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Eventos morfológicos e padrões de crescimento durante a ontogenia das primeiras fases do ciclo de vida das espécies de bagres marinhos (Ostariophisii – Ariidae) no estuário do Rio Goiana (PE/PB–Brasil).

André Ricardo de Araújo Lima

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Dissertação apresentada ao programa de pós-graduação em oceanografia da Universidade Federal de Pernambuco, como parte dos requisitos para obtenção do grau de Mestre em Ciências na área de Oceanografia.

Orientador: Dr. Mário Barletta

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SUMÁRIO

PÁG

RESUMO

Esse estudo apresenta os eventos morfológicos durante a ontogenia das espécies *Cathorops spixii* (Agassiz, 1829) e *C. agassizii* (Eigenmann & Eigenmann, 1888), usando os padrões de crescimento como resposta às necessidades anatômicas no momento das transições morfológicas (embriogênese e metamorfoses). Os espécimes de *C. spixii* e *C. agassizii* foram coletados nas três áreas (superior, media, inferior) do canal principal do estuário do Rio Goiana. Ovos, embriões livres e juvenis (< 40 mm) foram retirados da cavidade bucal do macho incubador de ambas as espécies. O desenvolvimento dos bagres ariideos foi estudado desde um embrião logo após a neurulação até a formação de um juvenil. Em geral, embriões de *C. agassizii* possuem sacos vitelínicos mais pesados comparado com *C. spixii* (0.27g ± 0.01 e 0.22g ± 0.02, respectivamente). As descrições revelam que o aparecimento das vértebras e dos otólitos, bem como a ossificação dos otólitos e do aparelho de Weber ocorre primeiro em embriões de *C. agassizii*. Antes da eclosão os embriões de ambas as espécies apresentam o esqueleto axial e apendicular bem ossificado. As divergências morfológicas externas são mais visíveis em embriões livres. Embriões livres de *C. agassizii* possuem olhos maiores em diâmetro e barbilhões maxilares mais curtos quando comparado com *C. spixii*; e o focinho e a cabeça são mais longos. Embriões livres de *C. spixii* possuem nadadeiras peitoral, dorsal, pélvica e anal localizadas numa posição mais posterior comparado com *C. agassizii*. Embriões livres de *C. agassizii* também possuem vitelos maiores. Durante o período juvenil, o principal caráter morfológico que difere as espécies é o diâmetro do olho e o comprimento do barbilhão maxilar, maiores em *C. agassizii*. Os embriões crescem lentamente no sentido longitudinal, mas padrões de crescimento acelerado [alométrico positivos (β_0>1)] foram observados para a largura da cabeça e o diâmetro do olho. Isto parece estar relacionado com o rápido desenvolvimento dos órgãos sensoriais como os otólitos, o aparelho de

Weber, o cristalino, as narinas e os barbilhões. Após a eclosão, embriões livres incubados na cavidade bucal dos machos crescem isometricamente (β_0=1). Padrões de crescimento lento [alométrico negativo (β_0<1)] foram observados na largura da cabeça e no diâmetro do olho durante o período de saco vitelínico, considerando que os órgãos sensoriais já estão formados. Os ossos da cabeça são bem reconhecidos em embriões livres, que se assemelham com juvenis. O final do período de saco vitelínico é caracterizado por uma mudança direta de embrião livre para juvenil, sem um período larval verdadeiro. O período juvenil é caracterizado por padrões de crescimento próximos ao isométrico em todas as regiões do corpo, sugerindo que os juvenis são altamente desenvolvidos e apresentam a maioria das características de peixes adultos.

Palavras-chave: crescimento; ontogênese; estuário; embrião; cuidado parental; otophisi; Siluriformes.

ABSTRACT

This study presents an overview of the morphological events during the early ontogeny of the species *Cathorops spixii* (Agassiz, 1829) and *C. agassizii* (Eigenmann & Eigenmann, 1888), using growth patterns as response to anatomical needs in the moment of morphological transitions (embryogenesis and metamorphosis). Specimens of *C. spixii* and *C. agassizii* were taken in the three areas (upper, middle and lower) of the main channel of Goiana River estuary. Eggs, free-embryos and young-juveniles (< 4 cm) were collected from the buccal cavity of the mouth-brooding adult male of both species. The development and allometric growth of the ariid catfishes were studied from newly neurula embryo to juveniles. Generally, embryos of *C. agassizii* have a larger yolk mass compared with *C. spixii* (0.27g ± 0.01 e 0.22g ± 0.02, respectively). The descriptions revealed that vertebrae and otolith appearance and the ossification of the otoliths and Weberian apparatus occurs first in embryos of *C. agassizii*. In contrast, the external morphological divergence is stronger in free-embryos. Free-embryos of *C. agassizii* have larger eyes and shorter maxillary barbells when compared to *C. spixii*; and their snouts and head are longer. Free-embryos of *C. spixii* have pectoral, dorsal, pelvic and anal fins placed in a more posterior position compared with *C. agassizii*. Differences in head angle and width were not detected. Free-embryos of *C. agassizii* also have larger yolk-sacs. During the juvenile period the main morphological characters that differ between species are the eye diameter and the length of the maxillary barbells, reaching higher values in *C. agassizii*. Prior to hatching embryos of both species are well-developed with axial and appendicular skeleton well ossified. Embryos grow slowly longitudinally, but accelerated growth patterns [positively allometric (β_0>1)] were observed in head width and eye diameter. It seems to be related to the fast development of sensory organs such as otoliths, Weberian apparatus, lens, nostrils and barbells during the embryonic period. After hatching, mouth-brooded free-

embryos grow isometrically (β_0=1), except in the snout length that presents a fast growth pattern related to the end of the endogenous feeding. Slow growth patterns [negatively allometric (β_0<1)] were observed in head width and eye diameter during the yolk-sac period, take into account that sensory organs are already formed. Head bones are easily-recognizable in free-embryos resembling adult fishes. The end of the yolk-sac period is characterized by a direct change from free-embryo to a juvenile, without a true larva period. The juvenile period is characterized by growth patterns nearly to the isometric in all body regions, what suggests that juveniles of *C. spixii* are highly developed and resemble most characteristic of adult fishes.

Key words: growth; ontogenesis; estuary; free-embryo; parental care; otophysan; Siluriformes.

INTRODUÇÃO

Ontogenia se refere à descrição dos eventos morfológicos que ocorrem desde a fertilização até a formação de um individuo adulto. Maior parte das pesquisas em desenvolvimento ontogenético de peixes se restringe as descrições das primeiras fases de desenvolvimento, considerando que é neste período onde ocorrem as principais mudanças morfológicas (Fukuhara, 1988[1]). Esses estudos fornecem informações sobre como as mudanças de habitat, preferência alimentar, necessidades ecológicas e comportamento durante as fases iniciais estão relacionadas com a seqüência do desenvolvimento anatômico de órgãos e estruturas apendiculares (Koumoundouros *et al*., 2001[2]). O conhecimento da organogênese e dos padrões de crescimento é muito importante para compreender a biologia e as necessidades funcionais de uma espécie (Geerinckx *et al*., 2008[4]). Em bagres, por exemplo, o desenvolvimento precoce dos órgãos sensoriais reflete a grande adaptação dessas espécies a estuários tropicais e subtropicais. A ontogenia também pode auxiliar na comparação entre espécies que apresentam características morfológicas comuns mesmo durante as primeiras fases do ciclo de vida (Verreth *et al*., 1992[3]).

As espécies de bagres ariideos *Cathorops spixii* (Agassiz, 1829) e *C. agassizii* (Eigenmann & Eigenmann, 1888) são peixes marinhos de alta capacidade termohalina pertencentes à ordem Siluriformes (Ostariophysi), são encontrados habitando regiões estuarinas e procurando áreas menos salinas (lagoas e bocas de rios) no período de

[1]Fukuhara, O. (1988). Morphological and functional development of larval and juvenile *Limanda yokohamae* (Pisces: Pleuronectidae) reared in the laboratory. *Marine Biology* **99**, 271–281.
[2]Koumoundouros, G., Divanach, P., Kentouri, M. (2001). Osteological development of Dentex dentex (Osteichthyes: Sparidae): dorsal, anal, paired fins and squamation. *Marine Biology* **138**, 399–406.
[3]Verreth, J., Torreele, E., Spazier, E., Sluiszen, V., Rombout, J., Booms, R. (1992). The development of a functional digestive system in the African catfish Clarias gariepinus (Burchell). *Journal of the World Aquaculture* **Soc. 23**, 286–298.
[4]Geerinckx, T., Verhaegen, Y., Adriaens, D. (2008). Ontogenetic allometries and shape changes in the suckermouth armoured catfish *Ancistrus cf. triradiatus* Eigenmann (Loricariidae, Siluriformes), related to suckermouth attachment and yolk-sac size. *Journal of fish Biology* **72**, 803–814.

reprodução (Barletta *et al*., 2010[5]). Estão distribuídas na linha da costa Nordeste da America do Sul da Guiana até o Brasil (Marceniuk & Menezes, 2007[6]). Essas espécies, como os demais otophisi, são caracterizadas pela presença do aparelho de Weber, uma série de pequenos ossos e tendões que conectam a bexiga natatória e os otólitos do ouvido interno, permitindo uma melhor percepção de sons; e pelo cuidado parental, quando os machos incubam os ovos na cavidade bucal até que se complete o desenvolvimento dos juvenis (Burgess, 1989[7]).

A presença do aparelho de Weber e a incubação oral das fases iniciais de vida são considerados os principais eventos responsáveis pelo sucesso do grupo (Sanger & McCune, 2002[8]). Enquanto que para muitos peixes, os períodos críticos de desenvolvimento ocorrem durante as transições ecológicas e de desenvolvimento quando as larvas estão mais vulneráveis à influencias externas (Sifa & Mathias, 1987[9]), os bagres Ariideos possuem os principais órgãos sensoriais e a capacidade de forrageio quase completamente formados logo nos seus primeiros estágios. Isso sugere que embriões de bagres são altamente desenvolvidos antes que a alimentação externa seja iniciada, reduzindo consideravelmente a vulnerabilidade e tornando os bagres mais abundantes em estuários de regiões tropicais e subtropicais (Barletta & Blaber, 2007[10]).

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[5]Barletta, M. *et al*. (2010). Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. *Journal of Fish Biology* **76**, 2118–2176.

[6]Marceniuk, A. P. & Menezes, N. A. (2007). Systematics of the family Ariidae (Ostariophysi, Siluriformes), with a redefinition of the genera. *Zootaxa* **1416**, 01–126.

[7]Burgess, W. E. (1989). *An Atlas of Freshwater and Marine Catfishes. A Preliminary Survey of the Siluriformes*. T.H.F. Publications, Inc. Neptune City, N.J.

[8]Sanger, T. J. & Mccune, A. R. (2002). Comparative osteology of the *Danio* (Cyprinidae: Ostariophysi) axial skeleton with comments on *Danio* relationships based on molecules and morphology. *Zoological Journal of the Linnean Society* **135,** 529–546.

[9]Sifa, L. & Mathias, J. A. (1987). The critical period of high mortality of larvae fish. A discussion based on current research. *Chin. J. Oceanol. Limnol*. Vol 5. No. 1.

[10]Barletta, M. & Blaber, J. M. (2007). Comparison of fish assemblages and guilds in tropical habitats of the Embley (Indo-West Pacific) and Caeté (Western Atlantic) estuaries. *Bulletin of Marine Science* **80 (3)**, 647–680.

a Família Ariidae aparece como a mais abundante (Dantas *et al*., 2010[11]). Segundo os autores, para o canal principal desse estuário as espécies *C. spixii* e *C. agassizii* corresponderam a 53% da captura em número, e 63% em peso. No estuário do Rio Caeté (região tropical, Amazônia Oriental - norte do Brasil) (Barletta *et al*., 2005[12]) e no complexo estuarino de Paranaguá (transição entre as regiões tropical e subtropical, sul do Brasil) (Barletta *et al*., 2008[13]) a Família Ariidae também foi a mais abundante. Sendo assim, *C. spixii* foi apontado como possível espécie bioindicadora da qualidade ambiental e suas mudanças, devido a ser a mais abundante em número e peso, nos canais principais desses estuários (Barletta *et al*., 2005[12]; Barletta *et al*., 2008[13]).

A abundância destas espécies nos estuários do Rio Goiana indica a importância ecológica (espécie chave) dessas espécies neste local, enfatizando a necessidade de estudos sobre a sua ontogenia e ecologia, como ferramentas para a compreensão da função desses habitats como berçários. Além disso, a análise dos even tos morfológicos durante a ontogenia pode facilitar na diagnose específica durante os períodos embrionário e juvenil, uma vez que indivíduos de *C. spixii* e *C. agassizii* são morfologicalmente muito semelhantes, sobretudo durante as fases iniciais do ciclo de vida.

[11]Dantas, D. V., Barletta, M., Costa, M. F., Barbosa-Cintra, S. C. T.; Possatto, F. E., Ramos, J. A. A., Lima, A. R. A., Saint-paul, U. (2010). Movement patterns of catfishes (Ariidae) in a tropical semi-arid estuary. *Journal of Fish Biology* **76**, 2540–2557.
[12]Barletta, M., Barletta-Bergan, A., Saint-Paul, U., Hubold, G. (2005). The role of salinity in structuring the fishing assemblages in a tropical estuary. *Journal of Fish Biology* **66**, 1–28.
[13]Barletta, M., Amaral,C. S., Corrêa, M. F. M., Guebert, F., Dantas, D. V., Lorenzi, L., Saint-Paul, U. (2008). Factors affecting seasonal variations in the demersal fish assemblages at an ecocline in a tropical-subtropical estuary. *Journal of Fish Biology* **73**, 1314–1337.

OBJETIVO GERAL

Este trabalho tem por objetivo determinar os diferentes períodos e fases ontogenéticas e os padrões de crescimento das espécies *C. spixii* e *C. agassizii* de ovo a juvenil.

OBJETIVOS ESPECÍFICOS

1. Descrever os principais eventos morfológicos durante o desenvolvimento ontogenético de embriões (pré e pós-eclosão) e juvenis de *C. spixii* e *C. agassizii*.

2. Comparar os padrões de crescimento alométrico entre os diferentes períodos de desenvolvimento e relacioná-los com as necessidades anatômicas durante as transições morfológicas de cada espécie.

MATERIAL E MÉTODOS

As amostras foram coletadas nas três áreas (superior, intermediária e inferior) do canal principal do estuário do Rio Goiana (Nordeste – Brasil) entre 2005 e 2009 (Fig. 1). Os espécimes foram coletados com rede de arrasto (puxada por uma embarcação) seguindo a metodologia proposta por Barletta *et al.* (2005[12]) e Barletta *et al.* (2008[13]). Foi utilizada uma rede de 7,72 m de abertura e 8,72 m de comprimento (tralha superior

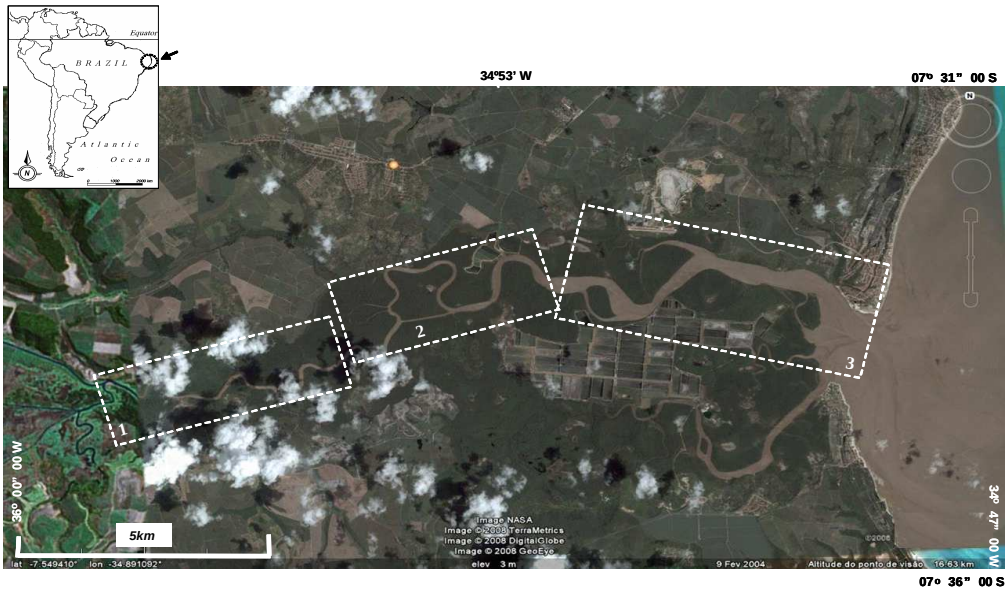

Figura 1. Estuário do Rio Goiana. = porções (1) superior, (2) intermediária e (3) inferior.

com 7,1 m e inferior com 8,5 m). A malha da rede varia entre 35 mm nas asas e 22 mm no saco (entre nós). Para obter-se uma amostragem representativa para todas as classes de tamanho dos espécimes de peixes, um sobre-saco de malha menor (5 mm) foi usado dentro do saco. Ovos, embriões livres e juvenis (>35–40 mm CT) foram coletados da cavidade oral dos machos incubadores, anestesiados com tricaine-metano-sulfonato (MS 222) e armazenados em formol tamponado à 4%.

Setenta ovos e sessenta embriões livres de ambas as espécies; e cinquenta e cinco juvenis de *C. spixii* e quarenta e sete juvenis de *C. agassizii* foram analisados para este estudo. O desenvolvimento de *C. spixii* e *C. agassizii* foi descrito seguindo eventos morfológicos categóricos no sentido de agrupar fases e períodos em uma sequência lógica de intervalos hierárquicos propostas por Balon (1990[14]). O período embrionário compreende o estágio do ovo (Ø<10 mm), desde um embrião fusiforme formado logo após a neurulação até a eclosão. O período de saco-vitelínico, caracterizado pela contribuição nutricional do vitelo, acaba quando a alimentação exógena é iniciada. O período juvenil, imediatamente após o consume do vitelo, é caracterizada pela alimentação autônoma.

Para comparar o desenvolvimento ontogenético entre períodos e espécies, foram feitas mensurações digitais para indivíduos pequenos com um estéreo microscópio trinocular - ZEISS; STEMI 2000-C – e o software AxioVision Release 4.7.2 (captador de imagens calibrado com escala milimétrica em todos os aumentos do macrômetro que converte os pixels da imagens captadas pela câmera digital – Canon; Powershot G10 – em milímetros). Um paquímetro digital – 799 Starrett/range: 6″/150 mm – foi usado para espécimes maiores (>23 mm CT). As variáveis morfométricas mensuradas foram:

[14] Balon, E. K. (1990). Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. *Guelph Ichthyology Reviews* **1**, 1–42.

comprimento total (CT), diâmetro do olho (DO), comprimento do focinho (CF), comprimento pré-peitoral (CPP), comprimento pré-dorsal (CPD), comprimento pré-pélvico (CPV), comprimento pré-anal (CPA), comprimento da cabeça (CC), largura da cabeça (LC) (Fig. 2a,b). O angulo (α°) entre o eixo do corpo e a ponta superior do focinho foi mensurado em todos os espécimes (Fig. 2c) e os dados merísticos (número de espinhos e raios das nadadeiras peitorais, dorsal, pélvica e anal; e o número de miômeros pré e pós-anais) foram quantificados.

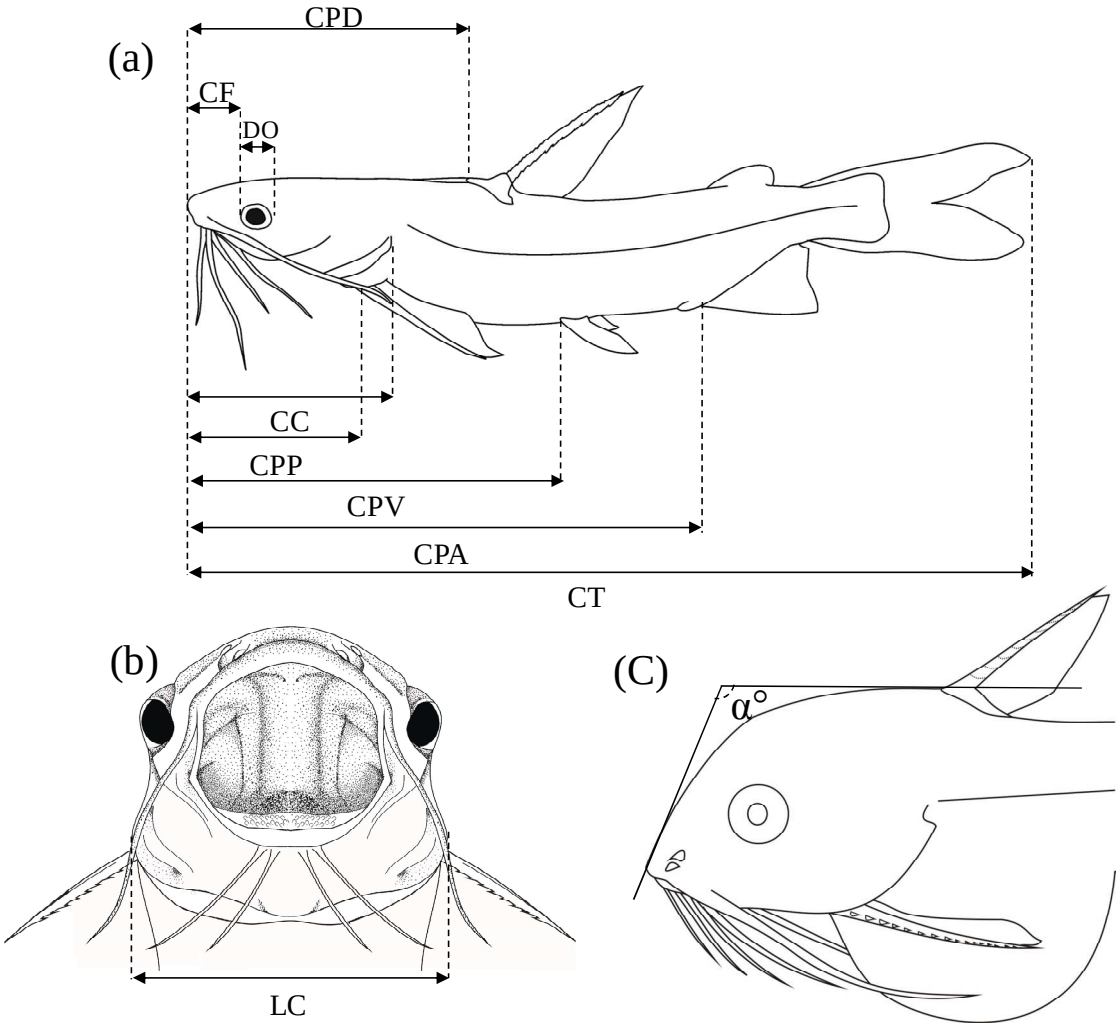

Figura 2. Variáveis morfométricas. (a) CT, comprimento total; DO, diâmetro do olho; CF, comprimento do focinho; CPD, comprimento pré-dorsal; CPP, comprimento pré-peitoral; CPV, comprimento pré-pélvico; CPA, comprimento pré-anal; CC, comprimento da cabeça. (b) LC, largura da cabeça. (c) α°, ângulo da cabeça.

A série ontogenética foi analisada pela técnica de diafanização, garantindo a transparência dos tecidos e a visualização do esqueleto para revelar caracteres e comparar os padrões gerais de ossificação entre espécies (Dingerkus & Uhler, 1977[15]; Potthoff, 1984[16]).

O crescimento alométrico foi calculado como uma função potência do CT de acordo com o modelo $Y = \beta_0 CT^{\beta 1} + \varepsilon$, onde Y é a variável dependente, CT a variável independente, β_0 o intercepto e β_1 o coeficiente de crescimento (Huxley, 1924[17]). Para cada período, o coeficiente de crescimento do modelo de regressão foi calculado e sua robustez foi mensurada pelo calculo do r^2 (coeficiente de determinação) (Zar, 1984[18]). No crescimento isométrico, β_1 é 1 para comprimentos e 3 para volume (van Snik *et al.*, 1997[19]). Quando β_1 é menor que a curva isométrica, é conhecido com crescimento alométrico negativo; quando maior, crescimento alométrico positivo (van Snik *et al.*, 1997[19]). Teste-*F* (Fisher) com 5% de nível de significância foi realizado para comparar os coeficientes de crescimento dos períodos ontogenéticos entre espécies e para verificar diferenças nos padrões de crescimento entre os períodos embrionário e de saco-vitelínico; e entre os períodos de saco-vitelínico e juvenil para ambas as espécies (Sokal & Rohlf, 1995[20]).

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[15]Dingerkus, G. & Uhler, L. D. (1977). Enzime clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology* **52,** 22 –232.
[16]Potthoff, T. (1984). *Clearing and staining techniques*. In H.G. Moser; W.J. Richards; D.M. Cohen; M.P. Fahay; A.W. Kendall jr. & S.L. richardson (Eds). *Ontogeny and systematics of fishes*. La Jolla, Spec. Publ. Amer. Soc. Ichthyol. Herpetol.
[17]Huxley, J.S. (1924). Constant differential growth-ratios and their significance. *Nature* **114**, 895-896.
[18]Zar, J. H. (1996). Bioestatistical analysis. 3rd ed. Prentice-Hall, New Jersey, 662 pp.
[19]van Snik, G. M. J., van den Boogaart, J. G. M. & Osse, J. W. M. (1997). Larval growth patterns in *Cyprinus carpio* and *Clarias gariepinus* with attention to finfold. *Journal of Fish Biology* **50,** 1339–1352.
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RESULTADOS

Os resultados do presente estudo permitiram agrupar as diferentes fases ontogenéticas de *C. spixii* (ANEXO I) e *C. agassizii* (ANEXO II) em uma seqüência hierárquica de acordo com o surgimento de caracteres morfológicos categóricos. O período embrionário compreende o estágio do ovo, desde o surgimento de um embrião logo após a neurulação até a eclosão. Após a eclosão, surgem os embriões livres, sem um estágio larval verdadeiro, caracterizado pela contribuição nutricional do saco vitelínico e compreendendo o período de saco vitelínico. O período juvenil inicia logo após a absorção do saco vitelínico, quando a alimentação exógena é iniciada (ANEXOS I e II).

O estudo corrobora a hipótese de que bagres ariideos possuem um desenvolvimento precoce durante o período embrionário. Embriões de *C. spixii* e *C. agassizii* são considerados altamente desenvolvidos por possuírem todas as nadadeiras diferenciadas e bem desenvolvidas, órgãos sensoriais funcionais e esqueletos axial e apendicular formados.

O aparecimento das vértebras e otólitos, bem como a ossificação dos otólitos e do aparelho de Weber ocorre primeiro em *C. agassizii* na fase II (11mm ± 0.5) quando comparado com *C. spixii* onde esses eventos ocorrem em embriões na fase III (14.3mm ± 0.43).

O presente estudo revelou que, assim como em peixes adultos, embriões livre e juvenis dessas espécies podem também ser distinguidos por divergências morfológicas dos olhos maiores em diâmetro de *C. agassizii* e os barbilhões maxilares mais longos de *C. spixii*.

As espécies *C. spixii* e *C. agassizii* compartilham variação similar nos padrões de crescimento durante as transições morfológicas. As variáveis morfométricas diâmetro do olho e largura da cabeça diferiram significativamente entre as transições de

embrião para embrião livre e de embrião livre para juvenil em ambas as espécies. Em geral, os embriões apresentaram um rápido crescimento destas variáveis, mostrando que os bagres ariideos utilizam as reservas energéticas do vitelo principalmente para o desenvolvimento precoce de órgãos sensoriais localizados na região da cabeça (*ex*: olhos, otólitos, narinas, barbilhões) durante o período embrionário. Após a eclosão, o crescimento destas variáveis em embriões livres é mais lento, uma vez que os indivíduos já apresentam órgãos sensoriais bem desenvolvidos muito antes da eclosão.

As medidas morfométricas relacionadas ao crescimento longitudinal crescem lentamente durante o período embrionário, acelerando para próximo ao isométrico nos períodos de saco vitelínico e juvenil. Para ambas as espécies, o comprimento da cabeça diferiu significativamente durante todas as transições morfológicas, enquanto que os comprimentos pré-peitoral e pré-anal só apresentaram diferenças durante a transição de embrião para embrião livre.

Em *C. spixii* o comprimento pré-dorsal não diferiu, enquanto que o comprimento pré-pélvico diferiu entre as transições morfológicas. Em *C. agassizii* o comprimento pré-dorsal diferiu somente durante a transição de embrião para embrião livre, enquanto que o comprimento pré-pélvico não mostrou diferenças entre as transições.

O comprimento do focinho não diferiu entre as transições morfológicas de ambas as espécies, crescendo sempre próximo à isometria, exceto no período de saco vitelínico de *C. spixii* que apresentou crescimento alométrico positivo.

Estas características de padrões de crescimento parecem estar relacionadas com uma mudança na estratégia de desenvolvimento das espécies, onde as necessidades anatômicas após a eclosão estão relacionadas com o gasto de energia para o crescimento longitudinal nos períodos de saco vitelínico (embrião livre) e juvenil.

A depleção vitelínica diferiu significativamente durante a transição de embriões para embriões livres nas duas espécies. O consumo do vitelo por embriões antes da

eclosão é muito lento enquanto que embriões livres apresentam uma taxa de consumação mais acelerada. Isto está relacionado com outra mudança nas necessidades anatômicas durante a transição de embriões livres para juvenis, pois, apesar das nadadeiras estarem bem desenvolvidas após a eclosão, os indivíduos com saco vitelínico não possuem boa habilidade para natação, sendo assim necessária uma rápida consumação da massa vitelínica antes que os juvenis sejam liberados pelos machos incubadores no ambiente.

A migração da boca durante a ontogenia das espécies estudadas também pode explicar a necessidade de uma boca rostral para o inicio da alimentação exógena. Em geral, a migração do ângulo da cabeça é mais rápida no período embrionário e mais lento no período de saco vitelínico. Durante o período juvenil, não são observadas muitas mudanças no ângulo da cabeça, o que indica que a boca subterminal já migrou da posição ventral para a posição rostral, marcando o início da alimentação autônoma por juvenis de ambas as espécies.

CONCLUSÃO

A principal conclusão deste estudo é que os principais órgãos sensoriais e a capacidade forrageira dos bagres estão quase que completamente formados antes mesmo da eclosão, sugerindo que embriões de bagres são altamente desenvolvidos antes do inicio da alimentação exógena, reduzindo a vulnerabilidades e os tornando abundantes em regiões estuarinas tropicais e sub-tropicais (ANEXOS I e II).

O desenvolvimento do esqueleto axial (crânio e coluna vertebral) e apendicular (raios e espinhos das nadadeiras), bem como sua ossificação ocorre primeiro em *C. agassizii* quando comparado com *C. spixii* na mesma classe de comprimento. Esse fato pode ser corroborado por uma taxa de consumo de vitelo maior em *C. agassizii* durante o período embrionário o que sugere um metabolismo mais acelerado para este período.

Além disso, ambas as espécies realizam uma estratégia de desenvolvimento similar na qual os embriões priorizam o desenvolvimento dos principais órgãos sensoriais, como os olhos, pelo rápido crescimento de seus diâmetros; os otólitos, pelo rápido crescimento da largura da cabeça; e as narinas e barbilhões, pelo rápido crescimento do focinho. Após a eclosão os embriões-livres priorizam o crescimento longitudinal.

C. spixii e *C. agassizii* apresentam um longo período de cuidado parental e os juvenis liberados (>35–40 mm) no ambiente são altamente desenvolvidos. Por isso, estes bagres apresentam estratégias de sobrevivências que incentiva seus usos como espécies bioindicadoras de qualidade ambiental de estuários onde vivem.

Para uma melhor avaliação da função deste habitat para estas espécies, destaca-se a necessidade da realização de um estudo para compreender como os diferentes períodos de desenvolvimento ontogenético utilizam este ambiente e quais são os requisitos ecológicos necessários para completar o ciclo de vida.

ANEXOS

ANEXO I

Early development and allometric shifts during the ontogeny of a marine catfish (*Cathorops spixii* - Ariidae)

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ABSTRACT

The precocious development and allometric growth of the ariid catfish *Cathorops spixii* (Agassiz, 1829) were studied from newly neurula embryo to juveniles. The results show that prior to hatching embryos are well-developed with axial and appendicular skeleton densely ossified. Embryos grew slowly longitudinally, but positive allometric growth patterns were observed in head width and eye diameter. This seems to be related to the fast development of sensory organs such as otoliths, Weberian apparatus, lens, nostrils and barbells during the embryonic period. After hatching, mouth-brooded free-embryos grew longitudinally isometric, except the snout length that presents a fast growth pattern related to the end of the endogenous feeding. Slow growth patterns were observed in head width and eye diameter during the yolk-sac period, taking into account that sensory organs are already formed. Head bones are easily recognizable in free-embryos resembling juvenile fish. The end of the yolk-sac period is characterized by a direct change from free-embryo to juvenile, without a true larval period. The juvenile period is characterized by growth patterns nearly isometric in all body regions, suggesting that juveniles of *C. spixii* are highly developed and bear most of the characteristic of adult fish.

Key words: precocious development; allometric growth; ontogenetic periods; tropical estuary; free-embryo; parental care; otophysi.

INTRODUCTION

Studies on the ontogeny of fishes are important to characterize their early development phases, allowing, among other things, a better diagnosis among species that present common morphological features even during embryonic and larval periods (Arezo *et al*., 2005; Ortíz-Galindo *et al*., 2008; Osman *et al*., 2008). These studies provide information about the developing abilities of fish and the anatomical needs of developing organs, being important for understanding the biology, functional trends and environmental preferences of the developing stages of a species (Galis *et al*., 1994; Koumoundouros *et al*., 2001; Wainwright *et al*., 2006). Also, osteological studies during the early life stages represent an important tool for clarifying the relations between skeletal ontogeny and the developing habit of a fish (Adriaens & Verraes, 1998). In addition, the knowledge of the embryonic and larval developing sequence serve as a model for comparisons when normal patterns of development are altered by factors such as mutations and changes in temperature (Meijide & Guerrero, 2000).

Researches on systematics (Marceniuk & Menezes, 2007), ecology (Araújo, 1988; Azevedo *et al*., 1999; Barletta *et al*., 2003; Barletta *et al*., 2005; Barletta *et al.*, 2008; Dantas *et al*., 2010) and biology (Fávaro e*t al*., 2005) of *Cathorops spixii* (Agassiz, 1829) have been done considering adult fish. However, there is no study related to the ontogenetic development of this species during the early stages of their life cycle.

The yellow catfish *C. spixii* inhabits shallow costal waters and brackish estuaries, seeking for lagoons and river mouths for spawning (Barletta *et al*., 2005; Barletta *et al*., 2008, Dantas *et al*., 2010). They are distributed along the northeast coast of South America, from Guiana to Brazil (Marceniuk & Menezes, 2007). This Ostariophysi species belongs to the Order Siluriformes – Family Ariidae which is

characterized by the presence of the Weberian apparatus, a series of small tendons and bones that connect the swimming bladder and otoliths of the inner ear (Sanger & McCune, 2002). This apparatus allows catfishes to have a better perception of sounds and consequently a good adaptation to turbid waters (Rosen & Greenwood, 1970; Burgess, 1989). As reported for the family, after spawning, males incubate fertilized eggs in their mouth until a young-juvenile is developed (Rimmer & Merrick, 1983; Rimmer, 1985c; Burgess, 1989; Acero 2002).

Studies in the Goiana Estuary (tropical semi-arid region – Northeast Brazil) revealed that the Ariidae Family is the most abundant (Barletta *et al*., 2010; Dantas *et al*., 2010). The Family corresponds to 53% of the catch in number and 63% in weight for the main channel of this estuary. *C. spixii* was the most abundant in terms of density (individuals m^{-2}) and biomass (g m^{-2}). In the Caeté Estuary (Eastern Amazon – North Brazil) (Barletta *et al*., 2005) and in the Paranaguá Estuary (transition between tropical and subtropical regions – South Brazil) (Barletta *et al*., 2008) *C. spixii* was also reported as the most abundant. For that reason, Costa *et al*. (2004) suggested that this species could be considered a bioindicator of environmental quality regarding their high abundance and ecological guild in these estuaries.

Ontogenetic, as well as ecological studies are fundamental to understand the function of these habitats as nursery areas for catfishes and other fish species. These studies are important not only to describe morphological adaptations that a species may develop to reduce vulnerability to predators, but also to understand how different developmental periods utilize the available habitats and which are the requirements to complete their life cycle. (Barletta-Bergan *et al*., 2002; Brown *et al*., 2004). Based on this information, this study has as objective to describe the different ontogenetic periods, considering the growth patterns of *C. spixii* in an estuarine ecosystem.

MATERIALS AND METHODS

Specimens of *C. spixii* were collected from the main channel of Goiana river estuary (Northeast - Brazil) from 2005 to 2009. Fish samples were taken from three areas (upper, middle and lower) of the main channel (Dantas *et al*., 2010) by an otter trawl net following the methodology proposed by Barletta *et al*., 2005 and Barletta *et al*., 2008 (Fig. 1). The net was 8.72m long with an opening size of 7.72 m (ground-rope with 8.5m and head-rope with 7.1m). The mesh-size varied between 35mm in the body and 22mm in the cod-end (between knots). In order to obtain a representative sample for the entire size range of fish, a cover with a smaller mesh-size (5 mm) was used inside the cod-end. Eggs, free-embryos and juveniles (>35–40 mm Total Length, TL) were collected from the oral cavity of the mouth-brooding adult males, anesthetized using tricaine methanesulfonate (MS 222) and stored in 4% buffered formalin.

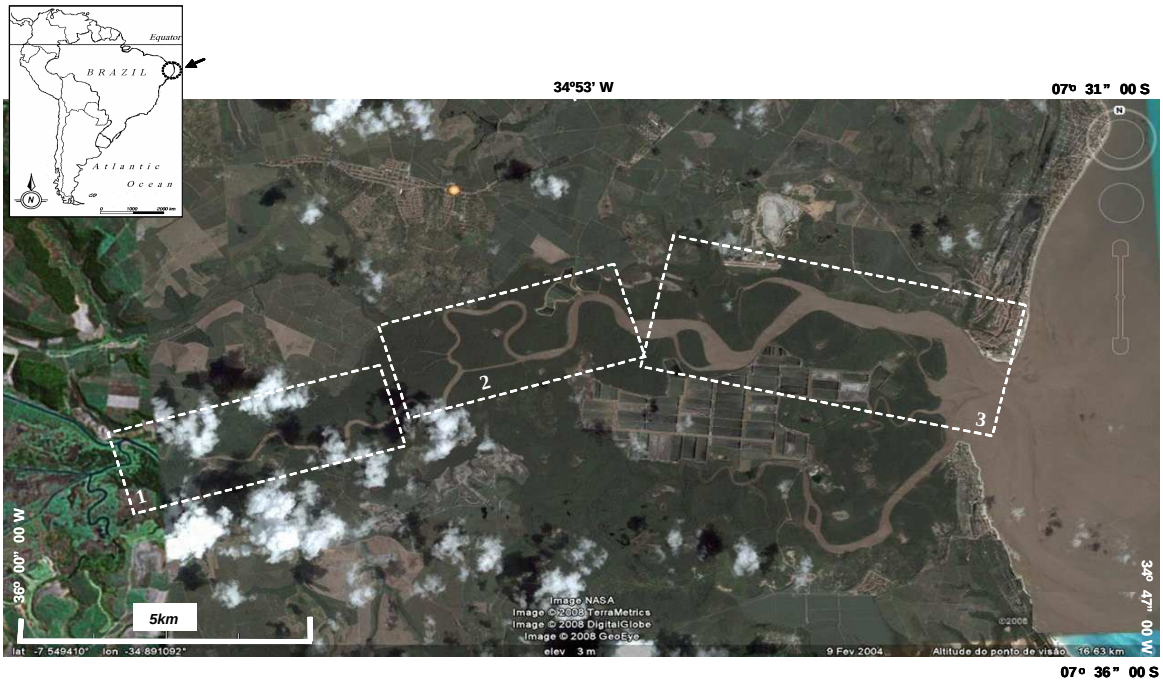

Figure 1. Goiana Estuary. = (1) Upper, (2) Middle and (3) Lower portions of the estuary.

Eighty eggs, sixty free-embryos and fifty five juveniles were analyzed for this study. The precocious development of *C. spixii* was described following categorical morphological events in order to group phases and periods in a logical sequence of three steps following the hierarchy of intervals proposed by Balon (1990). The embryonic period comprise the egg stage (Ø<10 mm), ranging from a newly neurula streak-shaped embryo to hatching (<23 mm TL). The yolk-sac period (>23–35 mm TL), characterized by the nutritional contribution of the yolk-sac, ends when external feeding is initiated. The Juvenile period (>35–82 mm TL), immediately after the yolk-sac total consumption, is characterized by the beginning of autonomous feeding.

In order to compare the ontogenetic development between periods and phases, digital measurements were made with the aid of a trinocular stereo microscope - ZEISS; STEMI 2000-C - and the software AxioVision Release 4.7.2 (an image capturer calibrated with a millimeter scale in all micrometer zooms that converts the image pixels obtained from the digital camera – Canon; Powershot G10 – in millimeter) for small specimens. A digital caliper – 799 Starrett/range: 6″/150 mm – was used for larger specimens (>2.3 cm TL). The morphometric variables measured were: total length (TL), standard length (SL), eye diameter (ED), snout length (SNL), pre-pectoral length (PPL), pre-dorsal length (PDL), pre-pelvic length (PVL), pre-anal length (PAL), head length (HL), head width (HW) (Fig. 2a,b). The angle (α°) between the body axis and the upper lip surface was measured in all specimens (Fig. 2c) and meristic data (number of spines and rays of pectoral, dorsal, pelvic, anal and caudal fins; and the number of pre and post-anal miomers) were also counted. The ontogenetic series of the species was analyzed by clearing and staining, assuring transparency to tissues and skeleton visualization that reveals skeletal characters and patterns of ossification (Dingerkus & Uhler, 1977; Potthoff, 1984).

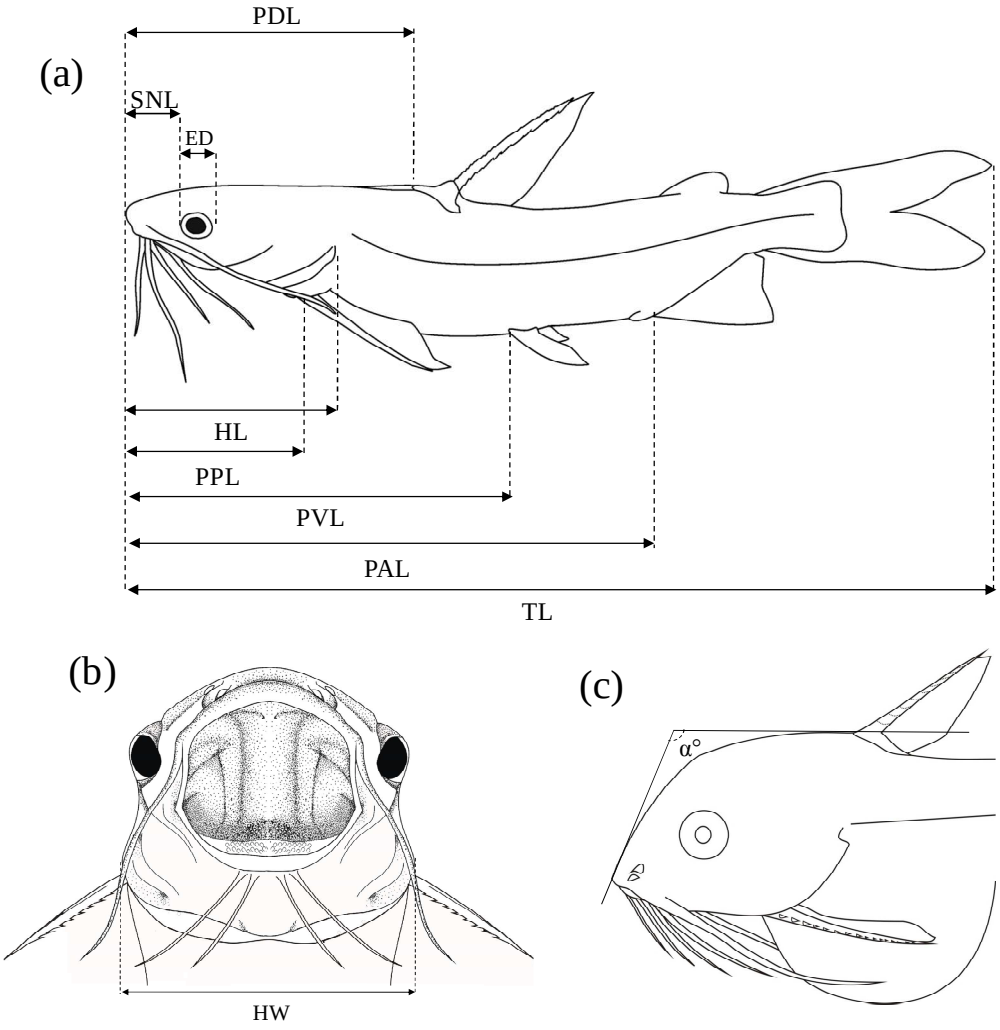

Figure 2. Morphometric variables. (a) TL, total length; ED, eye diameter; SND, snout length; PDL, pre-dorsal length; PPL, pre-pectoral length; PVL, pre-pelvic length; PAL, pre-anal length; HL, head length. (b) HW, head width. (c) α°, head angle.

Allometric growth was calculated as a power function of TL according to the model $Y = \beta_0 TL^{\beta 1} + \varepsilon$ (Huxley, 1924). The linearization of the model was performed on log-transformed data resulting in regressions curves of the type $\log Y = \log \beta_0 + \beta_1 \log TL + \log \varepsilon$, where Y is the dependent variable, TL the independent variable, β_0 the intercept and β_1 the slope or growth coefficient. For each period, the growth coefficient of the regression model was calculated and its robustness was measured by calculating the r^2 (coefficient of determination) (Zar, 1996). In isometric growth, the slope β_1 is 1

for length and 3 for volume. When the slope β_1 is smaller than the isometric slope, it is known as negative allometric growth; when higher, positive allometric growth (van Snik *et al*., 1997). *F*-tests (Fisher) with a 5% level of significance were performed to verify differences in growth patterns between embryonic and yolk-sac period; and yolk-sac and juvenile period (Sokal & Rohlf, 1995).

RESULTS

EMBRYONIC PERIOD

C. spixii eggs are spheres relatively large with an average diameter of 8±0.71mm surrounded by a thick envelope, the chorion. The developing embryo is located on a large orange-colored yolk sphere located in the center of each egg. This period was subdivided in five ontogenetic phases:

Phase I. *Otic vesicle formation and notochord in pre-flexion*

This phase is characterized by a fusiform embryo with V-shaped miomers measuring 6.99±0.69mm (*n*=30) total length (Fig. 3a.1,3). The optic vesicle has a marginal pigmentation and differentiates to form the lens. The small otic vesicles appear, but they still lack otoliths. Three regions of the brain are already well-defined, forebrain, midbrain and hindbrain (Fig. 3a.2), initiating the cephalization process. In the head is observed the presence of four developing branchiostegal arcs. The notochord is seen as a median line along the body axis and marks the pre-flexion phase (Fig. 3a.3). The median fin begins right after the head, continue through the dorsal extension of the body and go forward the ventral portion. There are no skeletal structures at this phase (Fig. 5a). The mouth is also absent. Maxillary barbell buds appear at the lateral edges of the future upper maxilla. The head angle (α°) has 12.89±5.41°, explaining why the snout migrates from a sub-terminal ventral position to a rostral position in the following phases.

Phase II. *Anlage of skeletal structures and notochord flexion*

Embryos now have an average total length of 8.3±0.73mm (*n*=10). The three regions of the brain are further developed and grow according to head growth (Fig. 3b.2). Formation of the operculum starts. Maxillary barbells keep growing. The anlage of skeletal cartilaginous structures appears at the head (Fig. 5b.2). The notochord marks the flexion phase (Fig. 5b.3 and Fig. 3b.3). The lens begins to form in the optic vesicles. The pectoral fin bud protrudes from the body trunk. The head angle has an average of 31.11±3.19°, indicating that the migration of the snout begins before mouth formation.

Phase III. *Skeletal cartilaginous structures differentiation and mouth development*

The embryo has an average total length of 11.8±0.47mm (*n*=10). The head grows disproportionally in relation to the body. The mouth appears in the ventral sub-terminal position of the head at the same time of a single nostril. The head angle increases (37.43±6.25°). The optic vesicle is pigmented and lenses are formed. The three regions of the brain grow and become more developed (Fig. 3c.2). The otic vesicle is well-developed, but otoliths remain absent (Fig. 5c.2). The operculum and the branchiostegal arcs are now partially developed. The maxillary barbells keep growing and the mental barbells appear. Skeletal cartilaginous structures appear to initiate the formation of the axial and appendicular skeleton. The anlage of the Weberian apparatus appears at the four anterior-most vertebrae as a bud (Fig. 5c.2). The cartilaginous pectoral fin has between 4–5 rays and a flexible spine (Table I). The dorsal fin lacks rays, but has a cartilaginous spine in formation. The cartilaginous caudal fin has between 14 – 18 rays (Table I) and initiates the formation of the hypural and para-hypural bones (Fig. 5a.3). Neural and haemal spines appear as cartilaginous structures along the notochord, marking a post-flexion phase (Fig. 5c.3 and Fig. 3c.3).

Phase IV. *Vertebrae formation and otolith appearance*

The embryo has an average total length of 14.3±0.43mm (*n*=10). The miomers change from V-shaped to W-shaped (Fig. 3d.3). Otoliths appear as two conglomerates of small granules in the now well-expanded otocyst (Fig. 5d.2 and Fig. 3d.2). The head angle keeps increasing (60.92±8.19°). Notochord begins to be replaced by bony vertebrae at the same time neural and haemal spines initiate their ossification process (Fig. 5d.1,2). The process of ossification of the Weberian apparatus and of pectoral (10 rays) and dorsal (7 rays) fins also initiates (Fig. 5d.1,2,4). The spines are still flexible. The pelvic (6 rays) and anal (19 –22 rays) fins are cartilaginous (Fig. 5d.5). The cartilaginous caudal fin (22–26 rays) has hypural and para-hypural bones well-developed (Fig 5d.3). The epural bone appears as a cartilaginous structure. The number of rays of pectoral, dorsal, and pelvic fins do not vary in the following phases (Table I).

Phase V. *Bifurcation and ossification of caudal fin and hatching*

The embryo has an average total length of 20.33±1.87mm (*n*=20) and it is curved with the caudal fin above the head (Fig. 3e.1). Ossification and differentiation of the head bones initiate (Fig. 5e.1). The head has an average angle of 88.04±8.08° indicating that the mouth keeps migrating to a sub-terminal rostral position. The otoliths keep increasing in size. The vertebrae and the Weberian apparatus are formed and ossified (Fig. 5e.2). The cartilaginous premaxillary tooth plate appears. Neural and haemal spines and the dentary bone are in ossification (Fig. 5e.1,3). The pelvic and anal (22–25 rays) fins initiate the ossification process (Fig. 5e.5). The caudal fin (31–46 rays) bifurcates (Fig. 3e.3). In larger specimens of this phase dentary bones are well-ossified (Fig. 5f.1). Hypural and para-hypural bones and rays of the caudal fin initiate the ossification process (Fig. 5f.3). At the end of this phase hatching occurs (Fig. 3f.1).

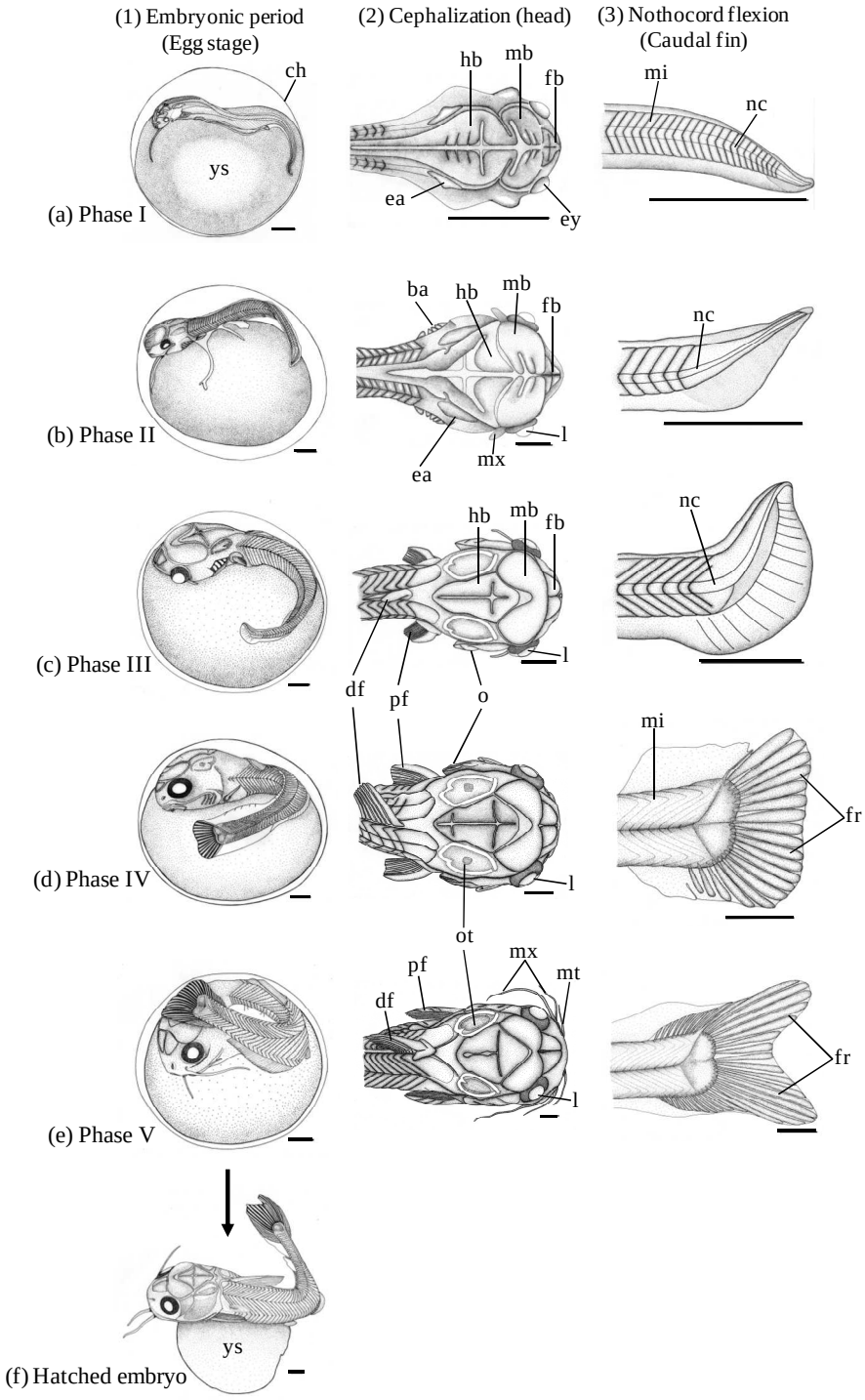

Figure 3. Ontogenetic phases of the embryonic period of *C. spixii*. ch, chorion; df, dorsal fin; ea, otic vesicle (ear); ey, optic vesicle (eye); fb, forebrain; fr, fin rays; ba, branchiostegal arcs; hb, hindbrain; l, lens; mb, midbrain; mi, miomer; mt, mental barbell; mx, maxillary barbell; nc, notochord; o, operculum; ot, otolith; pf, pectoral fin; ys, yolk-sac. Scale bars: 1 mm. Arrow indicates moment of hatching.

YOLK-SAC PERIOD

Free-embryo stage

Mouth-brooded free-embryos (Fig. 4a) have an average total length of 27.24±3.57mm (*n*=60). Nostrils are close together and separated by a narrow septum. Otoliths are formed and ossified. The dorsal and lateral pigmentation are first visible in newly hatched individuals. Head bones (frontal, mesethmoid, lateral ethmoid, sphenotic, pterotico, extrascapular, supraocciptal process and the pre-dorsal plate) are well-recognizable and moderately ossified (Fig. 5g.1,2). The head angle increases reaching 114.822±19.38°. Anal fin rays vary between 24–25 and the caudal ones between 47–60 (Table I). The maxillary barbells overreach the pectoral fin base. Premaxillary tooth plates are well-developed and the dentary bone is completely ossified (Fig. 5g.3). This period is characterized by a wider variation in fish development. Moderately developed fish have a large yolk-sac in the ventral portion of the body and underdeveloped fins while highly developed fish present only a trace of yolk-sac and fins almost completely developed. At 3 cm TL, food particles started to be observed in the stomachs of some individuals, marking the onset of exogenous feeding still within the adult male buccal cavity.

JUVENILE PERIOD

Juveniles have an average total length of 53.38±14.43mm (*n*=55). The body is densely pigmented. The head angle increases but does not vary much (163.88±4.76°). Pectoral and dorsal fin rays and spines, epural, hypural and para-hypural bones are ossified (Fig. 5h.1,2,3,4,5). Anal fin rays vary between 22–25 and caudal rays between 50–62 (Table I). Axial and appendicular skeleton are now well ossified (Fig. 5h.1,2,3,4,5). Head bones are fully differentiated (Fig. 5h.1,2). This period is characterized by mouth-brooded juveniles (>35–40 mm TL) resembling most

characteristics of free-embryos (Fig. 4b); and free-swimming juveniles (>40 mm TL) that at 80 mm TL resemble almost all characteristics of adult fish (Fig. 4c)

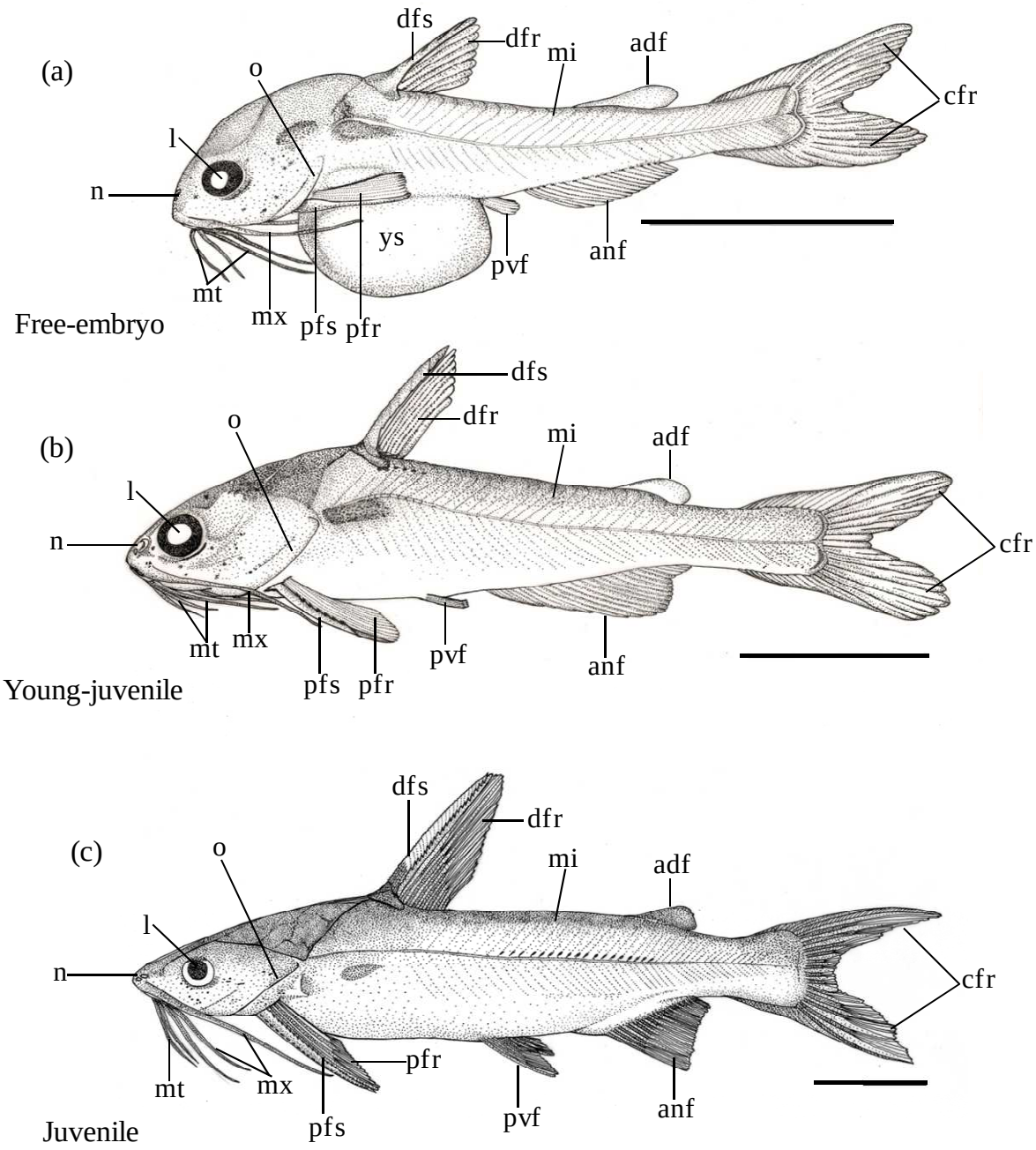

Figure 4. (a) Free-embryo and (b) young-juvenile (c) juvenile of *C. spixii*. adf, adipous fin; anf, anal fin; cfr, caudal fin rays; dfr, dorsal fin rays; dfs, dorsal fin spine; l, lens; mi, miomer; mt, mental barbell; mx, maxilary barbell; n, narine; o, operculum; pfr, peitoral fin rays; pfs, peitoral fin spine; pvf, pelvic fin; ys, yolk-sac. Scale bars: 10 mm.

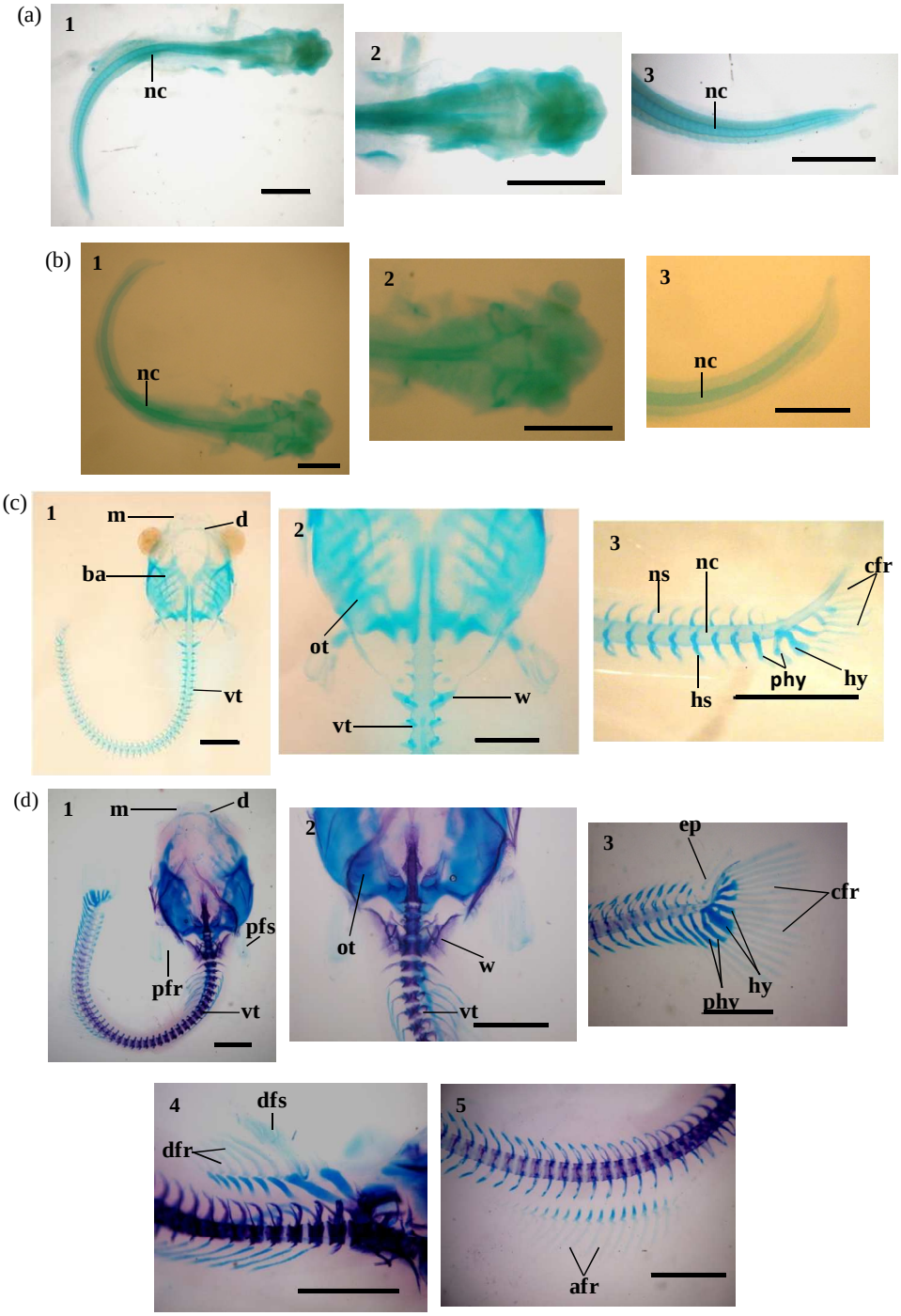

Figure 5. Ontogenetic shifts in ossification patterns of *C. spixii*. (a)–(e) Phases I to V of the embryonic period; (f) newly hatched embryo; (g) free-embryo; and (h) juvenile. afr, anal fin rays; ba, branchiostegal arc; cfr, caudal fin rays; d, dentary bone; dfr, dorsal fin rays; dfs, dorsal fin spines; ef, sphenotic bone; ep, epural bone; et, lateral ethmoid bone; fr, frontal bone; hs, haemal spine; hy, hypural bones; m, maxilla; met, mesethmoid bone; nc, notocord ns, neural spine; ot, otolith; pd, pre-dorsal plate; pfr, pectoral fin rays; pfs, pectoral fin spine; phy, parahypural bones; pm, pre-maxillary plate; pto, pterotico; sp, supraocciptal process; vt, vertebrae; w, Weberian apparatus. Scale bars: 1 mm.

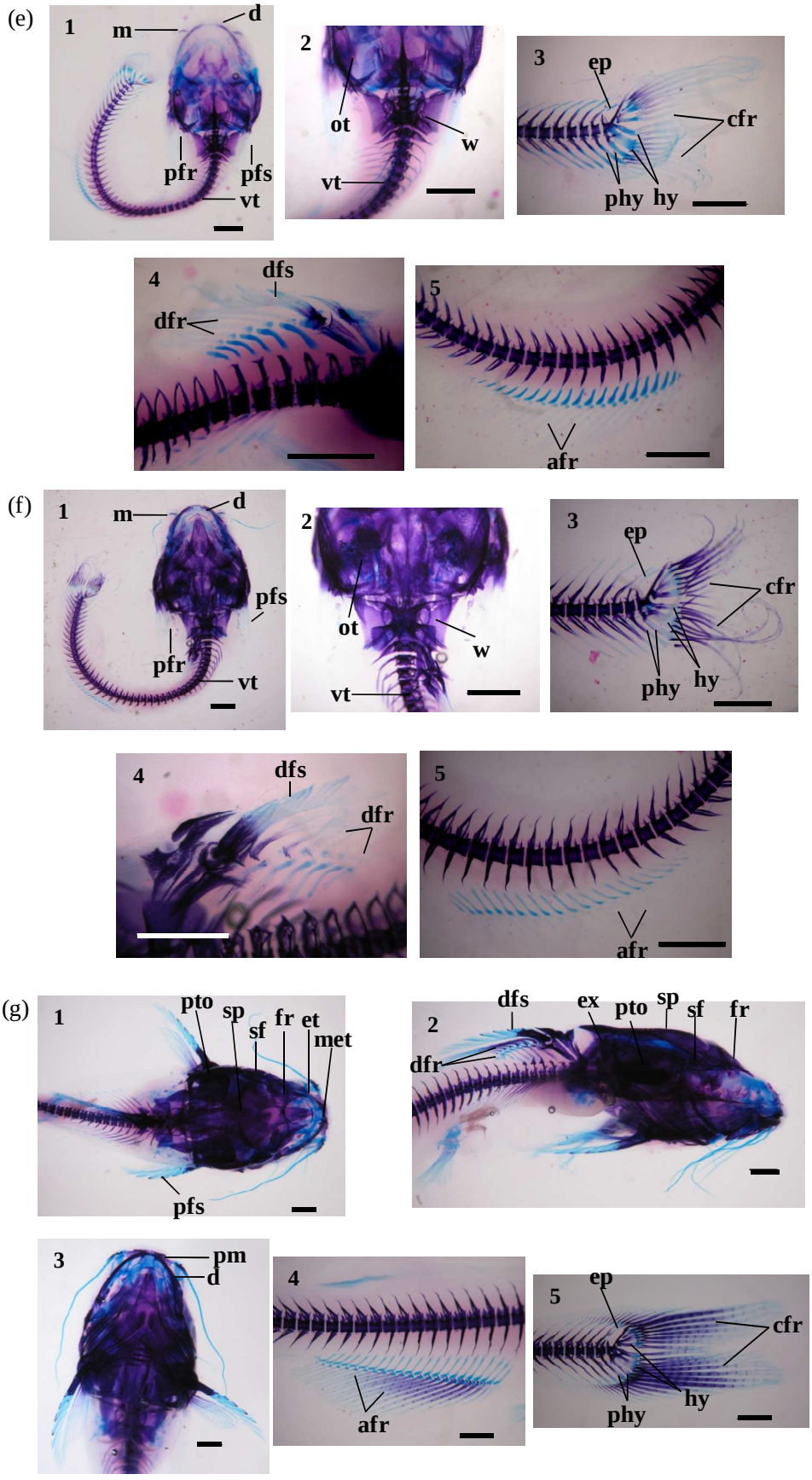

Figure 5. (Continued)

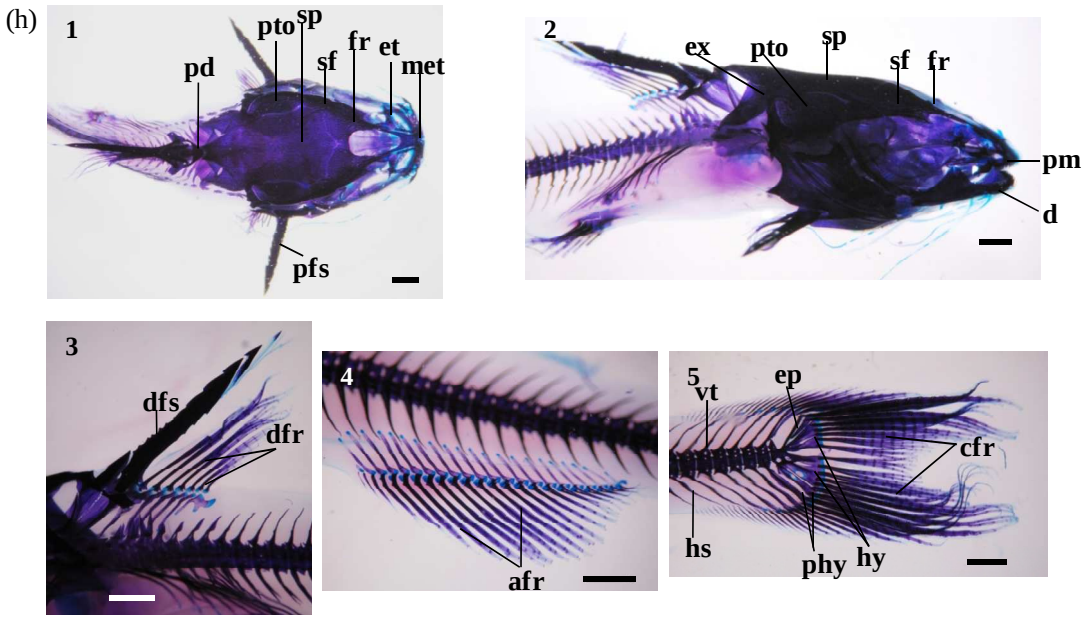

Figure 5. (Continued)

GROWTH PATTERNS

Growth patterns of *C. spixii* were divided in three periods: Prior to hatching (embryonic period), from hatching to yolk-sac consumption (yolk-sac period) and subsequent to yolk-sac consumption (juvenile period). Body proportions related to longitudinal growth showed patterns varying from negatively allometric ($\beta_1<1$) to nearly isometric ($\beta_1=1$). Pre-pectoral (PPL), pre-dorsal (PDL), pre-pelvic (PVL), pre-anal (PAL) and head (HL) lengths grew slowly [negatively allometric ($\beta_1<1$)] during the embryonic period and accelerate during yolk-sac and juvenile periods (Table II, Fig. 6c-g). PPL growth differed between embryonic and yolk-sac periods ($\beta_1=0.43$ and 0.93, respectively), but grew negatively allometric growth during yolk-sac and juvenile periods ($\beta_1=0.93$ and 0.91) (Table II and III). PDL growth showed negatively allometric patterns and did not differ among the three periods ($\beta_1=0.81$, 0.96 and 0.94) (Tables II and III). PVL and HL growths differed among all periods (Table III), growing slowly during the embryonic period ($\beta_1=0.69$ PVL, 0.75 HL), near-isometric ($\beta_1\approx1$) during the yolk-sac period ($\beta_1=1.17$ PVL, 1.05 HL), negatively allometric for HL ($\beta_1=0.90$) and

near-isometric for PVL (β_1=0.97) during the juvenile period. Moreover, PAL growth was negatively allometric during the embryonic period (β_1=0.71) and differed from the isometric pattern of the yolk-sac period (β_1=1.04), which presented a similar growth to that of the juvenile period (β_1=1.06).

Eye diameter (ED), head width (HW), and snout length (SNL) showed accelerated growth patterns [positively allometric (β_1>1)] during the embryonic period. However, ED growth differed among all periods changing from a fast growth pattern (β_1=1.59) during the embryonic period to a very slow pattern in the yolk-sac period (β_1=0.26) and accelerating again to a near-isometric growth in the juvenile period (β_1=0.95). While, HW growth pattern differed among all periods, being fast during the embryonic period (β_1=1.65), slow during the yolk-sac period (β_1=0.45) and near-isometric during the juvenile period (β_1=1.16) (Tables II and III). SNL showed an accelerated growth pattern and did not differ among periods (Tables II and III).

The head angle (α°) grew fast during the embryonic period (β_1=1.74), which showed a similar pattern of that in the yolk-sac period when growth slowed down (β_1=1.15). During the juvenile period the head angle differed significantly of that in the yolk-sac period, changing to a marked slow growth (β_1=0.08) indicating that the upper lip surface of the sub-terminal mouth moved from a ventral position to the final rostral position (Fig. 6i and Tables II and III).

Yolk-sac depletion (YSD) showed a particular pattern. During the embryonic period the yolk-sac weight decreased slowly (β_1= – 0.38) emphasizing the importance of this structure for embryos. This pattern differed significantly of that showed in the yolk-sac period, when its weight decreased quite faster (β_1= – 2.64) due to the rapid consumption of the yolk-sac during this period (Fig. 6j and Table II and III). During the juvenile period the yolk-sac was absent.

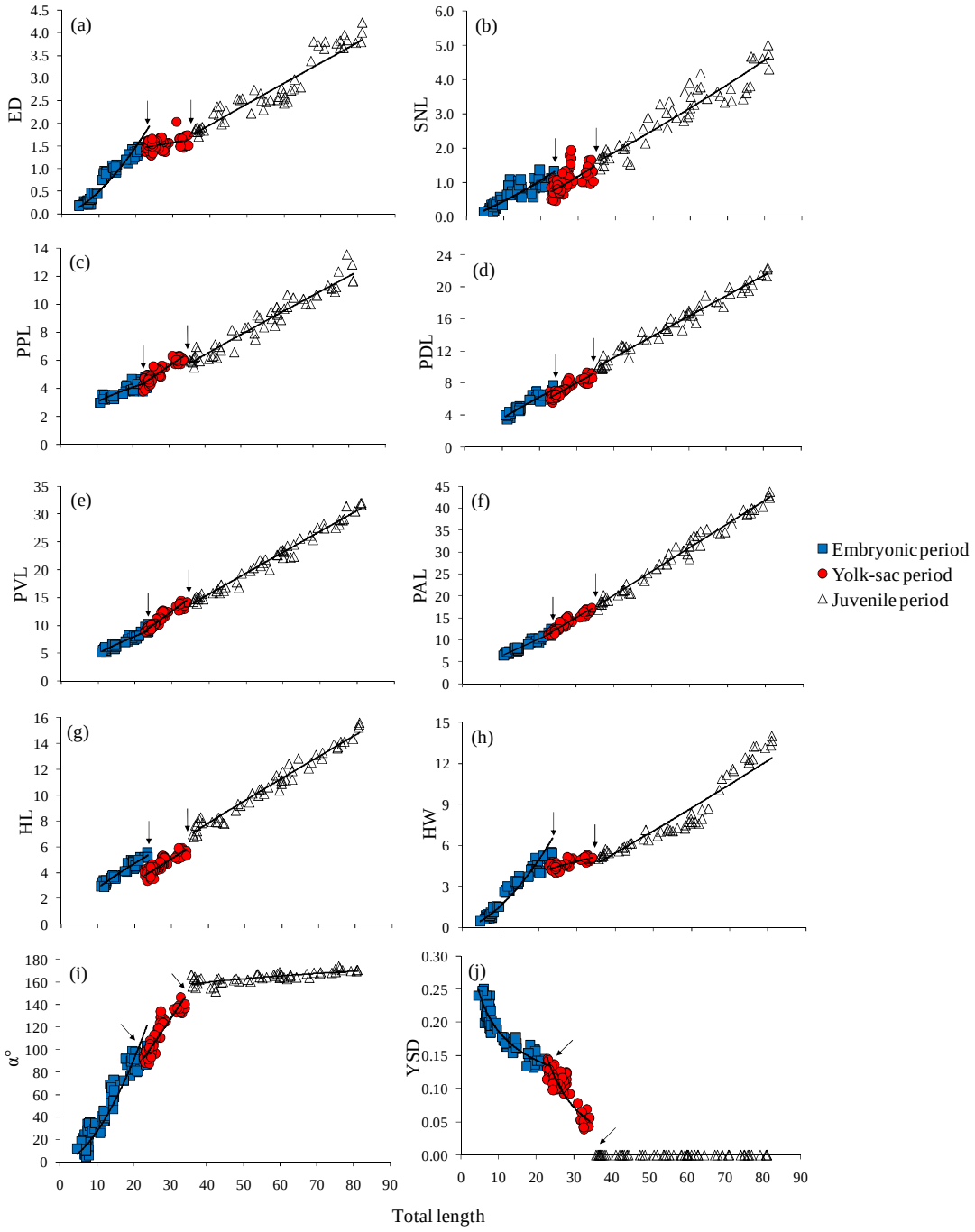

Figure 6. Relationships of the morphometric variables with total length (TL) for the embryonic period, Yolk-sac period and juvenile period. (a) ED, eye diameter; (b) SNL, snout length; (c) PPL, pre-pectoral length; (d) PDL, pre-dorsal length; (e) PVL, pre-pelvic length; (f) PAL, pre-anal length; (g) HL, head length; (h) HW, head width; (i) α°, head angle; (j) YSD, yolk-sac depletion. Arrows indicating the moment of hatching and yolk-sac depletion, respectively. The equations values are on Table II following the model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$.

TABLE I. Meristic frequency of ontogenetic periods of *C. spixii* classified according to the criteria defined on table

Ontogenetic periods	N° of pre and post anal miomers	N° of branchiostegal arcs	N° of spines and rays of pectoral fin	N° of spines and rays of dorsal fin	N° of spines and rays of pelvic fin	N° of spines and rays of anal fin	N° of spines and rays of caudal fin
Embryonic period							
Phase I	18-21 / 27-29	4	- / -	- / -	- / -	- / -	- / -
Phase II	18-20 / 26-32	4	- / -	- / -	- / -	- / -	- / -
Phase III	18-19 / 26-27	4	I / 4-5	I / -	- / -	- / -	- / 14-18
Phase IV	15-17 / 26-27	4	I / 10	I / 7	- / 6	- / 19-22	- / 22-26
Phase V	15-17 / 25-27	4	I / 10	I / 7	- / 6	- / 22-25	- / 31-46
Yolk-sac period	14-19 / 23-29	4	I / 10	I / 7	- / 6	- / 23-25	- / 43-60
Juvenile period	14-15 / 27-29	4	I / 10	I / 7	- / 6	- / 21-25	- / 48-62

TABLE II. Growth coefficients [slope (β_1)], intercept (β_0) and r^2 values of morphometric variables in 195 specimens of *C. spixii* regressed with total length (LT). Model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$. ED, eye diameter; SNL, snout length; PPL, pre-pectoral length; PDL, pre-dorsal length; PVL, pre-pelvic length; PAL, pre-anal length; HW, head width; HL, head length; α°, head angle; YSD, yolk-sac depletion

	Embryo (n = 80)			Free-embryo (n = 60)			Juvenile (n = 55)		
Variable	β_0	β_1	r^2	β_0	β_1	r^2	β_0	β_1	r^2
ED	-1.9060	1.5960±0.0496	0.9306 ***	-0.1869	0.2600±0.0754	0.1729 **	-1.2280	0.9492±0.0444	0.8980 ***
SNL	-1.6280	1.2700±0.0710	0.8060 ***	-2.4860	1.7300±0.2669	0.4242 ***	-1.7570	1.2690±0.0631	0.8861 ***
PPL	0.0503	0.4298±0.0425	0.7287 ***	-0.6274	0.9303±0.0612	0.8021 ***	-0.6509	0.9090±0.0327	0.9370 ***
PDL	-0.2625	0.8126±0.0453	0.8944 ***	-0.5136	0.9593±0.0585	0.8253 ***	-0.4530	0.9373±0.0232	0.9692 ***
PVL	0.0060	0.6919±0.0352	0.9106 ***	-0.6275	1.1690±0.0497	0.9066 ***	-0.3625	0.9710±0.0197	0.9791 ***
PAL	0.0832	0.7075±0.0275	0.9458 ***	-0.3542	1.0370±0.0404	0.9202 ***	-0.4019	1.0640±0.0200	0.9819 ***
HW	-1.4520	1.6500±0.0457	0.9441 ***	0.0241	0.4469±0.0444	0.6397 ***	-1.1340	1.1670±0.0471	0.9220 ***
HL	-0.2997	0.7477±0.0309	0.9388 ***	-0.8541	1.0550±0.0771	0.7666 ***	-0.5559	0.9047±0.0230	0.9674 ***
α°	-0.3086	1.7400±0.0984	0.8022 ***	0.4097	1.1460±0.0608	0.8616 ***	2.0640	0.0867±0.0096	0.6088 ***
YSD	-0.3467	-0.3825±0.0152	0.8911 ***	2.7590	-2.6420±0.1764	0.7974 ***			

NS, non-significant ($P > 0.05$); **$P < 0.01$; ***$P < 0.001$

TABLE III. Significance values of comparisons of regression slopes (β_1) (in relation to LT) of the morphometric variables between the embryonic and yolk-sac periods; and yolk-sac and juvenile periods in *C. spixii*. Model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$. ED, eye diameter; SNL, snout length; PPL, pre-pectoral length; PDL, pre-dorsal length; PVL, pre-pelvic length; PAL, pre-anal length; HW, head width; HL, head length; α°, head angle; YSD, yolk-sac depletion

	Embryo and yolk-sac periods		Yolk-sac and juvenile periods	
Variables	*F*-test		*F*-test	
ED	$F_{(1,134)}$	64.4195 ***	$F_{(1,109)}$	56.8992 ***
SNL	$F_{(1,134)}$	2.5259 ^{NS}	$F_{(1,109)}$	3.7752 ^{NS}
PPL	$F_{(1,95)}$	44.1289 ***	$F_{(1, 109)}$	0.0917 ^{NS}
PDL	$F_{(1,95)}$	3.7498 ^{NS}	$F_{(1, 109)}$	0.1386 ^{NS}
PVL	$F_{(1,95)}$	59.7367 ***	$F_{(1, 109)}$	15.4451 ***
PAL	$F_{(1,95)}$	44.5753 ***	$F_{(1, 109)}$	0.3804 ^{NS}
HW	$F_{(1,134)}$	65.0952 ***	$F_{(1,109)}$	79.7347 ***
HL	$F_{(1,95)}$	14.9013 ***	$F_{(1, 109)}$	4.3870 *
α°	$F_{(1,134)}$	3.5131 ^{NS}	$F_{(1,109)}$	423.7360 ***
YSD	$F_{(1,134)}$	311.3630 ***		

DISCUSSION

Research on the ontogeny of fish revealed that many catfishes lack a true larval period, switching from free-embryo (after hatching) directly to juvenile. This was described by Balon (1990) as a precocial (or direct) development. Examples from the literature are the clariid *Clarias gariepinus* (Osman *et al.*, 2008), the Loricariid *Ancistrus cf. triradiatus* (Geerinckx *et al*., 2007) and the ariids *Galeichthys feliceps* (Tilney & Hecht, 1993) and *Arius graeffei* (Rimmer, 1985c). The early ontogeny of *C. spixii* comprised three periods: embryonic period (egg stage), yolk-sac period (free-embryo stage) and juvenile period, supporting the idea that a true larval period is in fact absent.

In most teleosts fish, hatchlings have a median fin fold from which pectoral, dorsal, caudal, pelvic and anal fins will form later, and the skeleton lacks even

cartilaginous elements (Adriaens & Verraes, 1997; Koumoundouros *et al*., 2001; Arezo *et al*., 2005; Pinder & Gozlan, 2004; Iwamatsu, 2004; Meijide & Guerrero, 2000). However, prior to hatching, structures of the axial and appendicular skeleton of *C. spixii* are already well-developed and almost completely ossified. All fins are completely separated and fully rayed, the caudal fin is forked, and the mouth is open. This precocious development can be attributed to the large yolk mass and the long incubation time under the care of mouth-brooding males of the specie (Adriaens & Vanderwalle, 2003; Burgess, 1989; Acero 2002).

Studies have been done on the ontogeny of both cranial and postcranial skeleton of catfishes (Merriman, 1940; Bamford, 1948; Adriaens & Verraes, 1998; Sanger & McCune, 2002; Geerinckx *et al*., 2007) in order to determine the functional importance for feeding, respiration and sensory organs. Most works have focused on the ontogeny of the chondocranium and osteocranium, which in general, form a well-developed chondocranium in the embryonic period. Patterns of ossification are observed only at hatching [*e.g*. *Corydoras aeneus* (Huysentruyt *et al.*, 2008), *C. gariepinus* (Adriaens & Verraes, 1998) and *A. cf. triradiatus* (Geerinckx *et al*., 2007)]. In *C. spixii*, the anlage of cartilaginous skeletal structures appears at the head during the second ontogenetic phase. Along the following embryonic phases, maxillary bones change from a cartilaginous rudiment to an ossified, well-developed, bone at the end of the embryonic period. Fin rays appear as cartilaginous structures in the third embryonic phase, increasing in number and replacing the median fin fold as ossification starts inside the egg. Pectoral and dorsal spines are still flexible and their ossification begins just before hatching. At the third phase, cartilaginous neural and haemal spines appear along the notochord. Notochord begins to be replaced by ossified vertebrae at the fourth phase, when neural and haemal spines initiate the ossification process. Hypurals, para-hypurals

and epural bones are already visible in the embryo caudal fin. Otoliths are completely formed and ossified at the end of the embryonic period. The anlage of the Weberian apparatus appears at the fourth anterior-most vertebrae as a bud, being ossified and completely developed before hatching, as reported for Rosen & Greenwood (1970) for Otophysans (Ostariophysi excluding Gonorynchiformes). During the yolk-sac period, head bones begin to differentiate and the axial skeleton is completely ossified. Fin rays and spines keep ossifying, except the caudal fin that is already well-developed. In juveniles, head bones keep differentiating until the end of the period when they resembling almost every character to adult fish.

Sensory organs are also reported to have a fast development in catfishes even during the embryonic period (Rimmer, 1985c; Menon *et al*., 1989; Osman *et al*., 2008). In general, prehatching organogenesis in catfishes starts with the formation of the notochord, the brain, and the optic and otic vesicles (Osman *et al*., 2008; Tilney & Hecht, 1993). In *C. spixii*, the three regions of the brain (forebrain, midbrain and hindbrain) are already well-defined in the first embryonic phase. The otic vesicle appears as a small empty cup that change to a highly developed structure with a big otolith in the fifth embryonic phase. The lens begins to form in the optic vesicle during the second embryonic phase and it is completed before hatching. Nostrils are formed at the third phase. Maxillary and mental barbells are well recognizable in embryos, but keep increasing in length during yolk-sac and juvenile periods. The Weberian apparatus is ossified and completely developed before hatching. Similar patterns of sensory organs development is observed in *G. feliceps*, where the brain regions, otoliths, olfactory placodes (nostrils), as well as branchiostegal arcs, all form between 22 to 23 days after fertilization (Tilney & Hecht, 1993). A fast development is also present in the cyprinid sunbleak *Leucaspius delineates*, which initiate to develop sensory organs

before hatching, but hatch with morphologically immature features (Pinder & Gozlan, 2004). However, in *C. gariepinus* sensory organs start to develop later in newly hatched larvae, different from that normally observed in ariids (Osman *et al*., 2008; Mukai *et al.*, 2008).

The early development of the Weberian apparatus (Sanger & McCune, 2002) and the oral incubation of the first phases (Acero, 2002) may be responsible for the success of Ariidae. Parental care in *C. spixii* extends from newly fertilized eggs to the development of a juvenile between 3.5 and 4 cm TL. Tilney & Hecht (1993) also reported that juveniles (5.4±0.2cm TL) of the ariid *G. feliceps* are freed from mouth-brooders after 140 days. For many fishes, critical periods occur during ecological and developmental transitions when the developing larvae are more vulnerable to external influences (Sifa & Mathias, 1987; Chambers *et al*., 2001). However, principal sensory organs and foraging capacity of catfish are almost completely formed at the earliest stages. Catfish embryos are highly developed even before external feeding is initiated, significantly reducing vulnerability to predators (Osman *et al*., 2008; Arockiaraj *et al*., 2003). Moreover, the high tolerance to turbid water areas and low salinity make catfish more abundant in tropical and subtropical estuarine regions (Barbieri *et al*., 1992; Barletta-Bergan *et al*., 2002a).

During its early ontogeny, *C. spixii* body proportions and growth rates changed considerably. During the embryonic period, head width and eye diameter showed fast growth patterns during what seems to be related to the rapid development of the otic vesicle and consequently the otoliths. Morphometric variables related to longitudinal growth such as pre-pectoral, pre-dorsal, pre-pelvic, pre-anal and head lengths showed slow growth patterns. The exception being the snout length that grows nearly isometric. It is suggested here that the precocious development in embryos of *C. spixii* is

associated to a large energy supply (yolk mass) for the development of organs related to senses in parts of the head. Similar growth patterns were observed in the suckermouth armoured catfih *A. cf. triradiatus,* in which head width and snout length showed a fast positively allometric growth, although morphometric variables related to longitudinal growth (*e.g*. pre-pectoral, pre-dorsal and pre-pelvic lengths) showed patterns near isometric growth (Geerinckx *et al*., 2008).

The fast consumption of the yolk mass during the yolk-sac period can be related to the demand of energy for the development of abilities (*e.g*. swimming) and the end of parental care, when *C. spixii* juveniles leave the mouth of adult males. Differently from the embryonic period, the energy supply in the yolk-sac period (free-embryo) seems to be used in longitudinal growth. Pre-pectoral, pre-dorsal, pre-pelvic, pre-anal, head lengths growth patterns were nearly isometric. Head width and eye diameter showed very slow growth patterns, emphasizing that free-embryos already have well-developed sensory organs. Snout length growth showed a strongly accelerated pattern that can be explained by the transition from endogenous feeding to exogenous feeding at the end of this period. Geerinckx *et al*. (2008), also reported *A. cf. triradiatus* free-embryos to have the same near-isometric pattern in longitudinal growth, except the snout length that showed a fast allometric growth, related not to shifts in feeding behavior, but to the need of suckermouth attachment to substrata as soon as the embryo leaves the egg.

During the embryonic period head angle α° showed a fast growth, while in the yolk-sac period it slowed down. The sub-terminal mouth appears at the third ontogenetic phase in a ventral position. However, in the first two phases it was already possible to observe some developing lip structures. The head angle kept increasing following the mouth migration from the ventral position to the final sub-terminal rostral position at 180°. This fast growth pattern of the head angle in the early ontogenetic

phases of *C. spixii* embryos is possibly due to the need of a rostral mouth to initiate the exogenous feeding when free-embryos become juveniles and parental care finishes. For this reason, few shifts in head angle were observed in juveniles because they had already started with autonomous feeding. In *A. cf. triradiatus*, the same angle between the upper lip surface of the suckermouth and the body axis decreases while yolk-sac is reduced, until complete disapearence (Geerinckx *et al*., 2008). This pattern differs significantly of that observed for *C. spixii*, because in loricariid catfishes the sub-terminal mouth migrates from a rostral position to a ventral position which allows them to attach in the substrata.

The juvenile period is characterized by growth patterns nearly isometric for all body regions, what suggest that juveniles of *C. spixii* are highly developed and already resemble most characteristics of an adult fish. Fin rays are completely ossified and rayed, pectoral and dorsal fins have hard and bony spines, sensory organs and the foraging abilities are well-developed, and the axial and appendicular skeleton are fully developed. Thus, reducing vulnerability to predators and making them more abundant in estuarine regions.

According to Dantas *et al*. (2010), the Ariidae Family was mostly represented by juveniles in the main channel of Goiana estuary with high density values (1 600.9 individuals ha^{-2} and 18 814 g ha^{-2}). In addition, the authors suggested that due to the high eury-thermohaline capacity of catfishes, *C. spixii* uses principally the middle and lower estuary for juvenile recruitment during the late-rainy season when the freshwater input makes these regions less saline. Moreover, the high turbidity in these regions during this season could explain the preference for these areas by promoting refuge and avoidance of predation for juveniles (Barletta-Bergan *et al*., 2002*a*; Barletta *et al*., 2005;

Barletta *et al*., 2008), emphasizing the importance of estuaries for the development of the early ontogenetic phases of this species.

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ANEXO II

Allometric growth patterns as a response to developmental shifts during the early ontogeny of the Ariid catfish *Cathorops agassizii* (Siluriformes)

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ABSTRACT

Development and allometric growth patterns of the ariid catfish *Cathorops agassizii* (Eigenmann & Eigenmann, 1888) were studied from newly neurula embryos to juveniles. The ontogenetic sequence revealed that embryos are well-developed with axial and appendicular skeleton almost completely ossified before hatching. Embryos showed slow longitudinal growth patterns. During this phase, fast growth patterns were observed in head width and eye diameter related to precocious development of sensory organs as otoliths, Weberian apparatus, lens, nostrils and barbells. Posterior to hatching, mouth-brooded free-embryos grew isometric in regions related to longitudinal growth. Slow growth patterns were observed in head width and eye diameter during the yolk-sac period, when sensory organs are already formed. Ossified head bones are easily recognizable in free-embryos, resembling juvenile fishes. The descriptions indicate that *C. agassizii* changes directly from a free-embryo into a juvenile form, without a true larval period. Juveniles are characterized by near-isometric growth patterns in all body regions, reinforcing the idea that juveniles of *C. agassizii* are also highly developed and carry most characteristic of adult fishes.

Key words: precocious development; allometric growth; ontogenetic periods; tropical estuary; free-embryo; parental care; Ostariophysi.

INTRODUCTION

Research about the ontogeny of fishes is considered an important tool for evaluating responses to different patterns of development among teleosts (Iwamatsu, 2004; Verreth *et al*. 1992). It is important to know how developing organs are associated with functional trends and environmental preferences that arise in a fish, principally during early life stages (Fukuhara, 1988; Wainwright *et al*., 2006). Usual switches in behavior, feeding, ecological requirements, habitat, and abilities are observed in different developmental stages and these events are closely related with changes in body shape and formation of specific structures or organs, such as otoliths and fins (Galis *et al*., 1994; Koumoundouros *et al*., 2001; Pinder & Gozlan, 2004; Morgan & Vincent, 2007). The current knowledge about allometric growth patterns of selected body regions (*e.g*. head, trunk and tail) has been seen as a relevant implement for explaining the relations between developmental priorities and performance of a species (Gisbert, 1999; Gisbert *et al*., 2002; Choo & Liew, 2006; Geerinckx *et al*., 2008). Also, knowledge of organogenesis is quite important for understanding the biology, anatomical needs and species-specific adaptations of a developing fish (Meijide & Guerrero, 2000; Koumoundouros *et al*., 2001). Also, studies on ontogeny are fundamental not only to sequence the development of a fish, but also to permit a detailed comparison between species that present common morphological features (Verreth *et al*. 1992).

Cathorops agassizii (Eigenmann & Eigenmann, 1888) is an Ostariophysi (Order Siluriforms – Family Ariidae) marine catfish usually found inhabiting mangrove creeks and main channels of brackish estuaries, seeking for less saline areas (*e.g*. lagoons and river mouths) for spawning (Barletta *et al*., 2003; Barletta *et al*., 2005; Barletta *et al*., 2008, Dantas *et al*., 2010). This species shows a significant presence in the northeast

coast of South America (from Guiana to Northeast Brazil) (Froese & Pauly, 2005; Marceniuk, 2007; Marceniuk & Menezes, 2007). In order to reduce vulnerability, ariid mouth-brooder males display parental care by incubating fertilized eggs in the buccal cavity until the development of a young-juvenile is complete (Rimmer & Merrick, 1983; Rimmer, 1985c; Burgess, 1989; Acero, 2002; Nelson, 2006). Also, the presence of a series of small tendons and bones that connect the swimming bladder and otoliths of the inner ear, allow them to have a better perception of sounds (Otophysan Weberian apparatus) and has been thought to be responsible for the success of the group in turbid estuaries and coastal areas (Burgess, 1989; Rosen & Greenwood, 1970, Evans, 1925; Popper & Coombs, 1980; Sanger & McCune, 2002). The combination of both parental care and the Weberian apparatus contribute to the success of survival and growth of *C. agassizii*.

A study on the ecology of fish in the Goiana Estuary (tropical semi-arid Northeast Brazil) emphasizes that the Ariidae Family was the most abundant in terms of density (individuals m^{-2}) and biomass (g m^{-2}), being *C. agassizii* the second most abundant species of the family in number and weight (Dantas *et al*., 2010). Its high abundance was also reported by Barletta & Costa (2009) one of the reasons why traditional communities at the lower portion of that estuary capture catfishes for subsistence. Other reports about the high abundance of ariids for the Caeté Estuary (Eastern Amazon – North Brazil) (Barletta *et al*., 2005) and the Paranaguá Estuary (Tropical–Subtropical region – South Brazil) (Barletta *et al*., 2008) also exist. Their ecological status allows ariid species to be bioindicator of environmental quality stability and shifts in these estuaries, not only through density and biomass, but also by reflecting changes in their developmental patterns.

Authors have paid attention to systematics, phylogeny (Kailola, 2004; Marceniuk, 2007; Marceniuk & Menezes, 2007; Betancur-R, 2009) and ecology (Araújo, 1988; Azevedo *et al*., 1999; Barletta *et al*., 2003; Barletta *et al*., 2005; Barletta *et al.*, 2008) of species belonging to the Ariidae Family. However, studies on biology of ariid catfishes are scarce due to its low economical value and no importance for recreational activities (Adriaens & Vanderwalle, 2003).

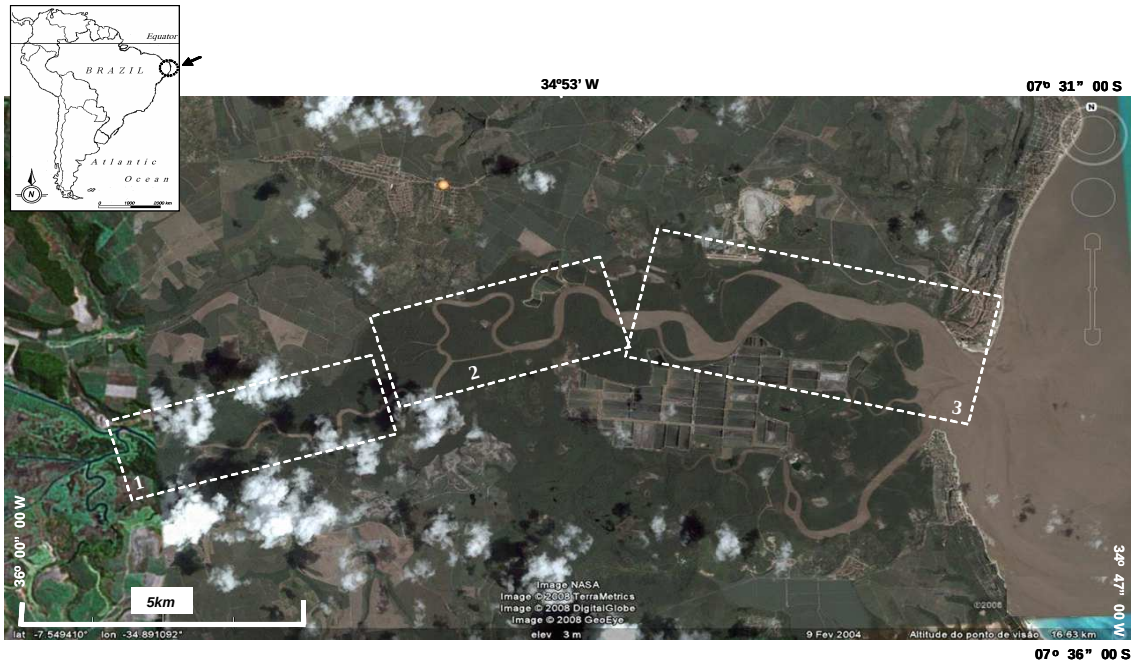
Even less is known about the early life stages of *C. agassizii.* For this reason, this paper presents an overview of the ontogeny concerning embryonic, free-embryo and juvenile development of *C. agassizii* and its relation with the allometric growth patterns in each one of these periods.

MATERIALS AND METHODS

Fish samples were taken in the three areas (upper, middle and lower) of the main channel of Goiana Estuary (Northeast- Brazil) between 2005 and 2009 (Fig. 1). Specimens were collected by otter trawling a net with the aid of a boat following the methodology proposed by Barletta *et al.*, 2005 and Barletta *et al.*, 2008. The net was 8.72m long with an opening of 7.72 m (ground-rope 8.5m and head-rope 7.1m). The mesh-size varied between 35mm in the body and 22mm at the cod-end (between knots). In order to obtain a representative sample for the entire size range of the fish specimens, a cover with a smaller mesh-size (5 mm) was used inside the cod-end. Eggs, free-embryos and juveniles (>35–40 mm Total Length, TL) were collected from the oral cavity of mouth-brooding adult male, anesthetized using tricaine methanesulfonate (MS 222) and stored in 4% buffered formalin.

Sixty eight eggs, sixty free-embryos and forty seven juveniles were analyzed. The precocious development of *C. agassizii* was described by following categorical

morphological events in order to group phases and stages in a logical sequence following the hierarchy of intervals proposed by Balon (1990) (Table IV). The embryonic period comprised the egg stage (Ø<10 mm), ranging from the newly neurula streak-shaped embryo to hatching (<22 mm total TL). The yolk-sac period (>22–36 mm TL), characterized by the nutritional contribution of the yolk-sac, ends when external



feeding is initiated. The Juvenile period (>36–89 mm TL), immediately after the yolk-sac consumption, is characterized by the autonomous feeding.

Figure 1. Goiana Estuary. ▭ = (1) Upper, (2) Middle and (3) Lower portions of the estuary.

In order to compare the ontogenetic development between periods, digital measurements for small individuals were made with a trinocular stereo microscopy - ZEISS; STEMI 2000-C - and the software AxioVision Release 4.7.2 (image capturer calibrated with a millimeter scale in all micrometer zooms that converts the image pixels obtained from the digital camera – Canon; Powershot G10 – into millimeter). A digital caliper – 799 Starrett/range: 6″/150 mm – was used for larger specimens (>2.3 cm TL). The morphometric variables measured were: total length (TL), standard length

(SL), eye diameter (ED), snout length (SNL), pre-pectoral length (PPL), pre-dorsal length (PDL), pre-pelvic length (PVL), pre-anal length (PAL), head length (HL), head width (HW) (Fig. 2a,b). The angle (α° in degrees) between the body axis and the upper lip surface was measured in all specimens (Fig. 2c) and meristic data (number of spines and rays of pectoral, dorsal, pelvic, anal and caudal fins; and the number of pre and post-anal miomers) were quantified. The ontogenetic series of the species was analyzed by clearing and staining, to assure transparency of tissues and skeleton visualization to reveal characters and to observe the general ossification patterns (Dingerkus & Uhler, 1977; Potthoff, 1984).

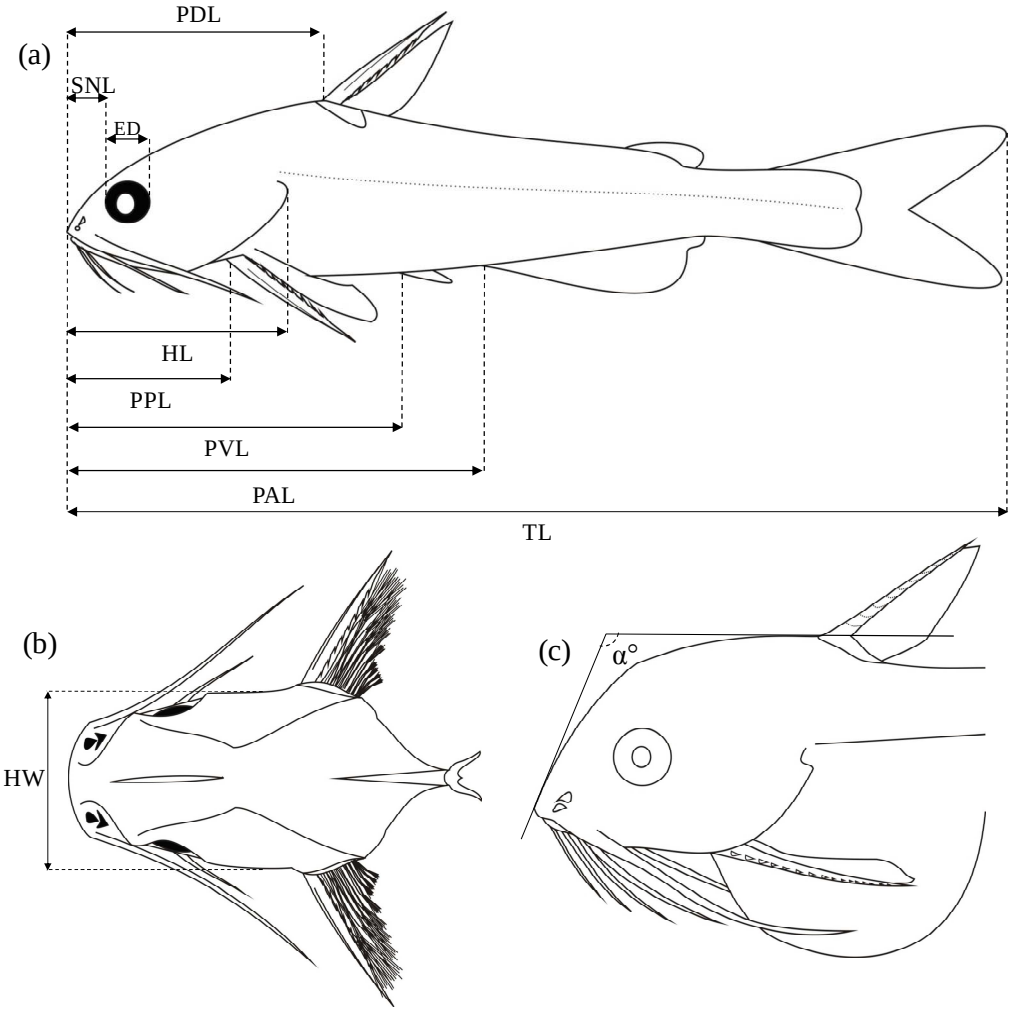


Figure 2. Morphometric variables. (a) TL, total length; ED, eye diameter; SND, snout length; PDL, pre-dorsal length; PPL, pre-pectoral length; PVL, pre-pelvic length; PAL, pre-anal length; HL, head length. (b) HW, head width. (c) α°, head angle.

Allometric growth was calculated as a power function of TL according to the model $Y = \beta_0 TL^{\beta 1} + \varepsilon$ (Huxley, 1924). The linearization of the model was performed on log-transformed data resulting in regression curves of the type $\log Y = \log \beta_0 + \beta_1 \log TL + \log \varepsilon$, where Y is the dependent variable, TL the independent variable, β_0 the intercept and β_1 the slope or growth coefficient. For each period, the growth coefficient of the regression model was calculated and its robustness was measured by calculating r^2 (coefficient of determination) (Zar, 1996). In isometric growth, the slope β_1 is 1 for length and 3 for volume. When the slope β_1 is smaller than the isometric slope, it is known as negative allometric growth; when higher, positive allometric growth (van Snik *et al*., 1997). *F*-tests (Fisher) with a 5% level of significance were performed to verify differences in growth patterns between embryonic and yolk-sac periods; and yolk-sac and juvenile periods (Sokal & Rohlf, 1995).

RESULTS

EMBRYONIC PERIOD

Females of *C. agassizii* produce big sphere-shaped eggs (Ø=8.88±0.42mm) with a wide orange-colored yolk mass located in their centers. The embryonic development occurs on the yolk sphere until hatching. The morphological descriptions revealed that the embryonic period comprises four ontogenetic phases:

Phase I. *Vesicle otic formation and notochord in pre-flexion*

Streak-shaped neurula embryos have an average total length of 7.93±0.95mm (*n*=29). V-shaped miomers are observed along the body varying from 18 to 21 in the pre-anal region and between 27 and 29 in the post-anal region (Fig. 3a.1,3 and Table I). The optic vesicle is well-recognizable, but lack lenses (Fig. 3a.2). A small otic vesicle is present, but there are no otoliths (Fig. 3a.2). Four developing branchiostegal arcs are observed where the operculum will form later on. The regions of the brain are well-

defined (forebrain, midbrain and hindbrain) (Fig. 3a. 2). The notochord is observed along the body axis and is pre-flexioned by the end of the caudal fin (Fig. 3a.3). The median fin fold circumvents the body on both dorsal and ventral portions. Skeletal structures are still not observed (Fig. 5a.1,2,3). Maxillary barbell buds are observed in the region where the upper maxilla will surge. The head angle (angle between the body axis and the upper lip surface) has an average 15.79±4.81°, indicating that the mouth migration has not initiated yet.

Phase II. *Mouth formation, otolith appearance and notochord in post-flexion*

Embryos have an average 11.31±0.49mm (*n*=10) in total length. The head is wide in relation to body and its angle (α°) has an average of 40.79±8.38°, showing that the sub-terminal ventral mouth is formed and keeps migrating to the rostral position (Fig. 3b.1,2,3). Developing nostrils are visible at the snout. Miomers acquire a W shape (Fig. 3.b3). Lenses form (Fig. 3b.2). The three regions of the brain grow and become more developed. The otic vesicle is partially developed and otoliths surge as two conglomerates of small granules (Fig. 3b.2 and Fig. 5b.2). The operculum is recognizable and branchiostegal arcs continue to develop. Maxillary barbells grow and mental barbells appear. Cartilaginous skeletal structures surge to initiate the formation of the upper maxilla, the dentary bone and the Weberian apparatus (Fig. 5b.1,2). The anlage of the Weberian apparatus appears at the fourth anterior-most vertebrae (Fig. 5b.2). Pectoral and dorsal fins (5–7 rays) surge as cartilaginous structures with flexible spines (Fig. 5b.1 and Table I). The cartilaginous caudal fin presents between 15 and 20 rays with hypural and para-hypural bones in development (Fig. 5b.3 and Table I). Neural and haemal spines surge along the post-flexioned notochord. Ossifying vertebrae begin to appear replacing the notochord (Fig. 5b.1,2).

Phase III. *Epural bone formation and ossification of the Weberian apparatus*

Embryos have an average total length of 14.2±0.26mm (*n*=10). The notochord is fully replaced by bony vertebrae (Fig. 5c.1). The head angle has an average of 62±5.69°. The Weberian apparatus, the dentary bone and the upper maxilla are ossified and well-developed (Fig. 5c.1,2). Pectoral (10 rays), dorsal (7 rays), pelvic (6 rays) and anal (20–22 rays) fins are moderately ossified, but still present flexible spines (Fig. 5c.1,4,5 and Table I). The caudal fin presents between 19 and 21 rays (Fig. 5c.3 and Table I). Hypural and para-hypural bones, neural and haemal spines and skeletal structures that will form the cranium initiate ossification (Fig. 5c.1,3). The cartilaginous epural bone appears (Fig. 5c.3). The number of rays of pectoral, dorsal and pelvic fins do not vary in the following phases (Table I).

Phase IV. *Bifurcation of caudal fin and hatching*

Embryos have an average total length of 18.64±1.54mm (*n*=19). The embryo curves the body and its caudal fin is on the head (Fig. 3d.1). The head angle increases to 103.6±11.21°, indicating that the sub-terminal mouth is migrating from a ventral to a rostral position. Head bones formation starts(Fig. 5d.1). The premaxillary tooth plate appears as a cartilaginous structure. Neural and haemal spines, epural, hypural and para-hypural bones keep ossifying (Fig. 5d.1 and e.3). Otoliths increase in size (Fig. 5d.2). The caudal fin (32–39 rays) bifurcates (Fig. 5e.3 and Table I). In larger specimens of this phase otoliths are relatively large and well-developed (Fig. 5e.1,2). At the end of this phase hatching occurs (Fig. 3e.1).

YOLK-SAC PERIOD

Free-embryo stage

Mouth-brooded free-embryos have 29.5±0.88mm (*n*=60) in total length (Fig. 4a). Otoliths are formed. Nostrils are close together and separated by a septum. Dorsal

and lateral pigmentation of the body appears first in newly hatched free-embryos. In the head is observed the differentiation and ossification of the head bones (frontal, mesethmoid, lateral ethmoid, sphenotic, pterotico, extrascapular, supraocciptal process and the pre-dorsal plate) (Fig. 5f.1,2). The head angle increases to 147.61±12.1°. Neuraland haemal spines, epural, hypural and para-hypural bones are ossified (Fig. 5f.5). Fins are densely ossified, but lack some cartilaginous structures. Anal fin present rays varying between 24–25 and the caudal fin between 45–54 (Fig. 5f.4,5 and Table I). The bony premaxillary tooth plate is now well-developed (Fig. 5f.3). Smaller specimens of this period are moderately developed with a large yolk-sac and under-developed fins, while larger specimens are highly developed with a trace of yolk-sac and fins completely developed. Food particles were observed in the stomachs of someindividuals (>3cm total length, TL), marking the onset of exogenous feeding even within the adult male mouth.

JUVENILE PERIOD

Juveniles have 57.13±18.15mm (*n*=47) in total length (Fig. 4b). The body is densely pigmented. The axial and appendicular skeletons are fully ossified (Fig. 5g.1,2,3,4,5,6). The head angle increases (160.52±5.68°) but do not vary much. Anal fin rays vary between 21 and 25, and on the caudal fin between 48 and 59 rays (Table I). Head bones are fully differentiated and easily-recognizable (Fig. 5g.1,2). Juveniles raging from 36 to 40 mm TL are mouth-brooded and resemble some characteristics of free-embryos. Juveniles are released from the mouth of the adult male at 4 cm TL. At 8.9 cm, juveniles resemble most characteristics of adult fishes (Fig. 4c).

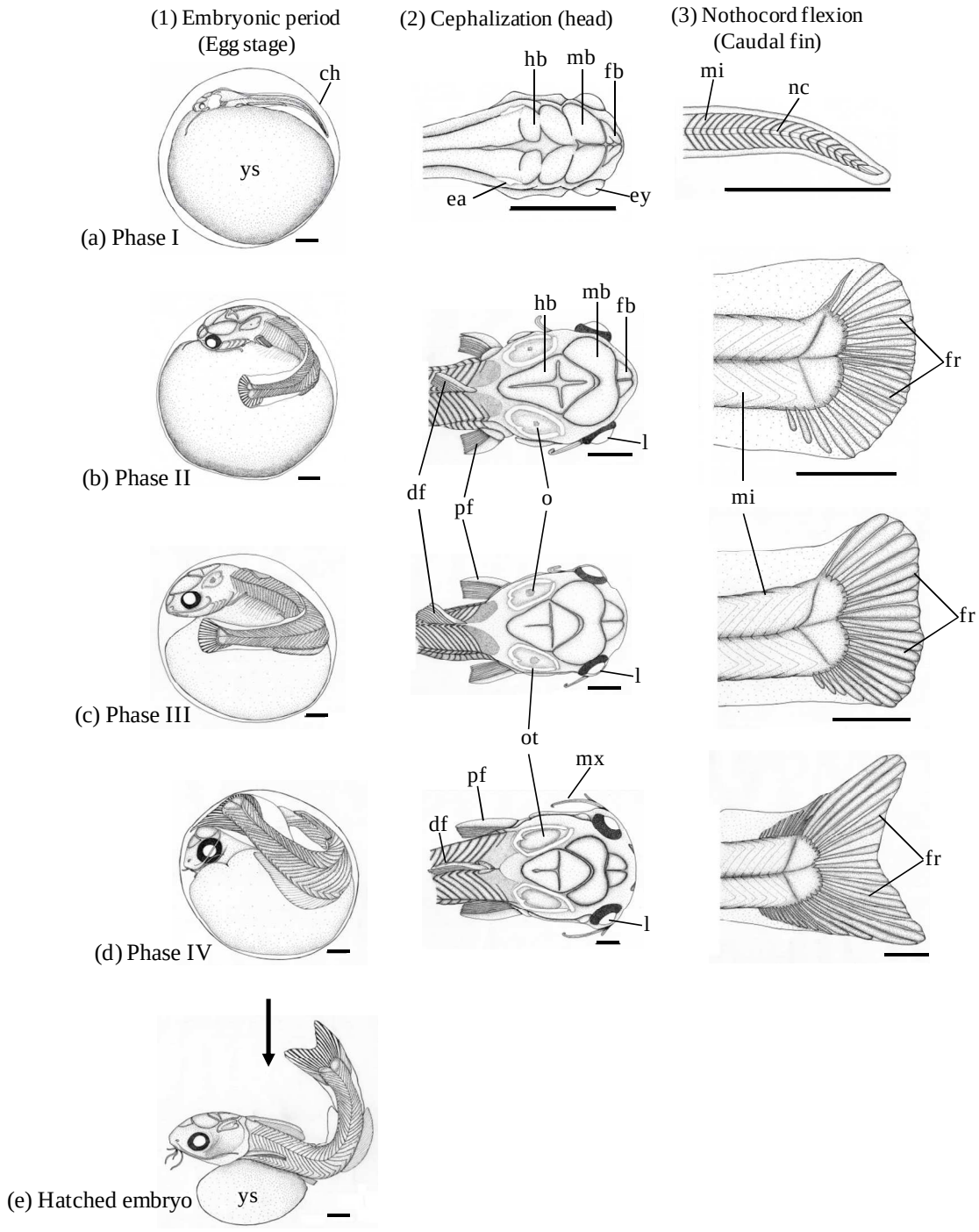


Figure 3. Ontogenetic phases of the embryonic period of *C. agassizii*. ch, chorion; df, dorsal fin; ea, otic vesicle (ear); ey, optic vesicle (eye); fb, forebrain; fr, fin rays; ba, branchiostegal arcs; hb, hindbrain; l, lens; mb, midbrain; mi, miomer; mt, mental barbell; mx, maxillary barbell; nc, notochord; o, operculum; ot, otolith; pf, pectoral fin; ys, yolk-sac. Scale bars: 1 mm. Arrow indicating the moment of hatching.

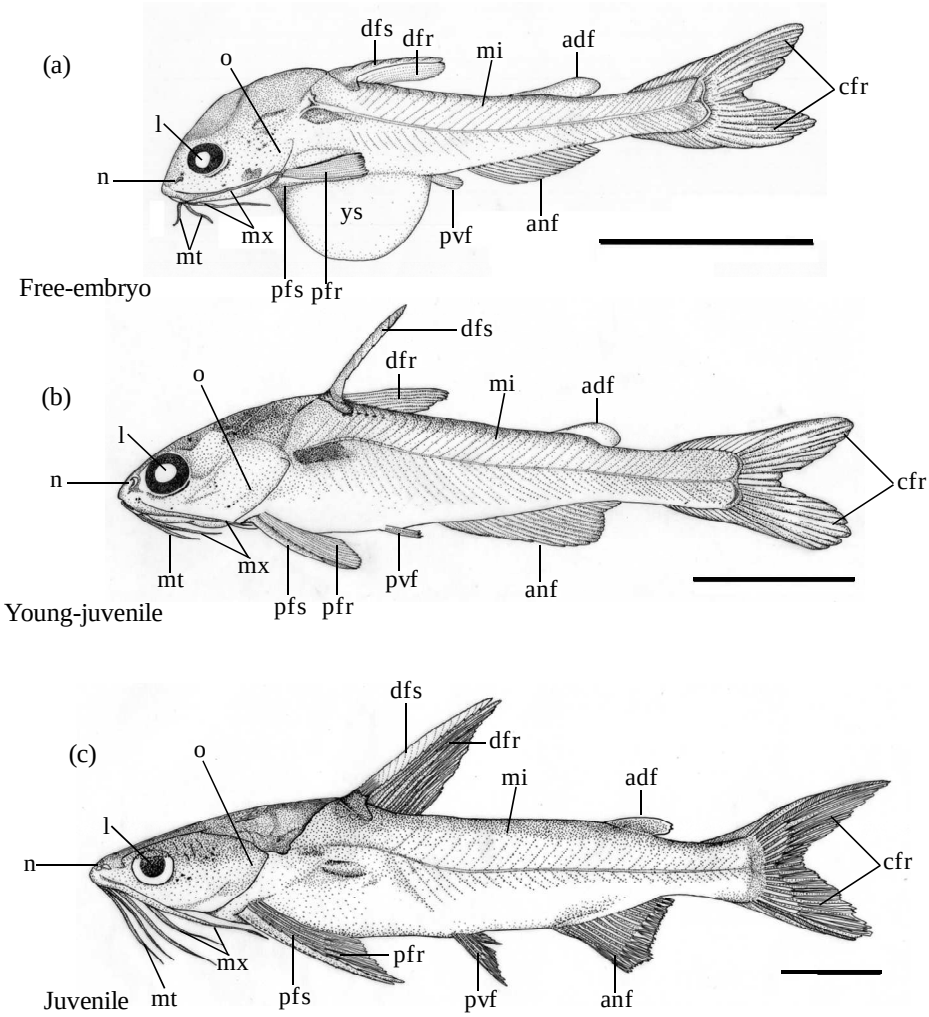


Figure 4. (a) Free-embryo and (b) Young-juvenile and (c) Juvenile of *C. agassizii*. adf, adipous fin; anf, anal fin; cfr, caudal fin rays; dfr, dorsal fin rays; dfs, dorsal fin spine; l, lens; mi, miomer; mt, mental barbell; mx, maxilary barbell; n, narine; o, operculum; pfr, peitoral fin rays; pfs, peitoral fin spine; pvf, pelvic fin; ys, yolk-sac. Scale bars: 10 mm.

GROWTH PATTERNS

The ontogenetic series of *C. agassizii* was divided in three periods. The embryonic period (egg stage) extends from neurula embryo to the moment of hatching; the yolk-sac period (free-embryo stage) comprises the newly hatched embryo until the full consumption of the yolk-sac; the juvenile period begins after yolk-sac consumption when individuals resemble most characteristics of adult fishes.

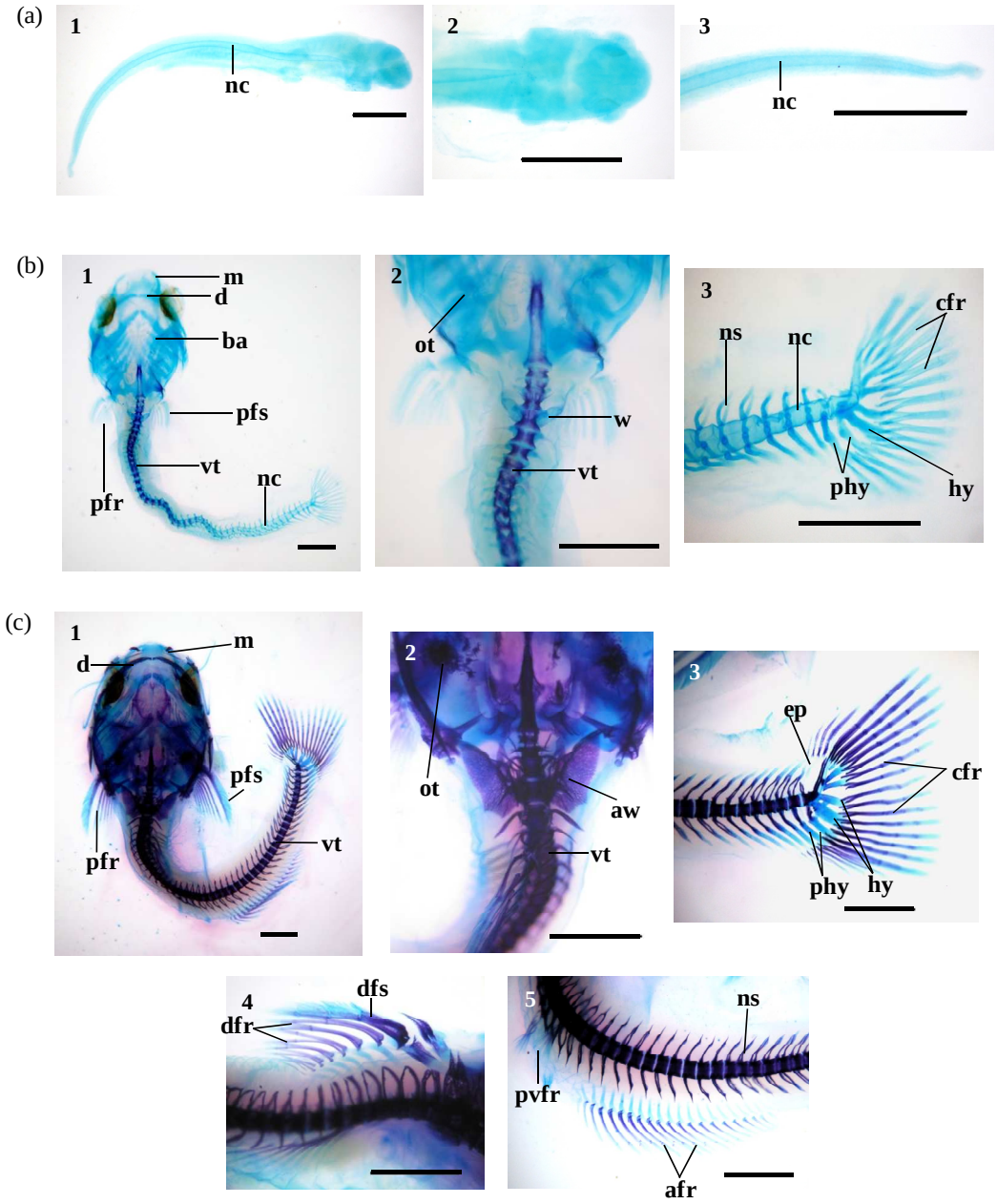


Figure 5. Ontogenetic shifts in ossification patterns of *C. agassizii*. (a)–(d) Phases I to IV of the embryonic period; (e) newly hatched embryo; (f) free-embryo; and (g) juvenile. afr, anal fin rays; ba, branchiostegal arc; cfr, caudal fin rays; d, dentary bone; dfr, dorsal fin rays; dfs, dorsal fin spines; ef, sphenotic bone; ep, epural bone; et, lateral ethmoid bone; fr, frontal bone; hs, haemal spine; hy, hypural bones; m, maxilla; met, mesethmoid bone; nc, notocord ns, neural spine; ot, otolith; pd, pre-dorsal plate; pfr, pectoral fin rays; pfs, pectoral fin spine; phy, parahypural bones; pm, pre-maxillary plate; pto, pterotico; pvfr, pelvic fin rays; sp, supraoccipital process; vt, vertebrae; w, Weberian apparatus. Scale bars: 1 mm.

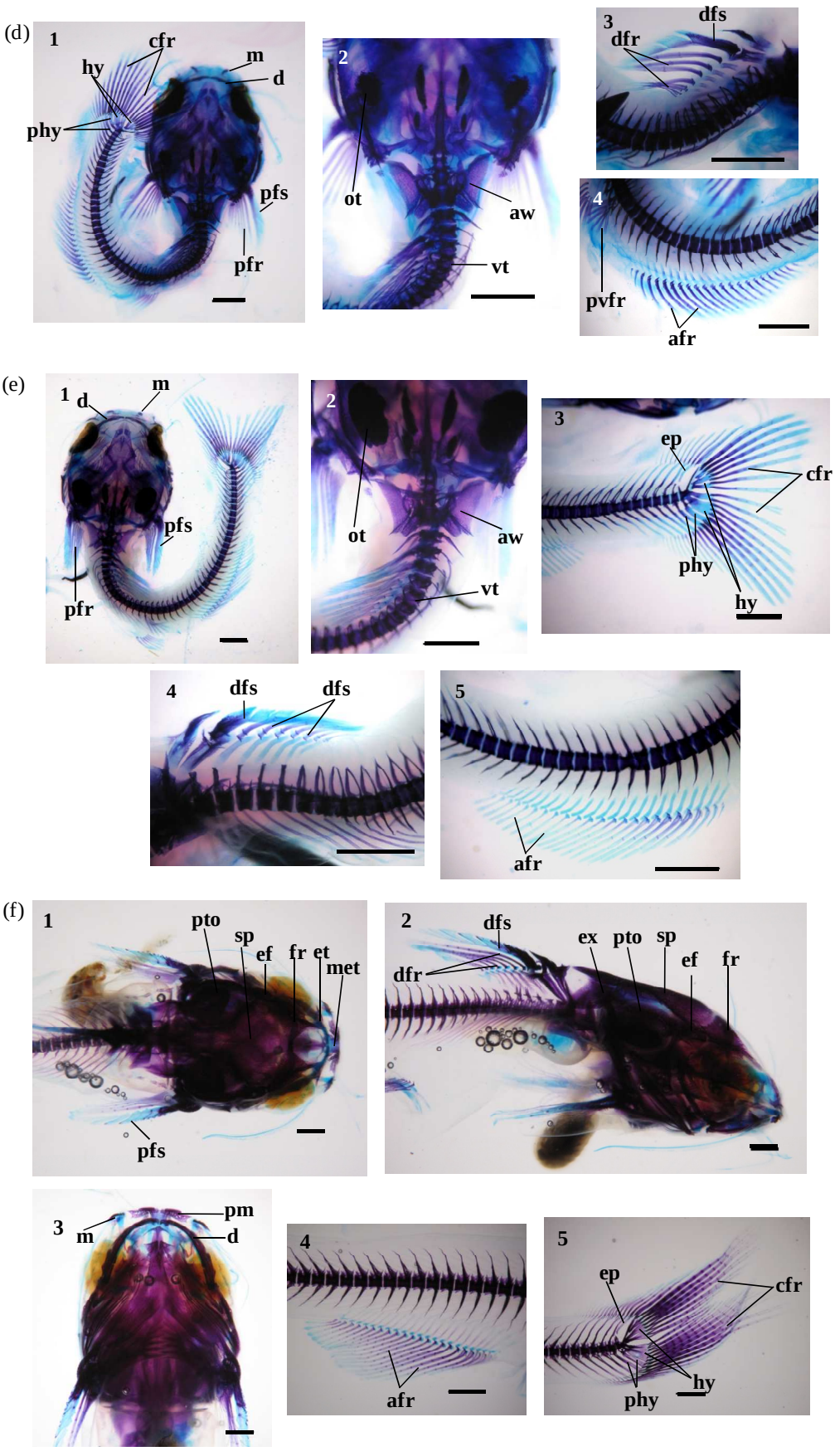


Figure 5. (Continued)

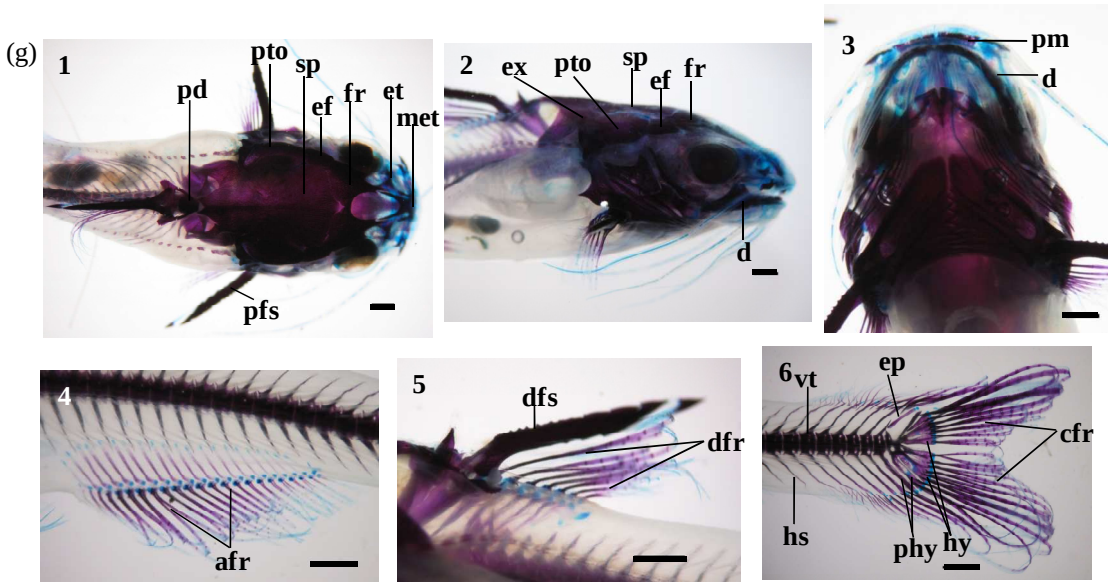


Figure 5. (Continued)

The analyses of growth patterns related to morphometric variables among all periods revealed that the embryonic period is characterized by fast growth patterns [positively allometric growth (β_1>1)] in eye diameter (ED), head width (HW) and snout length (SNL), and for slow growth patterns [negatively allometric growth (β_1<1)] in variables related to longitudinal growth [pre-pectoral (PPL), pre-dorsal (PDL), pre-pelvic (PVL), pre-anal (PAL) and head (HL) lengths] (Table II). ED and HW growth patterns differed significantly among all periods, changing from fast growth patterns during the embryonic period (β_1=1.57 and 1.75, respectively) to slow patterns during the yolk-sac period (β_1=0.35 and 0.54, respectively), and accelerating the growth to near-isometric ($\beta_1\approx1$) during the juvenile period (β_1=0.97 and 1.02, respectively) (Fig. 5a,h and Tables II and III). SNL growth pattern did not differ among periods, growing allometricaly positive (β_1=1.34) during the embryonic period and near to isometric patterns during yolk-sac and juvenile periods (β_1=1.18 and 0.96, respectively) (Tables II and III).

TABLE I. Meristic frequency in the different ontogenetic periods of *C. agassizii* classified according to the criteria defined on table

Ontogenetic periods	N° of pre and post anal miomers	N° of branchiostegal arcs	N° of spines and rays of pectoral fin	N° of spines and rays of dorsal fin	N° of spines and rays of pelvic fin	N° of spines and rays of anal fin	N° of spines and rays of caudal fin
Embryonic period							
Phase I	18-21 / 27-29	4	- / -	- / -	- / -	- / -	- / -
Phase II	17-18 / 24-28	4	I / 5-6	I / 5-7	- / -	- / -	- / 15-20
Phase III	15-19 / 24-28	4	I / 10	I / 7	- / 6	- / 20-22	- / 19-21
Phase IV	16-19 / 23-26	4	I / 10	I / 7	- / 6	- / 19-22	- / 31-45
Free-embryo period	13-18 / 24-30	4	I / 10	I / 7	- / 6	- / 22-25	- / 40-58
Juvenile period	12-16 / 25-29	4	I / 10	I / 7	- / 6	- / 21-25	- / 48-59

PPL and PAL grew slowly during the embryonic period (β_1=0.47 and 0.76, respectively), differing from the isometric growth patterns observed during the yolk-sac and juvenile periods. PDL growth showed a slow pattern during the embryonic period (β_1=0.63) and differed from the negatively allometric pattern observed during the yolk-sac period (β_1=0.87), which in turn did not differ from the near-isometric growth in the juvenile period. The growth pattern observed in PVL did not differ among periods, which grew negatively allometric during the embryonic period (β_1=0.80) and near isometric during the yolk-sac and juvenile periods (β_1=0.93 and 0.98, respectively). HL showed a slow growth pattern during the embryonic period, differing from the positively allometric growth observed in the yolk-sac period (β_1=1.31), and from the isometric pattern during the juvenile period (β_1=1.02) (Tables II and III).

The growth pattern observed in the head angle (α°) during the embryonic period was fast (β_1=2.16), emphasizing the accelerated migration of the sub-terminal ventral mouth to the rostral position (Fig. 6i and Table II). This pattern differed of that observed in the yolk-sac period, which had a slow growth pattern (β_1=0.59) (Tables II and III). The head angle growth pattern also differed between yolk-sac and juvenile periods, which slowed significantly during the transition from free-embryo to juvenile (β_1=0.09), indicating that the mouth migration to the rostral position was almost finished (Fig. 6i and Tables II and III).

The yolk-sac depletion (YSD) presented a particular pattern. During the embryonic period the yolk-sac consumption was slow (β_1= –0.45), taking into account the importance of this energy supply for embryos (Fig. 6j and Table II). However, during the yolk-sac period the yolk-sac consumption was very fast (β_1= –1.88), due to the need of swimming abilities (restricted by the presence of the yolk-sac) before being released by adult males (Fig. 6j and Table II).

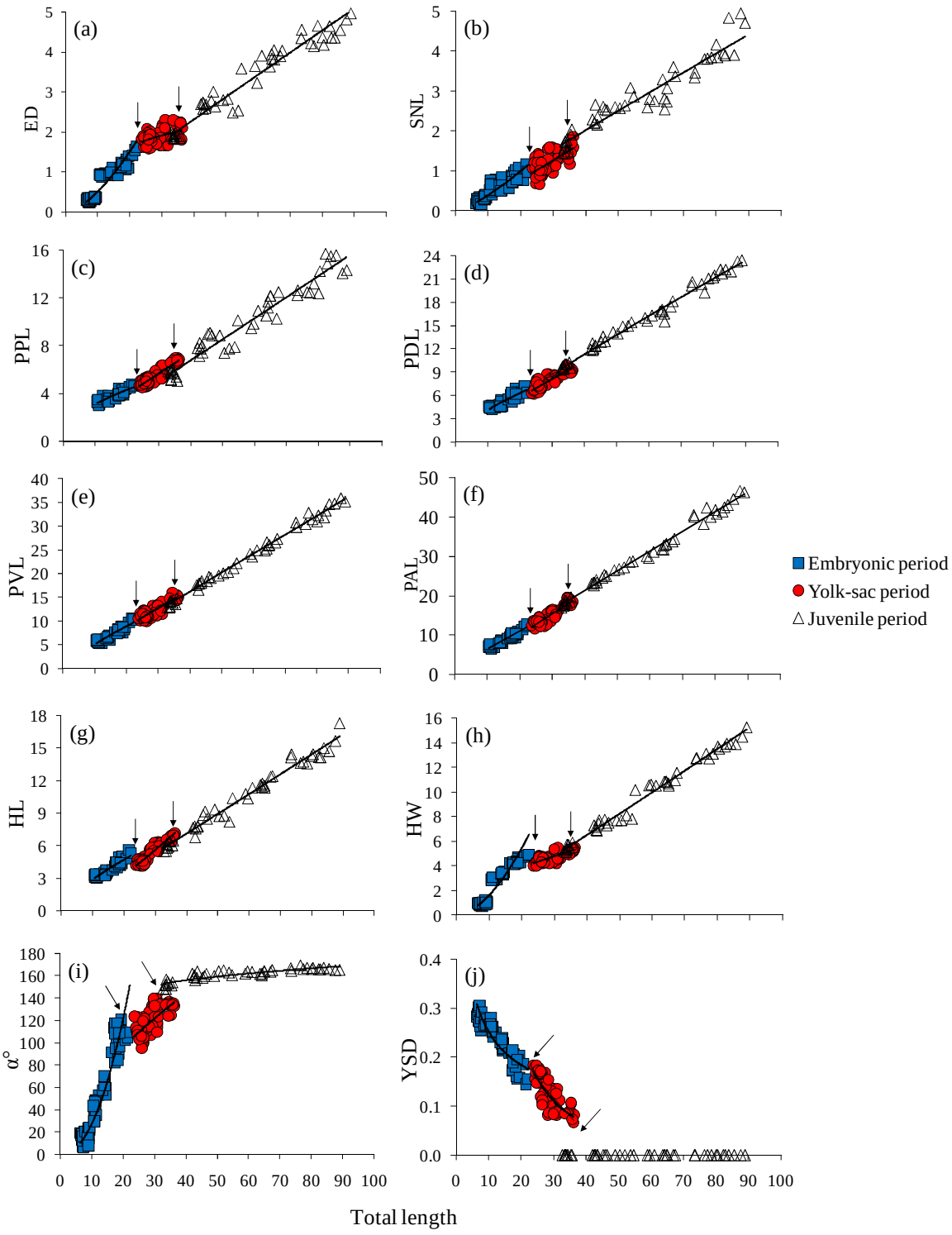


Figure 6. Relationships of the morphometric variables with total length (LT) for the embryonic period, Yolk-sac period and juvenile period. (a) ED, eye diameter; (b) SNL, snout length; (c) PPL, pre-pectoral length; (d) PDL, pre-dorsal length; (e) PVL, pre-pelvic length; (f) PAL, pre-anal length; (g) HL, head length; (h) HW, head width; (i) α°, head angle; (j) YSD, yolk-sac depletion. Arrows indicating the moment of hatching and yolk-sac depletion, respectively. The equations values are on Table II following the model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$.

TABLE II. Growth coefficients [slope (β_1)], intercept (β_0) and r^2 values of morphometric variables in 175 specimens of *C. agassizii* regressed with total length (TL). Model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$. ED, eye diameter; SNL, snout length; PPL, pre-pectoral length; PDL, pre-dorsal length; PVL, pre-pelvic length; PAL, pre-anal length; HW, head width; HL, head length; α°, head angle; YSD, yolk-sac depletion.

	Embryo (n = 68)			Free-embryo (n = 60)			Juvenile (n = 47)		
Variable	β_0	β_1	r^2	β_0	β_1	r^2	β_0	β_1	r^2
ED	-1.8630	1.5700±0.0726	0.8761 ***	-0.2398	0.3527±0.0920	0.2021 ***	-1.1920	0.9718±0.0344	0.9466 ***
SNL	-1.7520	1.3450±0.0703	0.8472 ***	-1.6380	1.1780±0.1877	0.4042 ***	-1.2420	0.9652±0.0434	0.9167 ***
PPL	0.0138	0.4755±0.0384	0.8055 ***	-0.6066	0.9206±0.0602	0.8015 ***	-0.8169	1.0280±0.0390	0.9391 ***
PDL	-0.0197	0.6277±0.0495	0.8130 ***	-0.3813	0.8721±0.0655	0.7536 ***	-0.3938	0.9020±0.0153	0.9872 ***
PVL	-0.0991	0.8058±0.0425	0.9066 ***	-0.2666	0.9279±0.0663	0.7716 ***	-0.3755	0.9891±0.0131	0.9922 ***
PAL	0.0479	0.7596±0.0479	0.9284 ***	-0.3128	1.0150±0.0659	0.8035 ***	-0.1912	0.9493±0.0132	0.9914 ***
HW	-1.5380	1.7480±0.0738	0.8946 ***	-0.1164	0.5393±0.0603	0.5797 ***	-0.8714	1.0510±0.0200	0.9840 ***
HL	-0.2543	0.7130±0.0436	0.8785 ***	-1.1920	1.3140±0.0651	0.8754 ***	-0.7821	1.0200±0.0233	0.9771 ***
α°	-0.7302	2.1610±0.1007	0.8747 ***	1.2140	0.5898±0.0826	0.4675 ***	2.0390	0.0957±0.0080	0.7599 ***
YSD	-0.1427	-0.4552±0.0254	0.8289 ***	1.8210	-1.8790±0.1894	0.6292 ***			

NS, non-significant ($P > 0.05$); **$P < 0.01$; ***$P < 0.001$

TABLE III. Significance values of comparisons of regression slopes (β_1) (in relation to TL) of the morphometric variables between the embryonic and yolk-sac periods; and yolk-sac and juvenile periods in *C. agassizii*. Model: $Y = \beta_0 LT^{\beta 1} + \varepsilon$. ED, eye diameter; SNL, snout length; PPL, pre-pectoral length; PDL, pre-dorsal length; PVL, pre-pelvic length; PAL, pre-anal length; HW, head width; HL, head length; α°, head angle; YSD, yolk-sac depletion.

	Embryo and yolk-sac periods		Yolk-sac and juvenile periods	
Variables	*F*-test		*F*-test	
ED	$F_{(1,124)}$	39.3230 ***	$F_{(1,103)}$	42.3149 ***
SNL	$F_{(1,124)}$	0.5666 ^{NS}	$F_{(1,103)}$	1.5650 ^{NS}
PPL	$F_{(1,95)}$	39.2164 ***	$F_{(1,103)}$	1.6936 ^{NS}
PDL	$F_{(1,95)}$	8.7145 *	$F_{(1,103)}$	0.2526 ^{NS}
PVL	$F_{(1,95)}$	2.4212 ^{NS}	$F_{(1,103)}$	1.0991 ^{NS}
PAL	$F_{(1,95)}$	12.1895 ***	$F_{(1,103)}$	1.2821 ^{NS}
HW	$F_{(1,124)}$	40.2455 ***	$F_{(1,103)}$	73.3981 ***
HL	$F_{(1,95)}$	58.8727 ***	$F_{(1,103)}$	19.6510 ***
α°	$F_{(1,124)}$	36.5717 ***	$F_{(1,103)}$	51.7833 ***
YSD	$F_{(1,124)}$	90.4564 ***		

NS, non-significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

TABLE IV. Summary of the main morphological events during the ontogeny of *C. agassizii*.

Ontogenetic period	Phases	Morphological events
Embryonic	Phase I	Notochord in pre-flexion; V-shaped miomers; optic vesicle recognizable; formation maxillary barbell buds; three regions of the brain well-defined.
	Phase II	Notochord in post-flexion; W-shaped miomers; appearance of the lenses, mouth and nostrils; formation of otoliths (conglomerate of small granules); anlage of the Weberian apparatus as a cartilaginous bud; formation of pectoral, dorsal and caudal fins; hypural and para-hypural bones in development; replacement of the notochord by ossified vertebrae.
	Phase III	Notochord fully replaced by vertebrae; Weberian apparatus, dentary bone and upper maxillary ossified; pectoral, dorsal, pelvic and anal fins moderately ossified; initiate the ossification of caudal fin; formation of epural bone; initiate the ossification of hypural and para-hypural bones, and neural and haemal spines.
	Phase IV	Initiate the head bones formation; otoliths increase in size; caudal fin bifurcates; hatching at the end of the phase.
Yolk-sac		Otoliths formed; Nostrils close together, separated by a septum; initiate the dorsal and lateral pigmentation; neural and haemal spines, epural, hypural and para-hupural bones ossified; bony premaxillary tooth plate well-developed; onset of exogenous feeding at the end of this period.
Juvenile		Body densely pigmented; axial and appendicular skeleton fully ossified; head bones fully differentiated; releasing of the buccal cavity of the adult male at 4 cm TL.

DISCUSSION

Different growth patterns during early life of fishes are the result of specific combinations of factors interacting dynamically according to functional demands that each species requires (Geerinckx *et al.*, 2008; Gomes *et al.*, 2010). This period is marked by rapid development and growth while many morphological changes are occurring (Fuiman & Higgs, 1997). For many fishes, critical periods occur during the ecological and developmental transitions (*e.g.* the transition from endogenous to exogenous feeding) when larvae are more vulnerable to external influences, increasing vulnerability to predation and also resulting in higher mortalities (Chambers & Leggett, 1987; Chambers *et al.*, 2001). However, whereas principal sensory organs and foraging capacity of the ariid catfish *C. agassizii* are almost completely formed in the earliest stages, studies suggest that catfish embryos are highly developed before external feeding is initiated, what reduces vulnerability (Rimmer, 1985c; Menon *et al*., 1989; Arockiaraj *et al.*, 2003; Osman *et al.*, 2008). Studies in estuaries of tropical (Cervigón, 1985, Barletta & Blaber, 2007; Barletta *et al*., 2010; Dantas *et al.*, 2010), and tropical-subtropical transitional (Barletta *et al.*, 2008) latitudes of South America suggest that the different ontogenetic phases of ariids are well adapted to live in different reaches of these estuaries due to the early development of the Weberian apparatus (Sanger & McCune, 2002) and the high thermohaline capacity (Barletta & Blaber, 2007).

Different from most teleost, which hatchlings are underdeveloped (Richards, 2006), embryos of *C. agassizii* are highly developed long before hatching. This precocious development could be explained by their long incubation periods, once the purpose is to release developed individuals. A long incubation period was also reported by Tilney and Hecht (1993) for *Galeichthys feliceps* Valenciennes, 1840, with an incubation period of twenty-weeks and juveniles being freed (from the mouth) by adult

males at 5.4cm ± 0.2 TL. The brooding period of *Neoarius graeffei* (Kner & Steindachner, 1867) lasted from 6 to 8 weeks and juveniles were released when they were up to 5.9 cm TL (Rimmer, 1985c). The incubation period of *C. agassizii* is about three months and parental care also extends from newly fertilized eggs to the development of a juvenile measuring 3.6–4cm TL (Dantas *et al*., 2010). Moreover, the large yolk mass present in Ariid eggs seems to be the principal energy supply permitting a fast development even inside the egg envelope (Adriaens & Vanderwalle, 2003). Also, the ontogenesis of *C. agassizii* supports the idea that, as many catfishes, they lack a true larval period, changing from free-embryos (after hatching) directly into juveniles (Balon, 1990). The same patterns of direct development in catfishes were reported for the clariid *Clarias gariepinus* (Burchell, 1822) (Osman *et al.*, 2008), the Loricariid *Ancistrus cf. triradiatus* Eigenmann, 1918 (Geerinckx *et al.*, 2007) and the ariid *G. feliceps* (Tilney & Hecht, 1993).

Embryos of *C. agassizii* are characterized by the early ossification of the axial (vertebrae and cranial structures) and appendicular skeleton (fin rays and spines) (Higuchi, 1982). After hatching, free-embryos (yolk-sac period) are highly developed with the axial and appendicular skeletons completely ossified, except for the fin rays and spines that keep ossifying. Juveniles in turn, present few cartilaginous structures and keep growing until resemble almost all characters of adult fish. Similar patterns of ossification were observed in the ariid *G. feliceps*, in which the skeleton of newly hatched free-embryos is almost completely ossified (Tilney & Hecht, 1993).

Some teleost fishes start to develop their sensory organs before hatching but they hatch with morphologically immature features, such as reported for the cyprinid sunbleak *Leucaspius delineatus* (Heckel, 1843) (Pinder & Gozlan, 2004). Other fishes initiate the development of sensory organs before hatching, for example the Erythrinid

Hoplias lacerdae Miranda Ribeiro, 1908 (Gomes *et al.*, 2010) and the Clariid *C. gariepinus* (Osman *et al.*, 2008; Mukai *et al.*, 2008). However, sensory organs such as otoliths, nostrils, barbells and the Weberian apparatus in *C. agassizii* have also a fast, thus precocious development even during the embryonic period. Similar patterns of sensory organs development are observed in *G. feliceps*, where principal sensory organs develop during the embryonic period and are well-developed long before hatching (Tilney & Hecht, 1993).

The early development of *C. agassizii* is characterized by shifts in body proportions and growth patterns. The fast growth patterns of head width and eye diameter and the positively allometric of the snout length seem to be associated with the availability of a large energy supply (yolk mass) for de development of sensory organs during the embryonic period; whereas patterns related to longitudinal growth were slow. The rapid growth of the head width supports the idea of having a wide space in the head for the development of large otoliths. Also, most of the organs related to sensing are located in the head, such as nostrils (snout), eye and barbells. Geerinckx *et al.*, (2008) also reported the suckermouth armoured catfih *A. cf. triradiatus* to have a similar growth pattern to that observed in *C. agassizii*, in which head width and snout length showed a fast positively allometric growth, although morphometric variables related to longitudinal growth (*e.g.* pre-pectoral, pre-dorsal and pre-pelvic lengths) showed near-isometric patterns.

Different from the embryonic period, the growth patterns of pre-pectoral, pre-dorsal, pre-pelvic and head lengths accelerated to near-isometric and positively allometric during the yolk-sac period what suggests that the energy supply in free-embryos seems to be related to longitudinal growth. Head width slowed the growth patterns to a strongly negatively allometric during this period; and eye diameter of both

species does not grow much in relation total length, suggesting that free-embryos already have well-developed sensory organs.

In free-embryos, the abilities to swimming are interfered by the presence of the yolk sac. The fast consumption of the yolk mass during the yolk-sac period is the most important event during the transition of free-embryos into juveniles, giving to *C. agassizii* juveniles the ability to swim and leave the mouth of adult males. Similar patterns of near-isometric growth in longitudinal lengths was observed in *A. cf. triradiatus* free-embryos, except in the snout length that showed a fast allometric growth related to the need of suckermouth attachment to substrata as soon as the embryo leaves the egg (Geerinckx *et al.*, 2008).

One of the most important event during the ontogeny of the species studied could be considered the migration of the sub-terminal mouth from the ventral to the rostral position, marking the beginning of exogenous feeding. The relation between body axis and the upper lip surface of the mouth (head angle α°) was very fast during the embryonic period, while in the yolk-sac period it slowed to a negatively allometric growth in *C. agassizii*. The head angle kept increasing following the mouth migration until the final rostral position at approximately 180°. Few shifts in head angle were observed in juveniles, when they start autonomous feeding. In loricariid catfishes the sub-terminal mouth migrates from a rostral position to a ventral position allowing them to attach to the substrata. This peculiar characteristic significantly differs of that observed in *C. agassizii* because the same angle between the upper lip surface of the suckermouth and the body axis decreases, reaching zero such as in juveniles of *A. cf. triradiatus* (Geerinckx *et al.*, 2008).

Juveniles are characterized by growth patterns nearly to the isometric in all body parts, emphasizing that they already resemble most characteristics of adult fishes. The

fact that they are highly developed suggests that they are ready to be freed by mouth-brooding males. Mouth-brooder males seek for the salt edge border in the upper estuary of the Goiana Estuary during the late-dry season when salinity reach 0 to probably avoid predators which avoid this portion of the estuary (Barletta *et al*., 2005; Barletta & Blaber, 2007; Barletta *et al*., 2008; Dantas *et al.*, 2010). The release of individuals of *C. agassizii* occurs during the early-rainy season, when juveniles migrate from the upper to the middle portion of the Goiana Estuary for reproduction and juvenile recruitment (Dantas *et al.*, 2010). These strategies also help reducing vulnerability to predators and adverse environmental conditions (Dantas *et al.*, 2010).

The precocious development, the high eury-thermohalin capacity and the high tolerance to turbid areas are important to promote refuge and avoidance of predation by juveniles of *C. agassizii* (Barletta & Blaber, 2007; Dantas *et al.*, 2010). All of these events during the development of this species support the idea of wide distribution and high abundance of Ariids in tropical and subtropical estuarine regions of South America. These affirmations emphasize the importance of the different habitats of the estuarine ecosystem for the development of the early ontogenetic periods of this species.

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