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COMPOSIÇÃO DO CONTEÚDO GASTRINTESTINAL E ENZIMAS DIGESTIVAS EM TELEÓSTEOS COM DIFERENTES HÁBITOS ALIMENTARES

DISSERTAÇÃO DE MESTRADO

Ana Paula Gottlieb Almeida

Santa Maria, RS, Brasil

2014

COMPOSIÇÃO DO CONTEÚDO GASTRINTESTINAL E ENZIMAS DIGESTIVAS EM TELEÓSTEOS COM DIFERENTES HÁBITOS ALIMENTARES

Ana Paula Gottlieb Almeida

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Biodiversidade Animal, Área de Concentração em Bioecologia e Conservação de Peixes, da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para obtenção do grau de **Mestre em Ciências Biológicas – Área de Biodiversidade Animal**

Orientador: Prof. Dr. Bernardo Baldisserotto
Co-orientador: Prof. Dr. Everton Behr

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elaborada por
Ana Paula Gottlieb Almeida

como requisito parcial para obtenção do grau de
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Pela minha vida e pela segunda chance de viver.

Aos meus avós,
Por tudo que fizeram por mim e pela confiança.

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Veja
Não diga que a canção está perdida
Tenha fé em Deus, tenha fé na vida
Tente outra vez

Beba
Pois a água viva ainda está na fonte
Você tem dois pés para cruzar a ponte
Nada acabou

Tente
Levante sua mão sedenta e recomece a andar
Não pense que a cabeça aguenta se você parar,
Há uma voz que canta,
Uma voz que dança,
Uma voz que gira
Bailando no ar

Queira
Basta ser sincero e desejar profundo
Você será capaz de sacudir o mundo, vai
Tente outra vez

Tente
E não diga que a vitória está perdida
Se é de batalhas que se vive a vida
Tente outra vez

Tente outra vez – Raul Seixas

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Biodiversidade Animal
Universidade Federal de Santa Maria

COMPOSIÇÃO CENTESIMAL DO CONTEÚDO DO TRATO GASTRINTESTINAL E ATIVIDADE ENZIMÁTICA DIGESTIVA DE TELEÓSTEOS

AUTORA: ANA PAULA GOTTLIEB ALMEIDA
ORIENTADOR: BERNARDO BALDISSEROTTO
Data e Local da Defesa: Santa Maria, 18 de julho de 2014.

O objetivo desse estudo foi avaliar a relação entre a atividade enzimática digestiva e a composição centesimal do conteúdo do trato gastrintestinal e hábitos alimentares de quatro teleósteos de dois hábitos alimentares no verão e no inverno. Foram escolhidas duas espécies onívoras *Rhamdia quelen* e *Pimelodus maculatus* e duas espécies detritívoras *Hypostomus commersoni* e *Loricariichthys anus*. Os peixes foram coletados com o auxílio de rede de arrasto nos meses de março e julho de 2013, no canal São Gonçalo, Pelotas – RS. O trato digestório foi dividido em estômago, intestino anterior e posterior. No estômago foi ensaiada a atividade da pepsina e nas duas porções do intestino foram ensaiadas as atividades da tripsina, quimotripsina e lipase. Foi determinado o teor proteico e lipídico do conteúdo de cada porção do trato digestório. A atividade enzimática digestiva não está relacionada com o hábito alimentar e a composição centesimal do conteúdo do trato gastrintestinal. Espécies detritívoras apresentaram maior atividade das proteases alcalinas, o que pode ser uma adaptação para utilizar melhor o baixo teor proteico encontrado no conteúdo gastrintestinal dessas espécies.

Palavras-chave: Pepsina. Quimotripsina. Tripsina. Lipase. Proteína. Lipídios.

ABSTRACT

Master Dissertation
Post-Graduate Course in Animal Biodiversity
Federal University of Santa Maria

CENTESIMAL COMPOSITION OF GASTROINTESTINAL CONTENT AND DIGESTIVE ENZIMATIC ACTIVITY OF TELEOSTS

AUTHOR: ANA PAULA GOTTLIEB ALMEIDA
ADVISOR: BERNARDO BALDISSEROTTO
Date and place of the defense: Santa Maria, July 18th, 2014.

The aim of this study was to evaluate the relationship between the digestive enzyme activity and the centesimal composition of the contents of the gastrointestinal tract and food habits of four teleost two food habits in summer and winter. Two omnivorous species *Pimelodus maculatus* and *R. quelen* and two detritivorous species *Hypostomus commersoni* and *Loricariichthys anus* were chosen. Fish were collected with the aid of the trawl during March and July 2013 in São Gonçalo channel, Pelotas - RS. The digestive tract was divided into stomach, anterior and posterior intestine. Stomach was assayed the activity of pepsin and the two portions of the intestine were assayed the activities of trypsin, chymotrypsin and lipase. The protein and lipid content of the contents of each portion of the digestive tract was determined. Digestive enzyme activity is not related to the feeding habits and the centesimal composition of the contents of the gastrointestinal tract. Detritivorous species showed higher activity of alkaline proteases, which may be an adaptation to better utilize the low protein content found in the gastrointestinal tract of these species.

Keywords: Pepsin. Chymotrypsin. Trypsin. Lipase. Protein. Lipids.

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1 INTRODUÇÃO

Conhecer a dieta e os hábitos alimentares dos peixes é essencial para o entendimento da biologia desses animais assim como para a formulação de dietas para espécies cultivadas. Os peixes podem ser classificados como onívoros, detritívoros/iliófagos, herbívoros ou carnívoros/piscívoros (BALDISSEROTTO, 2013). Dentro de cada categoria, os peixes podem ser classificados em: generalistas, apresentam um amplo espectro alimentar; especialistas, apresentam uma dieta restrita a uma pequena variedade de alimentos e geralmente possuem adaptações morfológicas específicas; e oportunistas, que utilizam uma fonte abundante e incomum de alimento (ABELHA, AGOSTINHO & GOULART, 2011).

O hábito alimentar dos peixes pode variar durante o ciclo de vida, podendo o animal alimentar-se de zooplâncton durante a fase juvenil e apresentar hábito alimentar herbívoro durante a fase adulta. Isso também pode ser observado em peixes carnívoros, que em estágios iniciais do desenvolvimento ontogenético alimentam-se de zooplâncton, mudando sucessivamente para pupas e larvas de insetos na fase juvenil e finalmente para a ictiofagia durante a fase adulta. Além disso, peixes de mesmo hábito alimentar, mas pertencentes a famílias distintas, podem apresentar diferenças na organização do trato gastrintestinal, revelando a importância da filogenia.

A rápida adaptação digestiva às variações na qualidade e disponibilidade de nutrientes no ambiente é possível devido ao trato gastrintestinal ser funcionalmente dinâmico. Espécies que apresentam ampla adaptabilidade trófica são capazes de utilizar todos os recursos alimentares disponíveis no ambiente e que sejam adequados ao seu trato gastrintestinal e capacidade digestiva.

1.1 Morfologia do trato gastrintestinal

O trato gastrintestinal dos peixes consiste em um tubo com parede formada por quatro camadas distintas: mucosa, submucosa, muscular e serosa (GONÇALVES *et al*., 2013). Pode-se dividir o trato digestório dos peixes, modo geral, em: cavidade bucofaringeana, esôfago, estômago, intestino anterior e intestino posterior. Em espécie que apresentam o intestino longo, o mesmo pode ser dividido em três porções: anterior, média e posterior.

A cavidade bucofaringeana compreende a boca e a faringe. Nessa região estão presentes as estruturas responsáveis pela apreensão, busca, seleção e quebra do alimento. A

boca é o órgão por onde o alimento entra no sistema digestório, seu formato e posição pode dar uma ideia da condição diversificada dos níveis tróficos. Peixes com abertura bucal grande são, geralmente, carnívoros como, por exemplo, *Hoplias malabaricus*, *H. lacerdae* e *Salminus brasiliensis* que apresentam boca terminal, fenda bucal ampla e lábios delgados aderidos às respectivas maxilas (MENIN & MIMURA, 1991; RODRIGUES & MENIN, 2006a; MACIEL *et al.*, 2009). O onívoro *Pseuplatystoma corruscans* também apresenta a boca e a cavidade bucal com as mesmas características, o que reflete uma preferência à ictiofagia (RODRIGUES & MENIN, 2006b). Peixes com cavidade bucal pequena e abertura estreita são, frequentemente, fitoplanctófagos, bentófagos e herbívoros, o que auxilia na sucção e/ou raspagem do alimento como, por exemplo, o onívoro *Leporinus macrocephalus*, que apresenta boca terminal com fenda bucal pequena e lábios inferiores espessos e flexíveis (RODRIGUES *et al.*, 2006). Os lábios podem ser diferenciados em um órgão adesivo ou protrátil como nas espécies detritívoras *Rhinelepis aspera*, *Hypostomus regani*, *H. ternetzi*, *H. margaritifer*, *H. microstomus* e *Megalancistrus aculeatus*, que apresentam boca ventral com lábios espessos em forma de ventosa e papilas adesivas, o que possibilita aderir ao substrato (DELARIVA & AGOSTINHO, 2001). A língua dos peixes geralmente não é desenvolvida e pode possuir uma pequena movimentação em espécies carnívoras, auxiliando na apreensão da presa. Os dentes podem estar localizados na mandíbula, na maxila, no vômer, no palato, na língua, na faringe e nos lábios e podem ser cônicos, pontiagudos, esféricos, curvados, com formatos de caninos ou molares (GONÇALVES *et al*., 2013). Peixes carnívoros, geralmente, apresentam dentes mais desenvolvidos que peixes herbívoros devido ao tamanho da presa e a necessidade de prevenir o escape da mesma. Os dentes faringeanos estão envolvidos com a mastigação e quebra do material vegetal e são mais desenvolvidos em peixes herbívoros.

Os rastros branquiais são projeções ósseas ou cartilaginosas localizadas, geralmente, na superfície anterior dos arcos branquiais. No entanto, essas estruturas podem estar localizadas sobre as lamelas branquiais como nos detritívoros *Rhinelepis aspera*, *Hypostomus regani*, *H. ternetzi*, *H. margaritifer*, *H. microstomus* e *Megalancistrus aculeatus* (DELARIVA & AGOSTINHO, 2001) e *H. commersoni* (ALMEIDA *et al.*, 2013). Possuem função de filtrar, proteger os filamentos branquiais e prevenir o escape do alimento. Espécies filtradoras apresentam rastros branquiais longos, finos e próximos, os quais retêm as partículas de alimentos presentes na água que são direcionadas para o esôfago como em *Pimelodus nigribarbis* e *P. valenciennes*. Rastros branquiais pequenos, fortes, espaçados e pontiagudos são mais comuns em espécies carnívoras/piscívoras a atuam prevenindo o escape da presa

durante a deglutição como em *Hoplias malabaricus* e *Serrasalmus maculatus* (ALMEIDA *et al.*, 2013).

A região anterior compreende o esôfago e o estômago. O esôfago consiste em um tubo curto, reto e de paredes espessas por onde o alimento passa (WILSON & CASTRO, 2011). É um órgão altamente distensível em peixes predadores e menos distensível em espécies detritívoras e herbívoras (BALDISSEROTTO, 2013). MENIN & MIMURA (1993) estudaram a anatomia do esôfago de seis espécies com diferentes hábitos alimentares e constataram que o esôfago é curto e tubular em todas as espécies, independente do hábito alimentar. Além disso, os mesmos autores constataram que o esôfago dos onívoros *Pimelodus* sp. e *Brycon lundii* e do ictiófago *Hoplias malabaricus*, que ingerem presas inteiras, apresenta grande distensibilidade enquanto que as espécies iliófagas *Prochilodus marggravi* e *P. afins* e o onívoro *Leporinus reinhardti* apresentam esôfago pouco distensível, pois ingerem partículas menores de alimento. O estômago armazena temporariamente o alimento e desempenha funções químicas e mecânicas na digestão. Apresenta uma anatomia variável, mesmo em espécies com hábitos alimentares semelhantes (BALDISSEROTTO, 2013). O estômago pode ser classificado em três tipos: retilíneo, sifonal e cecal. O estômago retilíneo é raro e, em alguns casos, é atualmente um indicativo da ausência de estômago, como na espécie detritívora *Rhinelepis aspera* (DELARIVA & AGOSTINHO, 2001) (WILSON & CASTRO, 2011). O estômago sifonal é semelhante a um sifão, em forma de U ou J, com lúmen amplo. É tipo de estômago mais comum entre os peixes como, por exemplo, no onívoro *Pimelodus* sp. (MENIN & MIMURA, 1992a) e nos iliófagos *Prochilodus marggravi* e *P. afins* (MENIN & MIMURA, 1993c). O estômago cecal é similar a um saco em forma de Y e característico de espécies que ingerem grandes quantidades de alimento ou que ingerem presas grandes (GONÇALVES *et al.*, 2013). MENIN & MIMURA (1992a) encontraram estômago do tipo cecal em Y em duas espécies onívoras, *Leporinus reinhardti* e *Brycon lundii*. MENIN & MIMURA (1993b) estudaram a anatomia do estômago de três espécies ictiófagas e encontraram diferentes formatos de estômago mesmo em espécies com o mesmo hábito alimentar. *Acestrorhynchus lacustris* e *A. britskii* apresentam estômago cecal em Y, no entanto *Hoplias malabaricus* apresenta estômago com formato semelhante à letra L, o que os autores consideraram como um formato intermediário ao cecal e ao sifonal. Os detritívoros *Hypostomus regani*, *H. ternetzi*, *H. margaritifer* e *H. microstomus* possuem estômago pequeno, com as paredes finas e saculiformes, e *Megalancistrus aculeatus*, também detritívoro, possui estômago mais muscular e maior que as espécies anteriores (DELARIVA & AGOSTINHO, 2001). O estômago dos peixes pode ser dividido em duas regiões: a cárdia,

localizada anteriormente e que apresenta glândulas gástricas tubulares e secretam ácido clorídrico e pepsinogênio, a partir das células oxinticopépticas (WILSON & CASTRO, 2011) e a pilórica, localizada posteriormente e que apresenta uma camada muscular circular mais desenvolvida e tem a função de triturar o alimento em partículas menores, aumentando assim a área de superfície para a ação das enzimas digestivas (GONÇALVES *et al*., 2013).

A região mediana compreende o intestino propriamente dito. Em peixes com estômago ele inicia após o piloro e em peixes agástricos logo após o esôfago e pode formar um bulbo intestinal de armazenamento temporário em algumas espécies (WILSON & CASTRO, 2011). A função primária do intestino é completar o processo de digestão iniciado no estômago e absorver nutrientes, águas e íons (BALDISSEROTTO, 2013). O intestino pode ser divido em duas porções: proximal, muitas vezes subdividida em mais de um segmento, e distal, também denominada de reto. Essas regiões são diferenciadas pela presença da válvula íleo-retal, em algumas espécies, e/ou pela anatomia e coloração (RODRIGUES *et al.*, 2009). Ao contrário dos mamíferos, o intestino dos peixes, no lugar de vilosidades, apresentam pregas longitudinais, circulares ou reticulares (WILSON & CASTRO, 2011). Geralmente intestinos longos refletem a ingestão de alimentos pouco digeríveis, como espécies herbívoras e detritívoras, enquanto que intestinos curtos são encontrados em espécies carnívoras. Intestinos com comprimentos intermediários são encontrados em espécies onívoras (WILSON & CASTRO, 2011). BECKER *et al.* (2010) estudaram o intestino de espécies com diferentes hábitos alimentares utilizando o cálculo do quociente intestinal (QI), que é a razão entre o comprimento do intestino e o comprimento total do peixe, e através desse cálculo constataram que a espécie piscívora *Hoplias malabaricus* apresenta menor valor de QI (0,51) quando comparado com a espécie herbívora *Ctenopharyngodon idella* (2,78) enquanto que as espécies onívoras *Rhamdia quelen* e *Hoplosternum littorale* apresentaram valores de QI intermediários (respectivamente 0,65 e 1,36). No entanto, o comprimento intestinal isolado não deve ser utilizado como um indicador fidedigno do hábito alimentar, pois o mesmo pode ser influenciado por outros fatores não associados com a dieta como, por exemplo, a ontogenia e a filogenia (GONÇALVES *et al*., 2013). A área absortiva do intestino pode ser aumentada também com a presença dos cecos pilóricos, que são projeções de fundo cego localizadas no início do intestino, como ocorre em *Brycon orbignyanus* (SEIXAS FILHO *et al.*, 2000).

O trato digestório dos peixes também apresenta órgãos acessórios que auxiliam na digestão como o fígado, a vesícula biliar e o pâncreas. O fígado possui as funções de assimilar e armazenar nutrientes, produzir a bile, manter a homeostase corporal com processamento de

carboidratos, proteínas, lipídios e vitaminas. Também é um órgão de indicação do estado nutricional e fisiológico em peixes (CABALLERO *et al.*, 1999) contribuindo na desintoxicação de contaminantes orgânicos e inorgânicos. Entre as células hepáticas, os hepatócitos, encontram-se canalículos biliares que desembocam na vesícula biliar, onde a bile é estocada. A vesícula biliar é um órgão oco localizado adjacente ao fígado e perto da porção anterior do intestino. Sua função é armazenar e secretar a bile quando o alimento entra no intestino (WILSON & CASTRO, 2011). Os sais biliares presentes na bile emulsificam os lipídios, facilitando a ação das lipases e consequentemente auxiliando na digestão. O pâncreas, na maioria dos teleósteos, é um órgão difuso, podendo estar espalhado no tecido adiposo, no mesentério, entre o intestino, estômago, fígado e vesícula biliar. O tecido pancreático também pode se desenvolver no parênquima hepático e originar o hepatopâncreas. O pâncreas é constituído de dois grupos de células, exócrinas e endócrinas. O pâncreas endócrino é formado por células conhecidas como ilhotas de Langerhans que produzem os hormônios insulina, glucagon, somatostatina, peptídeo YY e os secretam na corrente sanguínea (GONÇALVES *et al*., 2013). O pâncreas exócrino é formado por células acinares, responsáveis pela produção, armazenamento e secreção de enzimas digestivas e bicarbonato, o qual neutraliza o pH ácido do quimo proveniente do estômago (BALDISSEROTTO, 2013).

1.2 Enzimas digestivas

As enzimas são proteínas catalizadoras que aumentam a velocidade das reações químicas. De todas as funções proteicas, a catálise talvez seja a mais importante. Na ausência das enzimas, a maioria das reações nos sistemas biológicos apresentaria velocidade excessivamente lenta para viabilizar produtos a um ritmo adequado para um organismo metabolizante. A enzima se liga ao substrato através do sítio ativo formando um complexo de transição que resulta em um determinado produto (Campbel, 2005).

As enzimas digestivas em vertebrados superiores são sintetizadas e secretadas ao longo do trato gastrintestinal. Em peixes, os processos digestivos são primários, mas o conjunto de enzimas é semelhante ao observado em outros vertebrados. A síntese e níveis apropriados de enzimas digestivas são regulados pela disponibilidade de nutrientes no ambiente, entre outros, os quais variam ao longo do tempo (LÓPEZ-VÁSQUEZ *et al*., 2009).

No estômago ocorre a produção de suco gástrico no qual está presente o ácido clorídrico e pepsinogênio que é a forma inativa da pepsina. O ácido clorídrico apresenta baixo

pH, que solubiliza as proteínas, abrindo sua estrutura para facilitar a ação da enzima proteolítica pepsina. Em pH baixo, o pepsinogênio é convertido em pepsina e a enzima torna-se ativa. A pepsina é uma endopeptidase, isto é, cliva as ligações peptídicas dentro da cadeia polipeptídica e apresenta alta afinidade por aminoácidos hidrofóbicos, como triptofano e a fenilalanina (BAKKE *et al*., 2011). O alimento, agora denominado de quimo, segue para o intestino e é misturado às secreções intestinais, pancreáticas e hepáticas. Primeiramente ocorre a liberação de bicarbonato (HCO_3^-) que neutraliza o pH ácido do quimo proveniente do estômago possibilitando a ação das enzimas digestivas que atuam na luz intestinal em pH alcalino (BALDISSEROTTO, 2013).

No intestino ocorre a ação das proteases alcalinas, as quais são produzidas e secretadas pelas células acinares do pâncreas exócrino. Essas células pancreáticas contêm, principalmente, grânulos de zimogênios das enzimas tripsina (tripsinogênio), quimotripsina (quimotripsinogênio), carboxipeptidases (procarboxipeptidase) e elastase (proelastase). A cascata de ativação dos zimogênios das enzimas proteolíticas é iniciada pela enteroquinase, que é secretada pelas células intestinais, que converte o tripsinogênio em tripsina, a qual converte os outros zimogênios em enzimas ativas, inclusive o próprio tripsinogênio (BAKKE, GLOVER, KRODGDAHL, 2011).

O amido é um polímero de moléculas de glicose unidas por ligações α-1,4 e α-1,6. Em mamíferos, a digestão do amido inicia na boca através da ação da amilase salivar (ptialina). No intestino, tanto de mamíferos quanto de peixes, o quimo sofre a ação da α-amilase pancreática. A α-amilase pancreática hidrolisa ligações α(1-4) glicosídicas, resultando em maltose e oligossacarídeos a partir do amido e do glicogênio. O amido é um componente incomum na dieta natural dos peixes, sendo mais utilizado em dietas artificiais em cultivos. A principal fonte de carboidratos é o glicogênio presente nas presas animais ingeridas (BAKKE, GLOVER, KRODGDAHL, 2011).

O pâncreas também produz e secreta as enzimas envolvidas na hidrólise dos lipídios, as lipases ester de colesterol hidrolase, colipase, lipase e fosfolipase. Essas enzimas “digerem” os triglicerídios, fosfolipídios e ésteres de colesterol em ácidos graxos, glicerol, monoglicerídeos e colesterol. Os lipídios são emulsificados pela ação dos ácidos biliares, que facilita a ação das enzimas. A hidrólise de triglicerídeos em mamíferos é realizada por duas lipases principais, o sistema lipase-colipase pancreática e a lipase com menor especificidade ativada por sais biliares. Os fosfolipídios constituem uma parte substancial do lipídio da dieta em peixes, porém existem poucos estudos sobre sua digestão no intestino. Provavelmente, os fosfolipídios são digeridos pelas fosfolipases pancreática resultando em 1-acil

lisoglicerofosfolipídios e ácidos graxos livres. Ainda não está claro se os peixes apresentam uma hidrolase éster de cera específica (TOCHER, 2003).A lipase pancreática é liberada no intestino na forma ativa. Essa enzima, em peixes, apresenta maior afinidade por glicerídeos com ácidos graxos de cadeia longa e altamente insaturados contrastando com a lipase dos mamíferos que apresentam maior atividade em ligações ésteres com ácidos graxos de cadeia com menos de 20 carbonos (BAKKE, GLOVER, KRODGDAHL, 2011).

As enzimas digestivas são indutivas, isto é, sua atividade responde à presença do substrato. Em peixes, espera-se que atividade enzimática digestiva reflita o hábito alimentar. Consequentemente, peixes herbívoros apresentariam maior atividade de carboidrases que peixes carnívoros, os quais apresentariam maior atividade proteolítica (STECH, CARNEIRO & PIZAURO Jr., 2009). Isso se deve a natureza do alimento que os peixes ingerem. Peixes herbívoros consomem alimentos de origem vegetal que apresentam maior teor de amido enquanto que peixes carnívoros consomem alimentos de origem animal com maior teor proteico. No entanto, estudos que relacionam os níveis enzimáticos com dietas de diferentes composições e estudos com espécies de diferentes hábitos alimentares, às vezes não confirmam esse paradigma. LUNDSTEDT, MELO & MORAES (2004) não detectaram alterações na atividade proteolítica no estômago de juvenis de *Pseudoplatystoma corruscans* alimentados com dietas contendo diferentes teores de proteína. No entanto, nesse mesmo estudo, os autores detectaram um aumento da atividade da amilase em peixes alimentados com dietas com níveis crescentes de amido. A atividade amilolítica de *Rachycentron canadum* apresenta uma relação direta com os níveis de amido na dieta (REN *et al*., 2011). CHAKRABARTI *et al*. (1995) estudou a atividade das enzimas amilase, esterase, celulase e proteases ácida e alcalinas em 11 espécies com hábitos alimentares diferentes (onívoros, carnívoros e herbívoro) e encontrou relação entre a atividade enzimática e o hábito alimentar.

1.3 Área de coleta e espécies em estudo

As espécies estudadas foram coletas no canal São Gonçalo em Pelotas – RS. Esse canal tem uma extensão de aproximadamente 75 km, 6 m de profundidade, 200 a 500 m de largura e conecta a Lagoa Mirim à Laguna dos Patos, formando o sistema lagunar Patos-Mirim, que apresenta uma bacia de drenagem de 263.876 km². Esse sistema está localizado na planície costeira do Rio Grande do Sul, embora parte da Lagoa Mirim esteja localizada no Uruguai (BURNS *et al*., 2006).

1.3.1 *Loricariichthys anus* (Valenciennes, 1836)

Espécie pertencente à ordem Siluriformes, família Loricariidae e subfamília Loricariinae. Distribui-se no baixo rio Paraná, rio da Prata, rio Uruguai, laguna dos Patos e rios costeiros do Estado do Rio Grande do Sul (GHAZZI & OYAKAWA, 2007). Conhecido popularmente como cascudo-viola (Figura 1), essa espécie pode ser distinguida de outros loricarídeos por apresentar um pedúnculo caudal e focinho deprimidos e com nadadeira adiposa ausente. Os machos com lábios inferiores ampliados do gênero *Loricariichthys* transportam os ovos fertilizados ligados ao lábio até a eclosão das larvas (FERRARIS Jr., 2003). Espécie bentônica que vive no fundo dos rios, se alimenta de detritos vegetais e animais e algas aderidas ao substrato revelando seu hábito alimentar detritívoro/iliófago (ALBRECHT & SILVEIRA, 2001).

Figura 1. *Loricariichthys anus* (Foto de C. D. Timm, retirada do FishBase)

1.3.2 *Hypostomus commersoni* (Valenciennes, 1836)

Espécie pertencente à ordem Siluriformes, família Loricariidae e subfamília Hypostominae. Distribui-se nas bacias do baixo e médio Paraná, do Uruguai e da laguna dos Patos (CARVALHO & BOCKMANN, 2007). Alimenta-se de detritos vegetais e animais e algas que se encontram aderidas ao substrato, seu hábito alimentar é detritívoro/iliófago (LONDON, 1983). É uma espécie bentônica que vive no fundo dos corpos d'águas. Apresenta o corpo revestido por placas ósseas com pequenos espinhos formando quilhas longitudinais (Figura 2). Sua coloração é pardo-amarelada e frequentemente apresenta manchas pequenas e arredondadas. Possui boca na posição ventral, os lábios são desenvolvidos os quais formam uma ventosa, geralmente com numerosos dentículos (KOCH, MILANI & GROSSER, 2000).

Figura 2. *Hypostomus commersoni* (Foto de A. G. Becker)

1.3.3 *Rhamdia quelen* (Quoy & Gaimard, 1824)

Espécie pertencente à ordem Siluriformes e família Heptapteridae. Distribui-se em praticamente todas as bacias hidrográficas brasileiras (BOCKMANN, 2007). Espécie bentônica que vive no fundo de rios, prefere ambientes com águas mais calmas com fundo arenoso ou lodoso. Apresenta hábitos noturnos e durante o dia se escondem entre pedras e troncos. É uma espécie onívora com preferência por peixes, insetos, crustáceos, detritos vegetais e animais. Apresenta corpo robusto e alongado com a presença de uma longa nadadeira adiposa (Figura 3). Não apresenta escamas, sendo chamando de peixe de couro. Sua coloração varia de marrom-avermelhado claro a cinza com a superfície ventral do corpo mais clara que a dorsal. Possui barbilhões junto à boca (GOMES *et al*., 2000).

Figura 3. *Rhamdia quelen* (Foto de A. G. Becker)

1.3.4 *Pimelodus maculatus* (LaCepède, 1803)

Essa espécie pertence à ordem Siluriformes e família Pimelodidae. Distribui-se nas bacias do São Francisco, do Paraná, do Paraguai e do Uruguai (SHIBATTA & BOCKMANN, 2007). Apresenta corpo robusto e alongado com a presença de nadadeira adiposa bem desenvolvida e três pares de barbilhões junto à boca (Figura 4) (WEBER, 2003). Apresenta coloração marrom-amarelada a cinza claro com manchas arredondadas escuras na região dorsal e o ventre branco (FISHER, PEREIRA & VIEIRA, 2004). Conhecido como mandi-amarelo, é uma espécie onívora. Estudos de análise estomacal dessa espécie revelaram a presença de larvas de insetos, microcrustáceos, algas e grãos de areia (LOLIS & ANDRIAN, 1996).

Figura 4. *Pimelodus maculatus* (Foto de A. G. Becker)

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2 OBJETIVOS

2.1 Objetivo geral

Determinar a relação entre a atividade enzimática digestiva e a composição centesimal do alimento ingerido de acordo com o hábito alimentar de *Pimelodus maculatus*, *Rhamdia quelen*, *Hypostomus commersoni* e *Loricariichthys anus* e sua variação em duas estações do ano.

2.2 Objetivos específicos

Determinar a composição centesimal do alimento ingerido pelos peixes.

Avaliar a relação entre a composição centesimal do conteúdo e a atividade enzimática digestiva.

3 MANUSCRITO

Gastrointestinal content composition and digestive enzymes activities in summer and winter of four freshwater teleosts with two different feeding habits

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Abstract

The aim this study was to evaluate the relationship between the digestive enzyme activity and centesimal composition of gastrointestinal content and feeding habits of four freshwater teleosts of two feeding habits. The enzyme activities are not related to centesimal composition of gastrointestinal content. Detritivorous species showed the highest alkaline proteases activities, which can be an adaptation to utilize the lowest protein level found in the gastrointestinal content of these species.

Key Words: pepsin, chymotrypsin, trypsin, lipase, protein, lipid

Introduction

The digestive enzymes in higher vertebrates are synthetized and secreted throughout the gastrointestinal tract. The fish have a high versatility of their digestive processes, which may vary according to species, size, age, stage of maturity, temperature, type of food ingested and feeding history. The appropriate synthesis and levels of digestive enzymes may be affected by environmental factors that vary over time (Garcia Carreño *et al*., 2002; López-Vásquez *et al*., 2009).

Fish are usually classified according to feeding habit and it is expected that digestive enzyme activities reflect the feeding habits and the diet of the fish (Fernández *et al*., 2001; Langeland *et al*., 2013). Usually herbivorous fish show highest carbohydrase activities than carnivorous fish, which exhibit highest proteolytic activities due to higher dietary carbohydrate and protein levels respectively (Chan *et al*., 2004). However, most studies dealing with digestive enzymes activities were performed with species raised in fish culture conditions and fed with the same diet for at least one month (García-Carreño, *et al*., 2002; Ren *et al*., 2011; Aguilera *et al*., 2012; Azarm *et al*., 2012; Leef *et al.*, 2012).

The studies of fish collected in the environment analysed only digestive enzyme activities without evaluating the centesimal composition of ingested food. López-Vásquez *et al.* (2009) studied eight species with different feeding habits and observed that the enzymatic activity contrasted with the expected: higher alkaline proteases activities were observed in the omnivorous *Osteoglossum bicirrhosum* (Cuvier, 1829) and the lowest activity in the carnivorous *Cichla monoculus* Agassiz, 1831 and the detritivorous fish presented higher amylase, maltase and alkaline protease activities.

The relationship between digestive enzymes activities and centesimal composition of the ingested food by the fish in the environment is important to understand the feeding biology of species and diets formulations to cultured species in fish farms. Four teleost species belonging to two trophic guilds, omnivorous and detritivorous, were chosen for this study. The omnivorous *Rhamdia quelen* (Quoy & Gaimard, 1824) (silver catfish) feeds mainly on fish, crustaceans, insects, plant remains and organic detritus (Gomes *et al.*, 2000) and *Pimelodus maculatus* LaCèpede, 1803 (mandi-amarelo) on insect larvae, micro crustaceans, algae and sand grains (Lolis & Andrian, 1996). The detritivorous *Hypostomus commersoni* Valenciennes, 1836 (cascudo) feeds mainly on algae, zooplankton and sediments (London, 1983) and *Loricariichthys anus* (Valenciennes, 1835) (cascudo-viola) on sediments and plant remains (Albrecht & Silveira, 2001).

Materials and Methods

Animals

Four species of fish (*Rhamdia quelen* - 40.7 ± 0.6 cm; 779.3 ± 73.4 g, *Pimelodus maculatus* - 29.7 ± 0.2 cm; 234.6 ± 7.0 g, *Loricariichthys anus* - 31.7 ± 0.7 cm; 165.1 ± 9.7 g and *Hypostomus commersoni* - 36.3 ± 2.5 cm; 403.4 ± 70.7 g) (n = 15 each) were collected on March (summer) and August (winter) at the freshwater site of the São Gonçalo channel, Pelotas, southern Brazil. They were collected using a shrimp trawl (10.5 m head rope, 0.5 cm bar mesh in the center, 1.3 cm bar mesh on the wings) deployed for 5 minutes between 5 and 8 m depth by a wood boat 60 Hp engine. Immediately after the collect, fishes were euthanized by severing the spinal cord and then weighed and measured. The gastrointestinal tract was removed and divided into the following segments: stomach, anterior and posterior intestine.

The contents of the gastrointestinal tract segments were collected separately, placed in plastic tubes and kept refrigerated, while the segments were placed in liquid nitrogen. The samples were taken to the Fish Physiology Laboratory at the Federal University of Santa Maria.

Tissue homogenates

Samples from stomach, anterior and posterior intestine were homogenized in an ice bath at ratio 1:10 (tissue: homogenization buffer) with an Ultraturrax. The homogenization buffer solution was 20 mM Tris/ 10 mM phosphate, pH 7.0 in 50% (v/v) glycerol. The extract was centrifuged and supernatant was utilized in assays as enzyme source.

Enzyme assays

Pepsin activity was assayed by the specific methods of Hidalgo et al. (1999). The pepsin substrate was 1.5% casein in 0.2 M KCl (pH 1.8). Reactions were carried out at 30 °C for 40 min, stopped with 15% TCA, and the optical density of the supernatant recorded at 280 nm against tyrosine as standard. Specific activity was expressed in µmol hydrolysed substrate min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$). Trypsin and chymotrypsin were assayed by the specific methods of Hummel (1959). The trypsin substrate was 1.04 mM TAME-HCl (α-p-toluene-sulfonyl-L-arginine methyl ester hydrochloride) in 0.01 M CaCl/0.2 M Tris-HCl (pH 8.1), incubated at 25°C and optical density followed at 247 nm for 60 s. Chymotrypsin substrate was 1mM BTEE (N-benzoyl-L-tyrosine ethyl ester) in methanol 2:3 (v/v), assayed in 0.1 M CaCl/0.1 M Tris-HCl (pH 7.8) at 30 °C, and optical density of supernatant followed at 256 nm for

60 s. Activities were expressed in µmol arginine min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$) and nmol tyrosine min^{-1} mg $protein^{-1}$ (mU mg $protein^{-1}$), respectively.

Lipase activity was assayed by the specific method of Gawlicka et al. (2000). Reaction was incubated with 0.4 mM p-nitrophenyl myristate in 24 nM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100 at 30 °C for 30 min. The reaction was stopped with 10 mM NaOH and the optical density followed at 405 nm. One unit was defined as µmol substrate hydrolysed per min and expressed per milligram protein (U mg $protein^{-1}$).

To establish the specific activities of the enzymes, protein concentrations were determined in the enzyme extracts by the method of Lowry et al. (1951), with bovine albumin as standard.

Determination of the centesimal composition of content of tract gastrointestinal

Protein and lipid levels were determined in stomach and intestinal contents. The protein level was determined according to the micro Kjeldahl method (method 920.52) according to AOAC (1995). The lipid level was extracted and quantified according to Bligh & Dyer (1959).

Statistical analysis

Data are presented as the mean ± S.E.M. The Levene test was used to verify the homogeneity of variances. If the data were homoscedastic, the relationship between species, seasons and digestive enzymatic activities were assessed by two-way ANOVA followed by Tukey's test. If the conditions for parametric ANOVA were not satisfied, then the non-parametric Sheirer-Ray-Hare extension of the Kruskal-Wallis test followed by the Nemenyi test was used. The relationship between species

and centesimal composition and the relationship between seasons and centesimal composition in the same species were assessed by one-way ANOVA followed by Tukey's test if the data were homoscedastic. Otherwise, the Kruskal-Wallis test followed by the comparison of the mean ranks was used. These analyses were performed with the Statistica 7.0 software. The correlation between digestive enzymatic activities and centesimal content was assessed by Pearson correlation using Sigma Plot 11.0 software. Differences were considered significant at $P < 0.05$.

Results

Enzymatic activity

The highest pepsin activity was detected in the stomach of *R. quelen* and *P. maculatus* followed by *L. anus* and *H. commersoni* in both seasons ($d.f.= 3$, $F= 131.416$, $P<0.001$). In the winter, pepsin activity was higher in *R. quelen* and *P. maculatus* and lower in *L. anus* compared to summer ($d.f.= 1$, $F= 8.206$, $P<0.01$) (Fig. 2A).

The highest trypsin activity was detected in the anterior intestine of *H. commersoni* followed by *R. quelen*, *L. anus* and *P. maculatus* in the summer. On the other hand, in the winter the highest enzymatic activity was found in *P. maculatus* followed by *L. anus* and *H. commersoni* and *R. quelen* that did not differ ($d.f.= 3$,$F=16.488$, $P<0.001$). There was a reduction in the trypsin activity of *R. quelen* and *H. commersoni* and an increase of *P. maculatus* and *L. anus* enzyme activity in the winter compared to summer ($d.f.= 1$, $F= 13.004$, $P<0.001$) (Fig. 2B). In the posterior intestine the highest trypsin activity in both seasons was detected in *L. anus*, *H. commersoni* and *P. maculatus* and the lowest in *R. quelen* ($d.f.= 3$, $F= 27.651$,

P<0.001). Trypsin activity was lower in the winter for all species compared to summer (d.f.= 1, F= 513.679, P<0.001) (Fig. 2C).

The highest chymotrypsin activity in the anterior intestine in the summer was detected in *H. commersoni* followed by *R. quelen* and the lowest in *P. maculatus* and *L. anus*. On the other hand, in the winter the highest activity was observed in *P. maculatus* followed by *L. anus* (d.f.= 3, F= 4.27, P<0.01). There was a reduction on chymotrypsin activity in the anterior intestine of *R. quelen* and *H. commersoni* and an increase in *P. maculatus* and *L. anus* in the winter compared to summer (d.f.= 1, F= 24.84, P<0.001) (Fig 2D). The highest chymotrypsin activity in the posterior intestine, in the summer was detected in *H. commersoni*, *P. maculatus* and *L. anus* and the lowest in *R. quelen*. The chymotrypsin activity in the winter was higher in *P. maculatus* than *R. quelen* (d.f.= 3, F= 32.89, P<0.001). The chymotrypsin activity was lower in posterior intestine of all species at winter, when compared to summer (d.f.= 1, F= 3558,98, P<0.001) (Fig. 2E).

The lipase activity was highest in the anterior intestine of *H. commersoni* and *R. quelen*, followed by *L. anus* and *P. maculatus*. In the winter, the highest lipase activity was found in *P. maculatus* followed by *L. anus*. The lowest activity was observed in *H. commersoni* and *R. quelen* (d.f.= 3, F= 76.187, P<0.001). There was a significant increase on lipase activity in *P. maculatus* and *L. anus* and a significant reduction in *H. commersoni* and *R. quelen* (d.f.= 1, F= 0.810, P= 0.371) in the winter compared to summer (Fig. 3A). The lipase activity of the posterior intestine in the summer was similar in all species. In the winter the lipase activity was lower in *L. anus* than *P. maculatus* and *R. quelen* (d.f.= 3, F= 6.57, P<0.001). The lipase activity of the posterior intestine of all species decreased significantly in the winter compared to summer (d.f.= 1, F= 2240.78, P<0.001) (Fig. 3B).

Centesimal composition of the gastrointestinal tract content

The *H. commersoni* specimens collected did not have any content in the stomach at both seasons, whereas *L. anus* did not show stomach and intestinal contents in the winter. The highest protein level in the stomach content in the summer was found in *R. quelen* and the lowest in *L. anus* and *P. maculatus* (d.f.= 2, F= 67.9344, P<0.001). In the winter, the highest protein level was found in *P. maculatus* (d.f.= 1, F= 49,4945, P<0.01). There was a significant reduction in the protein level of stomach content of *R. quelen* (d.f.= 1, F= 35.5192, P<0.01) and a significant increase in the content of *P. maculatus* (d.f.= 1, F= 2182, 654, P<0.001) in the winter compared to summer (Table 1).

In the anterior intestinal content the highest protein level in the summer was found in *H. commersoni* and the other species did not differ significantly between them (d.f.= 3, F= 5.6358, P<0.05). In the winter the highest protein level was detected in *R. quelen* and *P. maculatus* and the lowest in *H. commersoni* (d.f.= 2, F= 2.376, P=0.173774). There was a reduction in the protein level in the anterior intestinal content of *H. commersoni* (d.f.= 1, F= 11.3395) and an increase in *R. quelen* (d.f.= 1, F= 2.00821, P= 0.229412) and *P. maculatus* (d.f.= 1, F=6.14728) in the winter compared to summer (Table 1).

In the posterior intestinal content the highest protein level in the summer was found in *R. quelen* followed by *L. anus*, *P. maculatus* and *H. commersoni* (d.f.= 3, F= 258.5429, P<0.001). In the winter, the highest protein level was detected in *R. quelen* followed by *P. maculatus* and *H. commersoni* (d.f.= 2, F= 66.6164, P<0.001). In the winter, the protein level of the posterior intestinal content was lower in *R. quelen* (d.f.= 1, F= 115.6785, P<0.001) and higher in *P. maculatus* (d.f.= 1, F= 98.494, P<0.001) compared to summer (Table 1).

The highest lipid level in the stomach content in summer was found in *R. quelen* and the lowest in *P. maculatus* (d.f.= 1, F= 18182.05, P<0.001),while in the winter the contrary was observed (d.f.= 1, F= 38.3139, P<0.01). There was a reduction in the lipid level in the stomach of *R. quelen* (d.f.= 1, F= 9782.69, P<0.001) and an increase in *P. maculatus* (d.f.= 1, F= 87.3356, P<0.001) in the winter compared to summer. In the anterior intestinal content, the highest lipid level was found in *H. commersoni* in both seasons, without difference between seasons for this species. There was a significant increase in lipid level in the anterior intestinal content of *P. maculatus* (d.f.= 1, F= 9.2319, P<0.05) in the winter compared to summer (Table 1).

In the posterior intestinal content, the highest lipid level in summer was found in *H. commersoni* and the others did not differ between them (d.f.= 3, F= 4.87784, P<0.05). In the winter, there was no difference on lipid levels in the posterior intestinal content of all species that presented any content (d.f.= 2, F= 2.359229, P=0.175411). However, there was a reduction in the lipid level of the posterior intestinal content in *R. quelen* (d.f.= 1, F= 91.3402, P<0.001) and an increase in *P. maculatus* (d.f.= 1, F= 22.08358, P<0.01) in the winter compared to summer (Table 1).

Enzymatic activity x gastrointestinal tract centesimal composition

There was no relationship between the enzyme activities and the centesimal composition of the different segments. It was not possible to determine the relationship between the digestive enzymatic activity and the centesimal composition of ingested food in the stomach of *H. commersoni* in both seasons due to absence of stomach content. In winter, this relationship could not be determined in *L. anus* due

to absence of content in three segments of the gastrointestinal tract used in this study.

Discussion

The presence and quantity of appropriate digestive enzymes determine the utilization of nutrients. Several studies on activity of digestive enzymes in fish suggested that enzymatic activity is influenced by the diet ingested or by feeding habits (Fernández *et al*., 2001; Chan *et al*., 2004; Drewe *et al*., 2004; Langeland *et al*., 2013). However, enzyme activity in cultured juveniles of *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) was not influenced by the diet (Lundstedt *et al*., 2004). The diet also did not influence most digestive enzymes in *R. quelen*, except the intestinal protease (Moro *et al*., 2010). The results of this study also did not fully support the hypothesis that digestive enzymatic activity is determined by centesimal composition of the food or feeding habits.

Most studies demonstrating a relationship between diet and digestive enzyme activity were performed in cultivated species. The cultured *Colossoma macropomum* (Cuvier, 1816) showed higher lipase activity in the anterior intestine when fed with higher lipid levels (De Almeida *et al*., 2006) and *Monopterus albus* (Zuiew, 1793), presented higher trypsin activity when fed with higher protein levels (Ma *et al*., 2014). In the present study, the protein and lipid levels observed in the gastrointestinal tract did not show any relationship with proteolytic and lipase activities. This absence can be associated with the variation of nutrients availability over time in the environment as it was observed in the centesimal composition of food ingested by the fish in summer and winter. This indicates that probably these enzymes showed a constitutive character in some species, i. e., the digestive enzymes are always

available in the gastrointestinal tract to digest any food that may be found by the fish, as proposed by Lundstedt *et al*. (2004) to *P. corruscans*.

Consequently, the digestive enzyme activity seems to be unrelated to feeding habit. Similar results were found by López-Vásquez *et al*. (2009) that observed higher alkaline proteolytic activity in two detritivorous species and one omnivorous species than carnivorous species. The same authors supposed that the higher proteolytic activity in detritivorous species was due to feeding with detritus, which represent a poor source of nutrients. That may be the explanation for the higher activity of trypsin and chymotrypsin in the posterior intestine of *P. maculatus*, *L. anus* and *H. commersoni* since the protein content of the contents of this portion was lower in both seasons in these species. They could increase the digestive enzymes activities to use completely the low protein level of the food. Analysis of the digestive enzyme activities in 11 species distributed in three trophic categories (Chakrabarti *et al*., 1995) and four phylogenetically related species with two feeding habits (Chan *et al*., 2014) and the study of López-Vázques *et al*. (2009) also led to the same conclusion.

In conclusion, digestive enzyme activity cannot be used as the unique indicator of the feeding habit because in fish collected in the wild usually this relationship is not found.

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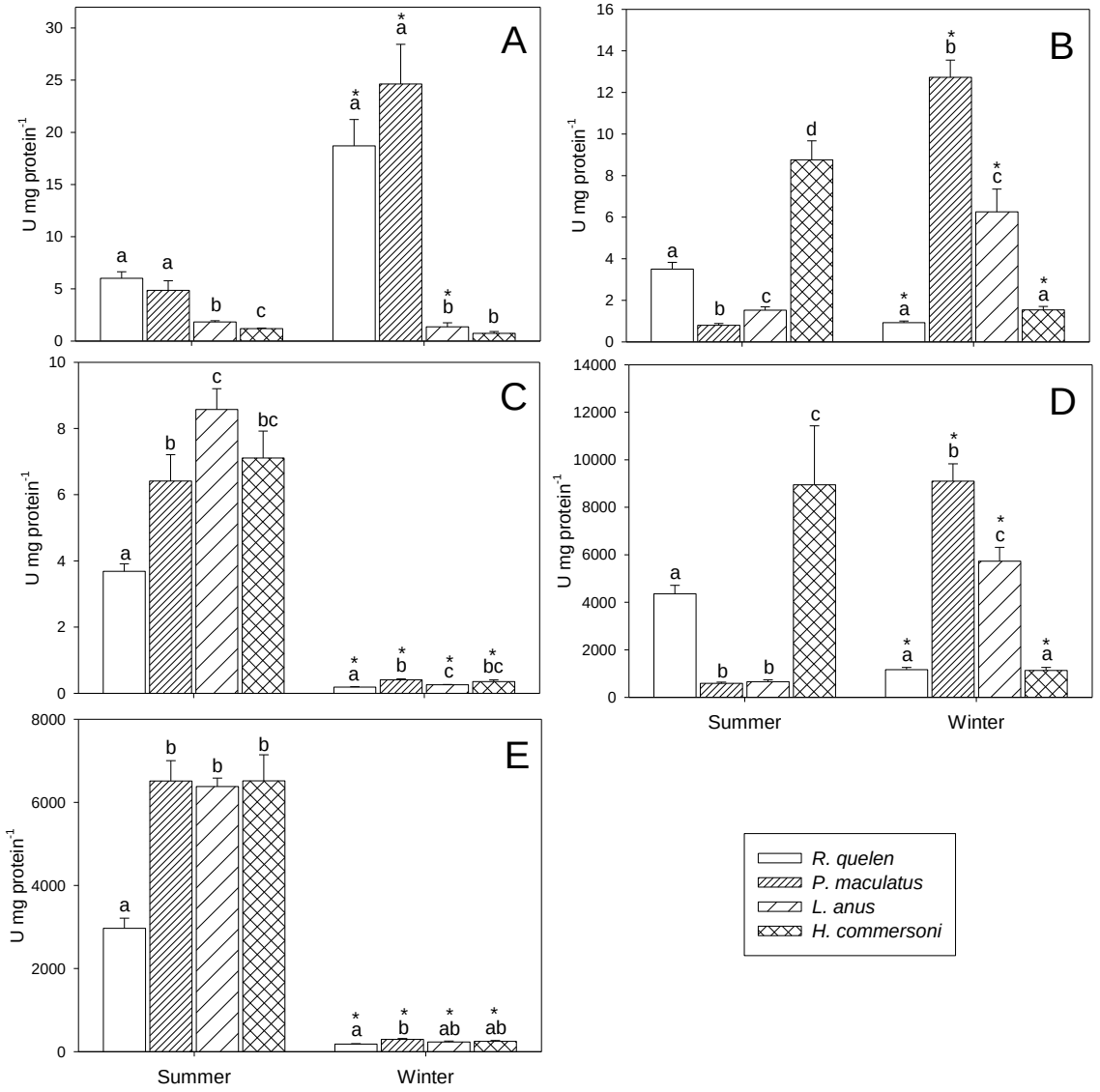

FIG. 1. Proteolytic enzymatic activities in the studied species in the summer and winter. A: pepsin, B: trypsin in the anterior intestine, C: trypsin in the posterior intestine, D: chymotrypsin in the anterior intestine, E: chymotrypsin in the posterior intestine. Different letters indicate significant differences between species in the same season. * indicate significant difference from summer ($P<0.05$). (U, a Caraway unit).

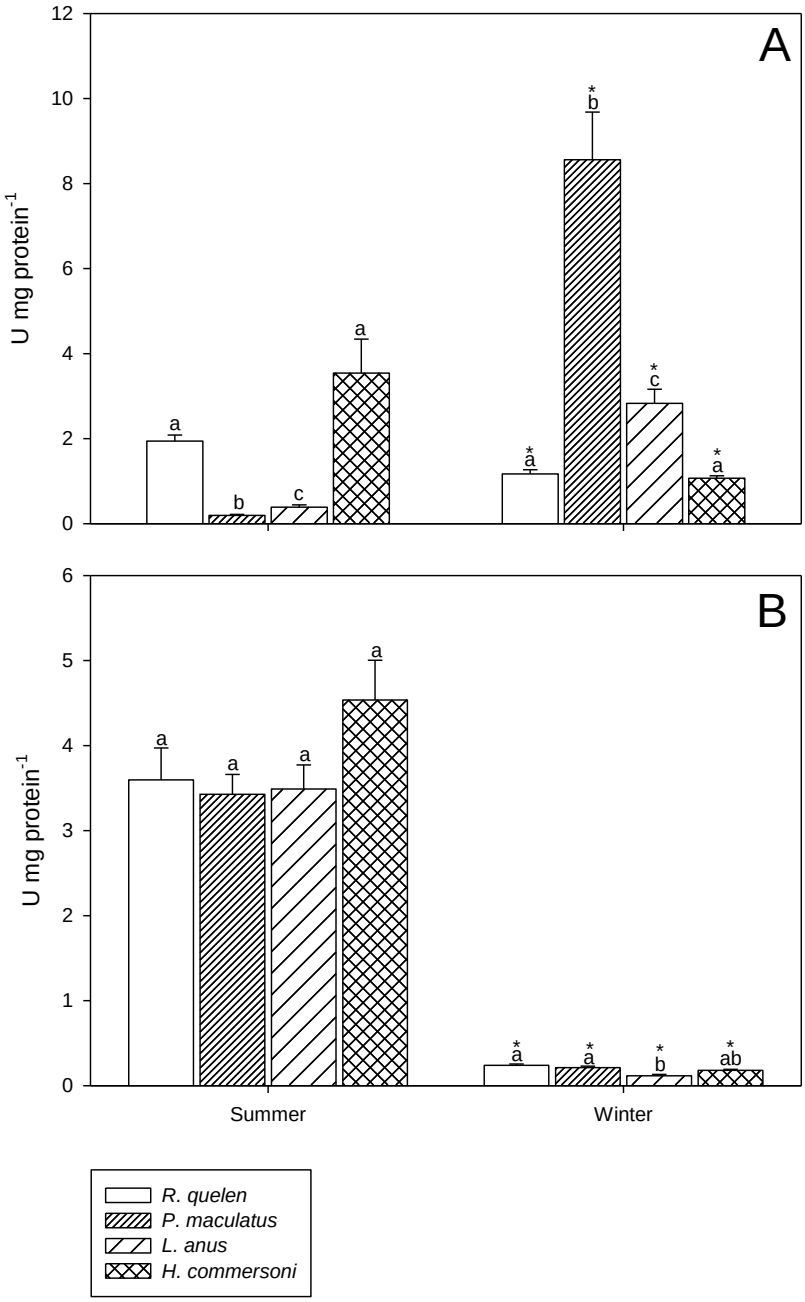

FIG. 2. Lipase activity in the A: anterior intestine and B: posterior intestine. Different letters indicate significant differences between species in the same season. * indicate significant difference from summer (P<0.05). (U, a Caraway unit).

Table I. Percentage (of dry matter) and range (between parenthesis) of protein and lipids in the content of the gastrointestinal tract of four teleost species collected in two seasons.

Species	Stomach		Anterior intestine		Posterior intestine	
	Summer	Winter	Summer	Winter	Summer	Winter
PROTEIN						
Rhamdia quelen	61.77 ± 10.81^{a} (50.95 – 72.58)	18.37 ± 6.49^{a*} (11.89 – 24.86)	11.21 ± 0.03^{a} (11. 18 – 11.25)	21.59 ± 12.69^{a*} (8.90 – 34.28)	52.92 ± 4.35^{a} (48.57 – 57.28)	25.87 ± 0.17^{a*} (25.70 – 26.04)
Pimelodus maculatus	9.05 ± 1.16^{b} (7.89 – 10.205)	44.86 ± 0.65^{b*} (44.21 – 45.51)	12.04 ± 0.95^{a} (11.09 – 12.99)	24.35 ± 8.54^{a*} (15.81 – 32.89)	9.45 ± 0.26^{b} (9.19 – 9.72)	17.66 ± 1.41^{b*} (16.26 – 19.07)
Loricariichthys anus	11.04 ± 0.04^{b} (10.99 – 11.08)	N	10.47 ± 0.19^{a} (10.28 – 10.66)	N	13.77 ± 1.45^{bc} (12.31 – 15.22)	N
Hypostomus commersoni	N	N	15.29 ± 2.94^{b} (12.35 – 18.23)	9.57 ± 0.14^{b*} (9.43 – 9.70)	7.05 ± 0.72^{bd} (6.34 – 7.77)	7.78 ± 3.01^{c} (4.76 – 10.79)
LIPIDS						
Rhamdia quelen	7.07 ± 0.08^{a} (6.98 – 7.15)	0.91 ± 0.07^{a*} (0.84 – 0.97)	0.71 ± 0.09^{a} (0.62 – 0.80)	0.51 ± 0.22^{a} (0.29 – 0.73)	1.94 ± 0.23^{a} (1.71 – 2.16)	0.64 ± 0.07^{a*} (0.56 – 0.71)
Pimelodus maculatus	0.41 ± 0.01^{b} (0.39 – 0.42)	1.96 ± 0.29^{b*} (1.68 – 2.25)	0.29 ± 0.02^{a} (0.27 – 0.31)	0.56 ± 0.15^{a*} (0.41 – 0.71)	0.27 ± 0.03^{a} (0.24 – 0.29)	1.67 ± 0.51^{a*} (1.15 – 2.18)
Loricariichthys anus	N	N	0.75 ± 0.08^{a} (0.67 – 0.83)	N	2.34 ± 2.28^{a} (0.06 – 4.62)	N
Hypostomus commersoni	N	N	4.81 ± 1.41^{b} (3.40 – 6.22)	7.36 ± 0.82^{b} (6.54 – 8.17)	3.82 ± 0.01^{b} (3.81 – 3.83)	2.72 ± 1.34^{a} (0.52 – 3.83)

N: not determined because specimens did not have any content in this portion. Different letters in the columns indicate significant difference between species.
* indicates significant difference from summer in the same portion.

4 CONCLUSÃO GERAL

Não foi identifica uma relação entre a atividade enzimática e a composição centesimal do conteúdo do trato gastrintestinal bem como entre a atividade enzimática e o hábito alimentar do peixe. Podemos concluir que:

1. Atividade enzimática digestiva não deve ser utilizada como único indicador do hábito alimentar porque em peixes coletados na natureza essa relação, às vezes, não é encontrada.

2. A composição centesimal do conteúdo do trato gastrintestinal, no ambiente natural, não apresenta uma relação direta com a atividade enzimática digestiva.

3. Os peixes podem apresentar uma relação inversa entre a composição centesimal do conteúdo gastrintestinal e a atividade das enzimas digestivas como uma adaptação ao baixo teor nutritivo do alimento ingerido.

ANEXO A - Normas da revista Journal of Fish Biology

INSTRUCTIONS FOR AUTHORS

1. *Journal of Fish Biology* welcomes research manuscripts containing new biological insight into any aspect of fish biology. We invite papers that report results and ideas of value to fish biology that will serve a wide international readership. Hence the novelty of the content of manuscripts should have relevance beyond a particular species or place in which the work was carried out. **All material submitted must be original and unpublished, and not under consideration for publication elsewhere.** If in doubt about overlap, please give details of any related work submitted or in press when submitting your manuscript. The *Journal* uses plagiarism detection software, so in submitting your manuscript you accept that it may be screened against previously published literature.

The Fisheries Society of the British Isles (FSBI) considers that scientists should avoid research threatening the conservation status of any species of fish, which is already regarded as threatened according to the IUCN Red List of Threatened Species a nd the associated Red List Categories and Criteria version 3.1 (http://www.iucnredlist.org/technical-documents/categories-and-criteria) or which is listed as such in a Red Data Book appropriate to the geographic area concerned. In accordance with this view, papers based on such research will not be accepted, unless the work had clear conservation objectives.

Authors are encouraged to place all species distribution records in a publicly accessible database such as the national Global Biodiversity Information Facility (GBIF) nodes (www.gbif.org) or data centres endorsed by GBIF, including BioFresh (www.freshwaterbiodiversity.eu/).

2. ***Submission of manuscripts***. We will consider: Regular papers (original research), Review papers, which will either be invited or agreed with an Associate Editor (see 17), Brief Communications (see 18), Letters (see 19), and Comments and Replies (see 20). Contributors to the *Journal of Fish Biology* should read the Editorial on submissions and authorship in *Journal of Fish Biology* **79,** 1-2 (2011) (available here) Manuscripts are submitted online at http://jfb.edmgr.com, where a user ID and password are assigned on the first visit. Full instructions and support are available on this site. **Authors are expected to suggest potential referees**, selected internationally, for their manuscripts in the 'Suggest Reviewers' section.

3. ***Preparation of manuscripts***. Authors should consult a recent issue of Journal of Fish Biology for details of style and presentation. **If their manuscript does not follow the format of the Journal, it will be returned to them unreviewed**. Manuscripts must be **double-spaced throughout,** all pages must be numbered and **line numbering set to continuous,** including tables, figure legends and reference lists**. Use a font size ≥ 12. Do not save files in PDF (portable document format) format.**

The first page must contain the following information: the title of the paper, name(s) (initials ONLY for forenames) and FULL academic address(es) of ALL author(s); if the address of any author has changed, it should be added as a footnote. Telephone number and email address for the corresponding author (**one only**) should be provided as a footnote. A concise running headline of not more than 45 characters inclusive of spaces should also be given on this page. For regular papers arrange sections in the following sequence: Title page (as a separate page), Abstract and Key Words (as a separate page), Introduction, Materials and Methods, Results, Discussion (**a combined Results and Discussion is not acceptable and Conclusions as a heading is only acceptable in Review Papers**), Acknowledgements (for individuals use initials only for forenames and no titles), References, Tables (with captions; see 6 below), Figure captions, Figures and Appendices. Within sections, subdivisions should not normally exceed two grades; decimal number classification of headings and subheadings should not be used (see recent past issues). Footnotes should not be used except in Tables. Spelling must be U.K. English, e.g. Concise Oxford English Dictionary (as distinct from American English) throughout, except in quotations and references. All Latin words (but excluding scientific words other than genus and species) should be in italics. **Do not write text in the first person**.

Do not duplicate information in tables and figures, or *vice versa* or in text and figures. Do not repeat table headings and figure legends in the text. Punctuation should be consistent and only a single space inserted between words and after punctuation. **Do not indicate positions of tables and figures in the text**. Two blank lines should be left after headings and between paragraphs. Text should be typed without end of line hyphenation, except for compound words. Lower case ‘l’ for ‘1’ or ‘O’ for ‘0’ should not be used.

4. ***Abstract***. This must be concise and summarize **only** the significant findings of the paper (*i.e.*not the background or methods). It should be followed by a list of **≤ 6 key words or key phrases that are not included in the title, with a maximum of 100 characters (including punctuation and spacing).**

5. ***Illustrations***. Photographs should be selected only to illustrate something that cannot adequately be displayed in any other manner. Magnification should be given in actual terms and all stains used should be described in full. Colour figures can be included; the first two will be produced free of charge, additional figures will be produced online free of charge, print production will be at the author's expense. Authors must complete a Colour Work Agreement Form for any colour figures requiring payment. This will be indicated on acceptance. The form can be downloaded as a PDF from the home page at http://jfb.edmgr.com, or by clicking here Please note that the Colour Work Agreement Form must be returned by post to the address provided on acceptance. Number figures consecutively using Arabic numerals [Fig. 1, 2, *etc*.: subdivide by (a), (b), *etc*.], in order of their mention in the text. A fully descriptive caption must be provided for every figure and the complete list of captions typed together on a separate page. Captions must not be included on the figures. All relevant information, *e.g*. keys to the symbols and formulae, should be included in the caption. The minimum reduction for the figures may be indicated. Artwork should be received in digital format. Line artwork (vector graphics) should be saved as Encapsulated PostScript (EPS) and bitmap files (half-tones or photographic images) as Tagged Image Format (TIFF). Native file formats should not be submitted. More detailed information on the submission of electronic artwork can be found at http://authorservices.wiley.com/bauthor/illustration.asp

6. ***Tables***. Number consecutively in Roman numerals (Table I, II, etc.), **in the order of their mention in the text**. Captions for tables should be **typed directly above each table**, not on a separate page. Footnotes to tables should be indicated by superscripts and typed at the bottom of the tables. Tables and figures must 'stand alone' and so all abbreviations must be defined in the figure captions and as footnotes in the tables. Tables, figures and figure captions should be saved in separate files from the main text of the manuscript. Tables should not be embedded in the text file in picture format.

7. ***Units and symbols***. Use metric units. Physical measurements should be in accordance with the Système International d'Unités (SI), *e.g*. mm, mm^3 , s, g, μg, m s^{-1}, g l^{-1}. Use joules not calories. Authors will find the following two publications helpful: *British Standard 1991: Part I: 1967 Recommendations for Letter Symbols, Signs and Abbreviations and Units, Symbols and Abbreviations. A Guide for Biological and Medical Editors and Authors (Baron, D.N., ed.) published by the Royal Society of Medicine, London.*

In mathematical expressions, single letters (italics) should be used for variables, qualifying them with subscripts (not italics) if required, e.g. length L, fork length L_F, standard length L_S,

index *I*, gonado-somatic index I_G, hepato-somatic index I_H, etc. The 24 hour clock should be used for time of day, *e.g.* 1435 hours, not 2.35 p.m. Calendar dates should be as, *e.g.* 15 June 1998. In the text, one-digit numbers should be spelt out unless they are used with units of measure (in which case they should not be hyphenated), *e.g.* five boxes, 5 cm. Numerals should be used for all numbers of two or more digits, *e.g.* 34 boxes. Use mass(es) rather than weight(s). Means and error (S.D., S.E., 95% C.L., *etc*.), should be to the same number of decimal places. Salinity is dimensionless with no units; do not use psu, ‰ or similar.

8. ***Statistics***. Present statistics as follows: name of test, test statistic with associated degrees of freedom (d.f.; note that an *F*-distribution has TWO d.f. values) and probability level (*P*). If data conform to all the assumptions of the statistical method used, precise *P*-values can be given, otherwise *P*-values should be >0.05, 0.05, 0.01 and 0.001. The *P*-values given by statistical packages assume that all the assumptions of the statistical method are fully met. Although ANOVA and regression are robust, the real *P*-values are likely to be different from the values printed by the package, because of violations of the assumptions. Provide confidence intervals (95% C.I.) for parameters estimated by ANOVA and regression analysis.Contributors to the*Journal of Fish Biology* should read the Editorial on reporting statistical results in *Journal of Fish Biology* **78,** 697-699 (2011) (available here)

9. ***Species nomenclauture***. On first mention of a species name in the main text, the common name of the species, if one is available, followed by the scientific species name (Latin binomial name, in italics) with the describing authority and date of authorship must be given. The common name should not be separated from the scientific name by a comma nor should the species name be in parentheses. The describing authority and date of authorship should not be separated by a comma. For example: the rainbow trout *Oncorhynchus mykiss* (Walbaum 1792); NOT, the rainbow trout, [*Oncorhynchus mykiss* (Walbaum, 1792)]. First use of species names in the title and Abstract should include common and scientific names as above, but do not require the describing authority and date of authorship.

Use standard sources for species common names, including: Wheeler, A. (1992). A list of the common and scientific names of fishes of the British Isles. *Journal of Fish Biology* **41**(Supplement A) (for British fishes); Wheeler, A.C., Merrett, N.R. & Quigley, D.T.G. (2004). Additional records and notes for Wheeler's (1992) *List of the Common and Scientific Names of Fishes of the British Isles*. *Journal of Fish Biology* **65**, Supplement B (for British fishes); Nelson, J.S., Crossman, E.J., Espinosa-P´erez, H., Findley, L.T., Gilbert, C.R., Lea, R.N. & Williams, J.D. (2004). *Common and scientific names of fishes from the United States, Canada, and Mexico*. Committee on Names of Fishes. 6th edn. Bethesda, MD, U.S.A.:

American Fisheries Society (for North American fishes; except those covered above for British fishes); Froese, R. & Pauly, D. (Eds) (2010). FishBase. World Wide Web electronic publication. www.fishbase.org.; *FAO Guides for Fisheries Purposes*.

When first using scientific species names the describing authority name appears in parentheses only if the binomial combination of the name has changed since the original description.*Oncorhynchus clarkii* (Richardson 1836) for example, includes the authority name in parentheses because Richardson initially described the species in the genus *Salmo*, under the name *Salmo clarkia*, whereas the name *Salmo marmoratus* Cuvier 1829 is currently recognized exactly as originally named by Cuvier. When the describing authority is Linnaeus, this should be abbreviated to L., e.g. *Cyprinus carpio* L. 1758. The citation for the original description of a species should not be included in the References unless additional specific details (*i.e.* more than just the species name) supplied by that publication are discussed in the manuscript. Use the online *Catalog of Fishes* as the standard authority for species nomenclature and date of description: Eschmeyer, W. N. (Ed.) *Catalog of Fishes* electronic version (5 January 2011).http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp After initial use of the species' common and scientific names, subsequent reference to the species should use the scientific name (without describing author or date) NOT the common name. The genus name should be abbreviated to a single letter (*e.g. C. carpio* and *O. mykiss*), except at the start of a sentence or where confusion may arise from multiple genera with the same first letter.

When listing synonyms for a species, the following style is required [based in part on Mincarone & Fernholm *Journal of Fish Biology* (2010) **77**, 779–801]:

Eptatretus cirrhatus (Forster 1801)

Homea banksii Fleming 1822: 375 (original description; type locality: South Seas; holotype: unknown)

Bdellostoma heptatrema Müller 1836: 79 (original description; type locality: South seas; holotype: unknown)

*Bdellostoma forster*i Müller 1836: 80 (original description; type locality: Queen Charlotte Sound, New Zealand; holotype: unknown). Conel, 1931: 76 *Bdellostoma forsteri* var. *heptatrema*. Müller, 1838: 174 (new combination) *Bdellostoma cirrhatum*. Günther, 1870: 511 (in part). Hutton, 1872: 87 (in part). Putnam, 1874: 160 (in part). Günther, 1880: 27

(Note that species names that are modifications of an existing binomial, rather than an original description, are separated from the author name by a full stop, *Bdellostoma cirrhatum*.

Günther, 1870: 511 (in part).

The plural ‘fish’ should be used for the same species, ‘fishes’ for more than one species. Any specimens used for taxonomic analyses should, wherever possible, be deposited in appropriate scientific collections (*e.g.* museums and university collections, or private collections when there is good evidence that these are adequately maintained), with identifying catalogue numbers, so that they are accessible to the scientific community for subsequent examination and taxonomic revision. N**amebearing type specimens of taxa that are described in the *Journal of Fish Biology* as new to science must be deposited in recognized national or international institutions that can meet Recommendations 72F.1-5 of the International Code of Zoological Nomenclature (ICZN, 1999; available here) for institutional responsibility**. The chosen institute for deposition of name-bearing type specimens should be able to meet these responsibilities into the foreseeable future. A paratype series may be distributed among more than one recognized national or international institution at the discretion of the authors. This is encouraged for paratype series that include numerous specimens, where the paratype series can be split into two or more representative samples, comprising several specimens that are deposited at different institutions. For examples of recognized national or international institutions see earlier taxonomic publications in the *Journal of Fish Biology*, or check institutions listed in Eschmeyer’s*Catalog of Fishes Online* (available here), and see Poss & Collette, *Copeia* **1995**, 48- 70, for U.S. and Canadian institutions. Institutional abbreviations used in manuscripts should follow standard code designations as given in Eschmeyer’s *Catalog of Fishes Online* (see link above). Contributors to the *Journal of Fish Biology* should read the Editorial on correct nomenclature in*Journal of Fish Biology* **78,** 1283-1290 (2011) (available here)

10. ***Genetic nomenclature***. The *Journal* uses the zebrafish system (see http://zfin.org/zf info/nomen.html) for genes and proteins of fish origin. Genes should be in italic lower case text and proteins in non-italic lower case text with the first letter capitalized. If the genes and proteins are of human origin, use the human nomenclature, with genes in upper case italic text and proteins in upper case non-italic text. Contributors to the *Journal of Fish Biology* should read the Editorial on correct nomenclature in *Journal of Fish Biology* **78,** 1283-1290 (2011) (available here)

11. ***Sequence data***. Manuscripts containing novel amino acid squences (*e.g.* primer sequences) will only be accepted if they carry an International Nucleotide Sequence Databases (INSD) accession number from the European Biology Laboratory (EMBL), GenBank Data Libraries (GenBank) or DNA Data Bank of Japan (DDBJ). The *Journal of*

Fish Biology strongly recommends that when authors deposit data in genetic data banks they include specimen catalogue numbers (for specimens preserved in collections), a note identifying sequences that are derived from type specimens (see 9) and collection locality data. The data base accession number must be given in the Materials and Methods section of the manuscript. For taxonomic papers that refer to sequences derived from specimens preserved in collections (see 9), authors should include a table that clearly links each sequence accession number with the specimen from which it was derived. Sequences from type specimens should also be clearly identified in this Table (***e.g.*given in bold text**). A nomenclature for genetic sequences for type and some non-type specimens has been proposed by Chakrabarty *et al*. (2013) [Chakrabarty, P., Warren, M., Page, L., Baldwin, C. (2013). GenSeq: An updated nomenclature for genetic sequences and a formal ranking of sequences from type and non-type sources. *Zookeys* **346**, 29–41, doi: 10.3897/zookeys.346.5753] and may be used (but is not obligatory): sequences from holotypes are identified as genseq-1, paratypes genseq-2, those from topotypes are genseq-3, and the genetic marker(s) used are incorporated into the nomenclature (*e.g.* genseq-2 ND2). Lengthy nucleotide sequences will only be published in the text if, in the judgement of the Editor-in-Chief, these results are of general interest and importance. **Where sequences are already published, reference to the original source will suffice.**

12. ***RAPD***. Data derived by RAPDs (randomly amplified polymorphic DNAs) technology are frequently not satisfactory and conclusions derived from them unreliable. Papers submitted to the*Journal* should not include data generated by this technique.

13. ***Acknowledgement of copyright***. Authors should obtain permission from the copyright owner (usually this is the publisher) to use any figure, table or extended quotation from material that has previously been published. Acknowledgements, however, should cite the author: ‘Reproduced with permission from Einstein (1975)’.

14. ***References*.** The list of references should be arranged alphabetically according to the surname of the first author and set out as follows:

Boisvert, C. A. (2005). The pelvic fin and girdle of *Panderichthys* and the origin of tetrapod locomotion. *Nature* **438**, 1145–1147.

Nagahama, Y., Yoshikuni, M., Yamashita, M., Tokumoto, T. & Katsu, Y. (1995). Regulation of oocyte growth and maturation in fish. In *Current Topics in Developmental Biology*, Vol. 30 (Pederson, R. A. & Schatten, G., eds), pp. 103–145. San Diego, CA: Academic Press.

Zar, J. H. (1999). *Biostatistical Analysis*, 4th edn. Upper Saddle River, NJ: Prentice Hall.

It is important to include the article's Digital Object Identifier (DOI) (see section 24) in the reference as volume and page information is not always available for articles published online. Please note the following example:

Song, J., Mathieu, A., Soper, R. F. & Popper, A. N. (2006). Structure of the inner ear of bluefin tuna Thunnus thynnus. *Journal of Fish Biology* **68**, 1767–1781.doi:10.1111/j.1095-8649.2006.01057.x

The order in the list should be:

(i). Single authors. Where more than one reference is given for a single author the publications should be listed chronologically.

(ii). Two authors. These should be arranged first alphabetically, then chronologically. For text citations, use the names of both authors and the year. Do not use *et al*. for two-author references.

(iii). Three or more authors. These should be arranged chronologically. For all text citations, use the surname of the first author only, followed by *et al*. and the date.

If more than one reference by the same author(s) published in the same year is cited, use *a*, *b*,*etc*. after the year in both text and list, *e.g*. (1963*a*). Text citations can be given in either of two ways: (a) with date in parentheses, 'as demonstrated by Jones (1956)'; (b) with names and date in parentheses, 'according to recent findings (Jones, 1956)'. **Where more than one reference is cited in the text these should be in chronological order**, *e.g*. Smith, 1975; Arnold, 1981; Jones, 1988. **Journal titles must be given in full**. Provide names and initials of **all** authors, the full title of the paper, the volume number and the page numbers.. **Authors should check that all citations in the text are in the list of references and *vice versa***, and that their dates match. Journal titles, book titles and any other material within the reference list which will be italicized in print should be italicized or underlined in the manuscript.

References must be available in the public domain, *e.g*. 'do not include grey' literature.

List electronic references separately, under the heading **Electronic References,** and set out as follows:

ICES (2001). Report of the Northern Pelagic and Blue Whiting Fisheries Working Group. *ICES CM 2001/ACFM:17*. Available at http://www.ices.dk/reports/acfm/2001/wgnpbw/wgnpbw01.pdf (last accessed 6 April 2010).

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17. ***Reviews***. Reviews should be concise, critical and creative. They should seek to stimulate topical debate and new research initiatives. Prospective authors are asked to submit a synopsis (two pages maximum) of their paper to an Associate Editor. The Editor-in-Chief can be consulted to advise on the appropriate Associate Editor to be approached. The synopsis should outline why the review is topical, its main points and objectives, and how it will stimulate debate and research. When the proposal has been accepted by an Associate Editor, he or she will invite the author to submit a manuscript, following the Instructions for Authors, within an agreed time limit.

18. ***Brief Communications***. A Brief Communication may be concerned with any subject within the scope of the *Journal of Fish Biology* but should be **confined to a single point or issue of progress**, such as an unusual occurrence, an interesting observation, or a topical and timely finding. The manuscript must, however, have some relevance beyond the species or locality under consideration. To qualify for inclusion as a Brief Communication a paper **must be short (five printed pages maximum; c. 2500 words)**. An abstract of not more than three sentences is required. **No subheadings or subdivisions should be included**. In other respects submitted manuscripts should comply with the instructions given above.

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publication. Include title page, key words (note no Abstract), main text and references (maximum four) (no tables or figures).

20. ***Occasional Comments***. Comments concerning recent published papers in the *Journal* may be considered by the Editor-in-Chief. The comments will be sent to the original authors to provide an opportunity for reply. Publication of the Comment and Reply will end the debate.

21. ***Acceptance of papers***. Papers will normally be critically reviewed by two or more independent experts in the relevant discipline and evaluated for publication by the Editors, but the Editors may return to authors without review any manuscripts deemed to be of inadequate quality or inappropriate for the *Journal of Fish Biology*. The final decision to accept a paper will be made by the Editor-in-Chief.

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