UNIVERSIDADE FEDERAL DE MINAS GERAIS
ESCOLA DE VETERINÁRIA

CAPTURA, ADAPTAÇÃO, REPRODUÇÃO E PERFIL DE AMINOÁCIDOS E ÁCIDOS GRAXOS DE OVOS E LARVAS DE PACAMÃ (*Lophiosilurus alexandri*)

DELIANE CRISTINA COSTA

BELO HORIZONTE

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unida pelo espírito e não pelo sangue.

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Laqua	Laboratório de Aquaculura da UFMG
LRE	Larvas recém eclodidas
LFL	Larvas no final do período lecitotrofico
NHL	New hatched larvae
LPL	End of the lecithotrofic larval period
IAA	Indispensable amino acids
DAA	Dispensable amino acids
FA	Fatty Acids
NL	Neutral lipids
PL	Polar Lipids
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
HUFA	Highly unsatured fatty acids

RESUMO

CAPTURA, ADAPTAÇÃO, REPRODUÇÃO E PERFIL DE AMINOÁCIDOS E ÁCIDOS GRAXOS DE OVOS E LARVAS DE PACAMÃ (*Lophiosilurus alexandri*)

O presente estudo objetivou analisar a captura, transporte, adaptação e reprodução de matrizes selvagens de *Lophiosilurus alexandri* às condições de laboratório. Foram realizadas duas campanhas para coletas de exemplares a partir de habitats naturais. Os animais sobreviventes da primeira (7) e segunda (17) captura foram divididos em quatro tanques de 5 m^3, sendo em dois deles adicionada camada de areia e mantidos a temperatura de 28ºC. As desovas ocorreram de forma natural 30 dias após o aumento da temperatura, mas somente nos tanques com fundo de areia. No primeiro período reprodutivo foram coletados 24 desovas nestes tanques. No segundo período reprodutivo os animais foram sexados por canulação e distribuídos novamente em quatro tanques, revestidos com areia no fundo e mantidos a temperatura de 28ºC. Em todos os tanques ocorreram desovas num total de 36. Durante a etapa de reprodução, foram coletadas amostras de ovos, larvas recém eclodidas (LRE) e larvas no final do período lecitotrófico (oito dias pós eclosão) (LFL) para determinação do perfil de aminoácidos e de ácidos graxos. A proteína bruta não apresentou variações no decorrer do desenvolvimento ($P > 0,05$) e a concentração de lipídeos foi superior em LRE ($P < 0,05$). O aminoácido (AA) encontrado em maior concentração em ovos, LRE e LFL foi a Glutamina. Os AA indispensáveis (IAA) Isoleucina, leucina e valina sofreram redução em (LFL), enquanto em ovos LRE os valores foram superiores e semelhantes entre si. A arginina e lisina apresentaram valores inferiores e semelhantes em ovos e LRE e aumentaram sua participação em LFL. Os AA dispensáveis (DAA) como ácido aspártico, tirosina e glicina aumentaram em LFL enquanto a alanina foi reduzida nesta fase de vida. Com relação aos lipídios neutros (NL) sua porcentagem aumentou nas larvas LFL. O ∑SFA (ácidos graxos saturados) reduziu durante o desenvolvimento ontogenético, enquanto ∑MUFA (ácidos graxos monoinsaturados) foi reduzido somente em LFL. O ∑PUFA (ácidos graxos poli-insaturados) foi maior em LFL provavelmente devido ao aumento nas porcentagens dos ácidos graxos Araquidônico (20:4n-6) (ARA), Eicosapentaenoico (C20:5n3) (EPA) e docosahexaenoico (C22:6 n3) (DHA). Os ácidos graxos polares (PL) foram encontrados em maiores porcentagens ovos e LRE e foram reduzidos em LFL. O ∑SFA reduz ao longo do desenvolvimento ontogenético enquanto o ∑MUFA é reduzido somente em LFL. O ∑PUFA foi superior em LFL, provavelmente devido ao aumento nas porcentagens de ARA e DHA. EPA manteve suas porcentagens inalteradas ao

longo do desenvolvimento. O pacamã se adapta e reproduz em condições de laboratório, com possibilidade de manipulação do período reprodutivo pelo controle da temperatura da água e alimentação. A proteína é mantida durante o desenvolvimento ontogenético embora tenha havido mudanças nas classes de aminoácidos, como redução (IAA) como isoleucina, leucina e valina em LPL. *L. alexandri* utilizam preferencialmente SAFA e MUFA como fonte de energia tanto da fração NL como PL durante seu desenvolvimento inicial.

Palavras-chave: Conservação, cuidado parental, nutrientes

ABSTRACT

CAPTURE, ADAPTATION, REPRODUCTION AND PROFILE OF AMINOACIDS AND FATTY ACIDS IN EGGS AND LARVAE OF PACAMÃ (*Lophiosilurus alexandri*)

This study aimed to analyse the capture, transport, adaptation and reproduction of *Lophiosilurus alexandri* wild breeders when moved to laboratory conditions. Two sampling sessions in natural habitats were carried out. The surviving animals from the first and second sampling sessions were placed into four 5 m^3 tanks. A layer of sand was added to two tanks and they were kept at a temperature of 28ºC. Spawning occurred naturally 30 days after temperature rise, but only in tanks with a sand bottom. In the first reproductive period, 24 spawnings were collected from these tanks. In the second reproductive period, the animals were sexed by cannulation and divided again into four tanks with sand on the bottom and kept at a temperature of 28ºC. Spawning occurred in all tanks, totalling 36. During reproduction, samples of eggs, newly hatched larvae (NHL), and larvae at the end of lecithotrophic period (eight days after hatching) (LPL) were collected to determine the amino acid and fatty acid profiles. Crude protein did not change throughout the experiment ($P > 0.05$) and concentration of lipids was highest in NHL ($P < 0.05$). Glutamine was the amino acid (AA) found in the highest concentrations in eggs, NHL, and LPL. The essential amino acids (EAA) isoleucine, leucine, and valine decreased in LPL, while in eggs and NHL, the values were higher and similar to each other. Arginine and lysine showed lower and similar amounts in eggs and NHL and increased in LPL. The dispensable amino acids (DAA), such as aspartic acid, tyrosine, and glycine increased in LPL while alanine decreased in this stage of life. The neutral lipids (NL) percentage increased in LPL larvae. The ΣSFA (saturated fatty acids) decreased during ontogeny, while ΣMUFA (monounsaturated fatty acids) decreased only in LPL. The ΣPUFA (polyunsaturated fatty acids) was highest in LPL, probably due to increased percentages of the fatty acids Arachidonic (20:4n-6) (ARA), Eicosapentaenoic (C20:5n3) (EPA), and Docosahexaenoic (C22:6 n3) (DHA). Polar fatty acids (PL) were found in larger percentages in eggs and NHL and decreased in LPL. The ΣSFA decreased during ontogenetic development, while ΣMUFA decreased only in LPL. The ΣPUFA was highest in LPL, probably due to the increased percentage of ARA and DHA. The percentage of EPA remained unchanged throughout the experiment. The "pacamã" is able to adapt and reproduce in laboratory conditions, and it is possible to manipulate its reproductive period by controlling the water temperature and food. Protein is kept during ontogenetic development although

there were changes in the amino acid classes, such as a decrease (IAA) of isoleucine, leucine, and valine in LPL. *L. alexandri* preferably use SAFAs and MUFAs as an energy source, from both the NL and PL fractions, during its early development.

Keywords: Conservation, nutrients, parental care

1. REVISÃO DE LITERATURA

1.1. O pacamã (*Lophiosilurus alexandri*)

O *Lophiosilurus alexandri* (Steindachner, 1876) é uma espécie carnívora, endêmica do rio São Francisco, conhecida popularmente como pacamã (Travassos, 1959). Pertence à Classe Actinopterygii, Ordem Siluriformes e família Pseudopimelodidae. É uma espécie carnívora (Meurer et al., 2010; Souza et al., 2014), bentônica e de hábito alimentar noturno (Tenório et al., 2006). Seu alto valor de mercado, a pressão da pesca esportiva e o represamento de rios têm reduzido às populações selvagens desta espécie colocando-a na lista de peixes ameaçados de extinção (BioBrasil, 2014).

A reprodução do pacamã ocorre naturalmente ou através de indução hormonal (Sato et al., 2003; Santos et al., 2013), de forma parcelada, em tanques com o fundo recoberto por areia, com os reprodutores formando ninhos sendo assim classificado como uma espécie psamófila (Sato et al., 2003) e apresenta cuidado parental com o macho cuidando da massa de ovos (Costa et al., 2015). A fecundidade do pacamã é considerada baixa, o que é característico de espécies sedentárias, especialmente as que apresentam cuidado parental (Godinho, 2007; Santos et al., 2013). A sexagem desta espécie pode ser realizada através da análise comparativa das papilas genitais (Lopes et al., 2013) ou por técnicas de celiotomia (incisão na cavidade abdominal) e celioscopia (exame visual da cavidade abdominal) (Mellilo-Filho, 2016).

Os ovócitos não hidratados do pacamã possuem o maior tamanho dentre 23 espécies de peixes da Bacia do Rio São Francisco estudadas (Sato et al., 2003) sendo classificados como adesivos e grandes (3.056,7 ± 154,2 μm) (Bazzoli e Godinho, 1997; Rizzo et al., 2002; Guimarães-Cruz et al., 2009) e com período de incubação em torno de 56,6 horas (Santos et al., 2013). Durante a incubação dos ovos, a aplicação de Florfenicol em concentrações menores que 309 mg L^{-1} pode ser utilizada como medida profilática aumentando a viabilidade dos ovos (Batista et al., 2014).

No momento da eclosão, as larvas apresentam em média 8,4 mm de comprimento e saco vitelino com grande quantidade de reserva endógena (Guimarães-Cruz et al., 2009; Santos et al., 2013) que pode durar até cerca de sete dias ou mais dependendo da temperatura da água como observado no Laboratório de Aquacultura da UFMG, até ser completamente absorvido. Larvas recém-eclodidas podem ser cultivadas em água doce ou salinizada (Santos

e Luz, 2009) na proporção de 2 à 4 g de sal L^{-1}, podendo esta salinidade ser aumentada até 6 g de sal L^{-1} em larvas com 12 dias após a eclosão (Luz e Santos, 2008), a tolerância a salinidade no entanto diminui com o aumento da temperatura (Martins et al., 2014).

Diferentes tipos e tamanhos de alimentos (Pedreira et al., 2008; Tenório et al., 2006) e diferentes concentrações de alimentos vivos (Santos e Luz, 2009) podem ser utilizados durante a larvicultura do pacamã que se inicia, em geral, no 8º dia após a eclosão. O uso de náuplios de artêmia como alimento vivo durante a larvicultura de pacamã tem proporcionado altos valores de sobrevivência, em torno de 76,6% á 97,7%, peso médio de 154,6 mg e 24,6 mm de comprimento (Luz et al., 2011; Cordeiro et al., 2016). A temperatura da água tem forte influência nesta fase de larvicultura com melhores resultados a temperaturas entre 29 e 32º (Takata et al., 2014). Sua larvicultura também pode ser realizada em condições de laboratório sob diferentes fluxos de água (Luz et al., 2011). O uso de tanques de cor marrom (Pedreira et al., 2012) ou ambientes com reduzida luminosidade (Tenório et al., 2006) mostram bons resultados. Na larvicultura intensiva, pode-se empregar densidades de até 300 larvas L^{-1} em sistema de recirculação de água durante a fase de alimento vivo (Cordeiro et al., 2015). O uso de anestésicos como tricaina (Ribeiro et al., 2013a) ou eugenol (Ribeiro et al., 2013b) em larvas e juvenis durante operações de manejo é recomendo com a finalidade de minimizar os efeitos nocivos do estresse.

A utilização da técnica de condicionamento alimentar usando diferentes proporções de coração bovino durante a transição do alimento vivo pelo alimento inerte é considerada um fator chave para produzir juvenis de pacamã se alimentado exclusivamente de ração formulada (Luz et al., 2011; Santos et al., 2012; Melillo Filho et al., 2014; Silva et al., 2014). Salaro et al. (2015) verificaram que o coração bovino utilizado durante o condicionamento alimentar pode ser substituído por uma mistura de ração comercial e gelatina sem afetar o desempenho e sobrevivência dos animais. O tamanho inicial e a densidade de estocagem são fatores que afetam o sucesso do condicionamento para *L. alexandri*, sendo recomendado o uso de densidades de até 40 juvenis L^{-1} (Cordeiro et al., 2016). Após este processo de transição, os juvenis respondem positivamente ao consumo exclusivo de ração, fator que o caracteriza como uma potencial espécie para produção em cativeiro (Luz et al., 2011; Melillo Filho et al., 2014; Silva et al., 2014; Cordeiro et al., 2016). Nesta fase de vida a manutenção da temperatura em torno de 28,8ºC favorece o consumo de alimento (Costa et al., 2014). Em estudo de comportamento e com alimentadores, esta espécie é preferencialmente noturna, embora tenham a plasticidade para adaptar á outros horários de alimentação sem prejuízos ao desempenho (Kitagawa et al., 2015). A utilização da salinidade de 2,5 g de sal L^{-1} é

recomendada durante a fase de juvenil sem interferir no desempenho dos (Matiolli, 2014) proporcionando aos animais uma forma de medida profilática.

Dietas contendo níveis médios de proteína bruta de 36,2% (Souza et al., 2013) e inclusão de até 15% de uma fonte de carboidrato (Figueiredo et al., 2015), promoveram aumentos nos índices de ganho de peso, taxa de crescimento específico e conversão alimentar aparente de juvenis de pacamã.

Grande parcela dos estudos realizados com pacamã foi conduzida com larvas e juvenis. Aspectos sobre o comportamento reprodutivo em condições de confinamento ainda se encontram incipientes e são de suma importância para viabilizar a criação desta espécie em condição de confinamento de modo a obter uma oferta constante de formas jovens visando abastecer o mercado futuro. A viabilidade da domesticação de uma espécie nativa depende da capacidade de adaptação e de reprodução que os exemplares selvagens (futuros reprodutores) irão apresentar em condições de confinamento. No Brasil há escassez de informações científicas que relatem a captura de reprodutores de peixes selvagens e adaptação dos animais em condição de laboratório. Estas informações são importantes visando o estudo da biologia reprodutiva e formação de um plantel de reprodutores. Geralmente, os produtores e alguns institutos de pesquisas possuem estas informações. Contudo, por falta de sistematização nas capturas, estas informações não são publicadas e divulgadas a comunidade interessada.

1.2. Captura, adaptação e reprodução de espécies selvagens

A reprodução de espécies selvagens em cativeiro é mundialmente utilizada como estratégia de conservação de peixes ameaçados de extinção e constitui-se como uma ferramenta útil para a preservação ou restauração de populações selvagens (Kelley et al., 2006; Blanchet et al., 2008; Saravanan et al., 2013). A falta de informações sobre a captura de animais adultos (potencial reprodutores), transporte para instalações em cativeiro e como lidar com os animais para garantir alta sobrevivência das espécies de áreas tropicais e sub-tropicais é preocupante.

O ponto de partida para a domesticação de novas espécies de peixes para a aquicultura é o domínio da biologia reprodutiva e o desenvolvimento de estratégias para a manutenção de reprodutores em condições de confinamento de modo que realizem com eficiência seu processo reprodutivo visando à produção de larvas e juvenis (Pankhurst e Fitzgibbon, 2006).

A captura dos exemplares selvagens é marcadamente a etapa mais agressiva do processo, pois os animais são submetidos a condições de estresse durante a captura por exercício físico (Baker et al., 2008), por meio da perseguição e injúrias físicas que podem ser causadas pelas redes (Vander Haegen et al., 2004) e também por condições de hipóxia (Clearwater e Pankhurst 1997).

Durante o transporte, as variações nos parâmetros de qualidade da água e acúmulo de níveis de amônia e dióxido de carbono, além da densidade inadequada causam prejuízos à homeostase dos animais (Carneiro et al., 2009; Harmon, 2009). Estas ações geram reações fisiológicas no peixe induzindo o animal ao estresse (Iversen et al., 2005; Portz et al., 2006; Harmon, 2009; Tort, 2011). A magnitude e duração do estresse durante estes eventos podem durar de horas a dias dependendo do tempo de exposição a estes fatores causadores (Bolasina, 2011; Fanouraki et al., 2011).

O resultado do estresse de captura/manuseio está associado, principalmente, ao aumento do cortisol plasmático e redução dos níveis plasmáticos de andrógenos e estrógenos promovendo dentre outros fatores a atresia folicular (Clearwater Pankhurst, 1997). A elevação do cortisol em machos de trutas (*Oncovhynchus mykiss*,) resultou em redução significativa do teor de gonadotropina da hipófise, para metade da concentração circulante de testosterona e redução significativa no tamanho dos testículos. Da mesma forma na fêmea, altas concentrações de cortisol causaram um decréscimo no conteúdo de gonadotropina pituitária, redução nos níveis circulantes de testosterona e estradiol, supressão acentuada da quantidade de vitelogenina em trânsito a partir do fígado para o ovário e redução significativa no tamanho do ovário (Pickering, 1992).

O cortisol pode afetar também o sistema imune dos animais, deixando-os mais susceptíveis a infecções (Bonga 1997; Tort, 2011), uma vez que a correlação entre níveis de cortisol sanguíneo e susceptibilidade a doenças em peixes é alta (Pickering, 1992). Dessa maneira, nem todos os peixes transferidos para ao laboratório se adaptam ao cativeiro ou são capazes de reproduzir ou sobreviver (Ashley, 2007; Murchie et al., 2009). Práticas de manejo aplicadas ao processo de captura como diferentes tipos e diâmetros das malhas de rede (Vander Haegen et al., 2004; Cooke et al., 2008) uso de puçás (Grutter e Pankhurst 2000), tipos de caixas de transporte (Harmon, 2009), meios de transporte (Iversen et al., 2005; Murchie et al., 2009) e substâncias com potencial anestésico contribuem para redução de injúrias e estresse nos animais facilitando a aclimatação ao cativeiro (King, 2008).

Ainda, para o sucesso reprodutivo, o conhecimento sobre características da espécie como época e local de desova (Hachero-Cruzado et al., 2007), características físico-químicas

do ambiente (Moyo, 2011) e o comportamento reprodutivo (Madhu e Madhu, 2014; Maricchiolo et al., 2016) são de suma importância para que se possa manipular adequadamente a reprodução desses animais em cativeiro e devem ser previamente estudadas. Além destes fatores, a adoção de algumas estratégias reprodutivas como a construção do ninho e cuidado parental devem ser cuidadosamente observadas e são cruciais para espécies que se reproduzem sem indução hormonal em cativeiro (Esteve, 2005, Clotfelter et al., 2006; Ota e Kohda, 2006). O cuidado parental mantém a qualidade e garante a proteção de ovos e larvas de *Pomatoschistus minutus* (Lissåker e Kvarnemo, 2006; Lehtonen et al., 2013). Nesta mesma espécie, a seleção sexual do parceiro ocorre através da avaliação do cuidado parental de modo que as fêmeas preferem acasalar com machos que fornecem maior cuidado parental (Lindström et al., 2006). Machos de *Neogobius melanostomus* necessitam da utilização de pedras como substratos para a formação de ninho e sem esta condição os animais não realizam a reprodução (Meunier et al., 2009). No entanto, caso a reprodução não ocorra, de forma espontânea, técnicas de indução hormonal deverão ser avaliadas (Cañavate et a., 2005; Agulleiro et al., 2006; Duncan et al., 2012)

Outro fator a ser considerado durante a adaptação dos animais selvagens capturados ao ambiente de confinamento é a dieta. Esta deve ser o semelhante ao perfil alimentar que os animais recebem na natureza (Rodríguez et al., 2004). A oferta de alimentos muito aquém da necessidade nutricional dos animais impede o correto desenvolvimento dos animais e podem influenciar vários parâmetros reprodutivos, como o desenvolvimento gonadal, quantidade e qualidade das desovas, eclodibilidade e qualidade larval (Salze et al., 2005; Ling et al, 2006; Hachero-Cruzado et al., 2009).

Trabalhos foram realizados visando comparar o perfil de ácidos graxos e aminoácidos de espécies selvagens e de espécies mantidas em cativeiro a fim de fornecer respostas sobre estimativas de deficiências das dietas dos animais, o que levaria a perda de qualidade nas desovas e mortalidade das larvas, bem como, em alguns casos, a falta de resposta à reprodução (Sheikh-Eldin et al., 1996; Lund et al., 2008; Rodriguez-Barreto, 2012).

Durante todo o desenvolvimento embrionário e larval a síntese dos nutrientes contidos nos oócitos é totalmente dependente do fornecimento dos nutrientes maternos (Finn e Fyhn, 2010). Os aminoácidos e ácidos graxos são fatores nutricionais diretamente relacionados à qualidade dos ovos e larvas e peixes (Yanes-Roca et al., 2009; Samaee et al., 2010; Fuiman e Ojanguren, 2011). Desta forma existe um elevado interesse em entender a importância do perfil de aminoácidos e de ácidos graxos e relacioná-los com possíveis deficiências na alimentação dos reprodutores. O perfil de aminoácidos e ácidos graxos também fornecem

informações relevantes para a compreensão das necessidades nutricionais das larvas no inicio da alimentação exógena (Saavedra et al., 2006; Gurure et al., 2009; Farhoudi et al., 2012).

1.3. Composição de aminoácidos e ácidos graxos em ovos e larvas de peixes

Durante a embriogênese, até o início da alimentação exógena, todos os nutrientes necessários para o crescimento, diferenciação celular e metabolismo dos órgãos se originam a partir das reservas de vitelo (Wiegand 1996; Tocher, 2010). Sendo assim, os ovos dos peixes devem conter todos os nutrientes essenciais necessários para o desenvolvimento do embrião e crescimento das larvas (Kamler, 2008; Rønnestad et al., 1999).

Os constituintes dos lipídios e proteínas, ácidos graxos e aminoácidos (AA) respectivamente, são os componentes mais abundantes encontrados nos ovos de peixes (Finn e Fyhn, 2010). As quantidades destes nutrientes presentes em ovos e larvas de peixes sofrem variações nas suas concentrações no decorrer do desenvolvimento inicial (Cruzado et al., 2013; Yeganeh, et al., 2014). Além disso, são de suma importância devido a sua participação no metabolismo (anabolismo e catabolismo) de estruturas e órgãos desses animais (Wiegand, 1996; Sargent et al., 1999; Finn e Fyhn, 2010). A quantidade e o catabolismo das proteínas e lipídios parecem variar de acordo com as exigências fisiológicas de cada espécie bem como a sequência exata de consumo destes nutrientes (Ohkubo e Matsubara, 2002; Ohkubo et al., 2006).

Fatores como a dieta fornecida aos reprodutores (Gunasekera et al., 1996; Pickova et al., 2007; Yanes-Roca et al., 2009; Sink et al., 2010), habitat onde os reprodutores se encontram (selvagem ou domesticado) (Gunasekera et al., 1999; Dantagnan et al., 2007; Araújo et al., 2012; Lanes et al., 2012) dentre outros fatores, são variáveis capazes de modificar o perfil dos ácidos graxos e aminoácidos em ovos e larvas de peixes marinhos e de água doce.

As proteínas são degradadas e reabsorvidas e os AA são utilizados para a formação de tecidos ou catabolizadas para a produção de energia (Rønnestad et al., 2003; Finn e Fyhn, 2010; Kaushik e Seiliez, 2010; Cruzado et al., 2013). O metabolismo proteico durante o desenvolvimento inicial dos peixes pode diferir bastante entre espécies, principalmente no que diz respeito à quantidade, composição dos aminoácidos antes do inicio da alimentação e também sobre as diferentes funções dos aminoácidos (Gunasekera et al., 1999; Samaee et al., 2010; Finn e Fyhn, 2010).

Os requisitos de AA para fases jovens de peixes são altos devido à rápida taxa de crescimento (Conceição et al., 1998; Rønnestad et al., 1999). Dessa forma, a determinação do perfil de AA em ovos e larvas após a eclosão também pode ser usado como um indicador da qualidade nutricional das larvas de peixes (Cara et al., 2007; Jaya-Ram et al., 2008; Grote et al., 2011) e para estimar índices de exigências para fases jovens (Saavedra et al., 2006). A composição do perfil de aminoácidos e ácidos graxos no tecido das larvas fornecem informações relevantes para a compreensão das necessidades nutricionais das larvas no inicio da alimentação exógena (Saavedra et al., 2006; Gurure et al., 2007; Farhoudi et al., 2012). Desta forma, é possível comparar, posteriormente, estes perfis de nutrientes das larvas e das dietas oferecidas e determinar possíveis desequilíbrios alimentares (Conceição et al., 2003; Saavedra et al., 2007; Garrido et al., 2012; Oberg e Fuiman 2015).

Assim, conhecendo as exigências nutricionais é possível evitar deficiências de AA e suplementar as dietas evitando os baixos índices de crescimento e deformidades esqueléticas (Cahu et al., 2003; Conceição et al., 2003; Luo et al., 2005; Saavedra et al., 2006).

Os lipídios e seus constituintes ácidos graxos são o segundo componente mais abundante do ovo após as proteínas, cuja função principal é o fornecimento de energia e constituintes de membranas (Henderson, 1996; Glencross, 2009; Tocher, 2010). Ácidos graxos saturados e moninsaturados são os substratos energéticos, preferencialmente, utilizados pelas larvas para produção de energia (Cejas 2004; Dantagnan et al., 2007; Araújo et al., 2012). Já os ácidos graxos de cadeias longas como os poliinsaturados (PUFA) e altamente insaturados (HUFA) são requeridos para a formação da visão, sistema nervoso, precursores de eicosanoides (Bell, et al., 1995; Sargent et al., 1999) e resistência ao estresse (Carrier et al., 2011). Devido a sua essencialidade, os ácidos graxos também podem influenciar o sucesso de eclosão e qualidade dos ovos (Pickova et al., 2007) sobrevivência e taxa de crescimento das larvas (Samaee et al., 2009; Fuiman e Ojanguren, 2011).

Larvas de peixes marinhos necessitam de suplementação de PUFA essenciais como ácido eicosapentaenóico (20:5 n-3; EPA), ácido docosa-hexaenóico (22:6 n-3; DHA) e ácido araquidônico (20:4 n-6 ARA) via dieta dos reprodutores ou diretamente na alimentação para apresentar crescimento satisfatório durante as fases de desenvolvimento inicial (Sargent et al., 1999; Bransden et al., 2005; Jaya-Ram et al., 2008). Este fato se deve a ausência ou baixa atividade das enzimas Δ-6, Δ-5 e Δ-4 dessaturases e elongasse, que torna as espécies marinhas sem capacidade em alongar e dessaturar 18:2 n-6 e 18:3 n-3 a ácidos graxos de cadeias maiores (Izquierdo, 1996; Tocher 2010). Evidências bioquímicas e moleculares suportam a hipótese de que espécies dulcícolas são capazes de produzir HUFA biologicamente ativo a

partir de PUFA com cadeias a partir de 18 carbonos através do processo denominado de bioconversão com atividade de enzimas dessaturases e elongases (Henderson, 1996; Zheng et al., 2004; Agaba et al., 2005; Ling et al., 2006; Tocher, 2010) desde que seus precursores estejam disponíveis em quantidades suficientes para a ocorrência de tal processo.

Mudanças no perfil dos aminoácidos e ácidos graxos em relação ao desenvolvimento de espécies de água doce (Mello et al., 2011; Portella et al., 2013) foram pouco estudados quando comparado a espécies marinhas caracterizando escassez de informações sobre alterações na composição destes constituintes em espécies neotropicais de água doce, indicando a necessidade de mais estudos em diferentes espécies e condições de criação.

2. REFERÊNCIAS BIBLIOGRÁFICAS

Agaba, M.K., Tocher, D.R., Zheng, X., Dickson, C.A., Dick, J.R., Teale, A.J. 2005. Cloning and functional characterization of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. *Comparative Biochemistry and Physiology*, *Part B*, **142**, 342-352.

Agulleiro, M. J., Anguis, V., Cañavate, J. P., Martínez-Rodríguez, G., Mylonasf, C. C., Cerdà, J. 2006. Induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using different administration methods for gonadotropin-releasing hormone agonist. *Aquaculture*, **257**, 511-524.

Araújo, B.C., Honji, R.M., Mello, P.H., Moreira, R.G. 2012. The influence of captive breeding on the fatty acid profiles of *Salminus hilarii* (Characiformes: Characidae) eggs and larvae. *Aquaculture Internacional*, **20**, 1161-1181

Ashley, P.J. 2007. Fish welfare: Current issues in aquaculture. *Applied Animal Behavior Science*, **104**, 199-235.

Baker, D.W., Peake, S.J., Kieffer, J.D. 2008. The Effect of Capture, Handling, and Tagging on Hematological Variables in Wild Adult Lake Sturgeon. *North American Journal of Fisheries Management*, **28**, 296-300.

Batista, A.L.D., Filho, R.M., Takata, R., Silva, W.S., Santos, A.E.H., Luz, R.K. 2014. Incubação de ovos de pacamã com florfenicol. *Pesquisa agropecuária brasileira*, **49**, 911-914.

Bazzoli, N., Godinho, H. P. Ovócitos vitelogênicos do surubim *Pseudoplastystoma coruscans* e do pacamã *Lophiosilurus alexandri*. In MIRANDA, M. O. T. (org.). Surubim. Belo Horizonte: IBAMA, 1997, p.81-90 (Coleção Meio Ambiente, Série Estudos Pesca, 19).

Bell, J.G., Sargent, J.R. 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, **218**, 491-499.

BIOBRASIL. Efetividade das ações de conservação de peixes ameaçadas de extinção. *Biodiversidade Brasileira revista cientifica* 2014.

Blanchet, S., Páez, D.J., Bernatchez, L., Dodson, J.J. 2008. An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): Implications for supportive breeding programs. *Biological Conservation*, **141**, 1989-1999.

Bolasina, N.S. 2011. Stress response of juvenile flounder (*Paralichthys orbignyanus*, Valenciennes 1839), to acute and chronic stressors. *Aquaculture*, **313**, 140-143.

Bonga, S.E.W. 1997. The Stress Response in Fish. *Physiological Reviews*, **77**, 591-623.

Bransden, M.P., Battaglene, S.C., Morehead, D.T., Dunstan, G.A., Nichols, P.D. 2005. Effect of dietary 22:6n-3 on growth, survival and tissue fatty acid profile of striped trumpeter (*Latris lineata*) larvae fed enriched Artemia. *Aquaculture*, **243**, 331-344.

Cahu, C., Infante, J.Z., Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, **227**, 245-258.

Cañavate, J.P., Anguis, V., Calvo, A., Manchado, M. 2005. Recent developments in the reproduction of the Senegal sole (*Solea senegalensis*) in captivity. European *Aquaculture Society Special Publication*, **35**, 154-155.

Cara, J.B., Moyano, F.J., Zambonino, J.L., Alarco, F.J. 2007. The whole amino acid profile as indicator of the nutritional condition in cultured marine fish larvae. *Aquaculture Nutrition*, **13**, 94-10.

Carneiro, P.C.F., Kaiseler, P.H.S., Swarofsky, E.A.C., Baldisserotto, B. 2009. Transport of jundiá Rhamdia quelen juveniles at different loading densities: water quality and blood parameters. *Neotropical Ichthyology*, **7**, 283–288.

Carrier III, J. K., Watanabe, W.O., Harel, M., Rezek, T.C., Seaton, P.J., Shafer, T.H. 2011. Effects of dietary arachidonic acid on larval performance, fatty acid profiles, stress resistance, and expression of Na+/K+ ATPase mRNA in black sea bass *Centropristis striata*. *Aquaculture*, **319**, 111-121.

Cejas, J. R., Almansa, E., Jérez, S., Bolanos, A., Felipe, B., Lorenzo, A. 2004. Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. *Comparative Biochemistry and Physiology*, **Part B 139**, 209-216.

Clearwater, S.J., Pankhurst, N.W. 1997. The response to capture and confinement stress of plasma cortisol, plasma sex steroids and vitellogenic oocytes in the marine teleost, red gurnard. *Journal of Fish Biology*, **50**, 429-441.

Clotfelter, E.D., Curren, L.J., Murphy, C.E. 2006. Mate Choice and Spawning Success in the Fighting Fish *Betta splendens*: the Importance of Body Size, Display Behavior and Nest Size. *Ethology*, **112**, 1170-1178.

Conceicão, L.E.C., Ozório, R.O.A., Suurd, E.A., Verreth, J.A.J., 1998. Amino acid profiles and amino acid utilization in larval African catfish (*Clarias gariepinus*): effects of ontogeny and temperature. *Fish Physiology and Biochemistry*, **19**, 43-57.

Conceição, L.E.C., Grasdalen, H., Rønnestad, I. 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. *Aquaculture*, **227**, 221-232.

Cooke, S.J., Suski, C.D., Danylchuk, S.E., Danylchuk, A.J., Donaldson, M.R., Pullen, C., Bultè, G., O'toole, A., Murchie, K.J., Koppelman, J.B., Shultz, A.D., Brooks, E., Goldberg, T.L. 2008. Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. *Journal of Fish Biology*, **73**, 1351-1375.

Cordeiro, N. I. S., Costa, D. C., Silva, W.S., Takata, R., Miranda-Filho, K. C, Luz, R. K. 2016. High stocking density during larviculture and effect of size and diet on production of juvenile *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae). *Journal of Applied Ichthyology*, **32**, 61-66.

Costa, D.P., Leme, F.O.P., Takata, R., Costa, D.C., Silva W.S., Filho, R.M., Alves, G. M., Luz, R.K. 2014. Effects of temperature on growth, survival and physiological parameters in juveniles of *Lophiosilurus alexandri*, a carnivorous neotropical catfish. *Aquaculture Research*, 1-10.early view

Costa, D.C.C., Silva, W. S., Melillo-Filho, R., Filho, K.M.C., Santos, J.C.E.S., Luz, R.K. 2015. Capture, adaptation and artificial control of reproduction of *Lophiosilurus alexandri*: A carnivorous freshwater species. *Animal Reproduction Science*, **159**, 148-154.

Cruzado, I.H., Rodríguez, E., Herrera, M., Lorenzo, A., Almansa, E. 2013. Changes in lipid classes, fatty acids, protein and amino acids during egg development and yolk-sac larvae stage in brill (*Scophthalmus rhombus* L.). *Aquaculture Reserach*, **44**, 1568-1577.

Czesny, S., Dabrowski, K. 1998. The effect of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). *Aquatic Living Resource*, **1**, 371-378.

Dantagnan, H., Bórquez, A.S., Valdebenito, I.N., Salgado, I.A., Serrano, E.A., Izquierdo, M.S. 2007. Lipid and fatty acid composition during embryo and larval development of puye

Galaxias maculatus Jenyns, 1842, obtained from estuarine, freshwater and cultured populations. *Journal of Fish Biology*, **70**, 770-781.

Duncan, N., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Valles, R., Mylonas, C. C. 2012. Reproductive development, GnRHa-induced spawning and egg quality of wild meagre (*Argyrosomus regius*) acclimatised to captivity. *Fish Physiology and Biochemistry*, **38**, 1273-1286.

Esteve, M. 2005. Observations of spawning behaviour in Salmoninae: Salmo, *Oncorhynchus* and *Salvelinus*. *Reviews in Fish Biology Fisheries*, **15**, 1-21.

Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M. 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. *General Comparative Endocrinology*, **173**, 313-322.

Farhoudi, A., Kenari A. A., Chamanara, V., Farsani, A.S. 2012. Ontogeny Changes in Fatty Acid and Amino Acid Profiles in Yellowfin Seabream (*Acanthopagrus latus*) Eggs and Larvae. *World Journal of Fish and Marine Sciences*, **4**, 290-296.

Figueiredo, R.A.C.R., Souza, R.C., Bezerra, K.S., Campeche, D.F.B., Campos, R.M.L., Souza, A.M., Melo, J.F.B. 2014. Relação proteína:carboidrato no desempenho e metabolismo de juvenis de pacamã (*Lophiosilurus alexandri*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **66**, 1567-1576.

Finn, R.N., Fyhn, H.J. 2010. Requirement for amino acids in ontogeny of fish. *Aquaculture Research*, **41**, 684-716.

Fuiman, L.A., Ojanguren, A.F. 2011. Fatty acid content of eggs determines antipredator performance of fish larvae. *Journal of Experimental Marine Biology and Ecology*, **407**, 155-165.

Garrido, S., Saiz, E., Peters, J., Ré, P., Alvarez, P., Cotano, U., Herrero, D.L., Martínez de Murguía, A., Irigoien, X. 2012. Effect of food type and concentration on growth and fatty acid composition of early larvae of the anchovy (*Engraulis encrasicolus*) reared under laboratory conditions. *Journal of Experimental Marine Biology and Ecology*, 434–435, 16-24.

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture*, **1**, 71-124.

Godinho, H.P. 2007. Estratégias reprodutivas de peixes aplicadas à aqüicultura: bases para o desenvolvimento de tecnologias de produção. *Revista Brasileira de Reprodução Animal*, **31**, 351-360.

Grote, B., Hagen, W., Lipinski, M.R.,Verheye, H.M.,Stenevik, E.K.,Ekau, W. 2011. Lipids and fatty acids as indicators of egg condition, larval feeding and maternal effects in Cape hakes (*Merluccius paradoxus* and *M. capensis*). *Marine Biology*, **158**, 1005-1017.

Grutter, A.S., Pankhurst, N.W. 2000. The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. *Journal of Fish Biology*, **57**, 391-401.

Guimarães-Cruz, R.J., Santos, J.E., Sato, Y., Veloso-Júnior, V.C. 2009. Early development stages of the catfish *Lophiosilurus alexandri* Steindachner, 1877 (Pisces: Pseudopimelodidae) from the São Francisco River basin Brazil. *Journal of Applied Ichthyology*, **25**, 321–327.

Gunasekera, R.M., Shim, K.F., Lam, T.J. 1996. Influence of protein content of broodstock diets on larval quality and performance in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture,* **I46**, 245-259.

Gunasekera R.M., De Silva, S.S., Ingram, B.A. 1999. The amino acid profiles in developing eggs and larvae of the freshwater Percichthyid fishes, trout cod, *Maccullochella macquariensis* and Murray cod, *Maccullochella peelii peelii*. *Aquatic Living Resource,* **12**, 255-261.

Gurure, R., Atkinson, J., Moccia, R.D. 2007. Amino acid composition of Arctic charr, *Salvelinus alpinus* (L.) and the prediction of dietary requirements for essential amino acids. *Aquaculture Nutrition,* **13**, 266-272.

Harmon, T.S. 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture,* **1**, 58-66.

Hachero-Cruzado, I., Gárcia-Lopez, A., Herrera, M., Vargas Chacoff L., Martínez-Rodriguez, G., Mancera, J. M., Navas, J. I. 2007. Reproductive performance and seasonal plasma sex steroid and metabolite levels in a captive wild broodstock of brill *Scophthalmus rhombus* L. *Aquaculture Research*, **38**, 1161-1174.

Hachero-Cruzado, I., Olmo, P., Sánchez, B., Herrera, M., Domingues, P., 2009. The effects of an artificial and a natural diet on growth, survival and reproductive performance of wild caught and reared brill (*Scophthalmus rhombus*). *Aquaculture*, **291**, 82-88.

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition,* **49**, 5-22.

Iversen, M., Finstad, B., McKinley, R.S., Eliassena, R.A., Carlsend, K.T., Evjen, T. 2005. Stress responses in Atlantic salmon (*Salmo salar* L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. *Aquaculture,* **243**, 373-382.

Izquierdo, M.S. 1996. Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutnrion,* **2,** 183-191.

Jaya-Ram, A., Kuah, M-K.,, Lim, P-S., Kolkovski , S., Shu-Chien, A.C. 2008. Influence of dietary HUFA levels on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs expression in female zebrafish *Danio rerio*. *Aquaculture*, **277**, 275-281.

Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Reviews in Fish Biology Fisheries*, **18,** 143-200.

Kaushik, S., Georga, I., Koumoundouros, G. 2011. Growth and body composition of Zebrafish (*Danio rerio*) larvae fed a compound feed from first feeding onward: Toward implications on nutrient requirements. *Zebrafish*, **8**, 87-95.

Kelley, J.L., Magurran, A.E., García, M.C. 2006. Captive breeding promotes aggression in an endangered Mexican fish. *Biological Conservation*, **133**, 169-177.

King, H.R. 2009. Fish transport in the aquaculture sector: An overview of the road transport of Atlantic salmon in Tasmania. *Journal of Veterinary Behavior*, **4**, 163-168.

Kitagawa, A.T., Costa, L.S., Paulino, R.R., Luz, R.K., Rosa, P.V., Guerra-Santos, B., Fortes-Silva, R. 2015. Feeding behavior and the effect of photoperiod on the performanceand hematological parameters of the pacamã catfish (*Lophiosilurus alexandri*). *Applied Animal Behaviour Science*, **171**, 211-218.

Lanes, C.F.C., Bizuayehu, T.T., Bolla, S., Martins, C., Fernandes, J.M.O., Bianchini, A., Kiron, V., Babiak, I. 2012. Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broodstocks. *Aquaculture*, **324-325**, 267–275.

Lehtonen, T.K., Lindström, K., Wong, B.B.M. 2013. Effect of egg predator on nest choice and nest construction in sand gobies. *Animal Behavior*, **86**, 867-871.

Lindström, K., Mary, C.M. St., Pampoulie, C. 2006. Sexual selection for male parental care in the sand goby, *Pomatoschistus minutus*. *Behavioral Ecology and Sociobiology*, **60**, 46-51.

Ling, S., Kuah, M-K., Muhammad, T.S.T., Kolkovski, S., Shu-Chien, A.C. 2006. Effect of dietary HUFA on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs in female swordtail *Xiphophorus helleri*. *Aquaculture*, **261**, 204-214.

Lissåker, M., Kvarnemo, C. 2006. Ventilation or nest defense parental care trade-offs in a fish with male care. *Behavioral Ecology and Sociobiology*, **60**, 864-873.

Lopes, J.P., França, F.L., Neto, M. A. S. 2013. O domínio na produção de alevinos de pacamã – Propagação na Chesf permite repovoamento no rio São Francisco. *Panorama da Aquacultura*, **23**, 24-29.

Lund, I., Steenfeldt, S. J., Suhr, K. I., Hansen, B. H. 2008. A comparison of fatty acid composition and quality aspects of eggs and larvae from cultured and wild broodstock of common sole (*Solea solea* L.). *Aquaculture Nutrition*, **14**, 544-555.

Luo, Z., Liu, Y., Mai, K., Tian, L., Yang, H., Tan, X., Liu, D. 2005. Dietary L-methionine requirement of juvenile grouper Epinephelus coioides at a constant dietary cystine level. *Aquaculture*, **249**, 409-418.

Luz, R.K., Santos, J.C.E. 2008. Avaliação da tolerância de larvas do pacamã *Lophiosilurus alexandri* Steindachner, 1877 (Pisces: Siluriformes) a diferentes salinidades. *Acta Scientica Biological Science*, **30**, 345-350.

Luz, R.K., Santos, J.C.E., Pedreira, M.M., Teixeira, E. A. 2011. Effect of water flow rate and feed training on "pacamã" (Siluriforme: Pseudopimelodidae) juvenile production. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **63**, 973-979.

Madhu, K., Madhu, R. 2014. Captive spawning and embryonic development of marine ornamental purple firefish *Nemateleotris decora* (Randall & Allen, 1973). *Aquacultur*e, **424-425**, 1-9.

Maricchiolo, G., Casella, G., Mancuso, M., Genovese, L. 2016. Report of spontaneous spawning of captive red scorpionfish, *Scorpaena scrofa* (Linnaeus, 1758) with special attention on capture and broodstock management. *Aquaculture Research*, **47**, 677-680.

Martins, Y.S., Melo, R.M.C., Campos-Junior, P.H.A., Santos, J.C.E., Luz, R.K., Rizzo, E., Bazzoli, N. 2014. Salinity and temperature variations reflecting on cellular PCNA, IGF-I and II expressions, body growth and muscle cellularity of a freshwater fish larvae. *General Comparative Endocrinology*, **202**, 50-58.

Mattioli, C.C. *Efeito da salinidade da água sobre juvenis de pacamã Lophiosilurus alexandri*. 2014. 66f. Dissertação (Mestrado em produção animal) – Universidade Federal de Minas Gerais.

Mejri, S., Audet, C., Vandenberg, G.W., Parrish, C.C., Tremblay, R. 2014. Biochemical egg quality in a captive walleye (*Sander vitreus*) broodstock population relative to ovulation timing following hormonal treatment. *Aquaculture*, **431**, 99-106.

Mellilo-Filho, R. Tese. *Técnicas para sexagem precoce em pacamã (lophiosilurus alexandri, steindachner, 1876).* 2016. Dissertação (Mestrado em produção animal) – Universidade Federal de Minas Gerais.

Melillo-Filho, R.,Takata1, R., Santos, A.E.H., Silva, W.S., Ikeda, A.L., Rodrigues, L.A., Santos, J.C.E., Salaro, A.L., Luz, R.K. 2014. Draining system and feeding rate during the initial development of *Lophiosilurus alexandri* (Steindachner, 1877), a carnivorous freshwater fish. *Aquaculture Research*, **45**, 1913-1920.

Mello, P.H., Araújo, B.C.,Venturieri, R.L.L., Moreira, R.G. 2011. Fatty acids as a tool to compare cachara (*Pseudoplatystoma reticulatum*) (Siluriformes: Pimelodidae) and hybrid (*Pseudoplatystoma corruscans* x *Pseudoplatystoma reticulatum*) larvae during early development. *Aquaculture International*, **20**, 1139-1160.

Meunier, B., Yavno, S., Ahmed, S., Corkum, L.D. 2009. First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus). Journal of Great Lakes Research*, **35**, 908-612.

Meurer, F., Oliveira, S.T.L., dos Santos, L.D., Colpini, L.M.S. 2010. Níveis de oferta de pós-larvas de tilápia do Nilo para alevinos de pacamã (*Lophiosilurus alexandri*). *Revista Brasileira de Ciências Agrárias*, **5**, 111-116.

Moyo, N.A.G 2011. An investigation into the factors affecting the natural reproduction of *Opsaridium peringueyi*. *Physics and Chemistry of the Earth*, **36**, 876-880.

Murchie, K.j., Danylchuk, S.E., Pullen, C.E., Brooks, E., Shultz, A.D., Suski, C.D., Danylchuk, A.J., Cooke, S.J. 2009. Strategies for the capture and transport of bonefish, *Albula vulpes*, from tidal creeks to a marine research laboratory for long-term holding. *Aquaculture Reserach*, **40**, 1538-1550.

Oberg, E.W., L.A. Fuiman, L.A. 2015. Linking fatty acids in the diet and tissues to quality of larval southern flounder (*Paralichthys lethostigma*). *Journal of Experimental Marine Biology and Ecology*, **467**, 7-15.

Ohkubo, N., Matsubara, T. 2002. Sequential utilization of free amino acids, yolk proteins and lipids in developing eggs and yolk-sac larvae of barfin flounder *Verasper moseri*. *Marine Biology*, **140**, 187-196.

Ohkubo, N., Sawaguchi, S.,Hamatsu, T., Matsubara, T. 2006. Utilization of free amino acids, yolk proteins and lipids in developing eggs and yolk-sac larvae of walleye Pollock *Theragra chalcogramma*. *Fisheries Science*, **72**, 620-630.

Ota, K., Kohda, M. 2006. Description of alternative male reproductive tactics in a shell-brooding cichlid, *Telmatochromis vittatus*, in Lake Tanganyika. *Journal of Ethology*, **24**, 9-15.

Pankhurst, N.W., Fitzgibbon, Q.P. 2006. Characteristics of spawning behaviour in cultured greenback flounder *Rhombosolea tapirina*. *Aquaculture,* **253**, 279- 289.

Pedreira, M.M., Santos, J.C.E., P, Sampaio, E.V., Pereira, F.N., Silva, J.L. 2008. Efeito do tamanho da presa e do acréscimo de ração na larvicultura de pacamã. *Revista Brasileira de Zootecnia*, **37**,1144-1150.

Pedreira, M.M., Sampaio, E.V., Santos, J.C.E., Pires, A.V. 2012. Larviculture of two neotropical species with different distributions in the water column in light- and dark-colored tanks. *Neotropical Ichthyology*, **10**, 439-444.

Pickering, A. D. 1992. Rainbow trout husbandry: management of the stress response. *Aquaculture*, **100**, p.125-139.

Pickova, J.; Brännäs, E.; Andersson, T. 2007. Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. *Aquaculture International,* **15**, 305-311.

Portella, M. C., Takata, R., Leitão, N. J., Menossi, O.C. 2013 Free amino acids in pacu, *Piaractus mesopotamicus*, eggs and larvae. *Journal of the World Aquaculture Society*, **44**, 425-434.

Portz, D.E., Woodley, C.M., Cech Jr., M.J. 2006. Stress-associated impacts of short-term holding on fishes. *Reviews in Fish Biological Fisheries*, **16**, 125-170.

Ribeiro, P.A.P., De Melo, D.C., Espirito Santo, A.H. Silva, W.S., Santos, A.E.H., Luz, R.K. 2013a. Tricaine as an anaesthetic for larvae and juveniles of *Lophiosilurus alexandri*, a carnivorous freshwater fish. *Aquaculture Research*, **44**, 1-5.

Ribeiro, P.A.P., Miranda-Filho, K.C., Melillo-Filho, R., Santos, A.E.H., Silva, W.S., Rodrigues, L.A., Luz, R.K. 2013b. Efeito anestésico do eugenol em juvenis de pacamã. *Pesquisa Agropecuária Brasileira*, **48**, 1136-1139.

Rizzo, E., Sato, Y., Barreto, B.P., Godinho, H.P. 2002. Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts. *Journal of Fish Biology*, **61**, 615-632.

Rodríguez, C., Acosta , C., Badía, P.,, Cejas, J. C., Santamaría, F.J.,Lorenzo, A. 2004. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. *Comparative Biochemistry and Physiology*, Part B **139**, 619 -629.

Rodríguez-Barreto, D., Jerez, S., Cejas, J. R., Martin, M.V., Acosta, N. G., Bolãnos, A., Lorenzo, A. 2012, Comparative study of lipid and fatty acid composition in different tissues

of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). *Aquaculture*, **360-361**, 1-9.

Rønnestad, I., Thorsen, A., Finn, R.N., 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. *Aquaculture*, **177**, 201-216.

Ronnestad, I., S.K. Tonheim, S.K., Fyhn, H.J., Rojas-García, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y., Conceição, L.E.C. 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. *Aquaculture*, **227**, 147-164.

Saavedra, M., Conceicao, L.E.C., Pousao-Ferreira, P., Dinus, M.T. 2006. Amino acid profiles of *Diplodus sargus* (L., 1758). *Aquaculture*, **261**, 587-593.

Saavedra M., Beltran, M., Pousão-Ferreira P., Dinis, M.T., Blasco, J., Conceição L.E.C. 2007. Evaluation of bioavailability of individual amino acids in *Diplodus puntazzo* larvae: Towards the ideal dietary amino acid profile. *Aquaculture*, **263**, 192-198.

Salaro, A. L., Junior, J. C. O., Lima, F. W., Ferraz, R. B., Pontes, M. D., Campelo, D. A. V., Zuanon, J. A. S., Luz, R. K. 2015. Gelatin in replacement of bovine heart in feed training of *Lophiosilurus alexandri* in different water salinities. *Anais da Academia Brasileira de Ciências*, **87**, 2281-2287.

Salze, G., Tocher, D., Roy, W. J., Robertson, D. A. 2005. Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research*, **36**, 1488-1499.

Samaee, S.M., Estévez, A., Giménez, G., Lahnsteiner, F. 2009. Evaluation of quantitative importance of egg lipids and fatty acids during embryos and larvae development in marine pelagophil teleosts: With an emphasis on *Dentex dentex*. *Journal Animal Science*, **311ª**, 735-751.

Samaee, S.M., Mente, E., Estévez, A., Giménez, G., Lahnsteiner, F. 2010. Embryo and larva development in common dentex (*Dentex dentex*), a pelagophil teleost: The quantitative composition of egg-free amino acids and their interrelations. *Theriogenology*, **73**, 909-919.

Santos, J.C.E., Luz, R.K. 2009. Effect of salinity and prey concentrations on *Pseudoplatystoma corruscans, Prochilodus costatus* and *Lophiosilurus alexandri* larviculture. *Aquaculture*, **287**, 324-328.

Santos, L.D. dos., Silva, L.C.R. da., Amorin, J.V.O., Balen, R.E., Meurer, F. 2012. Effect of food processing on the development of pacamã fingerlings (*Lophiosilurus alexandri*). *Arquivo de Ciências Veterinárias e Zoologia da UNIPAR*, **15**, 115-120.

Santos, H.B., Sampaio, E.V., Arantes, F.P., Sato, Y. 2013. Induced spawning and reproductive variables of the catfish *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae). *Neotropical ichthyology*, **11**, 607-614.

Saravanan, R., Vijayanand, P., Vagelli, A.A., Murugan, A., Shanker, S., Rajagopal, S., Balasubramanian, T. 2013. Breeding and rearing of the two striped cardinalfish, *Apogon quadrifasciatus* (Cuvier, 1828) in captive condition. *Animal reproduction science*, **137**, 237-244.

Sargent, J., Bell, G., Mc Evoy, L., Tocher, D., Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, **177**, 191-199.

Sato, Y., Fenerich-Verani, N., Nuner, A. P. O., Godinho, H. P., Verani, J. R. 2003: *Padrões reprodutivos de peixes da bacia do São Francisco*. In: Águas, peixes e pescadores do São Francisco das Minas Gerais. Org: H. P. Godinho, A. L. Godinho. PUC Minas, Belo Horizonte. 468 pp.

Sheikh-Eldin, M., De Silva, S. S., Anderson, T. A., Gooley, G. 1996. Comparison of fatty acid composition of muscle, liver, mature oocytes, and diets of wild and captive *Macquarie perch, Macquaria australasica*, broodfish. *Aquaculture*, **144**, 201-216.

Silva, W.S., Cordeiro, N.I.S. Costa, D.C., Takata, R., Luz, R.K. 2014. Frequência alimentar e taxa de arraçoamento durante o condicionamento alimentar de juvenis de pacamã. *Pesquisa agropecuária brasileira*, **49**, 648-651.

Sink, T.D., Rebecca T.L., Pohlenz, C., Buentello, A.., Gatlin, D. 2010. Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. *Aquaculture*, **298**, 251–259.

Souza, M. G., Seabra, A. G. L., Silva, L. C.R., Santos, L.D., Balen, R. E., Meurer, F. 2013. Exigência de proteína bruta para juvenis de pacamã. *Revista Brasileira de Saúde e Produção Animal*, 14, 362-370.

Souza, M.G., Costa, M.M., Seabra, A.G., Balen, R.E., Meurer, F. 2014. Alimento vivo e inerte para alevinos de pacamã. Live and artificial feed to pacamã fingerlings. *Revista Agrarian*, **7**, 360-364.

Takata, R., Silva, W.S., Costa, D.C., Filho, R.M., Luz, R.K. 2014. Effect of water temperature and prey concentrations on initial development of *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae), a freshwater fish. *Neotropical Ichthyology*, **12**, 853-859.

Tenório, R.A., Santos, A.J.G., Lopes, J.P., Nogueira, E.M.S. 2006. Crescimento do niquim (*Lophiosilurus alexandri* Steindachner 1876), em diferentes condições de luminosidade e tipos de alimento. *Acta Scientical Animal Science*, **28**, 305-309.

Tocher, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, **41**, 717-732.

Tort, L. 2011. Stress and immune modulation in fish. *Developmental & Comparative Immunology*, **35**, 1366–1375.

Travassos, H. 1959. Nótula sobre o pacamã *Lophiosilurus alexandri* Steindachner, 1876. *Atlas da Sociedade de Biologia*, **4**, 1-2.

Vander Haegen, G.E., Ashbrook, C.E., Yi, K.W., Dixon, J.F. 2004. Survival of spring chinook salmon captured and released in a selective commercial fishery using gill nets and tangle nets. *Fisheries research*, **68**, 123-133.

Wiegand, M.D. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology Fisheries*, **6**, 259-286.

Yanes-Roca, C., Rhody, N., Nystrom, M., Main, K.L. 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). *Aquaculture*, **287**, 335-340.

Yeganeh, S. 2014. Variations of fatty acids in Rainbow trout (*Oncorhynchus mykiss*) during eyed egg and larval development stages. *International Journal of Aquatic Science*, **5**, 167-180.

Zheng, X., Seiliez, I., Hastings, N., Tocher, D.R., Panserat, S., Dickson, C.A., Bergot, P., Teale, A.J. 2004. Characterization and comparison of fatty acyl D6 desaturase cDNAs from freshwater and marine teleost fish species. *Comparative Biochemistry Physiology*, **Part B 139**, 269-279.

3. OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o comportamento reprodutivo de *Lophiosilurus alexandri* em condições de laboratório e desenvolver manejos que maximizem a reprodução desta espécie em cativeiro, avaliar a composição bioquímica de ovos, larvas após a eclosão e larvas no final do período lecitotrófico.

3.2 OBJETIVOS ESPECÍFICOS

- Capturar exemplares selvagens na natureza para a observação do comportamento reprodutivo em condições de cativeiro;
- Avaliar alterações no período reprodutivo em comparação ao que ocorre na natureza;
- Determinar o perfil e a quantidade dos aminoácidos em ovos e larvas de pacamã;
- Determinar o perfil e a quantidade dos ácidos graxos presente em ovos e larvas de pacamã.

4. Artigo 1

CAPTURE, ADAPTATION AND ARTIFICIAL CONTROL OF REPRODUCTION OF “PACAMÃ”, A CARNIVOROUS FRESHWATER SPECIES

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Capture, adaptation and artificial control of reproduction of *Lophiosilurus alexandri*: A carnivorous freshwater species

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ABSTRACT

The present study describes the capture adaptation and reproduction of wild *Lophiosilurus alexandri* broodstock in laboratory conditions. There were two periods when capturing was performed in natural habitats. The animals were placed in four tanks of 5 m^3 with water temperatures at 28 °C with two tanks having sand bottoms. Thirty days after the temperature increased (during the winter) the first spawning occurred naturally, but only in tanks with sand on the bottom. During the breeding season, there were 24 spawning bouts with egg mass collections occurring as a result of the spawning bouts that occurred in the tanks. The hatching rates for eggs varied from 0% to 95%. The spawning bouts were mainly at night and on weekends. In the second reproductive period, the animals were sexed by cannulation and distributed in four tanks with all animals being maintained in tanks with sand on the bottom at 28 °C. During this phase, there were 36 spawning bouts. Findings in the present study contribute to the understanding of the reproductive biology of this endangered species during captivity.

1. Introduction

The reproduction of wild species in captivity is widely used around the world as a conservation strategy of endangered fish, and is a useful tool for the preservation or restoration of wild populations (Kelley et al., 2006; Blanchet et al., 2008; Saravanan et al., 2013). However, the task of adapting wild broodstock to laboratory conditions involves many processes, ranging from capture, transport, acclimation and maintenance of the animals in captivity (Moorhead and Zeng, 2010; Maricchiolo et al., 2014). Improper management of these actions may result in gonadal atresia and even lead to mortality of captured animals as a result of the stress response (Barton et al., 2003; Ashley, 2007; Murchie et al., 2009). Breeding in captivity also requires the study of reproductive biology of the species and the development of strategies for maintaining animals in confined conditions (Hunting Ford, 2004; Pankhurst and Fitzgibbon, 2006; Moorhead and Zeng, 2011). This is an important consideration because confined conditions can promote differences with respect to the natural behavior of the species.

The "pacamã" *Lophiosilurus alexandri* (Siluriforme: Pseudopimelodidae Steindachner, 1876) is a species that has been used for restocking the "São Francisco" River, Brazil. The reproduction of "pacamã" has been made in fish

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stations under natural conditions, using ponds. The market value, tasty meat and resulting fishing pressure have reduced wild populations of "pacamã" which is now an endangered species (Brasil, 2014). It is an endemic fish of the "São Francisco" river (Travassos, 1960), carnivorous, benthic in habitat preference and has nocturnal behavioral patterns (Shibatta, 2003; Tenório et al., 2006). Reproduction in this species occurs between October and February (Sato et al., 2003a; Barros et al., 2007) with the males of the species caring for the egg mass (Sato et al., 2003b). The fecundity of this species is less than for many aquatic species but consistent with fecundity rates of species with the sedentary nature of this species (Santos et al., 2013). The size of vitelogenic oocytes of *L. alexandri* are large and have highly adhesive characteristics (Bazzoli and Godinho, 1997; Rizzo et al., 2002; Guimarães-Cruz et al., 2009; Santos et al., 2013). However, reproduction in controlled conditions has not been studied for this species.

The present study was conducted to assess reproductive capacity of *L. alexandri* wild broodstock after capture, transport, and adaptation in laboratory conditions.

2. Materials and methods

The present research has followed the methodology approved by the Ethics Committee on Animal Use, Protocol. 25/2010 – CEUA/UFMG.

2.1. First capture of wild animals

To capture the animals, a license of Capture and Transport was obtained by the State Institute of Forest-MG Scientific Fishing license n°105/09. The first group of animals were captured in the region of "Pontal do Abaeté" near "Três Marias-Minas Gerais", Brazil (18° 20′ 16″S, 45° 49′ 58″W). The capture period was initiated at the 1800 h between 16 and 26 September 2009. A mesh of 8–20 mm was used for the capturing and animals were collected the next morning (0600 h). Specimens captured were transported in a 190 L box boat to the "Companhia de Desenvolvimento do Vale do São Francisco" (CODEVASF) where animals were kept in concrete tanks for a week. The fish were subsequently transported to the Aquaculture Laboratory (Laqua) of the "Universidade Federal Minas Gerais, Belo Horizonte, Minas Gerais", Brazil. This transport was conducted in a 1000 L box with aeration, water temperature at 22 °C, during 4 h (380 km) of transportation. In the laboratory, the animals were submitted to acclimation. Animals were housed in a single tank of 7 m^3 covered with geomembrane liner with water conditions as follow: temperature between 25° and 27 °C and dissolved oxygen >5.5 mg L^{-1}. At this time, each animal had a microchip implanted (Microchip Parteners) in the lumbar region for identification and data collection. Once a week about 50% of the water in the tank was exchanged.

In November of 2009, the animals were transferred to a new tank with the same management specifications being imposed. At this time, two more animals were captured and submitted to the same procedures as previously described. The animals were fed daily with frozen filets of tilapia and the remains were collected after 24 h.

2.2. Second capture of wild animals

The second capture occurred in the region of "Sao José do Buriti" in the reservoir of "Três Marias in the São Francisco" River Basin (18° 42′ 33.2″S 45° 09′ 13″W) in January of 2011. For the second capture period, changes in practices occurred to improve the efficiency of the process in comparison with the first capture period.

Nets with 1500 m and mesh ranging from 7 to 15 cm among knots were used starting at the 1800 h and the animals were captured the next morning at the 0600 h. The captured animals were transported in a 190 L box with aeration. At the time of landing, the fish were weighed, subjected to prophylactic antibiotics (sulfadoxine and trimethoprim 75 mg kg^{-1}) and immediately transported to the Laqua as described for the first capture period. The transport lasted about 3 h (distance of 269 km). Upon reaching the Laqua, the animals were submitted to a 30-min acclimation and treated with potassium permanganate bath (6 mg L^{-1}) for 15 min. The animals were subsequently allocated to two tanks of 5 m^3 covered with a geomembrane liner, maintained in a static system, with additional aeration (dissolved oxygen >5.5 mg L^{-1}) and at a controlled temperature (26.0 ± 1.0 °C). The water was salinized (1 g L^{-1}) in the first 2 days. Treatment with the potassium permanganate bath was conducted again 2 days after capture.

The animals were monitored daily. Once a week, about 50% of the volume of water was exchanged in each tank. The animals were fed two to three times per week with tilapia filet and a vitamin–mineral premix (600 mg Vitamin C and 30 mg of premix). The food was offered directly to the mouth of the animals utilizing a hook.

After 15 days, the animals were weighed on a digital scale (Digital instruments FG5020). At this time, a microchip was place in each specimen as performed in the first group of captured animals.

Due to the lack of methodology for sexual differentiation, the fish of the second capture group (17 animals) and surviving animals from the first capture group (seven animals) were combined in March of 2011 and then placed in one of four 5 m^3 tanks. The following conditions and densities were imposed: Tanks 1 and 4, eight animals with an average weight of 2.304 ± 1.33 kg and 2.55 ± 1.10 kg, respectively; Tanks 2 and 3; four animals weighing 1.67 ± 0.36 kg and 1.860 ± 0.43 kg, respectively. In Tanks 2 and 4, there was approximately 5–10 cm of sand (pool filter type) added to the bottom of the tank.

Tanks of 5 m^3 were maintained with a volume of 2–2.5 m^3. The volume was reduced to avoid fish jumping out of the tank. Initially the temperature of the tank was maintained at 26.0 ± 1.0 °C with a dissolved oxygen >5.5 mg L^{-1} and a photoperiod of 10 h light. About 50% of the volume of water was exchanged weekly.

2.2.1. First breeding period

In late June of 2011 (5 months after capturing the second group of animals) the water temperature was increased to 28 °C. During the period from June 2011 to April 2012, the tanks were inspected daily in the morning and afternoon for spawning verification. When spawning bouts

were detected, the animals were captured by netting, the microchip was read and the egg mass was collected. The egg mass was subsequently weighed and placed in 50 L tanks maintained at 28.0 ± 0.5 °C at an oxygen concentration of >5.0 mg L^{-1} for hatching with process lasting 24–48 h.

2.2.2. Resting period

Due to the lack of spawning between January and April of 2012, a reduced temperature was employed (22.0 ± 1.0 °C) in all tanks in May of 2012. The animals were fed once a week as described previously. This period of lesser water temperature was maintained for 1 year and 3 months because of reconstruction in the laboratory. In addition, the water in the tanks continued to be replaced at 50% of the total volume once a week.

2.2.3. Second breeding period

In August of 2013, the animals were sexed using the methodology described by Lopes et al. (2013) by detecting the oviduct on the females. For the second breeding period, the animals were prepared as follows: Tank 1: four females and two males weighing 2.47 ± 0.60 kg and 2.53 ± 0.83 kg, respectively; Tank 2: three females weighing 2.93 ± 0.74 kg and two males weighing 3.28 ± 0.02 kg; Tank 3: three females weighing 3.21 ± 1.23 kg and two males weighing 3.0 ± 0.62 kg; Tank 4: four females and two males weighing 2.84 ± 1.27 kg and 1.56 ± 0.98 kg, respectively. In Tanks 1 and 3, sand was added on the bottom as described for the Tanks 2 and 4. The biomass of each individual tank ranged from 14 to 15 kg.

The water temperature in the tank was increased to approximately 28 °C. The animals started to feed twice a week and 50% of the water was replaced once a week as previously described. During the period of August 2013 to April 2014, the tanks were inspected daily in the morning and afternoon to verify the occurrence of spawning.

3. Results

3.1. First capture

In the first campaign were captured a total of 12 fish (between 3.6 and 4.7 kg). Two animals died in the CODEVASF station and two animals died in the first week after being captured after being placed in the laboratory. These fish were dissected and identified as females. Another interesting point was the observation of a large amount of visceral fat. For 20 days, all the food offered was collected, indicating that animals were not feeding. From this time, the animals gradually started to feed. After 33 days of being housed in the laboratory, the animals were fed on the tank surface, eating out of the hand of the animal caretaker.

After being transferred to the new tanks, the animals did not eat for almost 6 months and one additional death occurred during this time. After the 6 month period, there was a return of the feeding behavior in the animals. No spawning bouts were observed during this time (from the first capture in September of 2009 to March of 2011).

3.2. Second capture

Twenty animals were captured in 2 days of collection (15 on the first day and five in the second day) with animals weighing between 0.8 and 2.5 kg. Once in the laboratory, three animals died jumping out of the tank. For this reason the volume of water in the tank was reduced. Animals began to feed 4 days after arriving at the laboratory.

3.2.1. First breeding period

After combining the fish from the two capture periods and having the water temperature increased, the first spawning occurred. Nests were established by the animals on the sandy bottom of the tanks. After spawning bouts, animal withdrawals from the nests were attempted and the animals expressing parental behavior reacted by attempting to continue to reside on the nest. This behavior was repeated in all subsequent spawn bouts. Only a single and at no time was there multiple animals observed caring for a single mass of eggs.

The first spawning occurred in Tank 2, 30 days after the increase in temperature (Table 1). It should be noted that this occurred during the winter (in July). By early December of 2011, there had been 11 spawning bouts in this tank with egg masses weighing from 46 to 191 g. The number of larvae varied from 240 to 4600. All spawning bouts were protected by a single individual identified by microchip. The average time between spawning bouts was approximately 14 days (6–28 days).

In Tank 4, the first spawning occurred in early August of 2011, approximately 60 days after the water temperature increased. In this tank, there were 13 spawning bouts with the egg mass weighing 55–150 g and containing 117–3500 larvae. From the total, 12 of the egg masses were protected by the same animal as identified by microchip observation. The interval between spawning bouts was 9.2 days (4–27 days).

Some spawning bouts resulted in a 0% hatching for unknown reasons. The spawning bouts were always found in the morning, suggesting that egg fertilization occurred at night and especially on weekends, when the activity in the laboratory and especially around the tanks was less.

In both tanks, it was noted that after the removal of egg masses, the fish remained on the nest for 1 or 2 days and became more aggressive. In the course of spawning period, when the animal that was protecting the egg mass was removed from the nest, other fish immediately moved to the nest to protect the egg mass.

Another important point is that there were spawning bouts only in the tanks with sand on the bottom (Tanks 2 and 4). In Tanks 1 and 3, without sand, there were not spawning bouts during the observation period.

Due to the adherence nature of the eggs and the large amount of sand in the egg mass, it was not possible to establish the relationship between fecundity and egg weight, and weight of the egg mass and egg number.

3.3. Resting period

The animals remained more than a year at the lesser temperature with no spawning bouts being registered.

Table 1
Number of egg masses in the breeding tanks of *Lophiosilurus alexandri* from June 2011 to April 2012.[a]

Months/year	Tank 1/without sand	Tank 2/with sand	Tank 3/without sand	Tank 4/with sand
June/2011	–	–	–	–
July/2011	–	1	–	–
August/2011	–	3	–	2
September/2011	–	2	–	2
October/2011	–	2	–	4
November/2011	–	2	–	5
December/2011	–	1	–	–
January, February, March, April/2012	–	–	–	–
Average weight of egg masses (g)	–	102.9 ± 36.6	–	126.9 ± 42.4

Tank 1: eight animals with average weight of 2.30 ± 1.33 kg.
Tank 2: four animals with average weight of 1.67 ± 0.36 kg.
Tank 3: four animals with average weight of 1.86 ± 0.43 kg.
Tank 4: eight animals with average weight of 2.55 ± 1.10 kg.
[a] In this breeding period the animals was not sexed.

Furthermore, although even with a constant supply of food, the breeders remained 6 months without search for food and no mortality was registered.

3.4. Second breeding period

In August 2013, after a revised arrangement of animals in the tanks (according to the sex, sand added in Tanks 1 and 3 and an increase in the temperature to 28 °C), spawning bouts were recorded in all tanks (Table 2).

The first spawning occurred in Tank 3, 24 days after increasing the temperature. In this tank, there were 12 spawning bouts (25–95 g) and the egg masses were always protected by the same animal. The average number of days between spawning bouts was 11 days (5–23 days).

The first spawning took place in Tank 4, 54 days after increasing the temperature. In this tank, there were 18 spawning bouts (8–153 g) recorded with three animals being identified as having spawned. The first six consecutive nests that resulted from the spawning were protected by the same individual (chip number: 40612). The nests from the three subsequent spawning bouts were protected by a different animal than the one protecting the initial six egg masses that were spawned in Tank 4 (chip number: 4237). The fish number 40612 returned to protect the egg masses resulting from three consecutive spawning bouts. Subsequently, fish number 4237 also protected an egg mass spawning. In the 14 spawning, the male fish was not identified by microchip reading. The subsequent egg mass resulting from a spawning was protected by another animal (chip number 19227). The last three nests were protected by the animal with chip number 40612. The average number of days between spawning bouts was 8.2 days (ranging from 3 to 16 days).

The first spawning bout (Tank 1) was in January 2014 when there were five spawning bouts and all of the nests resulting from these spawns were protected by the same individual (30–145 g). The average number of days between spawning bouts was 11.5 days (8–16 days). In Tank 2, there was only one spawning (42 g) in November 2013.

4. Discussion

Results of the present study indicate wild *L. alexandri* can be adapted to fully controlled conditions in captivity where they eat normally and reproduce. Findings indicate the possibility of manipulating the breeding period by controlling the water temperature and feeding.

The management adopted in the second capture by transporting on the same day after the time of capture and application of prophylactic treatments enhanced the adaptation of animals to laboratory conditions as evidenced by them starting to feed in 4 days while after the first time of capture fish it took more than 20 days before feeding was initiated after the time of capture. According to Bernier (2006), appetite reduction in fish occurs in response to acute or chronic stress. The transfer of animals from their natural habitat to captivity involves various management practices that promote physiological changes in the fish inducing animal stress (Iversen et al., 2005; Portz et al., 2006; Harmon, 2009). The magnitude and duration of stress during these events can last for hours to days (Bolasina, 2011; Fanouraki et al., 2011). Fish weight at the time of first and second captures differed (3.6–4.7 kg, and 0.80–2.5 kg, respectively). According to Meka and McCormick (2005), larger animals may be more susceptible to stress.

In the present study, fish spent long periods without food when temperature was maintained at 22 °C. Fasting in low temperatures was also demonstrated in other fish species (Sun et al., 2006; Oyugi et al., 2012; Wu et al., 2015). In *L. alexandri*, the large amount of visceral fat could be an energy resovoir on which animals draw during periods of fasting. These stores of fat were detected in animals that died and were dissected in the present study. In other species, the lipids stored as perivisceral fat function as a source of energy and can ensure the fish survival during fasting (Van Dijk et al., 2005; Arrington et al., 2006; Ibarz et al., 2010). In addition, prolonged fasting occurs in response to thermoregulatory mechanisms of fish (McCue, 2010).

In relation to water temperature and sand presence in the tank for breeding, the importance of these factors were evident in the present study. The spawning bouts were not observed when fish were maintained in tanks with water temperatures of 26 °C after the first capture and without

Table 2
Egg masses in the breeding tanks of *Lophiosilurus alexandri* between August of 2013 and April of 2014. All tanks had sand as a substrate on the bottom of the tanks for spawning.[a]

Months/year	Tank 1	Tank 2	Tank 3	Tank 4
August/2013	–	–	–	–
September/2013	–	–	1	–
October/2013	–	–	2	1
November/2013	–	1	4	4
December/2013	–	–	4	4
January/2014	1	–	1	5
February/2014	3	–	–	2
March/2014	1	–	–	2
April/2014	–	–	–	–
Average weight of egg masses (g)	81.0 ± 48.6	42.0	69.4 ± 23.1	71.2 ± 45.1

Tank 1: four females weighing 2.47 ± 0.60 kg and two males with 2.53 ± 0.83 kg.
Tank 2: three females weighing 2.93 ± 0.74 kg and two males with 3.28 ± 0.02 kg.
Tank 3: three females weighing 3.21 ± 1.23 kg and two males with 3.0 ± 0.62 kg.
Tank 4: four females weighing 2.84 ± 1.27 kg and two males with 1.56 ± 0.98 kg.
[a] The animals were sexed according to the methodology described by Lopes et al. (2013).

substrate even at 28 °C. It was determined that there were males and females in all tanks by subsequent sex detrminations during the preiod of the first reproductive cycle. In nature, *L. alexandri* reside on the sandy bottoms of water masses where they populate and remain buried most of the day. This is a protective and food capturing strategy for enhancing the survial of the animals (Ryer et al., 2008; Reig et al., 2010). Without substrate to bury, the animals have ventral injuries as reported in sole (*Solea solea*; Ellis et al., 1997) and in the present study. In addition, the construction of the nest for spawning is a common finding (Detrich et al., 2005; Esteve, 2005) so as to protect the egg masses from predators (Meunier et al., 2009; Lehtonen et al., 2013). The importance of sand inclusion in tanks was confirmed by spawning bouts in all tanks during the second breeding period of the present study.

L. alexandri displayed the behavioral characteristic of male parental care in the present study as validated by using chip numbers to identify specific animals and by sexing of the fish. The exception was Tank 4 in the second breeding period where three different males were caring for the egg masses. In this tank, however, there was dominance by a single male that protected 12 of 18 egg masses that resulted from spawning bouts in this tank. Only one female specimen classified during sexing contributed to the parental care of egg masses in the present study. However, it should be noted that the sexing technique in not as precise as ideally desired, thus, this animal may not have been a female. The parental care by males is common in several species (Thünken et al., 2010; Lehtonen, 2012; Saravanan et al., 2013) and is essential for offspring survival (Lissåker and Kvarnemo, 2006; Hanson et al., 2009; Dey et al., 2010). This ability can also be used as a means of male selection by females (Lindström et al., 2006; Hale and Mary, 2007), which may explain the dominance of one male in parental care of egg masses in the present study. In addition, an interesting behavior was observed when the dominant animal expressing parental care was removed from the tank in the present study. Other animals attempted to protect the egg mass after the dominant animal was removed. This behavior is reported in the literature as reproductive parasitism, that is, when another male takes the place in parental care of the orignial male that dominates in parental care (Ota and Kohda, 2006, 2011).

Despite the large number of spawning bouts in the present study, it was not possible to identify the female fish, because there was only one fish in the nest, causing doubt if these animals are monogamous. Thus, studies are needed to identify the females and to understand the reproductive behavior of this species.

The spawning times were primarily during the night. It has been shown that beyond melatonin, the hormones involved in reproduction, are GnRH and LH with concentrations of these hormones changing in daily rhythms. Spawning occurred in the evening, likely as a protective mechanism against egg mass predation (Bayarri et al., 2004; Meseguer et al., 2008; Migaud et al., 2010). The incidence of spawning increased over weekends when there were less human activity in the laboratory during the present study. According to Davidson et al. (2009), routine activities in the laboratories promote excessive noise, inducing a number of negative responses leading to temporary stress in animals (Smith et al., 2004; Wysocki et al., 2006). The reduced human activity during the weekends, therefore, most likely contributed to an enviroment that favored the increased incidence of spawning on weekends that was noted in the present study.

In both breeding seasons, the spawning bouts began a few days after the water temperature increase. The maturation and spawning is signaled by the action of steroid hormones, which are controlled by environmental factors such as water temperature (Pankhurst and King, 2010; Okuzawa and Gen, 2013). In this context, it is well established that the temperature promotes changes in sex steroid concentrations, affecting the process of reproductive phsiological and ultimately egg maturation (García-López et al., 2009; Arantes et al., 2011). Furthermore, there is the possibility of manipulating the reproductive period by temperature control of the water any time of year as described in previous (Tveiten and Johnsen, 1999; Utoh et al., 2013) and the present study.

Inconsistent with the finding in other fish species where food restriction reduced energy reserves and affected sexual maturation during vitellogenesis (Chatzifotis et al.,

2011) for *L. alexandri* the reproductive activities were initiated a short period of time after the temperature increase and proper nutrition was provided in the present study. Tian et al. (2010) found in *Cynoglossus semilaevis* (Günther, 1873) a rapid restoration of energy reserves after long periods of fasting and food supply.

In both reproductive periods the egg masses varied from 46 to 191 g and 8 to 153 g, respectively, and hatching rates varied from 0% to 95%. According to Bobe and Labbé (2010), food restriction and stress are some of the main factors involved in the quality of eggs that result from specific spawning bouts. The spawnings occurred at intervals ranging from 3 days to 28 days in two reproductive periods. This period may vary depending on the weight and age of the females (Macchi et al., 2004; Claramunt et al., 2007).

When temperatures were increased (28 °C) and proper food was supplied, spawning ceased after 6 months. There needs to be more studies with the species used in the present study to understand egg production capcity during one breeding cycle and how environmental factors such as temperature impact reproduction. This study provides initial evidence that reproduction can occur when *L. alexandri*s are in captivity. Further studies should be conducted, however, to understand the reproductive cycle of *L. alexandri*.

Results of the present study show the importance of management systems if *L. alexandri* are to going to reproduce in captivity. *L. alexandri* can be maintained in captivity and reproduce under controlled conditions especially by manipulating the water temperature. These results contribute to a greater understanding of the reproductive biology of this species and provide evidence to maximize the production of juveniles for restocking programs for this endangered species.

Conflict of interest

None declared.

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References

Arantes, F.P., Santos, H.B., Rizzo, E., Sato, Y., Bazzoli, N., 2011. Influence of water temperature on induced reproduction by hypophysation, sex steroids concentrations and final oocyte maturation of the "curimatã-pacu" *Prochilodus argenteus* (Pisces: Prochilodontidae). Gen. Comp. Endocrinol. 172, 400–408.

Arrington, D.A., Davidson, B.K., Winemiller, K.O., Layman, C.A., 2006. Influence of life history and seasonal hydrology on lipid storage in three neotropical fish species. Fish Biol. 68, 1347–1361.

Ashley, P.J., 2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. 104, 199–235.

Barros, M.D.M., Guimarães-Cruz, R.J., Veloso-Júnior, V.C., Santos, J.C.E., 2007. Reproductive apparatus and gametogenesis of *Lophiosilurus alexandri* Steindachner (Pisces, Teleostei, Siluriformes). Rev. Bras. Zool. 24, 213–221.

Barton, B.A., Haukenes, A.H., Parsons, B.G., Reed, J.R., 2003. Plasma cortisol and chloride stress responses in juvenile walleyes during capture, transport, and stocking procedures. N. Am. J. Aquacult. 65, 210–219.

Bayarri, M.J., Rodríguez, L., Zanuy, S., Madrid, J.A., Sánchez-Vázquez, F.J., Kagawa, H., Okuzawa, K., Carrillo, M., 2004. Effect of photoperiod manipulation on the daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrarchus labrax*). Gen. Comp. Endocrinol. 136, 72–81.

Bazzoli, N., Godinho, H.P., 1997. Ovócitos vitelogênicos do surubim *Pseudoplastystoma coruscans* e do pacamã *Lophiosilurus alexandri*. In: Miranda, M.O.T. (Ed.), Surubim. IBAMA (Coleção Meio Ambiente, Série Estudos Pesca, 19), Belo Horizonte. , pp. 81–90.

Bernier, N.J., 2006. The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. Gen. Comp. Endocrinol. 146, 45–55.

Bobe, J., Labbé, C., 2010. Egg and sperm quality in fish. Gen. Comp. Endocrinol. 165, 535–548.

Bolasina, N.S., 2011. Stress response of juvenile flounder (*Paralichthys orbignyanus*, Valenciennes 1839), to acute and chronic stressors. Aquaculture 313, 140–143.

Blanchet, S., Páez, D.J., Bernatchez, L., Dodson, J.J., 2008. An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): implications for supportive breeding programs. Biol. Conserv. 141, 1989–1999.

Brasil. Ministério do Meio Ambiente. Portaria MMA n° 445, de 17 de dezembro de 2014. Lista Nacional Oficial de Espécies da Fauna Ameaçadas de Extinção – Peixes e Invertebrados Aquáticos.

Chatzifotis, S., Papadaki, M., Despoti, S., Roufidou, C., Antonopoulou, E., 2011. Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (*Dicentrarchus labrax*). Aquaculture 316, 53–59.

Claramunt, G., Serra, R., Castro, L.R., Cubillos, L., 2007. Is the spawning frequency dependent on female size? Empirical evidence in *Sardinops sagax* and *Engraulis ringens* off northern Chile. Fish. Res. 85, 248–257.

Davidson, J., Bebak, J., Mazik, P., 2009. The effects of aquaculture production noise on the growth, condition factor, feed conversion, and survival of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 288, 337–343.

Detrich, H.W., Jones, C.D., Kim, S., North, A.W., Thurber, A., Vacchi, M., 2005. Nesting behavior of the icefish *Chaenocephalus aceratus* at Bouvetøya Island, Southern Ocean. Polar Biol. 28, 828–832.

Dey, C.J., O'Connor, C.M., Gilmour, K.M., Kraak, G.V.D., Cooke, S.J., 2010. Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. Horm. Behav. 58, 599–605.

Ellis, T., Howell, B.R., Hughes, R.N., 1997. The cryptic responses of hatchery-reared sole to a natural sand substratum. J. Fish Biol. 51, 389–401.

Esteve, M., 2005. Observations of spawning behaviour in Salmoninae: *Salmo*, *Oncorhynchus* and *Salvelinus*. Rev. Fish Biol. Fish. 15, 1–21.

Fanouraki, E., Mylonas, C.C., Papandroulakis, N., Pavlidis, M., 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. Gen. Comp. Endocrinol. 173, 313–322.

García-López, Á., Sarasquete, C., Rodríguez, M.G., 2009. Temperature manipulation stimulates gonadal maturation and sex steroid production in Senegalese sole *Solea senegalensis* Kaup kept under two different light regimes. Aquacult. Res. 40, 103–111.

Guimarães-Cruz, R.J., Santos, J.E., Sato, Y., Veloso-Júnior, V.C., 2009. Early development stages of the catfish *Lophiosilurus alexandri* Steindachner, 1877 (Pisces: Pseudopimelodidae) from the São Francisco River basin Brazil. J. Appl. Ichthyol. 25, 21–327.

Hale, E.R., Mary, C.M.S.T., 2007. Nest tending increases reproductive success, sometimes: environmental effects on paternal care and mate choice in flagfish. Anim. Behav. 74, 77–588.

Hanson, K.C., Abizaid, A., Cooke, S.J., 2009. Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*). Horm. Behav. 56, 503–509.

Harmon, T.S., 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Rev. Aquacult. 1, 58–66.

Hunting Ford, F.A., 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. J. Fish Biol. 65, 122–142.

Ibarz, A., Padrós, F., Gallardo, M.A., Fernández-Borrás, J., Blasco, J., Tort, L., 2010. Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'Winter Syndrome'. Rev. Fish Biol. Fish. 20, 539–556.

Iversen, M., Finstad, B., McKinley, R.S., Eliassena, R.A., Carlsend, K.T., Evjen, T., 2005. Stress responses in Atlantic salmon (*Salmo salar* L.)

smolts during commercial well boat transports, and effects on survival after transfer to sea. Aquaculture 243, 373–382.
Kelley, J.L., Magurran, A.E., García, M.C., 2006. Captive breeding promotes aggression in an endangered Mexican fish. Biol. Conserv. 133, 169–177.
Lehtonen, T.K., 2012. Signal value of male courtship effort in a fish with paternal care. Anim. Behav. 83, 1153–1161.
Lehtonen, T.K., Lindström, K., Wong, B.B.W., 2013. Effect of egg predator on nest choice and nest construction in sand gobies. Anim. Behav. 86, 867–871.
Lindström, K., Mary, C.M., Pampoulie, St.C., 2006. Sexual selection for male parental care in the sand goby, *Pomatoschistus minutus*. Behav. Ecol. Sociobiol. 60, 46–51.
Lissåker, M., Kvarnemo, C., 2006. Ventilation or nest defense-parental care trade-offs in a fish with male care. Behav. Ecol. Sociobiol. 60, 864–873.
Lopes, J.P., França, F.L., Neto, M.A.S., 2013. O domínio na produção de alevinos de pacamã–Propagação na permite repovoamento no rio São Francisco. Panor. Aquacult. 23, 24–29.
Macchi, G.J., Pájaro, M., Ehrlich, M., 2004. Seasonal egg production pattern of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). Fish. Res. 67, 25–38.
Maricchiolo, G., Casella, G., Mancuso, M., Genovese, L., 2014. Report of spontaneous spawning of captive red scorpionfish, *Scorpaena scrofa* (Linnaeus, 1758) with special attention on capture and broodstock management. Aquacult. Res., 1–4 (early view).
McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. 156, 1–18.
Meka, J.M., McCormick, S.D., 2005. Physiological response of wild rainbow trout to angling: impact of angling duration, fish size, body condition, and temperature. Fish. Res. 72, 311–322.
Meseguer, C., Ramos, J., Bayarri, M.J., Oliveira, C., Sanchez-Vázquez, F.J., 2008. Light synchronization of the daily spawning rhythms of gilthead sea bream (*Sparus aurata* L.) kept under different photoperiod and after shifting the LD cycle. Chronobiol. Int. 25, 666–679.
Meunier, B., Yavno, S., Ahmed, S., Corkum, L.D., 2009. First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostomus*). J. Great Lakes Res. 35, 608–612.
Migaud, H., Davie, A., Taylor, J.F., 2010. Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. J. Fish Biol. 76, 27–68.
Moorhead, J.A., Zeng, Z., 2010. Development of captive breeding techniques for marine ornamental fish. Rev. Fish. Sci. 18, 315–343.
Moorhead, J.A., Zeng, C., 2011. Breeding of the forktail blenny *Meiacanthus atrodorsalis*: Broodstock management and larval rearing. Aquaculture 318, 248–252.
Murchie, K.J., Danylchuk, S.E., Pullen, C.E., Brooks, E., Shultz, A.D., Suski, C.D., Danylchuk, A.J., Cooke, S.J., 2009. Strategies for the capture and transport of bonefish, *Albula vulpes*, from tidal creeks to a marine research laboratory for long-term holding. Aquacult. Res. 40, 1538–1550.
Okuzawa, K., Gen, K., 2013. High water temperature impairs ovarian activity and gene expression in the brain–pituitary–gonadal axis in female red seabream during the spawning season. Gen. Comp. Endocrinol. 194, 24–30.
Ota, K., Kohda, M., 2006. Nest use by territorial males in a shell-brooding cichlid: the effect of reproductive parasitism. J. Ethol. 24, 91–95.
Ota, K., Kohda, M., 2011. Social status-dependent nest choice of territorial males under reproductive parasitism in a Lake Tanganyika cichlid *Telmatochromis vittatus*. J. Fish Biol. 78, 700–712.
Oyugi, D.O., Cucherousset, J., Baker, D.J., Britton, J.R., 2012. Effects of temperature on the foraging and growth rate of juvenile common carp *Cyprinus carpio*. J. Therm. Biol. 37, 89–94.
Pankhurst, N.W., Fitzgibbon, Q.P., 2006. Characteristics of spawning behaviour in cultured greenback flounder *Rhombosolea tapirina*. Aquaculture 253, 279–289.
Pankhurst, N.W., King, H.R., 2010. Temperature and salmonid reproduction: implications for aquaculture. J. Fish Biol. 76, 69–85.
Portz, D.E., Woodley, C.M., Cech Jr., M.J., 2006. Stress-associated impacts of short-term holding on fishes. Rev. Fish Biol. Fish. 16, 125–170.
Reig, L., Duarte, S., Valero, J., Oca, J., 2010. Preference of cultured sole (*Solea senegalensis*) for different substrates differing in material, texture and colour. Aquacult. Eng. 42, 82–89.
Rizzo, E., Sato, Y., Barreto, B.P., Godinho, H.P., 2002. Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts. J. Fish Biol. 61, 615–632.
Ryer, C.H., Lemke, J.L., Boersma, K., Levas, S., 2008. Adaptive coloration, behavior and predation vulnerability in three juvenile north Pacific flatfishes. J. Exp. Mar. Biol. Ecol. 359, 62–66.
Santos, H.B., Sampaio, E.V., Arantes, F.P., Sato, I., 2013. Induced spawning and reproductive variables of the catfish *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae). Neotrop. Ichthyol. 11, 607–614.
Saravanan, R., Vijayanand, P., Vagelli, A.A., Murugan, A., Shanker, S., Rajagopal, S., Balasubramanian, T., 2013. Breeding and rearing of the two striped cardinalfish, *Apogon quadrifasciatus* (Cuvier, 1828) in captive condition. Anim. Reprod. Sci. 137, 237–244.
Sato, Y., Fenerich-Verani, N., Godinho, H.P., 2003a. Reprodução induzida de peixes da bacia do São Francisco. In: Godinho, H.P., Godinho, A.L. (Eds.), Águas, peixes e pescadores do São Francisco das Minas Gerais. Editora PUC Minas, Belo Horizonte, pp. 275–289.
Sato, Y., Fenerich-Verani, N., Nuñer, A.P.O., Godinho, H.P., Verani, J.R., 2003b. Padrões reprodutivos de peixes da bacia do São Francisco. In: Godinho, H.P., Godinho, A.L. (Eds.), Águas, peixes e pescadores do São Francisco das Minas Gerais. Editora PUC Minas, Belo Horizonte, pp. 229–274.
Shibatta, O.A., 2003. Family Pseudopimelodidae. In: Reis, R.E., Kullander, S.O., Ferraris Jr., C.J. (Eds.), Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, pp. 425–426.
Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise induced stress response and hearing loss in goldfish (*Carassius auratus*). J. Exp. Biol. 207, 427–435.
Sun, L., Chen, H., Huang, L., 2006. Effect of temperature on growth and energy budget of juvenile cobia (*Rachycentron canadum*). Aquaculture 261, 872–878.
Tenório, R.A., Santos, A.J.G., Lopes, J.P., Nogueira, E.M.S., 2006. Crescimento do niquim (*Lophiosilurus alexandri* Steindachner 1876), em diferentes condições de luminosidade e tipos de alimento. Acta Sci. Anim. Sci. 28, 305–309.
Thünken, T., Meuthena, D., Bakker, T.C.M., Kullmann, H., 2010. Parental investment in relation to offspring quality in the biparental cichlid fish *Pelvicachromis taeniatus*. Anim. Behav. 80, 69–74.
Tian, X., Fang, J., Dong, S., 2010. Effects of starvation and recovery on the growth, metabolism and energy budget of juvenile tongue sole (*Cynoglossus semilaevis*). Aquaculture 310, 122–129.
Travassos, H., 1960. Catálogo dos peixes do vale do Rio São Francisco. Bol. Soc. Cearense Agron. I, 36.
Tveiten, H., Johnsen, H.K., 1999. Temperature experienced during vitellogenesis influences ovarian maturation and the timing of ovulation in common wolfish. J. Fish Biol. 55, 809–819.
Utoh, T., Horie, N., Okamura, A., Mikawa, N., Yamada, Y., Tanaka, S., Oka, H.P.O., Tsukamoto, K., 2013. Water temperature manipulation can induce oocyte maturation and ovulation in the common Japanese conger, *Conger myriaster*. Aquaculture 392–395, 120–127.
Van Dijk, P.L.M., Hardewig, I., Hölker, F., 2005. Energy reserves during food deprivation and compensatory growth in juvenile roach: the importance of season and temperature. J. Fish Biol. 66, 167–181.
Wu, B., Luo, S., Wang, J., 2015. Effects of temperature and feeding frequency on ingestion and growth for rare minnow. Physiol. Behav. 140, 197–202.
Wysocki, L.E., Dittami, J.P., Ladich, F., 2006. Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.

5. Artigo 2

Profile of amino acids and fatty acids in eggs and larvae of *Lophiosilurus alexandri*, a carnivorous freshwater catfish

Highlights

1. During development, the Protein is saved during ontogeny of *Lophiosilurus alexandri*
2. The profile of amino acids showed variations during the development
3. The lipids were the main source of energy used during the development of *Lophiosilurus alexandri*
4. Saturated fatty acids and monounsaturated are preferably used as an energy source for larvae *Lophiosilurus alexandri*

Profile of amino acids and fatty acids in eggs and larvae of *Lophiosilurus alexandri*, a carnivorous freshwater catfish

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Abstract - The aim of this study was to evaluate the composition of moisture, crude protein, lipids and changes in the profile of amino acids (AA) and fatty acids (FA) during the ontogeny of *L. alexandri*. Samples of eggs, newly hatched larvae (NHL), and larvae at the end of lecithotrophic period (LPL) (eight days post-hatching) were obtained from three spawnings. The moisture values were highest for the eggs, intermediate for NHL, and lowest for LPL. Crude protein did not show any change during development ($P>0.05$). The concentration of lipids was highest in LPL ($P<0.05$). Glutamine was the amino acid (AA) found in the highest concentrations in eggs, NHL, and LPL. The essential amino acids (EAA) isoleucine, leucine, and valine decreased in LPL, while in eggs and NHL, the values were higher and similar to each other. Arginine and lysine showed lower and similar amounts in eggs and NHL and increased in LPL. The dispensable amino acids (DAA), such as aspartic acid, tyrosine, and glycine increased in LPL while alanine decreased in this stage of life. The neutral lipids (NL) percentage increased in LPL larvae. The ΣSFA (saturated fatty acids) decreased during ontogeny ($P < 0.0001$), while ΣMUFA (monounsaturated fatty acids) decreased only in LPL. The ΣPUFA (polyunsaturated fatty acids) was highest in LPL, probably due to increased percentages of the fatty acids Arachidonic (20:4n-6) (ARA), Eicosapentaenoic (C20:5n3) (EPA), and Docosahexaenoic (C22:6 n3) (DHA). Polar fatty acids (PL) were found in larger percentages in eggs and NHL and decreased in LPL. The ΣSFA decreased during ontogenetic development, while ΣMUFA decreased only in LPL. The ΣPUFA was highest in LPL, probably due to the increased percentage of ARA and DHA. The percentage of EPA remained unchanged throughout the experiment. The Protein is kept during ontogenetic development of pacamã although there were changes in the amino acid classes, such as a decrease (IAA) of isoleucine, leucine, and valine in LPL. *L. alexandri* preferably use SAFAs and MUFAs as an energy source, from both the NL and PL fractions, during its early development.

Keywords: essential amino acids, nutritional requirement, embryonic development, pacamã, hatchery.

1. Introduction

From embryogenesis until first feeding, all nutrients required for larvae growth, cell differentiation, and metabolism originate from the yolk sac reserves (Rønnestad et al., 2003; Finn e Fyhn, 2010; Tocher, 2010). Proteins and lipids, as well as their respective amino acids and fatty acids constituents, represent a considerable portion of the composition of fish eggs (Finn and Fyhn, 2010). However, the amount may differ considerably between species (Cross et al., 2013; Yeganeh, et al., 2014) during ontogenetic development (Samaee et al., 2009; Mello et al., 2011; Farhoudi et al., 2012), as a result of the diet provided by breeders (Pickova et al., 2007; Sink et al., 2010; Zakeri et al., 2011) and the habitat (Aragon et al., 2004; Dantagnan et al 2007; Lanes et al., 2012).

During organogenesis, proteins are degraded and absorbed, and the amino acids are used to either form tissues or are catabolised to generate metabolic energy (Li et al., 2009; Fyhn and Finn, 2010; Cross et al., 2013). The fatty acids are destined for energy production, and to become membrane constituents and eicosanoid precursors (Henderson, 1996; Glencross, 2009; Tocher, 2008; 2010).

Knowing the composition of the profile of amino acids and fatty acids in larval tissue provides relevant information for understanding the nutritional requirements of the larvae at the beginning of exogenous feeding (Saavedra et al., 2006; Gurure et al., 2009; Farhoudi et al., 2012). With this information, the nutrient profiles of larvae can be compared to their diet in order to determine possible imbalances (Conceição et al., 2003b; Saavedra et al., 2007; Garrido et al., 2012; Oberg and Fuiman, 2015).

The pacamã *Lophiosilurus alexandri* (Siluriformes: Pseudopimelodidae (Steindachner, 1876) is an endemic species to the São Francisco River, Brazil (Travassos, 1960), which is carnivorous, benthic, and nocturnal (Shibatta, 2003; Tenorio et al., 2006). As it is now an endangered species (Brazil, 2014), this species has been part of restocking programmes in the São Francisco River. Its reproduction occurs in batches (Sato et al., 2003a; Barros et al., 2007; Costa et al., 2015). In general, vitellogenic oocytes of this species are large and adhesive, with a long incubation period compared to other neotropical species (Bazzoli and Godinho, 1997; Rizzo et al., 2002; Guimarães-Cruz et al., 2009; Santos et al., 2013). Its fecundity is considered low (Santos et al., 2013). Despite this knowledge, the absence of nutritional management information for breeders is becoming an obstacle for large scale production of juveniles of this species in captivity. Until now, there is no available information on the composition of the amino acids (AA) and fatty acids (FA)

profiles in the eggs and larvae of pacamã, and this data is an important starting point for the determination of AA and FA requirements for this species.

The aim of this study was to determine the profile of amino acids and fatty acids of *Lophiosilurus alexandri* eggs, newly hatched larvae, and larvae at the end of lecithotrophic period.

2. Material and methods

The present research followed the methodology approved by the Ethics Committee on Animal Use, Protocol. 25/2010 - CEUA / UFMG.

2.1. Sampling

The egg samples used in this study came from breeders that were collected from nature in São Francisco River and were then adapted to captive conditions in the Laboratory of Aquaculture (Laqua) of the Federal University of Minas Gerais (UFMG) (Costa et al., 2015). The breeding animals were kept for about six months in 5 m^3 tanks containing a thin layer of sand (approximately 5 to 10 cm deep) at the bottom (pool filter type) with supplemental aeration (dissolved oxygen > 5.5 mg L^{-1}) and average temperature of 28 °C. The breeders, weighing about 2.5 kg, were fed twice a week with tilapia fillets containing a vitamin premix capsule (600 mg of vitamin C + 30 mg of premix).

In order to achieve this goal amino acids and fatty acids profiles of *Lophiosilurus alexandri* were determined in eggs, newly hatched larvae and larvae at the end of lecithotrophic period.

Eggs from three random spawnings obtained naturally in captivity were collected. The tanks were surveyed on a daily basis, and when spawning was detected, the eggs were collected and cleaned by removing sand particles and foiled eggs.

Eggs were placed in artificial incubators of 40 L^{-1}, hatching occurred 24 hour later and larvae were reared until day eight after hatching (at the end the end of the lecithotrophic period (LPL)).

Incubators were kept at an average temperature of 28 °C with constant aeration system maintaining an oxygen concentration > 5.5 mg L^{-1} (measured using a Hanna HI9146 unit), pH 7.05 (measured using a Hanna HI98129 combo device), and zero total ammonia (colorimetric

test Alfakit, Florianópolis, SC, Brasil). The water was changed once a day in order to maintain quality.

From each spawning, eggs, newly hatched larvae and LPL were collected for biochemical analyses. Samples were frozen in liquid nitrogen and stored in a freezer at -80 ° C for future lyophilisation.

2.2. Biochemical analysis

Protein and dry matter analyses were performed at the Nutrition Laboratory Veterinary/UFMG. The samples were lyophilised (lyophilizer LS 3000AISI 304., São Carlos, SP, Brasil) for about 36 hours. After drying, the samples were crushed for dry matter and nitrogen (protein) analysis. The moisture content was determined by drying the samples in an oven at 110 °C for 24 hours (A.O.A.C 1985). The nitrogen content was obtained using a LECO FP-528 nitrogen/protein analyser (St. Joseph, MI, USA). Subsequently, the nitrogen value found was multiplied by a correction factor of 6.25 to obtain the crude protein value.

2.3. Amino acid profile

Amino acid (AA) analyses were performed at the Protein Chemistry Centre of the Medical School of the University of São Paulo at Ribeirão Preto.

Protein-bound amino acid samples were hydrolysed in 6 M hydrochloric acid at 108 °C for 24h in nitrogen-flushed glass vials. Reversed-phase high pressure liquid chromatography (HPLC), in a Waters Pico-Tag amino acid analysis system that used norleucine as an internal standard, was used. The resulting chromatograms were analysed with Breeze software (Waters, USA). The results were expressed in (mg/g of protein).

2.4. Fatty acids profile

The analysis of fatty acids were carried out in the laboratory of Natural Resources, Institute of Ecology and Science Environmental, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Samples were analysed for total, polar, and neutral lipid contents as well as the fatty acid profile of total lipids. Lipids were extracted with chloroform:methanol (2:1) following the method described by Folch et al (1957) and quantified gravimetrically. Polar and neutral

lipids were separated using adsorption chromatography on silica cartridges (Sep-Pack, Waters S.A., Milford, MA, USA) as described by Juaneda and Rocquelin (1985). Fatty acid methyl esters were obtained using transesterification with methanol in sulphuric acid (Christie 1982) and separated and quantified using gas chromatography. During lipid and fatty acid analysis, samples were protected from oxidation by maintaining them under nitrogen gas and using butylated hydroxy toluene (100 mg L^{-1} of solvent). Fatty acid methyl esters were analysed using a gas chromatograph (Hewlett Packard 5890; Hewlett-Packard Company, Wilmington, DE, USA) equipped with a flame ionization detector and a Supelcowax fused silical capillary column (30 m x 0.32 mm ID, Supelco, Bellefonte, PA, USA) using nitrogen as a carrier. Samples were injected in split mode at 250°C. Column temperature was maintained at 180°C for 12 min, increasing afterwards to 212°C at a rate of 2°C min^{-1} nd maintained at 212°C for 13 min. Fatty acids were identified by comparing the retention times of methyl esters standards (Supelco) and by reference to a well characterised fish oil.

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Analyses System software package, version 8.0 (SAS). The data were submitted to a Cramér-von Mises normality test and Levene homoscedasticity. The data shown as percentages were previously transformed into arcsine for statistical analysis. Logarithmic transformation (natural logarithm) was used for the data that was not normal. Data were tested by one-way ANOVA and compared by Tukey at 5% probability.

5. Results

In Table 1 shows the biometric parameters length and weight of sample eggs, larvae newly hatched (NHL) and end of the period lecithotrophic (LPL).

Table 1. Biometrics of eggs (n= 175), newly hatched larvae (NHL) (n=170) and larvae at the end of lecitotrophic period (LPL) (n= 102).

Parameters	Eggs	NHL	LPL larvae
length (mm)	2,42 ± 0,11	6,48 ± 0,90	12,07 ± 1,28
weight (mg)	21,63 ± 2,65	7,77 ± 0,33	25,27 ± 0,48

The highest averages ($P<0.05$) of moisture were found for the eggs, intermediate values for NHL, and the lowest values for LPL (Table 2). Crude protein did not show any variation throughout development ($P>0.05$). The concentration of total lipids was highest in NHL, followed by LPL and eggs, which were similar ($P<0.05$). The neutral lipids NL percentages were higher in the LPL, lower and similar amounts percentages were observed in NHL and eggs ($P<0.0001$), and the PL percentages were lowest in the LPL, and similar higher values were found in eggs and NHL ($P <0.0001$).

Table 2. Mean (± SD) values of moisture (%), protein (% dry weight), total lipids (% dry weight), neutral lipids (NL) (% dry weight) and polar lipids (PL) (% dry weight) of fertilised eggs, newly hatched larvae (NHL), and larvae at the end of lecithotrophic period (LPL) of *Lophiosilurus alexandri*.

Parameters	Eggs	NHL	LPL
Moisture	81.91 ± 2.97^{a}	78.22 ± 0.97^{ab}	76.26 ± 0.52^{b}
Protein	61.01 ± 3.86^{a}	64.85 ± 2.31^{a}	60.28 ± 0.20^{a}
Total lipids	17.50 ± 0.65^{b}	$23.65 \pm 0,25^{a}$	16.64 ± 1.45^{b}
Neutral lipids	38.01 ± 0.53^{b}	39.04 ± 0.69^{b}	45.59 ± 0.74^{a}
Polar lipids	61.98 ± 0.53^{a}	61.16 ± 0.44^{a}	54.29 ± 0.86^{b}

Different letters indicate significant differences by Tukey test ($P<0.05$).

3.1 Amino acid contents

During the ontogenetic development of *L. alexandri*, 18 amino acids (AA) were quantified, ten indispensable amino acids (IAA) and eight dispensable amino acids (DAA) (Table 3). In eggs, the amino acids found with the highest concentrations were Glu (DAA) and Leu (IAA) that had values that were different to each other ($P < 0.0001$), followed by Ala and Asp (DAA) that were similar ($P > 0.05$). The least found AA was Cys (DAA). Additionally, low concentrations of many IAAs, such as His, Met, Phe, and Trp, were also found with values similar to each other ($P > 0.05$). In NHL, Glu and Leu also presented the highest concentrations and also differed to each other ($P < 0.0001$), and they were followed by Asp. Cys was the AA with the lowest concentration. Similar to that which was found in eggs, low concentrations of IAAs in NHL, such as His, Met, Phe, and Trp, were recorded with values similar to each other ($P > 0.05$). In LPL, Glu was still the largest constituent of the AAs, which was followed by Asp and Arg that differed to each other ($P < 0.0001$). The IDAAs, such as His, Ile, Met, Phe, and Trp, were found in low concentrations and the values were similar to each other ($P > 0.05$).

During the different stages of development, His, Met, Phe, Thr, Cys, and Pro showed similar concentrations ($P > 0.05$) (Table 3). Arg, Lys, Asp, Glu, Gly, and Tyr showed the highest concentrations in LPL ($P < 0.05$), while eggs and NHL showed lower concentrations that were similar to each other ($P > 0.05$). In contrast, LPL presented the lowest concentrations of Ile, Leu, Val, and Ala ($P < 0.05$). In eggs and NHL, concentrations of these AAs were higher and similar among these phases ($P > 0.05$). The concentration of Trp decreased throughout the development. Ser showed the highest concentration in eggs, intermediate values for NHL, and the lowest values for LPL.

Table 3. Mean (± SD) of the concentration of total (protein bound + free) amino acids content in eggs and newly hatched larvae (NHL), and larvae at the end of the lecithotrophic (LPL) period of *Lophiosilurus alexandri*. The statistical values of F and the probability are also presented.

Amino acids (mg/g of protein)	Eggs	NHL	LPL	F value	Prob.
Indispensable amino acids (IAA)					
Arginine (Arg)	71.0 ± 0.9^{Db}	75.2 ± 2.7^{Db}	81.9 ± 1.3^{Ca}	26.9	0.0010
Histidine (His)	29.0 ± 0.4^{Ga}	33.9 ± 3.9^{Ha}	30.3 ± 3.7^{Ha}	2.0	0.2156
Isoleucine (Ile)	55.9 ± 6.7^{EFa}	57.7 ± 0.4^{FGa}	35.2 ± 4.5^{Hb}	21.3	0.0019
Lysine (Lys)	54.4 ± 3.7^{EFb}	50.6 ± 4.1^{Gb}	63.8 ± 2.2^{DEa}	11.7	0.0085
Leucine (Leu)	104.2 ± 3.4^{Ba}	103.9 ± 2.6^{Ba}	74.6 ± 2.9^{CDb}	97.0	<0.0001
Metionine (Met)	$34.9 \pm 1,4^{Ga}$	33.9 ± 0.2^{Ha}	31.4 ± 4.2^{Ha}	1.5	0.2846
Phenylalanine (Phe)	$37.5 \pm 1,4^{Ga}$	36.5 ± 1.6^{Ha}	38.8 ± 3.3^{Ha}	0.7	0.5073
Treonine (Thr)	$51.9 \pm 1,9^{Fa}$	53.6 ± 0.1^{Ga}	51.8 ± 2.0^{FGa}	1.2	0.3585
Tryptophan (Trp)	$35.5 \pm 0,2^{Ga}$	32.6 ± 0.3^{Hb}	30.2 ± 0.1^{Hc}	189.0	<0.0001
Valine (Val)	$65.5 \pm 4,9^{Da}$	65.7 ± 0.9^{EFa}	49.2 ± 3.0^{FGb}	23.8	0.0014
Dispensable amino acids (DAA)					
Alanine (Ala)	86.4 ± 2.5^{Ca}	80.3 ± 3.1^{Da}	59.6 ± 3.0^{EFb}	72.8	<0.0001
Aspartic acid (Asp)	93.8 ± 2.7^{Cb}	91.8 ± 0.5^{Cb}	112.8 ± 5.5^{Ba}	32.5	0.0006
Cysteine (Cys)	3.6 ± 0.7^{Ha}	2.5 ± 0.9^{Ia}	4.5 ± 2.7^{Ia}	1.1	0.3806
Glutamine (Glu)	128.9 ± 1.0^{Ab}	131.3 ± 1.4^{Ab}	160.6 ± 6.7^{Aa}	58.2	<0.0001
Glycine (Gly)	30.3 ± 2.1^{Gb}	31.8 ± 1.0^{Hb}	56.6 ± 1.8^{EFa}	229.5	<0.0001
Proline (Pro)	54.0 ± 1.8^{Fa}	54.8 ± 1.8^{FGa}	55.9 ± 5.6^{EFa}	0.2	0.8091
Serine (Ser)	62.5 ± 4.6^{DEa}	60.4 ± 0.2^{EFab}	54.3 ± 0.2^{EFb}	7.8	0.0217
Tyrosine (Tyr)	36.3 ± 0.7^{Gb}	36.0 ± 0.2^{Hb}	38.8 ± 1.3^{GHa}	9.4	0.0140
F value	338.4	734.4	292.		
Prob.	<0.0001	<0.0001	<0.0001		

Lower case letters horizontally and vertically different capital letters indicate significant differences by Tukey test ($P<0.05$).

3.2 Fatty acids content

The fatty acids (FA) of composition of the Neutral lipids (NL) is shown in Table 4. During ontogeny, the percentages of the saturated FAs (SFA) C10:0, C 12:0, and C23:0 did not change in all stages of life. The C14:0 and C16:0 SFA were found in larger percentages in eggs and NHL. The C15: 0 and C17: 0 SFA were highest in eggs and lower in NHL and LPL, which were similar to each other. C18:0, C21:0 and C22:0 also showed the highest values in LPL, while in NHL and eggs, they presented lower values, which were similar to each other. The ΣSFA decreased throughout development from egg to LPL ($P<0.0001$).

The monounsaturated FA (MUFA) C14: 1 was highest in eggs, and lower in NHL and LPL, with values similar to each other. C16:1n-7 and C22:1n-7 showed the lowest percentages in LPL, and in eggs and NHL higher values were found that similar to each other (Table 4). The highest percentage of C16:1n-5 was found in eggs, with intermediate values in NHL, and the lowest values in LPL. NHL presented the highest percentages of C18:1n-9, followed by the eggs and LPL. The other MUFA had similar values during development. ΣMUFA values were highest and similar in eggs ($P<0.0001$) and NHL ($P<0.0001$) and lowest in LPL ($P<0.0001$).

The polyunsaturated FA (PUFA) C21:5n-3 was found in the highest percentages in LPL, with intermediate values in eggs, and the lowest values in NHL. C16:4n-1 was highest in NHL and similar and lower in eggs and LPL. The concentration of C18:2n-6 increased during ontogenetic development. As for C18:3n-3, the highest values were found in LPL, intermediate values in eggs, and the lowest values in NHL. The PUFAs C20:2n-9, C20:2n-6, C20:3n-6, C20:4n-6, C20:4n-3, C20:5n-3, C22:4n-6, C22:5n-6, C22:5n-3 and C22:6n-3 showed lower and similar amounts in eggs and NHL and the highest proportions in LPL. C16:2n-3, C18:3n-6, C18:4n-3, C20:3n-3, and C22:2n-6 showed similar results in the different phases. ΣPUFA was highest in LPL ($P<0.0001$) and lower and similar to each other in eggs and NHL.

The FAs of the n-9 series was least in LPL (Table 4). The n-3 ($P<0.0001$) and n-6 series ($P<0.0001$) were highest in LPL. The highly unsaturated FAs (HUFAs) of the n-3 ($P<0.0001$) and n-6 series ($P<0.0001$) were highest in LPL. The DHA/EPA ratio was highest in LPL. The DHA/ARA ratio remained the same during ontogenetic development ($P<0.0001$).

Table 4. FA Neutral lipids (NL) profile expressed as % of total FA in eggs, newly hatched larvae (NHL), and the end of the lecitotrophic larval period (LPL) of *Lophiosilurus alexandri*. (Mean (± SD).The statistical values of F and the probability are also presented.

% of total FA	**Eggs**	**NHL**	**LPL**	**F**	**P**
10:0	0.01±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}	1.50	0.2963
12:0	0.10±0.00^{a}	0.09±0.00^{a}	0.09±0.01^{a}	4.75	0.0580
13:0	1.26±0.27^{ab}	0.83±0.17^{b}	1.35±0.07^{a}	6.73	0.0293
14:0	4.95±0.24^{a}	5.12±0.05^{a}	2.68±0.21^{b}	193.67	<0.0001
15:0	0.89±0.10^{a}	0.63±0.05^{b}	0.46±0.06^{b}	30.79	0.0007
16:0	35.90±0.35^{a}	35.62±0.06^{a}	26.15±0.22^{b}	1775.18	<0.0001
17:0	0.84±0.09^{a}	0.58±0.01^{b}	0.60±0.01^{b}	21.50	0.0018
18:0	4.49±0.06^{b}	4.69±0.01^{b}	9.77±0.21^{a}	1110.63	<0.0001
21:0	0.04±0.01^{b}	0.04±0.01^{b}	0.12±0.01^{a}	19.70	0.0023
22:0	0.00±0.00^{b}	0.00±0.00^{b}	0.24±0.09^{a}	39.69	0.0003
23:0	0.18±0.08^{a}	0.13±0.02^{a}	0.19±0.02^{a}	0.50	0.6309
∑ SFA	49.05±0.07^{a}	48.38±0.03^{b}	42.68±0.28^{c}	2143.90	<0.0001
14:1n-5	0.11±0.02^{a}	0.09±0.01^{a}	0.10±0.02^{a}	1.95	0.2230
14:1	0.34±0.05^{a}	0.22±0.06^{b}	0.15±0.06^{b}	27.85	0.0009
15:1n-5	0.06±0.01^{a}	0.06±0.00^{a}	0.12±0.01^{a}	2.87	0.1332
16:1n-9	1.00±0.13^{a}	1.12±0.19^{a}	0.97±0.07^{a}	1.17	0.3715
16:1n-7	7.09±0.14^{a}	6.92±0.32^{a}	3.99±0.17^{b}	201.40	<0.0001
16:1n-5	0.35±0.07^{a}	0.28±0.06^{ab}	0.17±0.05^{b}	6.93	0.0275
17:1	0.08±0.01^{a}	0.06±0.01^{a}	0.07±0.00^{a}	2.55	0.1583
18:1n-11	0.15±0.02^{a}	0.17±0.01^{a}	0.17±0.00^{a}	3.55	0.0963
18:1n-9	24.76±0.65^{b}	26.34±0.48^{a}	21.29±0.02^{c}	92.18	<0.0001
18:1n-7	5.54±0.31^{a}	5.15±0.38^{a}	4.70±0.51^{a}	3.93	0.0811
18:1n-5	0.32±0.05^{a}	0.30±0.04^{a}	0.24±0.03^{a}	3.12	0.1180
20:1n-11	0.09±0.02^{a}	0.05±0.01^{a}	0.04±0.00^{a}	4.24	0.0711
20:1n-9	1.33±0.14^{a}	1.33±0.04^{a}	1.25±0.01^{a}	1.09	0.3940
20:1n-7	0.13±0.04^{a}	0.08±0.01^{a}	0.11±0.03^{a}	2.78	0.1400
22:1n-11	0.04±0.14^{a}	0.08±0.04^{a}	0.08±0.01^{a}	1.55	0.2869
22:1n-9	0.40±0.04^{a}	0.37±0.01^{a}	0.31±0.03^{a}	4.02	0.0780
22:1n-7	0.28±0.09^{a}	0.29±0.05^{a}	0.13±0.02^{b}	6.65	0.0301
∑ MUFA	42.07±0.44^{a}	42.91±0.03^{a}	33.89±0.83^{b}	401.59	<0.0001
16:2n-3	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.31	0.7461
16:4n-1	0.08±0.02^{b}	0.18±0.03^{a}	0.07±0.02^{b}	25,37	0.0012
18:2n-6	4.01±0.10^{c}	4.29±0.04^{b}	6.38±0.05^{a}	1172.77	<0.0001
18:3n-6	0.04±0.01^{a}	0.03±0.00^{a}	0.08±0.00^{a}	1.33	0.3316
18:3n-3	0.27±0.01^{b}	0.16±0.01^{c}	0.36±0.01^{a}	70.73	<0.0001
18:4n-3	0.08±0.01^{a}	0.08±0.02^{a}	0.11±0.01^{a}	4.94	0.0539
20:2n-9	0.28±0.02^{b}	0.29±0.02^{b}	0.41±0.02^{a}	56.48	0.0001
20:2n-6	0.32±0.03^{b}	0.35±0.03^{b}	0.83±0.01^{a}	498.91	<0.0001
20:3n-6	0.57±0.08^{b}	0.61±0.03^{b}	2.95±0.14^{a}	933.89	<0.0001
20:4n-6	0.31±0.02^{b}	0.31±0.05^{b}	3.60±0.32^{a}	592.47	<0.0001
20:3n-3	0.14±0.05^{a}	0.11±0.05^{a}	0.19±0.03^{a}	2.39	0.1722
20:4n-3	0.07±0.02^{b}	0.04±0.01^{b}	0.15±0.02^{a}	47.18	0.0002
20:5n-3	0.32±0.06^{b}	0.26±0.01^{b}	0.50±0.01^{a}	41.21	0.0003
22:2n-6	0.07±0.02^{a}	0.07±0.02^{a}	0.08±0.03^{a}	0.13	0.8810
21:5n-3	0.04±0.01^{ab}	0.00±0.00^{b}	0.12±0.00^{a}	7.89	0.0209

22:4n-6	0.15±0.05[b]	0.14±0.01[b]	0.83±0.05[a]	59.22	0.0001
22:5n-6	0.28±0.09[b]	0.32±0.04[b]	1.67±0.29[a]	111.28	<0.0001
22:5n-3	0.24±0.06[b]	0.23±0.07[b]	0.75±0.06[a]	78.35	<0.0001
22:6n-3	0.37±0.08[b]	0.31±0.02[b]	3.39±0.25[a]	663.50	<0.0001
∑ PUFA	7.67±0.36[b]	7.77±0.06[b]	22.51±1.20[a]	744.01	<0.0001
n-9	26.53±0.20[a]	27.16±0.15[a]	17.49±0.09[b]	659.56	<0.0001
n-3	1.54±0.14[b]	1.21±0.04[b]	5.59±0.27[a]	933.39	<0.0001
n-6	5.74±0.15[b]	6.12±0.06[b]	16.41±0.96[a]	635,60	<0.0001
n-3HUFA	1.19±0.12[b]	0.95±0.04[b]	5.11±0.28[a]	882.61	<0.0001
n-6HUFA	1.30±0.02[b]	1.38±0.04[b]	9.04±0.96[a]	399.03	<0.0001
n-3/n-6	0.27±0.02[b]	0.20±0.01[c]	0.34±0.00[a]	77.04	<0.0001
DHA/EPA	1.22±0.50[b]	1.20±0.10[b]	6.82±0.59[a]	219,40	<0.0001
DHA/ARA	1.22±0.32[a]	1.02±0.22[a]	0.94±0.01[a]	1.17	0,3722

Different letters indicate significant differences by Tukey test (P<0.05).Capric (C10:0), Lauric (C12: 0), Tridecanoic (C13:0), Myristic (C14:0), Pentadecanoic (C15:0), Palmitic (C16:0), Heptadecanoic (C17:0), Stearic (C18:0), Nonadecanoic (C19:0), Heneicosanóico (C21:0), Behenic (C22:0), Tricosanoic Methyl Ester (C23:0), Myristoleic (C14:1), Physeteric (14: 1 n5) 10-Pentadecenoic (C15:1n-5), Palmitoleic (C16:1), Palmitoleic (C16:1n-7), Acid Margaric (C17:1), Heptadecenoic (C17:1), Vaccenic (C18: 1 n11) Oleic (C18:1n-9), Vaccenic (C18:1n-7), 13-Octadecenoic (C18:1 n-5), 9-Eucosenoic (C20:1n-11), 11-Eucosenoic (C20:1 n-9), Paullinic (C20: n-7), Cetoleic (C22:1n-11), Erucic (C22:1n-9) 15-Docosenoic (C22:1 n-7), 9,12 Hexadecadienoic (16:2 n-4), 6,9,12,15-Hexadecatetraenoic (C16:4 n-1), Linoleic (C18:2 n-6), Gamma Linolenic (C18:3n-6), Linolenic (C18:3n3), Stearidonic (C18:4 n-3), 8,11-Eicosadienoic (C20:2 n-9), Eicosadienoic (C20:2n-6), Eicosatrienoic (C20:3 n-3), Dihomo-Gamma-Linolenic (C20:3n-6), Arachidonic (C20:4 n-6), Eicosatetraenoic (C20:4 n-3), Docosadienóicico (C22:2n-6), Eicosapentaenoic (C20:5n-3) (EPA), Heneicosapentaenoic (C21:5n-3), Docosadienoic (C22:2n-6), Adrenic (C22:4n-6), Docosapentaenoic (22:5n-6), Docosapentaenoic (C22:5n-3), Docosahexaenoic (C22:6n-3) (DHA).

The FA composition of Polar lipids (PL) is shown in Table 5. The SFA C10:0, C12:0, C16:0, and C18:0 were lowest in LPL and higher and similar to each other in eggs and NHL. C13:0, C22:0, and C23:0 were highest in LPL and lower and similar to each other in eggs and NHL. C14:0 and C15:0 decreased during ontogenetic development. The highest percentage of C17 was found in eggs. ΣSFA decreased during the development of eggs to LPL (P<0.0001).

The C14:1n-5, C15:1n-5, C16:1n-9, and C22:1n-11 MUFA were similar in the different development stages (Table 5). The eggs presented the highest percentages of C14:1 and were lower in NHL and LPL. The eggs and NHL showed the highest and similar percentages for C16:1n-7, C18:1n-9, C20:1n-9, C20:1n-7, and C22:1n-7. The C16:1n-5, C17:1, C18:1n-5, and C22:1n-9 FA values were lowest in LPL, intermediate in NHL, and highest in eggs. C18:1n-7 and C20:1n-11 decreased during the phases from eggs to LPL.

The C18:3n-6, C18:4n-3, C20:2n-9, C20:3n-3, C20:5n-3, and C21:5n-3 PUFA showed constant percentages throughout ontogenetic development. C16:2n-3 was lowest in LPL. C16:4n-1 and C18:2n-6 exhibited the highest values in NHL and smaller and similar values to

each other in eggs and LPL. The percentage of C18:3n-3 decreased during development. NHL had lower values of C20:4n-3 and C22:2n-6. The percentages of C20:2n-6 increased in NHL and LPL. The percentages of C20:3n-6 increased during development. The percentages of C20:4n-6, C22:4n-6, C22:5n-6, C22:5n-3 and C22:6n-3 were highest in LPL. ΣPUFA was highest in LPL ($P<0.0001$).

The n-9 percentages were lowest in LPL ($P<0.0001$) (Table 4). For n-3 and n-6, the highest values were observed in LPL ($P<0.0001$). The percentages of the n-3 and n-6 HUFAs were lower and similar in eggs and NHL and highest in LPL ($P<0.0001$). The n3/n6 ratio was highest in LPL, intermediate in eggs, and lowest in NHL ($P<0.0001$). The DHA/EPA and DHA/ARA ratios were highest in LPL ($P<0.0001$).

Table 5. FA Polar lipids (PL) expressed in % of total FA in eggs, newly hatched larvae (NHL), and the end of the lecitotrophic larval period (LPL) of *Lophiosilurus alexandri*. (Mean (± SD). The statistical values of F and the probability are also presented.

% of total FA	Eggs	NHL	LPL	F	P
10:0	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{b}	15.09	0.0046
12:0	0.03±0.00^{a}	0.03±0.00^{a}	0.01±0.00^{b}	29.29	0.0008
13:0	1.02±0.17^{b}	0.81±0.07^{b}	1.32±0.13^{a}	14.41	0.0051
14:0	1.43±0.05^{a}	1.26±0.02^{b}	0.82±0.01^{c}	342.67	<0.0001
15:0	0.57±0.05^{a}	0.46±0.04^{b}	0.21±0.01^{c}	73.85	<0.0001
16:0	24.26±0.99^{a}	23.44±0.11^{a}	20.26±0.16^{b}	40.12	0.0003
17:0	0.94±0.14^{a}	0.58±0.03^{b}	0.45±0.02^{b}	29.21	0.0008
18:0	17.50±0.43^{a}	17.94±0.51^{a}	15.07±0.03^{b}	47.80	0.0002
21:0	0.11±0.05^{a}	0.15±0.02^{a}	0.15±0.01^{a}	1.54	0.2884
22:0	0.11±0.02^{b}	0.13±0.01^{b}	0.27±0.03^{a}	10.07	0.0121
23:0	0.07±0.01^{b}	0.05±0.01^{b}	0.18±0.00^{a}	54.72	0.0001
∑SFA	46.77±0.60^{a}	45.36±0.69^{b}	39.25±0.13^{c}	172.66	<0.0001
14:1n-5	0.03±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	3.00	0,1250
14:1	0.10±0.00^{a}	0.05±0.00^{b}	0.04±0.00^{b}	22.46	0.0016
15:1n-5	0.04±0.01^{a}	0.03±0.00^{a}	0.02±0.00^{a}	4.75	0.0580
16:1n-9	0.81±0.03^{a}	0.64±0.18^{a}	0.63±0.05^{a}	2.54	0.1588
16:1n-7	3.10±0.25^{a}	2.88±0.17^{a}	1.38±0.03^{b}	83.13	<0.0001
16:1n-5	0.20±0.05^{a}	0.14±0.02^{ab}	0.09±0.01^{b}	11.47	0.0089
17:1	0.05±0.01^{a}	0.03±0.00^{ab}	0.02±0.00^{b}	5.25	0.0481
18:1n-11	0.18±0.01^{a}	0.17±0.02^{a}	0.10±0.01^{b}	16.76	0.0035
18:1n-9	23.78±0.49^{a}	24.58±0.39^{a}	15.48±0.08^{b}	583.02	<0.0001
18:1n-7	5.68±0.62^{a}	4.74±0.01^{b}	3.48±0.01^{c}	28.46	0.0009
18:1n-5	0.25±0.08^{a}	0.23±0.03^{ab}	0.11±0.00^{b}	7.48	0.0235
20:1n-11	0.07±0.00^{a}	0.06±0.01^{b}	0.01±0.00^{c}	57.00	0.0001
20:1n-9	1.28±0.16^{a}	1.40±0.03^{a}	0.85±0.00^{b}	29.79	0.0008
20:1n-7	0.09±0.00^{a}	0.09±0.00^{a}	0.06±0.00^{b}	12.50	0.0073
22:1n-11	0.07±0.02^{a}	0.06±0.02^{a}	0.04±0.00^{a}	2.54	0.1585
22:1n-9	0.43±0.02^{a}	0.37±0.02^{ab}	0.25±0.05^{b}	8.27	0.0188
22:1n-7	0.44±0.02^{a}	0.34±0.05^{a}	0.18±0.07^{b}	16.24	0.0038
∑MUFA	36.83±1.35^{a}	35.94±0.47^{a}	22.99±0.10^{b}	263.24	<0.0001
16:2n-3	0.01±0.00^{a}	0.02±0.00^{a}	0.00±0.00^{b}	10.94	0.0100
16:4n-1	0.04±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	5.85	0.0389
18:2n-6	4.88±0.43^{b}	6.27±0.13^{a}	4.92±0.12^{b}	24.35	0.0013
18:3n-6	0.16±0.01^{a}	0.14±0.03^{a}	0.12±0.01^{a}	4.32	0.0689
18:3n-3	0.24±0.01^{a}	0.18±0.02^{b}	0.14±0.00^{c}	38.67	0.0004
18:4n-3	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.26	0.7805
20:3n-6	1.72±0.36^{c}	2.50±0.12^{b}	4.41±0.26^{a}	94.48	<0.0001
20:2 n-9	0.23±0.05^{a}	0.27±0.03^{a}	0.27±0.04^{a}	0.93	0.4462
20:2 n-6	0.67± 0.09^{b}	0.86± 0.04^{a}	0.95±0.01^{a}	17.87	0.0030
20:4n-6	2.65±0.40^{b}	3.10±0.16^{a}	9.05±0.03^{a}	606.38	<0.0001
20:3n-3	0.12±0.03^{a}	0.10±0.02^{a}	0.12±0.01^{a}	1.06	0.4042
20:4n-3	0.07±0.02^{ab}	0.05±0.02^{b}	0.09±0.01^{a}	6.27	0.0338
20:5n-3	0.73±0.07^{a}	0.56±0.05^{a}	0.60±0.00^{a}	2.05	0.2092
22:2n-6	0.06±0.02^{a}	0.01±0.00^{b}	0.04±0.00^{ab}	6.45	0.0320
21:5n-3	0.03±0.00^{a}	0.00±0.00^{a}	0.02±0.00^{a}	2.25	0.1867

22:4n-6	0.39±0.10[b]	0.42±0.07[b]	1.48±0.03[a]	225.39	<0.0001
22:5n-6	0.78±0.26[b]	1.02±0.17[b]	4.03±0.08[a]	295.19	<0.0001
22:5n-3	0.52±0.03[b]	0.42±0.05[b]	1.39±0.04[a]	593.24	<0.0001
22:6n-3	2.31±0.37[b]	2.24±0.34[b]	9.57±0.02[a]	621.31	<0.0001
∑PUFA	15.70±1.84[b]	17.71±0.87[b]	37.35±0.23[a]	278.52	<0.0001
n-9	26.73±0.30[a]	27.53±0.56[a]	17.86±0.05[b]	259.56	<0.0001
n-3	4.08±0.37[b]	3.60±0.45[b]	11.97±0.02[a]	575.52	<0.0001
n-6	11.96±1.55[c]	15.18±0.46[b]	25.94±0.29[a]	175.30	<0.0001
n-3HUFA	3.78±0.37[b]	3.37±0.43[b]	11.80±0.02[a]	620.86	<0.0001
n-6HUFA	5.54±1.08[b]	7.04±0.34[b]	18.96±0.17[a]	377.91	<0.0001
n-3/n-6	0.36±0.03[b]	0.25±0.02[c]	0.48±0.00[a]	102.19	<0.0001
DHA/EPA	3.15±0.42[b]	3.97±0.54[b]	15.86±0.06[a]	963.74	<0.0001
DHA/ARA	0.87±0.05[b]	0.72±0.08[b]	1.06±0.00[a]	26.19	0.0011

Different letters indicate significant differences by Tukey test (P<0.05). Capric (C10:0), Lauric (C12: 0), Tridecanoic (C13:0), Myristic (C14:0), Pentadecanoic (C15:0), Palmitic (C16:0), Heptadecanoic (C17:0), Stearic (C18:0), Nonadecanoic (C19:0), Heneicosanóico (C21:0), Behenic (C22:0), Tricosanoic Methyl Ester (C23:0), Myristoleic (C14:1), Physeteric (14: 1 n5) 10-Pentadecenoic (C15:1n-5), Palmitoleic (C16:1), Palmitoleic (C16:1n-7), Acid Margaric (C17:1), Heptadecenoic (C17:1), Vaccenic (C18: 1 n11) Oleic (C18:1n-9), Vaccenic (C18:1n-7), 13-Octadecenoic (C18:1 n-5), 9-Eucosenoic (C20:1n-11), 11-Eucosenoic (C20:1 n-9), Paullinic (C20: n-7), Cetoleic (C22:1n-11), Erucic (C22:1n-9) 15-Docosenoic (C22:1 n-7), 9,12 Hexadecadienoic (16:2 n-4), 6,9,12,15-Hexadecatetraenoic (C16:4 n-1), Linoleic (C18:2 n-6), Gamma Linolenic (C18:3n-6), Linolenic (C18:3n3), Stearidonic (C18:4 n-3), 8,11-Eicosadienoic (C20:2 n-9), Eicosadienoic (C20:2n-6), Eicosatrienoic (C20:3 n-3), Dihomo-Gamma-Linolenic (C20:3n-6), Arachidonic (C20:4 n-6), Eicosatetraenoic (C20:4 n-3), Docosadienóicico (C22:2n-6), Eicosapentaenoic (C20:5n-3) (EPA), Heneicosapentaenoic (C21:5n-3), Docosadienoic (C22:2n-6), Adrenic (C22:4n-6), Docosapentaenoic (22:5n-6), Docosapentaenoic (C22:5n-3), Docosahexaenoic (C22:6n-3) (DHA).

4. Discussion

During embryogenesis up to the first feeding, all nutrients needed for growth, cell differentiation, and metabolism of organs originate from the yolk reserves (Wiegand 1996; Tocher, 2010). Thus, fish eggs should contain all the essential nutrients required for embryo development and larvae growth (Kamler, 2008; Rønnestad et al., 1999). The synthesis of nutrients contained in eggs is totally dependent on the supply of maternal nutrients (Finn and Fyhn, 2010). Hence, the diet provided by breeders is able to change the fatty acids and amino acid profiles in fish eggs and larvae (Gunasekera et al., 1996; Pickova et al., 2007; Yanes-Roca et al., 2009; Sink et al., 2010). As *L. alexandri* is a carnivorous species whose nutritional requirements for growth and reproductive success are, to date, not fully understood, we collected breeders from their natural environment and adapted them to laboratory conditions. They were fed tilapia fillets with a vitamin premix (600 mg of vitamin

C + 30 mg of premix). From the three spawnings collected, normal development of eggs and larvae was observed.

The eggs of *L. alexandri* showed the highest percentage of moisture, which decreased during the different stages. The high moisture value found in the eggs can be directly related to the absorption of water during the formation of the embryo cell (Fyhn and Finn, 2010).

Crude protein remained constant during the different stages of development, indicating that this component is preserved in the ontogenetic. Variation in the protein consumption content and sequence during development seems to differ among species (Gunasekera et al., 1999; Samaee et al., 2010; Finn and Fyhn, 2010). In species with carnivorous habits, such as *Maccullochella macquariensis*, *M. peelii peelii*, *Scophthalmus maximus*, and *Dicentrarchus labrax*, protein concentration decreases immediately after hatching, showing that this nutrient is an energy source during development (Finn et al., 1996; Rønnestad et al., 1998 Gunasekera et al., 1999a). The fact that the protein levels remain the same in *L. alexandri* may be a result of the mobilisation of this protein for the formation of structural tissues. On the other hand, the amino acid composition exhibited differences during ontogenetic development.

In *L. alexandri*, the His Met, Phe, and Thr IAA presented constant concentrations throughout development. IAA conservation is a nutritional strategy geared to maintaining larvae before they start exogenous feeding (Conceição et al., 2002). According to Li et al. (2009), His is preserved to the responsible for maintaining the body homeostasis and Met for being considered AA limiting (Zhou et al., 2011). Phe is the precursor for the synthesis of Tyr and is conservation for the synthesis of specific hormones (Li et al., 2009). However, Tyr increased in larvae after hatching. Tyr is the immediate precursor for the synthesis of several hormones including thyroxin (T4) and melatonin (Li et al., 2007). Conceição et al. (1997) suggested in *Scophthalmus maximus* that this AA retention or increase may be associated with early thyroid gland activity. Furthermore, fish thyroid hormones have a significant role in the pigmentation of skin and process and structure formation (Power et al., 2001; Li et al., 2007).

Concentrations of the IAA Ile, Leu, and Val decreased in LPL, while Trp decreased along of the development. This suggests that some of these AAs may have been mobilised, preferentially, as an energy source when the yolk reserves became depleted. During the development of *Scophthalmus maximus* larvae, the amino acid which was predominantly present in eggs and larvae was Leu. Hence, according to Conceição et al. (1997), larvae of this species may have higher Leu requirements than other species, since it is the AA with the highest concentration. Following this reasoning, *L. alexandri* larvae may also require more Leu, since this IAA was found predominantly in eggs and larvae. Leu is essential for growth

and works by stimulating protein synthesis in muscular tissues (Abidi & Khan, 2007). Trp is the main precursor of serotonin and is known for affecting food intake and aggression in fish (Hseu et al. 2003; Hoglund et al., 2005; Li et al., 2007). Portela et al. (2013) stated that in *Piaractus mesopotamicus* Trp was probably mainted for the synthesis of serotonin and melatonin. A similar behaviour throughout development was reported for *Latris lineata* (Brown et al., 2005). In *L. alexandri*, a sedentary species that occupies the bottom of rivers, this reduction in Trp levels during ontogeny may indicate that the concentration of this AA is reduced dramatically before the first feeding. One has to also consider that this species presents cannibalistic behaviour in the early stage of life and this can be stopped when a source of live food is offered (Santos and Luz 2009; Takata et al., 2014).

Val is involved in many metabolic pathways and is considered an indispensable AA. Its reduction may be associated with protein synthesis and fish growth (Abidi and Khan, 2004; Ahmed and Khan, 2006), which could explain the results of this study in which reduced quantities of LPL were found in *L. alexandri*.

In LPL, Arg and Lys were the only IAA whose proportions increased. Arg is an essential amino acid involved in several metabolic pathways, such as protein synthesis and production of urea and nitric oxide (Alam et al., 2002;. Li et al., 2009; Cheng et al., 2011.). In *M. macquariensis*, *M. peelii peelii* (Gunasekera et al., 1999a), and *Lates calcarifer* (Dayal et al., 2003), Arg decreased in larvae after yolk absorption, which can be an indication that this AA may be required as an energy source when the endogenous reserves are depleted. In *L. alexandri,* an increase in concentrations of this AA was observed in the LPL, showing that this AA may be being conservation and mobilised for protein synthesis. Similar behaviour was observed for the percentage of Lys, which is involved with growth and an increase of immune response in fish (May et al., 2006b;. Zhang et al., 2008).

The high concentrations of AA, such as Leu and Ala, found in the eggs and NHL are similar to those observed for other freshwater species such as *M. macquariensis*, *M. peelii peelii* (Gunasekera et al., 1999a), and *Ictalurus punctatus* (Sink et al., 2010). In *L. alexandri* LPL, this ratio is changed for Glu, Asp, and Leu. As recorded for *L. alexandri* in this study, high Glu concentrations have been observed throughout development in other species such as *Latris lineata*, *Salvelinus alpinus*, *Solea senegalensis,* and *Dicentrachus labrax* (Brown et al., 2005; Gurure et al., 2007; Cara et al., 2007). This is understandable since this AA has an important role as a structural component in the first stages of life although a higher proportion of it is required as the main source of energy during early development (Conceição et al., 2002; 2003; Li et al., 2007; Cheng et al., 2011). The proportions of Ala and Ser were

strikingly decreased in LPL of *L. alexandri*. These AAs are the main glucogenic precursors and are important energy substrates for fish that can be synthesised through certain biochemical pathways in the body itself (Conceição et al., 2003; Li et al., 2009).

AA requirements for the early life stages of fish are high due to fast growth rate (Conceição et al., 1998; Rønnestad et al., 1999). Thus, determination of the AA profile in eggs and larvae after hatching can be used as an indicator of the nutritional quality of the larvae (Guy et al., 2007; Jaya-Ram et al., 2008; Grote et al., 2011) and to estimate the requirements of the early stages of development (Saavedra et al., 2006). In *Diplodus sargus* (Saavedra et al., 2006) and *Sparus aurata* (Aragon et al., 2004), the comparison between the AA profile of the body tissue of larvae and rotifers, for example, showed a deficiency in the amount of certain amino acids in the live food, suggesting that the rotifers did not have the necessary amino acids to fulfil the nutritional needs of these species. This approach has been successfully adopted to study amino acid requirements in fish larvae (Tulli and Tibaldi, 1997; Oberg et al., 2015). Thus, based on data from this study, living organisms that meet the requirements of *L. alexandri* larvae should be used for successful larviculture or to employ live food enrichment techniques.

In *L. alexandri,* the concentration of total lipids was highest for NHL and lower in eggs and LPL, which were similar to each other. In *Pseudoplatystoma reticulatum* and its hybrid *Pseudoplatystoma corruscans* X *Pseudoplatystoma reticulatum*, carnivorous freshwater species, the total lipid percentages remained constant in eggs and larvae (Mello et al., 2012). The initial development of these species, however, is faster: approximately three days compared to the seven days of *L. alexandri* larvae. Several authors have reported that the FA composition of total lipids in eggs reflects the maternal diet (Izquierdo, 1996; Pickova et al., 2007; Sink et al., 2010; Zakeri et al., 2011). Thereby, the nutrition of breeders is very important for the production of quality embryos and larvae (Tocher, 2010). For Wiegand (1996), the maintenance of lipid content can be attributed to its storage and later use during a period of food restriction, when it can act as an energy reserve in larvae after the absorption of the yolk sac. In *L. alexandri,* the increase in lipid content immediately after hatching may indicate that this nutrient is being deposited during ontogenetic development because of its important function as a membrane component (Henderson, 1996; Glencross, 2009) and may have been consumed as a primary source of energy at the point of exhaustion of endogenous reserves. In *Carassius auratus* (Wiegand, 1996), *M. macquariensis* and *M. peelii peelii* (Gunasekera et al., 1999b) the reduction in the percentage of total lipids indicated the catabolism of this nutrient for energy production during ontogenetic development.

The percentage of NL in FA were higher in LPL, the lower and similar values were found in eggs and NHL. Neutral lipid is generally regarded primarily as an energy reserve (Wiegand, 1996), their percentages were reduced by in eggs and NHL probably due to their mobilization for energy production during embryonic development. For the PL fraction, the highest and similar percentages were found in eggs and NHL, while LPL had lower values. PL are found in greater proportions in the early life stages, due to its function as a structural constituent of organ and tissue (Sargent et al., 2002; Tocher 2010). As reported during the development of *C. auratus*, the depletion in the values of PL in FA may indicate that this class of FA has a role as the primary fuel for energy production rather than as a structural lipid (Wiegand, 1996). Tocher et al. (1985) stated that the consumption of this class of FA has the advantage of providing choline and phosphate for developing fish embryos, and for the synthesis of neurotransmitters and macromolecule, as well as of the other FA to energy production.

In general, in both NL and PL fractions, SFA decreased during development mainly due to the exhaustion of C16:0 and C15:0, indicating that these FAs are being preferably used as an energy source, as has been observed for other species, such as *Dentex dentex* (Tulli and Tibald, 1997), *Mackerel mackerel* (Ortega and Mourente, 2010), and *Salminus hilarii* (Araújo et al., 2012). Rainuzzo et al. (1991) reported that larvae containing a lower content of lipids mainly use C16:0 as the energy source, thus retaining n-3 HUFAs. According to Abi-Ayad et al. (2004), the reduction of this FA percentagens in *Sander lucioperca* larvae is an indication that these are the energy substrates preferably used by the larvae, as also observed during the development of *Diplodus sargus* (Cejas, 2004) and *S. mackerel* (Ortega & Mourente, 2010).

MUFA may be required preferably as a source of energy throughout the ontogenetic development (Tulli and Tibaldi, 1997; Abi-Ayad et al., 2004; Dantagnan et al., 2007). In eggs and NHL, these FAs were conservation and reduced in LPL, both in the NL and PL fractions. The decrease in the percentages of C16:1n-7, C16:1n-5, C18:1n-9, and C22:1n-7 only in the late lecithotrophic period of *L. alexandri* could be evidence that these nutrients are being catabolised as a source of energy in this phase, or that they are being mobilised to form other FAs in larger chains, since larvae in this period require more energy for swimming in order to find food because of the depletion of their endogenous reserves. A similar reduction of MUFAs in LPL has been reported for other freshwater fish species, such as *C. auratus* (Wiegand, 1996), *M. macquariensis*, and *M. peelii peelii* (Gunasekera et al., 1999b) and for marine species, such as *Scophthalmus maximus*, that, similar to *L. alexandri*, live buried in sandy bottoms (Silversand et al., 1996).

In *L. alexandri,* the decrease of SFAs and MUFAs were offset by an increase in PUFAs in the LPL in both the NL and PL portions. The increase in PUFAs in LPL could be related to the increase of n-6/n-3 fatty acids, such as C20:4n-6 and C22:6n-3, indicating the importance of these FAs to this stage of life.

Channa striata, a carnivorous freshwater fish species, is able to synthesise DHA from EPA and both genes responsible for this synthesis are regulated by the dietary level of C18 (Kuah et al., 2015). Freshwater species are capable of producing biologically active HUFA from PUFA C:18 a process called bioconversion through desaturase and elongase enzyme activity (Henderson, 1996; Ling et al., 2006; Jaya-Ram et al., 2008; Tocher, 2010). Studies have indicated that the expression of desaturase and elongase genes occurs shortly after hatching, indicating the possibility of longer chain PUFA synthesis at this time of development (Mitchell et al., 2012; Cunha et al., 2013.). In this study, EPA and DHA values increased considerably in LPL, that is, at mouth opening and the start of exogenous feeding.

The higher concentration of the HUFA C20:5n-3 (EPA) and C22:6n-3 (DHA) in *L. alexandri* LPL was followed by reduced levels of their precursor, the C18:3n-3 PUFA, found in the polar fraction, and the increase of ARA in LPL was followed by a reduction of its precursor, PUFA C18: 2n-6, which shows the bioconversion potential of this type of fatty acids, as reported for other species, such as the freshwater *S. hilarii* (Araújo et al., 2012), *Pseudoplatystoma reticulatum,* and the hybrid *Pseudoplatystoma corruscans* x *Pseudoplatystoma reticulatum* (Mello et al., 2011). The increase in DHA in LPL in both fractions of NL and PL is consistent with that found for *C. auratus* (Wiegand, 1991; 1996). DHA is the quantitatively most important FA of the group and has important structural roles in cell membranes, particularly in the neural tissue (Sargent 1999b). Likewise, EPA has an important physiological role in the modulation of the eicosanoid action and tends to be largely maintained during ontogenetic development (Tocher, 2010).

The PL ARA PL fraction increased soon after hatching and kept its constant percentages in NHL and LPL. According to Gunasekera (1999b), ARA may be maintained during development due to its importance in the further development of *M. macquariensis* and *M. peelii peelii* and also for its possible structural use. The ARA NL portion increased their percentages in LPL. The ARA FA has an essential role in growth, survival, and resistance to stress through production of eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes (Bell and Sargent, 2003; Glencross, 2009;. Villalta et al., 2008; Tocher, 2010). Different to the results of the present study, in freshwater species such as *Morone saxatilis* (Fu-Lin et al., 1995), *Sander lucioperca* (Abi-Ayad et al., 2000), and *Miss fluviatilis* (Abi-

Ayad et al., 2004), ARA levels in larvae showed a considerable reduction during development (Lin, Fu et al., 1995;. Abi-Ayad et al., 2000; Abi-Ayad et al., 2004). Figueiredo et al. (2012) reported that the consuming of ARA during the embryogenesis process, makes it necessary to add a food source that contains this nutrient.

The decrease of n-9 in LPL may indicate that the consumption of this specific class of PUFA meets some metabolic demand in this life stage, thus mainted n3 and n-6 FA. In *Thunnus thynnus* and *S. mackerel* (Ortega and Mourente, 2010), a decrease of this FA series was observed, together with a decrease in n-3 and n-6, indicating a high consumption of PUFAs in these species. On the other hand, in *L. alexandri*, there was a tendency for an increase of n-3 and n-6 in both NL and PL fractions for and also for the n-3 and n-6 HUFAs, suggesting their importance for larvae after depletion of their endogenous reserves, similar to *C. auratus* (Wiegand, 1996) and *M. macquariensis* (Gunasekera et al., 1999). This is due to fact that they act as eicosanoid precursors and structural constituents of the phospholipids of cell membranes, particularly neural and retina tissue (Masuda et al., 1999; Sargent et al., 1999b; Tocher, 2010). Zhu (1998) reported that the increase of PUFAs in the n-3 and n-6 series can be an indication that these FAs are stored for later use in the forming of new tissues, such as the nervous and visual systems as well as participation in the immune system and cell signalling.

Live food such as Artemia and rotifers are usually deficient in essential fatty acids such as EPA, DHA, and ARA and must be enriched when initially supplied to marine larvae (Sargent et al., 1999a; Hamre et al., 2005; Bransden, et al., 2005; Garrrido et al., 2012). However, it is well established that supplementing the diets of freshwater fish with EPA, DHA and ARA is not a common practice as they are able to obtain these HUFAs from smaller chain fatty acids, such as C18:3n3 and C18:2n-6 (Tocher, 2010). Therefore, the decrease in the percentages of C18:3n-3 and C18:2n-6 in LPL of *L. alexandri* suggests that foods that provide sufficient amounts of these fatty acids may be needed at the first exogenous feeding of *L. alexandri* to ensure proper intake of EPA, DHA, and ARA. The evaluation of the fatty acid profile of *Centropomus parallelus* eggs was of paramount importance in determining the choice of live food to be used at the first exogenous feeding of its larvae (Barroso et al., 2013). The supply of living organisms, such as Artemia nauplii, can secure a good supply of C18:3n-3 and C18:2n-6 (Rajkumar and Kumaraguru Vasagam, 2006; Barroso et al., 2013)

These results show that during the different stages of development of *L. alexandri* protein is conservation during ontogeny, although there were significant changes in the

composition of some AAs during development. Furthermore, the percentages of lipids decreased in LPL, showing their use as the main source of energy during this development phase. The consumption of SAFAs and MUFAs in developing embryos or larvae of *L. alexandri* allows temporary storage of essential fatty acids that can be mobilised for structural needs. The knowledge of the biochemical composition and changes in the AA and FA compositions of *L. alexandri* eggs and larvae during development can lead to a better understanding of the nutritional requirements of the larvae during the first feeding, as well as helping to improve the nutrition of breeders.

5. References

A.O.A.C. (1985) Official Methods of Analysis of the Association of the Analytical Chemists (14th edn), pp. 1018. Association of Official Anlytical Chemists, Washington, DC, USA.

Abi-Ayad, S.-M.E.-A., Kestemont, P., Mélard, C. 2000. Dynamics of total lipids and fatty acids during embryogenesis and larval development of Eurasian perch (*Perca fluviatilis*). *Fish Physiology and Biochemistry*, **23**, 233-243.

Abi-Ayad, S.-M.E.-A., Boutiba, Z., Mélard, C., Kestemont, P. 2004. Dynamics of total body fatty acids during early ontogeny of pikeperch (*Sander lucioperca*) larvae. *Fish Physiology and Biochemistry*, **30**, 129-136.

Abidi, S.F., Khan, M.A. 2004. Dietary valine requirement of Indian major carp, *Labeo rohita* (Hamilton) fry. *Jounal of Applied Ichthyology*, **20**, 118-122.

Abidi, S.F., Khan, M.A. 2007. Dietary leucine requirement of fingerling Indian major carp, *Labeo rohita* (Hamilton). *Aquaculture Research*, **38**, 478-486.

Ahmed, I., Khan, M.A., 2006. Dietary branched-chain amino acid valine, isoleucine and leucine requirements of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *British Journal Nutrition*, **96**, 450-460.

Alam, S., Teshima, S., Koshio, S., Ishikawa, M. 2002. Arginine requirement of juvenile Japanese flounder *Paralichthys olivaceus* estimated by growth and biochemical parameters. *Aquaculture*, **205**, 127-140.

Aragão C., Conceição L.E.C., Fyhn H.J., Dinis M.T. 2004a. Estimated amino acid requirements during early ontogeny in fish with different life styles: gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). *Aquaculture*, **242**, 589-605.

Aragão C., Conceição L.E.C., Dinisa, M.T., Fyhn, H.J. 2004b. Amino acid pools of rotifers and Artemia under different conditions: nutritional implications for fish larvae. *Aquaculture*, **234**, 429-445.

Araújo, B.C., Honji, R.M., Mello , P.H., Moreira, R.G. 2012. The influence of captive breeding on the fatty acid profiles of *Salminus hilarii* (Characiformes: Characidae) eggs and larvae. *Aquaculture Internacional*, **20**, 1161-1181.

Barroso, M.V., de Carvalho, C.V.A., Antoniassi, R., Cerqueira, V.R. 2013. Use of the copepod *Acartia tonsa* as thefirst live food for larvae of the fat snook *Centropomus parallelus*. *Aquaculture*, **388-391**, 153–158.

Bell, J.G., Sargent, J.R. 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture*, **218**, 491-499.

Bransden, M.P., Cobcroft, J.M., Battaglene, S.C., Morehead, D.T., Dunstan, G.A., Nichols, P.D., Kolkovski, S. 2005b. Dietary 22:6n-3 alters gut and liver structure and behaviour in larval striped trumpeter (*Latris lineata*). *Aquaculture*, **248**, 275-285.

Brown, M.R., Battaglene, S.C., Morehead, D.T., Brock, M. 2005. Ontogenetic changes in amino acid and vitamins during early larval stages of striped trumpeter (*Latris lineata*). *Aquaculture*, **248**, 263-274.

Cara, J.B., Moyano, F.J., Zambonino, J.L., Alarcón, F.J. 2007.The whole amino acid profile as indicator of the nutritional condition in cultured marine fish larvae. *Aquaculture Nutrition*, **13**, 94-103.

Cejas, J. R., Almansa, E., Jérez, S., Bolanos, A., Felipe, B., Lorenzo, A. 2004. Changes in lipid class and fatty acid composition during development in white seabream (*Diplodus sargus*) eggs and larvae. *Comparative Biochemistry and Physiology*, **Part B 139**, 209-216.

Cheng, Z., Buentello, A., Gatlin III, D.M. 2011. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, *Sciaenops ocellatus*. *Aquaculture*, **319**, 247-252.

Conceição, L.E.C., Van der Meeren, T., Verreth, J.A.J., Evjen, M.S., Houlihan, D.F., Fyhn, H.J., 1997. Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or Artemia. Mar. Biol. **129**, 255-265.

Conceição, L.E.C., Rønnestad, I. Tonheim, S. K. 2002. Metabolic budgets for lysine and glutamate in unfed herring (*Clupea harengus*) larvae. *Aquaculture*, **206**, 305- 312.

Conceição, L.E.C., Grasdalen, H., Rønnestad, I. 2003b. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. *Aquaculture*, **227**, 221-232.

Conceição, L.E.C., Ribeiro, L., Engrola, S., Aragão, C., Morais, S., Lacuisse, M., Soares, F., Dinis, M.T. 2007. Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). *Aquaculture*, **268**, 64-81.

Costa, D.C.C., Silva, W. S., Melillo-Filho, R., Filho, K.M.C., Santos, J.C.E.S., Luz, R.K. 2015. Capture, adaptation and artificial control of reproduction of *Lophiosilurus alexandri*: A carnivorous freshwater species. *Animal Reproduction Science*, **159**, 148-154.

Christie W.W. (1982) Lipid Analysis, pp. 207. Pergamon Press, Oxford, UK.

Cruzado, I.H., Rodríguez, E., Herrera, M., Lorenzo, A., Almansa, E. 2013. Changes in lipid classes, fatty acids, protein and amino acids during egg development and yolk-sac larvae stage in brill (*Scophthalmus rhombus* L.). *Aquaculture Research*, **44**, 1568-1577.

Cunha, I., Galante-Oliveira, S., Rocha, E., Planas, M., Urbatzka, R., Castro, L.F., 2013. Dynamics of PPARs, fatty acid metabolism genes and lipid classes in eggs and early larvae of a teleost. *Comparative Biochemistry and Physiology. Part B: Biochemistry an Molecular Biol*ogy, **164**, 247–258.

Dantagnan, H., Bórquez, A.S., Valdebenito, I.N., Salgado, I.A., Serrano, E.A., Izquierdo, M.S. 2007. Lipid and fatty acid composition during embryo and larval development of puye *Galaxias maculatus* Jenyns, 1842, obtained from estuarine, freshwater and cultured populations. *Journal of Fish Biology*, **70**, 770-781.

Desvilettes, C., Bourdier, G., Breton, J.C. 1997. Changes in lipid class and fatty acid composition during development in pike (*Esox lucius* L) eggs and larvae. *Fish Physiology and Biochemistry*, **16**, 381-393.

Farhoudi, A., Kenari A. A., Chamanara, V., Farsani, A.S. 2012. Ontogeny Changes in Fatty Acid and Amino Acid Profiles in Yellowfin Seabream (*Acanthopagrus latus*) Eggs and Larvae. *World Journal of Fish and Marine Sciences*, **4**, 290-296.

Figueiredo, J., Lin, J., Anto, J.,Narciso, L. 2012. The Consumption of DHA during Embryogenesis as an Indicative of the Need to Supply DHA during Early Larval Development: A Review. *Journal Aquaculture Research Development*, **3**, 1-7.

Finn, R.N., Fyhn, H.J. 2010. Requirement for amino acids in ontogeny of fish. *Aquaculture Research*, **41**, 684-716.

Fraser A.J., Gamble J.C. and Sargent J.R. 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). *Marine Biology*, **99**, 307-313.

Finn, R., N., Fyhn, H., J., Hendenon, R., J., Evjen, M., S. 1996. The sequence of catabolic substrate oxidation and enthalpy balance of developing embryos and yolksac larvae of turbot (*Scophalmus maximus* L.). *Comparative Biochemistry Physiology*, **115A**, 133-l51.

Folch L., Lees M., Sloane-Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497-509.

Fu-Lin, E.C., Ozkizilcik, S. 1995. Lipid and fatty acid composition of striped bass (*Morone saxatilis*) larvae during development. *Comparative Biochemistry and Physiology*, **111B**, 665-674.

Garrido, S., Saiz, E., Peters, J., Ré, P., Alvarez, P., Cotano, U., Herrero, D.L., Martínez de Murguía, A., Irigoien, X. 2012. Effect of food type and concentration on growth and fatty acid composition of early larvae of the anchovy (*Engraulis encrasicolus*) reared under laboratory conditions. *Journal of Experimental Marine Biology and Ecology*, **434–435**, 16-24.

Giménez, G., Estévez, A., Lahnsteiner, F., Zecevic, B., Bell, J.G., Henderson, R.J., Piñera, J.A., Sanchez-Prado, J.A. 2006. Egg quality criteria in common dentex (*Dentex dentex*). *Aquaculture*, **260**, 232-243.

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture*, **1**, 71-124.

Gunasekera R.M., Shim, K.F., Lam, T.J. 1997. Influence of dietary protein content on the distribution of amino acids in oocytes, serum muscle of Nile tilapia, *Oreochromis niloticus* (L). *Aquaculture*, **152**, p.205-221.

Gunasekera R.M., De Silva, S.S., Ingram, B.A. 1999a. The amino acid profiles in developing eggs and larvae of the freshwater Percichthyid fishes, trout cod, *Maccullochella macquariensis* and Murray cod, *Maccullochella peelii peelii*. *Aquatic Living Resource*, **12**, 255-261.

Gunasekera R.M., De Silva, S.S., Ingram, B.A. 1999b. Early ontogeny-related changes of the fatty acid composition in the Percichthyid fishes trout cod, *Maccullochella macquariensis* and Murray cod, *M. peelii peelii. Aquatic Living Resource*, **12**, p.219-227.

Gurure, R., Atkinson, J., Moccia, R.D. 2007. Amino acid composition of Arctic charr, *Salvelinus alpinus* (L.) and the prediction of dietary requirements for essential amino acids. *Aquaculture Nutrition*, **13**, 266-272.

Hamre, K., Moren, M., Solbakken, J., Opstad, I., Pittman, K. 2005. The impact of nutrition on metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, **250**, 555-565.

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition,* **49**, 5-22.

Hseu, J.R., Luc, F.I., Sud, H.M., Wange, L.S., Tsaie, C.L., Hwang, P.P. 2003. Effect of exogenous tryptophan on cannibalism, survival and growth in juvenile grouper, *Epinephelus coioides*. *Aquaculture*, 218, 251-263.

Hoglund, E., Bakke, J. M., Øverlic, Ø., Winberg, S., Nilsson, G.E. 2005. Suppression of aggressive behaviour in juvenile Atlantic cod (*Gadus morhua*) by l-tryptophan supplementation. *Aquaculture*, **249**, 525-531.

Izquierdo, M. S. 1996. Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition*, **2**, 183-191.

Jaya-Ram, A., Kuah, M-K., Lim, P-S., Kolkovski, S., Shu-Chien, A.C. 2008. Influence of dietary HUFA levels on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs expression in female zebrafish *Danio rerio*. *Aquaculture*, **277**, 275-281.

Juaneda P., Rocquelin G. 1985. Rapid and convenient separation of phospholipids and non-phosphorous lipids from rat using silica cartridges. *Lipids* **20**, 40-41.

Kuah, M.-K., Jaya-Ram, A., Shu-Chien, C.A. 2015. The capacity for long-chain polyunsaturated fatty acid synthesis in a carnivorous vertebrate: Functional characterisation and nutritional regulation of a Fads2 fatty acyl desaturase with Δ4 activity and an Elovl5 elongase in striped snakehead (*Channa striata*). *Biochimica et Biophysica Acta*, **1851**, 248-260.

Lanes, C.F.C., Bizuayehu, T.T., Bolla, S., Martins, C., Fernandes, J.M.O., Bianchini, A., Kiron, V., Babiak, I. 2012. Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and larvae obtained from farmed and wild broodstocks. *Aquaculture*, **324-325**, 267-275.

Li, P., Yin, Y., Li D., Kim, W.K., Wu, G. 2007. Amino acids and immune function. *British Journal of Nutrition*, **98**, 237-252.

Li, P., Mai, K., Trushenski, J., Wu, G. 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, **37**, 43-53.

Ling, S.,Kuah, M-K., Muhammad, T.S.T.,Kolkovski, S., Shu-Chien, A.C. 2006. Effect of dietary HUFA on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs in female swordtail *Xiphophorus helleri*. *Aquaculture*, **261**, 204-214.

Luo, Z., Liu, Y., Mai, K., Tian, L., Yang, H., Tan, X., Liu, D. 2005. Dietary L-methionine requirement of juvenile grouper *Epinephelus coioides* at a constant dietary cystine level. *Aquaculture*, **249**, 409-418.

Luz, R.K., Santos, J.E.C. 2008. Densidade de estocagem e salinidade da água na larvicultura do pacamã. *Pesquisa agropecuária brasileira*, **43**, 903-909.

Mai, K., Wan, J., Ai, Q., Xu, W., Liufu, Z., Zhang, L., Zhang, C., Li, H. 2006a. Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea*. *Aquaculture*, **253**, 564-572.

Mai, K., Mai, K., Ai, Q., Duan, Q., Zhang, C., Li, H., Wan, J., Liufu, Z. 2006b. Dietary lysine requirement of juvenile Japanese seabass, *Lateolabrax japonicas*. *Aquaculture*, **258**, 535-542.

Masuda, R., Takeuchi, T., Tsukamoto, K., Sato, H., Shimizu, K., Imaizumi, K., 1999. Incorporation of dietary docosahexaenoic acid into the central nervous system of the yellowtail *Seriola quinqueradiata*. *Brain Behavior Evolution*, **53**, 173–179.

Mello, P.H., Araújo, B.C.,Venturieri, R.L.L., Moreira, R.G. 2011. Fatty acids as a tool to compare cachara (*Pseudoplatystoma reticulatum*) (Siluriformes: Pimelodidae) and hybrid (*Pseudoplatystoma corruscans* x *Pseudoplatystoma reticulatum*) larvae during early development. *Aquaculture International*, **20**, 1139-1160.

Morais, S., Castanheira, F., Martinez-Rubio, L., Conceição, L.E.C., Tocher, D.R. 2012. Long chain polyunsaturated fatty acid synthesis in a marine vertebrate: ontogenetic and nutritional regulation of a fatty acyl desaturase with Δ4 activity. Biochim. Biophys. *Acta Mol. Cell Biol. Lipids*, **1821**, 660-671.

Oberg, E.W., L.A. Fuiman, L.A. 2015. Linking fatty acids in the diet and tissues to quality of larval southern flounder (*Paralichthys lethostigma*). *Journal of Experimental Marine Biology and Ecology*, **467**, 7-15.

Ortega, A., Mourente, G. 2010. Comparison of the lipid profiles from wild caught eggs and unfed larvae of two scombroid fish: northern Bluefin tuna (*Thunnus thynnus* L., 1758) and Atlantic bonito (*Sarda sarda* Bloch, 1793). *Fish Physiol Biochem*, **36**, 461-471. doi 10.1007/s10695-009-9316-8

Pickova, J.,Brannas, E., Andersson, T. 2007. Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. *Aquaculture International*, **15**, 305-311.

Power, D. M., Llewellyn, L., Faustinoa, M., Nowell, M. A., Bjornsson, B. Th., Einarsdottir, I. E., Canario, A.V.M.,Sweeney, G.E. 2001. Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology, Part C* **130**, 447-459.

Rayner, T. A., Jørgensen, N. O.G., Blanda, E., Wub, C.-H., Huang, C.-C. Mortensen, J., Hwang, J.-S., Hansen, B.W. 2015. Biochemical composition of the promising live feed tropical calanoid copepod *Pseudodiaptomus annandalei* (Sewell 1919) cultured in Taiwanese outdoor aquaculture ponds. *Aquaculture*, **441**, 25-34.

Rajkumar, M., Kumaraguru vasagam, K.P. 2006. Suitability of the copepod, Acartia clausi as a live feed for Seabass larvae (*Lates calcarifer* Bloch): Compared to traditional live-food organisms with special emphasis on the nutritional value. *Aquaculture*, **261**, 649-658.

Rønnestad, I., Koven, W., Tandler, A., Harel, M., Fyhn, H. J. 1998. Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **162**, 157-170.

Rønnestad, I., S.K. Tonheim, S.K., Fyhn, H.J., Rojas-García, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y., Conceição, L.E.C. 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. *Aquaculture*, **227**, 147-164.

Saavedra M., Conceição L.E.C., Pousão-Ferreira P., Dinis, M.T. 2006. Amino acid profiles of *Diplodus sargus* (L., 1758) larvae: Implications for feed formulation. *Aquaculture*, **261**, 587-593.

Saavedra M., Beltran, M., Pousão-Ferreira P., Dinis, M.T., Blasco, J., Conceição L.E.C. 2007. Evaluation of bioavailability of individual amino acids in *Diplodus puntazzo* larvae: Towards the ideal dietary amino acid profile. *Aquaculture*, **263**, 192-198.

Samaee, S.M., Estévez, A., Giménez, G., Lahnsteiner, F. 2009. Evaluation of quantitative importance of egg lipids and fatty acids during embryos and larvae development in marine pelagophil teleosts: with an emphasis on *Dentex dentex*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **311**, 735-751.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M.,Henderson, J., Tocher, D. 1999a. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, **179**, 217-229.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999b. Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, **177**, 191-199.

Sargent, J. R., Tocher, D.R., BELL, J.G. The lipids. pp. 181-257. **In**: *Fish Nutrition*, 3rd Edition, Ch.4. (Halver, J. E., Ed.). San Diego: Academic Press (2002).

Santos, L.D., Luz, R.K. 2009. Effect of salinity and prey concentrations on *Pseudoplatystoma corruscans*, *Prochilodus costatus* and *Lophiosilurus alexandri* larviculture. *Aquaculture*, 287, 324-328.

Silva, W.S., Cordeiro, N.I.S. Costa, D.C., Takata, R., Luz, R.K. 2014. Frequência alimentar e taxa de arraçoamento durante o condicionamento alimentar de juvenis de pacamã. *Pesquisa agropecuária brasileira*, **49**, 648-651.

Silversand, C., Norberg, B., Haux, C. 1996. Fatty-acid composition of ovulated eggs from wild and cultured turbot *(Scophthalmus maximus)* in relation to yolk and oil globule lipids. *Marine Biology*, **125**, 269-278.

Sink, T.D., Rebecca T.L., Pohlenz, C., Buentello, A.., Gatlin, D. 2010. Effects of dietary protein source and protein–lipid source interaction on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg production and quality, and fry hatching percentage and performance. *Aquaculture*, **298**, 251–259.

Takata, R., Silva, W.S., Costa, D.C., Filho, R.M., Luz, R.K. 2014.Effect of water temperature and prey concentrations on initial development of *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae), a freshwater fish. *Neotropical Ichthyology*, **12**, 853-859.

Tocher, D.R., Bendiksen, E.A., Campbell, P.J., Bell, J.G. 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, **280**, 21-34.

Tocher, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, **41**, 717-732.

Tulli, F., Tibaldi, E. 1997. Changes in amino acids and essential fatty acids during early larval rearing of dentex. *Aquaculture International*, **5**, 229-236.

Vazquez, R., González, S., Rodriguez, A., Mourente, G. 1994. Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae and firstfeeding larvae of the Senegal sole (*Solea senegalensis* Kaup). *Aquaculture*, **119,** 273-286.

Villalta, M., Estévez, A., Bransden, M.P., Bell, J.G. 2008. Arachidonic acid, arachidonic/eicosapentaenoic acid ratio, stearidonic acid and eicosanoids are involved in dietary-induced albinism in Senegal sole (*Solea senegalensis*) *Aquaculture Nutrition*, **14**, 120-128.

Wiegand, M. D. 1996. Utilization of yolk fatty acids by goldfish embryos and larvae. *Fish Physiology and Biochemistry*, **15**, 21-27.

Yeganeh, S. 2014. Variations of fatty acids in Rainbow trout (*Oncorhynchus mykiss*) during eyed egg and larval development stages. *International Journal of Aquatic Science*, **5**, 167-180.

Zakeri, M., Kochanian, P., Marammazi, J.G., Yavari, V., Savari, A., Haghi, M. 2011. Effects of dietary n-3 HUFA concentrations on spawning performance and fatty acids composition of broodstock, eggs and larvae in yellowfin sea bream, *Acanthopagrus latus*. *Aquaculture*, **310**, 388-394.

Zhang, C., Ai, Q., Mai, K., Tan, B., Li, H., Zhang, L. 2008. Dietary lysine requirement of large yellow croaker, *Pseudosciaena crocea* R. *Aquaculture*, **283**, 123-127.

Zhou F., Xiao, J.X., Hua, Y., Ngandzali, B.O., Shao, Q.J. 2011. Dietary l-methionine requirement of juvenile black sea bream (*Sparus macrocephalus*) at a constant dietary cystine level. *Aquaculture Nutrition*, **17**, 469-481.

Zhu P (1998) A study of biochemical composition in captive Atlantic halibut (*Hippoglossus hipoglossus*) eggs and larvae. Dissertation, University of Newfoundland

6. CONSIDERAÇÕES FINAIS

O pacamã é capaz de se adaptar e reproduzir em condições de cativeiro, desde que sejam aplicadas técnicas manejos durante o processo de captura de exemplares selvagens, transporte e aclimatação ás condições de cativeiro. Tanques recobertos com fundo de areia e manutenção da temperatura a 28ºC são fundamentais para que ocorra a reprodução natural desta espécie em cativeiro. O perfil de aminoácidos e ácidos graxos forneceram indicações importantes sobre as fontes de consumo de energia durante o desenvolvimento do pacamã e servem como indicação da qualidade nutricional das desovas.