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CRESCIMENTO, RESPOSTA FISIOLÓGICA E QUALIDADE DE FILÉ DE JUVENIS DE BEIJUPIRÁ (*Rachycentron canadum*) SUBMETIDOS A DIFERENTES DENSIDADES DE ESTOCAGEM

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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

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Orientador

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Victor Andrade da Silva

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Dedicatória

A Deus, aos meus pais Jerônimo Gomes e Amara Andrade e a todos os meus familiares por terem me conduzido a um caminho de grandes realizações e de felicidades

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Resumo

A densidade de estocagem é amplamente reconhecida como um fator crítico em aquicultura, uma vez que altas densidades representam uma potencial fonte de estresse crônico. Este estudo avaliou a resposta fisiológica, desempenho zootécnico e qualidade de filé de juvenis de beijupirá (*Rachycentron canadum*) submetidos a um estressor crônico, como altas densidades, visando à definição de condições de criação que levem à melhoria do bem estar animal e ao incremento da produtividade e qualidade do produto final. Os peixes foram testados por um período de 42 dias, em que juvenis de 46,32 ± 0,22 g (média ± erro padrão) foram aleatoriamente distribuídos em três densidades (3,30 ± 0,02; 6,67 ± 0,03; 13,15 ± 0,16 g L^{-1}), e em três repetições. Os tratamentos foram designados como baixa densidade (LD; 5 peixe/tanque), densidade média (MD; 10 peixe/tanque), e alta densidade (HD; 20 peixes/tanque), respectivamente. As concentrações de cortisol e lactato e a osmolalidade do plasma sanguíneo não foram afetadas pela densidade de estocagem, e seus valores estavam de acordo com as faixas basais previamente relatados para juvenis de beijupirá. No entanto, o ganho de peso e a taxa de crescimento específico foram significativamente maiores no grupo LD. A densidade de estocagem também influenciou o início e o desenvolvimento do rigor mortis. Juvenis de beijupirá criados na alta densidade apresentaram menor taxa de crescimento e qualidade de filé. Os resultados sugerem que juvenis de beijupirá criados nas altas densidades foram capazes de contrapor o estresse ao nível de composição do plasma. Contudo, energia da dieta e/ou reservas do corpo podem ter sido desviadas do crescimento como resposta à alta demanda metabólica causada pelo estresse. Dessa forma, o presente estudo sugere que juvenis de beijupirá devem ser mantidos em uma biomassa final de até 15 kg m^{-3}, aproximadamente para garantir máximo crescimento e melhor qualidade do produto final. Aparentemente, este estudo é o primeiro a abordar a influencia de um estresse crônico na resposta fisiológica e qualidade de filé de juvenis de beijupirá. Os resultados podem fornecer informações para avanços na criação dessa espécie, conduzindo a melhorias no bem estar animal, na qualidade do produto final e no aumento da produtividade.

Palavras-chave: Estresse crônico, rigor mortis, exsudação, qualidade de filé.

Abstract

Stocking density is recognized as a critical factor in aquaculture, since high densities represent a potential source of chronic stress. This study investigated the effects of a chronic stressor, such as high stocking densities, on growth, physiological responses, and flesh quality of juvenile cobia (*Rachycentron canadum*) tested for a period of 42 days. The trial was conducted in a recirculating aquaculture system equipped with tanks of 70 L tanks. Cobia of 46.32 ± 0.22 g (mean ± SEM) were randomly distributed into three stocking densities (3.30 ± 0.02; 6.67 ± 0.03; 13.15 ± 0.16 g L-1), and three replicates. Treatment groups were nominally assigned as low density (LD; 5 fish/tank), medium density (MD; 10 fish/tank), and high density (HD; 20 fish/tank), respectively. Plasma concentrations of cortisol, osmolality and lactate were not affected by density levels, and values were according with those reported as basal for juvenile cobia. However, growth rate were influenced by density levels, in which weight gain and specific growth rate were significantly higher in the LD group. Drip loss did not differ among density groups. However, stocking density influenced the onset and development of rigor mortis. Our findings indicated that rearing juvenile cobia at the high densities negatively affected growth rate and flesh quality. Results suggest that juvenile cobia reared at the high densities were able to counteract stress. However, energy from the diet and/or body reserves was diverted from growth as a response to the elevated metabolic demand caused by stress. In this regard, juvenile cobia should be maintained at a final biomass up to approximately 15 kg m^{-3} to ensure a maximal growth rate and an improved final product quality. To our knowledge, this is the first report assessing the influence of a chronic stressor on both physiological responses and flesh quality parameters in cobia. This may provide information to the advancement of cobia aquaculture, leading to improvements in fish welfare, final product quality and increased productivity.

Keywords: Chronic stress, rigor mortis, drip loss, flesh quality

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Sumário

1. Introdução

Nas últimas três décadas, a aquicultura mundial tem apresentado uma taxa de crescimento anual de 8,8%, sendo a atividade que mais cresce dentre os setores de produção animal (FAO, 2012a). Com o crescimento da indústria, produtores seguem uma tendência de maximizar a densidade de estocagem dos animais, visando o aumento nos níveis de produção. No entanto, altas densidades de estocagem podem provocar estresse, comprometendo não apenas o crescimento, mas também a reprodução, imunidade e bem estar dos peixes em cativeiro (ROWLAND et al., 2006; OBA et al., 2009), refletindo negativamente na produtividade e consequentemente na economia de empreendimentos aquícolas.

O beijupirá (*Rachycentron canadum*) é uma espécie emergente no cenário da aquicultura mundial, sendo apontado como um candidato à piscicultura marinha no Brasil (CAVALLI e HAMILTON, 2009). Em 2010, a produção mundial superou 40 mil toneladas (FAO, 2012b). Entre os principais países produtores dessa espécie se destacam a China e Taiwan. No Brasil, alguns estados já apresentam iniciativas de produção (CAVALLI et al., 2011; SAMPAIO et al., 2011). O beijupirá é comumente criado em duas fases. A primeira é realizada no continente, com as etapas de reprodução e larvicultura, enquanto que a segunda fase, denominada engorda, geralmente é realizada em gaiolas flutuantes instaladas em águas costeiras ou em mar aberto. Consequentemente, as opções de tratamento de doenças na fase de engorda são bastante restritas devido a questões logísticas e práticas, considerando principalmente a localização e eventualmente o tamanho das estruturas onde os peixes são criados. Por outro lado, a prevenção é a estratégia mais viável para o manejo de doenças na criação de beijupirá, e deve ser realizada de maneira a evitar a exposição dos peixes a situações estressantes (TRUSHENSKI et al., 2010). Deste modo, se torna imprescindível determinar a densidade de estocagem ótima nas fases iniciais da criação como forma de minimizar o estresse e, consequentemente, otimizar a produção.

Ashley (2007) comenta que a determinação da densidade ótima deve encontrar um ponto de equilíbrio entre a máxima produtividade e o mínimo de fatores que causem desordens fisiológicas e comportamentais nos peixes. Utilizando sistemas de recirculação de água, Liao et al. (2004) notaram que a densidade ótima para o beijupirá com peso entre 4 e 8 g seria de 370 peixes/m^3 ou cerca de 28 kg/m^3, enquanto Webb et al. (2007) não encontraram diferenças no crescimento quando peixes de quase 7 g foram criados durante 10 semanas em densidades variando de 40 a 440 g/m^3. No entanto, estudos sugerem que juvenis de beijupirá podem ser afetados por estressores associados à alta densidade em sistemas de recirculação (SCHWARZ et al., 2007) ou em gaiolas (BENETTI et al., 2008), em que infecções relacionadas ao estresse e infestações de *Pasteurella, Vibrio, Amyloodinium*, e *Benedenia spp*. são comuns (CHEN et al., 2001b; LOPEZ et al., 2002; CHI et al., 2003, KERBER et al., 2011).

A resposta primária ao estresse envolve o aumento de catecolaminas e cortisol (BARTON e IWAMA, 1991; BARTON, 2002). Esses hormônios induzem respostas secundárias que são caracterizadas por uma redução no glicogênio hepático, aumento nos níveis de glicose e lactato plasmáticos, assim como disfunção na osmoregulação. Portanto, o estresse pode levar a um maior consumo das reservas energéticas e esta relocação de energia metabólica pode interferir negativamente em outros processos fisiológicos, como crescimento, reprodução e imunidade (BARTON e IWAMA, 1991; WENDELAAR BONGA, 1997; MOMMSEN et al. 1999).

A literatura especializada ainda conta com poucos estudos tratando da resposta fisiológica do beijupirá ao estresse. Dois estudos (CNAANI e McLEAN, 2009; TRUSHENSKI et al., 2010) avaliaram a resposta de juvenis expostos a estressores experimentais agudos, como exposição ao ar ou ao baixo volume de água, e indicaram que os níveis de glicose, osmolalidade, cortisol e lactato no sangue aumentaram nas primeiras horas após a exposição aos estressores, retornando aos níveis normais em até 12 h.

Diversas espécies têm sido amplamente estudadas quanto às influências do estresse sobre a qualidade de filé, no entanto nenhuma informação se encontra disponível na literatura especializada a respeito do beijupirá. A maioria dos estudos tem abordado os efeitos de estressores agudos, que comumente podem ocorrer durante a despesca ou métodos de abate. Dois indicadores frequentemente utilizados para avalição da qualidade de filé são a perda de líquido por exsudação e o rigor mortis. Em diversas espécies, o aumento da atividade e ocorrência de estresse antes do abate, somado as subsequentes respostas endócrinas, podem resultar em uma rápida queda do pH muscular devido ao aumento de ácido lático oriundo do metabolismo anaeróbio do músculo. A redução do pH muscular resulta em um rápido início do rigor mortis, podendo causar uma redução na vida de prateleira e aumento na perda de liquido por exsudação (BERG et al., 1997; JERRETT et al., 1996; POLI et al., 2005).

A perda de líquido por exsudação, por sua vez, se refere a perdas de fluídos de conteúdo proteico do interior das células, denominado exsudato, podendo causar uma degeneração mais rápida do tecido muscular. Dessa forma, uma grande quantidade de exsudato pode influenciar a aparência, suculência, textura e sabor do filé, a qual, portanto, representa um fator de impacto comercial (KRISTOFFERSEN et al., 2007). Por outro lado, o rigor mortis é descrito como o primeiro processo post mortem que apresenta uma maior influencia sobre aparência e textura do filé. O início e desenvolvimento do rigor mortis são afetados por diversos fatores, como espécie, idade e tamanho dos peixes, procedimentos pré-abate e os métodos de abate (BERG et al., 1997; VAN DE VIS et al., 2003; POLI et al., 2005; SIMITZIS et al., 2013).

Dessa forma, o presente estudo avaliou a resposta fisiológica, desempenho zootécnico e qualidade de filé de juvenis de beijupirá submetidos a um estressor crônico, como altas densidades, visando a definição de condições de criação que levem à melhoria do bem estar animal e ao incremento da produtividade e qualidade do produto final.

2. Revisão de literatura

Atualmente, existem várias iniciativas de criação do beijupirá (*R. canadum*) em águas brasileiras, mais especificamente nos estados da Bahia, Espírito Santo, Pernambuco, São Paulo (CAVALLI et al., 2011) e Rio Grande do Norte. Apesar dessas iniciativas, a produção comercial de peixes marinhos no Brasil não tem registros nas estatísticas de produção de pescado (MPA, 2012).

O beijupirá é uma espécie pelágica e migratória de ampla distribuição geográfica, ocorrendo em águas tropicais e subtropicais em todos os continentes entre as latitudes de 32ºN e 28ºS, com exceção da porção leste do Oceano Pacífico (SHAFFER e NAKAMURA, 2006). Devido à ausência de uma vesícula gasosa, possui hábito natatório ativo similar ao observado nos tubarões de pequeno porte. Possui hábito alimentar predador, composto por peixes, crustáceos e, eventualmente bivalves (ARENDT et al., 2001) e lulas (FRANKS et al., 1996), embora no litoral Pernambucano sua alimentação seja composta por peixes ósseos demersais, ingerindo poucos crustáceos (DOMINGUES et al., 2007). O hábito alimentar indica, portanto, uma preferência alimentar carnívora associada à disponibilidade de alimento no ambiente natural ao longo do litoral.

Nos últimos anos, o beijupirá tem sido alvo de uma série de estudos, nos quais foi demonstrado ser um excelente candidato para a criação. Entre as principais características de interesse estão a alta taxa de crescimento (ARNOLD et al., 2002; LIAO et al., 2004), eficiência alimentar e bom valor comercial (CHOU et al., 2001; WANG et al., 2005), excelente qualidade de carne, ideal para a preparação de sashimi (CHEN, 2001[a]; CHOU et al., 2001; KAISER e HOLT, 2005), adaptação ao confinamento (SUN et al., 2006), relativa tolerância à baixas salinidades (FAULK e HOLT, 2006; RESLEY et al., 2006) e facilidade em desovar em cativeiro (ARNOLD et al., 2002; FAULK e HOLT, 2006).

Devido a estas características, diversos países têm demonstrado interesse em produzi-lo em escala comercial, sendo China e Taiwan os países que lideram a produção mundial, onde a criação ocorre de forma intensiva desde a década de 1990 (LIAO et al., 2004). Outros países também já figuram na lista de produtores de beijupirá, entre os quais Porto Rico e Vietnã (BENETTI, et al., 2007; NHU et al., 2011). Portanto, a maioria dos resultados positivos com a criação do beijupirá está concentrada no sudeste asiático, sendo que em Taiwan cerca de 80% das gaiolas são destinadas a criação do beijupirá (LIAO et al., 2004). É importante salientar que algumas regiões produtoras do sudeste asiático possuem um inverno rigoroso quando comparado ao Brasil. Segundo Yu e Ueng (2007), o beijupirá é criado em regiões com variação anual de temperatura de 15,5 a 30,5ºC, sendo o seu crescimento acelerado em temperaturas acima de 28ºC.

Em sistemas de criação em alto mar, o beijupirá pode crescer de 4 a 6 kg por ano (ARNOLD et al., 2002; CHOU et al., 2001; WANG et al., 2005) e até 10 quilos em um período de 12 a 14 meses (LIAO et al., 2004). Em Porto Rico, estudos mostraram que o peso final pode variar de 1,5 a 6 kg em 12 meses (BENETTI et al., 2007). O tamanho comercial no mercado mundial é de 6 a 8 kg, enquanto que no mercado interno de Taiwan há uma preferência por peixes variando entre 8 e 10 kg (MIAO et al., 2009).

A dieta utilizada é nutricionalmente balanceada, normalmente possuindo 45% de proteína bruta, de 15 a 16% de lipídio bruto e 11% de cinzas (CHOU et al., 2001; LIAO et al., 2004). No entanto, as dietas podem ser manipuladas para se obter uma maior concentração de lipídios na carne (GAYLORD e GATLIN, 2000), sem prejuízo na conversão alimentar aparente do beijupirá (CHOU et al., 2001). Apesar da exigência mínima de lipídio do beijupirá ser de 6% (CHOU et al., 2001), é possível utilizar uma dieta com maiores porcentagens de lipídio bruto (CRAIG et al., 2006). A maior vantagem do enriquecimento das dietas com lipídios está relacionada à presença dos ácidos graxos altamente insaturados (HUFAs), principalmente o EPA (ácido eicosapentanóico) e o DHA (ácido decosapentanóico), que juntos são responsáveis pela boa qualidade nutricional das dietas (WATANABE, 2002). O mercado japonês, por exemplo, exige um produto final com alta qualidade para seu consumo *in natura* na forma de sashimi (CRAIG et al., 2006), portanto sendo necessário uma suplementação de lipídios na dieta superior ao exigido pela espécie.

O fator de conversão alimentar (FCA) é relativamente baixo, variando entre 1,02 e 1,80, dependendo do tamanho dos peixes (SU et al., 2000), das condições do ambiente, principalmente a temperatura, e proporção de farinha de peixe utilizada nas dietas. Entretanto, normalmente são obtidos valores de FCA em torno de 2,0, com sobrevivência acima de 75% (BENETTI et al., 2007).

Na fase de berçário, as criações comerciais de beijupirá em Taiwan utilizam normalmente densidade de 13 peixes/m^3 (≈400 g/m^3), alcançando uma produtividade final de 8,4 kg/m^3 em um período que varia entre 4 e 5 meses de criação. Já na fase de engorda final, os peixes são transferidos para gaiolas maiores onde são criados com taxas de densidade de 2 peixes/m^3, alcançando uma produtividade de 14 kg/m^3 ao final de 6 a 8 meses de criação (LIAO et al., 2004). Em Porto Rico, a produtividade final numa criação em mar aberto variou entre 5 e 15 kg/m^3, mas o ganho de peso e a sobrevivência diminuíram nas densidades mais altas (BENETTI et al., 2010). A variabilidade na produtividade indica uma relação direta da densidade de estocagem inicial com o ganho de peso, mortalidade e conversão alimentar.

A densidade de estocagem é amplamente reconhecida como um fator crítico na aquicultura, uma vez que altas densidades podem representar uma potencial fonte de estresse nos peixes. A resposta primária ao estresse envolve o aumento de catecolaminas e cortisol (BARTON e IWAMA, 1991; BARTON, 2002). Esses hormônios induzem respostas secundárias que são caracterizadas por uma redução no glicogênio hepático, aumento nos níveis de glicose e lactato plasmáticos, assim como disfunção na osmoregulação. Portanto, o estresse pode levar a um maior consumo das reservas energéticas e esta relocação de energia metabólica pode interferir negativamente em outros processos fisiológicos, como crescimento, reprodução e imunidade (BARTON e IWAMA, 1991; WENDELAAR BONGA, 1997; MOMMSEN et al. 1999).

A literatura especializada ainda conta com poucos estudos tratando da resposta fisiológica do beijupirá ao estresse. Dois estudos (CNAANI e McLEAN, 2009; TRUSHENSKI et al., 2010) avaliaram a resposta de juvenis expostos a estressores experimentais agudos, como exposição ao ar ou ao baixo volume de água, e indicaram que os níveis de glicose, osmolalidade, cortisol e lactato no sangue aumentaram nas primeiras horas após a exposição aos estressores, retornando aos níveis normais em até 12 h.

Espécies como salmão do Atlântico, robalo europeu e pargo europeu são amplamente estudadas quanto as influencias do estresse sobre a qualidade de filé (ROTH et al., 2006; BAGNI et al., 2007; MØRKØRE et al., 2008), mas no entanto nenhuma informação se encontra disponível na literatura especializada a respeito do beijupirá. A maioria dos estudos tem abordado os efeitos de estressores agudos, que comumente podem ocorrer durante a despesca ou métodos de abate. Por exemplo, uma exaustiva atividade muscular antes do abate pode provocar uma drástica redução de ATP muscular e produção de ácido lático, este último resultante do excessivo consumo de glicogênio, e que provoca uma concomitante redução do pH muscular (BERG et al., 1997; JERRETT et al., 1996; POLI et al., 2005). Portanto, o aumento da atividade e o estresse antes do abate pode antecipar o desenvolvimento do rigor mortis, reduzir a vida de prateleira e aumentar a perda de liquido por exsudação.

Dessa forma, dois indicadores frequentemente utilizados para avalição da qualidade de filé são a perda de líquido por exsudação e o rigor mortis. A perda de líquido por exsudação se refere a perdas de fluídos de conteúdo proteico do interior das células, denominado exsudato, podendo causar uma degeneração mais rápida do tecido muscular. Dessa forma, uma grande quantidade de exsudato pode influenciar a aparência, suculência, textura e sabor do filé, a qual, portanto, representa um fator de importância comercial (KRISTOFFERSEN et al., 2007). Por outro lado, o rigor mortis é descrito como o primeiro processo post mortem que apresenta uma maior influencia sobre aparência e textura do filé. O início e desenvolvimento do rigor mortis são afetados por diversos fatores, como espécie, idade e tamanho dos peixes, procedimentos pré-abate e os métodos de abate (BERG et al., 1997; VAN DE VIS et al., 2003; POLI et al., 2005; BAGNI et al., 2007; SIMITZIS et al., 2013).

Nenhuma informação está disponível na literatura especializada a respeito dos efeitos de estressores crônicos sobre as respostas fisiológicas e qualidade do filé do beijupirá. Dessa forma, o presente estudo investigou a resposta fisiológica, desempenho zootécnico e qualidade de filé de juvenis de beijupirá submetidos a diferentes densidades, visando a definição de condições de criação que levem à melhoria do bem estar animal e ao incremento da produtividade e qualidade do produto final.

3. Referências bibliográficas

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4. Artigo Científico

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Effects of stocking density on growth, physiological responses and flesh quality in juvenile cobia (*Rachycentron canadum*)

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Abstract

Stocking density is recognized as a critical factor in aquaculture, since high densities represent a potential source of chronic stress. This study investigated the effects of stocking density on growth, physiological responses and flesh quality of juvenile cobia (*Rachycentron canadum*). The trial was conducted for 42 days in a recirculating aquaculture system equipped with 70-L tanks. Cobia of 46.32 ± 0.22 g (mean ± SEM) were randomly distributed into three stocking densities (3.30 ± 0.02; 6.67 ± 0.03; 13.15 ± 0.16 g L^{-1}), and three replicates. Treatment groups were nominally assigned as low density (LD; 5 fish/tank), medium density (MD; 10 fish/tank), and high density (HD; 20 fish/tank), respectively. Plasma concentrations of cortisol and lactate, and osmolality were not affected by density levels, and values were according with those reported as basal for juvenile cobia. Weight gain and specific growth rate were significantly higher in the LD group. Drip loss had no significant difference among density groups, but stocking density influenced the onset and development of rigor mortis. Our findings indicated that rearing juvenile cobia at the high densities negatively affected growth rate and flesh quality. Juvenile cobia reared at the high density were able to cope with stress in terms of plasma composition, but energy from the diet and/or body reserves were probably diverted from growth as a response to the elevated metabolic demand caused by stress. Juvenile cobia should be maintained at a final biomass of up to approximately 15 kg m^{-3} to ensure a maximal growth rate and an improved final product quality. To our knowledge, this is the first report assessing the influence of a chronic stressor on both physiological responses and flesh quality parameters in cobia.

Keywords: Fish, chronic stress, hematology, rigor mortis, drip loss.

1. Introduction

In the last three decades, aquaculture has expanded at an average annual rate of 8.8% (FAO, 2012). As the aquaculture industry grows, farmers tend to increase fish stocking densities in order to achieve higher production levels. However, increased stocking densities may induce stress, and lead not only to reduced survival and impaired growth rates, reproduction and immune status, but ultimately affect the profitability of the activity.

High stocking densities in fish grown in captivity represents a potential source of chronic stress, and it is widely recognized as a critical factor in aquaculture. Increases in the concentrations of plasma catecholamines and cortisol are described as primary responses to stress in fish. These hormones lead to secondary responses, which involve metabolic energy demand to cope with stress. Decrease in hepatic glycogen, and increase in concentrations of plasma glucose and lactate, as well as a temporary dysfunction in osmoregulation are described as secondary stress responses. Thus, stress may induce energy consumption, which negatively interferes on growth, reproduction and immunity (Barton & Iwama, 1991; Wendelaar Bonga, 1997; Mommsen et al., 1999; Barton, 2002). Therefore, the recognition of stress status is critical to ensure fish welfare and the success of any aquaculture operation. Many quantitative parameters, such as growth rate, condition factor, hepatosomatic index and blood composition (concentration of plasma cortisol, osmolality and lactic acid), may be used as indicators of stress in fish (Barton & Iwama, 1991; Montero et al., 1999).

Cobia (*Rachycentron canadum*) is an emerging species for aquaculture. In 2010, world production was estimated at around 40,000 MT (FAO, 2013). The main producing areas are in China and Taiwan, but several other countries in the Americas and Asia have demonstrated interest in cobia aquaculture. Commercial culture is basically divided in two major steps. The first one occurs in land-based rearing systems, where early juveniles are produced. In the second step, known as grow-out, fish are usually reared in cages placed in coastal or open sea waters. Under these conditions, disease treatment options are restricted due to practical and logistic limitations. As a result, strategies to avoid or minimize stress exposure and hence reduce the occurrence of diseases and parasites are an important management strategy.

Determining the optimum fish stocking density is one way to diminish stress. In this regard, it is essential to establish a balance between increasing productivity and minimizing factors that may lead to physiologic and behavior disorders (Conte, 2004; Ashley, 2007). Liao et al. (2004), Webb et al. (2007) and Benetti et al. (2010) evaluated the influence of stocking density on growth performance, but none of them considered the effects on the physiological responses of cobia. Currently, only two studies considering the effects of acute stress on cobia are available. Cnaani & McLean (2009) assessed the responses of juvenile cobia exposed to an experimental stressor (air exposure) over a period of 24 hours, while Trushenski et al. (2010) evaluated the hematological responses of cobia exposed to low water and air exposure over a 72-hour time frame.

While the effects of stress on flesh quality have been assessed in several fish species, again no information is available for cobia. Most studies have addressed the effects of acute stressors during pre-slaughter procedures or slaughtering methods. Exhaustive exercise and occurrence of severe stress during slaughter may result in a rapid drop in muscle pH due to the production of lactic acid derived from anaerobic metabolism of white muscle. Reduced muscle pH results in a rapid onset of rigor mortis, which may reduce shelf life, alter flesh texture and increase drip loss (Berg et al., 1997; Jerrett et al., 1996; Poli et al., 2005). Drip loss and rigor mortis are therefore two commonly used indicators of flesh quality. Accordingly, this study evaluated the growth performance, physiological responses and flesh quality of juvenile cobia exposed to a chronic stressor such as high stocking density.

2. Material and methods

The study was performed at the Virginia Tech-Virginia Seafood Agricultural Research and Extension Center (VSAREC) in Hampton, VA, USA. The trial was conducted for 42 days in a RAS containing 70-L tanks, fluidized-bed biofilters, bubble bead filters, UV sterilizers, immersion titanium heaters, protein skimmers for processing/removal of dissolved organics and small suspended solids, and a diffusion aeration system. Fish were fed a commercial carnivorous fish diet (Otohime, Marubeni Nisshin Feed Company, Japan; 48% protein, 13% lipids, 4 mm) twice daily to apparent satiation. Water temperature, salinity, and dissolved oxygen (DO) were monitored daily (YSI-85 series dissolved oxygen meter; YSI Inc., Yellow Springs, Ohio, USA). Total ammonia-, nitrite- and nitrate-nitrogen (Spectrophotometric analysis; Hach Inc., Loveland, Colorado, USA), pH (YSI pH100 meter; YSI Inc., Yellow Springs, Ohio, USA), and alkalinity (Bromocresol green methyl red titration method, Hach Inc., Loveland, Colorado, USA) were also quantified daily. All fish in this trial were obtained from the same spawn (F3 generation) to avoid eventual biological bias. Prior to stocking the system and beginning the trial, all fish had been fed the same commercial diet that was used throughout the trial.

Tank inflow rates were maintained constant at 12 L min^{-1}, photoperiod was kept on a 12:12 h light/dark cycle, and water quality conditions were maintained as follows (mean ± SEM): temperature = 26.11 ± 0.17 ºC; salinity = 17.6 ± 0.1 g L^{-1}; pH = 7.70 ± 0.03; dissolved oxygen =

6.17 ± 0.07 mg L^{-1}; total ammonia nitrogen = 0.73 ± 0.04 mg L^{-1}; nitrite-nitrogen = 0.31 ± 0.03 mg L^{-1}; nitrate-nitrogen = 35.56 ± 2.30 mg L^{-1} and alkalinity = 142.5 ± 3.20 mg L^{-1}.

Juvenile cobia of 46.32 ± 0.22 g (mean ± SEM) were randomly distributed into three stocking densities (3.30 ± 0.02, 6.67 ± 0.03, 13.15 ± 0.16 g L^{-1}; mean ± SEM), and tested in three replicates. Density groups were nominally assigned as low density (LD; 5 fish/tank), medium density (MD; 10 fish/tank), and high density (HD; 20 fish/tank), respectively.

2.1 Growth performance parameters

On day 42, all fish were counted and survival estimated. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (K), apparent feed intake (FI) and dress out (%) were estimated with the following formulae:

$$\text{WG }(\%) = 100 \times \frac{\text{average final weight} - \text{average initial weight}}{\text{average initial weight}}$$

$$\text{SGR }(\%\text{BW day}^{-1}) = 100 \times \frac{\log_e(\text{average final weight}) - \log_e(\text{average initial weight})}{\text{number of days}}$$

$$\text{FCR} = \frac{\text{average individual feed intake}}{\text{average individual weight gain}}$$

$$\text{K} = 1000 \times \frac{\text{individual weight}}{\text{individual lenght}^{3}}$$

$$\text{FI }(\%\text{BW day}^{-1}) = 100 \times \frac{\text{average individual feed intake}}{\frac{(\text{initial individual weight} \times \text{final individual weight})^{0.5}}{\text{days of feeding}}}$$

$$\text{Dress out }(\%) = 100 \times \frac{\text{skinless fillet weight}}{\text{whole body weight}}$$

2.2 Blood sampling

At the end of trial, feeding was suspended 24 h prior to the collection of blood. In each sampling, three fish per tank were netted in a single pass in order to minimize sampling effort and additive stress associated with repeat netting. Fish were immediately sedated until they could be safely handled in a 100 mg L^{-1} solution of tricaine methanesulfonate (MS-222, Finquel®; Argent Chemical Laboratories, Inc., Redmond, Washington, USA) in culture water. Blood samples were collected from the caudal vasculature using capillary blood collection (Safe-T-Fill®; Kabe Labortechnik, Germany). After samples were collected, fish were euthanized by single cranial pithing. All blood samples were collected within 5 min of capture and sedation to minimize the possibility of other confounding responses. Whole blood were centrifuged (10 min, 10 000 x *g*, 4ºC) and plasma samples were stored frozen (-80ºC) until shipment for later analysis of cortisol, lactate and osmolality.

After blood sampling, whole liver was dissected and weighed for calculation of hepatossomatic index (HSI), which was calculated following the formula $HSI=(W_{liver}/W_{body})x100$. Whereupon, W_{liver} represents liver weight, and W_{body} represents whole body weight. Subsequently, these fish were filleted and samples were collected from the fillet for drip loss assessment. Frozen plasma were packed in ice and transported to the Fisheries and Illinois Aquaculture Center (FIAC), in Carbondale, Illinois, USA, where samples were stored again at -80ºC prior to hematological and analysis.

2.3 Blood plasma analysis

Plasma samples were analyzed at FIAC, and the methodologies were the same as in Trushenski et al. (2010). Briefly, plasma cortisol concentrations were quantified using an immunoassay (Cortisol EIA, DRG International, Mountainside, NJ, USA) according to the manufacturer's instructions. Plasma osmolality concentrations were determined using a vapor pressure osmometer (Model 5100C; Wescor Incorporated, Logan, UT, USA) and plasma lactate concentrations were measured using human blood testing supplies (Accutrend® lactate meter; Roche, Mannheim, Germany).

2.4 Drip loss

All three fish per tank that had their blood collected were filleted, and a slice of approximately 10 g of muscle was sampled from the epixial part of the fillet, weighed (W_0), marked and wrapped in aluminum foil. Samples were stored at 4ºC for a period of 96 hours. Subsequently, samples were unwrapped and weighed again (W_f), and drip loss (DL) was calculated by the following formula: $DL = [(W_0 – W_f) / W_0] \times 100$.

2.5 Rigor mortis

Two fish from each tank were sampled for rigor mortis assessment. A commercial slaughter method was used. Fish were immersed in ice and water (3:1), and slaughtered by gill cutting. Rigor mortis was measured at 0, 1, 3, 5, 7, 9, 12, 24, 36, 48, 72 and 96 hours post mortem using Cuttingers Method (tail drop). The same fish were used for rigor mortis measurements at each time point. In between measurements, fish were maintained at 4ºC. Rigor index (Ir) was calculated by the following formula: $Ir = [(L0 - Lt) / L0] \times 100$ (Bito et al., 1983). Whereupon, L represents the vertical distance between a table surface and the caudal fin base, when half of the fish fork length is placed on the edge of a table. Therefore, L0 represents measurements at time zero, whereas Lt represents subsequent measurements throughout the period of time described above. In this regard, Ir = 100% indicates full rigor.

2.6 Statistical analysis

Although multiple fish were sampled from each tank, replicate tanks served as the experimental units for all statistical analyses (n=3). All production performance and plasma blood data, rigor mortis and drip loss were analyzed by one-way analysis of variance (ANOVA) using the Statistical Analysis System (version 9.1; SAS Institute, Cary, NC, USA) to determine the significance of difference among density treatment means. Duncan’s multiple-range test was then performed. Differences were considered significant at $p<0.05$.

3. Results

3.1 Growth performance and survival

Final weight, FCR, FI, HSI, K, and dress-out were not significantly different among density groups (Table 1). On the other hand, weight gain ($P = 0.04$) and SGR ($P = 0.045$) were significantly higher in cobia from the LD group than those from either MD or HD groups. Final fish biomass in the LD, MD and HD treatments increased to 15.04, 25.61 and 53.94 kg m^{-3}, respectively. No mortality was registered for fish from the LD and HD groups; however, two fish from the MD group jumped out of the tanks during the trial. Survival was not significantly different between treatments (Table 1)

3.2 Hematological responses

Mean (± SEM) plasma cortisol concentrations ranged from 23.28 to 83.89 ng mL^{-1}, and no significant differences were detected among density groups ($p=0.32$). Plasma osmolality ($p=0.06$) and the concentration of lactate ($p=0.92$) were not influenced by density levels, and values ranged from 366.78 to 390.33 mOsm kg^{-1} and 0.21 to 0.31 mmol L^{-1}, respectively.

3.3 Drip loss

Mean (± SEM) drip loss (%) ranged from 0.85 (± 0.21) to 1.74 (± 0.32). Although drip loss was not significantly different ($p=0.057$) among density groups, it seemed to be positively associated with stocking density (Figure 1).

3.4 Rigor mortis

At one hour post mortem, the rigor mortis index was significantly larger ($p=0.026$) in cobia from the HD group compared to those from the MD and LD groups (Figure 2), which indicates an earlier onset of rigor in juvenile cobia reared at the high density. Afterwards, no significant differences were detected among density groups within each time point. Nevertheless, the average peak for rigor mortis in fish from the HD group was in the range of 1 to 3 hours post mortem, whereas for cobia from the MD and LD groups it was in the range of 3 to 5 h.

4. Discussion

The increase in the concentrations of plasma cathecolamines and cortisol is the most common quantitative indicator of the primary stress responses in fish (Pickering, 1993; Barton, 2002). In this study, however, stocking density had no effect on the plasma cortisol concentrations in cobia after 42 days. Plasma cortisol concentrations may not increase in chronically stressed fish due to a feedback mechanism that causes a down regulation of the hypothalamic-pituitary-interrenal axis. Previous studies (Pickering 1993; Sumpter, 1997; Procarione et al., 1999) demonstrated that in instances of long term stress cortisol concentrations may eventually return to basal levels despite the continued stress, demonstrating habituation to the stressful event.

Temporary osmoregulatory dysfunction is a secondary effect of stress in fish, and increased osmolality would be expected in cobia reared in hypertonic solutions as well as under stress (Davis, 2006; Burkey et al., 2007, Trushenski et al., 2010). However, we found no differences in the osmolality among density groups, which were within values considered as basal for juveniles of this species (Trushenski et al., 2010). Similarly, plasma osmolality and the concentrations of cortisol and lactate did not differ during the a 14 day-long confinement of gilthead sea bream (*Sparus aurata*) at 6 kg m^{-3} and 26 kg m^{-3}, but differences in their hematology were evident after a subsequent exposure to an acute handling stress, indicating that fish experienced a chronic stress situation (Barton et al., 2005).

Increased plasma lactate concentrations is also a secondary response to stress. Although stocking density and plasma lactate correlated positively in previous studies (van de Nieuwegiessen et al., 2008, Costas et al., 2013), density had no effect on the concentration of lactate in juvenile cobia, and values were within a range considered as basal for this species (Trushenski et al., 2010). In the present study, plasma lactate concentrations are in line with those found in other fish species maintained at high densities (Barton et al., 2005; Herrera et al., 2009; Santos et al., 2010). However, Costas et al. (2013) have recently shown that a high protein diet may represent a metabolic advantage in stressful situations and may counteract immunosuppression in fish. These authors have found that a high (46%) protein diet decreased plasma lactate concentrations in Senegalese sole (*Solea senegalensis*) reared at high densities as well as induced improvements in its immune status. Thus, the relatively high protein diet used in the present study (48%) may also have influenced the present results.

The response of different fish species to the stress caused by increasing density levels varies according to the duration as well as the potency of the stressing factor (Santos et al., 2010). For example, wedge sole (*Dicologoglossa cuneata*) exposed to different stocking densities for a relatively short period of time (22 days) presented no significant differences in growth performance, yet plasma cortisol and osmolality levels were higher in fish at the highest density (Herrera et al., 2009). In contrast, hematological stress indicators (cortisol and proteinemia) and resistance to infection were not different in the European sea bass (*Dicentrarchus labrax*) reared for 63 days at increased density levels, but SGR and feed intake were lower in fish at the highest density (Sammouth et al., 2009). Santos et al. (2010) added that the evaluation of chronic stress is much more challenging than assessing acute stress. They argued that acute stress is often measured as a transient change in the metabolism in an attempt to counteract the stress event, which is not the case of long-term stressing factors.

Differences in weight gain (p=0.040) and SGR (p=0.045) in this study may not be overly substantial, but they do indicate that raising cobia juveniles at the high and medium densities impaired growth. Decreases in growth rate at increasing stocking densities were observed for several fish species (Canario et al., 1998; Ellis et al., 2002; Sammouth et al., 2009, Roque d'Orbcastel et al., 2010; Santos et al., 2010; Tolussi et al., 2010), including cobia (Liao et al., 2004;

Benetti et al., 2010). Although these negative effects may be due to poor water quality (Bianchini et al., 1996; Santos et al., 2010) and aggressive behavior (Ellis et al., 2002), this does not seem to be the case here as water quality variables were within levels considered appropriate to the development of cobia and no agonistic behavior was observed throughout the experimental period. Interestingly, final biomass in the LD group was around 15 kg m^{-3}, which is similar to what is usually achieved in commercial cobia operations in sea-cage systems (Liao et al., 2004, 2007; Benetti et al., 2010; Sampaio et al., 2011). As fish biomass in the HD and MD exceeded 15 kg m^{-3} during the experimental period, this suggests that the fish biomass may have reached a threshold after which growth would have been negatively affected.

Previous studies demonstrated that cortisol, lactate and osmolality are suitable indicators of acute stress in juvenile cobia (Cnaani & McLean, 2009; Trushenski et al., 2010). For example, Trushenski et al. (2010) analyzed the hematology of cobia exposed to the air and to low water level, and found that osmolality and the concentrations of cortisol, glucose and lactate increased within the first hour after exposure, but returned to basal levels up to 12 h. In this study, the chronic stress caused by stocking density had no effect in these indicators, and their concentrations were within values considered as basal for juvenile cobia. Therefore, if only the results of plasma indicators are considered, one may be led to the conclusion that juvenile cobia experienced no stress. On the other hand, the evaluation of growth performance indicates the opposite.

Studies with sea bass (*D. labrax*) demonstrated that stocking density had no effect on the concentration of plasma stress indicators at the end of the rearing period, but others stress parameters were affected (Di Marco et al., 2008; Santos et al., 2010). A possible explanation to this is the habituation to the stressful conditions by the reduction of physiological alterations during the time of exposure (Pickering 1993; Sumpter, 1997; Procarione et al., 1999; Jentoft et al., 2005; Basrur et al., 2010). The habituation of the stress response, however, does not exclude the effects on growth performance observed in the repeatedly stressed fish versus the unstressed fish (Jentoft et al., 2005). Accordingly, juvenile cobia from this study were able to cope with the chronic stressor, but this may have caused an important burden in its energy metabolism, since significant differences in growth performance were registered. Stress is an energy-demanding process to which fish usually respond through the mobilization of energy substrates, and this reallocation of metabolic energy negatively influences other physiological process, such as growth, reproduction and immunity (Barton & Iwama, 1991; Iwama, 1998; Mommsen et al., 1999). In *D. labrax (*Di Marco et al., 2008; Santos et al., 2010) and piabanha *Brycon insignis* (Tolussi et al., 2010), increased density levels impaired not only growth performance, but also their energy metabolism. Roque d'Orbcastel et al. (2010) found that increased density also reduced feed intake of *D. labrax*, but Santos et al. (2010) demonstrated that this was partially compensated by a decrease in energy requirements for maintenance. Accordingly, in the present study, differences in growth performance in cobia reared at increased density levels might have been caused by the differential partitioning of energy. It is possible that energy from the diet and/or body reserves was diverted from growth as a response to the elevated metabolic demand caused by stress.

Drip loss is a factor of commercial importance, since elevated levels may affect appearance, juiciness, texture and flavor of the fillet (Kristoffersen et al., 2007). Roth et al. (2006) reported that acute stressed fish had softer texture and an almost three-fold higher drip loss than rested fish. This was probably due to the physical stress of muscle fibrils or connective tissue, which may lead to the release of protease that, in turn, accelerates muscle degeneration. However, drip loss in this study was not affected by density, and values were within those reported for rested fish (Roth et al., 2006). Similar results were recorded for barramundi *Lates calcarifer* (Wilkinson et al., 2008) and king salmon *Oncorhynchus tshawytscha* (Fletcher et al. 2003), in which simulated stress during harvest procedures had no effect on drip loss of their fillets.

Rigor mortis is described as the first post mortem process that has a major influence on the appearance and structure of fish muscle (Berg et al., 1997; Wilkinson et al., 2008). Increased activity and severe stress prior to death may lead to an accelerated breakdown of ATP. Under this condition, fish use anaerobic energy, leading to a rapid drop of muscle pH due to an increased production of lactic acid. A reduction in muscle pH results in a rapid onset of rigor mortis (Berg et al., 1997; Jerrett et al., 1996; Poli et al., 2005; Bagni et al., 2007). The onset and development of rigor are hence affected by pre-slaughter procedures, and slaughtering methods. They are also influenced by others factors such as species, age and size of the specimen (Berg et al., 1997; Van de Vis et al., 2003; Poli et al., 2005; Bagni et al., 2007; Simitzis et al., 2013). In the present study, stocking density influenced the onset and development of rigor mortis, but the relation between the chronic stressor - stocking density - and flesh quality variables is not clear. No information concerning this topic is currently available on the literature.

Regardless of density level, time from slaughtering to the onset of rigor in cobia may be considered shorter when compared with other fish species exposed to acute stress events. Atlantic salmon (*Salmo solar*), sea bass (*D. labrax*) and sea bream (*S. aurata*) submitted to harvest procedures exhibited full rigor in the range of 6 and 24 h post-mortem (Roth et al., 2006; Bagni et al., 2007; Mørkøre et al., 2008). Nevertheless, our findings are similar to those in barramundi, in which fish submitted to acute stressing agents (air exposure and exercise prior to slaughter) during harvest procedures exhibited a significantly faster onset of rigor, with full rigor being registered 3 h post-mortem. However, as cobia is a tropical fish, differences in rearing temperature for this species and the previously mentioned ones as well as the different methods applied for rigor assessment in the different studies shall also be taken into account. In addition, the slaughter method in this study intended to simulate a commercial slaughter procedure usually applied for cobia. However, this method (Poli et al., 2005; Simitzis et al., 2013) and the one used for rigor measurement (Berg et al., 1997; Skjervold et al. 1999) may have contributed to the earlier onset and development of rigor in juvenile cobia.

In conclusion, stocking densities had no effect on the concentrations of hematological stress indicators, but growth rate and rigor mortis were influenced. Accordingly, juvenile cobia should be maintained at a final biomass up to approximately 15 kg m^{-3} to ensure fish welfare as well a maximal growth rate and an improved final product quality. To our knowledge, this is the first report assessing the effects of a chronic stressor on both physiological responses and flesh quality parameters in cobia. This may provide information to the advancement of cobia aquaculture, leading to improvements in welfare, final product quality and increased productivity.

5. Acknowledgements

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Table 1. Mean (± SEM) production performance parameters for cobia (*Rachycentron canadum*) reared at low (LD), medium (MD) and high densities (HD). Means with different letters are significantly different based on one-way ANOVA ($p<0.05$) followed by Duncan's multiple-range test.

	LD	MD	HD	*p*-value
Stocking density (fish L-^{1})	0.07	0.14	0.29	
Initial biomass (kg m^{-3})	3.30 ± 0.02^{a}	6.67 ± 0.03^{b}	13.15 ± 0.16^{c}	<0.001
Final biomass (kg m^{-3})	15.02 ± 0.38^{a}	25.61 ± 1.5^{b}	53.94 ± 0.73^{c}	<0.001
Initial weight (g)	46.27 ± 0.35	46.67 ± 0.24	46.03 ± 0.55	0.56
Final weight (g)	210.27 ± 5.27	192.01 ± 8.57	188.80 ± 2.55	0.09
Survival (%)	100 ± 0	93.3 ± 3.4	100 ± 0	0.36
Weight gain (%)	354.35 ± 7.95^{a}	311.30 ± 16.32^{b}	310.13 ± 1.45^{b}	0.04
SGR (% BW day^{-1})	3.60 ± 0.42^{a}	3.36 ± 0.09^{b}	3.36 ± 0.08^{b}	0.045
FCR	0.98 ± 0.01	1.04 ± 0.05	1.02 ± 0.01	0.42
Feed intake (% BW day^{-1})	3.88 ± 0.04	3.70 ± 0.05	3.70 ± 0.05	0.08
HSI	2.69 ± 0.08	2.57 ± 0.11	2.48 ± 0.05	0.28
K	6.83 ± 0.04	6.70 ± 0.21	6.61 ± 0.07	0.54
Dress-out (%)	36.93 ± 0.45	36.32 ± 1.30	35.92 ± 0.19	0.69

SGR = specific growth rate; BW = body weight; FCR = feed conversion ratio; HSI = hepatosomatic index; K = condition factor.

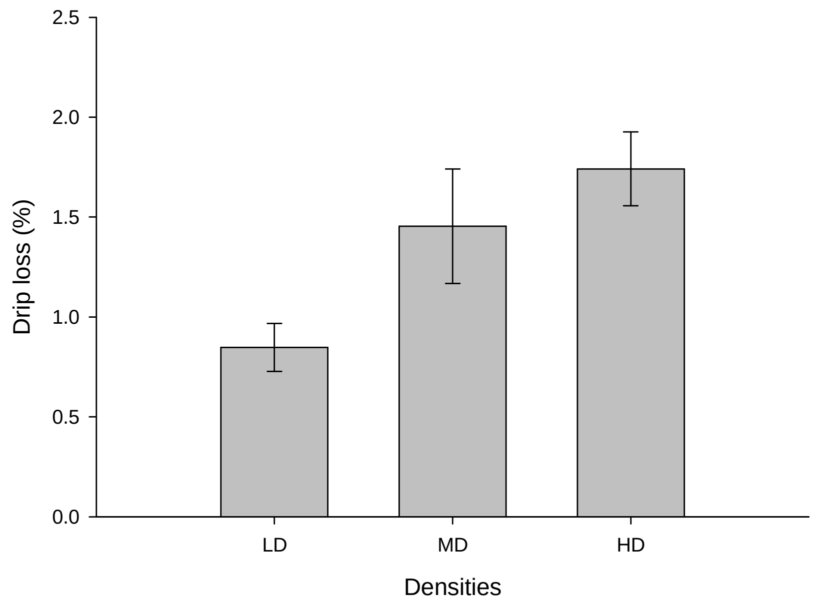

Figure 1. Mean (± SEM) drip loss (%) from flesh samples obtained from cobia (*Rachycentron canadum*) reared at low (LD), medium (MD) and high stocking densities (HD).

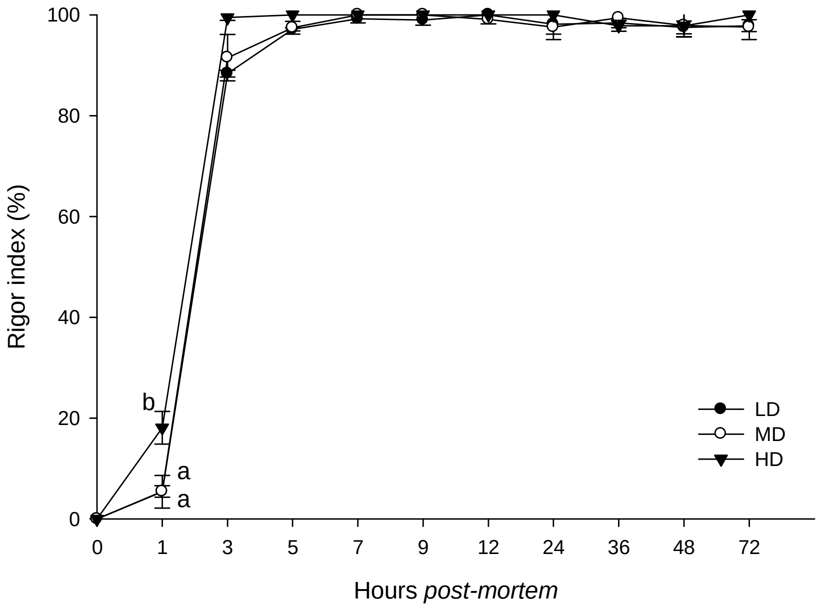

Figure 2. Mean (± SEM) rigor indices (%) for juvenile cobia (*Rachycentron canadum*) raised at low (LD), medium (MD) and high stocking densities (HD). Means with different letters are significantly different within each time point based on one-way ANOVA ($p<0.05$) with post hoc Duncan's multiple-range test.

5. Anexo (Normas para publicação na Aquaculture)

Introduction

Types of paper

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere. Articles are expected to contribute new information (e.g. novel methods of analysis with added new insights and impacts) to the knowledge base in the field, not just to confirm previously published work.
Review Articles can cover either narrow disciplinary subjects or broad issues requiring interdisciplinary discussion. They should provide objective critical evaluation of a defined subject. Reviews should not consist solely of a summary of published data. Evaluation of the quality of existing data, the status of knowledge, and the research required to advance knowledge of the subject are essential.
Short Communications are used to communicate results which represent a major breakthrough or startling new discovery and which should therefore be published quickly. They should not be used for preliminary results. Papers must contain sufficient data to establish that the research has achieved reliable and significant results.
Technical Papers should present new methods and procedures for either research methodology or culture-related techniques.
The *Letters to the Editor* section is intended to provide a forum for discussion of aquacultural science emanating from material published in the journal.
Contact details for submission

Papers for consideration should be submitted via the electronic submission system mentioned below to the appropriate Section Editor:
Nutrition:
D.M. Gatlin
The Nutrition Section welcomes high quality research papers presenting novel data as well as original reviews on various aspects of aquatic animal nutrition relevant to aquaculture. Manuscripts addressing the following areas of investigation are encouraged:
1) determination of dietary and metabolic requirements for various nutrients by representative aquatic species. Studies may include environmental/stress effects on animal's physiological responses and requirements at different developmental stages;
2) evaluation of novel or established feedstuffs as well as feed processing and manufacturing procedures with digestibility and growth trials. Such studies should provide comprehensive specifications of the process or evaluated ingredients including nutrients, potential anti-nutrients, and contaminants;
3) comparison of nutrient bioavailability from various ingredients or product forms as well as metabolic kinetics of nutrients, food borne anti-nutrients or toxins;
4) identification of key components in natural diets that influence attractability, palatability, metabolism, growth reproduction and/or immunity of cultured organisms;
5) optimization of diet formulations and feeding practices;
6) characterization of the actions of hormones, cytokines and/or components in intracellular signaling pathway(s) that influence nutrient and/or energy utilization.
7) evaluation of diet supplementation strategies to influence animal performance, metabolism, health and/or flesh quality.
Manuscripts concerning other areas of nutrition using novel or advanced methods are also welcome. Please note that in regard to various diet additives such as probiotics, prebiotics, herbal extracts, etc., a very large number of papers have already been published. Therefore, Aquaculture will not continue to accept manuscripts that present initial and preliminary investigations of such additives. Manuscripts addressing these and other feed additives will be accepted for review only if they are of the highest scientific quality and they represent a significant advance in our knowledge of the mechanisms involved in their metabolism. Manuscripts may also be considered if they present clinical efficacy data generated in large-scale trials and economic cost-benefit analysis of these applications.
Aquaculture Production Science:
B.Costa-Pierce
AQUACULTURE PRODUCTION SCIENCE (PS) is one of 5 sections of the international journal AQUACULTURE dedicated to research on improvements and innovations in aquatic food production.
worldwide dissemination of the results of innovative, globally important, scientific research on production methods for aquatic foods from fish, crustaceans, mollusks, amphibians, and all types of aquatic plants. Improvement of production

systems that results in greater efficiencies of resource usage in aquaculture. Effective applications of technologies and methods of aquaculture production for improved stocking regimes, the use of new species and species assemblages, and research on the efficient and sustainable usage of system space with the objective of minimizing resource usage in aquaculture. Investigations to minimize aquaculture wastes and improve water quality, technologies for nutrient recycling in aquaculture ecosystems, and the synergy of aquaculture and other food production systems using methods such as polyculture and integrated aquaculture.

Physiology and Endocrinology:
Fish: A. P. (Tony) Farrell
Invertebrate: J. Benzie
The Physiology Section welcomes high quality papers that present both novel research data and original reviews, on all aspects of the physiology of cultured aquatic animals and plants. Their content must be relevant to solving aquaculture problems.
Submitted manuscripts must have a valid hypothesis or objective, clearly state the relevance to aquaculture, have proper experimental design with appropriate controls and utilize appropriate statistical analysis. Mention of trade names is limited to the main text.
Relevant physiological topics include, but are not limited to:
- Reproductive physiology, including: Endocrine and environmental controls development; Induced ovulation and spermiation; Gamete quality, storage and cryopreservation; control of sex differentiation; Physiology and endocrinology of gynogenetic, triploid and transgenic organisms
- Molecular genetic assessment of physiological processes
- Larval physiology and ontogeny in relation to aquaculture, including metamorphosis, smolting (salmonids) and molting (crustacea)
- Nutritional physiology including endocrine and environmental regulation of growth
- Performance under variable culture conditions, including temperature optima and tolerances; Altered water quality and environmental variables; Stress and disease physiology; Rearing density
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