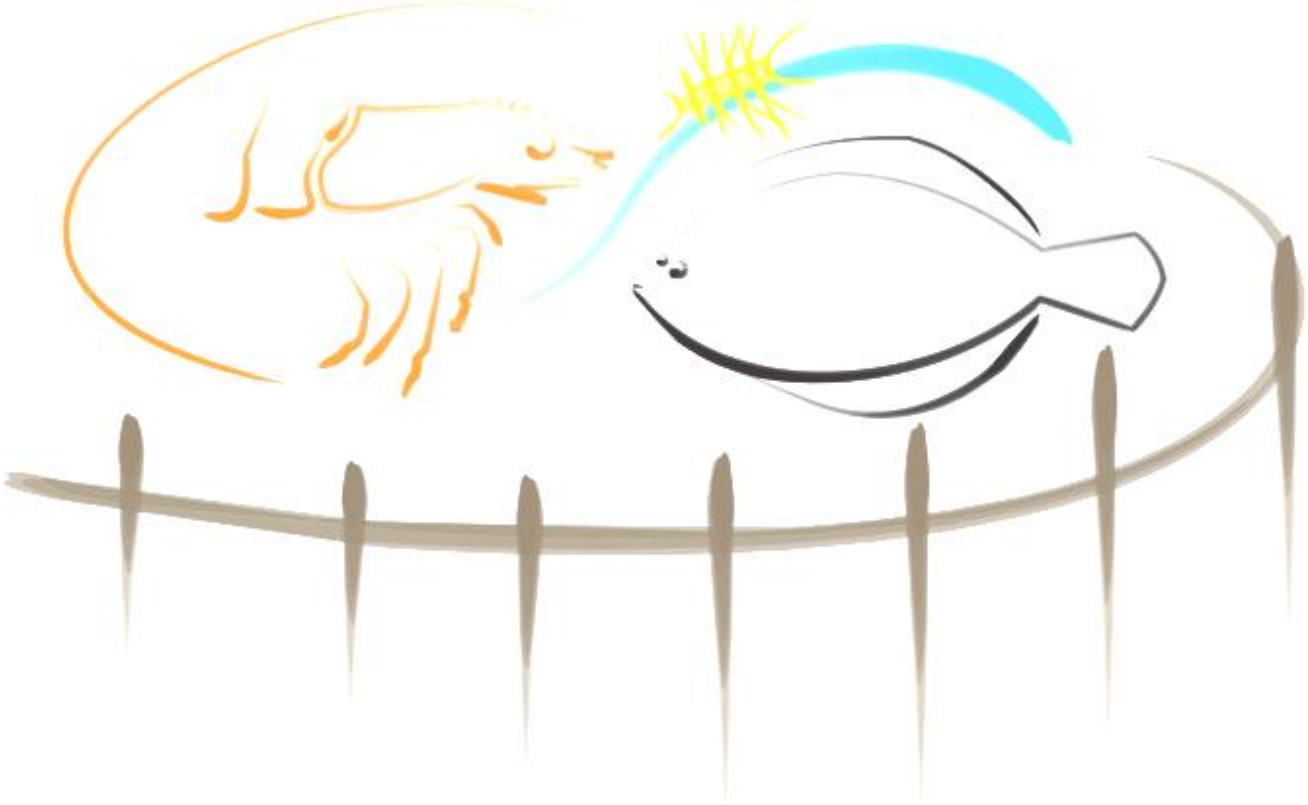
UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG

INSTITUTO DE OCEANOGRAFIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA



EFEITOS DA RESTRIÇÃO ALIMENTAR EM JUVENIS DO LINGUADO *Paralichthys orbignyanus*

FLORENCIA GABRIELA FÉOLA FARÍAS

FURG

RIO GRANDE, RS

MARÇO, 2015

Universidade Federal do Rio Grande - FURG

Instituto de Oceanografia

Programa de Pós-Graduação em Aqüicultura

EFEITOS DA RESTRIÇÃO ALIMENTAR EM JUVENIS DO LINGUADO *Paralichthys orbignyanus*

Dissertação apresentada como parte dos requisitos para obtenção do grau de Mestre em Aquicultura no Programa de Pós Graduação em Aquicultura da Universidade Federal do Rio Grande - FURG

Orientador: Dr. Luís André Sampaio

Co-Orientador: Dr. Ricardo Vieira Rodrigues

Rio Grande -RS- Brasil

Março, 2015

ÍNDICE

AGRADECIMENTOS

Para começar quero agradecer à OEA, à FURG e ao programa de Pós-graduação em Aquicultura por ter me dado a oportunidade de continuar com os meus estudos.

À CAPES pelo financiamento da bolsa.

Ao meu orientador Dr. Luís André Sampaio e co-orientador Dr. Ricardo Vieira Rodrigues por terem me aceitado como orientada.

Aos professores que formam parte do programa pelos conhecimentos adquiridos.

A todos aqueles que participaram das coletas e nas análises do meu experimento (Bianca, Diogo, Fabi, Jana, Marta, Rafa, Ricardo).

Ao Prof. Dr. José Monserrat por ter me ajudado com grande parte das análises e sempre ter tempo e vontade para ajudar e tirar dúvidas.

Aos laboratórios dirigidos pelo Dr. Luis A. Romano e o Dr. Martín Bessonart por terem me permitido realizar parte das análises.

As minhas amigas (Alejandra, Claudia, Natalia, Rosana, Sofía y Valentina).

A minha família por estar sempre presente.

Aos “meus peludos” Manya e Laika por fazer tudo mais fácil e alegrar a nossa vida.

Ao meu parceiro da vida, Esteban, por sempre estar do meu lado e por ter mudado a sua vida para me acompanhar durante estes dois anos.

1. RESUMO GERAL

O objetivo do presente estudo foi determinar os efeitos da restrição alimentar em juvenis do linguado *Paralichthys orbignyanus*. Para isso foram comparados peixes Alimentados (A) e peixes mantidos em Jejum (J) durante 8 semanas. Foram realizadas amostragens de seis peixes por tratamento nas semanas 0, 1, 2, 4 e 8 para comparação da utilização das reservas energéticas no fígado, plasma e músculo e o efeito da restrição alimentar na morfologia do rim e intestino. Os resultados mostraram que o jejum produziu variações nos índices biométricos e nas reservas energéticas (principalmente hepáticas) ao longo do tempo. O fator de condição diminuiu na 8 semana, enquanto o índice hepatossomático e o viscerossomático diminuíram a partir de 2 semanas. O colesterol plasmático aumentou enquanto o hepático diminuiu, indicando um possível transporte para a síntese de cortisol como resposta ao estresse. Níveis de glicose, proteínas e triglicerídeos plasmáticos foram mantidos ao longo do tempo, o mesmo foi observado para osmolalidade. O glicogênio plasmático apresentou aumento na semana 8, indicando possivelmente um acúmulo de glicose na forma de glicogênio. Já no músculo houve pico dos valores de glicose na semana 4 e aumento na quantidade de proteínas na semana 8. Os lipídeos musculares apresentaram variações ao longo do tempo similares em ambos os tratamentos, aumentando na semana 2 no tratamento A e na 4 no J; de forma antagônica a umidade diminuiu em ambos os tratamentos. No fígado ocorreu diminuição significativa no glicogênio e nos triglicerídeos na semana 2, e na glicose e nas proteínas na semana 4. Os triglicerídeos aumentaram nas semanas 4 e 8, possivelmente devido ao processo de re-esterificação dos ácidos graxos, formando lipoproteínas. Quanto aos parâmetros histológicos, não foram observadas diferenças no grau de deposição e tamanho dos melano-macrófagos no rim, nem nos parâmetros morfológicos intestinais. Os juvenis de

linguado sobrevivem durante oito semanas em jejum utilizando reservas energéticas hepáticas e sem sofrer modificações estruturais no rim e intestino.

Palavra-chave: *Paralichthys orbignyanus*, restrição alimentar, reservas energéticas.

6. ABSTRACT

The aim of this study was to determine the effects of starvation in *Paralichthys orbignyanus* juveniles. For that, two treatments were compared, where one group was Feeding (F) and the other was Starved (S). To compare between treatments, samples of six fish per treatment were taken in weeks 0, 1, 2, 4 and 8. There were compared the use of energetic reserves in liver, blood plasma and muscle, and the effect of starvation in the morphology of the kidney and the intestine of the fish. The results showed that starvation produced variations in biometric indexes and in energetic reserves (principally hepatic) along the time. The condition factor decreased at 8 weeks, while hepatosomatic and viscerosomatic indexes decreased both from week 2. Plasmatic cholesterol raised and the hepatic decreased, indicating a possible transport for cortisol synthesis as a stress response. Glucose, protein and triglyceride plasmatic levels were maintained along the time, corroborated by the constant levels of osmolality. Glycogen raised at week 8, indicating a possible glucose accumulation in the form of glycogen. The muscle suffered a glucose pike at week 4 and a rise in protein at week 8. Lipids showed variations along the time similar in both treatments, whit a rise at week 2 in F and in week 4 in S. In an antagonistic way, there was a moisture decrease in both treatments. The liver suffered a significant drop of glycogen and triglycerides at week 2, and in glucose and proteins at week 4. Triglycerides raised at weeks 4 and 8 possibly because of the process of fatty acid re esterification, leading to the formation on lipoproteins. With respect to histological parameters, there were not observed differences in the degree of deposition and the size of melano-macrophages, neither in the morphological parameters of the intestine. Although, *P. orbignyanus* juveniles can survive to 8 weeks of starvation using the hepatic energy stores without suffering structural changes in the kidney and in the intestine.

Key words: *Paralichthys orbignyanus*, starvation, energy stores.

3. INTRODUÇÃO GERAL

3.1 Distribuição de *Paralichthys orbignyanus*

Entre os 34ºS e os 47ºS da plataforma continental da América do Sul (litoral da Argentina, Brasil e Uruguai) ocorrem três espécies de *Paralichthys*: *P. isósceles* (Jordan, 1891), *P. orbignyanus* (Valenciennes, 1842) e *P. patagonicus* (Jordan, 1889) (Astarloa & Munroe, 1998), que apresentam diferenças na sua distribuição e morfologia, dentre outras características. *P. orbignyanus* particularmente ocorre em águas rasas e áreas de substrato macio, atingindo profundidades de até 45 metros (Diaz de Astarloa & Munroe, 1998).

Segundo Chao et al. (1985) é uma espécie marinho-estuarino dependente, já que habita águas marinhas e/ou estuarinas dependendo da fase de vida ou período anual. Ocorre comumente em áreas estuarinas como a Lagoa Mar Chiquita (Argentina) (Diaz de Astarloa, 1998), a Lagoa dos Patos (Brasil) (Chao et al., 1982) e a Lagoa de Rocha (Uruguai) (Pintos et al., 1988). Esses mesmos autores têm demonstrado que estas áreas são utilizadas como berçário, já que são protegidas e abundantes em alimento, e que por tanto, implicam um menor risco de predação para os juvenis de *P. orbignyanus*, que habitam profundidades menores do que 3 metros.

3.2 Morfologia e Biologia de *P. orbignyanus*

Comparando as três espécies mencionadas, *P. orbignyanus* atinge o maior comprimento, sendo 103 cm para fêmeas e 61 cm para machos (Diaz de Astarloa & Munroe, 1998). O lado ocular (esquerdo) apresenta coloração marrom com manchas claras e escamas ciclóides em ambos os lados do corpo, enquanto *P. Isósceles* (do lado ocular) e *P. Patagonicus* (em ambos os lados) apresentam escamas ctenóides. Além disso, *P. orbignyanus*

tem a menor quantidade de vértebras (entre 37 e 39) e de raios dorsais e anais, o maior número de arcos branquiais, e o menor diâmetro ocular (Diaz de Astarloa & Munroe, 1998).

São organismos predadores, posicionados nos níveis mais altos da rede alimentar (Rodríguez-Graña et al., 2008). Norbis & Galli (2004) determinaram que na Lagoa de Rocha, a dieta de *P. orbignyanus* é composta basicamente por peixes, sendo as principais espécies consumidas o peixe-rei *Odontesthes argentinensis* (Valenciennes, 1835), a corvina *Micropogonias furnieri* (Desmarest, 1823), a savelha *Brevoortia aurea* (Agassiz, 1829) e o próprio *P. orbignyanus*, entre outras espécies não identificadas.

Quanto à reprodução, as fêmeas apresentam desenvolvimento assincrônico dos ovócitos, o que leva a ocorrência de desovas seriadas (Mellito da Silveira et al., 1995). Desta forma, Mellito da Silveira et al. (1995) determinaram que o período reprodutivo de *P. orbignyanus* compreende os meses entre outubro e abril na zona costeira adjacente à Lagoa dos Patos no Brasil, enquanto López Cazorla (2005) determinou que no estuário da Bahia Blanca na Argentina ocorre entre novembro e janeiro. Os ovos são pelágicos, apresentando diâmetros entre 790 e 820 µm com uma única gota de óleo de aproximadamente 116-117 µm de diâmetro (Cerqueira et al., 1997).

3.3 Aquicultura de *P. orbignyanus*

P. orbignyanus é uma espécie que tolera uma ampla faixa de fatores ambientais como pH (entre 6,0 e 8,0) (Wasielesky et al., 1997), temperatura (8°C – 31°C) (Wasielesky et al., 1998) e salinidade (0-40‰) (Sampaio & Bianchini, 2002). Também tolera uma elevada concentração de compostos nitrogenados apresentando CL_{50}-96 h de 0,67 mg NH_3-N para amônia gasosa e 30,5 mg/L de nitrito (Bianchini et al., 1996). Além disso, apresenta elevado rendimento de filetagem e sua carne tem um elevado valor no mercado (Robaldo et al., 2012).

A informação mencionada acima faz com que *P. orbignyanus* seja uma espécie com um alto potencial para aquicultura. Estudos vêm sendo realizados na Argentina, no Brasil e no Uruguai com o objetivo de conhecer a biologia da espécie e determinar as condições ótimas para sua produção. Aspectos como reprodução e larvicultura são dominados (Bambill et al., 2006; Sampaio et al., 2007, 2008; Lanes et al., 2008; Radonic & Macchi, 2009; Rodrigues et al., 2012). Vários aspectos foram estudados, como fatores que induzem ao estresse como captura, transporte e densidade de estocagem (Bolasina, 2011), neuropeptídios associados à ingesta de alimento (Campos et al., 2010), criopreservação de sêmen (Ceccon Lanes et al., 2008), manipulação de fatores ambientais como fotoperíodo e temperatura para obtenção de desovas espontâneas (Bambill et al., 2006; Radonic et al., 2007; Sampaio et al., 2008), utilização de hormônios na indução a ovulação (Bambill et al., 2006; Sampaio et al., 2008), enriquecimento de artêmia com ácidos graxos da série n-3 HUFA durante as etapas larvais (Rodrigues et al., 2012), elaboração de dietas inertes que cumpram com as necessidades nutricionais nas diferentes etapas de vida dos peixes, principalmente durante o desmame (Féola et al., 2010; Salhi et al., 2010).

3.4 Restrição alimentar em peixes

De acordo com McCue (2010) a restrição alimentar é definida como uma condição biológica na qual alguma limitação extrínseca impossibilita a alimentação dos organismos. A ocorrência e duração desses eventos são variáveis; podem ser longos e frequentes quando causados por exemplo, por condições climáticas crônicas; longos e infrequentes provocados por mudanças estacionais; curtos e frequentes devido a ciclos diários; ou curtos e infrequentes causados por condições climáticas agudas. O mesmo autor considera que a restrição alimentar é um processo no qual não é possível distinguir fases discretas e que produz uma interrupção no equilíbrio entre o fluxo de energia e a massa corporal do organismo. Segundo Sokolova et

al. (2012) isso acontece pois o organismo necessita de mais energia do que em situações normais para manter a sua homeostase. O balanço energético tem importância na tolerância dos organismos ao estresse causado pelo ambiente e até na determinação dos limites da sua sobrevivência (Sokolova et al., 2012).

No curso do seu ciclo de vida, várias espécies de peixes sofrem períodos de restrição alimentar devido a fatores como a estação reprodutiva, a diminuição da disponibilidade de alimento e a migração (Hur et al., 2006). A capacidade dos peixes de suportar uma condição de jejum é muito variável, podendo ser desde dias até anos (Love, 1970), sendo que existem espécies que podem sobreviver por pelo menos dois anos nessa condição (Whyte et al., 1993). Na aquicultura a restrição alimentar também pode ocorrer (Park et al., 2012). As vezes os próprios criadores restringem a alimentação com a finalidade de melhorar a qualidade da água, ou reduzir efeitos negativos devido a doenças (Davis & Gaylord, 2011). Outro objetivo de submeter os peixes à falta de alimento, é o crescimento compensatório (CC). Esse processo foi definido como uma fase de crescimento rápido como resposta a uma realimentação adequada após uma perda de peso causada pela ausência de alimentação. Como resultado os organismos em questão podem atingir pesos mais elevados do que com uma alimentação contínua (Dobson & Holmes, 1984). Nos anos 80 começaram a ser realizados estudos de CC em espécies como a truta *Oncorhynchus mykiss*, obtendo resultados positivos (Dobson & Holmes, 1984). Além disso, o CC implica em vários benefícios para os aquicultores, como a diminuição da poluição, poupar tempo de trabalho, reduzir os custos devido ao menor uso de alimento e até o tempo de produção (Cho, 2005; Heide et al., 2006). Segundo Zhang et al. (2008) e Peres et al. (2011), o CC também poderia ser utilizado para manipular a composição final do músculo, com a finalidade de melhorar a qualidade da carne (evitando uma deposição de lipídeos em excesso, por exemplo) antes de ser enviada ao mercado.

A resposta aos períodos de restrição alimentar vai depender da espécie, do tamanho, estágio de desenvolvimento e idade dos peixes, da natureza e duração da restrição alimentar (Blasco et al., 1991; Kieffer & Tufts, 1998; Ali et al., 2003; Eroldog et al., 2006). Segundo Deng et al. (2004), o estresse produzido pelo jejum pode levar à diminuição na taxa metabólica, definido como depressão metabólica.

Para lidar com essa situação (tanto na natureza quanto em cativeiro), os peixes mantêm suas atividades vitais essenciais mediante o uso de reservas energéticas acumuladas (representadas como carboidratos, lipídeos e proteínas) no seu organismo, o que implica na utilização dos seus próprios tecidos corporais (Weatherley & Gill, 1987). Como resultado, ocorrem alterações na fisiologia e no metabolismo desses organismos que levam a mudanças tanto no nível estrutural quanto no bioquímico e/ou hematológico.

Alguns estudos têm demonstrado que em geral, a primeira fonte de energia utilizada frente a uma situação de restrição alimentar é o glicogênio, que é transportado pelo organismo como glicose (Barcellos et al., 2010). Contudo, existem casos em que são utilizados os lipídeos (Black & Love, 1986). Isso vai depender, por exemplo, do estado nutricional dos peixes (Echevarría et al., 1997).

A resposta do animal à falta de alimento também é influenciada pelo seu hábito alimentar, tanto que como os peixes carnívoros naturalmente apresentam uma ingesta de alimento menos frequente do que aqueles herbívoros ou onívoros, estariam melhor adaptados a enfrentar períodos de jejum (Bond, 1996).

As principais zonas de armazenamento de energia são o fígado, o músculo e a zona visceral (Riaño et al., 2011). Os principais órgãos para obtenção de energia variam entre as espécies (Brett et al., 1969; Stirling, 1976; Riaño et al., 2011). Também tem sido observado uma ordem na utilização dos tecidos: algumas espécies utilizam primeiro o fígado e

posteriormente o músculo (Stimpson, 1965; Johnston & Goldspink, 1973; Larsson & Lewander, 1973). Outras espécies começam utilizando reservas musculares, deixando as hepáticas para as etapas tardias do jejum evitando danos estruturais permanentes no tecido muscular devido ao consumo de reservas (Sant et al., 2009). Porém, no começo de um processo de restrição alimentar, geralmente o glicogênio hepático (que representa entre 1 a 6 % do peso do fígado) é a primeira reserva energética a ser mobilizada (Navarro & Gutiérrez, 1995; Hung et al., 1997; Barcellos et al., 2010; Davis & Gaylord, 2011). Contrariamente, o glicogênio muscular geralmente não sofre mudanças importantes frente à falta de alimento (Navarro & Gutiérrez, 1995). Esse glicogênio mobilizado é transformado em glicose através de atividade das enzimas glicogênio fosforilase e glucose-6-fosfatase, pelo processo denominado glicogenólise com a finalidade de manter a glicose circulante em níveis normais para o organismo. Davis & Gaylord (2011) determinaram que a glicogenólise ocorre durante aproximadamente duas semanas; posteriormente a glicose é gerada a partir da gliconeogênese.

A osmolalidade plasmática também pode variar frente a uma situação de restrição alimentar. Park et al. (2012) observaram que este parâmetro diminui ao longo do tempo, o que estaria indicando uma osmorregulação inadequada frente à restrição alimentar.

Outra resposta encontrada nos organismos é a perda de massa corporal. Segundo McCue (2010), é uma resposta comum e praticamente inevitável à restrição alimentar, diretamente proporcional ao gasto de energia já que tanto lipídeos quanto proteínas e carboidratos apresentam diferentes densidades energéticas. Isso tem influência no comportamento de índices como o fator de condição (FC) e os índices hepato e viscerosomático (IHS e IVS, respectivamente), que geralmente tendem a diminuir com o aumento do período de restrição alimentar.

3.4.1 Alterações morfológicas

A falta de alimento pode produzir mudanças em várias estruturas. No fígado, por exemplo, pode ocorrer alterações nos hepatócitos devido à perda de reservas energéticas (principalmente glicogênio) (Segner & Möller, 1984; Hur et al., 2006; Ostaszewska et al., 2006). No rim, pode ocorrer aumento no número de melano macrófagos (MMs) (Agius & Roberts 1981; Mizuno et al., 2002; Hur et al., 2006). Os MMs são células fagocíticas com pigmentação elevada, que podem ocorrer tanto livres quanto formando conjuntos de células (Centros Melano-macrófago; MMCs) (Meseguer et al., 1994). Pertencem ao sistema mononuclear fagocítico dos teleósteos, eliminando produtos da degradação celular e partículas estranhas por meio da fagocitose. Caraterísticas como a sua pigmentação, tamanho e/ou número podem ser influenciados por condições patológicas e/ou fisiológicas (Palmer et al, 1992) como por exemplo, a idade dos peixes (Brown & George, 1985) e a restrição alimentar (Micaele & Perdichizzi, 1990; Mizuno et al., 2002; Rios et al., 2007).

O intestino é um órgão que tem funções no balanço de água e eletrólitos (osmorregulação), absorção de nutrientes, regulação da digestão, dentre outras (Shaibani et al., 2013). Aqui, a restrição alimentar também ocasiona mudanças ou atrofias, que podem comprometer a atividade digestiva (Ostaszewska et al., 2006). Pode provocar a redução da altura e do número dos enterócitos, e até no comprimento das vilosidades; tudo isso leva à redução da área do epitélio consequentemente diminuindo a sua capacidade de absorção (e por tanto afetando o processo de osmorregulação) (Segner et al., 1987; Hall & Bellwood, 1995; Shaibani et al., 2013). Segundo Green & McCormick (1999), a redução na altura dos enterócitos é indicador de falta de alimento no trato digestório.

3.4.2 Alterações Bioquímicas

Alterações bioquímicas ocorrem tanto no músculo, como no fígado e no plasma de peixes submetidos ao jejum. Níveis de substratos energéticos como colesterol, glicogênio, glicose, lipídeos, proteínas e/ou triglicerídeos podem variar dependendo da espécie e da extensão do período de jejum (Boran & Yadav, 1996; Hung et al., 1997; Tripathi & Verma, 2003; Hur et al., 2006; Furné et al., 2012; Peres et al., 2013). Essa mobilização de reservas, precisamente de proteínas e lipídeos geralmente produz variações na quantidade de água dos tecidos; existe a tendência ao aumento no conteúdo de água em compensação à mobilização dessas reservas devido que os lipídeos apresentam um comportamento anfipático (Alliot et al., 1984; Black & Love, 1986; Hung et al., 1997).

É sabido que o perfil de ácidos graxos do músculo, fígado e gordura visceral são influenciados pela restrição alimentar. Sendo que a mudança desse perfil ocorre dependendo da espécie (De Silva et al., 1997). Contudo, foi demonstrada uma tendência à conservação/incremento do DHA (ácido docosahexaenóico) nos organismos em restrição alimentar por ser um ácido graxo essencial e componente importante das membranas biológicas (Tidwell et al., 1992; Einen et al., 1998). Também têm sido registradas variações como a diminuição nos níveis de PUFAs (ácidos graxos poli-insaturados) e MUFAs (ácidos graxos mono-insaturados), à utilização de alguns ácidos graxos individuais como o 18:1n9, ou o aumento dos ácidos graxos da sério n3, dentre outras (Tidwell et al., 1992; De Silva et al., 1997).

3.4.3 Alterações hematológicas

O hematócrito é um fator que pode ser influenciado pelo estresse, mas o momento em que essas variações acontecem (geralmente diminuições) vai depender da espécie (Rios et al.,

2002; Caruso et al., 2010). De acordo com Rios et al. (2005) a redução neste parâmetro poderia ser explicada por uma diminuição no tamanho dos eritrócitos.

Considerando que esse linguado apresenta potencial para aquicultura, o presente estudo pode servir como base para futuras pesquisas direcionadas ao seu manejo alimentar, permitindo determinar o momento no qual as mudanças tanto bioquímicas quanto morfológicas produzidas por um período de restrição alimentar ocorrem. Desta forma, o objetivo do presente estudo foi avaliar os efeitos da restrição alimentar em juvenis do linguado *P. orbignyanus*.

4. OBJETIVOS

4.1. Objetivo geral

Determinar os efeitos da restrição alimentar em juvenis do linguado *Paralichthys orbignyanus*.

4.2. Objetivos específicos

- Determinar quais são as principais fontes de mobilização de energia e órgãos de reserva e em qual momento do período de jejum começam a ser utilizadas e influenciados pelo jejum, respectivamente;
- Determinar as alterações morfológicas promovidas pela restrição alimentar no intestino e no rim de juvenis de linguado submetidos à restrição alimentar.

5. REFERÊNCIAS BIBLIOGRÁFICAS

AGIUS, C & RJ ROBERTS. 1981. Effects of starvation on the melano-macrophage centres of fish. J. Fish Biol., 19: 161-169.

ALI, M, A NICIEZA & RJ WOOTON. 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish., 4(2): 147-190.

ALLIOT, E, M DJABALI, A PASTOUREAD & H THEBAULT. 1984. Changes in the biochemical composition of tissues in juvenile sea bass during forced starvation. Bioch. Sys. Ecol., 12(2): 209-213.

BAMBILL, GA, O MASAKAZU, M RADONIC, AV LÓPEZ, MI MÜLLER, JJ BOCCANFUSO & FA BIANCA. 2006. Broodstock management and induced spawning of flounder *Paralichthys orbignyanus* (Valencienes, 1839) under a closed recirculated system. Rev. Biol. Mar. Oceanog., 41(1): 45-55.

BARCELLOS, LJG, A MARQUEZE, M TRAPP, RM QUEVEDO & D FERREIRA. 2010. The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá *Rhamdia quelen*. Aquaculture, 300: 231-236.

BIANCHINI, A, W WASIELESKY JR. & KC MIRANDA FILHO. 1996. Toxicity of nitrogenous compounds to juveniles of flatfish *Paralichthys orbignyanus*. Bull. Envirom. Contam. Toxicol., 56: 453-459.

BLACK, D & RM LOVE. 1986. The sequential mobilization and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. J. Comp. Physiol. B, 156: 469-479.

BLASCO, J, J FERNÁNDEZ & J GUTIÉRREZ. 1992. Fasting and refeeding in carp, *Cyprinus carpio* L.: the mobilization of reserves and plasma metabolite and hormone variations. J. Comp. Physiol. B, 162: 539-546.

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

BOND, CE.1996. Nervous and endocrine systems. In: Bond CE (ed) Biology of fishes. Saunders College Publishing, FortWorth, 241–258.

BORAH, S & RNS YADAV. 1996. Biochemical and haematological response to starvation in an air breathing freshwater teleost, *Heteropneustes fossilis* (Bloch). Indian J. Fish, 43: 307-310.

BRETT, JR, JE SHELBOURNE & CT SHOOP. 1969. Growth rate and composition of fingerling sockeye salmon, *Oncorhynchus merka*, in relation to temperature and ration size. J. Fish. Res. Bd Can., 26: 2363-2394.

BROWN, CL & CJ GEORGE. 1985. Age dependent accumulationof macrophage aggregates in the yellow perch, *Perca fluviatilis* (Mitchell). J. Fish Dis., 8: 135-138.

CAMPOS, VF, T COLLARES, JC DESCHAMPS, FK SEIXAS, OA DELLAGOSTIN, CFC LANES, J SANDRINI, LF MARINS, M O, LA SAMPAIO & RB ROBALDO. 2010. Identification, tissue distribution and evaluation of brain neuropeptide Y gene expression in the Brazilian flounder *Paralichthys orbignyanus*. J. Biosci., 35(3): 405-413.

CARUSO, G, G MARICCHIOLO, V MICALE, L GENOVESE, R CARUSO & MG DENARO. 2010. Physiological responses to starvation in the European eel (*Anguilla nguilla*): effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol. Biochem., 36: 71-83.

CECCON LANES, CF, M OKAMOTO, PV CAVALCANTI, T COLLARES, VF CAMPOS, JC DESCHAMPS, RB ROBALDO, LF MARINS & LA SAMPAIO. 2008. Cryopreservation of Brazilian flounder (*Paralichthys orbignyanus*) sperm. Aquaculture, 275: 361-365.

CERQUEIRA, VR, R MIOSO, JAG MACCHIAVELLO & AM BRUGGER. 1997. Ensaios de indução de desova do linguado, *Paralichthys orbignyanus* Valenciennes, 1839. Bol. Inst. Pesca, 24: 247-254.

CHAO, LN, LE PEREIRA, J PAEZ VIEIRA, M BENVENUTI & LPR CUNHA.1985. Relação preliminar dos peixes estuarinos e marinos da Lagoa dos Patos e região costeira adjacente. Rio Grande do Sul, Brasil. Atlântica, 5: 67-75.

CHO, SH. 2005. Compensatory Growth of Juvenile Flounder *Paralichthys olivaceus* L. and Changes in Biochemical Composition and Body Condition Indices during Starvation and after Refeeding in Winter Season. J. World Aquac. Soc., 36(4): 508-514.

DAVIS, KB & TG GAYLORD. 2011. Effect of fasting on body composition and responses to stress in sunshine bass. Comp. Biochem. Physiol. A, 158: 30-36.

DENG, L, WM ZHANG, HR LIN & CHK CHENG. 2004. Effects of food deprivation on expression of growth hormone receptor and proximate composition in liver of black seabream *Acanthopagrus schlegeli*. Comp. Biochem. Physiol. B, 137: 421-432.

DE SILVA, SS, RM GUNASEKERA & CM AUSTIN. 1997. Changes in the fatty acid profiles of hybrid red tilapia, *Oreochromis mossambicus* x *O. niloticus*, subjected to short-term starvation, and a comparison with changes in seawater raised fish. Aquaculture, 153: 273-290.

DÍAZ DE ASTARLOA, JM & TA MUNROE. 1998. Systematics, distribution and ecology of commercially important Paralichthiyd flounders occurring in Argentinean-Uruguayan waters (Paralichthys, Paralichthyidae): an overview. J. Sea Res., 39: 1-9.

DOBSON, SH & RM HOLMES. 1984. Compensatory growth in the rainbow trout, *Salmo gairdnieri* Richardson. J. Fish Biol., 25: 649-656.

ECHEVARRÍA, G, M MARTÍNEZ-BEBIÁ & S ZAMORA. 1997. Evolution of biometric indices and plasma metabolites during prolonged starvation in European Sea Bass (*Dicentrarchus labrax*, L.). Comp. Biochem, Physiol. A, 118(1): 111-123.

EINEN, O, W BORRE & MS THOMASSEN. 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition. Aquaculture, 166: 85-104.

EROLDOG, OT, M KUMLU, GA KIRIS & B SEZER. 2006. Compensatory growth response of *Sparus aurata* following different starvation and refeeding period. Aquacult. Nutr., 12: 203-210.

FÉOLA, F, M BESSONART, J GADEA, H KINOSHITA & M SALHI. 2010. Sustitución total de alimento vivo por microdietas experimentales en la alimentación de larvas de *Paralichthys orbignyanus*. In: I Congreso Uruguayo de Zoología, X Jornadas de Zoologia del Uruguay "Prof. Federico Achaval". Montevideo. Resúmenes del I Congreso Uruguayo de Zoología. Pp. 186.

FURNÉ, M, AE MORALES, CE TRENZADO, M GARCÍA-GALLEGO, MC HIDALGO, A DOMEZIAN & AS RUS. 2012. The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. J. Comp. Physiol. B, 182: 63-76.

GREEN, BS & MI MCCORMICK. 1999. Influence of larval feeding history on the body condition of *Amphiprion melanops*. J. Fish Biol., 55: 1273–1289.

HALL, KC & DR BELLWOOD. 1995. Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. J. Fish Biol., 47: 438-454.

HEIDE, A, A FOSS, AO STEFANSSON, J MAYER, B NORBERG, B ROTH, MD JENSSEN, R NORTVEDT & AK IMSLAND. 2006. Compensatory growth and fillet

crude composition in juvenile Atlantic halibut: Effects of short term starvation periods and subsequent feeding. Aquaculture, 261: 109-117.

HUNG, SOS, W LIU, H LI, T STOREBAKKEN & Y CUI. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. Aquaculture, 151: 357-363.

HUR, JW, JO HEE & IS PARK. 2006. Effects of long-term starvation on hepatocyte ultrastructure of olive flounder *Paralichthys olivaceus*. Ichthyol. Res., 53: 306-310.

JOHNSTON, IA & G GOLDSPINK. 1973. Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice *Pleuronectes platessa*. Mar. Biol., 19: 348-353.

LANES, CFC, M OKAMOTO, PV CAVALCANTI, T COLLARES, VF CAMPOS, JC DESCHAMPS, RB ROBALDO & LF MARINS. 2008 Cryopreservation of Brazilian flounder (*Paralichthys orbignyanus*) sperm. Aquaculture, 275: 361–365.

LARSEN, A & K LEWANDER. 1973. Metabolic effects of starvation in the eel, *Anguilla nguilla* L. Comp. Biochem, Physiol. A, 44: 367-374.

LÓPEZ CAZORLA, A. 2005. On the age and growth of flounder *Paralichthys orbignyanus* (Jenyns, 1842) in Bahia Blanca Estuary, Argentina. Hydrobiologia, 537: 81-87.

LOVE, RM. 1970. The Chemical Biology of Fishes: with a key to the chemical literature. Academic Press, London and New York, pp. 547.

McCUE, MD. 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A., 156: 1-18.

McLESSE, JM & TW MOON. 1989. Seasonal changes in the intestinal mucosa of winter flounder *Pseudopleuronectes americanus* (Walbaum) from Passamaquoddy Bay, New Brunswick. J. Fish Biol., 35: 381-393.

MELLITO DA SILVEIRA, MP, JCB COUSIN & M HAIMOVICI. 1995. Estrutura ovárica e testicular do linguado *Paralichthys orbignyanus* (Valenciennes, 1839). Atlântica, 17: 135-152.

MESEGUER, J, A LÓPEZ-RUIZ & MA ESTEBAN. 1994. Melano-macrophages of the seawater teleosts, sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*): morphology, formation and possible function. Cell Tissue Res., 277: 1-10.

MICHAELE, V & F PERDICHIZI. 1990. A quantitative and histochemical study of melano-macrophage centers in the spleen of the teleost fish *Diplodus annularis* L. J. Fish Biol., 37: 191-197.

MIZUNO, S, N MISAKA, Y MIYAKOSHI, K TAKEUCHI & N KASAHARA. 2002. Effects of starvation on melano-macrophages in the kidney of masu salmon (*Oncorhynchus masou*). Aquaculture, 209: 247-255.

NAVARRO, I & J GUTIÉRREZ. 1995. Fasting and starvation. Hochachka & Mommsen (eds.), Biochemistry and molecular biology of fishes. Vol. 4. Chapter 17. 393-434.

NORBIS, WC & O GALLI. 2004. Hábitos de alimentación del lenguado *Paralichthys orbignyanus* (Valenciennes, 1842) en una laguna costera somera del Atlántico Sur: Rocha, Uruguay. Ciencias Marinas, 30: 619-626.

OSTASZEWSKA, T, M KORWIN-KOSSAKOWSKI & J WOLNICKI. 2006. Morphological changes of digestive structures in starved tench *Tinca tinca* (L.) juveniles. Aquacult. Int., 14: 113-126.

PALMER, R, RH SOUTAR, EJ BRANSON, PJ SOUTHGATE, E DRIVAN, RH FICHARDS & RO COLLINS. 1992. Mortality in the Atlantic salmon, *Salmo salar* L., associated with pathology of the melano-macrophage and haemopoietic systems. J. Fish Dis., 15: 207-210.

PARK, LS, HUR JW & W CHOI. 2012. Hematological responses, survival, and respiratory exchange in the olive flounder, *Paralichthys olivaceus*, during starvation. Asian-Aust. J. Anim. Sci., 25(9): 1276-1284.

PERES, H, S SANTOS & A OLIVA-TELES. 2011. Lack of compensatory growth response in gilthead seabream (*Sparus aurata*) juveniles following starvation and subsequent refeeding. Aquaculture, 318: 384-388.

PERES, H, S SANTOS & A OLIVA-TELES. 2013. Selected plasma biochemistry parameters in gilthead seabream (*Sparus aurata*) juveniles. J. Appl. Ichthyol., 29: 630-636.

PINTOS, W, R SOMMARUGA, D CONDE, R DE LEON & G CHALAR. 1988. Antecedentes y nuevos aportes al conocimiento de la Laguna de Rocha. Depto. Hidrob., Sec. Limnol., Fat. Hum. Y Cs., Univ. De la Republica, Montevideo, 9 pp.

RADONIC, M, MI MÜLLER, AV LÓPEZ, GA BAMBILL, M SPINEDI & JJ BOCCANFUSO. 2007. Avances en la reproducción controlada del lenguado *Paralichthys orbignyanus* en Argentina. Ciencias Marinas, 33(2): 187-196.

RADONIC, M & GJ MACCHI. 2009. Gonadal sex differentiation in cultured juvenile flounder, *Paralichthys orbignyanus* (Valenciennes, 1839). J. World Aquacult. Soc., 40(1): 129-133.

RIAÑO, FY, MA LANDINES & GJ DIAZ. 2011. Efecto de la restricción alimenticia y la realimentación sobre la composición del músculo blanco de *Piaractus brachypomus*. Med. Vet. Zoot., 58(II): 84-98.

RIOS, FS, AL KALININ & FT RANTIN. 2002. The effects of long-term food deprivation on respiration and hematology of the neotropical fish *Hoplias malabaricus*. J. Fish Biol., 61: 85-95.

RIOS, FS, ET OBA & MN FERNANDES. 2005. Erythrocyte senescence and hematological changes induced by starvation in the neotropical fish traíra, *Hoplias malabaricus* (Characiformes, Erythrinidae). Comp. Biochem. Physiol. A, 140(3): 281-287.

RIOS, FS, L DONATTI, MN FERNANDES, AL KALININ & FT RANTIN. 2007. Liver histopathology and accumulation of melano-macrophage centers in *Hoplias malabaricus* after long-term food deprivation and re-feeding. J. Fish Biol., 71: 1393-1406.

ROBALDO, RB, RV RODRIGUES, MH OKAMOTO & LA SAMPAIO. 2012. Processing yield of wild-caught and indoor-reared Brazilian flounder *Paralichthys orbignyanus*. J. Appl. Ichthyol., 28: 815-817.

RODRIGUES, RV, LS FREITAS, RB ROBALDO & LA SAMPAIO. 2012. Crescimento e sobrevivência de juvenis do linguado *Paralichthys orbignyanus*: efeitos do enriquecimento da *Artemia* sp. com n-3 HUFA. Atlântica, 34(2): 121-127.

RODRÍGUEZ-GRAÑA, L, D CALLIARI, D CONDE, J SELLAMEN & R URRUTIA. 2008. Food web of a SW Atlantic shallow coastal lagoon: spatial environmental variability does not impose substantial changes in the trophic structure. Mar. Ecol. Prog. Ser., 362: 69-83.

SALHI, M, M BESSONART, F FÉOLA, M TAKATSUKA & J GADEA. 2010. Efecto de la relación DHA/EPA en microdietas sobre el crecimiento y supervivencia de larvas de *Paralichthys orbignyanus*. In: I Congreso Uruguayo de Zoología, X Jornadas de Zoologia del Uruguay "Prof. Federico Achaval". Montevideo. Resúmenes del I Congreso Uruguayo de Zoología. Pp. 256.

SAMPAIO, LA & A BIANCHINI. 2002. Salinity effects on osmoregulation and growth of the eurihaline flounder *Paralichthys orbignyanus*. J. Exp. Mar. Biol. Ecol., 269: 187-196.

SAMPAIO, LA, LS FREITAS, M OKAMOTO, LR LUOZADA, RV RODRIGUES & RB ROBALDO. 2007. Effects of salinity on Brazilian flounder *Paralichthys orbignyanus* from fertilization to juvenile settlement. Aquaculture, 262: 340-346.

SAMPAIO, LA, RB ROBALDO & A BIANCHINI. 2008. Hormone-induced ovulation, natural spawning and larviculture of Brazilian flounder *Paralichthys orbignyanus* (Valenciennes, 1839). Aquacult. Res., 39: 712-717.

SANT, F, A RIOS, L DONATTI, MN FERNANDES, AL KALININ & FT RANTIN. 2009. Effects of food deprivation in muscle structure and composition of traíra (*Hoplias malabaricus*): potential implications on flesh quality. Braz. Arch. Biol. Technol., 52(2): 465-471.

SEGNER, H, P BERKHARDT, EM AVILA, JV JUARIO & V STORCH. 1987. Nutrition-related histopathology of the intestine of milkfish *Chanos chanos* fry. Dis. Aquat. Org., 2: 99-107.

SEGNER, H & H MÖLLER. 1984. Electron microscopical investigations on starvation-induced liver pathology in flounders *Platichthys flesus*. Mar. Ecol. Prog. Ser., 19: 193-196.

SHAIBANI, ME, BM AMIRI & S KHODABANDEH. 2013. Starvation and refeeding effects on pyloric caeca structure of Caspian salmon (*Salmo trutta caspius*, Kessler 1877) juvenile. Tissue and Cell, 45: 204-210.

SOKOLOVA, IM, M FREDERICH, R BAGWE, G LANNING & AA SUKHOTIN. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Marine Environ. Res., 79: 1-15.

STIMPSON, JH. 1965. Comparative aspects of the control of glycogen utilization in vertebrate liver. Comp. Biochem. Physiol., 15: 187-197.

STIRLING, HP. 1976. Effects of experimental feeding and starvation on the proximate composition of the European bass *Dicentrarchus labrax*. Mar. Biol., 34: 85-91.

TIDWELL, JH, CD WEBSTER & JA CLARK. 1992. Effects of feeding, starvation and refeeding on the fatty acid composition of channel catfish, *Ictalurus punctatus*, tissues. Comp. Biochem. Physiol., 103A (2): 365-368.

TRIPATHI, G & P VERMA. 2003. Starvation-induced impairment of metabolism in a freshwater catfish. Z. Naturforschung., 58: 446-451.

WASIELESKY JR., W, A BIANCHINI, M SANTOS & L POERSCH. 1997. Tolerance of juvenile flatfish *Paralichthys orbignyanus* to acid stress. J. World Aquacult. Soc., 28: 202-204.

WASIELESKY JR., W, A BIANCHINI & K MIRANDA.1998. Tolerancia a la temperatura de juveniles de lenguado *Paralichthys orbignyanus*. Frente Marítimo, 17: 43-48.

WEATHERLEY, AH & HS GILL. 1987. The biology of fish growth. Protein, lipid and caloric contents. Academic Press, London, 4: 139-146.

WHYTE, JNC, RJ BEAMISH, NG GINTHER & CE NEVILLE. 1993. Nutritional condition of the pacific lamprey (*Lampetra tridentate*) deprived of food for periods of up to two years. Can. J. Fish. Aquatic Sci., 50(3): 591-599.

ZHANG, XD, YF ZHU, LS CAI & TX WU. 2008. Effects of fasting on the meat quality and antioxidant defenses of market-size farmed large yellow croaker (*Pseudosciaena crocea*). Aquaculture, 280: 136-139.

6. ARTIGO EM ANEXO

EFFECTS OF STARVATION ON JUVENILE FLOUNDER *Paralichthys orbignyanus*

ABSTRACT

The aim of this work was to determine the effects of starvation in *Paralichthys orbignyanus* juveniles. For this, two treatments were compared, Feeding (A) and Starvation (S); samples of six fish per treatment were taken in weeks 0, 1, 2, 4 and 8. The results showed that the lack of food leads to the diminution of the K, HSI and VSI, due to the decreased of hepatic reserves: glycogen and triglycerides at week 2, and glucose and proteins at week 4. There was a rise in triglycerides between weeks 4 and 8, possibly due to a fatty acid re-esterification process. The hepatic cholesterol decreased, while the plasmatic raised, thus indicating its possible transport for cortisol synthesis. The plasma glycogen and the muscular protein raised in the last week, the former possibly due to a glucose accumulation and the later because glucose would be stimulating protein synthesis. Starvation did not affect the size and the deposition degree of melano-macrophages, neither the intestinal structures. Then *P. orbignyanus* juveniles can survive to eight weeks of starvation by using basically hepatic energy stores and without structural changes neither in the kidney nor in the intestine.

Key words: *Paralichthys orbignyanus*, starvation, energy stores.

1. INTRODUCTION

The flounder *Paralichthys orbignyanus* (Valenciennes, 1842) is distributed in coastal and estuarine areas between Brazil and Argentina, up to 45 m deep (Diaz de Astarloa & Munroe, 1998). It is a marine/estuarine dependent species (Chao et al, 1985), and tolerates a wide range of environmental factors such as temperature (8 - 31 °C) (Wasielesky et al., 1998), salinity (0 - 40 ‰) (Sampaio & Bianchini, 2002), and pH (between 6.0 and 8.0) (Wasielesky et al., 1997). Besides that, it presents a high fillet yield and it is highly appreciated in the market (Robaldo et al., 2012). All these characteristics make *P. orbignyanus* a species with a

high potential for aquaculture. Studies have been conducted in Argentina, Brazil and Uruguay aiming to determine the optimum conditions for the production of this species in captivity. Aspects such as breeding and larviculture techniques are dominated (Bambill et al., 2006; Sampaio et al., 2007; 2008; Lanes et al., 2008; Radonic & Macchi, 2009; Rodrigues et al., 2012).

Several fish species are forced to undergo periods of fasting during their life cycle due to the decrease in food availability, migration, and breeding season (Hur et al., 2006). Fish farmers also can submit fish to food deprivation for different purposes including a compensatory growth response (Cho, 2005). In order to survive, organisms rely on energy reserves stored in the body (carbohydrates, proteins, and lipids), which implies in the catabolism of their own tissues (Weatherley & Gill, 1987). Therefore, metabolic changes occur, and the nature of these changes will depend, among other factors, on the species, age of the organism, and the starvation period (Blasco et al., 1992; Kieffer & Tufts, 1998). Major energy sources and their storage places (compounds and organs) and the order in which they are used during starvation vary between species (Black & Love, 1986).

Biochemical alterations have been observed in muscle, liver and plasma of unfed fishes. For example, the reduction of hepatic glycogen (Segner & Möller, 1984; Borah & Yadav, 1996; Hur et al., 2006), protein and glucose levels of liver and muscle (Borah & Yadav, 1996; Tripathi & Verma, 2003). In bass (*Dicentrarchus labrax*), it was observed a decrease of muscular glucose and triglycerides (Zammit & Newsholme, 1979), while juvenile *Sparus aurata* underwent a reduction of plasmatic glucose, cholesterol and protein after two weeks of starvation (Peres et al., 2013).

Muscle, liver and visceral fat content can also be affected (De Silva et al., 1997). In hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) starved for 45 days, there

was observed an increase in the proportion of fatty acids (µg/mg of lipids) and in the percentage of PUFA in liver and muscle. DHA percentage (of total lipids) showed a significant rise in liver with starvation, while in muscle it did not vary significantly (De Silva et al., 1997).

Histological changes have been observed in different organs of fish when subjected to food restriction. Some parameters for the evaluation of this situation are: A- the increase in the number of melano-macrophages (MMs) in the kidney (Michaele & Perdichizzi, 1990), which are structures that act on the metabolism of toxic substances and the immune system of fish (Agius & Roberts, 1981). B- a decrease in the thickness of the intestinal mucosa and reduction of the intestinal microvilli, leading to a decrease in the absorption capacity of the epithelium (Segner et al., 1987; Hall & Bellwood, 1995). C- changes in the hepatocyte structure, as a reduction of glycogen reserves, in the diameter of the nucleus, larger mitochondria, and the lack of Golgi bodies and lipids in the cytoplasm (Hur et al., 2006).

In some species the hematocrit and plasma osmolality are also influenced; the former generally experienced a decrease (Rios et al., 2002; Caruso et al., 2010), but it can also remain stable as well (Caruso et al., 2011). The last underwent a significant reduction in *Paralichthys olivaceus* fasted for four weeks (Park et al., 2012).

Therefore, the objective of this study was to evaluate the effects of food restriction in juvenile flounder *P. orbignyanus*. Considering that this species has a potential for aquaculture, this kind of research could be important, being useful as a basis for future studies with feed management of the species, allowing to determine the point at which biochemical and structural changes caused by food restriction occurs for flounder.

2. MATERIAL AND METHODS

2.1 *Experimental fish*

Juvenile Brazilian flounder *P. orbignyanus* were reared according to the protocol described by Sampaio et al. (2008).

2.2 *Experimental design*

Four recirculating aquaculture systems were used in the experiment. Each system was composed by 3 tanks (300 L) coupled to a sump with a moving biological filter for ammonia oxidation, a sand filter (Sibrape, Model S50, Brazil), a protein skimmer (Plaspiral, Brazil), and a pump (Sibrape, FIT-33, 1/3 hp, Brazil) for water recirculation. Water sterilization was obtained using UV lamp (Sibrape, 95 W, Pond Clean, Brazil).

Seven fish (26.4 ± 1.2 cm; 234.2 ± 28.1 g) were placed in each tank (n=84), and acclimated under controlled conditions of photoperiod (12h L:12h D) and temperature (23.7 ±0.7 °C) and constant aeration, for one month prior to the experiment. They were hand fed with a commercial diet (50% protein and 10% lipids, Nicoluzzi - Brazil) once a day until satiation. Then, two treatments were compared during 8 weeks: 1) STARVATION (S) in which the fish of two systems (chosen randomly) were not fed during the experimental period; 2) FEEDING (F; used as a control group) in which the fish of the other two systems were fed using the same protocol of the acclimation period.

Water quality parameters were measured daily during the acclimation and the experimental period: salinity was measured using a portable refractometer (Atago, Japan), dissolved oxygen and temperature were measured with an oxymeter (YSI, Model 550A, USA) and pH with a pHmeter (Toledo, Brazil). Alkalinity was measured by titration accordingly to APHA (1998). Ammonia and nitrate were determined according to the

methods presented by UNESCO (1983), and nitrite following the methodology of Strickland & Parsons (1972).

One fish of each tank was sampled at the beginning of the experiment (t=0) and on weeks 1 (t=1), 2 (t=2), 4 (t=4) and 8 (t=8). Thus, in each sampling time, a total of 12 fish were sampled, 6 per treatment. Feeding of fish in the Control treatment was stopped 24 h before each sampling period.

Fish were anesthetized with benzocaine (50 ppm). First, body mass and total length were measured using a digital balance (Marte, BL3200H, Brazil 0.01g) and an ichthyometer (0.1 cm), respectively.

2.3 *Hematology*

Firstly, blood samples were collected from the caudal vein using heparinized 1 mL syringe. Immediately, the hematocrit was determined using heparinized capillary tubes by centrifugation during 10 min at 12.000g using a centrifuge (HT, H-240, China). The remaining of the blood was centrifuged for 10 minutes at 4°C and 1000g (SOLAB, SL-703) and plasma was stored at -80°C.

2.4 *Biochemical analyses*

After blood collection, the fish were submitted to euthanasia by immersion in a benzocaine bath (300 ppm), and the weight of the viscera and liver were registered. Samples of muscle and liver were immersed in liquid nitrogen and then stored at -80°C. Concentration of cholesterol, glucose, glycogen, total proteins and triglycerides were measured in blood plasma, liver and muscle using commercial kits (Enzymatic Cholesterol, Enzymatic Glucose, Enzymatic Total Protein and Enzymatic Triglycerides by Doles, Brazil). The extractions for

cholesterol, glucose, glycogen and triglycerides were obtained using the homogenized solution for metabolite extraction. Protein extraction for liver and muscle was made using the methodology employed by Amado et al. (2006). The glycogen was measured using the Carr & Neff (1984) method, modified by Nery & Santos (1993). The glycogen was broken into glucose, which was measured using the commercial kit Enzymatic Glucose. In all cases (muscle, liver and blood plasma) the determination of components were made by spectrophotometer (BioTek, EL 808, Spain) at 490 nm using the software Gen5 1.08. Muscle total lipids were extracted using the method described by Folch et al. (1957) and fatty acid methyl esters (FAMEs) were separated using gas chromatography (Hewlett Packard 5890, USA). Osmolality was determined using a vapor-pressure osmometer (Vapro 5600, Wercor Inc., Logan, UT, USA).

2.5 *Histological analyses*

Samples of liver, intestine and kidney were fixed in 10% buffered formalin for subsequent histological evaluation. The samples were dehydrated in a graded series of ethanol, embedded in Paraplast and sectioned at 5µm. The slides of intestine and kidney were stained with haematoxilin and eosin, and liver slides were stained with PAS technique. The slides were observed in an optical microscope (Carl Zeiss, Primo Star GmbH, Germany) and photomicrographs were taken with a digital camera (Carl Zeiss, Axiocam ERc 5s, Germany) (Figure 1). The estimation of numbers and relative surface areas of melano-macrophage centers were made following the methodology described by Weibel & Gomez (1962). Intestinal histology was performed accordingly to Peng et al. (2013). Villus height was measured from the lowest point between two longitudinal villus to the fold top; enterocyte height was measured from the base of to the top of enterocyte. Ten measures per fish were

made for both parameters. All measurements mentioned above were made using the software AxioVision Rel. 4.8.

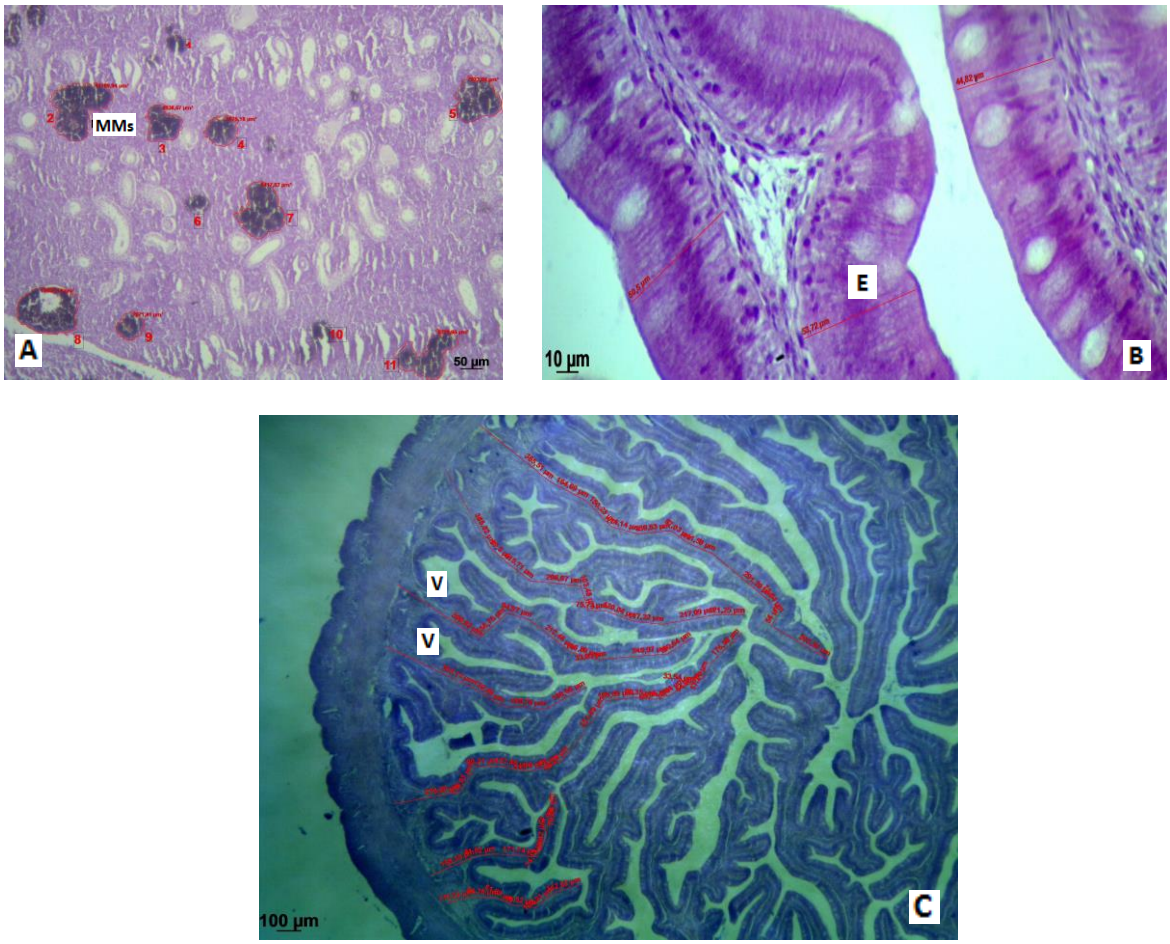


Figure 1. Photomicrographs of kidney (A) and transversal section of intestine (B and C) showing the measures (µm) of melano-macrophages (a; 10×), enterocyte height (b 40×) and villi`s length (d; 4×) of juvenile *Paralichthys orbignyanus*. MMs=melano-macrophages, E=enterocyte and V=villi.

2.6 *Biometric indexes*

Using the biometric data mentioned above, Viscerosomatic (VSI) and Hepatosomatic (HSI) indexes, and condition factor (CF) were calculated as follows:

- VSI = (VW/TW)*100

- HSI = (LW/TW)*100

- CF = (TW/TL^3)*100

Where VW=viscera weight (g), LW= liver weight (g), TW= total fish weight (g), e TL= total fish length (cm).

2.7 *Statistical analyses*

All results are expressed in mean ± standard error. Differences between treatments were determined using two-way ANOVA, followed by the Test of Newman-Keuls or orthogonal planned comparisons. The significance level adopted was 95%.

3. RESULTS

No fish died along the acclimation and experimental time. The water quality parameters measured presented values within the range tolerated for *P. orbignyanus*. The dissolved oxygen was 7.09±0.24 mgO_2/L, pH was 8.33±0.09, and salinity, ammonia, nitrite, nitrate and alkalinity were, 24.3±1.1 ‰, 0.065±0.10 mg NH_3-N /L, 0.13±0.37 mg NO_2-N /L 7.7±3.9 mg NO_3-N /L and 238±56 mg $CaCO_3$/L, respectively.

Fish weight was not affected by starvation, while those fish continuously fed showed a significant rise in weight after 8 weeks ($P<0.05$). Weight of fish in both treatments were significantly different ($P<0.05$) at week 8 (Table 1). Condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) were not affected in fish that were normally fed. On the other hand, these parameters were reduced along the time, presenting significant differences ($P<0.05$) in fish maintained in starvation (S) during 8 weeks. VSI and HSI were

reduced after 2 weeks of starvation, while CF was affected only after 8 weeks of starvation. Significant differences between treatments were observed after 1, 2 and 8 weeks of starvation for HSI, VSI and CF, respectively (Table 1).

Table 1. Weight (W), Condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) (Mean ± SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks.

		Time (weeks)					P value		
	Treatment	**0**	**1**	**2**	**4**	**8**	**Treatment**	**Time**	**Treatment*Time**
W (g)	F	234.17±9.82a	235.96±16.50a	220.65±10.44a	237.31±17.23a	289.76±16.99b*	0.004*	0.022*	0.049*
	S	232.69±8.10	221.90±11.18	219.62±18.30	186.38±11.26	223.86±4.04			
CF	**F**	1.27± 0.007	1.26 ± 0.05	1.25 ± 0.12	1.28 ± 0.07	1.35 ± 0.19*	0.000*	0.504	0.001*
	S	1.29± 0.10a	1.17 ± 0.03ab	1.18 ± 0.08ab	1.20 ± 0.04ab	1.06 ± 0.04b			
VSI	**F**	3.56 ± 0.42	3.81 ± 0.41	3.49 ± 0.47*	3.51 ± 0.66*	3.40 ± 0.32*	0.000*	0.000*	0.015*
	S	3.52 ± 0.42a	3.21 ± 0.36ab	2.72 ± 0.34bc	2.56 ± 0.21bc	2.26 ± 0.22c			
HIS	**F**	1.45 ± 0.25	1.77 ± 0.30*	1.50 ± 0.41	1.65 ± 0.53*	1.57 ± 0.27*	0.000*	0.013*	0.002*
	S	1.58 ± 0.34a	1.22 ± 0.26ab	1.01 ± 0.26abc	0.85 ± 0.16bc	0.69 ± 0.07c			

Asterisk represents significant differences ($P<0.05$) between treatments at the same experimental time, and different letters indicate significant differences along the time in the Starvation treatment.

Blood Plasma

Cholesterol (Figure 2A) and glycogen (Figure 2B) levels in blood plasma were not affected in the F treatment. Up to 4 weeks of starvation, cholesterol and glycogen levels remained unchanged, but further on, it was observed a significant raise ($P<0.05$) of both components compared to that of fed fish.

It was observed that the levels of plasmatic glucose, protein and triglycerides were not affected by starvation, neither was osmolality. Only proteins and triglycerides of the F treatment presented significant variations ($P<0.05$); the former decreased between weeks 1 and 2 (determined by orthogonal planned comparisons), and the later a rise between weeks 0 and 4, but then returned to values similar to all others observed before (Table 2).

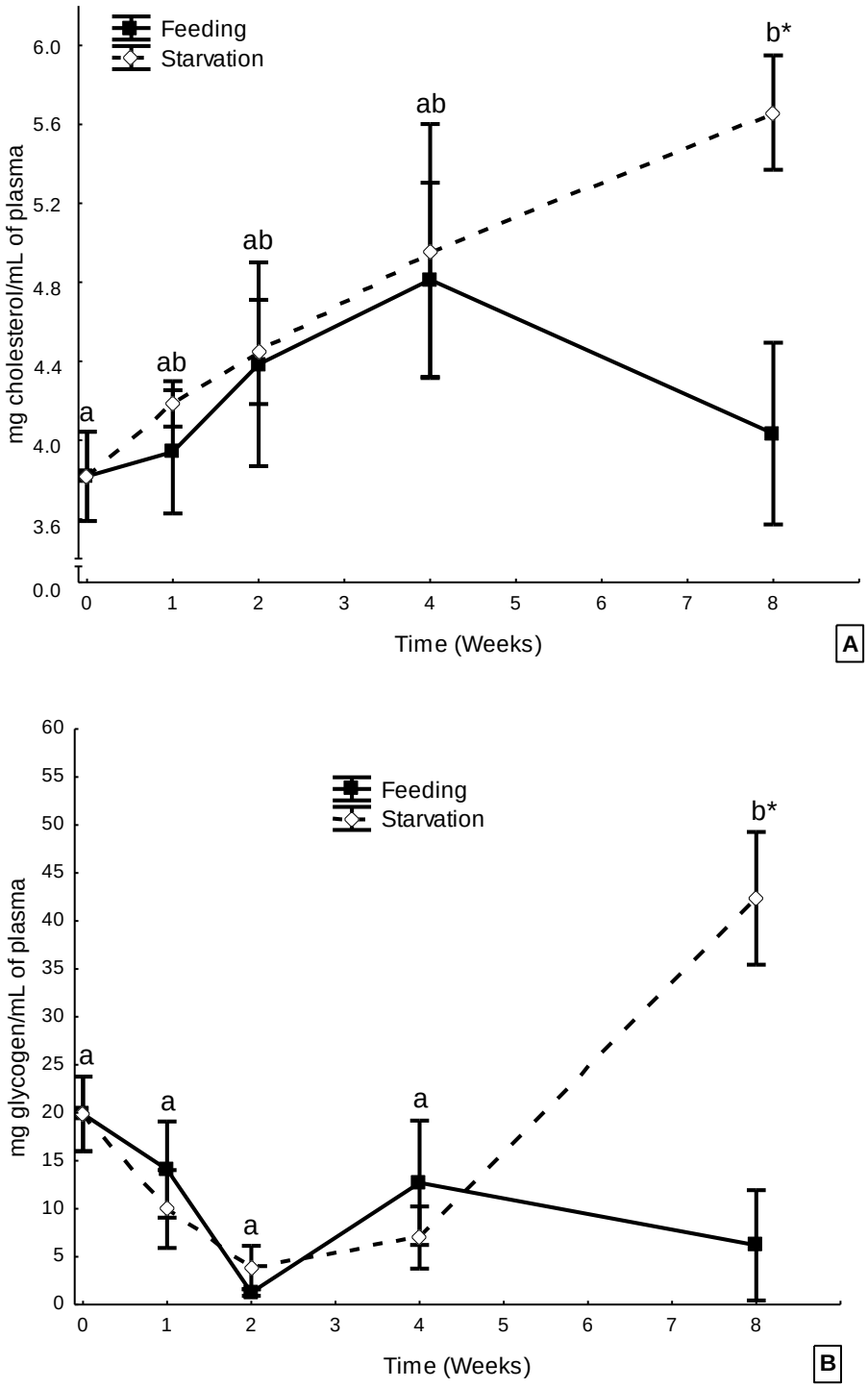


Figure 2. Cholesterol (A) and glycogen (B) plasma blood levels (Mean±SE) of *Paralichthys orbignyanus*) fed (F) or starved (S) for up to 8 weeks. Asterisk represents significant differences ($P<0.05$) between treatments at the same experimental time, different letters indicate significant differences over time in the same treatment.

Table 2. Plasma blood glucose, protein, triglycerides and osmolality (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks.

		Time (weeks)					P value		
	Treatment	0	1	2	4	8	Treatment	Time	Treatment*Time
Plasma Glucose (mg/mL)	**F**	0.25 ± 0.06	0.33 ± 0.04	0.28 ± 0.03	0.33 ± 0.05	0.33 ± 0.07	0.422	0.614	0.864
	S	0.28 ± 0.12	0.25 ± 0.05	0.28 ± 0.03	0.28 ± 0.03	0.34 ± 0.06			
Plasma protein (mg/mL)	**F**	45.58 ± 1.92	56.81 ± 7.03	47.85± 1.78	51.62 ± 3.46	53.27 ± 3.05	0.006*	0.177	0.453
	S	47.01 ± 3.51	48.51 ± 1.35	44.84 ± 1.53	43.87 ± 3.09	45.01 ± 2.07			
Plasma triglyceride (mg/mL)	**F**	3.43 ± 0.30 A	5.06± 1.17 AB	3.46 ± 0.51 AB	6.21 ± 1.43 B	4.24 ± 0.66 AB	0.014*	0.166	0.165
	S	3.57 ± 0.43	3.58 ± 0.29	3.21 ± 0.32	3.32 ± 0.50	3.66 ± 0.43			
Osmolality (mmol/kg)	**F**	327.00 ± 4.83	351.83 ± 20.45	322.0 ± 9.56	330.67 ± 9.39	369.83 ± 15.43	0.311	0.012*	0.351
	S	328.33 ± 1.45	321.83 ± 14.05	329.67 ± 3.04	336.33 ± 8.35	352.33 ± 13.48			

Capital letters indicate significant differences over time in the Feeding treatment. Circle represents significant differences determined by orthogonal planned comparisons.

Capital letters indicate significant differences over time in the Feeding treatment. Circle represents significant differences determined by orthogonal planned comparisons.

Hematology

With respect to the hematocrit, it remained stable along time for fed fish, there was a significant drop at week 4, but it rose up to the initial level at the end of the experiment. Starved fish, in contrast, presented a significant drop in hematocrit levels since the second week of starvation. Besides, both treatments were different in the last week ($P<0.05$) (Figure 3).

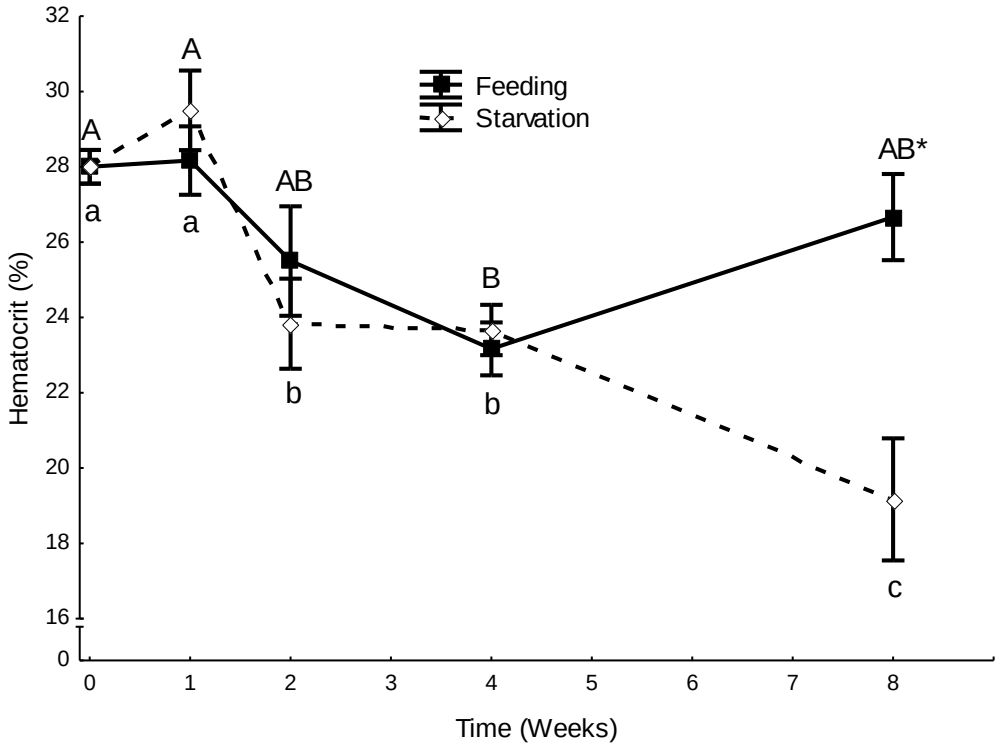


Figure 3. Hematocrit levels (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks. Asterisk represents significant differences ($P<0.05$) between treatments at the same experimental time. Different capital letters indicate significant differences over time for fed fish, while lower case letters resemble starved fish.

Muscle

Muscle cholesterol is not reported here because levels were below the detection limit of the method employed. Neither glucose (Figure 4A), nor total proteins levels (Figure 4B) were affected in fish normally fed. In starved fish, glucose level maintained low levels along the experiment, but it was significantly higher ($p<0.05$) at week 4. Differences between treatments occurred in week 4 ($p<0.05$); this could be due to an error in the analysis explained by the wide range of the standard error. However, the concentration of muscular protein had a significant rise between weeks 4 and 8 of starvation ($p<0.05$), but these values were not different from the initial levels. There were no significant differences between treatments for this parameter.

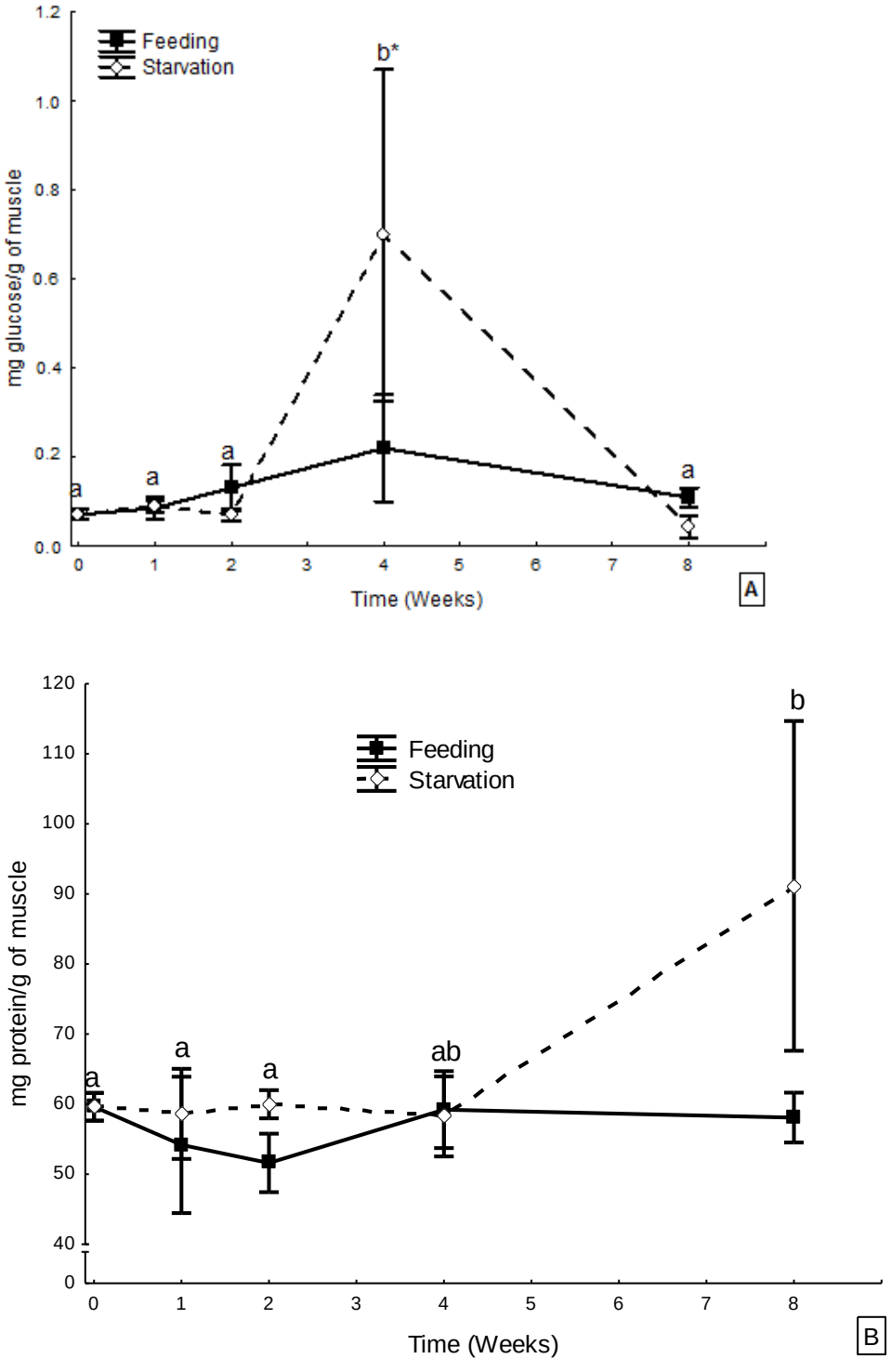


Figure 4. Muscle glucose (A) and protein (B) (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks. Different letters indicate significant differences over time in the S treatment.

In the case of muscle lipid levels (Figure 5A) and moisture (Figure 5B) both treatments were affected along the experimental time. It was observed an increase in lipids levels that became significant ($P<0.05$) at 2 and 4 weeks for F and S, respectively. Orthogonal planned comparisons showed significant differences at 2 weeks between treatments ($P<0.05$). Muscle moisture experienced a decrease along time, becoming significant in 2 and 4 weeks in the S and F treatment, respectively ($P<0.05$).

Generally, the percentage of fatty acids (of total lipids), individual fatty acids (16:0, 18:1n9, 18:2n6, 20:3n3, 20:4n6, 20:5n3, 22:5n3, 22:6n3) sums (SAFA, MUFA, PUFA, n3, n6 e n9) and ratios (n6/n3, DHA/EPA, EPA/AA), presented the same behavior for both treatments. Only the ratio DHA/AA showed a significant decreased in the S treatment along the experimental time, that was significantly different of the F treatment at week 8 ($p<0.05$). The most abundant fatty acids were: 16:0, 18:1n9, 18:2n6, 22:6n3. PUFAs and MUFAs presented similar values and, in general bigger than that of the SAFAs; and the n9 series generally showed superior levels than n3 and n6 (Table 3).

It was observed that muscular glycogen and triglycerides were not affected either by feeding or by starvation (Table 4).

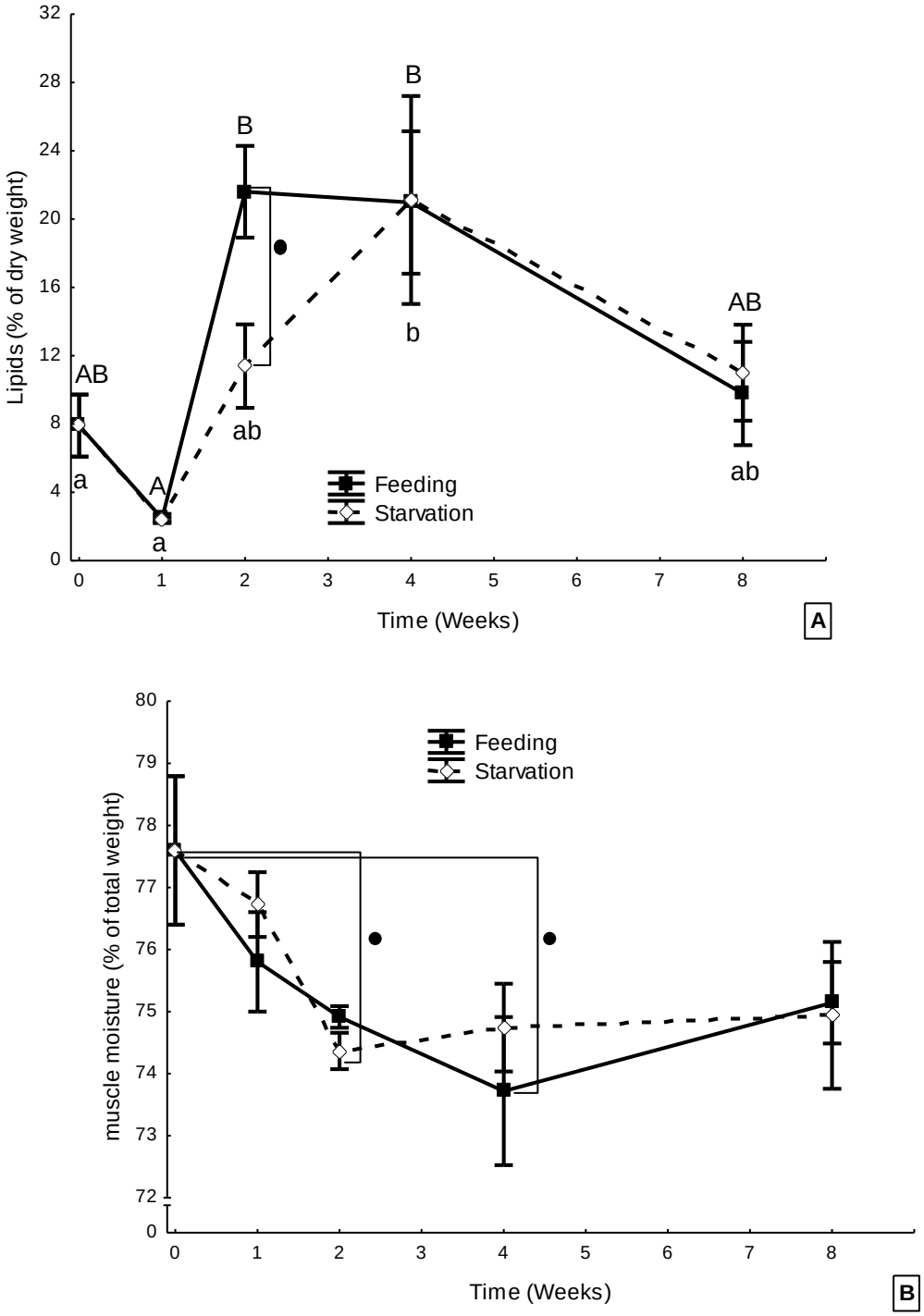


Figure 5. Muscle lipids (A) and moisture (B) (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks. Different letters indicate significant differences ($P<0.05$) over time in the same treatment (capital letter: Feeding (F); lower case: Starvation (S)) and circle represents significant differences determined by orthogonal planned comparisons.

Table 3. Main fatty acids composition (mg/g of total FA; mean± SE) of white muscle of *Paralichthys orbignyanus* Fed (F) or Starved (S) for up to 8 weeks.

Asterisk represents significant differences (P<0.05) between treatments at the same experimental time, and different letters indicate significant differences over time in the same treatment (capital letter: Feeding (F); lower case: Starvation (S)). Differences at week 2 were determined by orthogonal planned comparisons.

	Feeding					Starvation						
Weeks	0	1	2	4	8	1	2	4	8	Treatment	Time	Treatment*Time
14:0	1.30±0.98 Aa	0.19± 0.09 A	5.22± 1.60B*	4.63± 2.75B	3.05±1.95AB	0.19± 0.06a	2.40±1.38ab	4.87 ± 0.65b	2.59±1.80ab	0.223	0.000*	0.249
16:0	10.74±6.52Aa	3.03 ± 0.79 A	33.95± 11.22B*	31.16±17.56B	19.47± 11.33AB	3.14± 0.70a	16.24±8.48ab	32.56±23.56b	17.36±11.50ab	0.232	0.000*	0.259
16:1n-7	3.07±2.46Aa	0.43 ± 0.25 A	12.86± 3.96B*	11.47± 6.86B	7.40± 4.83AB	0.43± 0.17a	5.78± 3.39ab	12.03±9.25b	6.21±4.48ab	0.214	0.000*	0.249
18:0	2.30±1.38ACab	0.87±0.19 A	6.33±2.13B*	5.89±3.31BC	3.59±1.92ABC	0.82±0.13a	3.11±1.49ab	5.67±3.85b	3.29±2.09ab	0.183	0.000*	0.294
18:1n-9	12.99±9.03Aa	2,33±0.99a	54.56±19.91B*	49.66±30.08B	28.26±17.21AB	2.46±0.81a	24.44±15.10ab	51.27±39.92b	24.91± 17.58ab	0.232	0.000*	0.259
18:2n-6	10.76±7.85Aa	2.10±0.78a	40.14±11.98B*	35.70±21.22B	22.39±13.90AB	2.14±0.55a	18.72±10.38ab	39.11±29.59b	19.25±13.15ab	0.273	0.000*	0.243
18:3n-3	0.71±0.59Aa	0.09±0.05a	2.86±0.88B*	2.60±1.59B	1.67±1.10AB	0.09±0.03a	1.27±0.72ab	2.92±2.03b	1.29±0.92ab	0.234	0.000*	0.199
20:4n-6	0.85±0.49ABab	0.41±0.08a	1.86±0.50B*	1.73± 0.83B	1.14±0.53AB	0.42±0.09a	1.06±0.43ab	1.84±1.16b	1.00±0.53ab	0.337	0.000*	0.338
20:3n-3	0.085±0.08Aa	0.02±0.01a	0.37±0.13B*	0.34±0.20B	0.21±0.13AB	0.01±0.01a	0.18±0.09ab	0.35±0.27b	0.18±0.12ab	0.234	0.000*	0.358
20:5n-3	1.54±0.99Aa	0.41±0.12a	4.94±1.50B*	4.56±2.65B	3.13±1.92AB	0.43±0.08a	2.40±1.26b	4.78±3.56b	2.19±0.15ab	0.178	0.000*	0.290
22:5n-3	0.93±0.58Aa	0.29±0.06a	2.90±0.65B*	2.74±1.60B	1.90±1.14AB	0.30±0.06a	1.53±0.81ab	2.98±2.23b	1.40±0.89ab	0.269	0.000*	0.354
22:6n-3	4.25±2.63ABab	2.16±0.35a	8.80±2.67B*	8.62±4.26AB	6.25±2.96AB	2.11±0.28a	4.96±1.99ab	8.70±5.56b	4.32±2.37ab	0.176	0.000*	0.391
SAFA	15.62±9.50Aab	4.50±1.14a	48.91±15.86B*	45.12±25.50B	28.29±16.35AB	4.57±0.91a	23.57±12.24ab	46.55±33.51b	25.07±16.55ab	0.231	0.000*	0.284
MUFA	19.60±14.48Aa	3.42±1.38a	76.91±25.77B*	68.72±40.76B	43.02±26.74AB	3.60±0.92a	35.01±20.04ab	73.23±55.86b	37.24±25.91ab	0.247	0.000*	0.247
PUFA	23.26±14.24ABab	6.98±1.6326a	63.67±26.83B	64.45±36.44B	42.69±24.63AB	7.07±1.16a	34.93±17.78ab	69.52±50.74b	34.35±21.78ab	0.351	0.000*	0.507
n-9	13.97±9.65Aa	2.52±1.04a	57.80±21.23B*	52.70±31.87B	30.10±18.27AB	2.82±0.82a	26.05±16.03ab	54.66±42.52b	26.58±18.61ab	0.243	0.000*	0.262
n-6	12.67±8.79ABab	2.85±0.90a	38.06±20.88B	40.32±23.60B	25.46±15.47AB	2.91±0.68a	21.44±11.63ab	44,06±32.97b	21.84±14.66ab	0.464	0.000*	0.624
n-3	7.70±4.50ABab	2.98±0.55a	20.61±5.99B	19.51±10.69AB	13.69±7.61AB	2.96±0.43a	10.67±5.04ab	20.51±14.26b	9.70±6.02ab	0.192	0.000*	0.311
n-3 HUFA	5.19±3.06ABab	2.45±0.40a	11.72±3.29AB	11.36±5.86B	8.16±4.10AB	2.41±0.33a	6.50±2.79ab	11.74±7.84b	5.73±3.26ab	0.698	0.000*	0.934
n-6/n-3	1.58±0.46ABab	0.94±0.13a	1.81±0.75B	1.92±0.44B	1.74±0.33B	0.97±0.10a	1.90±0.37b	1.94±0.53b	2.14±0.28b	0.384	0.000*	0.793
DHA/EPA	3.08±1.07Bab	5.40±0.77a	1.79±0.14B	2.38±1.30B	2.30±0.92B	5.01±0.51a	2.38±0.81b	2.40±1.32b	2.32±0.80b	0.516	0.000*	0.937
DHA/AA	5.03±0.31Aba	5.28±0,22AB	4.72±0.29a	5.02±0.32AB	5.43±0.15B*	5.14±0.42a	4.77±0.50ab	4.73±0.39ab	4.25±0.23b	0.001*	0.000*	0.001*
EPA/AA	1.79±0.54ABb	0.99±0.14a	2.64±0.20B	2.41±0.67B	2.48±0.76B	1.04±0.16a	2,13±0.46b	2.30±0.74b	1.99±0.54b	0.086	0.000*	0.638

Table 4. Muscle glycogen and triglycerides (Mean ± SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks.

	Treatment	Weeks					p		
		0	1	2	4	8	Treatment	Time	Treatment*Time
Glycogen	F	40.94 ± 29.11	30.91 ± 20.02	41.35 ± 21.47	55.65 ± 48.48	147.51 ± 145.28	0.185	0.349	0.788
(mg/g)	S	24.02 ± 12.23	30.22 ± 14.82	8.84 ± 4.42	35.82 ± 15.82	53.84 ± 48.32			
Triglycerides	F	0.36 ± 0.04	0.49 ± 0.15	0.37 ± 0.08	0.31 ± 0.08	0.38 ± 0.07	0.088	0.733	0.249
(mg/g)	S	0.32 ± 0.06	0.20 ± 0.05	0.25 ± 0.05	0.30 ± 0.07	0.42 ± 0.07			

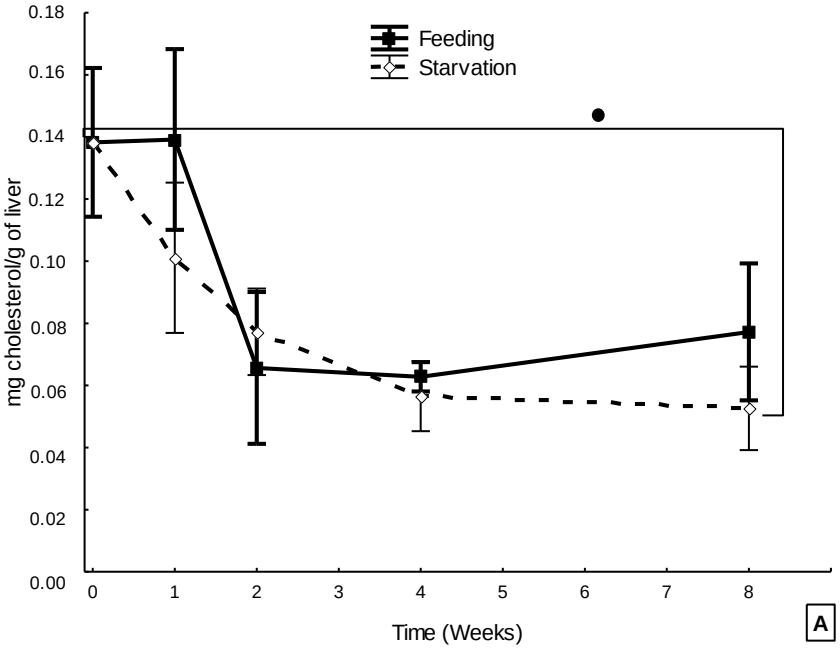
Liver

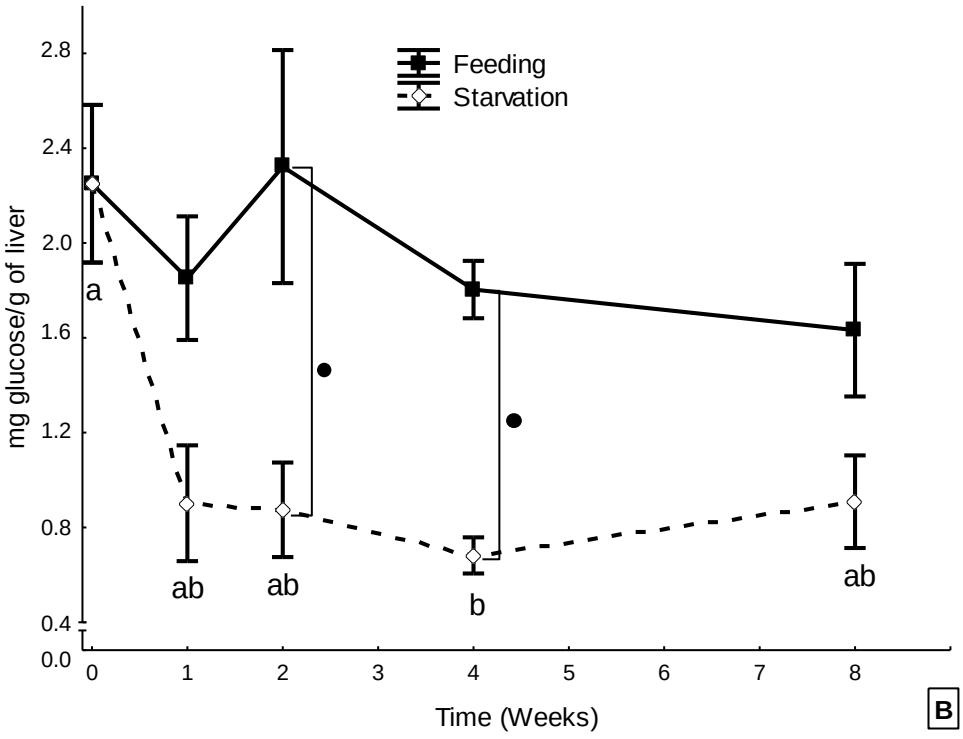
The content of cholesterol in liver of unfed fish showed a significant reduction ($P<0.05$) from the beginning to the end of the experiment (Figure 6A). Besides, there was a significant decrease in liver glucose between weeks 0 and 4 ($P<0.05$) (Figure 6B); differences between treatments occurred in weeks 2 and 4 ($P<0.05$; determined by orthogonal planned comparisons).

Starved fish presented a significant reduction of hepatic glycogen ($P<0.05$) between the firsts two weeks of starvation and remained low until the end of the experiment (Figure 6C).

On the other hand, the concentration of hepatic protein (Figure 7A) showed a significant decrease ($P<0.05$) between weeks 1 and 4, and remained at this level until the end of the experiment; differences between treatments were found at weeks 4 and 8 ($P<0.05$). Liver of fish starved showed a significant reduction on triglyceride level ($P<0.05$) between weeks 0 and 2, but afterwards triglyceride levels rose to the same concentration of the fish at the beginning of the experiment (Figure 7B). Comparing liver of fed and starved fish, there were significant differences between treatments in weeks 1 and 2 ($P<0.05$), determined by orthogonal planned comparisons and Newman-Keuls, respectively. The hepatic levels of all parameters mentioned above were not affected in fed fish.

In figures 8 A and B it is observed the change in the glycogen stores of the liver between weeks 0 and 2, respectively.


Feeding
Starvation
mg cholesterol/g of liver
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
1
2
3
4
5
6
7
8
Time (Weeks)
A


Feeding
Starvation
mg glucose/g of liver
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
a
ab
ab
b
ab
0
1
2
3
4
5
6
7
8
Time (Weeks)
B

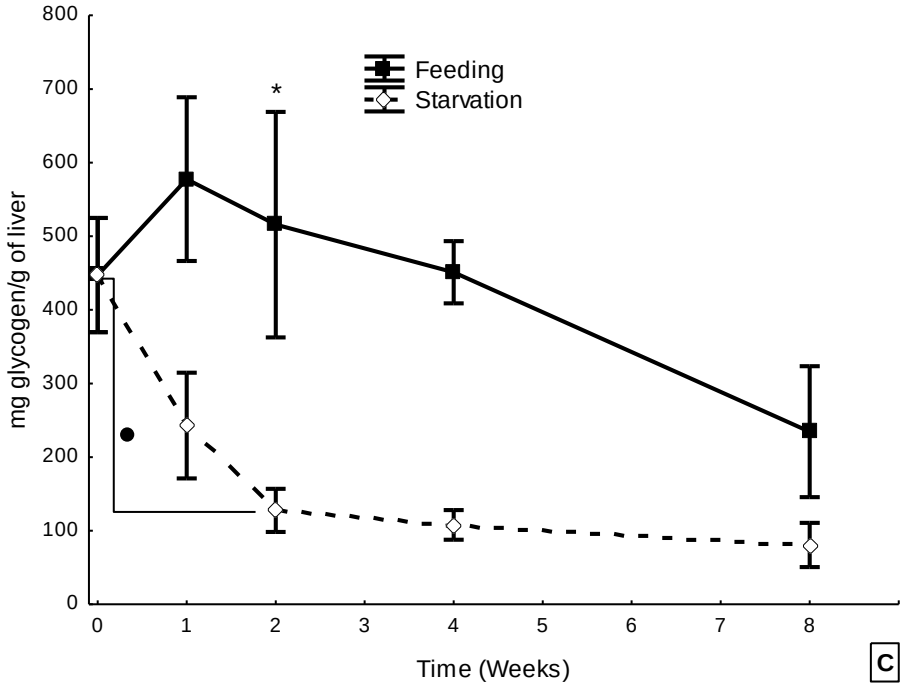


Figure 6. Hepatic cholesterol (A), glucose (B) and glycogen (C) (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks. Asterisk represents significant differences ($P<0.05$) between treatments at the same experimental time, different letters indicate significant differences over time in the S treatment, and circle represents significant differences determined by orthogonal planned comparisons.

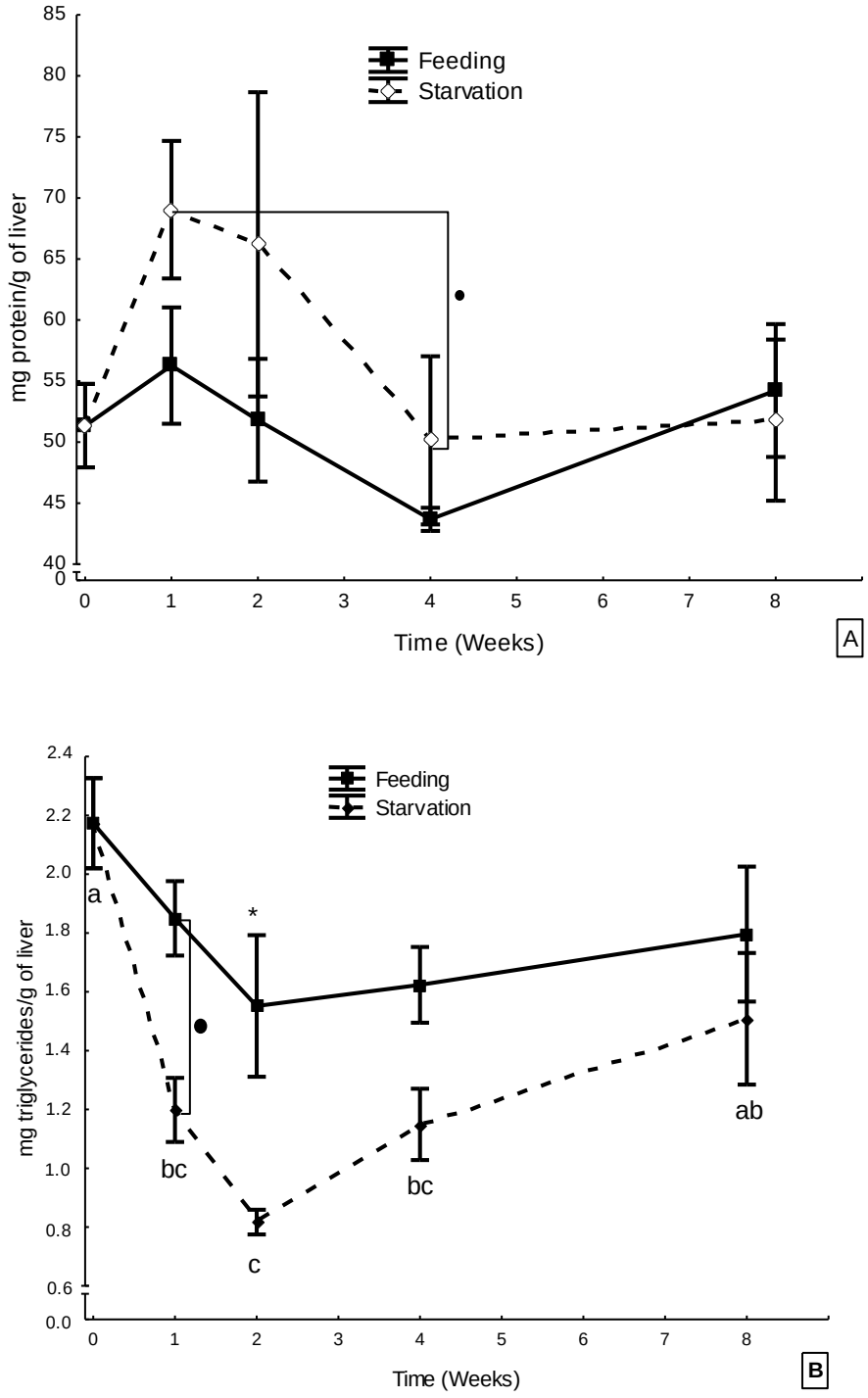


Figure 7. Hepatic total protein (A) and triglyceride (B) levels (Mean±SE) of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks. Asterisk represents significant differences (P<0.05) between treatments at the same experimental time, different letters indicate significant differences over time in the S treatment, and circle represents significant differences determined by orthogonal planned comparisons.

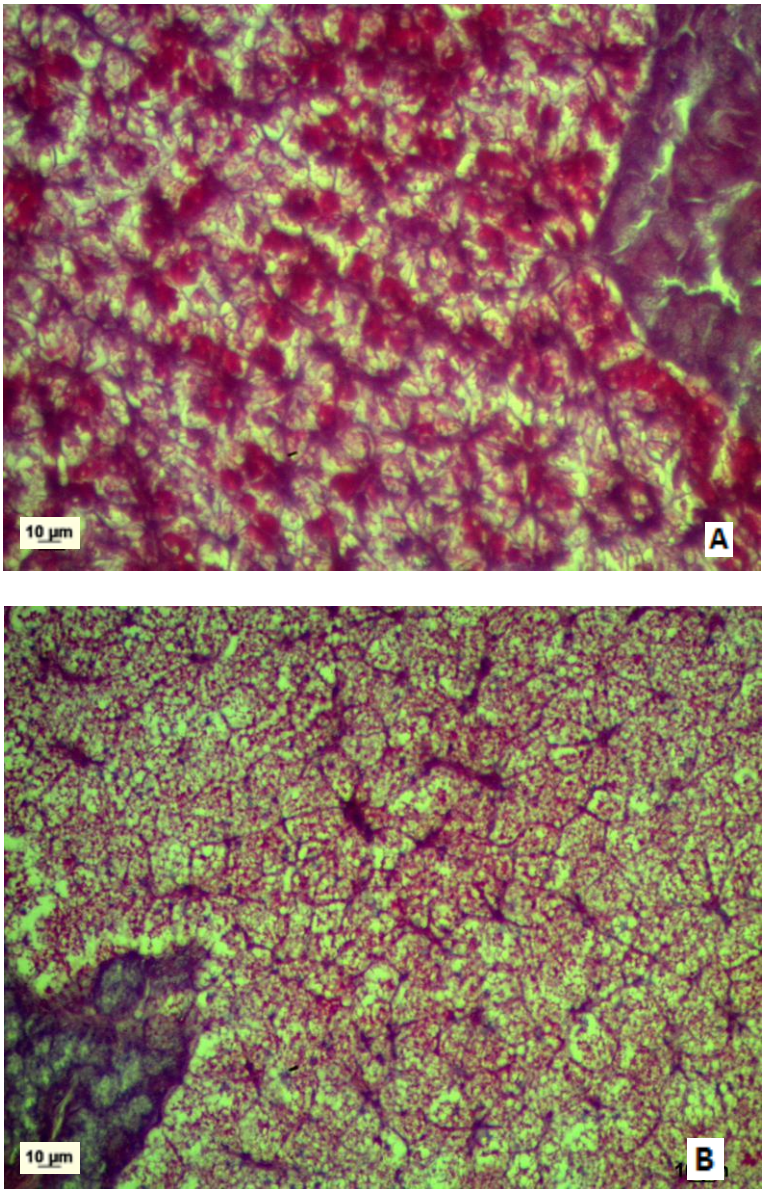


Figure 8. Images of the hepatic glycogen storage (PAS technique) at weeks 0 (A) and 2 (B) (40×) of starved *Paralichthys orbignyanus*.

Histology

Regarding to morphological parameters, the MMs number per kidney area and the MMs mean area were not affected by starvation, neither by feeding along the experimental period (Table 5). There were not detected variations along the time in both treatments with

respect to the enterocyte height and villi`s length, but in the later the F was always higher than the S, been significantly higher only at 2 weeks (Table 4).

Table 5. Mean (±SE) measures of MMs kidney deposition, MMs mean area, microvilli`s height, enterocyte height and villi`s length of *Paralichthys orbignyanus* fed (F) or starved (S) for up to 8 weeks.

		Time (weeks)					p		
	Treatment	0	1	2	4	8	Treatment	Time	Treatment*Time
MMs kidney deposition (MMs/µm²) (*10000)	F	1.76±0.20	1.64±0.14	1.72±0.11	1.9±0.21	1.63±0.17	0.039*	0.990	0.519
	S	1.87±0.19	1.99±0.11	2.03±0.09	1.80±0.14	1.97±0.12			
MMs mean area (µm²)	F	4650.58±367.13	5404.66±570.86	5034.55±650.46	5264.73±662.63	6472.69±1665.37	0.177	0.168	0.970
	S	4178.92±352.03	4842.26±539.21	4894.15±472.88	4979.12±412.03	5497.84±407.82			
Enterocyte height (µm)	F	46.06±1.31	46.01±1.74	44.5±1.6	43.81±1.71	43.27±1.55	0.551	0.109	0.150
	S	44.24±0.24	43.37±0.66	48.15±0.95	41.71±1.81	43.65±1.30			
Villi`s lenght (µm)	F	1207.50±70.01	1182.87±71.29	1268.35±125.03	1387.61±96.07	1139.91±71.29	0.021*	0.280	0.423
	S	1230.10±90.28	1109.45±98.77	990.09±97.98	1172.52±97.10	1014.04±74.41			

Asterisk represents significant differences (P<0.05) between treatments at the same experimental time, different letters indicate significant differences over time in the same treatment (capital letter: Feeding (F); lower case: Starvation (S)). Circle indicates significant differences determined by orthogonal planned comparisons.

DISCUSSION

Fish submitted to food restriction is a common feature in nature, more than in aquaculture. Even though, fish farmers can withheld food in order to reduce handling stress and negative effects because of diseases or reduced water quality (Davis & Gaylord, 2011). Fish can also be fasted to obtain a compensatory growth after returning to a normal feeding regime (Cho, 2005). Weight loss is a common answer to starvation, because of the use of energetic storages, influencing biometric indices that generally decrease over the starvation period (McCue, 2010). Three indexes (K, HSI and VSI) decreased significantly along the time for *P. orbignyanus*. Similar behavior was observed for the flounder *Pleuronectes platessa*, the seabass *D. labrax* and hybrid striped bass *Morone chrysops* x *Morone saxatilis* (Moon & Johnston, 1980; Alliot et al., 1984; Davis & Gaylord, 2011). According to Hung et al. (1997), the decreases in HSI and in VSI suggest that liver and viscera are the main sites of nutrient mobilization to cope with food restriction. In *P. orbignyanus*, these variations could be explained by the decrease in energy stores, principally from the liver.

As in *P. orbignyanus*, a rise in plasma cholesterol and maintenance in glucose level was observed in *D. labrax* submitted to 3 months of food restriction (Chatzifotis et al., 2011). In accord with Chatzifotis et al. (2011), the first could be explained because cholesterol is a precursor of steroid hormones like cortisol, which in stress situations acts stimulating the gluconeogenic process (Wendelaar Bonga, 1997). The second is maintained by gluconeogenesis, muscular proteolysis (setting free amino acids for hepatic gluconeogenesis) (Smutná et al., 2002), glycogenolisis and triglycerides breakdown. The rise in glycogen in the last week could be due to an accumulation of glucose.

On the other hand, the lack of changes in protein in *P. orbignyanus* indicates that there is an equilibrium between the rates of protein mobilization and consumption, as was observed

in *Anguilla anguilla*, *D. labrax* and *Gadus morhua* (Dave et al., 1974; Black & Love, 1986; Echevarría et al., 1997). This could indicate an elevated liver activity because there occurs the plasmatic protein synthesis (Harper, 1971). Further, the maintained levels of glucose, protein and triglycerides are consistent with osmolality, which was almost constant along the 8 weeks of starvation.

No changes in muscular protein levels were observed in *P. platessa*, *D. labrax* and *Dentex dentex* submitted to 30, 21, and 5 weeks of starvation, respectively (Patterson et al., 1971; Echevarría et al., 1997; Pérez-Jiménez et al., 2012). According to Pérez-Jiménez et al. (2012), these fish have a high capacity to deal with prolonged fasting periods avoiding protein hydrolysis in muscle, as it was also observed for *P. orbignyanus*. The last rise could be explained by the fact that glucose can reduce protein breakdown or stimulate protein synthesis during starvation as was demonstrated in humans; this could happen due to an amino acids internal recycling (Sim et al., 1979).

Starved and fed Atlantic halibut (*Hippoglossus hippoglussus*) showed similar lipid content during 5 weeks (Heide et al., 2006). It is known that wild halibut uses its hepatic energy sources during gonadal development and not that of the carcass (Haug & Gulliksen, 1988), so these could explain the behavior observed during starvation. This could also explain why lipid content in muscle did not change, implying that this energy reserve is not use during the fasting period in *P. orbignyanus*. The same could be applied to the fatty acids profile (individuals, sums, ratios and total), because it showed the same behavior of total lipids and remained unchanged during the fasting period, and as such, it was an evidence of no selective consumption of individual fatty acids. Besides, fatty acids had the same behavior in *Morone saxatilis* larvae submitted to different levels of starvation (Martin et al., 1984). In starved tilapia (*Oreochromis mossambicus x Oreochromis niloticus*), De Silva et al. (1997)

found that the main fatty acids were the same observed in *P. orbignyanus*: 16:0, 18:1n9, 18:2n6 e 22:6n3.

Generally, when fish begin to use muscular proteins and/or lipids, they accumulate water (Moon & Johnston, 1980; Alliot et al., 1984; Echevarría et al., 1997; Hung et al., 1997). In opposition to this, muscle moisture decreased in both *P. orbignyanus* fed and starved; this behavior could be due probably to the lipids rise, because their amphipathic behavior. The mentioned changes in lipid and fatty acid levels in both treatments could be due because most of them belong to triglycerides, so this would be hidden the behavior of polar lipids; the study of this lipid class will tell us if *P. orbignyanus* is using lipids (another than triglycerides) and if it has some preference in the utilization of fatty acids during food deprivation.

D. labrax (Chatzifotis et al., 2011), *Rhamdia quelen* (Barcellos et al., 2010) and *P. orbignyanus* in this case, did not have glycogen changes in muscle, indicating that it is not used as an energy source during the experimental period at least. According to Navarro & Gutiérrez (1995), muscular glycogen is involved in muscular activity mainly, so it would not be use against a stressful situation, or that it could also be maintained from glucose produced by the liver.

Muscular triglycerides are generally used as an energy source (Dave et al., 1975; Alliot et al., 1984). However, unchanged levels of muscular triglycerides for *P. orbignyanus* indicates that this lipid class does not act as an energy source during these 8 weeks of starvation.

The decrease of hepatic cholesterol in *P. orbignyanus* indicates a mobilization and transport by the blood stream for cortisol synthesis (Chatzifotis et al., 2011). The rapid decrease in glucose could be due to its fast synthesis and transport for blood stream to maintain stable levels of plasmatic glucose.

P. orbignyanus presented a drastic drop in hepatic glycogen content; the same was registered for *D. labrax* and *M. chrysops X M. saxatilis* after 3 and 1 week of food restriction (Alliot et al., 1984; Davis & Gaylord, 2011). According to Navarro & Gutiérrez (1995), glycogen is the first substrate used in starvation.

As in *P. orbignyanus*, the initial decrease in protein was shown in *Acipenser naccarii* and *Oncorhynchus mykiis* after 5 days of starvation and then remained stable (Furné et al., 2012). This could be due by the hydrolysis of hepatic proteins, and the resulting amino acids were used for the glucose synthesis in that organ.

According to Navarro & Gutiérrez (1995), triglycerides are the more accessible reserves within the lipids. The reduction of hepatic triglycerides at the beginning of the starvation period was also observed in starved *D. labrax* and *A. naccarii* after 1 week and 2 days (Alliot et al., 1984; Furné et al., 2010). According to Davis & Gaylord (2011), lipid stores are mobilized along with glycogen when an individual is fasted, as happened with *P. orbignyanus*. The subsequent increase was also recorded in *S. auratus* and *D. dentex* (Polakof et al., 2006; Pérez-Jiménez et al., 2012). That could happen because a fatty acid re-esterification resulting from adipose tissue hydrolysis that would lead to the formation of lipoproteins of low density that would be accumulated in the liver (Deng et al., 2004). Another explanation could be an interorgan lipid transport (Webster et al., 1994)

The observed changes in the first two weeks of starvation could suggest that the flounders are passing by a metabolic depression to maintain energy, but to determine this it should have been determined at least the oxygen consumption. The decrease in respiratory rate and/or in oxygen consumption (indicators of the metabolic state) showed that *P. olivaceus* (Park et al., 2012) and *Cynoglossus semilaevis* (Tian et al., 2010) presented a metabolic decrease along 12 and 5 weeks of starvation, respectively.

In respect to blood parameters, the hematocrit fell in *P. orbignyanus* and *Hoplias malabaricus* (26.5 to 22.69%) (Rios et al., 2005), indicating that starvation can cause erythrocyte changes, as a decreased in their number or volume (Rios et al., 2005), or diminish the erythropoiesis capacity of the organisms (Love, 1970).

The MMs deposition grade in the kidney (MMs/μm^2 of kidney) is a tool generally used to explain the nutritional status in fishes (Mizuno et al., 2002; Hur et al., 2006). In *Oncorhynchus masou* kidney and *H. malabaricus* liver, this parameter showed a rise after 8 and 6 weeks of starvation, respectively (Mizuno et al., 2002; Rios et al., 2007). Aversely, these parameters were not affected in *P. orbignyanus*; this could indicate that 8 weeks are not enough time to affect the MMs characteristics and therefore, to produce an immune response.

According to Ostaszewska et al. (2006), a long-term starvation can cause changes in structures of the digestive tract. Decrease of enterocyte height and villi`s length were observed for *Salmo trutta caspius* fasted for 3 and 6 weeks (Shaibani et al., 2013). Rapid enterocyte degeneration was also observed for *Tinca tinca* (Ostaszewska et al., 2006). For Hall & Bellwood (1995), that variation could be due to the formation of little cells because the lack of food or by the utilization of intestinal lipids stocked in vacuoles, which are situated in the enterocyte apical zone (Gisbert & Doroshov, 2003). All this could reduce the epithelial area, affecting the nutrient absorption efficiency (Shaibani et al., 2013). On the other hand, the intestinal structures studied for fasted Brazilian flounder were not influenced by the length of the fasting period, thus indicating these structures are not affected by food restriction, within the period of exposition in this work.

CONCLUSIONS

Eight weeks of food deprivation affected hepatic stores and hematocrit, thus indicating that *Paralichthys orbignyanus* could maintain its body energy requirements using hepatic sources mainly, but with erythropoiesis process being affected (decreasing the number or volume of the red blood cells). However, this period was not enough to affect muscular energy stores, plasma osmolality, the size and degree of deposition of MMs in the kidney and intestine morphology. At the beginning, this could suggest that juvenile *P. orbignyanus* can survive to eight weeks of starvation without changes.

REFERENCES

AGIUS, C & RJ ROBERTS. 1981. Effects of starvation on the melano-macrophage centers of fish. J. Fish Biol., 19: 161-169.

ALLIOT, E, M DJABALI, A PASTOUREAUD & H THEBAULT. 1984. Changes in the biochemical composition of tissues in juvenile sea bass during forced starvation. Biochem. Syst. Ecol., 12(2): 209-213.

AMADO, LL, RB ROBALDO, L GERACITANO, JM MONSERRAT & A BIANCHINI. 2006. Biomarkers of exposure and effect in the Brazilian flounder *Paralichthys orbignyanus* (Teleostei: Paralichthyidae) from the Patos Lagoon estuary (Southern Brazil). Mar. Poll. Bull., 52: 207-213.

AMANO, H, T FUJIYOSHI & H NODA. 1988. Changes in body components of whitefish *Coregonus muksun* after transplantation in reservoir. Nippon Suisan Gakkaishi. 54(3): 529-536.

BAMBILL, GA, O MASAKAZU, M RADONIC, AV LÓPEZ, MI MÜLLER, JJ BOCCANFUSO & FA BIANCA. 2006. Broodstock management and induced spawning

of flounder *Paralichthys orbignyanus* (Valencienes, 1839) under a closed recirculated system. Rev. Biol. Mar. Oceanog. 41(1): 45-55.

BARCELLOS, LJG, A MARQUEZE, M TRAPP, RM QUEVEDO & D FERREIRA. 2010. The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá *Rhamdia quelen*. Aquaculture, 300: 231-236.

BELLAMY, D, RA LEONARD & K DULIEN. 1968. Hepatic gluconeogenesis in rats treated with cortisol. Gen. Compar. Endocr., 10: 434-37.

BLACK, D & RM LOVE. 1986. The sequential mobilization and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. J. Comp. Physiol. B, 156: 469-479.

BLASCO, J, J FERNÁNDEZ & J GUTIÉRREZ. 1992. Fasting and refeeding in carp, *Cyprinus carpio* L.: the mobilization of reserves and plasma metabolite and hormone variations. J. Comp. Physiol. B., 162: 539-546.

BORAH, S & RNS YADAV. 1996. Biochemical and hematological response to starvation in an air breathing freshwater teleost, *Heteropneustes fossilis* (Bloch). Indian J. Fish, 43: 307-310.

CARR, RS & JM NEFF. 1984. Quantitative semiautomated enzymatic assay for the tissue glycogen. Comp. Biochem. Physiol. B, 77: 447-449.

CARUSO, G, G MARICCHIOLO, V MICALE, L GENOVESE, R CARUSO & MG DENARO. 2010. Physiological responses to starvation in the European eel (*Anguilla anguilla*): effects on hematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol. Biochem., 36: 71-83.

CARUSO, G, AG DENARO, R CARUSO, F MANCARI, L GENOVESE & G MARICCHIOLO. 2011. Response to short term starvation of growth, hematological,

biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*). Mar. Environ. Res., 72: 46-52.

CHAO, LN, LE PEREIRA, J PAEZ VIEIRA, M BENVENUTI & LPR CUNHA.1985. Relação preliminar dos peixes estuarinos e marinos da Lagoa dos Patos e região costeira adjacente. Rio Grande do Sul, Brasil. Atlântica, 5: 67-75.

CHATZIFOTIS, S, M PAPADAKI, S DESPOTI, C ROUFIDOU & E ANTONOPOULOU. 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.

CHO, SH. 2005. Compensatory growth of juvenile flounder *Paralichthys olivaceus* L. and changes in biochemical composition and body condition indices during starvation and after refeeding in winter season. J. World Aquacult. Soc., 36(4): 508-514.

DAVE, G, ML JOHANSSON-SJOBECK, K LARSSON, K LEWANDER & U LIDMAN. 1975. Metabolic and hematological effects starvation in the european ell, *Anguilla anguilla* L. I. Carbohydrate, lipid, protein and inorganic ion metabolism. Comp. Biochem. Physiol. A, 62(3): 423-430.

DAVIS, KB & TG GAYLORD. 2011. Effect of fasting on body composition and responses to stress in sunshine bass. Comp. Biochem. Physiol. A, 158: 30-36.

DENG, L, WM ZHANG, HR LIN & CHK CHENG. 2004. Effects of food deprivation on expression of growth hormone receptor and proximate composition in liver of black seabream *Acanthopagrus schlegeli*. Comp. Biochem. Physiol. B, 137: 421-432.

DE SILVA, SS, RM GUNASEKERA & CM AUSTIN. 1997. Changes in the fatty acid profiles of hybrid red tilapia, *Oreochromis mossambicus x O. niloticus*, subjected to

short-term starvation, and a comparison with changes in seawater raised fish. Aquaculture, 153: 273-290.

DÍAZ DE ASTARLOA, JM & TA MUNROE. 1998. Systematics, distribution and ecology of commercially important paralichthyid flounders occurring in Argentinean-Uruguayan waters (Paralichthys, Paralichthyidae): an overview. J. Sea Res., 39: 1-9.

ECHEVARRÍA, G, M MARTÍNEZ-BEBIÁ & S ZAMORA. 1997. Evolution of biometric indices and plasma metabolites during prolonged starvation in European sea bass (*Dicentrarchus labrax*, L.). Comp. Biochem, Physiol. A, 118(1): 111-123.

EINEN, O, B WAAGAN & MS THOMASSEN. 1998. Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). I. Effects on weight loss, body shape, slaughter- and fillet-yield, proximate and fatty acid composition. Aquaculture, 166: 85-104.

FOLCH, J, M LEES & GHS STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 266: 497-509.

FURNÉ, M, AE MORALES, CE TRENZADO, M GARCÍA-GALLEGO, MC HIDALGO, A DOMEZIAN & AS RUS. 2012. The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. J. Comp. Physiol. B, 182: 63-76.

GISBERT, E & SI DOROSHOV. 2003. Histology of the developing system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*). Aquat. Living Resour., 16(2): 77-89.

GUPPY, M, CJ FUERY & JE FLANIGAN. 1994. Biochemical principles of metabolic depression. Comp. Biochem. Physiol. B, 109(2/3): 175-189.

HALL, KC & DR BELLWOOD. 1995. Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. J. Fish Biol., 47: 438-454.

HARPER, HA. 1971. Review of Physiological Chemistry. Lange Medical Publications, Los Altos, California.

HAUG, T & B GULLISKEN. 1988. Variation in liver- and body condition during gonadal development of Atlantic halibut, *Hippoglossus hippoglossus*. Fiskeridir. Skr., 18: 351-363.

HEIDE, A, A FOSS, AO STEFANSSON, J MAYER, B NORBERG, B ROTH, MD JENSSEN, R NORTVEDT & AK IMSLAND. 2006. Compensatory growth and fillet crude composition in juvenile Atlantic halibut: Effects of short term starvation periods and subsequent feeding. Aquaculture, 261: 109-117.

HUNG, SSO, W LIU, H LI, T STOREBAKKEN & Y CUI. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. Aquaculture, 151: 357-363

HUR, JW, JH JO & PARK IS. 2006. Effects of long-term starvation on hepatocyte ultrastructure of olive flounder *Paralichthys olivaceus*. Ichthyol. Res., 53: 306-310.

JOHNSTON, IA & G GOLDSPINK. 1973. Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice *Pleuronectes platessa*. Marine Biol., 19: 348-353.

KIEFFER, JD & BL TUFTS. 1998. Effects of food deprivation on white muscle energy reserves in rainbow trout (*Oncorhynchus mykiss*): the relationship with body size and temperature. Fish Physiol. Biochem., 19: 239-245.

LANES, CFC, M OKAMOTO, PV CAVALCANTI, T COLLARES, VF CAMPOS, JC DESCHAMPS, RB ROBALDO & LF MARINS. 2008 Cryopreservation of Brazilian flounder (*Paralichthys orbignyanus*) sperm. Aquaculture, 275: 361-365.

LOVE, RM. 1970. The chemical biology of fishes. London: Academic Press. 222-257.

McCUE, MD. 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A, 156: 1-18.

MARTIN, FD, DA WRIGHT & JC MEANS. 1984. Fatty acids and starvation in larval striped bass (*Morone saxatilis*). Comp. Biochem. Physiol. B, 77(4): 785-790.

MICHAELE, V & F PERDICHIZI, 1990. A quantitative and histochemical study of melano-macrophage centers in the spleen of the teleost fish *Diplodus annularis* L. J. Fish Biol., 37: 191-197.

MIZUNO, S, N MISAKA, Y MIYAKOSHI, K TAKEUCHI & N KASAHARA. 2002. Effects of starvation on melano-macrophages in the kidney of masu salmon (*Oncorhynchus masou*). Aquaculture, 209: 247-255.

MOON, TW & IA JOHNSTON. 1980. Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa.* J. Comp. Physiol., 136: 31-38.

NAVARRO, I & J GUTIÉRREZ. 1995. Fasting and starvation. Biochem. Mol. Biol. Fishes, 4: 393-434.

NERY, LEM & EA SANTOS. 1993. Carbohydrate metabolism during osmoregulation in *Chasmagnathus granulata* Dana, 1851 (Crustacea, Decapoda). Comp. Biochem. Physiol. B, 106: 747-753.

OSTASZEWSKA, T, M KORWIN-KOSSAKOWSKI & J WOLNICKI. 2006. Morphological changes of digestive structures in starved tench *Tinca tinca* (L.) juveniles. Aquacult. Int., 14: 113-126.

PARK, LS, HUR JW & W CHOI. 2012. Hematological responses, survival, and respiratory exchange in the olive flounder, *Paralichthys olivaceus*, during starvation. Asian-Aust. J. Anim. Sci. 25(9): 1276-1284.

PATTERSON, S, IA JOHNSTON & G GOLDSPINK. 1974. The effect of starvation on the chemical composition of red and white muscles in the plaice (*Pleuronectes platessa*). Experientia, 30(8):892-894.

PENG, M, W XU, Q AI, K MAI, Z LIUFU & K ZHANG. 2013. Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). Aquaculture 392-395: 51-58.

PERES, H, S SANTOS & A OLIVA-TELES. 2013. Selected plasma biochemistry parameters in gilthead sea bream (*Sparus aurata*) juveniles. J. Appl. Ichthyol., 29: 630-636.

PÉREZ-JIMÉNEZ, A, G CARDENETE, MC HIDALGO, A GARCÍA-ALCÁZAR, E ABELLÁN & AE MORALES. 2012. Metabolic adjustments of *Dentex dentex* to prolonged starvation and refeeding. Fish. Physiol. Biochem., 38: 1145-1157.

POLAKOF, S, FJ ARJONA, S SANGAIO-ALVALLEROS, MPM DEL RIO, JM MANCERA & JL SOENGAS. 2006. Food deprivation alters osmorregulatory and metabolic responses to salinity acclimation in gilthead sea bream *Sparus auratus*. J. Comp. Physiol. B, 176: 441-452.

RADONIC, M & GJ MACCHI. 2009. Gonadal sex differentiation in cultured juvenile flounder, *Paralichthys orbignyanus* (Valenciennes, 1839). J. World Aqualcult. Soc., 40(1): 129-133.

RIOS, FS, AL KALININ & FT RANTIN. 2002. The effects of long-term food deprivation on respiration and haematology of the neotropical fish *Hoplias malabaricus*. J. Fish Biol., 61: 85-95.

RIOS, FS, ET OBA, MN FERNANDES, AL KALININ & FT RANTIN. 2005. Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish

traira, *Hoplias malabaricus* (Characiformes, Erythrinidae). Comp. Bioch. Physiol. A. 140: 281-287.

RIOS, FS, L DONATTI, MN FERNANDES, AL KALININ & FT RANTIN. 2007. Liver histopathology and accumulation of melano-macrophage centers in *Hoplias malabaricus* after long-term food deprivation and re-feeding. J. Fish Biol., 71: 1393-1406.

ROBALDO, RB, RV RODRIGUES, MH OKAMOTO & LA SAMPAIO. 2012. Processing yield of wild-caught and indoor-reared Brazilian flounder *Paralichthys orbignyanus*. J. Appl. Ichthyol., 28: 815-817.

RODRIGUES, RV, LS FREITAS, RB ROBALDO & LA SAMPAIO. 2012. Crescimento e sobrevivência de juvenis do linguado *Paralichthys orbignyanus*: efeitos do enriquecimento da *Artemia* sp. com n-3 HUFA. Atlântica, 34(2): 121-127.

SAMPAIO, LA & A BIANCHINI. 2002. Salinity effects on osmoregulation and growth of the eurihaline flounder *Paralichthys orbignyanus*. J. Exp. Mar. Biol. Ecol., 269: 187-196.

SAMPAIO, LA, LS FREITAS, M OKAMOTO, LR LUOZADA, RV RODRIGUES & RB ROBALDO. 2007. Effects of salinity on Brazilian flounder *Paralichthys orbignyanus* from fertilization to juvenile settlement. Aquaculture, 262: 340-346.

SAMPAIO, LA, RB ROBALDO & A BIANCHINI. 2008. Hormone-induced ovulation, natural spawning and larviculture of Brazilian flounder *Paralichthys orbignyanus* (Valenciennes, 1839). Aquacult. Res., 39: 712-717.

SEGNER, H, P BERKHARDT, EM AVILA, JV JUARIO & V STORCH. 1987. Nutrition-related histopathology of the intestine of milkfish *Chanos chanos* fry. Dis. Aquat. Org., 2: 99-107.

SEGNER, H & H MÖLLER. 1984. Electron microscopical investigations on starvation-induced liver pathology in flounders *Platichthys flesus*. Mar. Ecol. Prog. Ser., 19: 193-196.

SHAIBANI, ME, BM AMIRI & S KHODABANDEH. 2013. Starvation and refeeding effects on pyloric caeca structure of Caspian salmon (*Salmo trutta caspius*, Kessler 1877) juvenile. Tissue & Cell, 45: 204-210.

SHERIDAN, MA & TP MOMMSEN. 1991. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 81: 473–483

SIM, AJW, VR YOUNG, BM WOLF, D CLARKE & FD MOORE. 1979. Glucose promotes whole-body protein synthesis from infused aminoacids in fasting man. The Lancet, January 13, 68-71.

SMUTNÁ, M, L VORLOVA & Z SVOBODOVÁ. 2002. Phatobiochemistry of ammonia in the internal environment of fish (Review). Acta Vet. Brno., 71: 169-181.

STRICKLAND, JDH & TR PARSONS. 1972. A practical handbook of seawater analysis, Ottawa: Fish. Res. bd. Canada, 310.

TIAN, X, J FANG & S DONG. 2010. Effects of starvation and recovery on the growth, metabolism and energy budget of juvenile tongue sole (*Cynoglossus semilaevis*). Aquaculture, 310: 122-129.

TIDWELL, JH, CD WEBSTER & JA CLARK. 1992. Effects of feeding, starvation, and refeeding on the fatty acid composition of channel catfish, *Ictalurus punctuatus*, tissues. Comp. Biochem. Physiol. A, 103(2): 365-368.

TRIPATHI, G & P VERMA. 2003. Starvation-induced impairment of metabolism in a freshwater catfish. Verlag der Zeitschrift für Naturforschung, Tübingen, pp. 446-451.

UNESCO. 1983. Chemical methods for use in marine environmental monitoring. Manual and Guides 12, IOC. Paris, France.

VIGLIANO, FA, MI QUIROGA & JM NIETO. 2002. Metabolic adaptation to food deprivation and refeeding in fish. Ver. Ictiol., 10(1/2): 79-108.

WASIELESKY JR., W, A BIANCHINI, M SANTOS & L POERSCH. 1997. Tolerance of juvenile flatfish *Paralichthys orbignyanus* to acid stress. J. World Aquacult. Soc., 28: 202-204.

WASIELESKY JR., W, A BIANCHINI & K MIRANDA.1998. Tolerancia a la temperatura de juveniles de lenguado *Paralichthys orbignyanus*. Frente Marítimo, 17: 43-48.

WEATHERLEY, AH & HS GILL. 1987. The biology of fish growth. Protein, lipid and caloric contents. Academic Press, London, 4: 139-146.

WEBSTER, CD, JH TIDWELL, LS GOODGAME & DH YANCEY. 1994. Effects of fasting on fatty acid composition of muscle, liver and abdominal fat in channel catfish *Ictalurus punctatus*. J. World. Aquacult. Soc., 25(1): 126-134.

WEIBEL, ER, & DM GOMEZ. 1962. A principle for counting tissue structures on random section. J. Appl. Physiol., 17: 343-348.

BONGA, SW. 1997. The stress response in fish. Physiological reviews, *77*(3), 591-625.

XIAOBO, W, K YAOMEI & Z KAIYA. 2007. Starvation on changes in growth and fatty acid composition of juvenile res swamp crawfish, *Procambarus clarkii*. Chin. J. Oceanol. Limnol., 25(1): 97-105.

ZAMMIT, VA & EA NEWSHOLME. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobrach fish. Biochem. J., 194: 313-322.

7. CONCLUSÃO GERAL

- Juvenis de *P. orbignyanus* conseguem lidar com oito semanas de restrição alimentar utilizando apenas as reservas energéticas estocadas no fígado (colesterol, glicogênio, proteínas e triglicerídeos), sendo o processo de eritropoiese afetado (diminuição do hematócrito). Porém, esse período não foi suficiente produzir modificações:
    a- nos níveis de reservas energéticas no tecido muscular;
    b- nos componentes plasmáticos (glicose, proteínas e triglicerídeos), o que corrobora com a manutenção da osmolalidade;
    c- no tamanho e grau de deposição dos melano-macrófagos do rim;
    d- na morfologia do intestino.

Por tanto, juvenis de *Paralichthys orbignyanus* sobrevivem a oito semanas de jejum basicamente utilizando as reservas do fígado, sem apresentar modificações estruturais.

8. PERSPECTIVAS FUTURAS

- Para complementar o presente estudo poderiam ter sido registrados os níveis de cortisol e a atividade enzimática com a finalidade de determinar o nível de estresse produzido e as vias metabólicas utilizadas durante essas oito semanas de restrição alimentar.
- Estudos com períodos de restrição alimentar mais prolongados poderiam ser realizados para determinar em qual momento os juvenis de *Paralichthys orbignyanus* começam a utilizar as reservas musculares e a sofrer mudanças estruturais, e outros de realimentação com a finalidade de saber quanto tempo estes peixes podem permanecer sem alimento e posteriormente recuperar tanto os níveis de reservas utilizadas quanto as mudanças estruturais sofridas (a partir da realimentação).