



Physiological Responses in *Sciades passany* (Siluriformes, Ariidae): potential for Environmental Biomonitoring

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Abstract

This study investigated the physiological responses (metabolites and enzymes) of the estuarine catfish *Sciades passany* (Siluriformes: Ariidae), a sentinel species for ecophysiological biomonitoring in Maracá-Jipiôca Island, located in the Amazon estuary. Following capture, specimens were anesthetized with benzocaine (100 mg/L, and blood samples were collected via dorsal caudal vessel puncture using disposable needles containing disodium EDTA anticoagulant ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ - Sigma). Biometric measurements (body length and biomass) were recorded, and after euthanasia, gastrointestinal organs were removed for calculation of the viscerosomatic index (VSI) and hepatosomatic index (HSI). The length-weight relationship (LWR) was estimated using log-transformed linear regression according to Le Cren (1951), and the relative condition factor (Kn) was calculated for each individual. Plasma energy substrates—including glucose, total proteins, and total lipids—along with transaminase enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) were analyzed during two sampling periods: September 2018 and February 2019. Results revealed detectable physiological responses in *S. passany*, with variations in metabolites and somatic indices. Notably, hyperglycemia (278.51 ± 56.62 mg/dL) and hypertriglyceridemia (171.77 ± 82.94 mg/dL) were observed, along with positive allometric growth ($b = 3.161$, $r^2 = 0.88$) and elevated hepatosomatic index values (1.41 ± 0.4) during the dry season. The absence of significant enzymatic variations—typically indicative of environmental disturbances suggests that the observed changes primarily reflect intrinsic physiological modulations of the species. These findings underscore the potential of *Sciades passany* as a valuable bioindicator for monitoring estuarine conditions. Its ability to exhibit measurable physiological responses, such as variations in energy substrates and somatic indices, makes it a useful tool for assessing ecosystem health. Further research is needed to deepen understanding of this species' physiological dynamics and its relationship with estuarine ecosystem fluctuations.

Keywords: Amazonian Siluriforms; Amazon catfish; seasonal influence; Amapa delta.

Resumo - Respostas fisiológicas em *Sciades passany*: potencial para biomonitoramento ambiental

Este estudo investigou as respostas fisiológicas (metabólitos e enzimas) do bagre estuarino *Sciades passany* (Siluriformes: Ariidae), uma espécie sentinela para biomonitoramento ecofisiológico na Ilha Maracá-Jipiôca, no estuário amazônico. Após a captura, os animais foram anestesiados com benzocaína (100 mg/L) e o sangue coletado por punção dos vasos sanguíneos dorsocaudais com agulhas descartáveis contendo anticoagulante EDTA dissódico ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ - Sigma). Foram realizadas medições biométricas (comprimento corporal e biomassa) e após eutanásia, os órgãos do trato gastrointestinal foram removidos para cálculo dos índices viscerossomático (IVS) e hepatossomático (IHS). A relação peso-comprimento (LWR) foi estimada por regressão linear após transformação logarítmica dos dados, conforme Le Cren (1951) e o fator de condição

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relativo (Kn) de cada peixe foi calculado. Além disso, foram analisados os substratos energéticos plasmáticos, incluindo glicose, proteínas totais e lipídios totais, bem como as enzimas transaminases (alanina aminotransferase, aspartato aminotransferase e fosfatase alcalina) coletados nos meses de setembro/2018 e fevereiro/2019. Os resultados demonstraram que o *Sciades passany* apresenta respostas fisiológicas detectáveis, com variações em seus metabólitos e índices somáticos. Especificamente, foram observadas hiperglicemia (278.51 ± 56.62) e hipertrigliceridemia (171.77 ± 82.94), juntamente com crescimento alométrico positivo ($b = 3.161$, $r^2 = 0.88$) e valores elevados do índice hepatossomático (1.41 ± 0.4) na estação seca. A ausência de variações enzimáticas significativas, geralmente indicativas de distúrbios ambientais, sugere que as mudanças observadas refletem principalmente modulações fisiológicas intrínsecas da espécie. Esses achados demonstram o potencial de *Sciades passany* como um bioindicador valioso para monitorar as condições estuarinas. A capacidade da espécie de apresentar respostas fisiológicas mensuráveis, como variações nos níveis de substratos energéticos e índices somáticos, a torna uma ferramenta útil para avaliar a saúde do ecossistema. Estudos futuros são necessários para aprofundar a compreensão das respostas fisiológicas desta espécie e sua relação com a dinâmica do ecossistema estuarino.

Palavras-chave: Siluriformes amazônicos, bagre amazônico; influência sazonal; delta do Amapá.

Resumen - Respuestas fisiológicas en *Sciades passany*: potencial para la biomonitorización ambiental

Este estudio investigó las respuestas fisiológicas (metabolitos y enzimas) del bagre estuarino *Sciades passany* (Siluriformes: Ariidae), una especie centinela utilizada para el biomonitoreo ecofisiológico en la Isla Maracá-Jipióca, ubicada en el estuario amazónico. Tras la captura, los ejemplares fueron anestesiados con benzocaína (100 mg/L, y se recolectaron muestras de sangre mediante punción de los vasos dorsocaudales con agujas desechables que contenían EDTA disódico como anticoagulante ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ - Sigma). Se realizaron mediciones biométricas (longitud corporal y biomasa), y tras la eutanasia, se extrajeron los órganos del tracto gastrointestinal para calcular los índices viscerosomático (IVS) y hepatossomático (IHS). La relación longitud-peso (LWR) se estimó mediante regresión lineal con transformación logarítmica de los datos, según Le Cren (1951), y se calculó el factor de condición relativo (Kn) para cada individuo. Se analizaron los sustratos energéticos plasmáticos—glucosa, proteínas totales y lípidos totales, junto con las enzimas transaminasas (alanina aminotransferasa, aspartato aminotransferasa y fosfatasa alcalina), recolectadas en septiembre de 2018 y febrero de 2019. Los resultados mostraron que *S. passany* presenta respuestas fisiológicas detectables, con variaciones en sus metabolitos e índices somáticos. Se observaron hiperglucemia (278.51 ± 56.62 mg/dL) e hipertrigliceridemia (171.77 ± 82.94 mg/dL), además de crecimiento alométrico positivo ($b = 3.161$, $r^2 = 0.88$) y valores elevados del índice hepatossomático (1.41 ± 0.4) durante la estación seca. La ausencia de variaciones significativas enzimáticas, comúnmente asociadas a perturbaciones ambientales, sugiere que los cambios observados reflejan principalmente modulaciones fisiológicas intrínsecas de la especie. Estos hallazgos destacan el potencial de *Sciades passany* como bioindicador valioso para el monitoreo de las condiciones estuarinas. Su capacidad para presentar respuestas fisiológicas mensurables, como variaciones en los niveles de sustratos energéticos e índices somáticos, lo convierte en una herramienta útil para evaluar la salud del ecosistema. Se requieren estudios futuros para profundizar en la comprensión de sus respuestas fisiológicas y su relación con la dinámica del ecosistema estuarino.

Palabras clave: Siluriformes amazónicos, bagre amazónico; influencia estacional; delta de Amapá.

Introduction

Teleost fish, commonly known as catfish, belong to the order Siluriformes, which encompasses several freshwater and saltwater families, notably the Ariidae (Malabarba and Malabarba, 2020). While predominantly marine, some Ariidae species inhabit estuarine and freshwater environments. These species are recognized as significant fishing resources in regions such as Africa (Simier et al., 2021); and the Brazilian Amazon (Lutz et al., 2023). Despite their abundance and ecological importance, these fish remain understudied compared to other freshwater Siluriforms (Denadai et al., 2013). Given the increasing pressure on marine fish stocks and the crucial role of these species in local artisanal fisheries, a deeper understanding of their biology and ecology is essential (Correia et al., 2020).

The Brazilian government, through its Ministry of Agriculture, Livestock and Food Supply, has acknowledged the relevance of fish belonging to this family and implemented Recovery Plans for marine catfish, as well as for gillbacker sea catfish species *Sciades Parkeri* (Brazil, 2015;2018). It was done to help recovering fish stocks and organizing the fishing activity. The current study emphasizes the need of both

conducting studies and monitoring activities at Maracá-Jipiôca Ecological Station (ES) by highlighting the Amazonian region. The coastal-marine estuarine ecosystem at (ES) hosts several ariidae species, such as *Ampharius rugispinis*, *Bagre bagre*, *Cathorops spixii*, *Sciades passany*, Esteves-Silva et al. (2020) and *Sciades parkeri* ICMBio (2017). The aforementioned ES is an Amazonian environment influenced by rainy/dry season alternations, besides being susceptible to environmental contamination. In addition, it is a biodiversity hotspot Marchese (2015) given its rich biodiversity and fishing resources. Thus, it is essential conducting biomonitoring activity in this ecosystem. Biomonitoring is a tool to assess the health of ecosystems, as well as of organisms living in them, based on using sentinel species likely facing any environmental change Bani et al. (2022). An ecophysiological approach, employing biochemical, biometric, and morphological biomarkers, can effectively determine the integrity of the biotope and aquatic biocenosis.

The plasma profile used in fish biomonitoring activities enables inferring several physiological processes and detecting fish exposure to environmental stressors. Blood biochemistry has been utilized as a clinical diagnostic tool (Peres et al., 2014; Pérez-Rojas et al., 2022), highlighting the importance of establishing reference values to understand species-ecosystem interactions (Shahsavani et al., 2010). Therefore, establishing basal plasma values for energy substrates (glucose, proteins, total lipids) and non-plasma- specific enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]), in conjunction with biometric indices (Guedes and Correia, 2021), is essential.

Species *Sciades passany* (Valenciennes, 1840), which belongs to family Ariidae, meets the requirements to be sentinel species in the investigated ES, given its abundance and representativeness in the local biome. However, there is limited information on its physiological, ecological, and reproductive characteristics. This study aims to investigate the plasma profile of energy metabolites and enzymes in *S. passany* to provide foundational physiological data and establish a baseline for future research contributing to ichthyofauna conservation in the Maracá-Jipiôca Island estuarine ecosystem.

Material and Methods

The study site is located on Maracá Island, Amapá municipality, Amapá State. It lies over 320 km from the State's capital (Macapá City), in the Far North Brazilian Amazon (Figure 1). This island is an Integral-Protection Conservation Unit managed by Chico Mendes Institute for Biodiversity Preservation (ICMBio - Instituto Chico Mendes de Preservação da biodiversidade) and it hosts the Maracá-Jipiôca Ecological Station (ES) (01°49'51" and 02°12'54" N, 50°11'2" and 50°34'22" W).

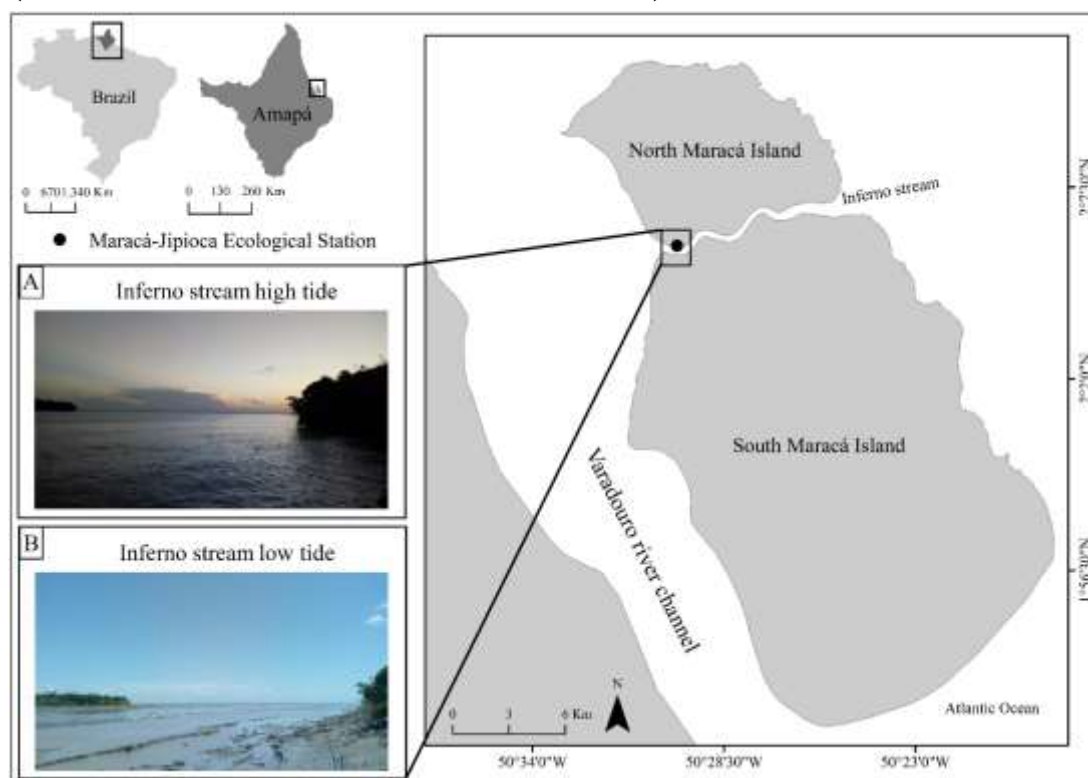


Figure 1. ESEC - Ecological Station of Maracá-Jipiôca. Black dot shows the location of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) research base.

The Northern and Eastern sides of the island are mostly oceanic environments. The Southern side of it is marked by the Turluri channel and the Western side of the island is separated from the continent by the Varadouro channel (Carapori). This channel, in its turn, stretches from Flechal River mouth to Capabe Stream Iranildo Coutinho 2021- personal communication). It is possible saying that the investigated ES is a coastal-estuarine ecosystem formed by mangroves, as well as by floodplain fields and forests, given the aforementioned fluvial-marine features.

Climate pattern in the investigated ES is featured by two seasons, only, namely: the rainy season, from December to July, and the dry season, from August to November ICMBio (2017). Three expeditions were held (seven days, each) over one year, and they covered the following seasonal abiotic cycle at ES: rainy season (April 2018), dry season (September 2018) and rainy season (February 2019). *S. passany* specimens were captured on Inferno stream banks with the aid of fishing rods and nets (30m/40mm) during the tidal change (from low to high tide) in each expedition. *S. passany*'s taxonomic identification was carried out based on the identification key used for Ariidae provided by Marceniuk (2005).

Fish (voucher number: BioFisio-UNIFAP #002) were anesthetized with 100 mg L-1 benzocaine, right after their collection. Blood samples were obtained by puncturing their dorsal-caudal blood vessels with disposable needles (40 x 1.2 mm) attached to 5-ml syringes – Labor Import (SC, BRAZIL). EDTA disodium salt (C₁₀H₁₄N₂Na₂O₈.2H₂O – Sigma) was used as anticoagulant agent. Blood was centrifuged in portable microcentrifuge (Global Trade, 8011154 model, SP, Brazil) at 6,000 RPM, for 5 minutes, to obtain plasma, as well as frozen and stored in freezer at - 20 °C, right away, until its subsequent use. Animals were euthanized through cranial-medial incision. Biometric measurements were taken to get standard and total body length, and biomass values. Organs in the gastrointestinal tract, including the liver, were eviscerated to calculate the following indices: celomic fat for viscerosomatic index (VSI, %): viscera weight (g)/total weight (g)*100; and hepatosomatic index (HSI, %) = liver weight/total weight*100. Fish length: weight ratio (LWR) was estimated through linear regression (Le Cren, 1951) after data logarithmic transformation, based on using the following formula: $W = aL^b$ (W = total body weight (g), L = total length (cm), a = linear coefficient (it represents the biomass increase/fattening degree), and b = angular coefficient (it represents growth type). Therefore, growth type was classified as isometric ($b = 3$), positive allometric ($b > 3$) and negative allometric ($b < 3$). The relative condition factor (K_n) of each fish was calculated: $K_n = (W/L^b)$; $K_n = 1.00$ was the adopted standard (Le Cren, 1951).

Animal collection at ES was authorized by the Sisbio/ICMBio/MMA system, under process n. 65929-1. Study conduction and laboratory analyses were authorized by the Research Ethics Committee on Animal Use of Federal University of Amapá (Ceua/Unifap), under process n. 009/2016. Analyses applied to plasma energy substrates, such as total proteins, triglycerides and glucose, as well as to enzymes, like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were carried out with the aid of commercial kits for humans (Life Biotechnologies®, MG, Brazil).

Statistical analyses were carried out in SigmaStat software, version 3.5. Results were expressed as mean and standard deviation ($\pm$), at 95% confidence interval ($p < 0.05$). Shapiro-Wilk test was applied to check data normality. It was followed by Student's t and Mann-Whitney tests for parametric and non-parametric analysis purposes, respectively. It was done to compare animals sampled in the dry season to those sampled in the rainy season.

Results and discussion

Results observed for these two seasons were compiled and descriptively presented to represent the effect of seasonality on fish annual life cycle.

Three expeditions were held to collect fish ($n = 70$). However, the samples herein used were ($n = 24$) those collected in the dry season, in September 2018 ($n=12$: 66% females, 8% males and 26% without identification) and in the rainy season, in February 2019 ($n = 12$: 25% males and 75% without identification). Female gonadal development stage (data not shown) suggested that fish collected in the dry season were at early maturation stages. All presented results are preliminary, because of the study's small sample size.

Based on the current findings (Table 1), the comparison between fish collected in the dry and rainy seasons did not evidence differences in total body length (cm) and body biomass (g) ($P = 0.322$ and $P = 0.595$, respectively). However, with respect to biometric indices, fish collected in the dry season recorded the highest HSI ($P = 0.023$), whereas those collected in the rainy season recorded the highest VSI ($P = 0.028$). Relative

Table 1. Biometric analysis and body index during the seasonal cycle. Results are expressed as mean and standard deviation. Different symbols indicate statistical differences.

Metrics	Dry season (September 2018)	Rainy season (April 2019)	Seasonal cycle
Total length (cm)	57.50 ± 5.25	56.58 ± 13.85	54.00 ± 8.06
St. length (cm)	47.39 ± 3.52	47.00 ± 9.49	47.70 ± 7.43
Weight (g)	1,655.56 ± 370.00	1,530.45 ± 828.01	1,586.75 ± 650.05
HSI (%)	1.41 ± 0.4*	0.84 ± 0.24 [#]	1.11 ± 0.43
VSI (%)	5.06 ± 1.02*	6.36 ± 1.40 [#]	5.77 ± 1.54
Kn	1.00 ± 0.08	1.01 ± 0.17	1.00 ± 0.14
Min-Max	0.86 - 1.12	0.82 - 1.42	0.78 - 2332
Growth coefficient (b)	3.161	2.821	2.869
Growth type	Positive allometry	Negative allometry	Negative allometry

condition factor remained unchanged between seasons (Kn = 1.0), as well as in the seasonal cycle (P = 0.878). LWR recorded positive allometric growth for fish collected in the dry season (b = 3.161, Strong correlation to $r^2 = 0.88$) and negative allometric growth for fish collected in the rainy season (b = 2.821, $r^2 = 0.93$) and in the seasonal cycle (b = 2.870, $r^2 = 0.92$). There was a significantly close correlation between these parameters in both cases. Analysis results did not point out plasma differences in total proteins (P = 0.473). However, fish sampled in the dry season recorded higher triglycerides (P = 0.008) and glucose (P = 0.009) concentrations than those sampled in the rainy season. ALT and ALP levels did not show a statistically significant difference between seasons (P = 0.076 and P = 0.546, respectively), whereas AST recorded the highest level in fish sampled in the rainy season (P = 0.016). Table 2 presents biochemical biomarkers, such as proteins, lipids and glucose, which were used as energy metabolism indicators, as well as liver enzymes found in plasma, such as ALT, AST and ALP, which were adopted as non-specific environmental stressor markers.

Table 2. Concentration of plasma energetic substrates and plasma enzymes, such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Results were expressed as mean and standard deviation. Different symbols indicate statistical differences.

Biomarkers	Dry season (September 2018)	Rainy season (April 2019)	Seasonal cycle
Proteins (g/dL)	2.73 ± 0.41	2.40 ± 0.22	2.70 ± 0.61
Glucose (mg/dL)	278.51 ± 56.62*	113.70 ± 21.63 [#]	192.20 ± 156.90
Triglycerides (mg/dL)	171.77 ± 82.94*	73.00 ± 41.33 [#]	125.29 ± 82.27
ALT (UL ⁻¹)	11.05 ± 4.95	12.02 ± 4.32	12.18 ± 1.32
AST (UL ⁻¹)	67.98 ± 14.35*	163.69 ± 50.20 [#]	95.32 ± 23.01
ALP (UL ⁻¹)	135.01 ± 59.83	131.77 ± 22.42	132.96 ± 16.03

The investigated ES does not have a detailed inventory about its ichthyofauna, so far. Yet, the structure and updated status of its fishing resources remain poorly understood. *S. passany* stands out for its potential to be used in local artisanal fishing, as well as for presenting features required for ecophysiological biomonitoring. Based on the current findings, we present this species' allometric growth and the mobilization of energy substrates in plasma, along with biometric indices. Additionally, plasma enzymes did not indicate any fish-health impairment that could point to any type of anthropogenic stressor.

To the best of our knowledge, the present study is among the pioneers in using fish physiological aspects as tools for biomonitoring aquatic ecosystems in remote regions like the ES. However, despite this, several

logistical obstacles still exist for conducting research in Amapá State, which negatively impacted the September 2018 expedition. Fish physiology studies require specific protocols for sample collection, preservation, and transport. According to Carvalho et al. (2023), logistics and accessibility strongly influence research results in remote Amazonian areas, which are susceptible to ecological pressures and poorly understood environmental impacts. Biomonitoring-based physiological approaches enable defining a species' biochemical profile in the ES, establishing baselines for ecological prospection and fishing status, and detecting impacts on populations or communities (Guedes & Correia, 2021).

Basic information about *S. passany* biology and reproduction is scarce. Given its relevance, it is important to understand the physiological responses of this species. HSI values recorded in fish indicate liver participation in reproductive processes, as this index can be used as a marker. The current findings, although preliminary, highlight aspects of fish physiology. Variations in lipid and carbohydrate concentrations in these animals are influenced by physiological aspects and environmental conditions (Manzoor et al., 2014). Thus, increased lipid and carbohydrate concentrations in *S. passany* suggest energy resource allocation. Studies on rainbow trout (*Oncorhynchus mykiss*) have shown changes in these metabolites during reproduction (Kocaman et al., 2005). Plasma glucose is a nutritional status indicator and a non-specific stressor marker (Ferri et al., 2022), while plasma triglycerides are not reliably correlated with dietary status (Wagner & Congleton, 2004). Further studies with robust approaches are needed to understand the context, given the lack of plasma reference values for *S. passany* and its congeners on Maracá Island.

Transaminase enzymes detect homeostasis disruption in the presence of stressors (Lima et al., 2018). The absence of a joint response from enzymatic biomarkers suggests that *S. passany* does not show evidence of homeostasis disruption from anthropogenic stressors. Though AST increase was recorded, it is associated with amino acid metabolism and energy demand in stress (Samanta et al., 2014) and can vary under different physiological conditions (Asadi et al., 2006). Thus, it is clear that the physiological behavior of fish in the dry season (September 2018) was different from that in the rainy season (February 2019). Moreover, although this species' reproduction season in the estuary of Maracá Island is yet to be confirmed, it is possible drawing a parallel between the current findings and data available in the literature about other estuaries. Accordingly, immature fish or those in early stage of gonadal development belonging to the family Ariidae, such as *Sciades hersbergii*, were recorded from August to October (Queiroga et al., 2012). Research in coastal-marine Amazonian areas faces logistical obstacles.

These preliminary results contribute to reference physiology values for *S. passany*. The adopted biomarkers show potential for ecophysiological and health status biomonitoring of Ariidae fish on Maracá-Jipióca Island. More physiological and reproductive information is needed to inform fishing and environmental resource management on this island.

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