

UNIVERSIDADE FEDERAL DA BAHIA
PROGRAMA DE DOUTORADO EM ZOOTECNIA

**SISTEMAS DE AUTO-ALIMENTAÇÃO PARA AVALIAR O CONSUMO
DE NUTRIENTES E RITMOS DIÁRIOS DA ATIVIDADE ALIMENTAR EM
PIRARUCU (*Arapaima gigas*)**

BRUNO OLIVETTI DE MATTOS

SALVADOR - BAHIA

FEVEREIRO – 2018



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Tese apresentada ao Programa de Doutorado em Zootecnia, da Universidade Federal da Bahia, como requisito parcial para obtenção do título de Doutor em Zootecnia.

Área de Concentração: Produção Animal

Orientador: Prof. Dr. Rodrigo Fortes da Silva
Coorientador: Prof. Dr. Luiz Vítor Oliveira Vidal

SALVADOR – BA
FEVEREIRO – 2018

Sistema de Bibliotecas – SIBI/UFBA

M435 Mattos, Bruno Olivetti.

Sistemas de auto-alimentação para avaliar o consumo de nutrientes e ritmos diários da atividade alimentar em pirarucu (*Arapaima gigas*) / Bruno Olivetti Mattos.- Salvador, 2018.

117 p.

Orientador: Rodrigo Fortes-Silva.

Coorientador: Luiz Vítor Oliveira Vidal.

Tese (Doutorado - Zootecnia) -- Universidade Federal da Bahia, Escola de Medicina Veterinária e Zootecnia, 2018.

1. Animais - Alimentos. 2. Pirarucu (Peixe). 3. Animais - Comportamento. I. Silva, Rodrigo Fortes da. II. Universidade Federal da Bahia. Escola de Medicina Veterinária e Zootecnia. III. Título.

CDD: 597.5

CDU: 591.5

**SISTEMAS DE AUTO-ALIMENTAÇÃO PARA AVALIAR O
CONSUMO DE NUTRIENTES E RITMOS DIÁRIOS DA ATIVIDADE
ALIMENTAR EM PIRARUCU (*Arapaima gigas*)**

Bruno Olivetti de Mattos

Tese defendida e aprovada para obtenção do grau de
Doutor em Zootecnia

Salvador, 20 de fevereiro de 2018

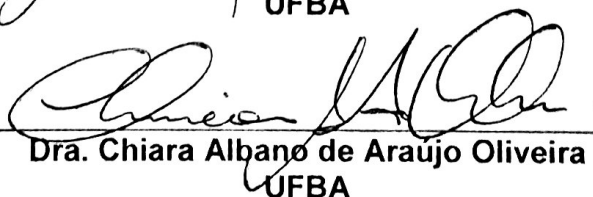
Comissão examinadora:



Dr. Luiz Vitor Oliveira Vidal
UFBA
Co-Orientador / Presidente



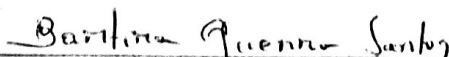
Dra. Manuela Silva Libânio Tosto
UFBA



Dra. Chiara Albano de Araujo Oliveira
UFBA



Dr. Ricardo Castelo Branco Albinati
UFBA



Dra. Bartira Guerra Santos
UFBA

BIBLIOGRAFIA DO AUTOR

BRUNO OLIVETTI DE MATTOS - filho de João Lúcio Pimenta de Mattos e Elizabete Antônia Olivetti de Mattos, nasceu em São Paulo, São Paulo, no dia 07 de junho de 1985. Em fevereiro de 1992, iniciou o ensino básico no Colégio Ibituruna, em Governador Valadares, MG. Em fevereiro de 1996, iniciou o ensino fundamental no Colégio Ibituruna, em Governador Valadares, MG. Em fevereiro de 2000, iniciou o ensino médio no Colégio Ibituruna, em Governador Valadares, MG. Em 05 de maio de 2003, ingressou no curso de graduação de Engenharia de Pesca da Universidade Estadual do Oeste do Paraná - UNIOESTE – Campus de Toledo, PR. No dia 09 de novembro de 2007, submeteu-se à banca para defesa do Trabalho de Conclusão de Curso, para obtenção do título de Bacharel em Engenharia de Pesca, Toledo, PR. Em 02 de março de 2009 iniciou o Mestrado em Zootecnia pela Universidade Federal de Lavras, MG. No dia 03 de novembro de 2010, submeteu-se a banca para a defesa da Dissertação de Mestrado em Zootecnia, Lavras, MG. Em 25 de Agosto de 2014 iniciou o Doutorado em Zootecnia pela Universidade Federal da Bahia, Salvador, BA. No dia 12 de julho de 2015, iniciou atividades do Programa Ciênicas sem Fronteiras na Espanha, na Universidad de Murcia, durante os 12 meses seguintes. Em 06 de setembro de 2017, submeteu-se a banca para a qualificação da Tese de Doutorado, Salvador, BA. No dia 20 de fevereiro de 2018, submeteu-se a banca para a defesa da Tese de Doutorado em Zootecnia, Salvador, BA.

EPÍGRAFE

Mesmo que o hoje te dê um não, lembre-se que há um amanhã melhor. A certeza de que os nossos caminhos devemos traçar ao lado de quem nos ama, com amor, paz, confiança e felicidade, é a base para se recomeçar.

Um recomeço, pra pensar no que fazer agora, acreditando em si mesmo, na busca do que será prioridade daqui pra frente.

Planos? Pra que os fizemos, já que o amanhã é mistério? A qualquer momento pode ser tempo, de revisar os conceitos e ações, e concluir, que tudo aquilo que você viveu marcou, porém não foi suficiente pra que continuasse.

As lembranças passadas ficam, tudo que vivemos era pra ser vivido.

O destino é como um livro do qual nós somos os autores, ele não vem pronto. Antes de nascermos ele está em branco. Ao nascermos introduzimos as primeiras passagens, um começo. Com o tempo, através das escolhas, vamos escrevendo-o página por página, rabiscadas, rasgadas ou marcadas, onde encontramos obstáculos, onde indicarão a melhor hora pra recomeçar, nos últimos dias de vida concluiremos, e no final deixamos nossas historias marcadas no coração daqueles, que sempre farão parte de nossa historia, onde quer que estejam.

Recomeçar é dar uma nova chance a si mesmo, é renovar as esperanças na vida e o mais importante, acreditar em você de novo.

A

meu pai João Lúcio Pimenta de Mattos
e minha mãe Elizabete Antônia Olivetti de Mattos
que foram o início de tudo e grandes
incentivadores.

A

meu irmão Vitor Olivetti de Mattos,
pelo estímulo que meu deu
pelos momentos que passamos juntos
e por ser especial na minha vida.

A

minha vovó Odila Vitrio Olivetti
que eu amo muito, minha grande companheira,
que sempre me deu forças para seguir em frente.

A

Stefanie Alvarenga Santos
que me incentivou para estar nesse momento,
me oportunizando a ilusão de que poderia alcançar esse objetivo.

DEDICO

AGRADECIMENTOS

À Deus pelo dom da vida.

À Universidade Federal da Bahia (UFBA) pela oportunidade oferecida de ingressar no Programa de Doutorado em Zootecnia.

À Universidade Federal do Recôncavo da Bahia (UFRB), pelo apoio e oportunidade de realizar a pesquisa.

À Universidade Federal de Lavras (UFLA), pelo apoio e oportunidade de realizar as análises centesimais das dietas experimentais.

À Universidad de Murcia (UMU) por proporcionar ensinamentos e conhecimentos durante minha estadia na Espanha.

Aos professores da Universidade Federal da Bahia (UFBA) do curso de Pós-Graduação em Zootecnia, pelos valiosos ensinamentos e oportunidade oferecidos.

Ao Prof. Dr. Rodrigo Fortes da Silva, pela dedicada orientação, ensinamentos, estímulo e amizade.

Ao Prof. Dr. Luiz Vítor Oliveira Vidal, pelo apoio, ensinamentos, amizade, conselhos e por compartilhar seus conhecimentos em aquicultura.

Ao Prof. Dr. Francisco Javier Sánchez-Vázquez, por ter recebido em sua Universidade e ter proporcionado novos conhecimentos e novas visões do que podemos aplicar na aquicultura brasileira.

A Prof. Dra. Maria Emília de Sousa Gomes Pimenta, pelo apoio, ensinamentos e amizade.

A Aquavale Piscicultura pela doação dos juvenis de pirarucu (*Arapaima gigas*) utilizados nos experimentos. Obrigado por todo o apoio.

A Poytara Alimentos pela doação de ingredientes utilizados na elaboração das dietas experimentais. Obrigado por todo o apoio.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, pela concessão da bolsa de estudos no Brasil e pela oportunidade de participar do Programa Ciências sem Fronteiras na Espanha, na Universidad de Murcia, proporcionando novos conhecimentos e por conceder bolsas de estudos nesse país, processo nº 99999.003219/2015-01.

A Fundação de Amparo à Pesquisa do Estado da Bahia – Fapesb, pelo financiamento do projeto de pesquisa, processo RED nº0004/2013 e PNE nº013/2014.

Aos amigos do AQUAUFRB, Adenize Silva, Alice Baião, Alison Eduardo, Bartira Guerra, Charlle Lima, Deise Sampaio, Denise Soledade, Jefferson Cartaxo, Jéssica Cerqueira, Mara Chane, Mariana Sampaio, Ricardo Uriel, Silvan Vianna e Yane Reis, pela amizade, apoio e demonstração de companheirismo.

Aos amigos do NEPESCA/UFLA, Marcos Rosa, Renata Fiorin, Elaine Costa Pereira e Karine Canestri Ribeiro e Constantina Torres Braga Maria, que muito contribuíram para a realização desta pesquisa.

Aos amigos da Universidad de Murcia, em especial Oli, Manolo, José Fernando, Andreina, Juan Fernando, Ariana, Viviana, Carol e Dani, que ajudaram em todos os momentos vividos na Espanha e por terem apoiado nos momentos difíceis. A Francisco Morales de Maya pela amizade e parceria no período que estive na Espanha, seu companheirismo foi fundamental para a estância na Espanha. Pela amizade que construímos, e por ser esse hermano espectacular, em que no momento mais difícil de minha vida, estava ao meu lado.

Aos funcionários da UFRB, Edmilson da Jardinagem, Edmilson da Água e Ronaldo da Manutenção, pela cordialidade e por terem disponibilizado todas as ferramentas necessárias e condições de realizarmos o trabalho na UFRB.

Aos amigos que sem eles nada disso seria possível, em especial a Aline dos Anjos Santos e Kayck Amaral Barreto, por nossa amizade e por serem fundamentais para a realização de todo o trabalho, que em todos os momentos de dificuldades e facilidades estavam presentes para auxiliar e desenvolver de forma eficiente a pesquisa.

À Renato Silva Leal, Guilherme Cleto de Carvalho e Jeicielle Pereira Macedo pela nossa eterna amizade e por ter ajudado nos momentos cruciais do trabalho e terem se mostrado grandes amigos em todas as ocasiões.

Ao grande amigo Baiano, Eduardo César Teixeira Nascimento Filho, que foi mais que um amigo, foi um irmão, sem sua ajuda nada disso teria acontecido, obrigado por tudo, cada minuto que sofremos na estufa valeu a pena, muito obrigado por tudo, sem você nada disso seria possível. Ao grande amigo Mineiro, Eric Marcio Balbino, que foi fundamental para que ficássemos em condições de trabalhar tranquilamente em Cruz das Almas, um grande amigo, sua amizade, seus conselhos, seus ensinamentos foram muito importantes para todo o sucesso deste trabalho. E aos grandes amigos sul-matogrossenses Camila Mendonça e André Nogueira, que estiveram ao meu lado no período de vivência no exterior, e foram fundamentais para uma estância tranquila e de muito aprendizado, o que culminou em uma amizade verdadeira e duradoura. Ainda, agradeço aos amigos Eduardo César Teixeira Nascimento Filho, Estela Maria Moreira Gregório Teixeira, Ricardo Uriel Pedrosa e Tamara Damasceno, que no momento mais difícil de minha vida, onde passei pelas maiores provas, eles foram mais que amigos, foram uma família, família esta que não tinha na Bahia, muito obrigado por tudo, um dia irei recompensar todo esse carinho, proteção e amor que tiveram comigo.

A meu pai e minha mãe, que saíram da casa onde vivem, deslocando de Minas para Bahia, largando suas rotinas e praticamente se dedicaram o tempo que estiveram na Bahia (90 dias) para levantarem uma estrutura que possibilitou o desenvolvimento desta pesquisa e também de outras que já foram feitas e outras que virão. Cada gota de sangue e suor valeu a pena, cada fio instalado, cada cano montado, cada buraco perfurado, tudo isso foi uma grande lição para todos nós, que basta querer e ter força de vontade e ser honesto que o trabalho é recompensado. Obrigado por tudo pai e mãe.

A todos que, direta ou indiretamente contribuíram para a realização deste trabalho e também por todas as pessoas que passaram em minha vida e fizeram com que o conhecimento adquirido ao longo dos anos, proporcionasse que eu chegasse neste momento.

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1. INTRODUÇÃO GERAL

As pesquisas de bem-estar, estão cada vez mais atuantes, e no caso de peixes se torna fundamental o conhecimento acerca de seu comportamento (Volpato et al., 2007). Com este conceito, têm-se buscado aprimorar o manejo alimentar, identificando na natureza o padrão que os animais desenvolvem e assim replicar estes conhecimentos para melhor alimentação (Alanärä et al., 2001) e saber as necessidades biológicas e metabólicas, porém, o comportamento alimentar é um conceito que se deve levar em conta (Brännäs e Strand, 2015), e é neste sentido, que se torna indispensável este entendimento, uma vez que, técnicas que buscam desenvolver o conhecimento alimentar podem proporcionar melhorias no manejo e no cultivo desses organismos (Fortes-Silva e Vieira-Rosa, 2012).

Dentre estas técnicas existentes, a auto-alimentação dos peixes configura-se como importante ferramenta neste processo, segundo Azzaydi et al., (1998), essa técnica, é a habilidade que os peixes possuem na assimilação do aprendizado de selecionar os alimentos. O sistema de auto alimentação, quando bem regulado, diminui o desperdício de alimento, além de fornecer o alimento no horário exato que o peixe deseja se alimentar, pois são os próprios peixes que acionam os alimentadores (Fortes-Silva e Vieira-Rosa, 2012).

Em estudos com diferentes espécies de peixes, foi possível observar a capacidade dos animais em ativar o alimentador de auto demanda e assim compor um ritmo específico de alimentação circadiano (Madrid et al., 2001), porém, em algumas estações do ano o ritmo alimentar pode variar (Bolliet et al, 2001). *Oncorhynchus masou masou* apresentou ritmo diruno com alguns picos nos períodos crepusculares (Flood, et al., 2011), *Dicentrarchus labrax* têm a capacidade de ritmo de alimentação diurna e noturna (Sánchez-Vázquez et al., 1994), *Tinca tinca*, *Oreochromis niloticus* e *Silurus glanis* apresentaram comportamento alimentar noturno (Herrero et al., 2005; Fortes-silva et al., 2010a; Boujard et al., 1995) e *Trachinotus carolinus*, goldfish e *Nothobranchius korthausae* são peixes com ritmo alimentar diurno (Heilman & Spieler, 1999; Sánchez-Vázquez et al., 1996; Lucas-Sánchez et al., 2011). Apesar de vários estudos demonstrarem a capacidade dos peixes em apresentarem ritmos circadianos de alimentação, ainda são escassos estudos com peixes tropicais de água

doce, como é o caso do Pirarucu (*Arapaima gigas*), que pouco se sabe sobre seu hábito alimentar e horários de alimentação.

Em relação ao comportamento alimentar dos peixes, este está diretamente relacionado com dois fatores, o ecológico e o comportamental (Volkoff e Peter, 2006), sendo que cada espécie ou grupo de peixe apresenta um hábito alimentar específico. De acordo com essa variabilidade de hábitos, os peixes são modelos experimentais de estudo para regulação do comportamento alimentar (Volkoff e Peter, 2006). Segundo Simpson e Raubenheimer (2001), os peixes desenvolveram capacidade de buscar de acordo com suas necessidades de ingestão, nutrientes específicos que possam compor sua dieta. Essa capacidade é certificada em estudos, em que peixes compuseram dietas nutricionalmente equilibradas e regularam a ingestão energética usando alimentadores de auto demanda (Sánchez-Vázquez et al., 1999), demonstrando a habilidade em selecionar dietas a partir de combinações de macronutrientes, obtendo assim os nutrientes necessários para seu metabolismo (Simpson e Raubenheimer, 2001).

Seleção de dietas para peixes foi pesquisado inicialmente utilizando alimentadores a demanda (Adron, 1973), oportunizando aos peixes selecionar macronutrientes específicos. Segundo Simpson e Raubenheimer (2001), esta é uma técnica importante para avaliar a preferência nutricional em peixes. Estudos com *Solea senegalensis* foi possível observar a capacidade de seleção de dietas contendo 68,00% de proteína (P), 16,30 % de carboidrato (C) e 15,70 % de lipídio (L) (Rubio, et al., 2009), já para a *Oncorhynchus mykiss* foi observado a seleção de 63,80% de P, 17,70% de C e 18,50% de L (Sánchez-Vázquez et al., 1999) e para o *Dicentrarchus labrax* foi constatado 55,00% de P, 23,00% de C e 22,00% de L (Rubio, et al., 2003), sendo essas espécies de peixes carnívoras. Quando trata-se de peixes onívoros, a seleção de dietas modifica seu perfil, segundo Fortes-Silva e Sánchez-Vázquez (2012) a espécie de peixe *Oreochromis niloticus* demonstrou seleção de 45,40% de P, 32,20% de C e 22,40% de L, enquanto o *Carassius auratus* selecionou 18,90% de P, 47,40% de C e 33,80% de L (Sánchez-Vázquez, et al., 1998a). Apesar de vários estudos demonstrarem a capacidade dos peixes em selecionar dietas, poucos avaliam os efeitos da amplitude das dietas na seleção, assim como são escassos estudos com peixes tropicais de água doce, como é o caso do Pirarucu (*Arapaima gigas*).

O Pirarucu é considerado carnívoro e um dos maiores exemplares de peixes de água doce do planeta e sua criação em cativeiro é cada vez mais explorada (Queiroz et al., 2002). Este peixe possui características específicas de crescimento podendo atingir peso superior a 10kg em 1 ano (Imbiriba, 2001). Apesar de possuir grande potencial para a aquicultura, há poucos estudos sobre a nutrição, o hábito, as preferências e os ritmos alimentar e locomotor desta espécie, sendo determinantes para o sucesso da criação deste peixe (Ono, et al., 2008).

O objetivo do presente trabalho é avaliar a capacidade do pirarucu (*Arapaima gigas*) em auto demandar rações a partir de alimentadores automáticos e determinar o ritmo diário alimentar e locomotor desta espécie. Ainda, avaliar não só a sua capacidade de regular o consumo de macronutrientes (meta nutricional), mas também a sua capacidade de defender essa meta diante dos desafios nutricionais (diluição e restrição protéica).

2. REVISÃO DE LITERATURA GERAL

2.1. Cronobiologia

Cronobiologia é a ciência que estuda a ritmicidade biológica em seres vivos e analisa o equilíbrio entre a ritmicidade endógena e a reação a estímulos ambientais. Tendo como objetivo, investigar os processos relacionados aos ritmos e suas relações com o ambiente, verificando os relógios biológicos e os responsáveis pelos padrões de ritmicidades dos organismos.

Segundo Lent (2005), de acordo com o ambiente, os relógios biológicos dos organismos podem ser influenciados pelas células sensoriais, fazendo com que haja sincronização com os ciclos naturais. Entretanto, esse conceito, demorou a ser aceito como é preconizado nos dias atuais, diversos autores, desde o século XVIII, buscavam relacionar o comportamento dos animais e plantas com o relógio biológico, como foi o caso do astrônomo Mairan, que observou diariamente os movimentos das folhas de Mimosa em ambiente totalmente ausente de luz e concluiu que apesar da total escuridão a folha apresentava movimentos, o que poderia ser justificado pelo relógio biológico (Daan, 1982).

Após inúmeros autores terem relatado suas experiências em favor dos relógios biológicos (Semon, 1905; Kleinhoonte, 1928; Brown, 1960; Brown, 1972), Pittendrigh (1981), comprovou que a ritmicidade advém de osciladores, demonstrando que as experiências anteriores estavam corretas sobre os relógios biológicos. Corroborando com Aschoff (1960), que naquela época, já tinha confirmado que os ritmos biológicos eram endógenos, ou seja, de funcionamento livre, independentemente do ambiente em que estão inseridos, mesmo este estando privado da exposição ambiental em laboratório. Assim, os ritmos sendo “geridos” pelos fatores endógenos, permite que os organismos tenham uma preparação fisiológica e comportamental para quaisquer eventualidade ambiental (Enright, 1970). Sendo esses fatores influenciados pelos osciladores, que sincronizam os organismos com os ciclos ambientais, proporcionando uma maior interação temporal ao ambiente (Moore-Ede et al., 1984).

Com o conceito determinado, inúmeras pesquisas voltadas para a medicina humana foram desenvolvidas, afim de compreender o ato de dormir e de despertar, assim como os padrões circadianos envolvidos nos processos fisiológicos e na

sensibilidade humana a medicamentos (Naylor, 2005). Entretanto, com o passar dos anos, buscou-se o entendimento do comportamento animal e das variáveis que poderiam afetar os ritmos biológicos. Nesse caso, dentre os animais de produção, estudos com peixes se tornou cada vez mais importantes, devido a gama de fatores envolvidos em seu ambiente e a importância de se conhecer o comportamento e os ritmos que impactam esses organismos.

2.1.1. Ritmos biológicos

Ritmos biológicos são acontecimentos biológicos que apresentam uma certa repetição em um determinado período de tempo, sendo em geral sincronizados com os ciclos da natureza (Pittendrigh, 1960). Assim, os ritmos biológicos podem ser classificados em: circadiano, lunar, mareal e anual (Halberg, 1959; Palmer, 1995; Naylor, 2002; Naylor, 2001).

2.1.1.1. Ritmos circadianos

O ritmo diário de luz e da escuridão desde o aparecimento da vida no planeta estabelece os ciclos sazonais de mudanças climáticas. A alternância do dia e da noite tem grande influência nas transformações adaptativas dos organismos, a diferença entre os ciclos biológicos e dos ciclos geofísicos caracteriza os ritmos circadianos (Cymborowski, 2010).

Ritmos circadianos são denominados dessa forma, pois, o tempo de duração desse ritmo ao longo de um dia, é aproximadamente 24 horas (Halberg, 1959). Ainda, segundo o mesmo autor, mesmo um ritmo diário estando ativo sem quaisquer influência ambiental, ou seja, o ritmo estando em curso livre, quem o controla é o oscilador do período intrínseco. Assim, as informações fornecidas sobre a hora do dia e também o tempo relacionado ao período do ano, podem ser transpassadas através de um hormônio, a melatonina, que também está envolvida na integração do fotoperíodo e coordenação das atividades rítmicas internas, importante no desempenho do sistema circadiano (Bayarri et al., 2004).

Os ritmos circadianos apresentam algumas propriedades, dentre as principais, destacam-se o fator endógeno de suas atividades, o método de sincronização com o

ambiente em que estão inseridos e a compensação nas variações de temperatura ambiental (Marques et al., 2003).

Ritmos circadianos estão presentes em diversos organismos vivos, uma vez que esse evento se torna essencial para desenvolver o comportamento das diversas espécies (Pittendrigh, 1960). Segundo Dunlap et al (2004), a ocorrência da geração desses ritmos está direcionada nas moléculas desses organismos, consistindo em alças de retroalimentação de transcrição e tradução gênica, formando um “relógio molecular”, apesar de que podem ocorrer algumas variações entre espécies.

Assim, de acordo com essas variações, os organismos podem apresentar diferentes padrões de comportamento, tanto locomotor como alimentar. Assim, como este estudo está direcionado a uma espécie específica de peixe, podemos descrever um breve detalhamento a cerca desse assunto em peixes. Alguns autores (Linner et al., 1990; Fraser & Metcalfe, 1997; Toguyeni et al., 1997; Bremset, 2000; Railsback et al., 2005; Flood et al., 2011) relataram o comportamento diurno de peixes, já autores como Fortes-Silva et al (2010a; 2010b) e Del Pozo et al (2011) observaram o padrão noturno. Ainda, Sánchez Vázquez-et al (1994), observaram uma variação deste comportamento, ocorrendo movimentações nos dois períodos.

Assim, podemos compreender que alguns fatores influenciam o comportamento de determinada espécie, como é o caso do marco-passo circadiano e o fotoperíodo, que geram automaticamente oscilações nas atividades dos peixes. Para as espécies diurnas os marca-passos circadianos estão sincronizados com a fase de iluminação do ciclo luz/escuro, onde a luz impulsiona o seu comportamento alimentar (Fortes-Silva e Vieira-Rosa, 2012).

2.1.1.2. Ritmos lunares

Os ritmos lunares, como o próprio nome já diz, está associado ao efeito da lua sobre os relógios endógenos dos organismos, sendo a movimentação da Lua em torno da Terra a principal ação existente para a ocorrência deste ciclo. Apesar de ter poucas informações a cerca deste ritmo, as alterações provocados por este, influencia a sincronização das atividade biológicas, como a locomoção, migração e reprodução (Hsiao e Meier, 1989;. Motohashi et al 2010).

Segundo Gliwicz (1986), em ambientes marinhos o ritmo lunar está associado as diversas condições de luz, temperatura e flutuações de correntes, muito por causa da atração magnética existente entre a Terra e as fases lunares.

A ação desse ritmo está relacionada com os diversos ciclos lunares existentes, dentre os quais se destacam o ciclo lunar, o ciclo semilunar e o ciclo das marés (este será detalhado em outro tópico). Assim, o ciclo lunar apresenta modificações contínuas em espaço de tempo de 30 dias em 30 dias, apresentando pico de atividades no momento que a fase lunar se completa. Já para o ciclo semilunar o espaçamento de tempo é em torno de 14,7 dias, apresentando até dois picos de atividades biológicas em um mês (Ikegami et al., 2009).

Assim, os ritmos lunares podem afetar o comportamento de várias espécies, de modo que estas podem sofrer influências no processo de migração, reprodução, alimentação, auto defesa, locomoção e demais atividades biológicas (Gliwicz 1986; Leatherland et al., 1992; Srisurichan et al., 2005; Lang et al., 2006). No caso da reprodução, o ritmo lunar por meio dos ciclos lunares influenciam diretamente a migração reprodutiva de algumas espécies de peixes, como é o caso da *Anguilla anguilla*, que inicia todo seu processo reprodutivo de acordo com o período lunar (Miyai et al., 2004). Segundo Lee et al (2002), garoupas desovam durante os picos do ciclo lunar, enquanto o coral *Plectropomus leopardus* libera seus gametas na fase de luna nova.

Já em ambientes de água doce, o brilho da lua pode afetar as atividades de diversos organismos que apresentam comportamento alimentar e locomotor noturno, de modo que essa sincronização irá depender das fases lunares (Neumann, 2013).

Alguns autores estudando a ingestão de alimentos para a *Salmo trutta*, *Oncorhynchus kisutch* e *Salvelinus alpinus*, durante os períodos lunares, observaram ritmicidade para esses peixes (Brown, 1946; Farbridge e Leatherland, 1987a, 1987b; Dabrowski et al., 1992). Apesar de estudos terem demonstrado que os peixes apresentaram comportamento alimentar nos períodos lunares e que na natureza os ritmos que estão relacionados com as fases lunares são os migratórios e os reprodutivos, os sinais relacionados para essa sincronização ainda não estão confirmados e são desconhecidos, necessitando uma maior investigação acerca desse assunto (López-Olmeda e Sánchez-Vásquez, 2010).

2.1.1.3. Rítmos mareais

Os ritmos mareais estão diretamente relacionados com os ritmos lunares, apresentando um ciclo mareal diário, sendo direcionados pelas forças gravitacionais exercidas pela lua, pelo sol e pela rotação e da rotação da Terra (Leatherland et al., 1992).

A ação deste ritmo nos organismos são de difícil mensuração, uma vez que, além das forças atuantes, diversas variáveis são imputadas, como as correntes oceânicas (direção e extensão), a profundidade das bacias oceânicas, o terreno topográfico e todo o sistema oceânico envolvido (Neumann, 2013). Segundo o mesmo autor, ainda, ao longo do planeta, cada costa oceânica apresenta diferentes formas e formatos, proporcionando diferentes ações nos organismos. Apesar das dificuldades encontradas para padronizar e basear os estudos a cerca destas variáveis atuantes, foi criado por meio da análise de Fourier, um conjunto de 100 componentes para prever qualquer situação de maré nas diferentes costas oceânicas existentes, normatizando dessa maneira todo o estudo envolvido nesta temática.

Dessa maneira, foi possível classificar os ciclos de mares atuantes (Defant, 1961; Barnwell, 1976):

- maré semi-diurnas: o período de duração dessas marés são metade de um dia corrente, com dois pontos de alta e dois pontos de baixa.
- maré diurna: tem um período ou ciclo de aproximadamente um dia, com um ponto de alta e um de baixa durante um dia corrente;
- maré mista: caracterizada por uma desigualdade diurna evidente com pontos de alta superiores ao pontes de baixa, e também ao contrário. Estes termos são definidos em pontos de referência de maré. Além disso, uma maré mista pode ser pensado como uma maré de transição que ocorre entre as áreas de marés semi-diurnas e diurnas.

Conhecimento dos padrões de movimento e das atividade de uma espécie oriuda das costas oceânicas podem fornecer informações úteis sobre a utilização desses recursos, requerimentos do habitat, interações com possíveis predadores,

competição alimentar, sistema locomotor e hábito alimentar (Turchin, 1998; Pittman e McAlpine, 2001).

Estudos sobre o comportamento de peixes considerando os ritmos mareais, mostram que o nível de atividade desses organismos tendem a oscilar com o ciclo das marés, sendo menor na maré baixa (Gibson 1967). Estudos com caranguejo da espécie *Carcinus maenas* demonstrou ritmicidade no período em que a maré estava alta (Naylor, 1963; McGaw e Naylor, 1992; Styrihave et al., 2003). Alguns estudos referentes a migrações desta mesma espécie de caranguejo, demonstrou migração vertical nos momentos de subida e descida das marés (Rewitz et al, 2004).

Embora os estudos sobre o ritmo de maré demonstram que em condições de laboratório constantes, os organismos mantêm ritmicidade, ainda assim, esses estudos fornecem pouca visibilidade ou até confiabilidade, pois no ambiente natural, os estímulos ambientais são inúmeros e são eficientes para sincronizar as atividades e/ou modificar o comportamento por meio dos ciclos das máres (Faria e Almeida, 2008). Ainda assim, os ritmos mareais podem ser de cunho endógeno, pois, persistem por períodos de tempo variáveis nas condições laboratoriais constantes (Gibson, 1982).

2.1.1.4. Ritmos anuais

Os ritmos anuais são descritos como eventos que apresentam duração de aproximadamente um ano, quando este não é afetado por questões ambientais (Halberg et al, 1965). Sendo os sinais reguladores dos organismos (Zeitgeber) influenciados pelo fotoperíodo, comprimento do dia (Nelson et al., 2010; Foster e Kreitzman, 2009; Johnsson, 2008; Bradshaw e Holzapfel, 2007), temperatura, chuva e/ou disponibilidade de alimento, sendo que cada espécie e ambiente apresenta variações diferentes (Bradshaw e Holzapfel, 2010; Immelmann, 1971). Na natureza têm-se o exemplo de ritmos anuais a temporada de hibernação de animais, período reprodutivo, migração de aves e o tempo de floração de determinadas plantas (Lincoln et al., 2006; Gwinner, 1996,1986; Pengelley, 1974).

Para avaliar o comportamento anual dos organismos é necessário realizar experimentos de longa duração ao longo de vários anos, para que dessa forma se tenha um padrão de atividade considerado anual, como é o caso de trabalhos de padrões de

germinação de sementes secas (Bünning, 1951) e da hibernação a eclosão de insetos (Gwinner, 1986).

Em peixes a relação dos ritmos anuais também se faz presente, pois, como esses organismos são pecilotérmicos, a ingestão de alimentos está diretamente relacionada com as variações sazonais que ocorre no corpo hídrico, uma vez que dependendo dessa variação, que também pode ocorrer com a temperatura e o comprimento do período luminoso do dia, seu metabolismo pode ser afetado, influenciando no processo alimentar (López-Olmeda e Sánchez-Vásquez, 2010). Autores como Komourdjian et al (1976) e Higgins e Talbot (1985), observaram que no período da primavera em que o dia está mais longo, os peixes se alimentam com maior intensidade em relação ao período outono, em que os dias estão mais curtos.

Variações sazonais ao longo do ano são fatores que influenciam o comportamento alimentar dos peixes, como observado por Müller (1978) e Jørgensen e Jobling (1989; 1990), para as espécies de peixe *Lota lota*, *Cottus carolinae* e *Salvelinus alpinus*. Segundo Eriksson (1978), essas variações apresentam dois padrões, como o comportamento crepuscular (peixe se alimenta no anoitecer e no amanhecer) e o comportamento bifásico (inversão do seu sistema circadiano).

Muitos estudos relatam esses tipos de comportamento, porém, não se tem conhecimento dos mecanismos biológicos que ativam e proporciona este tipo de conduta. Entretanto, em peixes do ártico, submetidos a condições extremas as alternâncias da condução alimentar se dá devido grandes variações do fotoperíodo (López-Olmeda e Sánchez-Vásquez, 2010; Butler et al., 2010; Paul et al., 2008; Bradshaw e Holzapfel, 2007). Já peixes de clima temperado, como o *Dicentrarchus labrax*, em que se tem conhecimento de sua alternância alimentar, exibindo padrão diurno na primavera ao outono e noturno no inverno (Sánchez-Vásquez et al., 1998b), os fatores bióticos como disponibilidade de alimentos, juntamente com outros fatores abióticos, podem ocasionar essas variações comportamentais.

2.2. Comportamento alimentar em peixes

O comportamento dos peixes é regido inicialmente por suas demandas diárias, como a busca por alimentos (Adams et al., 1988; Hara, 1993; Ferreira et al. 2004), a procriação da espécie com diferentes estratégias (Jamieson, 1991; Vazzoler, 1996;

Pecio et al., 2007; Azevedo et al., 2010) e a sobrevivência (Gerking, 1994; Maher & Lott 1995; Ferreira et al. 1998). Esse comportamento é relacionado da mesma forma que para os outros animais, ou seja, o peixe apesar de estar em ambiente aquático, em nada difere da sua essência comportamental animal (Sabino, 1999). Como o foco principal deste estudo é a questão alimentar, iremos neste item descrever sobre tal comportamento.

Assim sendo, a questão alimentar é de grande importância para os peixes, pois relaciona-se com todas atividades e desenvolvimento desses organismos (Dill, 1983; Wootton, 1999). Segundo Volkoff e Peter (2006), a ingestão dos alimentos, os hábitos alimentares, os mecanismos de detecção de alimentos, a preferência alimentar e a frequência de alimentação são fatores que estão intimamente correlacionados com o complexo comportamento alimentar. Dessa maneira, temos que, para a busca por alimentos os peixes desenvolveram três sistemas de conhecimento alimentar, sendo estes, o aprendizado a curto prazo, a memória intermediária através de efeitos parental e a memória ancestral que engloba o efeito genético no fenótipo (Fortes-Silva et al., 2016).

A memória alimentar, segundo Fortes-Silva et al. (2016), advém da carga genética dos organismos, por meio de uma carga gerada e de expectativas, por exemplo, sobre os tipos de alimentos que podem ser encontrados. Porém, esse conhecimento alimentar segundo Forbes (2001), potencializa a memorização de experiências positivas e/ou negativas, proporcionando aos organismos a associação de uma percepção alimentar de acordo com suas necessidades intrínsecas. Essas experiências permitem aos organismos avaliarem o quão um alimento satisfaz ou não as demandas nutricionais, proporcionando a estes a escolha por determinados alimentos, o que influi diretamente em sua alimentação (Fortes-Silva et al., 2016).

Segundo Simpson e Raubenheimer (1996) e Berthoud e Seeley (2000), existem três tipos de aprendizados que os organismos podem associar a consequências nutricionais: a aprendizagem de associações positivas, a aprendizagem de aversões e respostas não associativas. Assim, o conhecimento adquirido pode influenciar nas estratégias de buscas por alimentos, como relatado por Dill (1983), que infere que o estado nutricional dos organismos pode influenciar no consumo de determinados itens

alimentares, fazendo com que a busca por alimento não seja por necessidades mas sim por oportunidades, mesmo que altere o gasto energético.

Dessa forma, o meio oportuniza aos organismos aquáticos diversas conformações alimentares, seja pela oscilação dos recursos, seja pelas alterações ambientais espaciais. Assim sendo, os organismos aquáticos podem alterar seu comportamento alimentar, ou seja, o ambiente favorece a determinadas espécies a plasticidade trófica (Abelha et al., 2001), como descrito por Hahn et al. (1997), em que a espécie de peixe *Plagioscion squamosissimus* alterou os itens de sua dieta de acordo com a disponibilidade alimentar no ambiente, da mesma forma que Goulding (1980), que relatou esse tipo de comportamento para as espécies de peixes redondas amazônicas (*Colossoma macropomum*, *Mylossoma* spp. e *Myleus* spp.) em determinadas épocas do ano. Contudo, existem espécies que preferem manter seu padrão nutricional ao ter que alterar a estratégia alimentar (Bartumeus et al., 2005). Dessa maneira, nessas situações, o comportamento dos peixes está diretamente relacionado com a capacidade de assimilar e compreender as variações ambientais dos itens alimentares, podendo ser benéfico ou não para determinadas espécies, dependendo do hábito e comportamento alimentário (Holt e Johnston 2011).

Diante deste cenário, Gerking (1994) sugeriu a seguinte classificação alimentar aos peixes: generalistas que não apresentam preferência alimentar (*Oligosarcus jenynsii* - Nunes & Hartz, 2006; *Trachelyopterus lucenai* - Moresco e Bemvenuti, 2005; *Plagioscion squamosissimus* - Hahn et al., 1997), especialistas que detêm uma dieta restrita com adaptações morfológicas tróficas (*Schizodon nasutus*, *Schizodon intermedius*, *Steindachnerina insculpta* - Bennemann et al., 2000; *Bryconamericus stramineus* - Casatti e Castro, 1998; *Bryconops giacopinii* - Sabino e Zuanon, 1998) e oportunistas que se alimentam de itens fora de seu leque nutricional e/ou de itens em abundância (*Bryconamericus microcephalus* - Rezende e Mazzoni, 2003; e *Deuterodon* sp. - Mazzoni & Rezende, 2003; *Astyanax rivularis* - Casatti e Castro, 1998; *Deuterodon langei* e *Astyanax* spp. - Aranha et al., 1998; *Knodus moenkhausii* - Ceneviva-Bastos e Casatti, 2007). Entretanto, essa classificação pode ser alterada devido a plasticidade trófica das espécies e as variações ambientais (Abelha et al., 2001; Vazzoler, 1996; Keenleyside, 1979), porém, esta forma de categorização dos peixes se torna importante para o estudo nutricional, uma vez que, a partir deste ponto,

pode-se desenvolver pesquisas e monitoramento de seu comportamento alimentar, proporcionando melhores indicadores para a nutrição dos peixes e/ou práticas de preservação ambiental em diferentes corpos hídricos, favorecendo políticas públicas visando a melhoria nos índices produtivos e/ou conservação ambiental.

2.3. Aprendizagem dos peixes em auto-selecionar dietas

Como relatado anteriormente, o comportamento alimentar está baseado em um tripé (Fortes-Silva et al., 2016). Porém, nesse tópico será abordado com maior grau de importância a aprendizagem e o mecanismo para auto-seleção de dietas. Apesar da importância de outros temas, como memória parental e memória ancestral, neste referido trabalho, o aprendizado dos peixes em auto-selecionar dietas foi realizado de modo efetivo, afim de elucidar o comportamento alimentar do pirarucu.

Os peixes advindos da aquicultura exibem uma ampla variedade de comportamento alimentar em comparação com os animais terrestres. Os peixes são considerados bons modelos experimentais para estudar a regulação da ingestão de nutrientes (Volkoff e Peter, 2006) e possuem considerável plasticidade para ritmos de alimentação (Sánchez-Vázquez et al., 1996). De acordo com Simpson e Raubenheimer, (2001), os peixes são capazes de regular a ingestão de nutrientes e defender um alvo nutricional.

Assim sendo, segundo os autores Fortes-Silva et al (2016), a aprendizagem em auto-selecionar dietas por meio da busca e do auto-conhecimento, faz com que os peixes possam avaliar se determinados alimentos satisfazem ou não suas necessidades nutricionais, promovendo um conhecimento apurado.

Essa capacidade baseia-se na "sabedoria nutricional" observado em estudos realizados com diferentes espécies de peixes carnívoros, como *Solea senegalensis* (Rubio et al., 2009), *Oncorhynchus mykiss* (Sánchez-Vázquez et al., 1999) e *Dicentrarchus labrax* (Rubio; Sánchez-Vázquez; Madrid, 2003a) e peixes onívoros como *Oreochromis niloticus* (Fortes-Silva e Sánchez-Vázquez, 2012) e *Carassius auratus* (Sánchez-Vázquez et al., 1998a). A auto-seleção de nutrientes poderia ser usada para otimizar a composição de dietas dos peixes de cultivo (Brännäs e Strand, 2015), e permitir o desenvolvimento de programas mais eficientes de alimentação (Kitagawa et al., 2015). Além disso, os estudos que contemplam os peixes como guia

para projetar dietas, podem fornecer dados sobre mecanismos de regulação da ingestão de nutrientes (Fortes-Silva; Martínez; Sánchez-Vázquez, 2011; Fortes-Silva; Sánchez-Vázquez, 2012), e também sobre o bem-estar dos peixes de acordo com opções de seleção de alimentos livres (Kulczykowska; Sánchez Vázquez, 2010; Volpato; Gonçalves-De-Freitas; Fernandes-De-Castilho, 2007).

Corroborando com este pensamento, Simpson e Raubenheimer (1996) e Berthoud e Seeley (2000), relataram três sistemas de aprendizado:

- aprendizagem de associações positivas;
- aprendizagem de aversões;
- respostas não associativas

Aliado a esse processo, o conhecimento alimentar dos peixes pode ser um ponto importante na aprendizagem, uma vez que a busca pelo alimento se torna mais específica ao invés de ser aleatória (Viswanathan et al 1999; Bartumeus et al., 2005). Assim sendo, alguns mecanismos como o alimentador a demanda, se tornaram ferramentas importantes para a auto-seleção de dietas e conseqüentemente favoreceram a técnica de aprendizado aos peixes, de modo que investigações a cerca do comportamento alimentar de peixes se tornaram viáveis.

De acordo com Simpson e Raubenheimer (2001), devido ao desenvolvimento do alimentador a demanda, foi possível que peixes selecionassem dietas de sua preferência alimentar por meio do aprendizado nutricional e a ação de demanda, por meio da escolha. Assim sendo, esta metodologia se tornou uma maneira de facilitar o entendimento a cerca do aprendizado dos peixes e ainda proporcionou avaliações do comportamento alimentar em peixes.

Dessa maneira, o sistema de alimentação por alimentadores de auto demanda configurou-se como um importante instrumento para estudos dos ritmos de alimentação (Azzaydi et al., 1998). Este sistema permite ainda, diminuir o desperdício de alimento, além de fornecer a dieta no horário exato que o peixe deseja se alimentar (Fortes-Silva e Vieira-Rosa, 2012). Vários dispositivos de alimentação foram desenvolvidos e evoluindo com o tempo, como o sensor de toque (Adron et al, 1973; Landless, 1976; Grove et al., 1978), uma roda de catraca com pequenos furos que contém pastilhas alimentares individuais (Takahashi et al., 1981), a rotação de um disco com buracos perfurados em torno da periferia e operados por um motor síncrono

(Beach et al., 1986), sensor elétrico (Boujard et al., 1992), sensor de estiramento (Sánchez-Vázquez, et al., 1994) e sensor de presença – fotocélula com luz infravermelha (Kitagawa et al., 2015). Esses dispositivos são acoplados a um microcomputador que permite a gravação contínua do comportamento alimentar e o horário de preferência de alimentação dos peixes. Desse modo, o desenvolvimento de sistemas de auto-alimentação é um importante instrumento para avaliações do comportamento alimentar de peixes (Cho, 1992; Fortes-Silva et al., 2011).

Diversos autores trabalharam com esse sistema afim de buscar o a padrão de seleção de dietas de espécies de importância produtiva, como foi o caso de:

- *Carassius auratus* (Sánchez-Vázquez et al., 1998a);
- *Dicentrarchus labrax* (Aranda et al., 2000);
- *Dicentrarchus labrax* (Rubio et al., 2003);
- *Dicentrarchus labrax* (Vivas et al., 2003);
- *Diplodus puntazzo* (Vivas et al., 2006);
- *Oncorhynchus masou masou* (Flood et al., 2011);
- *Oncorhynchus mykiss* (Railsback et al., 2005);
- *Oncorhynchus mykiss* (Sánchez-Vázquez et al., 1999);
- *Oreochromis niloticus* (Fortes-Silva e Sánchez-Vázquez, 2012);
- *Salmo salar* (Fraser & Metcalfe, 1997, Bremset, 2000);
- *Salmo trutta* (Bremset, 2000);
- *Salvelinus alpinus* (Linnér et al., 1990);
- *Solea senegalensis* (Rubio et. al., 2009);
- *Sparus aurata* (Montoya et al., 2012).

2.4. Alimentadores de auto-demanda “self-feeders” e suas implicações

As pesquisas com o uso de alimentadores a demanda estão cada vez mais atuantes (Attia et al., 2012; Davie e Kopf, 2006; EFSA, 2009; Huntingford, 2006; Oldfield, 2011; Sloman et al., 2011), e no caso de peixes se torna fundamental o conhecimento acerca de seu comportamento utilizando esta técnica (Bayarri et al., 2004; Del Pozo et al., 2012; Faria e Almada, 2008; Farner, 1985; Helm et al., 2013; Hurd et al., 1998; Kumar Baghel et al., 2015; Noble et al., 2007; Vera et al., 2009,

2009), dentre estes o alimentar (Fast et al., 1997; Herrero et al., 2005; Mattos et al., 2016a, 2016b; Navarro-Guillén et al., 2017; Sánchez-Vázquez et al., 1996; Sanchez-Vazquez e Tabata, 1998), de modo que testes de preferência de alimentação se tornam importantes elos do conhecimento para entendimento desses animais (Ashley, 2007; Attia et al., 2012; Fortes-Silva et al., 2016; Huntingford, 2006; Poli et al., 2005; Volpato et al., 2007).

Com este conceito, têm-se buscado aprimorar o manejo alimentar, identificando na natureza o padrão que os animais desenvolvem, replicando esses conhecimentos para a melhor alimentação e investigando as necessidades biológicas e metabólicas (Arjona et al., 2009; Costas et al., 2011; Gaye-Siessegger et al., 2007; Li et al., 2017; Navarro-Guillén et al., 2017; Paredes et al., 2014; Polakof et al., 2012). Assim, o comportamento alimentar é um conceito que se deve levar em conta (Brännäs e Strand, 2015; Carlberg et al., 2015; Kentouri, 1999; Luz et al., 2017; Twibell et al., 2012), e é neste sentido, que se torna indispensável este entendimento, uma vez que, técnicas que buscam desenvolver o conhecimento alimentar podem proporcionar melhorias no manejo e no cultivo desses organismos, como é o caso da auto-alimentação, que configura-se uma importante ferramenta neste processo, pois, é a habilidade que os peixes possuem na assimilação do aprendizado de selecionar os alimentos (Almaida-Pagán et al., 2006; Flood et al., 2010, 2011; Fortes-Silva et al., 2011; Mattos et al., 2016b; Navarro et al., 2009; Rubio et al., 2004; Rubio et al., 2003a; Santos et al., 2016).

Portanto, o sistema de auto alimentação tornou-se uma ferramenta útil para avaliar os ritmos alimentares de peixes e preferências alimentares (Azzaydi et al., 1998; Montoya et al., 2012). Diferentes autores têm desenvolvido vários dispositivos acoplados a computadores para tais pesquisas, usando sensores eletrônicos (Boujard et al., 1992), sensores de estiramento (Sánchez-Vázquez et al., 1994) e fotocélulas infravermelho para detectar demandas alimentares (Kitagawa et al., 2015). Ao longo dos anos, esses sistemas têm auxiliado a desenvolver protocolos de alimentação com design eficaz, evitando o desperdício de ração e melhorando a utilização dos alimentos pelos peixes (Cho, 1992; Fortes-Silva et al., 2016).

Dessa forma, essa técnica permite aos peixes, regular e diminuir o desperdício de ração, pois fornece alimento no horário exato que o peixe deseja se alimentar,

efetivando o consumo e os índices produtivos, pois com o maior número de alimentações ao longo do dia, os índices de digestibilidade dos nutrientes são otimizados, influenciado de modo positivo a eficiência dos nutrientes no metabolismo animal (Flood et al., 2010, 2011; Fortes-Silva e Sánchez-Vázquez, 2012; Heilman e Spieler, 1999; Mattos et al., 2016b; Montoya et al., 2012; Navarro et al., 2009; Paspatis e Boujard, 1996; Rubio et al., 2004; Shi et al., 2016; Yamamoto et al., 2002).

Portanto, em estudos com diferentes espécies de peixes, foi possível observar a capacidade dos animais em ativar o alimentador de auto demanda e assim compor um ritmo específico de alimentação (Aranda et al., 2000; Biswas et al., 2016; Cuenca e De La Higuera, 1994; Fast et al., 1997; Madrid et al., 2001; Mattos et al., 2016a; Sánchez-Vázquez et al., 1994, 1998a), porém, em algumas estações do ano o ritmo alimentar pode variar (Bolliet et al., 2001; Boujard e Leatherland, 1992; Kulczykowska, 2010; Reeb, 2002; Volkoff et al., 2010). *Oncorhynchus masou masou* apresentou ritmo diurno com alguns picos nos períodos crepusculares (Flood et al., 2011), já *Dicentrarchus labrax* apresentou ritmo de alimentação diurno e noturno (Sánchez-Vázquez et al., 1998b; Sanchez-Vázquez et al., 1995), *Tinca tinca*, *Oreochromis niloticus* e *Silurus glanis* apresentaram comportamento alimentar noturno (Boujard, 1995; Fortes-Silva et al., 2010; Herrero et al., 2005) e *Trachinotus carolinus*, *Arapaima gigas*, *Carassius auratus* e *Nothobranchius korthausae* são peixes com ritmo alimentar diurno (Heilman e Spieler, 1999; Lucas-Sánchez et al., 2011; Mattos et al., 2016a; Sánchez-Vázquez et al., 1996).

Em relação ao comportamento alimentar dos peixes, este está diretamente relacionado a três fatores, o ecológico, o comportamental e a regulação endócrina (Mackenzie et al., 1998; Volkoff et al., 2010; Volkoff e Peter, 2006), sendo que cada espécie ou grupo de peixe apresenta um hábito alimentar específico. Segundo os mesmos autores, de acordo com essa variabilidade de hábitos, os peixes são modelos experimentais de estudo para regulação do comportamento alimentar. Os peixes desenvolveram capacidade de buscar de acordo com suas necessidades de ingestão, nutrientes específicos que possam compor sua dieta (Raubenheimer e Simpson, 1999; Simpson et al., 2004; Simpson e Raubenheimer, 2001). Essa capacidade é certificada em estudos, já citados nesta revisão, em que peixes compõem dietas nutricionalmente equilibradas e regulam a ingestão energética usando alimentadores de auto demanda,

demonstrando a habilidade em selecionar dietas a partir de combinações de macronutrientes, obtendo assim os nutrientes necessários para seu desenvolvimento (Raubenheimer e Simpson, 1999; Simpson et al., 2004; Simpson e Raubenheimer, 2001).

Assim sendo, a seleção de dietas foi pesquisado inicialmente utilizando alimentadores a demanda (Adron et al., 1973), oportunizando aos peixes selecionarem macronutrientes específicos. Adaptações ao longo dos anos foram necessárias, para possibilitar melhor adequação do método as diferentes espécies de peixes e também para peixes de menor tamanho, que são incapazes de acionar os alimentadores. Ainda, a popularização do uso de computadores nos anos oitenta e noventa, permitiu que novos sistemas fossem desenvolvidos para registrar com precisão a atividade de demanda alimentar dos peixes, assegurando um monitoramento da preferência e comportamento alimentar eficiente (Cuenca e De La Higuera, 1994; Sánchez-Vázquez et al., 1994).

De acordo com Simpson e Raubenheimer, (2001), a técnica de auto-alimentação é uma prática bem sucedida e aplicável aos estudos de seleção de dietas, sendo uma estratégia importante para investigação da preferência nutricional em peixes, na qual se baseiam na proposição de Richter, (1922), denominada “sabedoria nutricional”. Essa proposição esta relacionada ao ato dos animais selecionarem dietas que atendam as necessidades nutricionais e que não seja um fenômeno aleatório, mantendo o equilíbrio metabólico do animal. Assim, a escolha de uma dieta está relacionada aos efeitos benéficos que esta pode promover, fazendo com que a seleção seja determinada pelo *status* metabólico.

Por meio dessa estratégia, realizaram-se estudos investigando em muitas espécies de peixes a habilidade em selecionar dietas, em função de seus hábitos alimentares. Assim, diversas pesquisas foram realizadas utilizando esta técnica, como estudos com o *Solea senegalensis*, que foi possível observar a capacidade de seleção de dietas deste peixe, contendo 68,00% de proteína (P), 16,30 % de carboidrato (C) e 15,70 % de lipídio (L) (Rubio et al., 2009), já para a *Oncorhynchus mykiss* foi observado a seleção de 63,80% de P, 17,70% de C e 18,50% de L (Sánchez-Vázquez et al., 1999) e para o *Dicentrarchus labrax* foi constatado 55,00% de P, 23,00% de C e 22,00% de L (Rubio et al., 2003b), sendo essas espécies de peixes carnívoras. Quando

pesquisado o comportamento alimentar de espécies onívoras, a seleção das dietas pelos peixes, apresenta alvo nutricional diferente em relação aos carnívoros, como é o caso da *Oreochromis niloticus*, que demonstrou selecionar 45,40% de P, 32,20% de C e 22,40% de L (Fortes-Silva e Sánchez-Vázquez, 2012), e o *Carassius auratus*, que selecionou 18,90% de P, 47,40% de C e 33,80% de L (Sánchez-Vázquez et al., 1998a).

Diante de todos os benefícios descritos no uso do sistema de auto-alimentação, nota-se sua importância na nutrição de peixes. Contudo, na utilização deste sistema, pode-se verificar algumas desvantagens, como o custo elevado para a instalação, a participação de mão de obra especializada para condução dos trabalhos, a grande variedade de espécies de peixe que podem comprometer o uso de determinados alimentadores, devido os diferentes hábitos alimentares, promovendo reestruturação dos sistemas, ainda, podemos citar o uso desta metodologia para peixes de menor porte, pois este não apresentam capacidade de acionamento de determinados alimentadores, fazendo com que seja estabelecida uma outra forma de condução dos trabalhos, o que pode acarretar em maiores custos e provável redução na eficiência, e por fim, o treinamento dos peixes que pode comprometer o funcionamento, caso não seja realizado de maneira correta.

2.5. Pirarucu (*Arapaima gigas*)

A espécie de peixe *Arapaima gigas*, comumente chamada de Pirarucu, pertence a família Arapaimatidae e a ordem Osteoglossiforme (Ferraris, 2003). Sendo esta ordem dos peixes actinoptérgeos, que são peixes com nadadeiras suportadas por lepidotríquias, esqueleto interno tipicamente calcificado e aberturas branquiais protegidas por um opérculo ósseo. Essa ordem apresenta apenas três espécies de peixes, na qual todas estão presentes na América do Sul, mais especificamente na Bacia Amazônica (Imbiriba et al., 1996; Rebaza et al., 1999) e são caracterizadas pela língua óssea e áspera, com escamas grandes e grossas e fortemente afixadas como forma de mosaico (Venturieri e Bernardino, 1999; Ferraris, 2003). No Brasil, o pirarucu distribui-se em toda a Bacia Amazônica e também na Bacia Tocantins-Araguaia (Imbiriba et al., 1996).

O pirarucu é considerado o maior peixe de escama de água doce do Brasil. Apresenta corpo em formato cilíndrico, cabeça achatada e mandíbulas em evidência, seus olhos são amarelados e de pupila azulada, sua coloração é marrom-esverdeada, escura no dorso e avermelhada na porção final, sendo a intensidade variável de acordo com o período reprodutivo (Borghetti e Da Silva, 2008).

A respiração é um fator de importância para esse peixe, pois, caracteriza-se por possuir dois aparelhos respiratórios, as brânquias que realizam a respiração aquática e a bexiga natatória, que atua como pulmão, qualificando assim a respiração “obrigatória” desta espécie (Bard e Imbiriba, 1986; Romero, 1960), uma vez que a bexiga natatória é modificada e opera como um sistema de estoque de oxigênio, apresentando uma estrutura tipo “pulmão” (Soares et al., 2006), fazendo com que o peixe busque na superfície do corpo hídrico oxigênio a cada 10-20 minutos (Imbiriba, 2001; Lüling, 1964). Essa estrutura é caracterizada por uma câmara única subdividida por septos em pequenas câmaras cujas paredes são altamente vascularizadas, posicionadas na região do dorso acima da cavidade abdominal (Val e Almeida-Val, 1995). Outros autores como Pontes (1977), afirma que essa modificação apresentada na bexiga natatória é pelo fato de que as paredes internas desenvolveram um importante tecido vascularizado, proporcionando o aumento da superfície para a troca gasosa entre o oxigênio e o sangue circulante, conforme ocorre nos pulmões por meio das capilaridades.

Essa situação o condiciona para a criação em cativeiro, sendo possível seu cultivo em quaisquer condições, mesmo em ambientes com déficit de oxigênio (Fontenele, 1948; Souza e Val, 1990) e em elevadas densidades de estocagem, uma vez que a qualidade de água não se torna um impedimento, pois, a busca pelo oxigênio se dá pelo ar atmosférico e não em trocas gasosas com o meio aquático (Cavero et al., 2003; Ono et al., 2004).

Apesar dessas características favoráveis, é recomendável que a qualidade de água esteja controlada e dentro dos padrões recomendados (Sipaúba-Tavares, 1995; Proença e Bittencourt, 1994), pois a produção dos resíduos nitrogenados podem impactar de forma negativa a criação (mesmo que esta espécie de peixe tolere níveis elevados destes parâmetros), uma vez que ambientes com altos níveis de compostos nitrogenados, principalmente a amônia, comprometem o desempenho produtivo

(Esteves, 2011; Albanez e Matos, 2007; Vinatea- Arana, 2004), pelo fato de desaminar proteínas e quebrar o processo oxidativo para a formação de ATP (Parker e Davis, 1981), assim como o nitrito que oxida a hemoglobina em metahemoglobina, fazendo com que o organismo seja incapaz de transportar oxigênio (Albanez e Matos, 2007; Vinatea-Arana, 2004).

Na natureza, essa característica (respiração aérea) o torna suscetível a captura, sendo a pesca predatória com arpão um risco para a espécie (Val e Honczaryk, 1995). Para reduzir essa exploração, o Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), adotou medidas de manejo e conservação, estabelecendo tamanho mínimo de captura e captura zero no período do defeso reprodutivo.

Como relatado acima, o pirarucu apresenta grande potencial produtivo, sendo que esse potencial se dá além de sua característica respiratória, como também pela rusticidade, aceitação de ração extrusada (Crescêncio, 2005), resistência ao manejo, fácil adaptação às condições de cultivo, rápido crescimento, podendo atingir 10 a 12 kg em um ano de cultivo (Ono, et al., 2004) e ainda, possui excelente qualidade de carne, o que proporciona ótima aceitação no mercado, permitindo alcançar ótimos preços de venda. Alguns autores como Pereira-Filho et al (2003) e Sanguino et al (2007), observaram os parâmetros produtivos desta espécie e também corroboram com Ono et al (2004), afirmando seu grande potencial produtivo. Em sistemas mais intensivos, como tanque-rede, têm-se observado importantes resultados produtivos, contudo, algo ainda incipiente.

Segundo Ono et al (2004), apesar dessas características favoráveis, é uma atividade de grande risco, pois, não se tem um pacote tecnológico definido para a criação, como a dificuldade de se obter formas jovens em escala comercial (Ono, et al., 2011) e o saber da nutrição e de suas exigências, não se tem conhecimento, havendo apenas alguns resultados isolados de pesquisas (Stickney, 2005; Ono et al., 2011), entretanto, é um nicho de mercado com grandes perspectivas.

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Chapter 01: Self-feeder systems and infrared sensors to evaluate the daily feeding and locomotor rhythms of Pirarucu (*Arapaima gigas*) cultivated in outdoor tanks

Aquaculture 457 (2016) 118–123



Contents lists available at ScienceDirect

Aquaculture

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Short communication

Self-feeder systems and infrared sensors to evaluate the daily feeding and locomotor rhythms of Pirarucu (*Arapaima gigas*) cultivated in outdoor tanks



Bruno Olivetti de Mattos^{a,*}, Eduardo César Teixeira Nascimento Filho^a, Kayck Amaral Barreto^b, Luis Gustavo Tavares Braga^c, Rodrigo Fortes-Silva^{b,*}

^a Department of Animal Science and Veterinary Medicine, Campus Salvador, Federal University of Bahia (UFBA), 40170-110, Bahia, Brazil

^b Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Bahia (UFRB), 44380-000, Bahia, Brazil

^c Department of Agricultural and Environmental Sciences, Campus Ilhéus, State University of Bahia (UESC), 45662-900, Bahia, Brazil

ARTICLE INFO

Article history:

Received 5 November 2015
Received in revised form 15 February 2016
Accepted 17 February 2016
Available online 18 February 2016

Keywords:

Feeding behavior
Feeding schedule
Self-feeders
Pirarucu

ABSTRACT

This study evaluated the ability of Pirarucu (*Arapaima gigas*) to feed through self-demand feeders, and also determined daily feeding rhythm and locomotor activity. Twenty-four fish (312.58 ± 9.55 g, mean \pm SEM) were distributed in six 250-liter outdoor tanks. Each tank was equipped with a feeder adapted to allow fish to self-feed. Self-feeders and occupancy sensors were connected to a computer to allow measurement of feeding and locomotor activity. First, the ability of Pirarucu to use the self-feeding system was assessed. The daily rhythm of feeding activity and locomotion of Pirarucu was then evaluated. The fish learned to trigger the feeders and feeding demand stabilized at 158.79 activations per day. Predominant daytime feeding activity was observed, with 110.86 ± 0.27 counts/day (70% of the total actions) seen during the day, versus 47.93 ± 0.11 counts/day (30%) at night. However, no rhythm in this daily locomotor activity was observed. These study results were based on food being freely available and should be taken into account to improve the supply schedule of the species.

Statement of relevance: The use of an automatic feeder system activated by the fish could allow the animal to feed at their preferred time and hence reduces waste and improve the food intake of Pirarucu raised for aquaculture. Moreover, the optimum feeding strategies, including times, schedules and regimes, should be determined from detailed investigations of feeding rhythms.

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1. Introduction

Feeding, including feed type, ration size, feeding frequency, feed intake and the ability to absorb nutrients, can have a significant impact on the success of aquaculture fish (Xie et al., 2011; Tian et al., 2015). The approach usually adopted is to feed to "near-satiety" with a pre-determined number of feeds provided per day (Cho, 1992). However, this can be very subjective. Cho et al. describe that most available feeding charts tend to over-estimate feed requirements resulting in overfeeding, poor feed efficiency under most husbandry conditions, feed waste, pollution of the environment and therefore economic disadvantages for fish farmers. Fish feeding schedules are frequently designed by hatchery operators, which might give preferential consideration to staff working hours rather

than biological or technological factors (Kotani and Fushimi, 2011). Feeding of fish continues to be seen as an "art form" with the farmer, not the fish, deciding when and how often they are fed and estimating "satiety or near-satiety" (Cho, 1992). Conclusive indicators for the determination of feeding schedules for tropical fish production and rearing are lacking.

The self-demand feeder system is an important tool for studying feeding rhythms (Adron et al., 1973; Landless, 1976; Grove et al., 1978; Takahashi et al., 1981; Beach et al., 1986; Boujard et al., 1992; Sánchez-Vázquez et al., 1994; Azzaydi et al., 1998; Kitagawa et al., 2015). The system also can reduce food waste by providing food only when the fish wants to eat (Montoya et al., 2012). Measurement devices can be coupled to a computer, allowing continuous recording of feeding behavior including pattern and frequency of feeding preferences. Therefore, developing self-feeding systems based on scientific knowledge is a prerequisite to improving fish nutrition and husbandry and avoid wasting expensive feeds (Cho, 1992; Fortes-Silva et al., 2011).

* Corresponding authors.

E-mail addresses: mattos.bo@gmail.com (B.O. Mattos), fortes@ufrb.edu.br, fortesrs@yahoo.com.br (R. Fortes-Silva).

Abstract

This study evaluated the ability of Pirarucu (*Arapaima gigas*) to feed through self-demand feeders, and determining daily feeding rhythm and locomotor activity. Twenty-four fish ($312.58 \pm 9,55\text{g}$, mean \pm SEM) were distributed in six 250-liter outdoor tanks. Each tank was equipped with a feeder adapted to allow fish to self-feed. Self-feeders and occupancy sensors were connected to a computer to allow measurement of feeding and locomotor activity. First, the ability of Pirarucu to use the self-feeding system was assessed. The daily rhythm of feeding activity and locomotion of Pirarucu was then evaluated. The fish learned to trigger the feeders and feeding demand stabilized at 158.79 activations per day. Predominant daytime feeding activity was observed, with 110.86 ± 0.27 counts / day (70% of the total actions) seen during the day, versus 47.93 ± 0.11 counts / day (30%) at night. However, no rhythm in this daily locomotor activity was observed. These study results were based on food being freely available and should be taken into account to improve the supply schedule of the species.

Keywords: Feeding behavior, feeding schedule, Self-feeders, Pirarucu

1. Introduction

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The self-demand feeder system is an important tool for studying feeding rhythms (Adron et al., 1973; Landless, 1976; Grove et al., 1978; Takahashi et al., 1981; Beach et al., 1986; Boujard et al., 1992; Sánchez-Vázquez, et al., 1994; Azzaydi et al., 1998; Kitagawa et al., 2015). The system also can reduce food waste by providing food only when the fish wants to eat (Montoya et al., 2012). Measurement devices can be coupled to a computer, allowing continuous recording of feeding behavior including pattern and frequency of feeding preferences. Therefore, developing self-feeding systems based on scientific knowledge is a prerequisite to improving fish nutrition and husbandry and avoid wasting expensive feeds (Cho, 1992; Fortes-Silva et al., 2011).

Pirarucu (*A. gigas*) is considered a relatively new and important species for Brazilian aquaculture (Cavole et al., 2015; Martins et al., 2015; Torres et al., 2015). Currently, little published data exists about the optimal feeding management, ingestion rate and nutrition requirements of Pirarucu (Crescêncio et al., 2005). From the Arapaimidae family, also known as the giant of the Amazon, Pirarucu is endemic to the Amazon River basin system, which has excellent biological and zootechnical characteristics (Malheiros et al., 2016).

The aim of this study was to evaluate the ability of the Pirarucu (*Arapaima gigas*) to self-feed using self-demand feeders and to determine their feeding activity and locomotor daily rhythm.

2. Material and methods

2.1. Housing conditions

Procedures and management followed protocol approved by the ethics committee on animal use. Juvenile Pirarucu were provided by AguaVale fish farm (Bahia, Brazil).

The experiment was carried out at the Fish Nutrition and Feeding Behaviour Laboratory, Universidade Federal do Recôncavo da Bahia (Cruz das Almas, Brazil) in an outdoor system with controlled water temperature (29 ± 0.8 °C).

Water was recirculated within the system which also comprised of mechanical filters, ceramic ring biofilters and UV light (60 W intensity). The experiment was conducted from February to March 2015. Luminous intensity was measured throughout the day and night using a portable digital light meter probe with the photo-sensor (Luximeter, São Paulo, Brazil) placed above the tank. Fish received approximately 13h light and 11h dark photoperiod. The light period began at 06:00, when fish received a light intensity starting from 1 lux, which was the same as at the start of the evening period, at 19:00. Water parameters such as temperature, pH, oxygen and ammonia were measured daily, and the values 29 ± 0.8 °C, 6.55 ± 0.5 , 7.80 ± 0.6 mg/l and 5.00 ± 0.5 mg/l respectively, were considered appropriate for this species (Cavero et al., 2004).

2.2. Experimental set-up: Self-feeding and locomotor system

Each tank was equipped with a feeder (Igarapé Feeders, Barueri, Brazil), adapted to allow fish to self feed (Sanchez-Vazquez et al., 1994; Fortes-Silva et al., 2010a). Stretch sensors with a small rubber tip were connected to a switch, which was connected to the electrical part of the feeder. Sensors were placed 3 cm below the water surface and the feed was placed into the rubber tips to attract the fish. Each time the sensor was touched, feeders were triggered. Each self-feeder was connected to a computer with specific software (DIO98USB, University of Murcia, Spain) which measured the feeding activity of the fish (Figure 1).

Each tank included a system for measuring the locomotive activity of the fish, which allowed the rate of feeding activity to be measured. This was achieved by placing “Presence sensors”, photocells with infrared light (Omron E3S-AD62 model,

Japan), 25 cm from the bottom of the tank. These presence sensors were connected to the same computer system as the stretch sensors. Each time a fish passed the sensors, information was sent to the computer, generating a database throughout the experimental period where both feeding and locomotor activity data were recorded every 10 minutes and stored for later evaluation (Kitagawa et al., 2015).

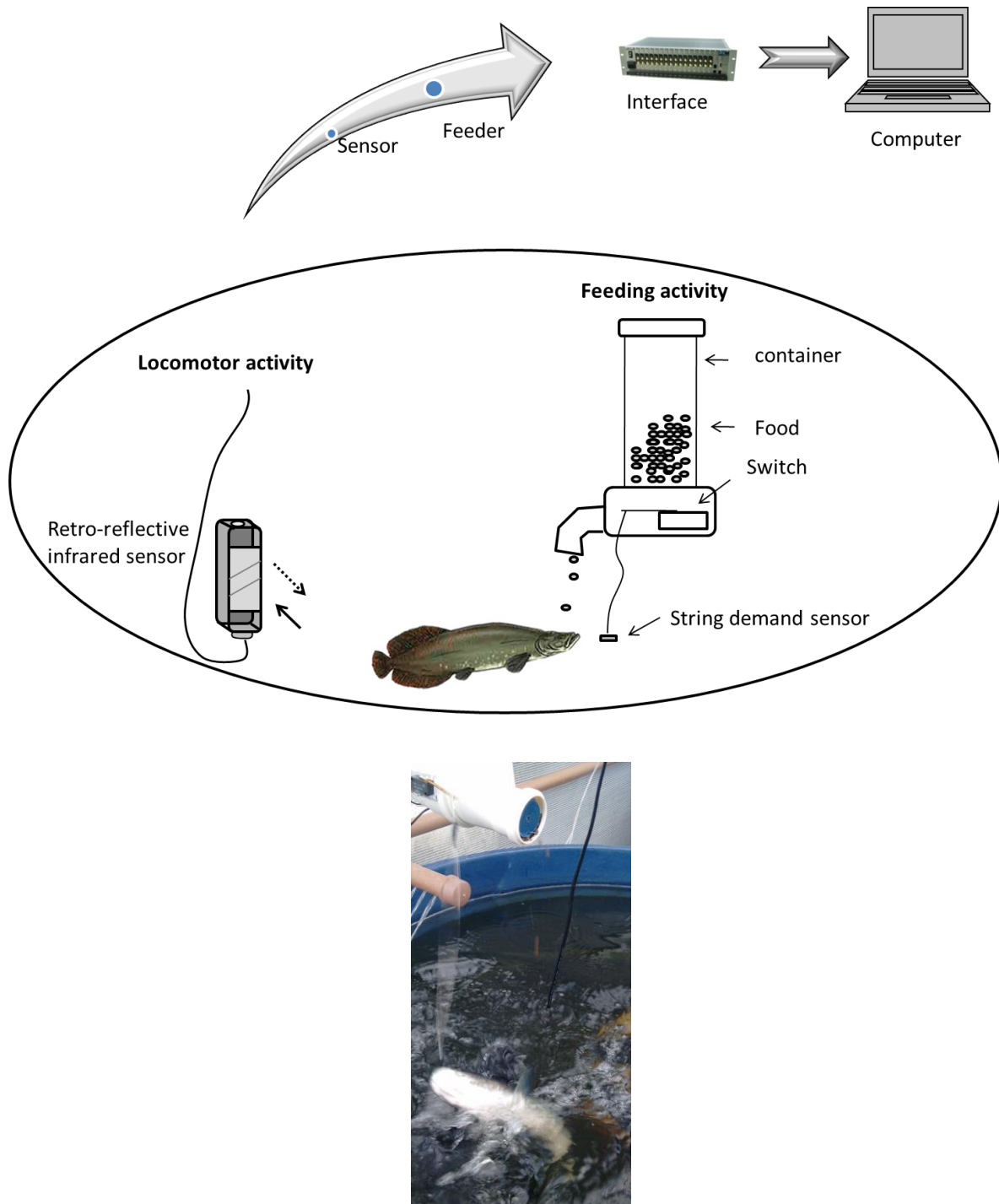


Figure 1 - Feeding system, feeding and locomotor rhythm follow-up.

2.3. Experimental design

Twenty four juvenile fish (mean of initial weight \pm SEM: 312.58 ± 9.55 g) were distributed in six 250 L outdoor tanks (four fish per tank). The experiments were divided into two steps.

2.3.1. Step 1: Learning of self-feeding

Step 1 (30 days) consisted of assessing the degree to which the fish adapted to the new feeding conditions. This was performed as the learning process varies by species e.g. as shown for *Carassius auratus* (Sanchez-Vazquez et al., 1998), *Oncorhynchus mykiss* (Sánchez-Vázquez et al., 1999) and *Sparus aurata* (Montoya et al., 2012). Each day, 200g of commercial diet (45% crude protein, 12% ether extract, 24% carbohydrate, 14% ash, 4 mm-grain-size pellets (Presence[®], São Paulo, Brazil) were placed in each feeder. Consumption was calculated by weighing the remaining feed the next day, and subtracting this from the initial feed weight. During this process, the performance parameters weight gain, the total feed intake and feed conversion were recorded. After confirming learning of self-feeding and stabilization of the feed demand, the second experimental stage was started.

2.3.2. Step 2: Recording fish feeding and locomotor activity patterns

Daily feeding and locomotor activity of Pirarucu was tested. The amount of food offered was adjusted on demand, based on the demand amount observed in step 1 (0.36 ± 0.02 g / demand), taking into account the biomass of each tank. Therefore, during this step (60 days), no residual food was observed in the tanks.

2.4. Data analysis

For data analysis, software was used to plot feeding and locomotor activity actograms (Temps, v.1, 179 Dr. Diez Noguera, Barcelona), which were doubly plotted for better visualization. Data from self-learning process was transferred to the computer and exported to Microsoft Excel for analysis and plotting. The results are expressed as the mean of six tanks \pm standard error of the mean (SEM). Food rhythm and locomotor activity were analyzed post-hoc using one-way ANOVA to determine significance. Statistical significance was considered to be $p < 0.05$. Data were analyzed using PROC GLM of SAS (version 9.2).

3. Results

3.1 Learning of self-feeding

From day one, activation of the feeders by fish was observed, with an initially increasing demand, which stabilized at 158.79 demands per day (Figure 2). There were no food leftovers for the entire experimental period, demonstrating adaptation of the animals to the self-demand feed system and control of food intake. There was no mortality or pathology during the experimental period. The final body weight of fish was 656.67 ± 53.25 g (mean \pm SEM), and their daily feed intake was 23.50 ± 2.18 (g/kg BW/day) with a food conversion rate of 1.04 ± 0.09 over the 30 day experimental period.

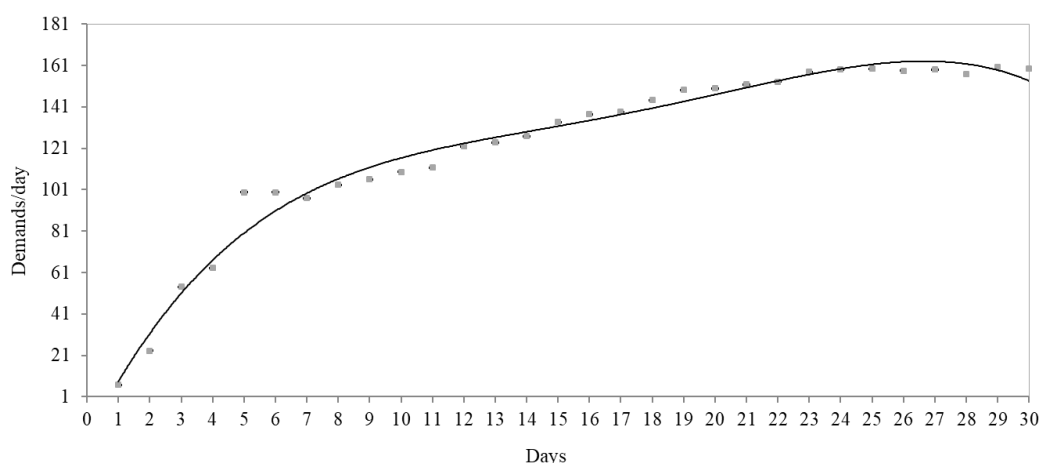


Figure 2 - Learning of demand feeding by Pirarucu (*Arapaima gigas*).

3.2. Fish feeding and locomotor activity patterns

Fish began the trial period able to trigger stretch sensors in demand feeders. Actograms and waveform analysis of feeding behaviour showed Pirarucu to have a daytime feeding activity of 110.86 ± 0.27 counts/day, which comprised 70% of total activity, versus 47.93 ± 0.11 counts/day (30% of total activity) overnight (figure 3a and 3b). The amount of daytime demand was higher than at night [F (1,718) = 354.384, $P < 0.0001$].

However, rhythm was not observed for daily locomotor activity, either in actograms or waveform (figure 4a and 4b), confirmed with one-way ANOVA [F (1,242) = 4.707, $P = 0.031$].

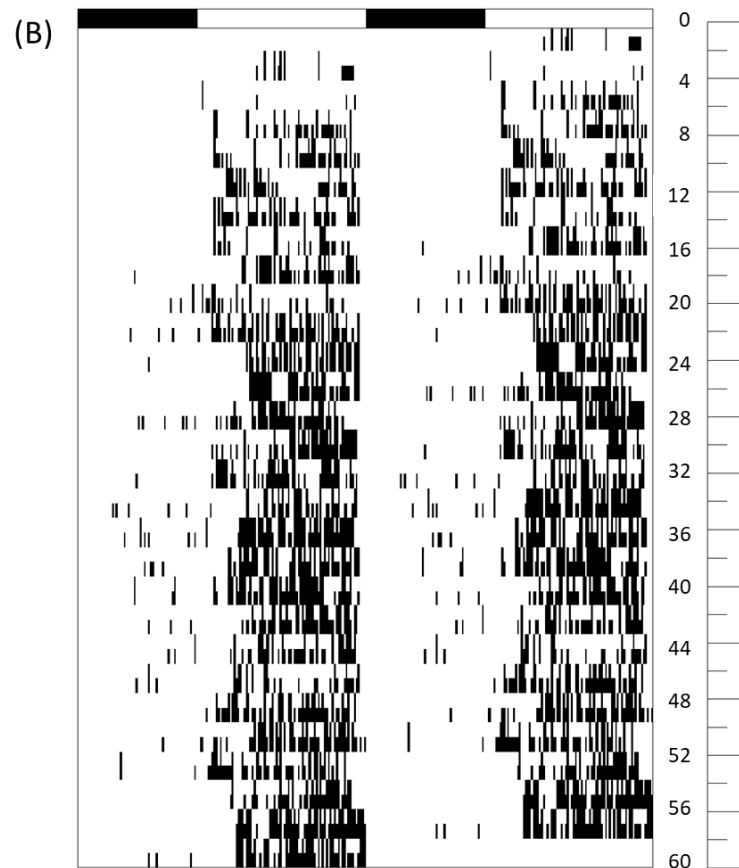
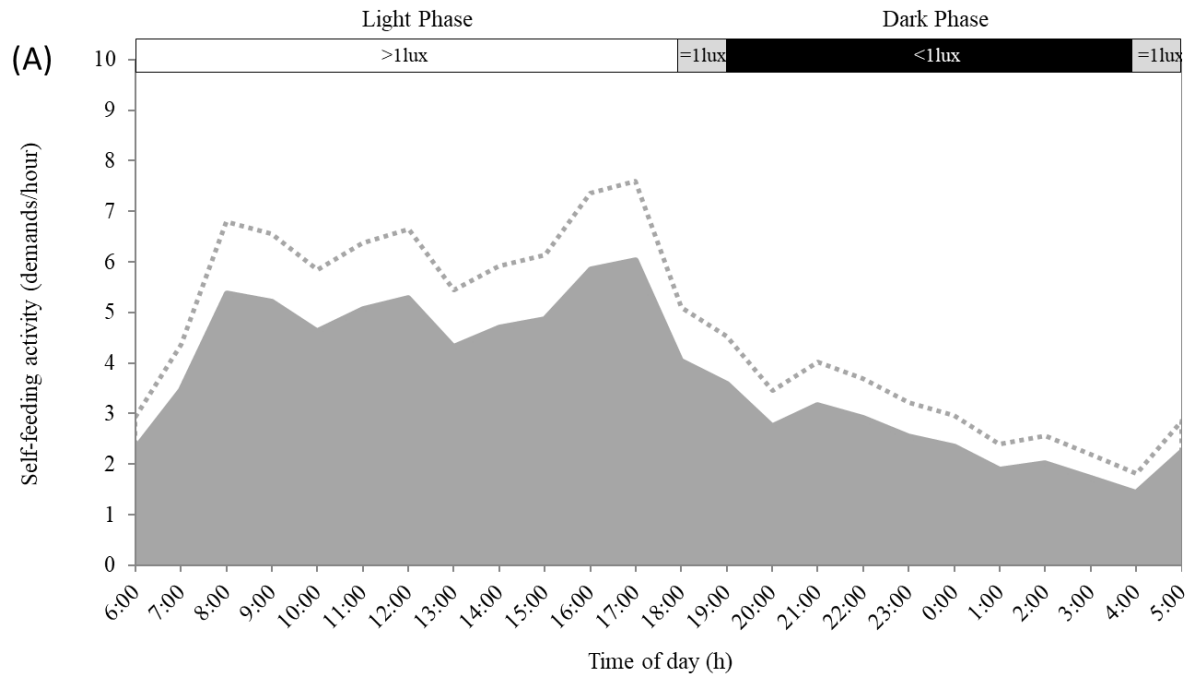


Figure 3 - Average daily waveforms (+ S.D. dotted line) (a) and actogram (b) for Pirarucu feeding activity rhythms (*Arapaima gigas*) during outdoor activities. The black and white bars on the top of graphics indicate the duration of dark (11:00h) and light (13:00h) phases respectively.

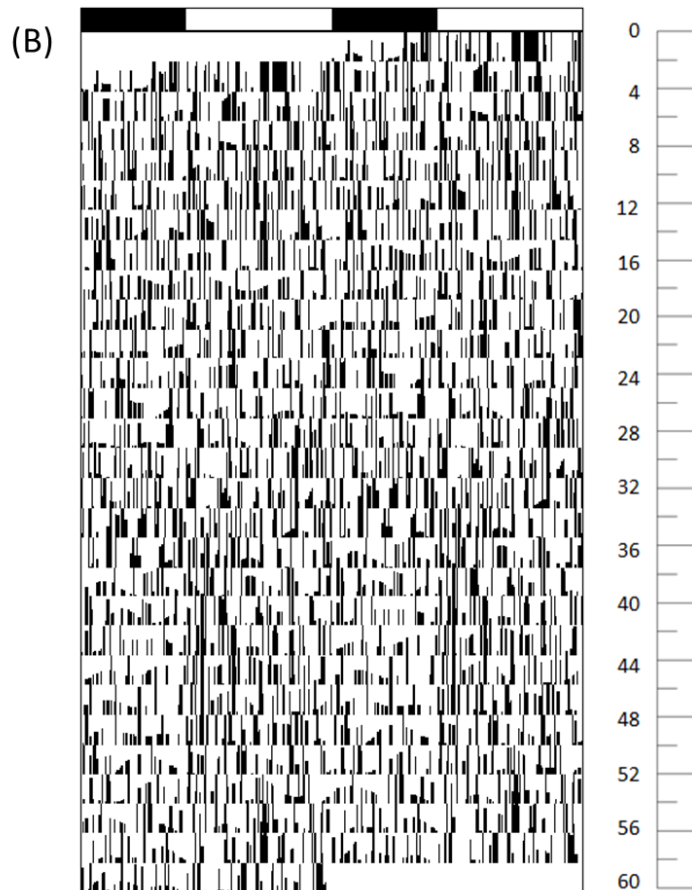
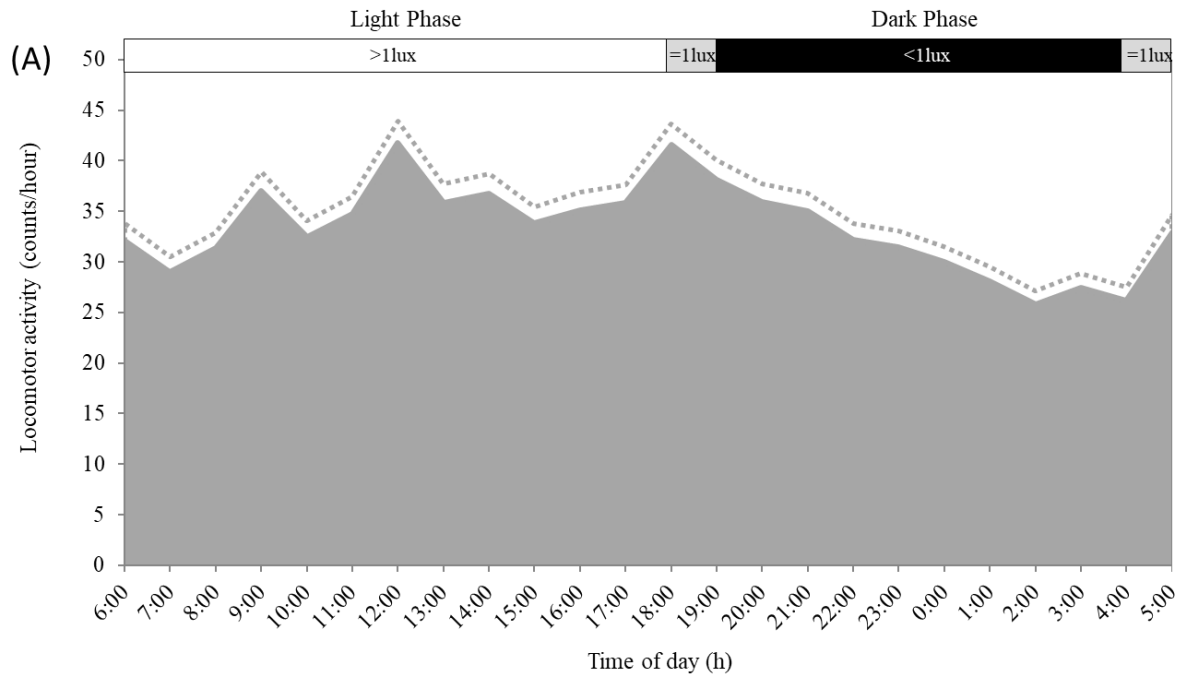


Figure 4 - Average daily waveforms (+ S.D. dotted line) (a) and actogram (b) for Pirarucu locomotor rhythm (*Arapaima gigas*) during outdoor activities. The black and white bars on the top of graphics indicate the duration of dark (11:00h) and light (13:00h) phases respectively.

4. Discussion

This study showed that juvenile *Arapaima gigas* used the self-feeding system efficiently, demonstrated by a clear feeding activity rhythm. In addition, fish had satisfactory growth, achieved without having leftover food. Our findings are in agreement with Kotani and Fushimi (2011), who suggest that optimum feeding strategies, including times, schedules and regimes should be determined from detailed investigations of feeding rhythms.

The fish were able to repeatedly trigger the self-feeders from the first day of experiment. Pirarucu maintained the triggering pattern without wasting feed from the self-feeders. This behavior is referred to as “learning associated with reward” (Forbes, 2001). According to Alanärä and Brännäs (1996), this system allows us to more appropriately manage cultivated species. Self-feeding is based on the learning ability of fish and therefore may improve performance and reduce waste levels since food is delivered depending on appetite (Azzaydi et al., 1998). In self-feeding systems it is assumed that fish can precisely control their feeding by simply activating the "trigger" inserted into water as sensors (Alanärä and Brännäs, 1996).

Studies related to the feeding rhythm of Pirarucu are scarce. Crescêncio et al. (2005) adopted four fixed times for supplying Pirarucu feed (daytime: 09:00 and 15:00, at night: 21:00 and 03:00). These authors concluded Pirarucu preferred feeding at night, but had better growth when fed during day. This preferred time of feeding conflicts with our results. However, it may be explained by the fixed feeding protocol, rather than feeding by choice, as recommended by Volpato et al., 2007. The diurnal feeding behavior of Pirarucu has also been observed with other carnivorous species such as *Salvelinus alpinus* (Linner et al., 1990), *Salmo salar* (Fraser and Metcalfe, 1997; Bremset, 2000), *Salmo trutta* (Bremset, 2000), *Oncorhynchus mykiss* (Railsback et al., 2005) and *Oncorhynchus masou masou* (Flood et al., 2011) when free choice was allowed by the provision of self-feeder systems. On the other hand, some authors have described high plasticity of fish feeding behavior (Kitagawa et al., 2015), describing it to be influenced by several factors such as the presence of predators or food (Fortes-Silva et al., 2010b). According to Fortes-Silva et al., (2010ab), tilapia showed diurnal, nocturnal and crepuscular feeding behavior, showing behavioural plasticity. Dual phasing feeding behavior in fish was first reported in European sea

bass (*Dicentrarchus labrax*) (Sánchez-Vázquez *et al.*, 1994). In our results, the locomotor rhythm showed no rhythmicity, with 57% movement during the day and 43% seen at night (Figure 4b). According to the Sánchez-Vázquez *et al.* (1996), the rate should be more than 65% of activations in goldfish (*Carassius auratus*) to define the activity. This behavior of pirarucu can be explained by the respiratory action of this fish type, which has bimodal breathing provided by the modified swim bladder, allowing oxygen taken at the water surface every ten or twenty minutes to capture atmospheric oxygen (Sawaya, 1946; Lüling, 1964; Lefevre *et al.*, 2015).

Although the goal of this work is not primarily to evaluate productive performance, the fish had good productive parameters compared to other studies. At first the animals showed a high feed intake of 23.50 ± 2.18 g / kg BW/day. However, one important factor in the production of fish is feed conversion, which in this experiment was 1.04 ± 0.09 , better than that reported by Crescêncio *et al.*, (2005) which varied from 1.28 to 1.90 in 313-gram animals fed at fixed times, both day and night. Some authors have reported a positive association of increased digestive enzymes and feeding time preference (Greenwood and Metcalfe, 1998; López-Vázquez *et al.*, 2009). This could explain the good performance of self-feeding fish. Therefore, predetermined amounts of feed, even if given in evenly spaced intervals at time, can be seen as a form of restricted feeding (Cho, 1992). None self-feeding systems use maximum body weight gain as their sole objective, and therefore are inherently uneconomical and will also not promote a lasting cohabitation of sustainable aquaculture in a cleaner environment.

5. Conclusions

This study revealed a strictly diurnal feeding activity for Pirarucu, without locomotion associated rhythm, likely explained by its bimodal breathing. Previous studies investigating feeding behaviour of Pirarucu were influenced by fixed schedule feeding experimental design. Self-feeding systems may promote better feed performance and reduce food wastage for tropical species in aquaculture systems.

Acknowledgements

The authors of this research thank AguaVale Aquaculture for supplying juvenile Pirarucu, the Poytara for supplying food ingredients and the Higher Education Personnel Improvement Coordination (CAPES) for granting doctoral scholarships. This work was supported by the National Scientific and Technological Development (CNPq, Grant number 477408/2013-3) and REDES/FAPESB (RED0004/2013) grants awarded to Dr. Rodrigo Fortes. The authors wish to acknowledge João Lúcio Pimenta de Mattos for his kind support and assistance during the study.

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Chapter 02: Daily self-feeding activity rhythms and dietary self-selection of pirarucu (*Arapaima gigas*)

Aquaculture 465 (2016) 152–157



Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Daily self-feeding activity rhythms and dietary self-selection of pirarucu (*Arapaima gigas*)



Bruno Olivetti de Mattos^{a,*}, Eduardo César Teixeira Nascimento-Filho^a, Aline dos Anjos-Santos^b, Francisco Javier Sánchez-Vázquez^c, Rodrigo Fortes-Silva^b

^a Department of Animal Science and Veterinary Medicine, Campus Salvador, Federal University of Bahia (UFBA), 40170-110, Bahia, Brazil

^b Laboratory of Feeding Behavior and Fish Nutrition, Center of Agricultural Sciences, Environmental and Biological, Campus Cruz das Almas, Federal University of Bahia (UFBR), 44380-000, Bahia, Brazil

^c Department of Physiology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain

ARTICLE INFO

Article history:

Received 18 February 2016
Received in revised form 31 August 2016
Accepted 2 September 2016
Available online 07 September 2016

Keywords:

Feeding behaviour
Feeding schedule
Food preference
Nutritional challenge
Nutritional wisdom

ABSTRACT

Daily feeding rhythms and the ability of pirarucu (*Arapaima gigas*) to compose a balanced diet through macronutrient self-selection were evaluated. Twelve fish (1573.3 ± 74.4 g) were distributed in six tanks of 250 l, two fish per tank. First, three experimental diets were prepared using an incomplete mixture of macronutrients (75% protein/25% carbohydrate-PC, 75% protein/25% fat-PF and 10% protein/45% fat/45% carbohydrate-PFC). These diets were provided to fish through a self-feeding system connected to a computer to record feeding activity. After this procedure, fish were challenged with a 50% protein dilution (diets: PC50 and PF50). The results showed that pirarucu exhibited a strict diurnal feeding pattern with 95.4% of daily feeding activity observed in the day-time. Fish selected 56.3% P, 24.2% C, 19.5% F, and consumed 150–151 kJ/kg BW/day of energy. After protein dilution, fish sustained energy intake by increasing the consumption of PC50 and PF50 to maintain the target protein intake of the previous stage. When fish were protein-restricted, they failed to sustain previous energy intake. These findings can be used to design feeding regimes and for formulating aquafeed for pirarucu. **Statement of relevance:** The use of an automatic feeder system activated by the fish could allow the animal to feed at their preferred time and hence reduces waste and improve the intake. Studies that consider the fish preference as a guide in designing diets can provide data on mechanisms of nutrient intake regulation for the development of aquafeeds for new species with potential for aquaculture.

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1. Introduction

The different fish species are not active both in the daytime and at night, but show daily behaviour rhythms (Sánchez et al., 2009). Some environmental events are unpredictable (e.g., weather changes), while others (e.g., daily light cycles) occur repeatedly because they are associated with periodical geophysical cycles, such as the Earth's rotation on its axis. Fish, as most animals, have biological rhythms synchronised to these dependable cycles. Thus they display feeding rhythms (López-Olmeda and Sánchez-Vázquez, 2010). The demand-feeding system has become a useful tool for assessing fish feeding rhythms and food preferences (Azzaydi et al., 1998; Montoya et al., 2012). Different researchers have developed various devices coupled to computers to conduct such research using electric sensors (Boujard et al., 1992), stretch sensors (Sánchez-Vázquez et al., 1994) and infrared photocells (Kitagawa et al., 2015). Over the years these systems have helped to

design effective feeding protocols that avoid feed wastage and improve feed utilisation in fish (Cho, 1992; Fortes-Silva et al., 2011a).

Aquaculture fish display a wide variety of feeding behaviours compared to terrestrial livestock, are considered good experimental models to study nutrient intake regulation (Volkoff and Peter, 2006), and possess considerable plasticity for feeding rhythms (Sánchez-Vázquez et al., 1996). According to Simpson and Raubenheimer (2001), fish are able to regulate nutrient intake and defend a nutritional target. This ability is based on the "nutritional wisdom" observed in studies conducted with different carnivorous fish species, such as *Solea senegalensis* (Rubio et al., 2009), *Oncorhynchus mykiss* (Sánchez-vázquez et al., 1999) and *Dicentrarchus labrax* (Rubio et al., 2003), and omnivore fish like *Oreochromis niloticus* (Fortes-Silva and Sánchez-Vázquez, 2012) and *Carassius auratus* (Sánchez-Vázquez et al., 1998). Nutrient self-selection could be used to optimize the diet composition of farmed fish (Fortes-Silva et al., 2016), and to enable the development of more efficient feeding schedules for fish species in aquaculture (Kitagawa et al., 2015). The studies that contemplate fish as a guide to design diets can provide data on mechanisms of nutrient intake regulation (Fortes-Silva et al., 2011b; Fortes-Silva and Sánchez-Vázquez, 2012), and on fish welfare according

* Corresponding author.
E-mail addresses: mattos.bo@gmail.com (B.O. Mattos), fortes@ufbr.edu.br, fortesrs@yahoo.com.br (R. Fortes-Silva).

Abstract

Daily feeding rhythms and the ability of pirarucu (*Arapaima gigas*) to compose a balanced diet through macronutrient self-selection were evaluated. Twelve fish (1573.3 ± 74.4 g) were distributed in six tanks of 250 l, two fish per tank. First, three experimental diets were prepared using an incomplete mixture of macronutrients (75%protein/25%carbohydrate-PC, 75%protein/25%fat-PF and 10% protein/45%fat/45%carbohydrate-PFC). These diets were provided to fish through a self-feeding system connected to a computer to record feeding activity. After this procedure, fish were challenged with a 50% protein dilution (diets: PC50 and PF50). The results showed that pirarucu exhibited a strict diurnal feeding pattern with 95.4% of daily feeding activity observed in the daytime. Fish selected 56.3% P, 24.2% C, 19.5% F, and consumed 150-151 kJ/kg BW/day of energy. After protein dilution, fish sustained energy intake by increasing the consumption of PC50 and PF50 to maintain the target protein intake of the previous stage. When fish were protein-restricted, they failed to sustain previous energy intake. These findings can be used to design feeding regimes and for formulating aquafeed for pirarucu.

Keywords: Feeding behaviour; Feeding schedule; Food preference; Nutritional challenge; Nutritional wisdom.

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Pirarucu (*Arapaima gigas*) is one of the largest freshwater fish species in the Amazon basin whose captive breeding is being increasingly exploited (Núñez et al., 2011). Pirarucu farming in the Brazilian Amazon is an important economic activity that has grown in the last few years (Malheiros et al., 2016). Its juveniles display high specific growth rates, and can reach a weight of over 10 kg in 1 year (Imbiriba, 2001). Pereira-Filho et al. (2003) obtained the best biomass growth for pirarucu on extruded diets (40% crude protein, 3.400 Kcal/kg diet). Young pirarucu (body weight between 11 g and 108 g) fed on extruded diets (45% crude protein) showed the best feed conversion of 0.8 (Cavero et al. 2003). When verifying the effect of four protein levels on pirarucu growth (32.7%, 39.3%, 43.4% and 48.6% crude protein), Ituassú et al. (2005) found that these levels have no effect on feed conversion and protein efficiency ratios, but the dietary protein level required for maximum growth was 48.6% for juveniles with 120.7 ± 3.5 g of weight. These results reveal that more nutritional studies are needed. Despite their great potential for aquaculture, there are no studies on this species' feeding behaviour and food preferences, which are key issues for successfully farming this fish species (Ono, et al., 2008).

The present paper aimed to assess the daily feeding rhythms of pirarucu, and to evaluate not only its ability to self-compose a balanced diet (nutritional target) based on incomplete mixtures of macronutrients, but also its capacity to defend such a target following nutritional challenges (i.e. protein dilution and deprivation).

2. Material and methods

2.1. Animal housing

This research was conducted in the Fish Nutrition and Feeding Behavior Laboratory (AQUAUFRB-NEPA), Federal University of Recôncavo of Bahia (UFRB, Cruz das Almas, Bahia, Brazil). Twelve juvenile pirarucu, kindly provided by the AguaVale fish farm (Bahia, Brazil), with a body weight of $1,573.3 \pm 74.4$ g (mean \pm SEM) were distributed in six 250-litre tanks (2 fish per tank). The system contained water recirculation, which comprised mechanical filters, biofilter ceramic rings and UV light (60 W). The experiment was conducted from May to July 2015. Light intensity was measured by a portable digital light meter probe with a photosensor (Luximeter, São Paulo, Brazil) placed above the tank both night and day. Fish received approximately 12 h of light and 12 h of darkness. The photoperiod was regarded as the beginning of the light period at 6 am, and received light intensity that started from 1 lux and gradually increased during the day to then return to the same 1 lux at the start of the evening period at 6 pm. Water parameters, such as temperature, pH, oxygen and ammonia, were measured daily, and the obtained values $29.0 \pm 0.8^\circ\text{C}$, 6.6 ± 0.5 , 7.8 ± 0.6 mg/l and 5.0 ± 0.5 mg/l respectively, were considered appropriate for this species (Cavero et al., 2004). This research was in line with the ethics principles in Animal Experimentation of the Ethics Committee of the UFRB.

2.2. Experimental diets

Five diets were prepared with incomplete mixtures of macronutrients (Table 1). This protocol was performed according to the recommendations for carnivorous fish by Vivas et al. (2006). The experimental design was the same used by Aranda et al. (2001), in which fish were simultaneously provided with three feeds of two or three macronutrients (Table 1): protein/carbohydrate (PC: 55.40% + 18.50%, corresponding as total macronutrients: 75% P and 25% C), protein/fat (PF: 55.40% + 18.50%, corresponding to 75% P and 25% F) and protein/fat/carbohydrate (PFC: 7.40% + 33.25% + 33.25%, corresponding to 10% P, 45% C and 45% F). PFC contained 7.40% of protein because we were unable to obtain factory pellets with only carbohydrate and lipid as ingredients. Casein and gelatin (5:1) were used as protein sources, dextrin was the carbohydrate source, and a mixture of fish and soybean oil (3:1) was the fat source.

Each diet was supplemented with an equal quantity of vitamins and minerals, sodium alginate as a binder, and cellulose as the filler. Ingredients were thoroughly mixed and moistened with 40% water in a blender (SAMMICH, MP3000) and then made into pellets by forcing the mixture through a meat press to be cut into approximately 4-mm length pellets. These pellets were dried and stored in a freezer (5°C) until use.

Table 1 - Composition of experimental paired diets.

Ingredients (g/100 g of diet)	PC	PF	PFC	PC50	PF50
Casein:gelatin (5:1)	55.40	55.40	7.40	27.70	27.70
Dextrin	18.50	0.00	33.25	9.30	0.00
Fish oil:soybean oil (3:1)	0.00	18.50	33.25	0.00	9.30
Vitamin and mineral mix ^a	2.00	2.00	2.00	2.00	2.00
CaCO ₃ /CaPO ₄	4.00	4.00	4.00	4.00	4.00
Cellulose	15.00	15.00	15.00	51.90	51.90
BHT	0.50	0.50	0.50	0.50	0.50
Binder (sodium alginate)	4.60	4.60	4.60	4.60	4.60
Gross energy (kJ/g) ^b	16.20	20.30	20.20	8.10	10.20
Proximate analysis (%)					
Dry matter	92.29	92.73	92.60	92.26	92.62
Crude protein (N x 6.25%)	53.11	54.60	7.31	26.92	27.13
Crude fat	0.57	16.68	32.49	0.58	8.10
NFE	17.08	0.75	32.59	8.86	0.83
Ash	7.26	8.75	9.28	9.10	8.99

^a Vitamins and minerals (mg/kg diet): Vit. A (min) 1000000 UI, Vit. D3 (min) 250000 UI, Vit. E (min) 12500 UI, Vit. K3 (min) 1250 mg, Vit. B1 (min) 1875 mg, Vit. B2 (min) 1875 mg, Vit. B6 (min) 1250 mg, Vit. B12 (min) 2500 mcg, Vit. C (min) 12.5 g, Pantotenic Acid(min) 5000 mg, Niacine (min) 10.0 g, Folic Acid (min) 625 mg, Biotine (min) 62.5 mg, Coline (min) 50 g, Copper (min) 625 mg, Iron (min) 6250 mg, Manganese (min) 1875 mg, Cobalt (min) 12.5 mg, Iodo (min) 62.5 mg, Zinc (min) 6250 mg, Selenium (min) 12.5 mg, Inositol (min) 12.5 g.

^b Calculated from energy intake using the following energy coefficients: 23.6 kJ/g for protein; 38.9 kJ/g for fat; and 16.7 kJ/g for carbohydrate (Miglav and Jobling, 1989).

The energy content of experimental diets was calculated by the following estimated metabolisable energy (ME) coefficients: 23.6 kJ/g for protein, 38.9 kJ/g for fat and 16.7 kJ/g for carbohydrates, which are the values found in the literature for carnivorous fish (Miglav and Jobling, 1989). The proximate composition of diets was determined by standard methods of the Association of Official Analytical Chemists (AOAC, 2012): the following were determined: content moisture by drying for 24 h at

110°C to constant weight; protein by the Kjeldahl method ($N \times 6.25$); crude fat by diethyl ether extraction; ash by heating at 450°C for 24 h; nitrogen-free extract (NFE) as the remainder of crude protein, crude fat and ash.

2.3. Experimental design

The purposes of Phase 1 were to evaluate the feeding activity by self-feeders system and also the intake target of macronutrients by pirarucu. Moreover, we assess whether the pattern of macronutrients intake keeps after challenge of diet dilution (phase 2 and 3). Data collected of macronutrient intake target during the experimental phases were used for comparative purposes. The feeding activity pattern was measured only in the phase 1 due dilution or restriction of macronutrients in the following phases that could lead to a bias in feeding activity. Initially fish were subjected to a 1-week acclimatisation period to adapt to the new experimental conditions, which included the assessment of fish's ability to trigger demand feeders. Three feeders were fitted (Igarapé, Barueri, Brazil feeders), and each contained one of the three different diets fed in each tank. Fish could trigger stretch sensors (placed 3 cm below the water surface), which were individually connected to their corresponding feeder. Each self-feeder was connected to a computer that used specific software (DIO98USB, University of Murcia, Spain) to record the animals' feeding activity.

For the diet self-selection trial (Phase 1), fish were had free access to the experimental diets (PC, PF and PFC) for 23 days. To evaluate feeding preferences, fish were provided with some challenges. On day 18, the same experimental diets (PC, PF and PFC) were switched between feeders to test if there were any preferences for a particular feeder, rather than a particular diet. On day 24 of the protein dilution trials (phase 2), fish were challenged according to Vivas et al. (2006) and Rubio et al (2009), to diet protein dilution by adding 50% cellulose to the PC50 and PF50 diets (Table 1). Thus, fish had access to PC50, PF50 and PFC. Finally on day 43, fish were challenged with a 7.40% protein restriction diet (phase 3) until day 64 to test their ability to compensate energy intake with fat and carbohydrate. To this end, fish were offered only one diet-PFC (Table 1) and the other two self-feeders with PC50 and PF50 were removed. Any food remains on the bottom of tanks were checked daily to ensure that fish used feeders efficiently and that there were no leftovers.

2.4. Data analysis

Feeding activity records were analysed with software (Temps, v.1, 179 Dr. Díez Noguera, Barcelona) for plotting the daily mean waveforms and actograms, which were double-plotted for better viewing purposes. Differences were analysed by a one-way ANOVA. The purified diets selection results are presented as 100% with their corresponding standard error (S.E.M). Percentage values were transformed into arcsine ($\sqrt{}$), and used to achieve homogeneity of variance. The significant differences between the average macronutrient selections, expressed as percentages, were assessed by a one-way ANOVA. Average gross energy and feed intake were subjected to a one-way ANOVA and the Bonferroni test for the *post hoc* determination of significant differences. Statistical significance was considered at $P < 0.05$. Data were analysed by PROC GLM of SAS (version 9.2).

3. Results

3.1 Daily self-feeding behaviour rhythms

Final body weight was 1606.3 ± 100.8 g (mean \pm SEM). The actograms and waveform analyses of feeding behaviour revealed that pirarucu has almost only diurnal feeding activity as 95.4% of their total daily activity took place in the daytime (Figure 5a). The total amount of food demands was significantly higher in the daytime than at night time [$F(1,136) = 80.857$, $P < 0.0001$]. Feeding activity was not evenly distributed during the day, but was concentrated around 09:00-12:00 h (Figure 5b).

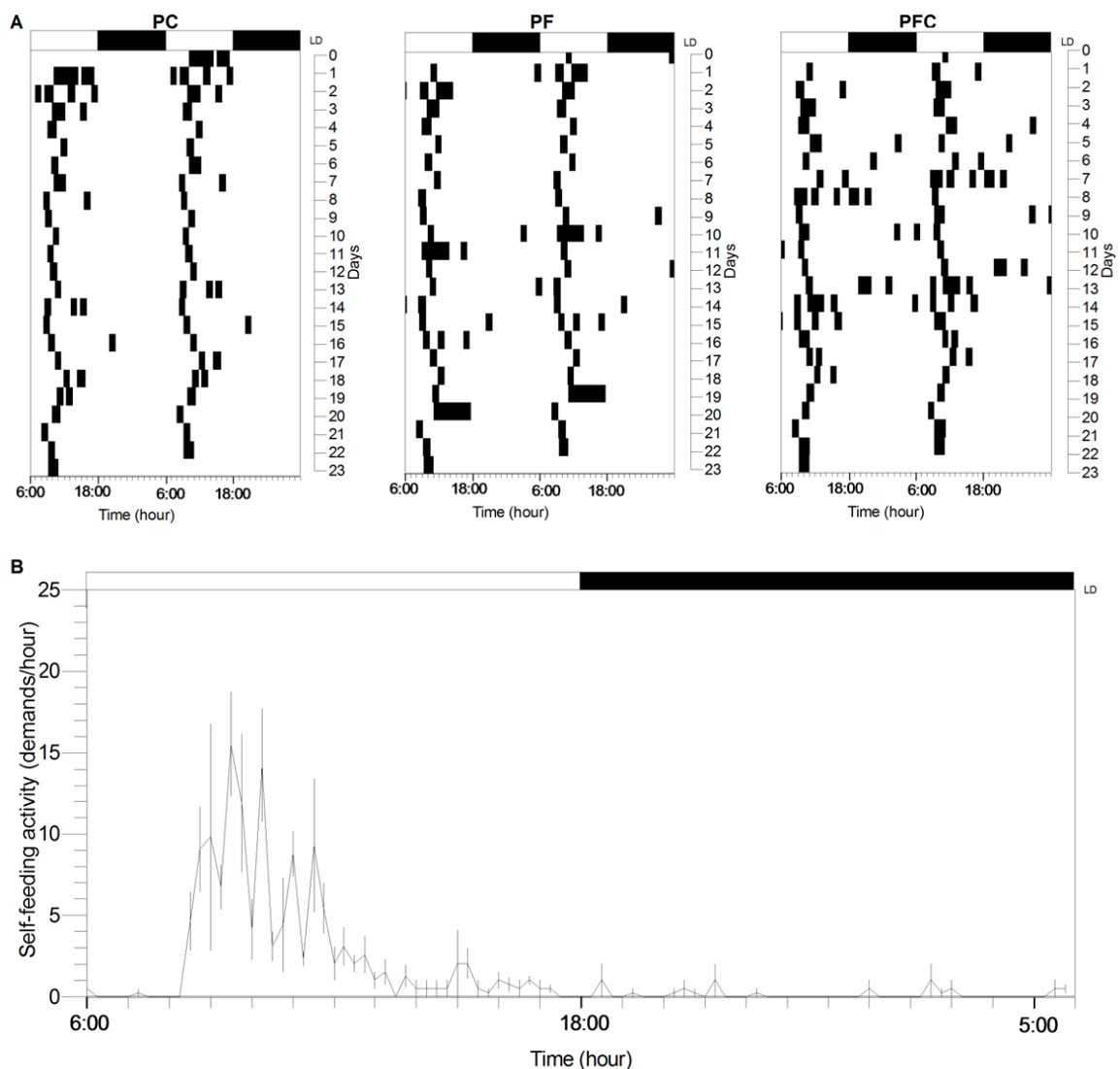


Figure 5 - Actograms (a) of dietary demand (protein+carbohydrate-PC, Protein+fat-PF and Protein+Fat+Carbohydrate-PFC) and average daily waveforms (b) for pirarucu feeding activity rhythms (*Arapaima gigas*) in the first 23 days of the experiment. The black and white bars at the top of graphics indicate the duration of the dark and light phases, respectively.

3.2. Macronutrient self-selection

In experimental phase 1, that is, from the very beginning of the trial, fish sustained diet selection with the following mean composition: $56.1 \pm 2.0\%$ protein, $22.8 \pm 1.0\%$ carbohydrates and $21.1 \pm 1.2\%$ lipids (Figure 6). On day 18, and after switching over diets among feeders, fish did not change their feeding preferences, and selected a similar diet: $55.3 \pm 2.8\%$ protein, $25.4 \pm 1.7\%$ carbohydrates and $19.3 \pm 1.8\%$ lipids. When the dietary protein was diluted by 50% (phase 2), pirarucu increased their food demand from a diluted diet (PC50 and PF50) to maintain the protein intake target and the previous selection pattern ($56.9 \pm 1.7\%$ protein, $25.0 \pm 0.9\%$ carbohydrates and $18.1 \pm 1.1\%$ lipids) (Figure 6).

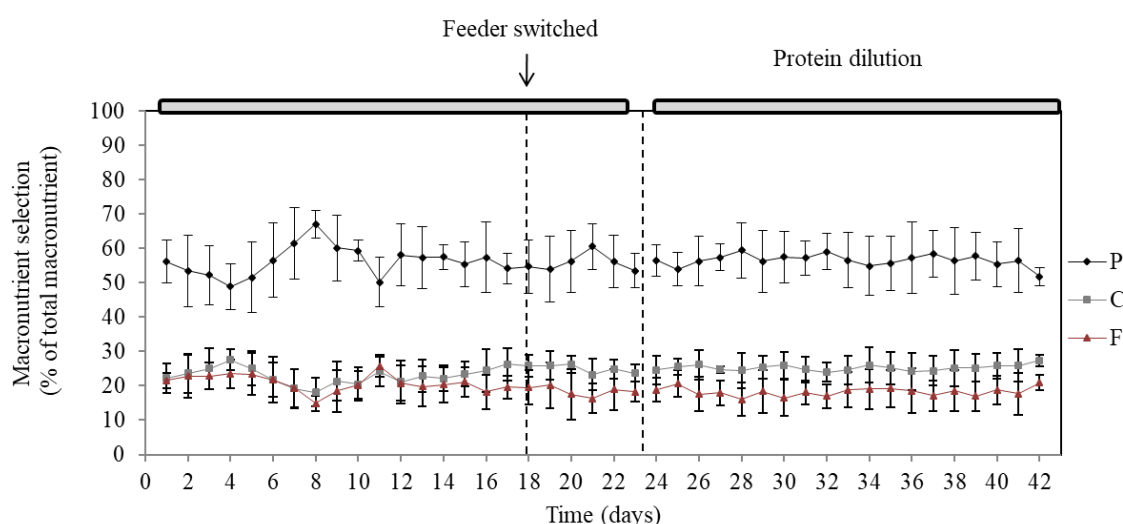


Figure 6 - Daily evolution of macronutrient self-selection. The vertical dotted line indicates the different experimental phases: time upon change in diet between feeders (Phase 1) and diluted diets PC50 and PF50 (Phase 2). Values (S.E.M.) represent the percentage of total macronutrients selected as 100% of juvenile pirarucu, $n=6$, $P<0.05$ (RM-ANOVA).

This macronutrient selection pattern was maintained between phases 1 and 2, and no significant differences were found [$F(1,262) = 0.097$, $P = 0.7556$]. The mean average composition of macronutrient selection was $56.3 \pm 1.2\%$ protein, $24.2 \pm 0.7\%$ carbohydrates and $19.5 \pm 0.7\%$ fat (Figure 7).

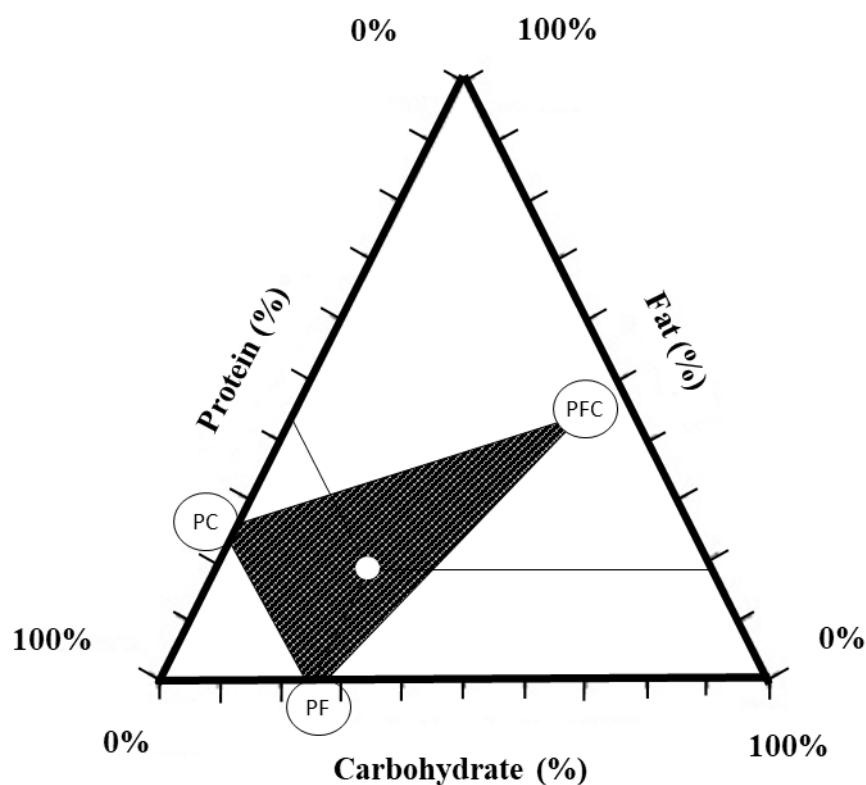


Figure 7 - The white circle inside the triangle represents the mean of the final diet selected by pirarucu in Phases 1 and 2 considering three dimensions (Protein, Carbohydrate and Fat). In the triangle the shaded areas indicate the composition (given as the percentage of each pure macronutrient) of all the possible diets that fish can select by combining the experimental diets of macronutrients.

The food intake and energy intakes showed some daily fluctuations at the beginning of phase 1, which stabilised on day 11 to 9.4 ± 1.0 g/kg BW/day and 150.8 ± 16.8 kJ/kg BW/day for food intake and energy, respectively (Figure 8). Considering the total energy intake in the first phase, 82.3 ± 8.4 kJ/kg BW/day came from the protein, 25.91 ± 2.80 kJ/kg BW/day from carbohydrate and 42.56 ± 16.8 kJ/kg BW/day from lipids respectively. After protein dilution (Phase 2), fish initially maintained their previous food intake, but energy intake dropped. On day 31, fish increased their food intake (15.2 ± 1.2 g/kg BW/day diet) to maintain protein intake, as previously mentioned, and also energy intake (151.0 ± 12.8 kJ/kg BW/day) (Figure 8). At the end of the phases, no significant differences in energy intake were observed

between Phases 1 and 2 ($P = 1.000$). In Phase 3 (protein restriction), their food intake significantly lowered to 2.1 ± 0.4 g/kg BW/day and energy intake to 39.3 ± 8.0 kJ/kg BW/day, with significant differences in both the food and energy intakes between Phases 1 and 3 ($P < 0.0001$).

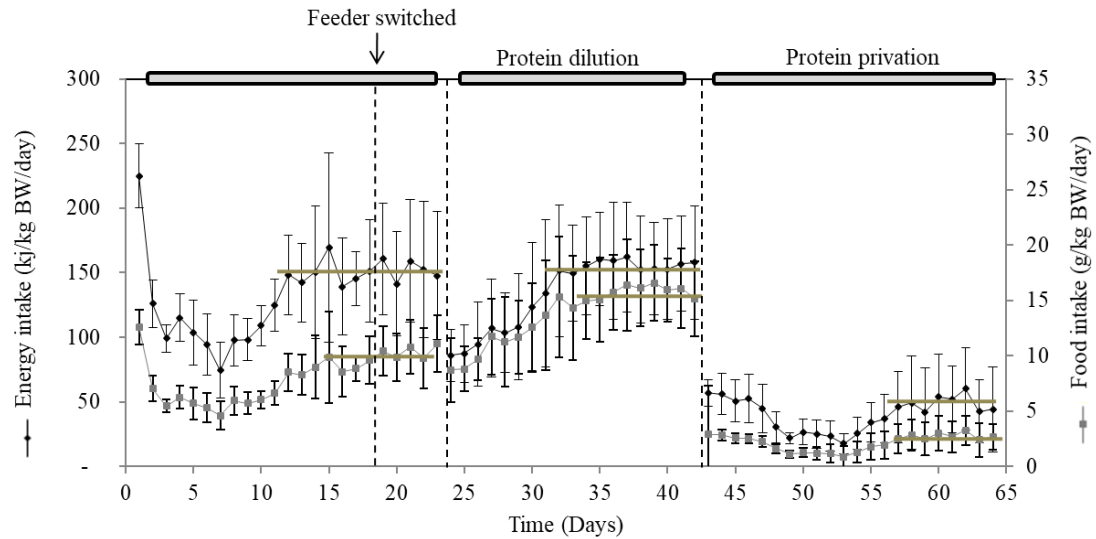


Figure 8 - Average daily energy intake (, left axis) and food intake (, right axis) of the pirarucu fed pairs diets: impact of dietary dilution (PC50 PF50, Phase 2) and restriction (PFC, Phase 3). The vertical broken line indicates the beginning of availability of diluted diets or restriction protein content. Values represent the mean \pm S.E.M. of juvenile pirarucu, $n = 6$, $P < 0.05$ (RMANOVA).

4. Discussion

Our results showed that juvenile pirarucu efficiently used self-feeders and displayed a consistent diurnal feeding rhythm. Very few studies on feeding schedules for pirarucu are available. Crescêncio et al. (2005) supplied diets at fixed times, and concluded that pirarucu preferred to feed at night, although growth rates were better when pirarucu were fed in the daytime. In our trials, pirarucu showed diurnal self-feeding behaviour, which agrees with other studies that have used self-feeders in salmonids such as *Salvelinus alpinus* (Linnér et al., 1990), *Salmo salar* (Fraser and Metcalfe, 1997; Bremset, 2000), *Salmo trutta* (Bremset, 2000), *Oncorhynchus mykiss* (Railsback et al., 2005) and *Oncorhynchus masou masou* (Flood et al., 2011). Our results corroborate those of Mattos et al. (2016), who observed predominant daytime feeding activity, with 110.86 ± 0.27 counts/day, 70% of total actions versus 47.93 ± 0.11 counts/day, 30% at night in pirarucu. However, plasticity of feeding behaviour should be observed in some species; for instance, European sea bass (*Dicentrarchus labrax*) present daytime feeding from spring to autumn, but change to nocturnal feeding behaviour in winter (Sanchez-Vazquez et al., 1994); Nile tilapia (*Oreochromis niloticus*) also display daytime (Toguyeni et al., 1997), nocturnal (Fortes-Silva et al., 2010a), or even twilight, feeding behaviour (Fortes-Silva et al., 2010b).

In the macronutrient selection trials, pirarucu fish's ability to defend their nutrient target was challenged. This capability has been previously shown in fish (Simpson and Raubemheimer, 2001). Given the option of choice, fish had more energy intake from protein and subsequently from lipids. After challenging with 50% protein dilution, pirarucu juveniles sustained their previous protein selection percentage (56%). This feeding behaviour has also been observed for carnivorous fish like *Dicentrarchus labrax*, which selected a diet rich in protein 59% P, 22% C and 19% F (Aranda et al., 2000), *Diplodus puntazzo* at 63% P, 21% C and 16% F (Vivas et al., 2006) and *Oncorhynchus mykiss* 64% P, 18% C and 18% F (Sánchez-Vázquez et al., 1999). Self-selection studies of diets in omnivore fish, such as tilapia (*Oreochromis niloticus*) or goldfish (*Carassius auratus*), have shown that they select a lower protein percentage (45.4% and 18.8%, respectively) (Fortes-Silva et al., 2011b; Sanchez-Vazquez et al., 1998). Our findings revealed that pirarucu preferred high protein intake, which supports the working hypothesis that different fish species have

“nutritional wisdom”, and thus defend a diet based in pure macronutrient, that best matches their intake target (Rubio et al., 2003; Almada-Pagan et al., 2008; Fortes-Silva et al., 2011b; Fortes-Silva, et al, 2012). This behaviour is in line with other studies that have reported a similar energy intake adjustment pattern after diets were diluted (Rubio et al, 2009; Almada-Pagán et al, 2006; Vivas et al., 2006). The hypothesis proposed for regulating nutrient intake in fish is similar to that in mammals (Badman and Flier, 2005). Many regulatory substances, such as leptin, cortisol, glucose and insulin, are integrated into the food intake regulation centres in the hypothalamus, and monoamines and neuropeptides play a central role in transmitting signals (anorexigenic and orexigenic messages) (Kulczykowska and Sanchez-Vazquez, 2010). These substances are able to modify feeding behaviour in fish according to eating habits and nutritional targets (Simpson and Raubenheimer, 2001).

When protein was restricted, pirarucu failed to maintain their previous energy intakes by increasing carbohydrate and fat intakes. This finding confirms that carnivorous fish require higher percentages of protein in their diet. Studies on European sea bass (*Dicentrarchus labrax*) have also demonstrated that when fish were protein-deprived and fed only fat and carbohydrates, they stopped eating, which indicates their strict carnivorous habit (Vivas et al., 2003). The energy intake of gilthead sea bream (*Sparus aurata*) continued, however, when fish were deprived of protein (Montoya et al., 2012). Omnivorous (generalist-feeding) fish are able to maintain energy intake using non-protein energy sources, such as dietary carbohydrates (Shiau and Chen, 1993). This finding has also been observed in studies with tilapia (*Oreochromis niloticus*) (Fortes-Silva et al., 2011b) and sharpnose sea bream (*Diplodus puntazzo*) (Vivas et. al., 2006). Indeed, omnivorous animals exhibit better behavioural and physiological flexibility when challenged to nutrient imbalance, which specialist animals cannot cope with (Raubenheimer and Simpson, 2003). Occurrence of a flexible diet is a striking aspect of tropical riverine ichthyofauna, where most species switch from one food item to another depending on the relative abundance of a feeding resource during a given season and in a spatial environment. These considerations highlight the difficulty of setting up reliable specific species feeding patterns in tropical teleosts (Abelha et al., 2001).

5. Conclusions

The results obtained herein provide data on the feeding behaviour of pirarucu (*Arapaima gigas*) and show their food preference for a daytime diet rich in protein for the first time. After nutritional challenge, fish defended their nutritional intake target: 56.3% protein, 24.2% carbohydrates, 19.5% fat and 150-151 kJ/kg BW/Day energy. After protein deprivation, pirarucu were unable to sustain their previous energy intakes, which reflects their strict carnivorous feeding habits. These findings agree with Fortes-Silva et al (2015), who studied diet self-selection by using animals as a guide to provide a useful tool for the aquaculture industry in order to develop feeding nutritionally sound protocols and aquafeed for new species with a potential for aquaculture.

Acknowledgements

The authors of this research thank AguaVale Aquaculture for supplying juvenile pirarucu, the Poytara for supplying food ingredients and the Higher Education Personnel Improvement Coordination (CAPES) for granting doctoral scholarships. This work was supported by the National Scientific and Technological Development (CNPq, Grant no. 477408/2013-3) and FAPESB (RED0004/2013 and PNE013/2014) grants awarded to Dr. Rodrigo Fortes. The authors wish to acknowledge João Lúcio Pimenta de Mattos for kind supporting and assisting with the study.

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Chapter 03: A new approach to feed frequency studies and protein intake regulation in juvenile pirarucu



Anais da Academia Brasileira de Ciências (2017) 89(2): 1243-1250
 (Annals of the Brazilian Academy of Sciences)
 Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
<http://dx.doi.org/10.1590/0001-3765201720160349>
www.scielo.br/aabc

A new approach to feed frequency studies and protein intake regulation in juvenile pirarucu

BRUNO O. DE MATTOS¹, EDUARDO C.T. NASCIMENTO FILHO¹, ALINE A. SANTOS², KAYCK A. BARRETO², FRANCISCO J. SÁNCHEZ-VÁZQUEZ³ and RODRIGO FORTES-SILVA²

¹Departamento de Zootecnia e Medicina Veterinária, Campus Ondina, Universidade Federal da Bahia/UFBA, Avenida Adhemar de Barros, s/n, Ondina, 40170-110 Salvador, BA, Brazil

²Laboratório de Comportamento Alimentar e Nutrição de Peixes, Centro de Ciências Agrárias, Ambientais e Biológicas, Campus Cruz das Almas, Universidade Federal do Recôncavo da Bahia/UFRB, Rua Rui Barbosa, 710, Centro, 44380-000 Cruz das Almas, BA, Brazil

³Department of Physiology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100, Murcia, Spain

Manuscript received on June 3, 2016; accepted for publication on December 8, 2016

ABSTRACT

This study aimed to investigate pirarucu's (*Arapaima gigas*) ability to trigger a self-feeding system to regulate protein intake between two standard diets that contained 39% and 49% of crude protein. The same system allowed the evaluation of daily feeding and locomotor activity rhythms. Eighteen fish (654.44±26.85g) were distributed into six 250 L tanks (3 fish/tank). Fish had free access to both diets (39% vs. 49% protein) by feeders (2 per tank), adapted to be activated by fish themselves. This system was connected to a computer system. After an adaptation period, fish learned to activate feeders and the mean food intake recorded was 2.14% of their body weight on a daily basis. Fish showed feeding (72.48%) and locomotor (72.49%) activity predominantly during the daytime, and daily variations of choice between diets, but fixed a protein intake feeding target at 44.53%. These results should be considered when discussing feeding behavior, feeding schedules and diet intake regulations.

Key words: *Arapaima gigas*, feeding behavior, protein intake, self-feeding.

INTRODUCTION

Fish show greater variety of eating behaviors compared to terrestrial animals. Given this variability, they are considered good experimental models for studying nutrient intake regulation (Volkoff and Peter 2006). Several studies

have demonstrated fish's ability to regulate nutrient intake, such as vitamins (*Oncorhynchus mykiss*), zinc (*Oncorhynchus mykiss*), taurine (*Dicentrarchus labrax*), oil sources (*Oreochromis niloticus*), and methionine/threonine/tryptophan (*Oreochromis niloticus*). Other studies have shown fish's ability to regulate macronutrient intake based in post-absorptive signals, e.g. proteins, lipids and carbohydrates (Fortes-Silva et al. 2016).

Correspondence to: Bruno Olivetti de Mattos
 E-mail: fortes@ufirb.edu.br

ABSTRACT

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1. INTRODUCTION

Fish show greater variety of eating behaviours compared to terrestrial animals. Given this variability, they are considered good experimental models for studying nutrient intake regulation (Volkoff and Peter 2006). Several studies have demonstrated fish's ability to regulate nutrient intake, such as vitamins (*Oncorhynchus mykiss*), zinc (*Oncorhynchus mykiss*), taurine (*Dicentrarchus labrax*), oil sources (*Oreochromis niloticus*), and methionine/threonine/tryptophan (*Oreochromis niloticus*). Other studies have shown fish's ability to regulate macronutrient intake based in post-absorptive signals, e.g. proteins, lipids and carbohydrates (Fortes-Silva et al. 2016).

Research into fish's free choice is essential to learn fish feeding behaviours that have not been fully explored at aquaculture levels. This practice may also contribute to animal welfare as it provides animals with self-select diets (Volpato et al. 2007). This approach could help to understand the relations between feeding behavior and nutrition (Forbes 2001; Simpson and Raubenheimer 2001), fish metabolism (Boujard and Leatherland 1992; López-Vázquez et al. 2009), and also animal growth (Bolliet et al. 2000). Although several studies have demonstrated fish's ability to select diets, very few studies have addressed this issue in freshwater tropical fish.

The self feeding system, in turn, also allowed us to measure feeding activity, daily rhythm and fish's preferential feeding hours. Variation in feeding patterns may exist among different fish species and their development stages, as well as a high degree of flexibility or behavioural change (Boujard et al. 1992). For example, *Oreochromis niloticus* has been described as a fish with plasticity to feeding behaviour and with a varying feeding period, which is considered daily for this species (Toguyeni et al. 1997; Vera et al. 2009; Fortes-Silva et al. 2010a). The self-feeding system allowed feeding to be fixed at the exact time that fish need food, which reduces the possible bias of a fixed time feeding schedule (Sánchez-Vázquez et al. 1996). Unlike manual feeding or automatic feeders, this methodology allowed fish themselves to trigger dispensing and self-feeding (Fortes-Silva et al. 2016).

This study aimed to investigate in pirarucu's (*Arapaima gigas*) ability to trigger a self-feeding system to regulate protein intake between two standard diets that contained 39% and 49% of crude protein. The same system allowed the evaluation of daily feeding and locomotor activity rhythms.

2. MATERIALS AND METHODS

2.1. Animal Housing

The work was carried out at the Fish Feeding Behavior and Nutrition Laboratory (AQUAUFRB), Universidade Federal do Recôncavo da Bahia (Cruz das Almas, Bahia, Brazil) during March/April of 2016. Eighteen juvenile pirarucus (654.44 ± 26.85 g), provided by AguaVale Fish Farming (Bahia, Brazil), were distributed into six 250 L tanks (3 fish per tank). The outdoor system, assembled in water recirculation, was equipped with mechanical filters, ceramic ring biofilters and 60 W UV light. The light intensity in the tanks was measured with a portable digital photometer (Luxímetro, São Paulo, Brazil). Fish were placed under a natural light photoperiod, with about 13 h of light (with threshold value of 900 lux occurring at midday) and 11 h of darkness (≤ 1 lux). Water parameters, temperature ($29.00 \pm 0.70^\circ\text{C}$), pH (6.55 ± 0.50), oxygen (7.80 ± 0.60 mg/L) and ammonia (5.00 ± 0.50 mg/l), were measured daily throughout the experiment and were kept in an acceptable range for the specie (Cavero et al. 2004). The experiment was conducted according to the principles of ethics in animal experimentation of the Ethics Commission on the Use of Animals (CEUA-UFRB) case number n° 23007.005093/2016-70.

2.2. Experimental Diets

Two experimental diets were used, one with 39% crude protein (in dry matter basis) and another 49% (Table 2), following a protein consumption range commonly observed on carnivorous fish (Gatlin 1999). Diets were extruded by the Pratigi Alimentos Company (Bahia, Brazil) and were analysed by standard Official Analytical Chemists Association (AOAC, 2012) methods. Content moisture was determined by drying for 24 h at 110°C to constant weight, crude protein by the Kjeldahl method ($\text{N} \times 6.25\%$), crude fat by diethyl ether extraction, ash by heating at 450°C for 24 h, and nitrogen-free extract (NFE) as the remainder of crude protein, crude fat and ash.

Table 2 - Composition of experimental diets.

Ingredients (g 100 ⁻¹ g of diet)	Diet 1 (39% of crude protein)	Diet 2 (49% of crude protein)
Fish meal	29.25	22.46
Poultry viscera flour	15.95	31.91
Soy bran	23.00	23.00
Corn bran	6.00	6.00
Corn grain	14.95	6.53
Fish oil	9.25	8.50
Dicalcium phosphate	0.50	0.50
Vitamin and mineral mix ^a	1.00	1.00
BHT	0.10	0.10
Proximate analysis (%)		
Dry matter	93,12	92,02
Crude protein (N x 6.25%)	39,22	49,26
Crude fat	20,95	14,35
NFE	25,26	17,02
Ash	12,44	17,22

^a Vitamins and minerals (mg/kg diet): Vit. A (min) = 1200.000 UI; Vit. D3 (min) = 200.000 UI; Vit. E (min) = 12.000 mg; Vit. K3 (min) = 2.400 mg; Vit. B1 (min) = 4.800 mg; Vit. B2 (min) = 4.800 mg; Vit. B6 (min) = 4.000 mg; Vit. B12 (min) = 4.800 mg; Vitamina C = 48.000 mg; Folic acid (min) = 1.200 mg; Pantotenic Acid (min) = 12.000 mg; Biotin (min) = 48 mg; Colin (min) = 65.000 mg; Niacina (min) = 24.000 mg; Iron (min) = 10.000 mg; Copper (min) = 600 mg; Manganes (min) = 4.000 mg; Zinc (min) = 6.000 mg; Iodo (min) = 20 mg; Cobalt (min) = 2 mg; Selenium (min) = 20 mg;

2.3. Experimental Design

Fish were initially submitted to a 1-week acclimatization period to adapt to their new experimental conditions. Commercial automatic feeders (Igarapé, São Paulo), were adapted with a trigger “steel line that works as a switch” placed (3 cm below water surface) (Almáida-Pagan et al. 2008; Mattos et al. 2016). Every time fish bite the trigger of a feeder, about 5 pellets of the corresponding experimental diet were released (on average 0.26 g). Feeders were previously adjusted to avoid overfeeding based in preliminary observations of appetite and feed intake. Feeding activity was measured as described by Fortes-Silva and coworkers (2016) using a software to acquire data from the feeders (DIO98USB, University of Murcia, Spain). After this adjustment period, experimental diets (39% and 49%) were offered in separate feeders so that fish could select protein percentages. To evaluate intake, 200 g of the experimental diets were placed daily in each feeder. Daily feed intake was calculated

from the weight considering the remainder of food placed in the feeders and the possible feed losses left in water.

Simultaneously to the feeders triggering activity, data acquired from infrared photocells (Omron, Model AD62-E3S, Japan) were recorded to measure locomotor activity, using the same software and hardware equipment. Two tanks have received an infrared photocell each, which was placed 25 cm from the tank's bottom. Every time fish passed the sensors, information was collected and sent to the computer (Kitagawa et al. 2015). Information was recorded every 10 minutes throughout the trial period. Thus a database was generated during the experimental period with both the feeding activity and locomotor activity rates.

At the end of the experiment, weight gain, daily feed intake and feed conversion were calculated for simple system validation purposes.

2.4. Data Analysis

The feeding and locomotor activity data were assessed by software (Temps, v.1, 179 Dr. Diez Noguera, Barcelona, Spain) to plot actograms and wave charts. Actograms were doubly plotted for better viewing. Those data were transformed to percentage to subsequent analysis.

The diet selection results are shown at 100% with the corresponding standard error. All percentage values were arcsine-transformed ($\sqrt{\cdot}$), which was used to achieve variance homogeneity and for the subsequent statistical analysis. Protein intake was expressed as \pm average standard error.

Data were evaluated by Student's t-test test for feeding and locomotor activity, diet selection and one-way ANOVA, Tukey test for protein intake between the days of experiment. Statistical significance was set at $p < 0.05$., and analysed by the SPSS program, version 15.0.

3. RESULTS

Although the main objective of this work was to evaluate a new feeding methodology for the species under study, in order to obtain feeding behaviour data, some performance parameters were considered to validate this methodology. Fish quickly learned to associate triggering feeders with food reward. The feed intake using this system stabilized around 21.42 ± 0.75 g/kg body weight/day. This consumption allowed fish to accomplish a feed conversion of 1.00 ± 0.06 and a weight gain of 609.44 ± 52.46 g within 28 days of experiment. There were no food leftovers, which indicated that fish adapted to this self-demand system.

3.1. Feeding And Locomotor Activity Rhythms

Fish started the trial period perfectly able to trigger the stretch sensors of the demand feeders. The wave chart and actogram showed a strict daytime feeding activity for pirarucu with 72.48% of total activity ($P < 0.05$), occurring during the light phase (Fig. 9A and 9B).

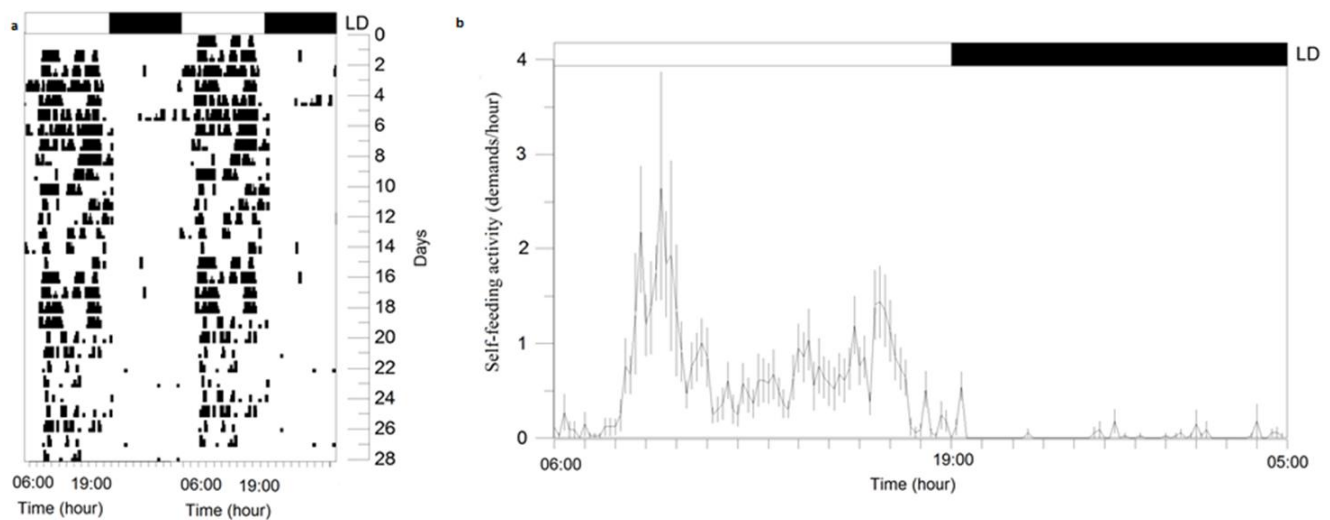


Figure 9 - Actogram (A) and average daily waveforms (B) for pirarucu's (*Arapaima gigas*) feeding activity rhythms measured by a self-feeding system. The white and black bars at the top of the graph indicate the duration of the light (13 h) and night (11 h) phases, respectively.

A similar behaviour to feeding activity was noted for locomotor activity, with 72.49% of activity occurring in the daytime (Fig. 10A and 10B). The statistical analysis revealed the predominance of daytime activity ($P < 0.05$).

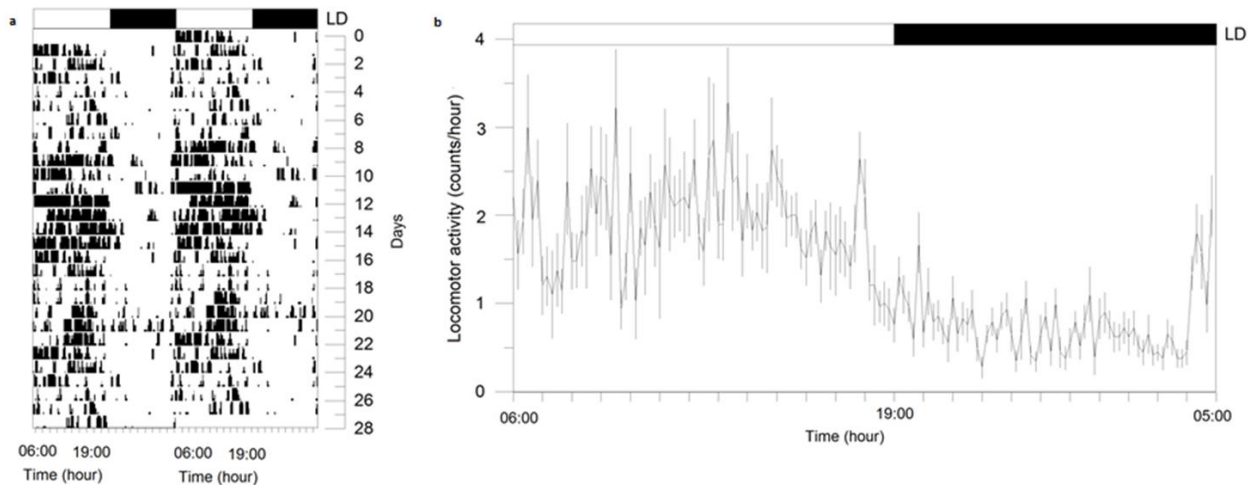


Figure 10 - Actogram (A) and average daily waveforms (B) for Pirarucu's (*Arapaima gigas*) locomotor rhythm measured by infrared photocells. The white and black bars at the top of the graphic indicate the duration of the light (13 h) and night (11 h) phases, respectively.

3.2. Protein Consumption Regulation

The daily selection patterns of the two diets showed fluctuations, which alternated throughout the experimental period. Thus, there were differences in the diet selection in the days 04, 15, 25, 26 ($P < 0.05$). On the other hand, the fish' protein intake remained constant ($44.53 \pm 0.26\%$), and no significant differences were found in protein consumption for the whole experimental phase ($P > 0.05$) (Fig. 11).

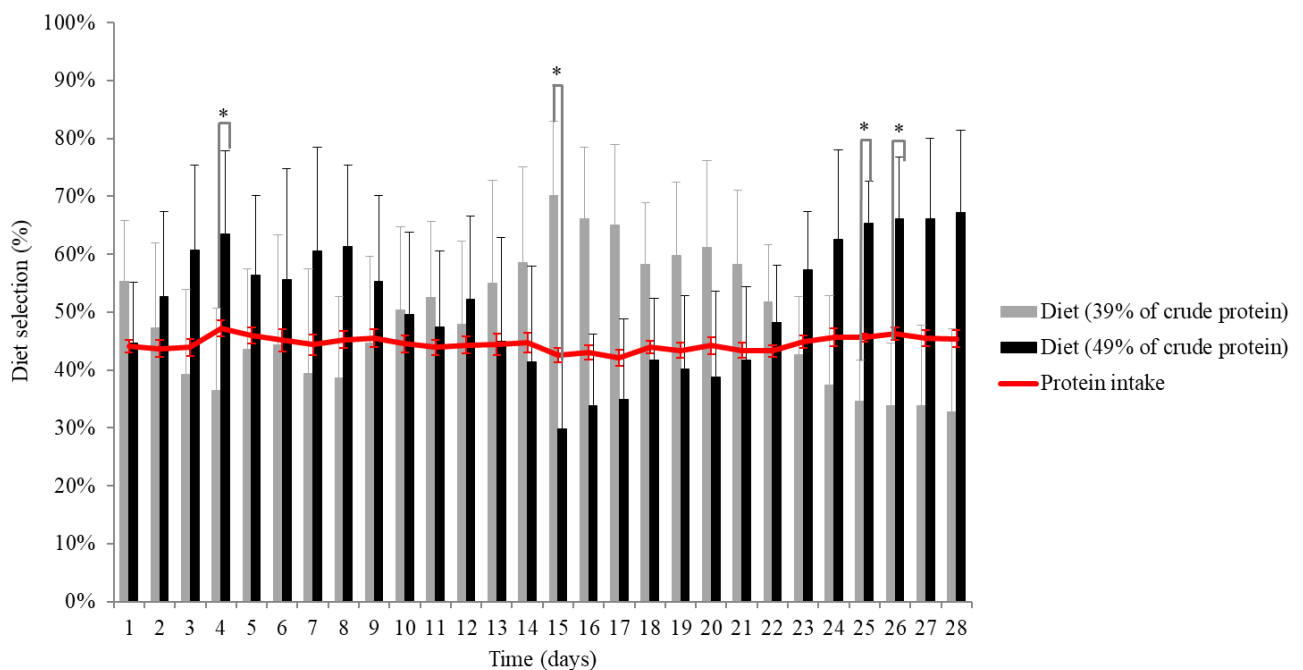


Figure 11 - Daily evolution of experimental diets selection (39% and 49% crude protein) and protein intake. Values of diets selection with asterisk are significantly different, ANOVA, $P < 0.05$, $n=6$.

4. DISCUSSION

The results showed that juvenile pirarucu (*Arapaima gigas*) used demand feeders efficiently and they displayed clearly a daytime feeding habit consistently and similarly to locomotor activity. This suggests that demand feeder methods are efficient tools to measure the diet preferences and protein intake regulation of this species. Although very few studies have evaluated the dietary behaviour of pirarucu. Crescêncio et al. (2005), observed the preference of pirarucu for nighttime feeding when they fixed feeding times, however, fish showed better performance when fed in the daytime. However, given the choice fish preferred feeding during the daytime. These findings bring to light discussions on the use of self-feeding tool under experiments of food intake, feeding behavior, and in turn on feed schedule. The fixed feeding schedules in aquaculture present a bias since fish is usually feed at specific times according to the handler's convenience, which may contribute to waste accumulation and poor use of food (Cho 1992; Nunes et al. 1996; Lima et al. 2009).

The data on pirarucu feeding activity obtained herein were similar to those reported by Mattos et al. (2016), who used the same self-feeding methodology, but we also noted a diurnal pattern for locomotor activity, which was not observed in previous studies. Even though pirarucu has mandatory bimodal respiration, which implies having to constantly move up to the water surface to breathe (Graham, 1997; Fernandes et al. 2012; Jiang et al. 2016; Lefevre et al. 2016), the diurnal pattern noted for locomotor activity was evident. According to Del Pozo et al. (2011), zebra fish present nocturnal feeding behaviour and diurnal locomotor behaviour. Fortes-Silva et al. (2010b) also observed this dualistic behaviour in tilapia (*Oreochromis niloticus*). It seems logical that the feeding activity pace is similar to locomotor activity. However, fish's behavioural plasticity makes them a very important experimental model.

Our results showed that juvenile pirarucu (*Arapaima gigas*) used demand feeders efficiently to maintain the ingested protein target. The value obtained for protein intake remained constant throughout the experiment period, and was similar to that reported by Ituassú et al. (2005) and Del Risco et al. (2008) for the species.

Although the animals in our study maintained a stable protein intake, we observed a strong demand rotation between the different diets throughout the experimental period. This behaviour (demand) can be described as a compromise rule,

where animals attempt to increase the consumption of a particular nutrient, but will be subject to suffer the metabolic consequences of excessively consuming other nutrients. This effect has been described by Raubenheimer and Simpson (1999) in studies that have addressed nutritional geometric analyses, and have been applied in nutritional studies on insects (*Manduca sexta* L.) (Thompson and Redak 2005). Studies that have used this approach first started to do so in experimental models, such as insects. Raubenheimer and Simpson (2003) reported that when locusts (*Locusta migratoria* - expert in one type of grass, and *Schistocerca gregaria* - herbivore in general) were confined and restricted to a nutritionally unbalanced diet, they had to regulate their intake of nutrients in order to mitigate nutrient excess or deficit.

These studies are considered pioneering and are examples for other species of farm animals. The same geometrical approach in animal nutrition has been considered valid to study diet selection in fish (Simpson and Raubenheimer, 2001). So when given the choice, fish have the ability to regulate nutrient intake, and to not suffer metabolic consequences of any excess or lack of essential nutrients that could condition their survival in nature (Fortes-Silva et al. 2016). Some authors call this innate ability “fish nutritional wisdom” (Rubio et al. 2003; Almaida-Pagan et al. 2008).

The primary goal of this study was not to assess fish performance parameters, but to assess the new feeding methodology for feeding behaviour studies in pirarucu. However, food consumption and conversion allowed us to gain an idea about the efficiency of this system. According to Crescêncio et al. (2005), the food conversion for pirarucu was 1.90 ± 0.06 and weight gain 692 ± 31 g over their 60-day experiment. With the self-feeding system, the food conversion result was 1.00 ± 0.06 and weight gain was 609.44 ± 52.46 g over 28 days. These data suggest better performance with free access to food supply. The way in which the feed is delivered mainly influences the growth rates and feed efficiency (Shi et al. 2016). According to Montoya et al. (2010), self-feeding systems allow fish to synchronise locomotor activity with metabolic and daily endocrine rhythms on the production of digestible enzymes for feeding times. Although for maximum benefit to be gained from the diet, feeding strategies should reflect the feeding rhythms of the fish (Azzaydi et al. 1998).

5. CONCLUSIONS

In conclusion, pirarucu was able to use the self-feeding system and showed strictly diurnal feeding and locomotor rhythms. Juvenile pirarucu used the feeders to adjust protein intake and to maintain a stable pattern of 44% crude protein. The self-feeding method can be considered a tool to help investigate food intake regulation, feeding behavior and feeding schedule.

ACKNOWLEDGEMENTS

The authors of this research thank AguaVale Aquaculture for supplying juvenile Pirarucu, the Pratigi Alimentos for supplying diets and the Higher Education Personnel Improvement Coordination (CAPES) for granting doctoral scholarships. This work was supported by the National Council for Scientific and Technological Development (CNPq, Grant number 477408/2013-3) and FAPESB (RED0004/2013 and PNE013/2014) grants awarded to Dr. Rodrigo Fortes. The authors wish to acknowledge João Lúcio Pimenta de Mattos for his kind support and assistance during the study.

RESUMO

Este estudo teve como objetivo investigar a capacidade do pirarucu (*Arapaima gigas*) em acionar um sistema de auto-alimentação para regular a ingestão de proteína entre duas dietas padrão que continham 39% e 49% de proteína bruta. O mesmo sistema permitiu a avaliação dos ritmos diários de atividade alimentar e locomotor. Dezoito peixes ($654,44 \pm 26,85\text{g}$) foram distribuídos em seis tanques de 250 L (3 peixes/tanque). Os pirarucu tiveram acesso livre as duas rações (39% vs 49% de proteína) por meio de dois alimentadores adaptados para serem acionados pelos próprios peixes conectados ao um sistema computacional de registro. Após um período de adaptação, os peixes aprenderam a ativar os alimentadores e o consumo médio registrado foi de 2,14% do seu peso corporal diariamente. O sistema foi capaz de captar a atividade alimentar (72,48%) e locomotora (72,49%) predominantemente no período diurno. Com relação à seleção da dieta pelos peixes, os mesmos apresentaram variações de escolha ao longo do período experimental, entretanto, o consumo de proteína foi constante nesse período, com o alvo protéico 44,53%. Esses resultados devem ser considerados quando se discute comportamento alimentar, cronograma de alimentação e metas nutricionais.

Palavras-chave: *Arapaima gigas*, comportamento alimentar, consumo protéico, auto-alimentação.

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CONSIDERAÇÕES FINAIS E IMPLICAÇÕES

A utilização do sistema de auto demanda para alimentação do pirarucu foi eficiente, promovendo um bom desempenho dos peixes e reduzindo o desperdício de alimento, uma vez que não foram encontradas sobras em todo o período experimental. Este sistema permitiu também a avaliação correta de seu hábito alimentar carnívoro. Quando ofertada uma dieta com base em misturas incompletas de macronutrientes e também dieta equilibrada, o pirarucu selecionou uma porcentagem fixa de proteína bruta. Após o desafio nutricional de restrição proteica, o peixe foi incapaz de sustentar a ingestão de proteína subindo o consumo da dieta.

Ainda, o sistema de autoalimentação conectado ao sistema computacional demonstrou que o pirarucu apresentou ritmo alimentar estritamente diurno. Essa metodologia, poderá auxiliar a aquicultura, no que tange as estratégias de alimentação desta espécie, incluindo horários e cronogramas alimentares precisos.

Por fim, os pirarucus apresentaram em dois momentos distintos do trabalho diferentes ritmos locomotores, o que pode ser explicado pelo tamanho dos peixes, pois, animais com menor tamanho apresentam maior mobilidade que animais maiores. Também este fato pode ser devido aos períodos (verão e outono), uma vez que, dependendo da estação, os peixes podem apresentar diferentes ritmos.