo real. No primeiro eixo da pesquisa, observou-se que a atividade alimentar foi severamente suprimida em temperaturas iguais ou inferiores a 24,1 °C, enquanto o desempenho máximo ocorreu na faixa entre 27,5 °C e 31,3 °C. Além do consumo, a temperatura alterou a dinâmica temporal: em águas quentes, a atividade é intensa e concentrada nos minutos iniciais, enquanto no frio ela se torna mais estável e reduzida. Na segunda parte, focada em quatros níveis de oxigênio (5, 3, 2 e 1 mg/L), reduzidos de forma gradual e abrupta com injeção de nitrogenio. Os resultados demonstraram que o método de redução do OD influenciou diretamente o comportamento: sob hipóxia gradual, os camarões mantiveram a alimentação estatisticamente estável até 3 mg/L, com queda crítica em 1 mg/L; já sob redução abrupta, o declínio dependeu da concentração e ocorreu de forma mais linear. O PAM revelou ainda que, em condições de hipoxia, os animais apresentaram um pico de atividade precoce seguido de rápida cessação, caracterizando uma estratégia de compensação e conservação de energia. A pesquisa conclui que o monitoramento acústico é uma ferramenta precisa para detectar estresse ambiental e pode ser integrado a sistemas de alimentação automática para otimizar a eficiência produtiva na carcinicultura.

Palavras-chave: Camarão Cinza; Monitoramento acústico passivo; Comportamento alimentar; Temperatura; Oxigênio dissolvido.

Abstract

The intensification of modern shrimp farming requires the improvement of feeding strategies and the rigorous control of environmental variables, as conventional visual monitoring methods are often unfeasible in intensive and turbid systems. Given the need for technological solutions that optimize productivity and sustainability, the use of Passive Acoustic Monitoring (PAM) emerges as an innovative alternative to assess animal welfare and behavior in real time. This thesis investigated the acoustic response and feed consumption of the Pacific white shrimp, *Penaeus vannamei*, under different temperature and dissolved oxygen (DO) conditions, using PAM as a central tool. Acoustic activity and feed consumption were monitored via hydrophones (PAM) in specimens subjected to thermal gradients ranging from 22.1, 24.1, 27.5, and 31.3 °C, as well as different DO levels (5, 3, 2, and 1 mg/L), including gradual and abrupt hypoxia, to identify critical levels of feed suppression and patterns of mandibular 'click' emissions under environmental stress. The study validated that mandibular 'click' emissions have a strong positive correlation with actual feed consumption, allowing for the non-invasive, real-time tracking of feeding behavior. In the first axis of the research, it was observed that feeding activity was severely suppressed at temperatures equal to or below 24.1 °C, while maximum performance occurred in the range between 27.5 °C and 31.3 °C. In addition to consumption, temperature altered temporal dynamics: in warm waters, activity is intense and concentrated in the initial minutes, whereas in cold waters, it becomes more stable and reduced. The second part focused on four oxygen levels (5, 3, 2, and 1 mg/L), reduced both gradually and abruptly through nitrogen injection. Results demonstrated that the method of DO reduction directly influenced behavior: under gradual hypoxia, the shrimp maintained statistically stable feeding until 3 mg/L, with a critical drop at 1 mg/L; under abrupt reduction, the decline was concentration-dependent and occurred more linearly. PAM further revealed that, under hypoxic conditions, the animals exhibited an early peak in activity followed by rapid cessation, characterizing a strategy of compensation and energy conservation. The research concludes that acoustic monitoring is a precise tool for detecting environmental stress and can be integrated into automated feeding systems to optimize production efficiency in aquaculture.

Key words: Whiteleg shrimp; Passive acoustic monitoring; Feeding behavior; Temperature; Dissolved oxygen.

Lista de figuras

Página

Lista de tabelas

Sumário

Página

1- Introdução

1.1- Contextualização da pesquisa

A criação de camarões é uma atividade aquícola de relevância global, com destaque para o camarão-branco-do-Pacífico, *Penaeus vannamei* (Boone, 1931), espécie dominante devido ao rápido crescimento, resistência a doenças e adaptabilidade a diferentes condições ambientais (Kamaruzzaman et al., 2018). Em 2022, a produção mundial dessa espécie alcançou cerca de 6,8 milhões de toneladas, impulsionando a aquicultura a superar a pesca em volume de produção e evidenciando sua importância econômica e social, especialmente no que diz respeito à segurança alimentar, à oferta de alimentos ricos em micronutrientes e à geração de empregos em áreas rurais com oportunidades limitadas (FAO, 2024).

No Brasil, o *P. vannamei* demonstrou excelente desempenho produtivo sob as condições climáticas locais, registrando uma produção anual de 113,3 mil toneladas e receita de 2,2 bilhões de reais (IBGE, 2024). Esse cenário reforça a necessidade contínua de aprimoramento das estratégias de cultivo, com foco em maior sustentabilidade e produtividade, especialmente por meio de avanços em seleção genética para resistência a doenças como WSSV e EMS (Fernandes et al., 2019).

Além dos avanços genéticos, os avanços na automação do manejo alimentar na carcinicultura tornaram-se essenciais para otimizar a produção e reduzir os custos operacionais (Reis et al., 2020). Contudo, em sistemas com alta turbidez da água, o monitoramento visual da atividade alimentar, comum em outras espécies, torna-se inviável (Reis et al., 2022). Em estudos etológicos de laboratório, a avaliação comportamental de camarões baseia-se tradicionalmente na observação visual direta ou no uso de vídeo gravações acopladas a softwares de imagem para análise posterior (Bardera et al., 2021). O problema é que a eficácia dessas metodologias esbarra em uma limitação severa: a dependência estrita do campo visual. Essa necessidade de água translúcida para as câmeras afasta o desenho experimental das condições turvas da realidade dos cultivos intensivos (Smith & Tabrett, 2013; Bardera et al., 2021). Além disso, um esforço amostral exaustivo é exigido para decodificar horas de vídeo manualmente (Bardera et al., 2020). Diante dessa limitação, o Monitoramento Acústico Passivo (Passive Acoustic Monitoring – PAM) surge como uma solução inovadora. Essa tecnologia utiliza hidrofones para captar os sons de "cliques" gerados pela oclusão das mandíbulas dos camarões durante a alimentação (Reis et al., 2022).

A identificação dessas emissões acústicas do *P. vannamei* possibilitou o desenvolvimento de alimentadores automáticos responsivos à demanda real, que dispensam ração em tempo real conforme a atividade alimentar de camarões detectada via PAM (Ullman et al., 2019b; Reis et al., 2022). Estudos têm demonstrado que sistemas de alimentação baseados em feedback acústico para camarões superam métodos tradicionais, como a alimentação manual ou o uso de alimentadores com tempos pré-programados (Ullman et al., 2019a, 2019b; Reis et al., 2021). Tais sistemas proporcionam maiores taxas de crescimento, peso final e rendimento zootécnico do *P. vannamei*, ao mesmo tempo em que permitem elevação nas taxas de arraçoamento sem comprometer a conversão alimentar (Reis et al., 2021; Ullman et al., 2019a, 2019b).

Ao contrário dos sistemas programados, que fornecem ração em horários fixos, os sistemas acústicos com feedback ajustam automaticamente a oferta alimentar conforme a demanda comportamental e fisiológica dos camarões (Reis et al., 2020, 2022). Em ambientes laboratoriais, a técnica PAM tem sido amplamente empregada para investigar o comportamento alimentar de *P. vannamei*, caracterizando assinaturas acústicas associadas à alimentação com diferentes tipos de ração (Silva et al., 2019; Peixoto et al., 2020a). A precisão e não invasividade da técnica posicionam-na como uma ferramenta eficaz na carcinicultura moderna, superando métodos convencionais como a análise de bandejas de alimentação (Ullman et al., 2019b).

Adicionalmente, o PAM tem sido utilizado para avaliar o impacto de diversos fatores sobre a emissão sonora, como classes de tamanho dos camarões (Hamilton et al., 2024), diâmetro dos pellets (Peixoto et al., 2020b), densidades de estocagem (Hamilton et al., 2023) e dietas suplementadas com atrativos (Soares et al., 2021; Peixoto et al., 2022; Tabbara et al., 2024). Essas investigações fornecem subsídios importantes para o aprimoramento das estratégias alimentares, visando à eficiência produtiva e à sustentabilidade ambiental.

Entretanto, para que o PAM seja plenamente eficiente como ferramenta de manejo, é fundamental compreender como o padrão sonoro e a atividade alimentar são modulados pelo ambiente. Nesse contexto, variáveis como a temperatura da água exercem influência direta no crescimento, a sobrevivência e o comportamento do *P. vannamei* (Wang et al., 2023; Dayalan et al., 2024). Por se tratar de um organismo ectotérmico, o metabolismo do camarão é regulado pela temperatura, refletindo-se no consumo de oxigênio (Kir et al., 2023). A espécie possui ampla tolerância térmica, sobrevivendo entre 7,2 °C e 41,9 °C; entretanto, a faixa ideal para produção situa-se entre 25 °C e 30 °C, garantindo estabilidade fisiológica (Kir et al., 2023). Estudos apontam que os melhores resultados produtivos ocorrem entre 28 °C e 30 °C, com salinidades de 33 ‰ a 40 ‰ (Ponce-palafox et al., 1997). Fora dessa faixa, o camarão apresenta sinais de estresse. Em temperaturas abaixo de 18 °C, observa-se uma redução acentuada da atividade alimentar,, o que representa um ponto crítico do ponto de vista comportamental (Wang et al., 2023). O frio também acarreta danos histológicos e eleva o custo metabólico da osmorregulação (Wang et al., 2023). Por outro lado, a maior tolerância à hipóxia foi observada em 22 °C e salinidade de 16,6 ‰, indicando que as condições ótimas para lidar com estressores fisiológicos nem sempre coincidem com aquelas que maximizam o crescimento (Bett & Vinatea, 2009). Em altas temperaturas, *P. vannamei* tolera incrementos graduais até 37 °C; choques térmicos abruptos para 40 °C, contudo, resultam em estresse severo e mortalidade total (Dayalan et al., 2024).

Outro fator determinante é o oxigênio dissolvido (OD), apontado como a principal variável limitante na aquicultura intensiva (Jasmin et al., 2022; Li et al., 2007). A manutenção de níveis adequados de OD é fundamental para a saúde e desempenho dos camarões, uma vez que condições de hipóxia reduzem o crescimento, aumentam a suscetibilidade a doenças e comprometem a viabilidade econômica (Araujo et al., 2024). Concentrações inferiores a 2 mg/L já afetam negativamente o crescimento, sendo 1 mg/L considerado próximo do limite letal (Hopkins et al., 1991; Seidman & Lawrence, 1985). Taxas de sobrevivência são significativamente maiores com OD acima de 4 mg/L (92,22%) em comparação com níveis abaixo de 2 mg/L (56,67%) (Lehmann et al., 2011).

A hipóxia atua como estressor químico, suprimindo o sistema imunológico o camarão (Li et al., 2007). Parâmetros como a contagem de hemócitos de *P. vannamei* são significativamente superior sob condições com OD acima de 4 mg/L (Lehmann et al., 2011). Também foi observado que a hipóxia reduz a resistência de *Penaeus monodon* ao *Vibrio harveyi* (Wuertz et al., 2023) e força uma mudança metabólica de lipídios e proteínas para proteínas exclusivamente, indicando uma adaptação ao estresse (Rosas et al., 1999). O manejo de OD está intimamente relacionado à alimentação do camarão, uma vez que a oferta de ração influencia diretamente a demanda por oxigênio. Por exemplo, para manter um OD mínimo de 3,0 mg/L, recomenda-se um limite de até 16 kg de ração por unidade de potência de aeração (Hopkins et al., 1991). Embora maiores níveis de aeração não garantam desempenho zootécnico superior, aumentam os custos com eletricidade, indicando que níveis intermediários, como 2,5 mg/L, podem ser mais econômicos, embora com maior risco (Araujo et al., 2024).

Diante da relevância desses fatores ambientais e tecnológicos, o presente trabalho tem como objetivo avaliar o comportamento alimentar do camarão *P. vannamei* sob diferentes condições de temperatura e níveis de oxigênio dissolvido, utilizando o Monitoramento Acústico Passivo (PAM).

1.2- Objetivos

Objetivo Geral:

Avaliar os efeitos da variação de temperatura e da concentração de oxigênio dissolvido sobre a atividade alimentar acústica e o consumo de ração do camarão-branco-do-pacífico, *Penaeus vannamei*, utilizando o método de Monitoramento Acústico Passivo (PAM).

Objetivos Específicos:

Caracterizar a atividade alimentar acústica e quantificar o consumo de ração do *P. vannamei* quando submetido a cinco diferentes temperaturas (32, 30, 27, 24 e 22 °C), abrangendo desde a faixa ótima até condições de estresse térmico.

Analisar a atividade alimentar acústica e o consumo de ração do *P. vannamei* quando exposto a quatro concentrações de oxigênio dissolvido (5, 3, 2 e 1 mg/L), representando condições de normóxia a hipóxia severa em dois métodos de redução gradual: remoção da oxigenação e remoção abrupta por meio de injeção de nitrogênio (N).

Correlacionar os padrões acústicos com o consumo real de ração para validar a eficácia do PAM como ferramenta de monitoramento do comportamento alimentar sob diferentes condições de estresse ambiental.

2- Artigos cientificos

Os resultados obtidos durante o trabalho experimental desta dissertação estão apresentados em dois capítulos referents aos artigos intitulados: “Thermal modulation of feeding behavior in *Penaeus vannamei* revealed by passive acoustic monitoring” e “Gasping for air: The impact of gradual versus abrupt hypoxia on the acoustic feeding behavior of *Penaeus vannamei*.”

2-Capitulo 1 Thermal modulation of feeding behavior in *Penaeus vannamei* revealed by passive acoustic monitoring

Artigo científico a Publicado https://doi.org/10.1016/j.aquaculture.2026.743631. a Revista – Aquaculture (ISSN: 1873-5622). Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela (https://www.sciencedirect.com/journal/aquaculture). referida revista

Thermal modulation of feeding behavior in *Penaeus vannamei* revealed by passive acoustic monitoring

Fábio Costa Filho[a], Indira Medina Torres[b], Roberta Borda Soares[a], D. Allen Davis[b], Silvio Peixoto[a]

[a] Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco, Recife, 52171-900, PE, Brazil.

[b] Auburn University - School of Fisheries, Aquaculture and Aquatic Sciences, Auburn, AL 36849, USA

Fábio Costa Filho; e-mail: ramosfabio22@gmail.com; ORCID: 0000-0002-8188-5327 (corresponding author)

Indira Medina Torres; e-mail: ipm0008@auburn.edu; ORCID:

Roberta Borda Soares; e-mail: beta.ufrpe@ufrpe.br; ORCID: 0000-0003-3952-5819

D. Allen Davis; e-mail: davisda@auburn.edu; ORCID:

Silvio Peixoto; e-mail: silvio.peixoto@gmail.com; ORCID: 0000-0002-2192-3854

Abstract

This study evaluated the effect of water temperature on the feeding activity of *Penaeus vannamei* using passive acoustic monitoring (PAM) as a noninvasive tool for real-time behavioral assessment. Juveniles (25.16 ± 1.38 g) were exposed to five temperature treatments (22.1, 24.1, 27.5, 29.4, and 31.3 °C) under controlled laboratory conditions. Feeding activity was monitored and quantified through acoustic detection of clicks emitted by shrimp mandibles and food consumption (FC) during 30-minute feeding periods. Both click production and feed intake peaked at 27.5 to 31.3 °C but declined significantly at cold temperatures of 22.1 and 24.1 °C. Acoustic activity correlated strongly with feed intake ($r = 0.65$, $p < 0.001$), validating PAM as a quantitative indicator of feeding intensity. Analysis of Covariance models revealed a significant interaction between time and temperature, indicating that thermal variation affected not only the magnitude but also the temporal dynamics of feeding. These findings confirm that ~ 28 - 30 °C range represents the optimal thermal window for feeding activity in *P. vannamei* and demonstrates the potential of PAM to support precision feeding and temperature-responsive management in shrimp aquaculture.

Keywords: Penaeid; clicks; feeding activity; temperature.

1. Introduction

Aquaculture has emerged as one of the main sources of animal protein production worldwide, driven by the growing demand for healthy and sustainable food. In this context, the Pacific white shrimp, *Penaeus vannamei* (Boone, 1931), stands out as the most economically important species in global shrimp farming, accounting for more than 6.8 million tons produced in 2022 (FAO, 2024). The success of *P. vannamei* farming is closely linked to the stability and quality of water parameters, especially in intensive systems. Among these factors, temperature is one of the most critical environmental variables, exerting a direct influence on metabolism, growth, survival, and feeding behavior of shrimp (Wyban et al., 1995).

As ectotherms, shrimp rely on external temperature to regulate core physiological processes, making this parameter a key determinant of productive performance (Giffard-Mena et al., 2024). Although *P. vannamei* has broad thermal tolerance 8.0 - 41.9 °C (Kır et al., 2023), growth performance is optimized between 25 and 30 °C (Ponce-Palafox et al., 1997; Kır et al., 2023). It has been reported that below this range, feed intake of this species declines and can cease under 18 °C, while cold exposure is also associated with epithelial disruption in gill lamellae and damage to the intestinal mucosa along with increased energetic costs of ion osmotic regulation (Wang et al., 2023). Moreover, above this optimal range, the thermal tolerance of *P. vannamei* drops sharply during unacclimated exposures above ~37 °C, overwhelming respiratory, enzymatic, and osmoregulatory capacity and causing rapid stress and high mortality (Dayalan et al., 2024). By contrast, gradual acclimation raises the upper lethal temperature for example, from about 34 °C under sudden exposure to about 37 °C with gradual warming (Kır et al., 2023; Dayalan et al., 2024).

Temperature is also closely related to *P. vannamei* feeding behavior, influencing the frequency, intensity, and duration of feeding activity. Thermal variations may affect appetite, ingestion capacity, and digestive efficiency, directly impacting feeding management and zootechnical performance of this species (Barajas-Sandoval et al., 2024). Although several studies have evaluated the effect of temperature on growth and survival of penaeid shrimp, few have objectively correlated temperature variation with feeding behavior over time, particularly under controlled conditions (Wyban et al., 1995; Tailly et al.,2021; Zhang et al., 2025). Accordingly, Passive Acoustic Monitoring (PAM) has recently emerged as a promising non-invasive approach for evaluating the feeding activity of *P. vannamei*, as it is possible to capture and record the characteristic clicking sounds produced by their mandibular occlusion (Silva et al., 2019). This technique has been widely used for behavioral studies of this species, with demonstrated efficiency in assessing feeding behavior in different situations, such as shrimp size, stocking density, feed frequency, as well as when using different additives and diet formulations, as reviewed by Peixoto and Soares (2025). Nevertheless, PAM was never applied to investigate the effects of water temperature on shrimp feeding behavior under laboratory conditions.

Although acoustic-based automated feeders are expanding in shrimp aquaculture, and temperature effects on feeding behavior are probably considered by their algorithms to improve feed management, this information is proprietary to the industry and not available in research using such acoustic systems (Reis et al., 2020, 2021; Walsh et al., 2022; Strebel et al., 2023). Therefore, the present study aims to use PAM to evaluate the effects of thermal variation on the feeding activity of *P. vannamei* under laboratory conditions, which can contribute not only to our understanding of shrimp feeding behavior, but also to improving feed management and acoustic-based automated equipment in shrimp farming.

2. Materials and methods

2.1. Experimental design

This study was conducted at the E.W. Shell Fisheries Center, Auburn University (AL, USA), in a temperature-controlled (27.5 °C) room with 20 glass aquaria (70 L each). Every four tanks were plumbed to one of five independent clean-water recirculating systems, each composed by a sump, biological filter media, and continuous aeration. Each of these systems corresponded to a specific temperature treatment: 22.1 °C (Very cold), 24.1 °C (Cold), 27.5 °C (Regular), 29.4 °C (Hot), and 31.3 °C (Very hot). Thermal stability was maintained using individual 300W heaters (ViaAqua) in each aquarium of warm temperature systems (29.4 and 31.3 °C) and chillers (Teco TK-2000h) installed in each sump of cooling systems (22.1 and 24.1 °C), while no heaters or chillers were needed at room temperature system (27.5 °C).

P. vannamei (25.16 ± 1.38 g) reared in the laboratory were randomly assigned to temperature systems (seven shrimp per aquarium) and acclimated for 24 h. During acclimation, shrimp were fed a commercial diet (Zeigler Bros Inc., USA; 2.4 mm, 35% CP) twice daily. Shrimp in each system underwent a 16-h fasting period, after which their feeding activity (acoustic data and food consumption) was recorded for 30-min. Water temperature was measured before each recording to confirm thermal conditions. Other water-quality parameters were measured daily in each system, including pH, salinity, dissolved oxygen (DO), total ammonia nitrogen (TAN-NH_4) and nitrite (NO^2). Values of pH, salinity and DO were measured with a YSI Professional Plus multiparameter meter (YSI Inc., Yellow Springs, OH, USA), whereas TAN and nitrite were determined colorimetrically using a YSI 9500 photometer (YSI Inc., Yellow Springs, OH, USA).

2.2. *Food consumption and passive acoustic monitoring*

Acoustic recordings (PAM) were conducted simultaneously with food consumption analysis in each aquarium, corresponding to four replicates per treatment. Recordings started when 3.5 g of the commercial feed was deposited at the center of each aquarium. After 30 min, uneaten pellets were siphoned and retained on pre-weighed cellulose filters (20 µm pore size), oven-dried at 100 °C for 24 h, and weighed. Feed intake was calculated as the difference between feed offered and the dry mass of recovered residues. To account for leaching losses, a control assay was conducted by placing 1.0 g (as is) of feed into twelve aquaria without shrimp, following the same recovery and drying protocol. These data were used to estimate feed leaching and correct intake values. Mean food consumption (FC) was calculated on a dry matter basis using the following formula:

$FC=Fo-(Fr/Fl)$

Where: FC = food consumption (g); Fo = dry weight of feed offered (g); Fr = dry weight of feed recovered (g); Fl = leaching correction factor (proportion of feed recovered from aquaria without shrimp, calculated as $F_l = F_r/F_o$)

Acoustic data were captured using omnidirectional AS-1 hydrophones (Aquarian Hydrophones, USA), with a flat frequency response between 1 Hz and 100 kHz and sensitivity of –208 dB re 1 V/µPa. Hydrophones were positioned at the center of each aquarium. Each hydrophone was connected to a 26 dB gain pre-amplifier (PA-4, Aquarian Hydrophones) and linked to a multitrack digital recorder (Zoom F6, USA), set to a 192 kHz sampling rate (16-bit resolution). The aeration in the aquarium was turned off to avoid acoustic interference during recordings.

Audio files were analyzed using Raven Pro 1.5 software (Cornell Lab of Ornithology, USA). A band-limited energy detector was used to automatically identify click sounds in the spectrogram (window type Hann, FFT size 512 samples, 3 dB filter bandwidth 135 Hz, 50% overlap), using the following settings: frequency range 15–90 kHz, signal duration 5–50 ms, and signal-to-noise ratio (SNR) above 12 dB. The Raven output included the timestamp of each detected click, which formed the database for subsequent statistical analysis. Click detection data (timestamps in milliseconds) from the acoustic recordings were processed in Microsoft Excel (Microsoft 365, Microsoft Corporation, USA) to calculate the frequency distribution of clicks per minute. A histogram analysis tool was applied, using click timestamps as the input range and 1-minute intervals over the 30-minute recording period (including the midpoint at 15 min) as bin widths. The output provided the number of clicks per minute for each recording.

2.3. Statistical analysis

The total click counts (15 and 30 min) and food consumption (FC) after 30 minutes were compared among temperature treatments using one-way Analysis of Variance (ANOVA). Prior to ANOVA, data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using Cochran's test. When significant differences were detected, treatment means were compared using Tukey’s Honest Significant Difference (HSD) post hoc test. ANOVA was also applied to compare water quality parameters among the different recirculation systems.

To assess the temporal dynamics of acoustic feeding activity, a multiple linear regression model was constructed using time (minutes) as a continuous predictor and temperature treatments as a categorical predictor. This model was interpreted as an Analysis of Covariance (ANCOVA), allowing evaluation of interaction effects between time and temperature on clicking rates. Following model fitting, pairwise comparisons of estimated marginal means (EMMs) among temperature treatments (Tukey-adjusted) were performed to resolve group differences; additionally, control-based contrasts were conducted using EMMs with Bonferroni adjustment. When the time × temperature interaction was significant, simple-effects comparisons were carried out at representative time points (15 and 30 min). Additionally, linear regression analyses were performed to investigate the correlation between total click counts (15 and 30 minutes) and food consumption using pooled data across temperature treatments.

All statistical analysis were conducted using R software (version 4.x) within the RStudio environment (RStudio Team, USA), adopting a significance level of $p < 0.05$ for all tests.

3. Results

Water temperature was maintained stable and differed significantly among the five preset treatments (22.1, 24.1, 27.5, 29.4, and 31.3 °C) to evaluate the feeding behavior of *P. vannamei*. All the other water quality parameters (pH, salinity, DO, TAN and nitrite) did not differ significantly among the treatments (Table 1).

Temperature significantly affected food consumption (FC) in *P. vannamei*. Consumption increased with temperature, being significantly lower at 22.1 and 24.1 °C when compared to the warmer temperatures of 29.4 and 31.3 °C (Figure 1A). Nevertheless, shrimp maintained at 27.5 °C showed intermediate mean FC value, which did not differ from those in colder (22.1 and 24.1 °C) and warmer (29.4 and 31.3 °C) temperatures (Figure 1A).

Overall, shrimp acoustic activity followed a similar pattern as food consumption, but with some peculiarities (Figures 1B-C). Within the first 15 minutes of recordings, shrimp at 29.4 °C produced significantly more clicks than those at 22.1 and 24.1 °C. However, clicking activity at 27.5 and 31.3 °C differed only from shrimp at the lowest temperature treatment (22.1 °C) (Figure 1B). Over the entire recording period (30 min), the total number of clicks displayed a similar trend with shrimp at 22.1 and 24.1 °C showing a significantly lower acoustic activity when compared to those maintained in the warmest temperatures (27.5, 29.4, and 31.3 °C), among which no significant differences were detected (Figure 1C). Additionally, significant positive correlations ($r = 0.65$, $p < 0.001$) were observed between the total number of acoustic clicks (30 min) and food consumption across aquaria pooled for all temperature treatments (Figure 2), confirming the relationship between these two variables and similarities observed in their results of feeding activity (Figure 1A and 1C).

The relationship between time and acoustic clicks was significantly influenced by water temperature, as revealed by ANCOVA models for both the 15 and 30-minute intervals (Table 2). In both analyses, the time, treatment, and time × treatment interaction terms were significant ($p < 0.05$). This interaction indicates that the rate of decline in clicking activity over time differed among temperatures. Relative to the 27.5 °C treatment, shrimp at 22.1 °C and 24.1 °C displayed significantly lower click rates. Conversely, pairwise comparisons of temperature treatments versus 27.5 °C (regular) did not show significant differences from clicking activity in warmer conditions (29.4 °C and 31.3 °C) for both 15 and 30 min (Tables 3 and 4, respectively). Overall, the decline in clicking activity was steeper under warmer temperatures, whereas shrimp in colder water maintained lower but more stable click rates throughout the observation period (Figure 3).

4. Discussion

The study demonstrated that water temperature has a significant influence on feeding behavior of *P. vannamei*, as evidenced by both food consumption and acoustic activity. The use of passive acoustic monitoring (PAM) proved effective in detecting variations in feeding behavior across temperature treatments, offering a non-invasive, real-time tool for shrimp behavioral studies. Overall, feeding activity measured by click emission and actual feed intake, was greater in warmer temperatures between 27.5 and 31.3 °C, but declined sharply at 24.1 and 22.1 °C.

As indicated by the present findings, the most dramatic behavioral suppression occurred under cold conditions. This reduction in feed intake reflects the ectothermic nature of shrimp, whose metabolic rate is dictated by ambient temperature (Dall et al., 1991; Wang et al.,2023). In our data, suboptimal low temperatures (≤ 24.1 °C) produced marked declines in both acoustic activity and food consumption (FC), consistent with thermal suppression of appetite and activity.

The oxygen consumption of *P. vannamei* varies with temperature (~15 - 32.5 °C), and activity level, providing a physiological basis for these behavioral shifts (Bett and Vinatea, 2009). It has been suggested that cold exposure depresses locomotor capacity, reducing feed encounter rates and decreasing feeding behavior, as observed in the foraging rhythm of *P. vannamei* (Dall et al., 1991; Ren et al., 2021). Furthermore, cold reduces digestive enzyme activity, hepatopancreatic enzyme activity, and total hemocyte count, limiting assimilation, immune function, and the desire to feed (Giffard-Mena et al., 2024; Hoffling et al., 2025). Therefore, it is suggested that the smaller number of clicks and stables feeding rhythm over time in shrimp maintained at colder temperatures (24.1 and 22.1 °C) are related to such physiological effects in behavior. From the acoustic point of view, *P. vannamei* feeding activity it is expected to occur in the first 10 - 15 min after food offering, resulting in steeper regressions lines of clicking rate in 30 min recordings, whereas gentle or flat slopes indicate abnormal behavior or lack of interest by shrimp, as observed in studies testing different diet formulations and attractants for *P. vannamei* (Peixoto et al., 2022; Tabbara et al., 2024).

In contrast to the potential metabolic and physiological constraints imposed by lower temperatures, the 27.5 to 31.3 °C range consistently appears to be the optimal range for feeding activity under our conditions, corroborating findings of lower feed conversion and higher growth within a similar range for *P. vannamei* (Wyban et al., 1995). Thermal optima for this species, however, are size-dependent: juveniles (~5 g) perform well up to ~30 °C, while larger shrimp (~16 g) are more sensitive above ~30 °C (Wyban et al., 1995). This pattern reflects size-dependent metabolic responses and restricted metabolic scope toward thermal edges (Dall et al., 1991; Bett and Vinatea, 2009). Therefore, we should consider that we used large shrimp (~25 g) in this study, and temperature-sensitive digestive enzymes, particularly in older/larger individuals, have been related to reduction in digestive efficiency of *P. vannamei* under heat stress (Al-Masqari et al., 2022). Ontogenetic shifts also affect osmoregulatory capacity and consequently shrimp growth performance under temperature-salinity interactions (Dall et al., 1991; Ren et al., 2021). Nevertheless, Ponce-Palafox et al. (1997) observed higher survival and feed intake for *P. vannamei* between 28 and 30 °C across different salinities, highlighting the robustness of this temperature window as thermal optimum for this species, which is supported by our acoustic and feed intake data.

A key innovation of this study was applying Passive Acoustic Monitoring (PAM) to track real-time feeding behavior across different temperatures. The acoustic activity correlated strongly with actual feed intake, validating PAM as a reliable proxy for feeding intensity and aligning with previous studies that linked click frequency to food consumption (Peixoto et al., 2022; Tabbara et al., 2024). This tool allowed us to uncover the distinct temporal dynamics of feeding: under optimal temperatures (27.5 to 31.3 °C), click activity rose rapidly and remained elevated, whereas cold conditions delayed initiation and led to early cessation. The significant time versus treatment interaction in ANCOVA models confirms that temperature alters not just the magnitude, but also the temporal profile of feeding behavior, which is an important information that would be probably missed under farming conditions. The characteristics of shrimp farming present several challenges for direct observation, as environmental factors (e.g., water depth and transparency) and culture conditions (such as tank or pond type, production system, stocking density, and shrimp size) often make tracking and visualizing feeding behavior impractical. However, these biological and operational constraints do not hinder the monitoring of feeding activity through PAM in detecting subtle responses to diet formulations or environmental cues (Smith and Tabrett, 2013; Reis et al., 2022; Peixoto and Soares, 2025). The clear link between temperature and feeding patterns shown in our data underscores the need to adapt feeding schedules to avoid inefficiency and waste in *P. vannamei* farming. By integrating

PAM outputs with real-time control algorithms, it becomes possible to develop demand-based feeding systems that automatically adapt to environmental fluctuations, supporting significant advances in precision feed management (Reis et al., 2022; Peixoto and Soares, 2025).

5. Conclusion

Water temperature exerts a strong influence on the feeding behavior of *Penaeus vannamei*, shaping both the intensity and temporal profile of acoustic activity and feed intake. Peak performance of these variables occurred consistently between 27.5 to 31.3 °C, while colder exposures (22.1 and 24.1 °C) produce feeding behavioral suppression. Beyond validating PAM through the strong correlation between click counts and feed intake, this study revealed how temperature dictates the temporal dynamics of feeding behavior. Optimal conditions prompted a rapid and intense response, whereas colder temperatures resulted in delayed and suppressed activity. This deeper understanding of behavioral dynamics is essential for designing truly adaptive, demand-based feeding systems. Importantly, because metabolic and physiological capacity change with body size and developmental stage, thermal optima feeding behavior should be evaluated across ontogeny in future studies, enabling stage-specific control rules for precision feeding and environmental management in shrimp aquaculture.

References

Al-Masqari, Z.A., Guo, H., Wang, R., Yan, H., Dong, P., Wang, G., 2022. Effects of high temperature on water quality, growth performance, enzyme activity and the gut bacterial community of shrimp (*Litopenaeus vannamei*). *Aquac. Res.* 53, 3283–3296.

Barajas-Sandoval, D.R., Escobedo-Fregoso, C., Quiroz-Guzmán, E., Tovar-Ramírez, D., Py, C.A., Peña-Rodríguez, A., et al., 2024. Effect of temporal thermal stress on *Penaeus vannamei*: growth performance and physiological plasticity. *Comp. Biochem. Physiol. A*, 111653.

Bett, C., Vinatea, L., 2009. Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. *Braz. J. Oceanogr.* 57, 305–314.

Dall, W., Hill, B.J., Rothlisberg, P.C., Sharples, D.J. (Eds.), 1991. *The Biology of the Penaeidae (Advances in Marine Biology, Vol. 27).* Academic Press.

Dayalan, V., Kasivelu, G., Raguraman, V., Nisa, A., 2024. Studies on temperature impact (sudden and gradual) of the white-leg shrimp *Litopenaeus vannamei*. *Environ. Sci. Pollut. Res.*

Tailly, J.-B.D., Keitel, J., Owen, M.A.G., Alcaraz-Calero, J.M., Alexander, M.E., Sloman, K.A., 2021. Monitoring methods of feeding behaviour to answer key questions in penaeid shrimp feeding. *Rev. Aquac.* 13, 1828–1843.

FAO, 2024. *The State of World Fisheries and Aquaculture 2024 — Blue Transformation in Action.* Rome: FAO.

Giffard-Mena, I., Ponce Rivas, E., Sigala-Andrade, H.M., Uganda-Solís, C., et al., 2024. Evaluation of the osmoregulatory capacity and three stress biomarkers in white shrimp *Penaeus vannamei* exposed to different temperature and salinity conditions: Na^+/K^+-ATPase, heat shock proteins and crustacean hyperglycemic hormones. *Comp. Biochem. Physiol. B* 271, 110942.

Hoffling, F.B., Miranda, C.S., de Araújo Mendes, M.H., Heindrickson, J., Pereira, S.A., Raggi, T., Morais, S., Seiffert, W.Q., Schleder, D.D., Vieira, F.B., 2025. The effects of a functional palatability enhancer on the growth, immune response and intestinal microbiota of *Penaeus vannamei* chronically exposed to a suboptimal temperature (22 °C). *Appl. Sci.* 15, 8132.

Kır, M., Sunar, M.C., Topuz, M., Sarıipek, M., 2023. Thermal acclimation capacity and standard metabolism of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) at different temperature and salinity combinations. *Journal of Thermal Biology* 112, 103429. https://doi.org/10.1016/j.jtherbio.2022.103429

Peixoto, S., Strebel, L., Soares, R., Davis, D.A., 2022. Acoustic feeding responses using marine chemoattractants in plant-based diets for naive and non-naive *Litopenaeus vannamei*. *Appl. Anim. Behav. Sci.* 257, 105792.

Peixoto, S., Soares, R., 2025. Advances in passive acoustic monitoring for behavioral assessment and feeding automation in shrimp aquaculture. Reviews in Aquaculture

Ponce-Palafox, J.T., Palacise, C.A.M., Ross, L.G., 1997. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone,

1931. *Aquaculture* 157, 107–115.

Ren, X., Wang, Q., Shao, H., Xu, Y., Liu, P., Li, J., 2021. Effects of low temperature on shrimp and crab physiology, behavior and growth: a review. *Front. Mar. Sci.* 8, 746177.

Reis, R., Novriadi, A., Swanepoel, G., Jingping, M., Rhodes, M., Davis, D.A., 2020. Optimizing feed automation: improving timer-feeders and on-demand systems in semi-intensive pond culture of shrimp *Litopenaeus vannamei*. *Aquaculture* 519, 734759.

Reis, A., Weldon, P., Ito, W., Stites, M., Rhodes, M., Davis, D.A., 2021. Automated feeding systems for shrimp: effects of feeding schedules and passive feedback feeding systems. *Aquaculture* 541, 736800.

Reis, J., Peixoto, S., Soares, R., Rhodes, M., Ching, C., Davis, D.A., 2022. Passive acoustic monitoring as a tool to assess feed response and growth of shrimp in ponds and research systems. *Aquaculture* 546, 737326.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S., 2019. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. *Aquaculture* 501, 76–81.

Strebel, L.M., Nguyen, K., Corby, T., Rhodes, M., Beck, B.H., Roy, L., Davis, D.A., 2023. On-demand feeding and the response of Pacific white shrimp (*Litopenaeus vannamei*) to varying dietary protein levels in semi-intensive pond production. *Aquaculture* 574, 739698.

Smith, D.V., Tabrett, S., 2013. The use of passive acoustics to measure feed consumption by *Penaeus monodon* (giant tiger prawn) in cultured systems. Aquac. Eng. 57, 38–47. https://doi.org/10.1016/j.aquaeng.2013.06.003.

Tabbara, M., Strebel, L., Peixoto, S., Soares, R., Morais, S., Davis, D.A., 2024. Use of passive acoustic monitoring to evaluate the effects of a feed effector on feeding behavior, growth performance and salinity stress tolerance of *Litopenaeus vannamei*. *Aquaculture* 582, 740499.

Walsh, S., Nguyen, K., Strebel, L., Rhodes, M., Davis, D.A., 2022. Utilising feed effectors and automated feeders for semi-intensive Pacific white shrimp (*Litopenaeus vannamei*) production. *Aquac. Fish Fish.* 2, 540–551.

Wang, Z., Liao, G., Chen, B., Fan, L., 2023. Impacts of acute cold-stress in Pacific white shrimp *Litopenaeus vannamei*: investigating the tissue-specific stress resistance response.

Aquac. Int. 31, 2649–2663.

Wyban, J., Walsh, W.A., Godin, D.M., 1995. Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquaculture* 138, 267–279.

Zhang, P., Jiang, Z., Zhang, Y., Leng, L., Yin, Z., He, W., Pan, D., Zeng, X., 2025. Changes in muscle quality and gut microbiota of whiteleg shrimp (*Penaeus vannamei*) within a live supply chain. *Animals* 15, 1431.

Table 1. Mean (± SD) water quality parameters of temperature (°C), pH, total ammonia nitrogen (TAN, mg/L), nitrite (mg/L), dissolved oxygen (DO, mg/L), and salinity (ppm) across five experimental temperature regimes (Very cold 22.1 °C; Cold 24.1 °C; Regular 27.5 °C; Hot 29.4 °C; Very hot 31.3 °C) to evaluate the feeding behavior of *Penaeus vannamei.*

Treatment	Temperature	pH	TAN	Nitrite	DO	Salinity
Very cold	22.1 ± 0.1^{e}	7.84 ± 0.08^{a}	0.62 ± 0.41^{a}	0.71 ± 0.50^{a}	5.90 ± 0.35^{a}	8.0 ± 0.2^{a}
Cold	24.1 ± 0.1^{d}	7.88 ± 0.09^{a}	0.59 ± 0.45^{a}	0.66 ± 0.47^{a}	5.70 ± 0.33^{a}	8.2 ± 0.1^{a}
Regular	27.5 ± 0.1^{c}	7.90 ± 0.10^{a}	0.68 ± 0.52^{a}	0.77 ± 0.56^{a}	5.50 ± 0.30^{a}	8.3 ± 0.6^{a}
Hot	29.4 ± 0.1^{b}	7.93 ± 0.11^{a}	0.74 ± 0.50^{a}	0.83 ± 0.58^{a}	5.40 ± 0.32^{a}	7.9 ± 0.3^{a}
Very hot	31.3 ± 0.1^{a}	7.96 ± 0.12^{a}	0.82 ± 0.55^{a}	0.89 ± 0.60^{a}	5.20 ± 0.38^{a}	8.3 ± 0.2^{a}

Table 2. Type III analysis of covariance (ANCOVA) for *Penaeus vannamei* clicks emitted during feeding activity under different temperature treatments. Temperatures tested were: 22.1 °C (Very cold), 24.1 °C (Cold), 27.5 °C (Regular), 29.4 °C (Hot), and 31.3 °C (Very hot). The model included the main effects of time and treatment, and their interaction. Significant values are marked by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001).

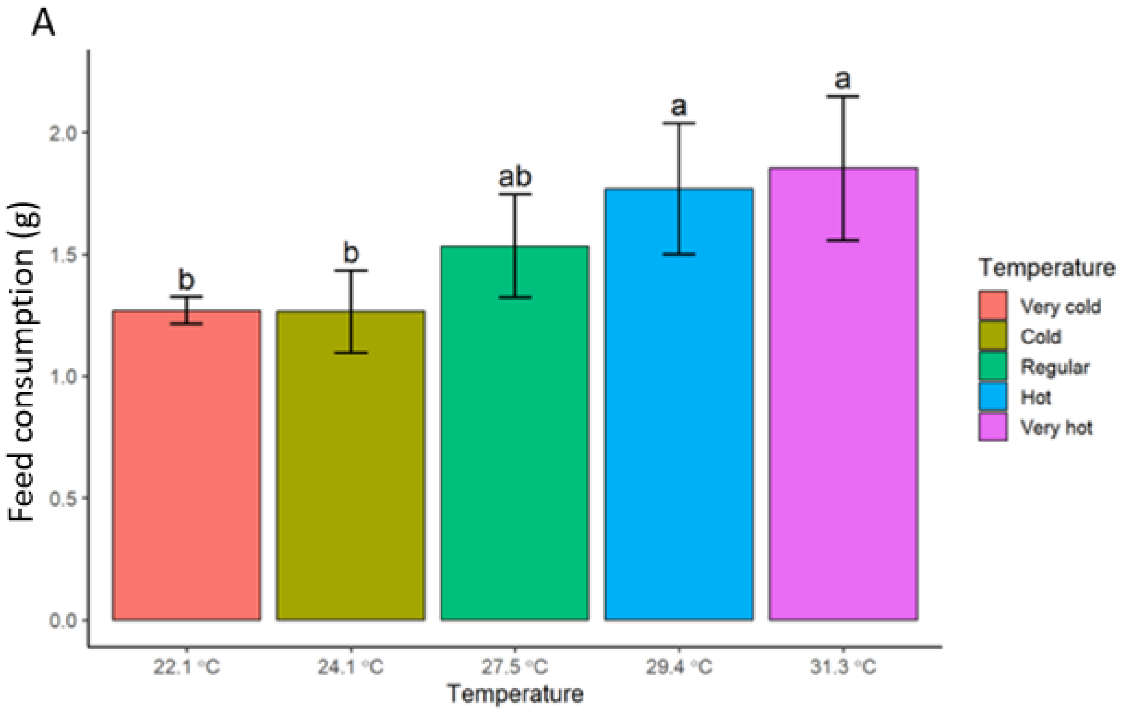
Source	Sum of Squares	Df	F value	Pr(>F)	Significance
Intercept	63670	1	248.743	< 2.2e-16	***
Time	22473	1	87.799	< 2.2e-16	***
Temperature	26779	4	26.154	< 2.2e-16	***
Time — Temperature	12867	4	12.567	1.93E-09	***
Residuals	74230	290			

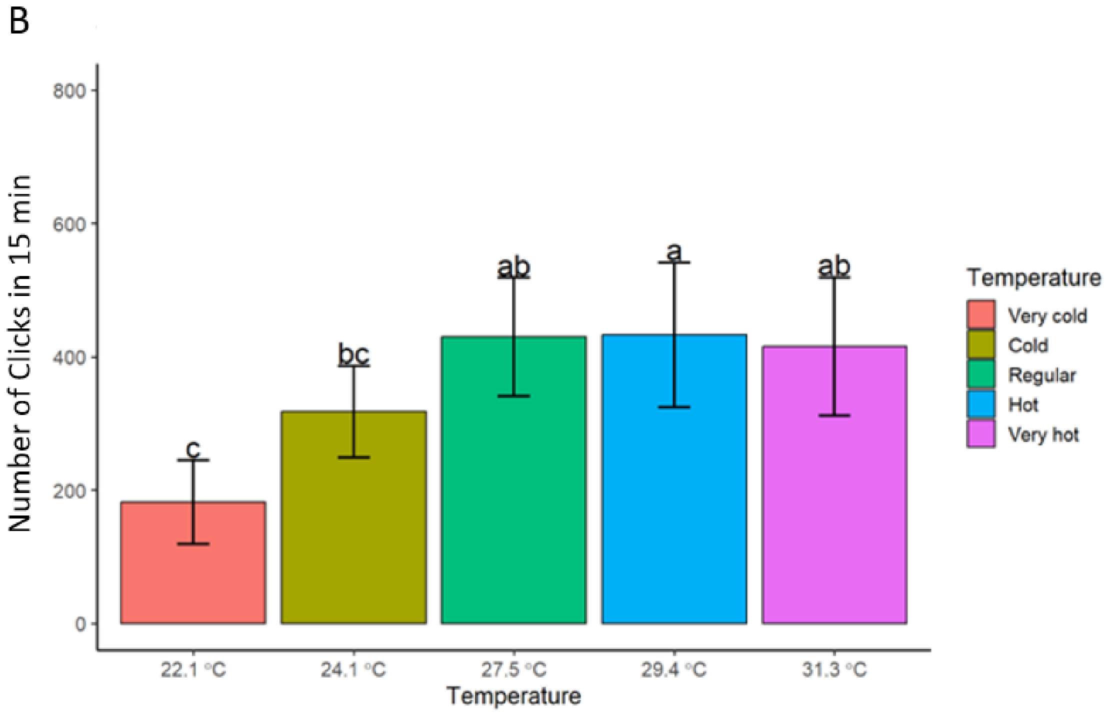
Table 3. Pairwise comparisons of temperature treatments versus the Regular temperature (27.5 °C) as a control, based on estimated marginal means (EMMs) with Bonferroni adjustment, using 15-min click recordings. Negative values indicate fewer clicks relative to the control. Temperatures tested were 22.1 °C (Very cold), 24.1 °C (Cold), 27.5 °C (Regular), 29.4 °C (Hot), and 31.3 °C (Very hot). Significant differences are denoted by asterisks (*p < 0.05).

Contrast	Estimate	SE	df	t-ratio	*p*-value
Very cold - Regular	-20.583	2.92	290	-7.047	<0.0001*
Cold - Regular	-12.617	2.92	290	- 4.319	0.0002*
Hot - Regular	-0.217	2.92	290	-0.074	1
Very hot - Regular	-5.017	2.92	290	-1.717	1

Table 4. Pairwise comparisons of temperature treatments versus the Regular temperature (27.5 °C) as a control, based on estimated marginal means (EMMs) with Bonferroni adjustment, using 30-min click recordings. Negative values indicate fewer clicks relative to the control. Temperatures tested were 22.1 °C (Very cold), 24.1 °C (Cold), 27.5 °C (Regular), 29.4 °C (Hot), and 31.3 °C (Very hot). Significant differences are denoted by asterisks (*p < 0.05).

Contrast	Estimate	SE	df	t-ratio	*p*-value
Very cold - Regular	-13.18	1.88	590	-6.997	<0.0001*
Cold - Regular	-7.8	1.88	590	-4.142	0.0002*
Hot - Regular	-0.1	1.88	590	-0.053	1
Very hot - Regular	-1.02	1.88	590	-0.54	1


A
Feed consumption (g)
2.0
1.5
1.0
0.5
0.0
b
b
ab
a
a
22.1 °C
24.1 °C
27.5 °C
29.4 °C
31.3 °C
Temperature
Temperature
Very cold
Cold
Regular
Hot
Very hot


B
Number of Clicks in 15 min
800
600
400
200
0
c
bc
ab
a
ab
22.1 °C
24.1 °C
27.5 °C
29.4 °C
31.3 °C
Temperature
Temperature
Very cold
Cold
Regular
Hot
Very hot

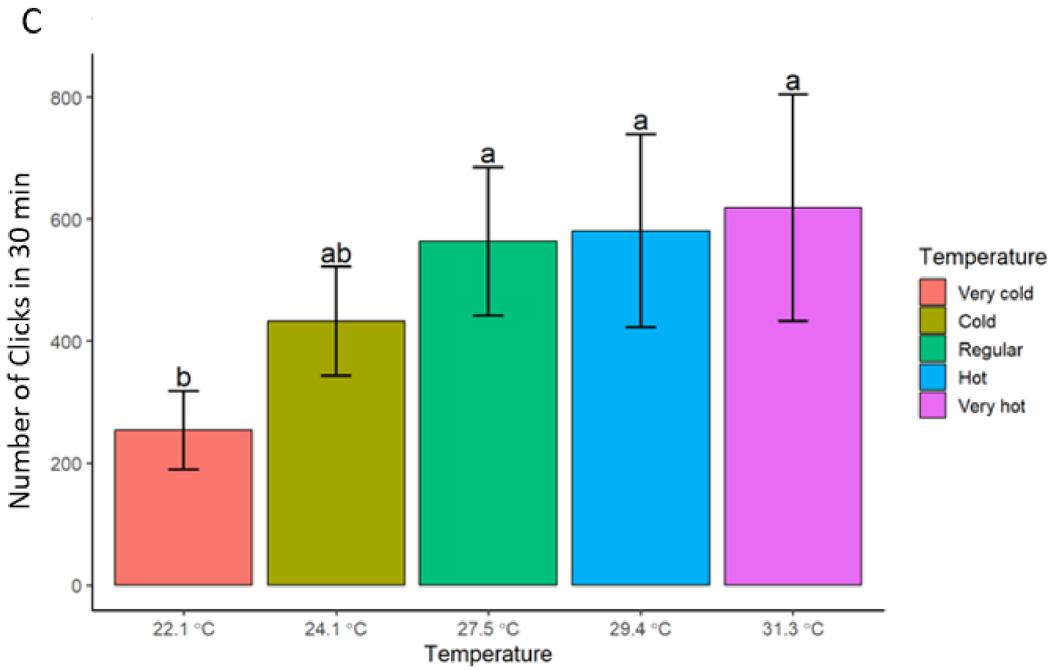


Figure 1. Effect of different water temperatures on the feeding behavior of *Penaeus vannamei* under controlled laboratory conditions. **(A)** Mean food consumption (g) per treatment after 30 minutes. **(B)** Mean number of acoustic clicks detected in the first 15 minutes of feeding activity. **(C)** Mean number of acoustic clicks detected over the full 30-minutes recordings of feeding activity. Temperatures tested were: 22.1 °C (Very cold), 24.1 °C (Cold), 27.5 °C (Regular), 29.4 °C (Hot), and 31.3 °C (Very hot). Bars represent mean values (± standard deviation) while different lowercase letters above indicate significant differences among treatments ($p < 0.05$).

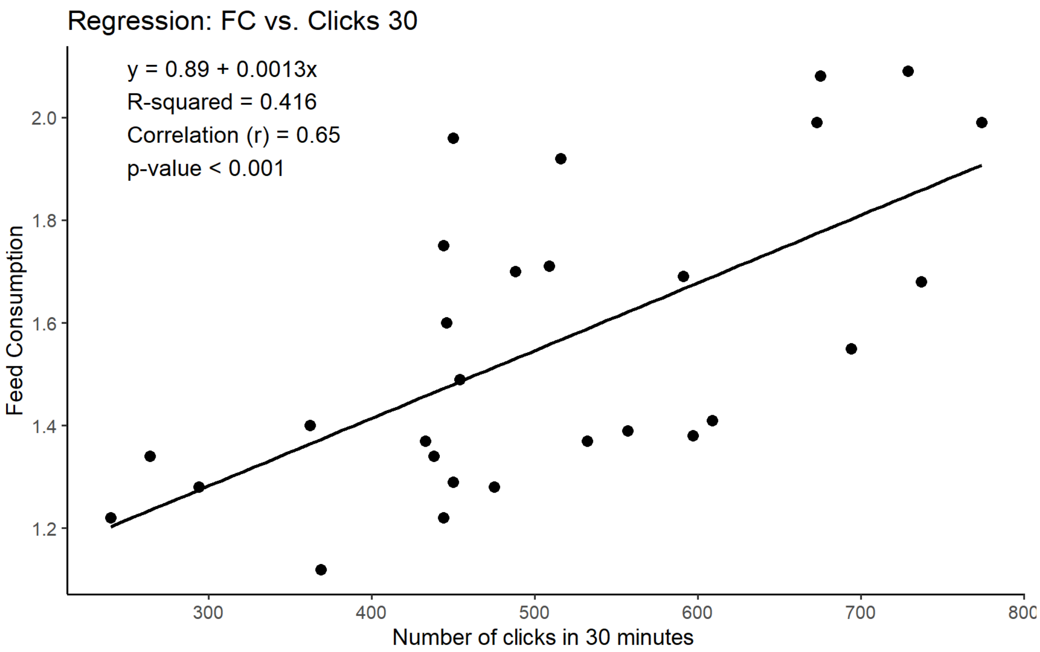


Figure 2. Linear regression between total number of acoustic clicks and food consumption of *Penaeus vannamei* across all treatments. Correlation between food consumption and number of clicks in 30 minutes ($r = 0.65$, $p < 0.001$). Each point represents the mean value from one aquarium replicate. The regression line illustrates the direction and strength of the relationship between acoustic activity and feed intake.

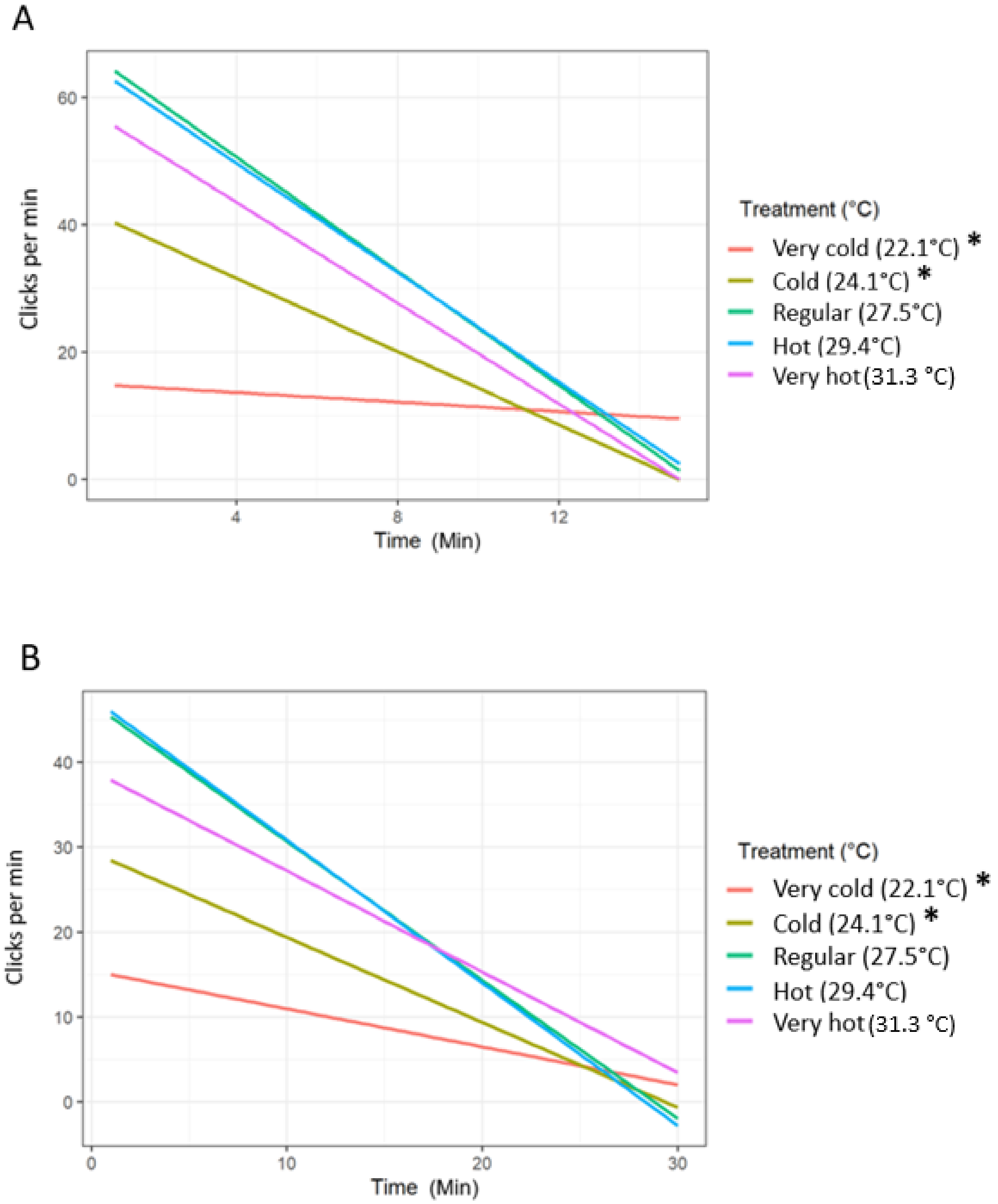


Figure 3. Linear trends in acoustic click activity of *Penaeus vannamei* across temperature treatments, derived from a Type III ANCOVA including time (min), treatment, and their interaction. X-axis: time (min), Y-axis: clicks per minute. (A) 15-min feeding period. (B) 30-min feeding period. Each line shows the model-fitted regression for a given temperature treatment.

Asterisks in the legend indicate treatments that differ from the Regular temperature condition (27.5 °C) at $p < 0.05$

3- Capitulo 2 Gasping for air: The impact of gradual versus abrupt hypoxia on the acoustic feeding behavior of *Penaeus vannamei*.

Artigo científico em revisão Revista – Aquaculture (ISSN: 1873-5622). Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela (https://www.sciencedirect.com/journal/aquaculture). referida revista

Fábio Costa Filho[a], Indira Medina Torres[b], Roberta Borda Soares[a], Silvio Peixoto[a], D. Allen Davis[b]

[a] Department of Fisheries and Aquaculture, Federal Rural University of Pernambuco, Recife, 52171-900, PE, Brazil.

[b] Auburn University - School of Fisheries, Aquaculture and Aquatic Sciences, Auburn, AL 36849, USA

Fábio Costa Filho; e-mail: ramosfabio22@gmail.com; ORCID: 0000-0002-8188-5327 (corresponding author)

Indira Medina Torres; e-mail: ipm0008@auburn.edu; ORCID:

Roberta Borda Soares; e-mail: beta.ufrpe@ufrpe.br; ORCID: 0000-0003-3952-5819

D. Allen Davis; e-mail: davisda@auburn.edu; ORCID:

Silvio Peixoto; e-mail: silvio.peixoto@gmail.com; ORCID: 0000-0002-2192-3854

Abstract

Dissolved oxygen (DO) is a critical limiting factor in intensive shrimp farming. It is well known that hypoxia can reduce feed intake, but accurately quantifying this behavioral response is challenging with traditional monitoring methods. Passive Acoustic Monitoring (PAM) offers a non-invasive, high-resolution alternative to assess shrimp behavior based on mandibular click emission during feeding activity. This study aimed to use PAM to evaluate the effect of progressive hypoxia on the feeding behavior of *Penaeus vannamei*. Groups of juvenile shrimps (16 g) were exposed to four DO levels of 5 (control), 3, 2, and 1 mg/L. The low levels (3, 2, and 1 mg/L) were achieved using two distinct methods: a “gradual reduction" by turning aeration off and a more "abrupt reduction" via nitrogen gas. Shrimp food consumption (FC) and acoustic click activity were measured over 30 min and analyzed using two-way ANOVA, linear regression, and Generalized Least Squares (GLS). Results showed that FC and click activity were strongly correlated (R = 0.807, *p*-value < 0.001) and the method of oxygen reduction significantly influenced both behavioral responses. Under gradual reduction, food consumption remained statistically similar at 5, 3, and 2 mg/L, but dropped significantly to 1 mg/L, accompanied by reduced click activity. Under abrupt reduction, food consumption showed a clearer DO-dependent decline, with the main significant drop occurring at 1 mg/L. The GLS indicated that under hypoxia, click activity peaked early and declined rapidly, whereas under aeration (5 mg/L) clicking rates remained more stable over time. These results suggest that the method of dissolved oxygen reduction (gradual vs. abrupt), rather than the final concentration alone, significantly influences the feeding behavior of *P. vannamei*. We validate PAM as a precise tool to detect hypoxic stress in real-time, offering a method to optimize feeding and aeration management.

Keywords: penaeid; clicks; feeding activity; dissolved oxygen.

1. Introduction

The intensification of *Penaeus vannamei* culture has shifted management focus toward the critical interplay between environmental stability and feeding efficiency. As stocking densities increase, dissolved oxygen (DO) emerges as the primary limiting factor, directly governing metabolic scope and growth performance (Bett and Vinatea, 2009; Mohammed et al., 2024). In high-density, low-exchange systems, water-quality parameters can deteriorate quickly, elevating health risks and undermining production sustainability (Hopkins et al., 1991; Mohammed et al., 2024). Among these parameters, DO is often the most sensitive and limiting, as it underpins respiration and energy metabolism and can modulate shrimp immune competence and survival (Bett and Vinatea, 2009; San Andres et al., 2025).

Hypoxia is frequently observed in rearing environments whenever biological oxygen demand exceeds the available supply a common occurrence near dawn in shrimp ponds, typically defined as DO concentrations below 3 mg/L (Ferreira et al., 2020; San Andres et al., 2025). While *P. vannamei* possesses physiological mechanisms that confer some tolerance to transient low-oxygen events, sustained or severe hypoxia triggers oxidative stress, immune suppression, and increased mortality (San Andres et al., 2025). Under such conditions, penaeid shrimp reduce feeding activity as an energy-conservation strategy, with concomitant decreases in locomotion and feed intake (Bett and Vinatea, 2009; Jasmin et al., 2022). Understanding the precise thresholds of these behavioral shifts across different hypoxia trajectories is therefore essential for optimizing aeration and feeding protocols in modern shrimp aquaculture.

While feed intake reduction is a response well-recognized under low DO levels in shrimp farming, evaluating the underlying mechanisms of this feeding behavior remains challenging. Conventional approaches of visual behavior assessments and feed-tray monitoring are labor-intensive, prone to observer bias, and ineffective under low-visibility or nighttime conditions

(Smith and Tabrett, 2013; Mohammed et al., 2024). Passive acoustic monitoring (PAM) offers a non-invasive alternative by detecting characteristic feeding sounds (clicks) produced during mandibular occlusion of shrimp (Smith and Tabrett, 2013; Silva et al., 2019). Therefore, PAM has been successfully applied to evaluate a range of behavioral responses in shrimp subjected to different management practices (e.g., stocking density, pellet size, and feed formulation), enabling continuous monitoring of feeding intensity with high temporal resolution (Peixoto and Soares, 2025). Moreover, this acoustic approach has recently been applied to investigate the effects of water temperature on the feeding activity of *P. vannamei*, demonstrating that different temperature levels (22.1, 24.1, 27.5, 29.4, and 31.3 °C) influence not only the overall magnitude of feeding activity but also its temporal pattern (Costa Filho et al., 2026).

Despite its proven effectiveness, the application of PAM to specifically quantify feeding behavior under varying levels of hypoxia has not been tested and remains a significant knowledge gap. Although the relationship between hypoxia and shrimp feeding is understood qualitatively, the present study aims to employ PAM to evaluate the effect of progressive hypoxia on real-time feeding behavior of *P. vannamei* using two distinct methods: gradual and abrupt (nitrogen-induced) reduction of DO levels.

2. Materials and methods

2.1. Experimental design

This study was conducted in a temperature-controlled room at the E.W. Shell Fisheries Center, Auburn University (AL, USA), using glass aquaria (70 L each) arranged in a closed clean-water recirculating system. This system consists of a common sump, biological filter media (fluidized bed and bead filters), circulation pump and continuous aeration. A total of 24 independently aerated aquaria were used. The experimental design was divided into two groups of 12 aquaria to evaluate two different hypoxia induction methods: (1) gradual reduction and (2) abrupt reduction. Four dissolved oxygen (DO) concentration treatments were established for each method: 5 (control), 3, 2, and 1 mg/L. Each treatment consisted of three replicates, resulting in a total of 12 aquaria per method. During the trials, recirculation was turned off, and the target concentrations were reached as follows: for gradual reduction, aeration was stopped to allow oxygen to decrease naturally; while for abrupt reduction, nitrogen gas (N_2) was injected into the water column to specifically evaluate rapid (abrupt) DO drops. For the gradual reduction method (aeration removal), the time required to reach the target DO levels dictated the recording sequence. It took approximately 2 hours to reach 3 mg/L, 4 hours for 2 mg/L, and 6 hours for 1 mg/L. Thus, recordings followed the order of 3, 2, and 1 mg/L. The abrupt reduction (N_2 injection) method followed the same procedural order but utilized nitrogen injection while monitoring until the desired DO level was reached. The aeration for the control group (5 mg/L) was also removed just prior to its recording session.

P. vannamei (mean weight 16.26 ± 1.00 g) were used in the experiment, with 10 shrimp stocked per aquarium (40 shrimps per m^2). The animals were previously reared under controlled laboratory conditions and acclimated to the experimental aquariums for 48 hours before data collection. A dedicated and independent group of shrimps was used for each DO treatment and reduction method (gradual and abrupt). The experiment was conducted over two days, as the gradual reduction method was applied on the first day, and the abrupt reduction on the second day. Throughout the experiment, shrimp were fed a commercial diet (2.4 mm pellets, CP 35%; Zeigler Bros Inc., USA).

To ensure precise control of the experimental treatments, dissolved oxygen (DO), water temperature and salinity were continuously monitored throughout the trials using a YSI Professional Plus digital meter (YSI Inc., Yellow Springs, OH, USA). Secondary parameters, such as pH and total ammonia nitrogen (TAN), were measured at the end of the trial, with TAN determined colorimetrically using a YSI 9500 photometer (YSI Inc., Yellow Springs, OH, USA).

2.2. Food consumption and acoustic analysis

During the trials, after a 16-h fasting period programmed according to the test sequence, each aquarium received 2.0 g of commercial feed at the first morning feeding, gently delivered at the tank center. Thus, after 30 min, the uneaten feed was siphoned onto pre-weighed cellulose filters (20 µm pore size), and the filters were oven-dried at 100 °C for 24 h to determine the dry mass recovered and compute consumption. To correct for leaching, a control assay was performed in twelve shrimp-free aquaria by adding 1.0 g (as is) of the same feed and applying the identical recovery and drying protocol; the proportion of feed recovered in these controls was used as a leaching correction factor in intake estimates. The mean food consumption (FC) on a dry-matter basis was calculated as $FC = Fo - (Fr / Fl)$, where Fo is the dry weight of feed offered, Fr is the dry weight of feed recovered, and Fl is the leaching correction factor (the proportion of feed recovered from shrimp-free controls, where $Fl = Fr / Fo$).

In parallel to food consumption analysis, passive acoustic monitoring (PAM) was conducted for each replicate aquarium. Acoustic data were captured using four omnidirectional AS-1 hydrophones (Aquarian Hydrophones, USA; flat frequency response 1 Hz–100 kHz; sensitivity −208 dBV), each connected to a 26 dB PA-4 preamplifier and a 6-channel multitrack recorder (Zoom F6, USA) set to a 192 kHz sampling rate and 16-bit resolution. Hydrophones were positioned at the center of each aquarium, and recordings began at feed offering and lasted 30 min.

Audio files were analyzed in Raven Pro 1.5 (Cornell Lab of Ornithology, USA) using a band-limited energy detector applied to the spectrogram (Hann window, FFT size 512 samples, 3 dB filter bandwidth 135 Hz, 50% overlap) with detection parameters of 15 – 90 kHz frequency range, 5 – 50 ms signal duration, and minimum signal-to-noise ratio of 12 dB. The Raven output provided the timestamp of each detected click, forming the database for subsequent calculations and statistical analyses. Thus, click data from each aquarium were processed in Microsoft Excel using a histogram analysis with the click occurrence timestamps to calculate the frequency of clicks per minute over the 30-minute recording period for each replicate.

2.3. Statistical Analysis

The treatment effects on food consumption (FC) and total click counts at 30 min were compared using a one-way analysis of variance (ANOVA), with dissolved oxygen level (5, 3, 2, and 1 mg/L) and reduction method (gradual and abrupt) as factors. Prior to analysis, normality and homogeneity of variances were verified using the Kolmogorov-Smirnov and Cochran's tests, respectively. When significant differences were detected, Tukey's HSD post hoc test was used for pairwise comparisons of treatment means. Additionally, to validate acoustic activity as an indicator of food consumption, linear regression analyses were performed to assess the correlation between total click counts (during 30 min) and FC values after 30 min.

The temporal dynamics of acoustic activity were evaluated using generalized least squares (GLS) models, which account for potential heteroscedasticity in the residuals. First, to compare the different DO treatments against the control (5 mg/L), a global model was constructed using time as a continuous predictor and DO level as a categorical predictor. Pairwise comparisons among the estimated marginal means (EMMs) were performed using Fisher's least significant difference (LSD) post-hoc test to identify specific differences between DO levels. Subsequently, to address the comparison between reduction pathways (gradual vs.

abrupt) within each specific DO level, separate GLS models were fitted for each DO concentration (3, 2, and 1 mg/L). In these subset analyses, time served as the covariate and the reduction method as the categorical factor. A significant Time × Treatment interaction indicated that the rate of activity decline differed significantly between the gradual and abrupt induction methods.

To examine the relationship between DO concentration and shrimp feeding metrics (food consumption and total acoustic clicks), linear regression models were applied. Pearson correlation coefficients (R) and their respective significance levels were calculated for each reduction method (gradual and abrupt) to determine the strength and direction of the association between DO levels and acoustic activity.

Acoustic click counts recorded at 1 mg/L dissolved oxygen were analyzed to compare the effects of gradual and abrupt hypoxia induction methods. For each method, the empirical cumulative distribution function (ECDF) was generated to visualize the cumulative probability distribution of click intensities. Differences between the two distributions were assessed using a two-sample Kolmogorov–Smirnov (K–S) test.

All statistical analyses were performed using R software (version 4.x) within the RStudio environment, adopting a significance level of $\alpha = 0.05$ for all tests.

3. Results

Throughout the experiment, water quality parameters were maintained at 28.5 ± 0.24 °C for temperature, 8.9 ± 0.68 for salinity, 7.41 ± 0.14 for pH, and 0.074 ± 0.082 mg/L for total ammonia nitrogen (TAN). Regarding the dissolved oxygen levels, the actual mean concentrations were 5.31 ± 0.20 mg/L for the Control, followed by 3.02 ± 0.09 mg/L, 1.94 ± 0.11 mg/L, and 1.13 ± 0.07 mg/L for treatments named as the 3, 2, and 1 mg/L, respectively.

Shrimp food consumption was significantly influenced by both the dissolved oxygen (DO) concentration and the method of oxygen reduction (Figure 1). Under the gradual reduction method, food consumption remained statistically similar among the 5 mg/L (control), 3 mg/L, and 2 mg/L groups (Figure 1A; Table 1). However, shrimp at the 1 mg/L treatment showed a significant reduction in feed intake compared to all other groups (Table 1). In the abrupt nitrogen-induced method, a clearer DO-dependent decline was observed, with the highest food consumption in the aerated control (5 mg/L), intermediate values at 3 and 2 mg/L, and the lowest values recorded at 1 mg/L (Figure 2A; Table 2). Accordingly, shrimp at the 1 mg/L treatment showed a significantly lower food consumption when compared to all other groups, while shrimp at 2 mg/L did not differ from the 3 mg/L treatment (Table 2).

Overall, shrimp clicking activity followed similar trends to food consumption in both dissolved oxygen reduction methods. In the gradual reduction method, the number of clicks recorded over 30 min was significantly lower in the 1 mg/L group compared to all others, except for the shrimp at 2 mg/L (Figure 1B; Table 1). For the nitrogen-induced (abrupt) method, shrimp in the aerated control (5 mg/L) exhibited a significantly higher clicking activity compared to the 1 mg/L group, but both did not differ from shrimp in intermediate values of 3 and 2 mg/L (Figure 2B; Table 2).

A significant positive linear relationship (*p*-value < 0.001) was observed between shrimp acoustic activity and food consumption. This regression analysis revealed a strong association (R = 0.807) between the total number of clicks recorded in 30 min and feed intake (Figure 3), confirming that clicking activity can be used as a predictor of food consumption under the conditions tested.

To evaluate the effect of DO levels and time on shrimp acoustic activity, a GLS analysis was performed for both DO reduction methods, considering time (min) as a covariate (Figure 4).

For the gradual reduction method, the analysis revealed that the main effects of DO treatment and time, as well as their interaction, were highly significant (Table 3). Pairwise comparisons confirmed that shrimp in the 2 mg/L and 1 mg/L groups exhibited significantly lower clicking activity over time compared to the control group (5 mg/L), while the 3 mg/L group did not differ significantly from the control (Table 4). Similarly, for the nitrogen-induced method, both the main effects of DO treatment and time had significant effects on shrimp clicking activity (Table 5). Remarkably, in this method, the interaction between Treatment and Time was not significant (Table 5), differing from the gradual method. Pairwise comparisons again revealed that shrimp in the 2 mg/L and 1 mg/L treatments had significantly reduced clicking activity compared to the aerated control, while the 3 mg/L treatment did not differ significantly from the control (Table 6).

Finally, the relationship between DO concentration and feeding metrics was modeled using linear regressions to compare the two hypoxia trajectories (Figure 5). For both gradual (Figures 5A, 5B) and abrupt (Figures 5C, 5D) methods, a significant positive correlation was observed between DO levels and both food consumption and acoustic activity (*p*-value < 0.001). The higher Pearson coefficients recorded for click activity (R = 0.782 and R = 0.773 for gradual and abrupt, respectively) compared to food consumption (R = 0.661 and R = 0.595) reinforce the precision of acoustic monitoring in reflecting feeding suppression across different oxygen reduction dynamics.

The distribution of acoustic clicks emitted by shrimp at 1 mg/L DO differ significantly between the DO reduction methods (Kolmogorov-Smirnov test). The ECDF analysis showed a clear shift to higher click intensities in the abrupt group compared to the gradual group (Figure 6). In the abrupt method, shrimp showed a median of 8 clicks/min, while in the gradual method the median was only 2.0 clicks/min. Furthermore, shrimp in the gradual reduction group recorded zero activity at the 25th percentile, while in the abrupt group they maintained the clicking activity at 2.0 clicks/min (Figure 6).

4. Discussion

The present study demonstrated that dissolved oxygen (DO) concentration plays a critical role in modulating the feeding behavior of *P. vannamei*, with both food consumption and acoustic activity significantly reduced under hypoxic conditions. Furthermore, the method of DO reduction whether gradual or abrupt (Nitrogen injection) influenced the magnitude of the shrimp's behavioral response. These findings were acquired through passive acoustic monitoring (PAM) and validated by direct feed intake, an approach applied for the first time to evaluate the effects of different DO levels, although it has already been successfully used in several PAM studies on shrimp feeding behavior, as reviewed by Peixoto and Soares (2025).

Under the gradual DO reduction, food consumption remained stable in the aerated control (5mg/L) and 3mg/L treatments, but dropped significantly to 1mg/L, indicating a clear behavioral threshold. This aligns with reports of reduced growth and feed efficiency in *P. vannamei* and *Penaeus monodon* reared at DO levels of 1.8 mg/L or lower, suggesting that while shrimp tolerate moderate hypoxia (~2 – 3 mg/L), levels near 1 mg/L exceed the physiological threshold for normal feeding behavior (Seidman and Lawrence, 1985; Dall et al., 1991). This is likely due to the energetic trade-off imposed by extreme hypoxia, forcing shrimp to shift from active feeding to energy conservation and metabolic depression (Zhang et al., 2006). Accordingly, such behavioral shift was confirmed for both food consumption and acoustic activity in shrimp when DO levels reached 1 mg/L. Nevertheless, at 1 mg/L, the ECDF analysis revealed a significant distributional shift (Figure 5), indicating that the probability of high-intensity clicking events differed between the gradual and abrupt reduction methods. This suggests that the dynamics of hypoxia induction not only the final DO concentration modulate shrimp acoustic behavior. In practical terms, abrupt DO drops can alter the pattern of behavioral suppression, emphasizing the critical importance of preventing dissolved oxygen from declining to 1 mg/L in *P. vannamei* farming.

Mirroring the food consumption results, click activity measured via PAM proved to be a precise proxy for feeding behavior, closely tracking feed intake patterns and showing clear reductions as DO decreased in both gradual and abrupt hypoxia scenarios. Importantly, while at moderate hypoxia (2 – 3 mg/L) both methods produced comparable patterns of shrimp feeding behavioral decline, at 1 mg/L the rate of reduction in clicking activity diverged between methods, highlighting that PAM is sensitive not only to identify behavior patterns at the lowest DO concentration, but also during its decline trajectory. This consistency reinforces its validity as a quantitative ethological approach for shrimp (Peixoto and Soares, 2025), as also indicated by the strong positive correlation ($R = 0.807$, p-value < 0.001) between FC and number of clicks of *P. vannamei* in the present study. Moreover, PAM revealed nuances in the temporal profile of feeding behavior under different DO levels, as indicated by the significant Time × Treatment interaction (GLS), showing that under air saturation clicking rates remained stable over time, whereas under hypoxia they peaked early and declined rapidly. This pattern is consistent with a hypoxia-compensation strategy of *P. vannamei*, characterized by an initial exploratory feeding response followed by energy-saving suppression driven by metabolic constraints (Nguyen et al., 2022).

The comprehensive behavioral suppression, observed in both food consumption and acoustic activity of *P. vannamei* in the present study, can also be interpreted through biochemical and immunological constraints. Hypoxic exposure is known to shift metabolism toward protein catabolism (lower O:N ratios) and reduce the energetic return on feeding in penaeid shrimp

(Rosas et al., 1999). In parallel, low DO significantly modulates immune indices (Li et al., 2007), reducing antibacterial activities (Jiang et al., 2005) and increasing susceptibility to vibriosis in penaeids (Le Moullac et al., 1998: He et al., 2024). Sudden drops in DO are known to trigger abrupt physiological shock, including elevated HIF-1 alpha expression and oxidative stress, which leads to behavioral suppression (He et al., 2024). These physiological limitations likely contributed to the reduced feeding activity observed in our study under low DO levels.

At the 3 mg/L level, no significant difference was detected in the *P. vannamei* behavioral response between the gradual and abrupt DO reduction pathways. This is consistent with field studies in which aeration administered at minimum DO levels of approximately 2.5 mg/L did not impair the growth, survival, or feed conversion of *P. vannamei* (Araujo et al., 2024). Similarly, recirculating systems with gradual, daily DO drops (occasionally < 2.5 mg/L) also did not negatively impact growth or gene expression of this species (San Andres et al., 2025). Taken together, these findings reinforce that not only the DO level but also the rate at which it changes determines the stability of feeding behavior in *P. vannamei*. Nevertheless, shrimp size should also be considered as an important factor when interpreting the present results. The shrimp used in our trials averaged ~16 g, which corresponds with a size class where body mass strongly shapes the feeding dynamics and overall energy turnover (Dall et al., 1991). Previous PAM studies observed that *P. vannamei* of ~10 g sustain a more persistent clicking rate, while smaller shrimp (~4 g) show a faster decline in click activity as they rapidly attain satiation (Peixoto and Soares, 2025). Therefore, the sharper behavioral suppression of clicking activity under hypoxia may reflect the higher absolute metabolic demands of ~16 g shrimp, making them less resilient to abrupt DO drops than juveniles (Dall et al.,1991).

Beyond these specific biological insights, one of the most significant contributions of this study is demonstrating that PAM provides an actionable, real-time window into shrimp feeding behavior, which is particularly valuable in farming systems where visual tracking is impractical and post observational feed response (e.g. feeding trays) is applied to estimate feeding rates (Reis et al., 2022). This technology can detect not only differences in feeding intensity but also behaviorally meaningful temporal shifts, allowing for early detection of hypoxic stress. This integration is critical because the feeding event itself is a primary driver of oxygen demand, and studies confirm that the oxygen consumption level of *P. vannamei* is significantly higher after feeding than before, as more oxygen is required to oxidize the feed nutrients (Budiardi et al., 2005). Although acoustics-based automated feeders have successfully improved feeding management (Reis et al., 2022; Peixoto and Soares, 2025), the present findings highlight their potential to prevent feed waste during hypoxic events. This is particularly critical given that shrimp aggregation around feeders can locally deplete oxygen levels. Therefore, alongside acoustic monitoring, strategic aerator positioning is required to ensure adequate water current across the feeding zone, thereby maintaining minimal water quality parameters and optimizing food consumption. Extending this integration toward DO management is therefore not just a plausible next step, but an essential one for managing the abrupt, feed-induced oxygen demand, if aerator noise and sensor placement are handled appropriately.

5. Conclusion

This study concludes that both the concentration and the rate of dissolved oxygen decline critically modulate the feeding behavior of *P. vannamei*. While shrimp maintained statistical parity in food consumption down to 2 mg/L under gradual hypoxia, abrupt DO drops suppressed feeding activity at 2 mg/L and became even more pronounced at 1 mg/L, highlighting the behavioral impact of sudden physiological shock. This research successfully validated passive acoustic monitoring (PAM) as a precise, real-time proxy for food consumption, confirmed by a strong linear correlation. Furthermore, PAM was sensitive enough to detect not only the intensity of feeding suppression but also the temporal dynamics, capturing the shift to an "early peak and rapid decline" clicking behavior under hypoxic stress. Since these results were obtained with 16 g shrimp, future studies should investigate how these behavioral responses to DO concentration and depletion rate evolve throughout the grow-out cycle. This offers a clear pathway for integrating environmental and feeding automation tailored to shrimp size and real-time behavior.

References

Araujo, A.N., San Andres, C.F., Nguyen, K.Q., Corby, T.L., Rhodes, M.A., García, J.C., Roy, L.A., Stoeckel, J., Davis, A.D., 2024. Effects of minimum dissolved oxygen setpoints for aeration in semi-intensive pond production of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture* 594, 741376.

Bett, C., Vinatea, L., 2009. Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. *Braz. J. Oceanogr.* 57, 305–314.

Budiardi, T., Batara, T., Wahjuningrum, D., 2005. Oxygen Consumption of White Shrimp (*Litopenaeus vannamei*) and Model of Oxygen Management in Intensive Culture Pond. Jurnal Akuakultur Indonesia 4, 89-96.

Costa Filho, F., Torres, I.M., Soares, R.B., Davis, D.A. Peixoto, S. 2026. Thermal modulation of feeding behavior in *Penaeus vannamei* revealed by passive acoustic monitoring. *Aquaculture*, 615, 743631.

Dall, W., Hill, B.J., Rothlisberg, P.C., Staples, D.J., 1991. The biology of the Penaeidae. Adv. Mar. Biol. 27, 1–489.

FAO, 2024. The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action. *Food and Agriculture Organization of the United Nations*, Rome.

He, H., Huang, S., Geng, N., Weng, S., He, J., Li, C., 2024. Acute hypoxia stress mediates HIF-

1α–Yki–Cactus axis to facilitate the infection of *Vibrio parahaemolyticus* in *Litopenaeus vannamei*. *Front. Immunol.* 15, 1476309.

Hopkins, J.S., Stokes, A.D., Browdy, C.L., Sandifer, P.A., 1991. The relationship between feeding rate, paddlewheel aeration rate and expected dawn dissolved oxygen in intensive shrimp ponds. *Aquac. Eng.* 10, 281–290.

Jasmin, A., Ramesh, P., Tanveer, M., 2022. An intelligent framework for prediction and forecasting of dissolved oxygen level and biofloc amount in a shrimp culture system using machine learning techniques. *Expert Syst. Appl.* 199, 117160.

Jiang, L.-X., Pan, L.-Q., Fang, B., 2005. Effect of dissolved oxygen on immune parameters of the white shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 18, 185–188.

Le Moullac, G., Soyez, C., Saulnier, D., Ansquer, D., Avarre, J.-C., Levy, P., 1998. Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. *Fish Shellfish Immunol.* 8, 621–629.

Mohammed, F., Gabbadon, P., Ajmal, T., Goodchild, M., 2024. A study on the effect of feed load on mortality of Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Fish Fish.* 4, e187.

Nguyen, T.V., Alfaro, A.C., Rodríguez, J., Bayot, B., Sonnenholzner, S., 2022. Changes in metabolic profiling of whiteleg shrimp (*Penaeus vannamei*) under hypoxic stress. Journal of Invertebrate Pathology 193, 107797.

Peixoto, S., Soares, R., 2025. Recent advances and applications of passive acoustic monitoring in assessing shrimp feeding behaviour under laboratory and farm conditions. *Rev. Aquac.*

Peixoto, S., Soares, R., Silva, J.F., Hamilton, S., Morey, A., Davis, D.A., 2020. Acoustic activity of *Litopenaeus vannamei* fed pelleted and extruded diets. *Aquaculture* 525, 735307.

Reis, J., Peixoto, S., Soares, R., Rhodes, M., Ching, C., Davis, D.A., 2022. Passive acoustic monitoring as a tool to assess feed response and growth of shrimp in ponds and research systems. *Aquaculture* 546, 737326.

Rosas, C., Martínez, E., Gaxiola, G., Brito, R., Díaz-Iglesia, E., Soto, L.A., 1999. The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of juvenile *Penaeus setiferus* (Linnaeus). *J. Exp. Mar. Biol. Ecol.* 234, 41–57.

San Andres, C.F., Araujo, A.N., Nguyen, K.Q., Corby, T., Rhodes, M.A., Roy, L.A., García, J.C., Abernathy, J.W., Sankappa, N.P., Saoud, I.P., Davis, D.A., 2025. Effects of diurnal oxygen variation on survival, growth, and stress gene expression in Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Res.* 8041189.

Seidman, E.R., Lawrence, A.L., 1985. Growth, feed digestibility and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved-oxygen levels. *J. World Aquac. Soc.* 16, 333–346.

Silva, J.F., Hamilton, S., Rocha, J.V., Borie, A., Travassos, P., Soares, R., Peixoto, S. (2019). Acoustic characterization of feeding activity of Litopenaeus vannamei in captivity. *Aquaculture*, 501, 76-83.

Smith, D.V., Tabrett, S., 2013. The use of passive acoustics to measure feed consumption by *Penaeus monodon* (giant tiger prawn) in cultured systems. *Aquac. Eng.* 57, 38–47.

Zhang, P., Li, J., Zhang, X., 2006. The effects of body weight, temperature, salinity, pH, light intensity and feeding condition on lethal DO levels of whiteleg shrimp *Litopenaeus vannamei*. *Aquaculture* 256, 579–587.

Table 1. Mean (±SE) food consumption (g) and acoustic activity (total clicks) of *Penaeus vannamei* recorded during the total 30-min duration across dissolved oxygen (DO) concentration treatments (5 Control, 3, 2 and 1 mg/L) under gradual reduction method. Different superscript letters within each column indicate significant differences among DO treatments based on One-way ANOVA with Tukey's post-hoc test (*p*-value < 0.05).

Treatment	Food consumption (g)	Clicks (30 min)
5 mg/L	1.23 ± 0.19^{a}	594.11 ± 155.75^{a}
3 mg/L	1.19 ± 0.03^{a}	540.67 ± 101.67^{a}
2 mg/L	0.92 ± 0.23^{a}	347.0 ± 186.0^{ab}
1 mg/L	0.45 ± 0.17^{b}	176.33 ± 94.87^{b}

Table 2. Mean (±SE) food consumption (g) and acoustic activity (total clicks) of *Penaeus vannamei* recorded during the total 30-min duration across dissolved oxygen (DO) concentration treatments (5 Control, 3, 2 and 1 mg/L) under abrupt reduction method. Different superscript letters within each column indicate significant differences among DO treatments based on One-way ANOVA with Tukey's post-hoc test (*p*-value < 0.05).

Treatment	Food consumption (g)	Clicks (30 min)
5 mg/L	1.38 ± 0.10^{a}	579.8 ± 149.17^{a}
3 mg/L	1.20 ± 0.07^{ab}	500.7 ± 81.94^{ab}
2 mg/L	1.07 ± 0.16^{b}	387.0 ± 20.52^{ab}
1 mg/L	0.78 ± 0.10^{c}	332.3 ± 48.79^{b}

Table 3. Type III analysis of deviance for the Generalized Least Squares (GLS) model fitted to the gradual dissolved oxygen (DO) reduction, evaluating the effects of DO concentration treatments (5 Control, 3, 2 and 1 mg/L) and time on acoustic activity (click rate) of *Penaeus vannamei*. Significant *p*-values (< 0.05) are marked by asterisks.

Source	Df	Chi-square (χ2)	*p*-value
(Intercept)	1	384.345	< 0.001
Treatment	3	28.357	< 0.001
Time	1	142.798	< 0.001
Treatment x Time	3	9.305	0.025

Table 4. Pairwise comparisons of *Penaeus vannamei* clicking activity in dissolved oxygen (DO) concentration treatments (3, 2 and 1 mg/L) versus control (5 mg/L) under the gradual DO reduction, based on Generalized Least Squares (GLS) adjusted for time. Significant *p*-values (< 0.05) are marked by asterisks.

Contrast	Estimate	SE	df	*t*-ratio	*p*-value
5 mg/L – 3 mg/L	1.547	2.889	99.334	0.535	0.594
5 mg/L – 2 mg/L	8.593	2.889	99.334	2.974	< 0.05 *
5 mg/L – 1 mg/L	13.754	2.889	99.334	4.760	< 0.05 *

Table 5. Type III analysis of deviance for the Generalized Least Squares (GLS) model fitted to the abrupt dissolved oxygen reduction (DO), evaluating the effects of DO concentration treatments (5 Control, 3, 2 and 1 mg/L) and time on *Penaeus vannamei* clicking activity. Significant *p*-values (< 0.05) are marked by asterisks.

Source	Df	Chi-square ($\chi 2$)	*p*-value
(Intercept)	1	350.895	< 0.001
Treatment	3	8.109	0.044
Time	1	130.343	< 0.001
Treatment x Time	3	2.224	0.527

Table 6. Pairwise comparisons of *Penaeus vannamei* clicking activity in dissolved oxygen (DO) concentration treatments (3, 2 and 1 mg/L) versus control (5 mg/L) under the abrupt DO reduction, based on Generalized Least Squares (GLS) adjusted for time. Significant *p*-values (< 0.05) are marked by asterisks.

Contrast	Estimate	SE	df	*t*-ratio	*p*-value
5 mg/L – 3 mg/L	2.899	3.024	99.517	0.959	0.340
5 mg/L – 2 mg/L	6.496	3.024	99.517	2.148	<0.05 *
5 mg/L – 1 mg/L	8.517	3.024	99.517	2.816	< 0.05 *

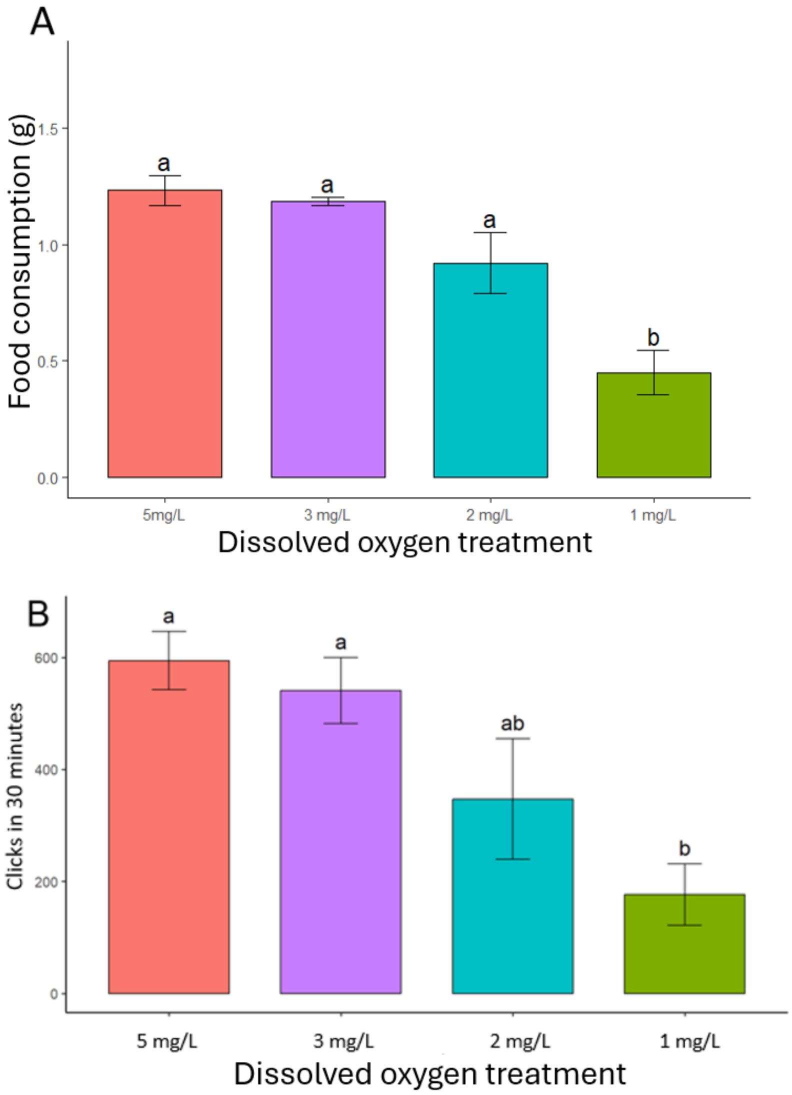


Figure 1. Effects of dissolved oxygen (DO) concentration on the feeding behavior of *Penaeus vannamei* under a gradual reduction method. (A) Mean food consumption (g) per treatment after 30 min. (B) Mean number of acoustic clicks detected over the entire 30-minute recording. The DO concentration treatments tested were: 5 (Control), 3, 2 and 1 mg/L. Bars represent mean values (± SE), and different lowercase letters indicate significant differences among treatments (One-way ANOVA with Tukey's post-hoc test, *p*-value < 0.05).

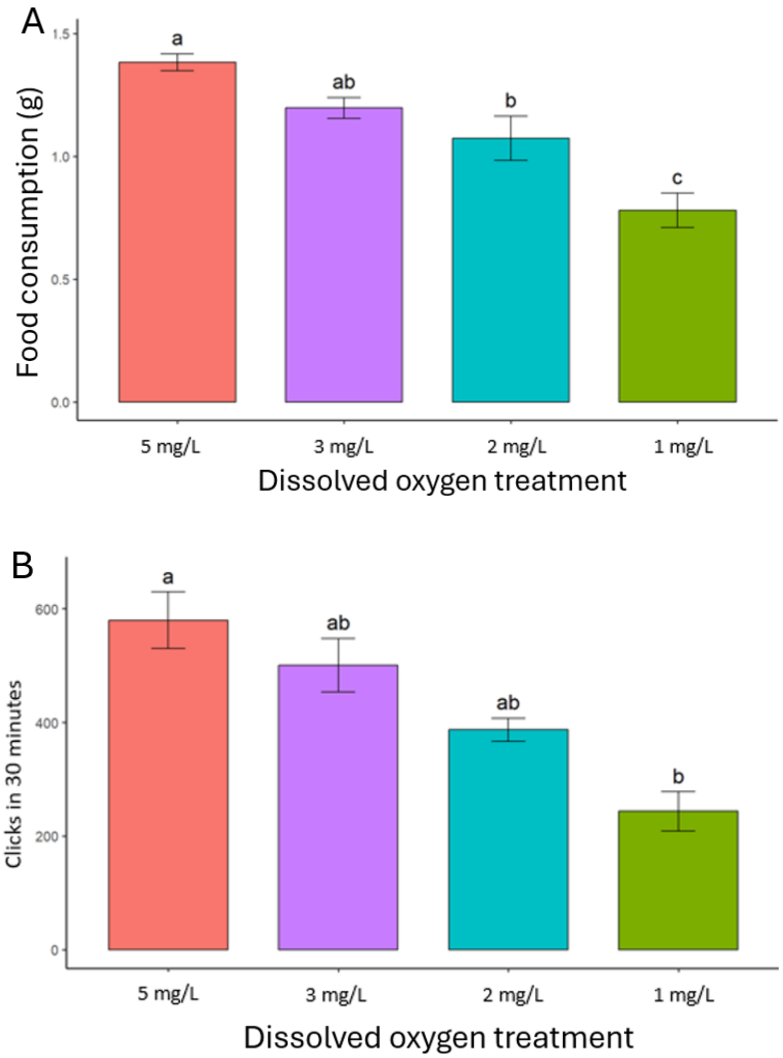


Figure 2. Effects of dissolved oxygen (DO) concentration on the feeding behavior of *Penaeus vannamei* under an abrupt reduction method. (A) Mean food consumption (g) per treatment after 30 min. (B) Mean number of acoustic clicks detected over the entire 30-minute recording. The DO concentration treatments tested were: 5 (Control), 3, 2 and 1 mg/L. Bars represent mean values (± standard error), and different lowercase letters indicate significant differences among treatments (One-way ANOVA with Tukey's post-hoc test, *p*-value < 0.05).

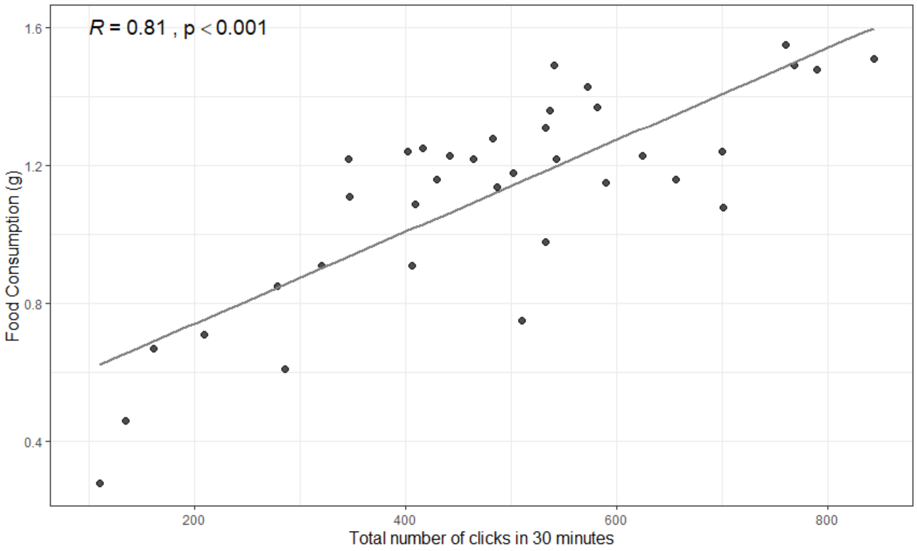


Figure 3. Linear regression between total number of acoustic clicks and food consumption (g) of *Penaeus vannamei* across the dissolved oxygen (DO) concentration treatments (5 Control, 3, 2 and 1 mg/L) during 30 min. Each point represents the value from one aquarium replicate.

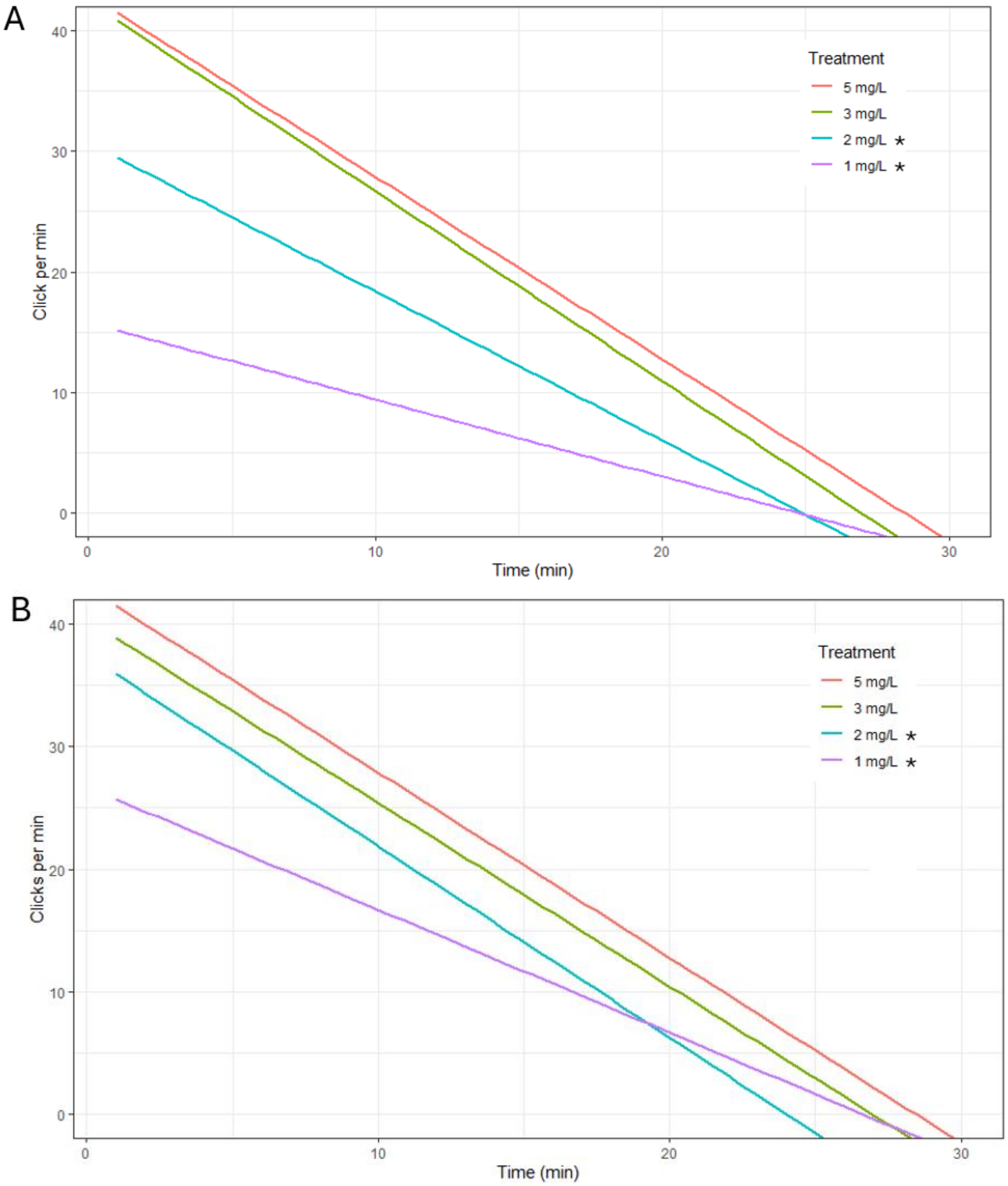


Figure 4. Linear trends in acoustic click activity of *Penaeus vannamei* across the dissolved oxygen (DO) concentration treatments (5 Control, 3, 2 and 1 mg/L) and reduction methods, derived from Generalized Least Squares (GLS) including time (min), DO treatment, reduction method, and their interactions. (A) Gradual reduction method. (B) Abrupt reduction method. Each line shows the model-fitted regression for a given DO treatment. Asterisks in the legend indicate treatments that differ significantly from the Control (5mg/L) based on GLS post hoc

tests (p-value < 0.01).

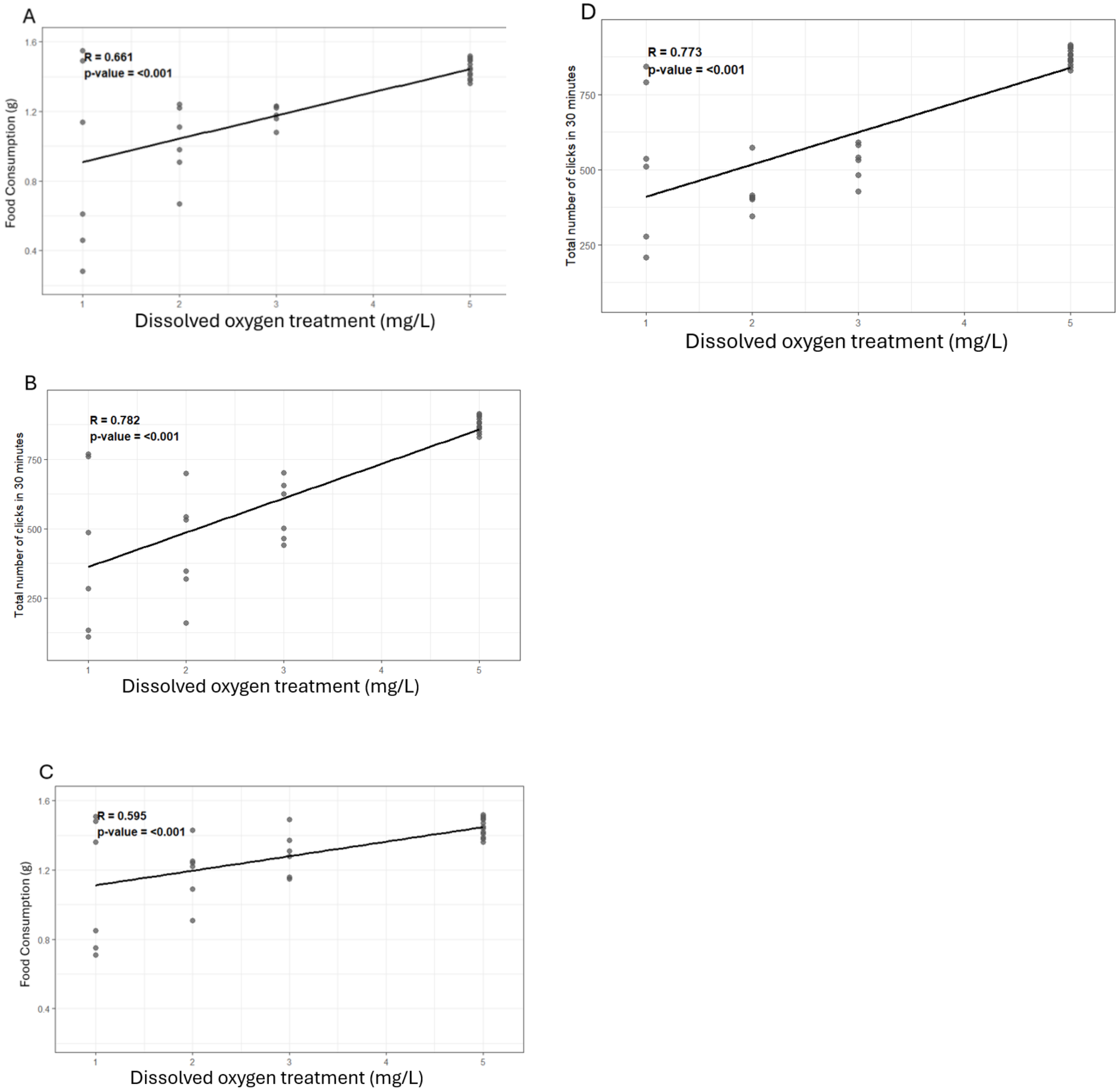

A
R = 0.661
p-value = <0.001
Food Consumption (g)
1.6
1.2
0.8
0.4
1
2
3
4
5
Dissolved oxygen treatment (mg/L)
D
R = 0.773
p-value = <0.001
Total number of clicks in 30 minutes
750
500
250
1
2
3
4
5
Dissolved oxygen treatment (mg/L)
B
R = 0.782
p-value = <0.001
Total number of clicks in 30 minutes
750
500
250
1
2
3
4
5
Dissolved oxygen treatment (mg/L)
C
R = 0.595
p-value = <0.001
Food Consumption (g)
1.6
1.2
0.8
0.4
1
2
3
4
5
Dissolved oxygen treatment (mg/L)

Figure 5. Relationship between dissolved oxygen (DO) concentration treatments (5 Control, 3, 2 and 1 mg/L) and feeding behavior of *Penaeus vannamei* based on food consumption and total number of acoustic clicks during the 30-min under two DO reduction methods: gradual (A, B) and abrupt (C, D). Solid lines represent the linear regression fit, with Pearson correlation coefficients (R) and significance levels indicated within each panel.

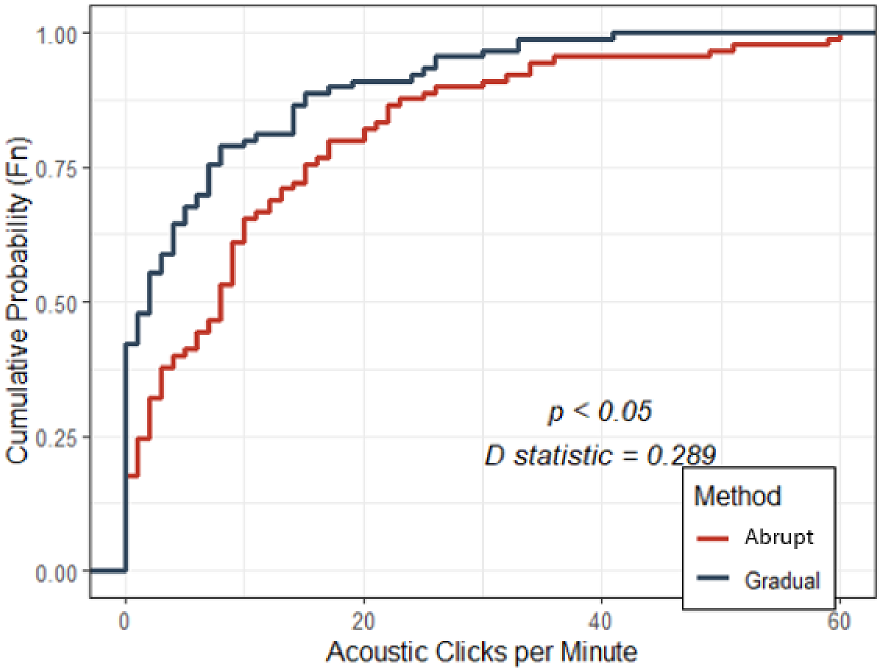


Figure 6. Empirical Cumulative Distribution Function (ECDF) of the acoustic behavior of *Penaeus vannamei* under severe hypoxia (1 mg/L). The graph displays the cumulative probability (Fn) of acoustic clicks per minute for two dissolved oxygen reduction methods: gradual (blue line) and abrupt (red line). Step lines represent the observed data distribution for each treatment (n = 90 observations per group). Statistical comparison was performed using the Kolmogorov-Smirnov test, with the D-statistic and significance level (p-value) indicated within the panel to highlight the distributional shift in behavioral intensity.

4- Considerações finais

A presente dissertação consolidou o Monitoramento Acústico Passivo (PAM) como uma ferramenta robusta, não invasiva e de alta precisão para a avaliação do comportamento alimentar do *Penaeus vannamei*. A forte associação positiva observada entre a emissão de cliques mandibulares e o consumo real de ração, tanto sob variações térmicas quanto sob diferentes concentrações de oxigênio, validando a técnica como um indicador confiável da ingestão de alimento em tempo real.

Em relação à influência térmica, concluiu-se que a temperatura da água atua como um regulador primário da intensidade e da dinâmica temporal da alimentação. A faixa entre 27,5 °C e 31,3 °C demonstrou ser a janela térmica ideal, promovendo o máximo desempenho alimentar caracterizado por uma resposta acústica intensa e imediata. Em contrapartida, condições de temperatura iguais ou inferiores a 24,1 °C resultaram em supressão comportamental significativa, onde a atividade alimentar se torna não apenas menor em magnitude, mas também mais lenta e estável, refletindo as limitações metabólicas impostas pelo frio.

Em relação aos níveis de oxigênio dissolvido, o estudo evidenciou que a resposta do *P. vannamei* é modulada tanto pela concentração final de oxigênio quanto pela velocidade de redução (gradual ou abrupta). Foi identificado um limiar crítico de comportamento em 1 mg/L, ponto no qual a atividade alimentar é severamente inibida, independentemente do método de indução. Contudo, a redução gradual mostrou-se mais impactante, gerando um declínio linear no consumo, enquanto a redução abrupta permitiu uma manutenção da estabilidade alimentar até concentrações próximas de 2 mg/L. O padrão acústico revelou ainda uma estratégia de "compensação e conservação" sob hipóxia, onde os animais apresentam um pico de atividade precoce seguido de rápida cessação para poupar energia.

Desta forma, os resultados aqui apresentados oferecem informações essenciais para o aprimoramento da automação na carcinicultura. A incorporação das variáveis ambientais e suas respectivas assinaturas acústicas nos algoritmos de alimentadores automáticos permite uma transição de sistemas baseados apenas em temporizadores para sistemas de alimentação de precisão baseados na demanda biológica real. Isso possibilita não apenas a otimização da conversão alimentar e a redução de desperdícios, mas também a detecção precoce de estresse ambiental, garantindo maior sustentabilidade e eficiência produtiva aos cultivos intensivos de *Penaeus vannamei*.

Recomenda-se que estudos futuros investiguem como essas respostas comportamentais variam ao longo da ontogenia da espécie, validando esses padrões para diferentes classes de tamanho e fases de cultivo.

Referencias

ARAUJO, A.N.; SAN ANDRES, C.F.; NGUYEN, K.Q.; CORBY, T.L.; RHODES, M.A.; GARCÍA, J.C.; ROY, L.A.; STOECKEL, J.; DAVIS, A.D. Effects of minimum dissolved oxygen setpoints for aeration in semi-intensive pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture, v.594, 741376, 2024.

BETT, C.; VINATEA, L. Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. Braz. J. Oceanogr., v.57, p.305–314, 2009.

DAYALAN, V.; KASIVELU, G.; RAGURAMAN, V.; NISA, A. Studies on temperature impact (sudden and gradual) of the white-leg shrimp *Litopenaeus vannamei*. Environ. Sci. Pollut. Res., 2024.

FAO. The State of World Fisheries and Aquaculture 2024 — Blue Transformation in Action. Rome: FAO, 2024.

FERNANDES, R.N.; BATISTA, F.M.; VALENTE, L.M.P. Genetic variation in disease resistance against White Spot Syndrome Virus (WSSV) in *Litopenaeus vannamei*. Frontiers in Genetics, v.10, p.1-10, 2019.

HAMILTON, S.; MOURA, G.J.B.; COSTA FILHO, F.; CRAVEIRO, C.; CASTRO-NETO, H.; ALMEIDA, M.; DUARTE-NETO, P.J.; SOARES, R.; PEIXOTO, S. Size matters: variability in the acoustic parameters during feeding activity of *Penaeus vannamei* in different size classes. Aquaculture, v.587, 2024.

HAMILTON, S.; COSTA FILHO, F.; SILVA, J.F.; DUARTE-NETO, P.J.; SOARES, R.; PEIXOTO, S. The loud crowd: interactions between stocking density and acoustic feeding activity of different size classes of *Litopenaeus vannamei*. Aquaculture, v.563, 2023.

HOPKINS, J.S.; STOKES, A.D.; BROWDY, C.L.; SANDIFER, P.A. The relationship between feeding rate, paddlewheel aeration rate and expected dawn dissolved oxygen in intensive shrimp ponds. Aquac. Eng., v.10, p.281–290, 1991.

IBGE. Produção da Pecuária Municipal 2023. Rio de Janeiro: IBGE, 2024.

JASMIN, A.; RAMESH, P.; TANVEER, M. An intelligent framework for prediction and forecasting of dissolved oxygen level and biofloc amount in a shrimp culture system using machine learning techniques. Expert Syst. Appl., v.199, 117160, 2022.

KAMARUZZAMAN, N.; NGUYEN, N.H.; KNIBB, W. Genetic improvement of feed efficiency traits in a plant-based diet environment for Pacific white shrimp *Penaeus vannamei.* Aquaculture, v.495, p.481-488, 2018.

KIR, M.; SUNAR, M.C.; TOPUZ, M.; SARIIPEK, M. Thermal acclimation capacity and standard metabolism of the Pacific white shrimp Litopenaeus vannamei (Boone, 1931) at different temperature and salinity combinations. Journal of Thermal Biology, v.112, 103429, 2023.

LI, E.; CHEN, L.; ZENG, C.; CHEN, X.; YU, N.; LAI, Q.; QIN, J.G. Growth, body composition, respiration and ambient ammonia nitrogen tolerance of juvenile white shrimp, *Litopenaeus vannamei*, at different salinities. Aquaculture, v.265, p.385-390, 2007.

LEHMANN, M.; SCHLEDER, D.D.; GUERTLER, C.; PERAZZOLO, L.M.; VINATEA, L. Hypoxia increases susceptibility of Pacific white shrimp to whitespot syndrome virus (WSSV). Arq. Bras. Med. Vet. Zootec., v.68, p.397–403, 2011.

PEIXOTO, S.; SOARES, R.; SILVA, J.F.; HAMILTON, S.; MOREY, A.; DAVIS, D.A. Acoustic activity of *Litopenaeus vannamei* fed pelleted and extruded diets. Aquaculture, v.525, 735307, 2020a.

PEIXOTO, S.; SILVA, J.F.; SOARES, R.; DAVIS, D.A. Size matters: Variability in the acoustic parameters during feeding activity of *Penaeus vannamei* in different size classes. Aquacultural Engineering, v.90, 102081, 2020b.

PEIXOTO, S.; STREBEL, L.; SOARES, R.; DAVIS, D.A. Acoustic feeding responses using marine chemoattractants in plant-based diets for naive and non-naive *Litopenaeus vannamei*. Appl. Anim. Behav. Sci., v.257, 105792, 2022.

PONCE-PALAFOX, J.T.; PALACISE, C.A.M.; ROSS, L.G. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture, v.157, p.107–115, 1997.

REIS, R.; NOVRIADI, A.; SWANEPOEL, G.; JINGPING, M.; RHODES, M.; DAVIS, D.A. Optimizing feed automation: improving timer-feeders and on-demand systems in semi-intensive pond culture of shrimp *Litopenaeus vannamei*. Aquaculture, v.519, 734759, 2020.

REIS, A.; WELDON, P.; ITO, W.; STITES, M.; RHODES, M.; DAVIS, D.A. Automated feeding systems for shrimp: effects of feeding schedules and passive feedback feeding systems. Aquaculture, v.541, 736800, 2021.

REIS, J.; PEIXOTO, S.; SOARES, R.; RHODES, M.; CHING, C.; DAVIS, D.A. Passive acoustic monitoring as a tool to assess feed response and growth of shrimp in ponds and research systems. Aquaculture, v.546, 737326, 2022.

ROSAS, C.; MARTÍNEZ, E.; GAXIOLA, G.; BRITO, R.; DÍAZ-IGLESIA, E.; SOTO, L.A. The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of juvenile *Penaeus setiferus* (Linnaeus). J. Exp. Mar. Biol. Ecol., v.234, p.41–57, 1999.

SEIDMAN, E.R.; LAWRENCE, A.L. Growth, feed digestibility and proximate body composition of juvenile *Penaeus vannamei* and Penaeus monodon grown at different dissolved-oxygen levels. J. World Aquac. Soc., v.16, p.333–346, 1985.

SILVA, J.F.; HAMILTON, S.; ROCHA, J.V.; BORIE, A.; TRAVASSOS, P.; SOARES, R.; PEIXOTO, S. Acoustic characterization of feeding activity of *Litopenaeus vannamei* in captivity. Aquaculture, v.501, p.76–81, 2019.

SOARES, R.; PEIXOTO, S.; DAVIS, D.A. Feeding behavior and growth of *Litopenaeus vannamei* fed soybean-based diets with added feeding effectors. Aquaculture, v.536, 736487, 2021.

TABBARA, M.; STREBEL, L.; PEIXOTO, S.; SOARES, R.; MORAIS, S.; DAVIS, D.A. Use of passive acoustic monitoring to evaluate the effects of a feed effector on feeding behavior, growth performance and salinity stress tolerance of *Litopenaeus vannamei*. Aquaculture, v.582, 740499, 2024.

ULLMAN, C.; RHODES, M.; DAVIS, D.A. Feed management and the use of automatic feeders in the pond production of Pacific white shrimp *Litopenaeus vannamei*. Aquaculture, v.498, p.44-49, 2019a.

ULLMAN, C.; SOARES, R.; PEIXOTO, S. Environmental factors modulate feeding behavior of Penaeus vannamei: insights from passive acoustic monitoring. Animals, v.9, n.1, 2019b.

WANG, Z.; LIAO, G.; CHEN, B.; FAN, L. Impacts of acute cold-stress in Pacific white shrimp *Litopenaeus vannamei*: investigating the tissue-specific stress resistance response. Aquac. Int., v.31, p.2649–2663, 2023.

WUERTZ, S.; BIERBACH, D.; BÖGNER, M. Welfare of decapod crustaceans with special emphasis on stress physiology. Aquac. Res., v.2023, 1307684, 2023.