UNIVERSIDADE FEDERAL DO PARANÁ

ISABELA PREVIATE

USO DE HABITAT E PADRÃO DE MOVIMENTO DE *CENTROPOMUS PARALLELUS* (N.V. ROBALO-PEVA) NO COMPLEXO ESTUARINO DE PARANAGUÁ, ESTADO DO PARANÁ, SUL DO BRASIL

CURITIBA
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USO DE HABITAT E PADRÃO DE MOVIMENTO DE *CENTROPOMUS PARALLELUS* (N.V. ROBALO-PEVA) NO COMPLEXO ESTUARINO DE PARANAGUÁ, ESTADO DO PARANÁ, SUL DO BRASIL

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Ecologia e Conservação. Programa de Pós Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Henry Louis Spach

Co-orientador: Alberto Teodorico Correia

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ISABELA PREVIATE**, intitulada: ""**USO DE HABITAT E PADRÃO DE MOVIMENTO DE *CENTROPOMUS PARALLELUS* (N.V. ROBALO-PEVA) NO COMPLEXO ESTUARINO DE PARANAGUÁ, ESTADO DO PARANÁ, SUL DO BRASIL**"", após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO.

Curitiba, 25 de Outubro de 2016.

Henry Louis Spach
HENRY LOUIS SPACH
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RESUMO

O robalo-peva (*Centropomus parallelus*) é uma espécie importante para a pesca comercial e recreativa no Brasil. Um estudo recente demonstrou que os juvenis desta espécie conseguem habitar ambientes de salinidade distinta ao longo do seu ciclo de vida. Contudo, uma vez que o conhecimento dos padrões migratórios e utilização do habitat por indivíduos são fundamentais na tomada de medidas adequadas para a conservação e manejo de uma espécie, é importante determinar se os indivíduos adultos desta espécie, sujeitos à exploração pesqueira, apresentam a mesma plasticidade ambiental. Nesse sentido, noventa adultos de robalo-peva foram coletados entre outubro de 2015 e março 2016 por meio da pesca por arpão e anzol no Complexo Estuarino de Paranaguá, Estado do Paraná, sul do Brasil. Três pontos de amostragem foram selecionados de acordo com um gradiente de salinidade conhecido: rio Cachoeira (ambiente oligohalino), rio Faisqueira (ambiente mesohalino) e Ponta do Poço (ambiente polialino). Os padrões de movimento foram inferidos a partir das concentrações de Sr:Ca e Ba:Ca obtidas ao longo do raio dos otólitos com o auxílio de uma microssonda de elétrons. A idade dos indivíduos foi estimada a partir da leitura dos anéis de crescimento anuais dos otólitos. Os dados sugerem oito diferentes padrões migratórios que mostram uma elevada plasticidade e adaptação ambiental a gradientes de salinidade distintos. Além disso, os dados também mostram que a maioria dos padrões migratórios incluem estuários como área de ocupação, isso evidencia a importância deste ambiente para a espécie, provavelmente devido à maior disponibilidade de alimentos e menor predação. Portanto a conservação desta espécie exige a preservação de ambientes de água doce e marinhos mas, principalmente, dos estuários, permitindo, desta forma, a conectividade entre habitats.

Palavras-chave: Centropomidae; microquímica de otólitos; conectividade entre habitats; gradiente de salinidade; conservação e manejo de recursos pesqueiros

ABSTRACT

The fat snook (*Centropomus parallelus*) is a species of importance to the commercial and recreational fisheries in Brazil. A recent study demonstrated that the early juveniles of this species can live in differently salinity environments throughout their lifecycle. Understanding of migratory patterns and habitat use by individuals are essential in the context of taking appropriate measures for the conservation and rational management of a species. Thus, it must be determined if the adults of this species, commercially exploited, have the same observed environmental plasticity. With this purpose ninety *C. parallelus* adults were collected between October 2015 and March 2016 using hook and spear fishing in Paranaguá Estuarine Complex, State of Paraná, South of Brazil. Three sampling sites were selected according to a known salinity gradient: Cachoeira River (oligohaline environment), Faisqueira River (mesohaline environment) and Ponta do Poço (polyhaline environment). The movement patterns were inferred from Sr:Ca and Ba:Ca concentrations recorded along the otoliths radius using a electron micro probe analyzer. The age of the individuals was estimated from the reading of the annual growth rings of otoliths. The data suggest eight different migration patterns that show a high plasticity and environmental adaptation to different salinity gradients. Furthermore, the data also show that almost migratory patterns include estuaries as an occupation area, suggesting the importance of these areas for the species, presumably due to the availability of food and lower predation pressure. Therefore the conservation of this species requires the preservation of freshwater and marine environments, but mainly estuaries, which allows the connectivity between habitats.

Keywords: Centropomidae; otoliths microchemistry; habitat connectivity; salinity gradient; conservation and rational management

APRESENTAÇÃO

Essa dissertação, integralmente escrita em inglês, foi elaborada no formato de artigo científico que será submetido a uma revista internacional indexada com arbitragem científica. O manuscrito será editado conforme as normas da revista que será selecionada posteriormente. Contudo parte dos resultados já foram apresentados sob a forma de comunicação oral no XIX Simpósio Ibérico de Estudos em Biologia Marinha que ocorreu de 5 a 9 de setembro de 2016, em Porto, Portugal.

O intuito deste artigo foi descrever os padrões de migração e conectividade entre habitats dos adultos de *Centropomus parallelus* que são importantes para a conservação e gestão sustentada da espécie. Para esta descrição foram utilizados 90 otólitos de peixes coletados de três diferentes locais: Rio Cachoeira, Rio Faisqueira e Ponta do Poço. Estas áreas foram selecionadas por suas características de salinidade: oligohalina, mesohalina e polihalina, respectivamente. Todos os locais de coleta localizam-se no Complexo Estuarino de Paranaguá situado no litoral do Estado do Paraná, Brasil.

Este estudo teve por base a análise dos elementos químicos (Sr, Ba e Ca) presentes nos otólitos dos exemplares coletados com o auxílio de uma microssonda de elétrons. A idade dos indivíduos também foi estimada pela leitura dos anéis de crescimento anuais. Os dados obtidos permitiram inferir acerca da migração dos indivíduos entre habitats de diferentes salinidades.

Os dados sugerem oito diferentes perfis de migração. Alguns dos quais estão descritos na literatura para os juvenis da mesma espécie. Outros, mais abundantes, que refletem maior mobilidade possivelmente estão relacionados à fuga de predadores ou à pesca artesanal. Podem, ainda, ocorrer devido procura de oportunidades de alimentação, mostrando sua alta capacidade de adaptação e plasticidade ambiental.

PREFÁCIO

Biologia da espécie

Os indivíduos das espécies que fazem parte da família Centropomidae caracterizam-se por apresentar a mandíbula inferior ultrapassando a superior. A linha lateral se estende até a nadadeira caudal que é profundamente bifurcada. A nadadeira dorsal, por sua vez, é separada por uma pequena abertura em duas partes, a primeira com oito espinhos e a segunda com um espinho e oito a onze raios macios. Além disso, seu comprimento máximo é cerca de dois metros (Nelson *et al*., 2016).

Esta família, anteriormente composta pelas subfamílias Latinae e Centropominae (Greenwood, 1976), atualmente é considerada monogenérica englobando os indivíduos do gênero *Centropomus* (Otero, 2004). Alguns exemplares deste grupo sobressaem-se não apenas pelo valor comercial (Chávez, 1963; Chávez-Caballero *et al.*, 2005), mas por sua importância na pesca recreativa (Muller & Taylor, 2012).

O gênero *Centropomus* é encontrado apenas na região americana do Atlântico e nas regiões tropical e subtropical do Pacífico. Este abriga doze espécies, seis delas em cada local mencionado pois nenhuma é decorrente de ambos. Estas espécies são simpátricas e sintópicas em seus respectivos oceanos. Além disso, nenhum híbrido foi documentado (Nelson *et al*., 2016).

As espécies deste gênero são eurihalinas, ou seja, capazes de sobreviver em ambientes nos quais ocorrem grandes variações de salinidade. Estão presentes em águas marinhas, dulcícolas e salobras, características de estuários, aonde o grupo é encontrado mais frequentemente (Seaman & Collins, 1983). Existem registros de cinco destas espécies para o Brasil: i) *Centropomus undecimalis*, ii) *C. ensiferus*, iii) *C. pectinatus*, iv) *C. mexicanus* e v) *C. parallelus*, habitando desde a região sul no Rio Grande do Sul até o Maranhão (Alvarez-Lajonchère & Tsuzuki, 2008).

C. parallelus, espécie alvo deste trabalho, é conhecido popularmente como robalo-peva e, em inglês, fat snook. Eles se distribuem do sul da Florida até a região de Santa Catarina no Brasil (Alvarez-Lajonchere & Tsuzuki, 2008). Os juvenis, por sua vez, são encontrados em uma ampla gama de fatores abióticos, tais quais: i) salinidade (0 a 30), ii) temperatura (24°C a 35°C) e iii) quantidade de oxigênio dissolvido na água (anóxica a altamente oxigenada). Eles tem preferência por habitats berçários de águas calmas e turvas, sobretudo nas proximidades de abrigos, como raízes de mangue, por exemplo (Aliaume *et al.*, 1997). Porém, esta variação de características abióticas não influencia na abundância da espécie, apenas determina o tamanho dos indivíduos (Chaves e Nogueira, 2013).

Sua alimentação baseia-se preferencialmente em peixes, quando adultos. Os juvenis, por sua vez, além de ictiófagos, ingerem pequenos crustáceos e larvas de insetos (Tonini *et al.*, 2007; Contente *et al.*, 2009). Seu comportamento com relação ao forrageio é considerado oportunista e com predileção por ambientes pelágicos, quando juvenil, porém bastante variável e dependente de disponibilidade de alimento e presença de predadores (Cerqueira & Tsuzuki, 2009).

Com relação à reprodução, a espécie apresenta um longo período reprodutivo, com variações de duração e datas e quantidade de picos. Registros de Vera Cruz indicam duração de oito meses (abril a dezembro) com picos de maio e junho e de outubro e novembro (Chávez, 1963). Na região sudeste do Brasil, em Cananéia-Iguape, São Paulo, há registros de períodos de desova com duração de oito a dez meses com picos na primavera e outono (Itagaki, 2005). Um terceiro registro, do litoral do Paraná, indica desova praticamente durante o ano todo com picos de outubro a janeiro (Daros *et al.*, 2016).

Os robalos-peva são considerados peixes hermafroditas protândricos, isto é, os órgãos sexuais masculinos atingem a maturidade e tornam-se ativos antes dos femininos (Alvarez-Lajonchère & Tsuzuki, 2008). Neles já foi observado gônadas em processo de mudança de sexo, com presença de remanescentes de dutos com esperma e lamelas ovígeras. A diferenciação fenotípica ocorre quando o indivíduo chega a 230 milímetros e 100 gramas aproximadamente, mediante a substituição das lamelas testiculares por oócitos perinucleares e conversão dos testículos em ovários (Alvarez-Lajonchère & Tsuzuki, 2008). Apesar disto, já foram registrados machos de maior comprimento, acima de 550 mm (Dutka-Gianelli, 2010), indicando que podem permanecer funcionais para o resto da vida e que a transição sexual não é obrigatória.

Otólito na ciência

Localizados no aparelho vestibular do ouvido interno de peixes ósseos teleósteos, os otólitos funcionam como receptores sensoriais concomitantemente com o sistema da linha lateral (Popper et al. 2005). São estruturas mineralizadas e acelulares formadas por carbonato de cálcio, em sua maioria sob forma de aragonita, embutido em uma matriz proteica, denominada otolina (Campana & Neilson, 1985). Eles estão presentes em três pares (*sagittae*, *lapilli* e a*sterisci*) que apresentam localização, função, tamanho, forma e microestrutura diferentes (Tresher, 1999).

Os *asterisci* são principalmente compostos por vaterita (Tresher, 1999; Popper *et al.*, 2005), um polimorfo de carbonato de cálcio assim como a aragonita. Localizam-se em um vestíbulo assim como os demais pares, denominado *lagenna* (saco ventral) e está mais próximo dos *sagittae*. Os *lapilli,* por sua vez, situam-se em sacos dorsais chamados de *utriculus* e estão mais distantes dos outros pares (Secor *et al.,* 1992; Wright *et al.*, 2002).

Os *sagittae*, também localizados em sacos ventrais, estes denominados *sacculus* (Secor *et al.,* 1992; Wright *et al.*, 2002), são os mais utilizados para estudos em Perciformes por serem os mais fáceis de remover e localizar, além de possuírem o maior eixo de crescimento e a maior massa (Tresher, 1999; Secor *et al.*, 1992). Tem formato elíptico e são comprimidos lateralmente. Além disso, apresentam uma pequena projeção anterior e ventral chamada de *rostrum* e um sulco na superfície medial (Wright *et al.*, 2002).

Os otólitos crescem por meio da contínua e concêntrica deposição de camadas de carbonato de cálcio, desta maneira, formam-se anéis de crescimento que apresentam periodicidade anual, sazonal e diária (Campana, 1999). Além da deposição de $CaCO_3$ e otolina, outros elementos são incorporados à matriz, em traços vestigiais, e por não sofrerem modificações químicas após esta incorporação, funcionam como marcadores ambientais (Campana *et al.*, 2000).

Devido a estas características, podem ser utilizados para estimar idade e crescimento (Francis e Campana 2004; Correia *et al.*, 2009), padrões de migração (Correia *et al.*, 2004; Gillanders, 2005; Albuquerque *et al.*, 2012), diferenciação de estoques pesqueiros (Correia et al. 2011), determinação de locais de nascimento (Di Franco *et al.*, 2011) e conectividade entre berçários e zonas de recrutamento costeiro (Reis-Santos *et al.*, 2012).

Para fazer essas inferências a respeito da história de vida de indivíduos e populações, diferentes ferramentas e metodologias são utilizadas para a exploração das informações contidas nos otólitos. A análise da forma do otólito é uma delas, na qual suas medidas de perímetro, área e outras são utilizadas para a diferenciação de estoques, por exemplo. Além disso, este tipo de análise pode fornecer dados a respeito de idade e sexo dos indivíduos (Campana & Casselman, 1993), pois os otólitos tendem a ser mais ornamentados com a aumento da idade (Panfili & Morales-Nin, 2002).

Uma outra ferramenta utilizada no estudo sobre populações de peixes, que utiliza a microquímica, é a ablação por laser. Devido à variação das concentrações dos elementos químicos incorporadas em pontos diferentes do mesmo otólito, ablações múltiplas são capazes de identificar diferenças entre diferentes espécies (Di Franco et al. 2014). Esse tipo de análise também é capaz de revelar informações no que concerne à dinâmica populacional de uma determinada espécie, bem como a sua capacidade de plasticidade em uso de habitats (Mai *et al.*, 2014).

Além da ablação por laser, existem outras maneiras de analisar a microquímica dos otólitos. O *solution based* consiste na dissolução do otólito na íntegra em ácido nítrico, essa solução contém informações sobre toda a história de vida do indivíduo, desde o momento do seu nascimento até sua captura. Este tipo de análise também revela dados sobre a estrutura da população (Correia *et al.*, 2011), incluindo evidências da existência de meta-populações (Correia *et al.*, 2014).

O EPMA (X-Ray Electron Probe Micro-Analyser) é uma ferramenta frequentemente usada para quantificar Sr e Ca em otólitos. Ela provou ser uma medida confiável destes elementos quando comparadas com outras metodologias (Campana *et al.*, 1997). Além disso, enquanto o *solution based* revela apenas informações como um todo a respeito da história de vida do indivíduo, o EPMA pode ser utilizado para análises de momentos específicos.

Diante das características apresentadas, o EPMA mostrou ser a alternativa que melhor cumpriria os propósitos deste trabalho, a metodologia que melhor quantificaria proporções de Sr e Ca, com análises pontuais, ambas importantes para o estudo de dinâmica populacional e ocupação de habitats.

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Introduction

Fish migration is a subject well documented and discussed since the middle of last century (Myers, 1949). Some of the features regarding the fish migration strategies vary not only between species, but within them, such as the partial migration, which configures a case of phenotypic plasticity (Secor, 2010). The fish choice among migrating, or not, is probably related to physiological processes with the aim of maximizing individual fitness under different environmental conditions. Furthermore it also appear to be a survival strategy that maximizes the use of feeding opportunities and predation escape (Jonsson & Jonsson 1993; Chapman *et al.*, 2012).

An example of family inserted in the context of fish migration is Centropomidae, that is a monogeneric family and includes only the *Centropomus* species (Otero, 2004). Some examples of Centropomidae, like *Centropomus undecimalis* (commom snook) and *Centropomus parallelus* (fat snook), are not only important in terms of commercial value due do its aquaculture potential (Chavez, 1963;. Chavez-Caballero *et al.*, 2005), but also because of the recreational fishing activities (Muller & Taylor, 2012). The genus *Centropomus* is found in the American Atlantic regions and tropical and subtropical Pacific regions. It includes twelve species, six of each ocean (Nelson *et al.*, 2016).

C. parallelus, the target species of this work, is an amphidromous fish popularly known as fat snook (robalo peva in Brazil). Individuals of the species are distributed from southern Florida to the region of Santa Catarina State (Alvarez-Lajonchere & Tsuzuki, 2008). Juveniles are found in a wide range of environments where abiotic factors can have extreme values and they have a preference for nursery areas with calm waters, especially near shelters such as mangrove roots (Aliaume *et al.*, 1997).

Concerning their feeding regimes, juveniles feed on fishes, small crustaceans and insect larvae, but as adults their diet is based on fish (Tonini *et al.*, 2007; Contente *et al.*, 2009). His foraging behavior is considered opportunistic, it means, that this behavior is highly variable, depending on food availability and presence of predators (Cerqueira & Tsuzuki, 2009).

This species has a protracted reproductive period, with some regional variations in terms of duration and peak occurrence of the spawning. Records of Vera Cruz, Mexico, indicate a spawning duration of eight months (April to December) with peaks in May, June, October and November (Chávez, 1963). In southeastern Brazil region, namely in a conservation unit of sustainable use, Cananéia-Iguape, São Paulo, there are records of *C. parallelus* spawning periods lasting eight to ten months with peaks in spring and autumn (Itagaki, 2005). Another record for southeastern Brazil region, in Rio de Janeiro, shows similar spawning duration but with peaks in December to March (Santos, 2014). Meanwhile, in the southern region of Brazil, in Paraná record shows that the spawning can occur almost all year with peaks from October to January in the Paranaguá Estuarine Complex (Daros *et al.*, 2016).

The *C. parallelus* is a protrandic hermaphrodites fish, it means that male's sexual organs reach maturity and become active before the females (Santos, 2014).

This species has a large aquaculture potential (Correa & Cerqueira, 2008) and is important for recreational and commercial fishing (Mendonça & Katsuragawa, 2001). However *C. parallelus* is considered a harmed species by overfishing, pollution of aquatic environments and loss of habitat (Rocha *et al.*, 2007, Feltrin-Contente *et al.*, 2009).

The migration of fish has been study using a wide range of tools, such as: (i) telemetry, (ii) mark-recapture and (iii) analysis of chemical elements in otoliths (Campana *et al.*, 1999; Trotter *et al.*, 2012; Liu *et al.*, 2014). Otoliths, for instance, could provide information about the migration patterns and habitat use of individuals that are critical for understanding the population dynamics of the species (Secor, 2010; Mai *et al.*, 2014; Daros *et al.*, 2016). Otolith microchemistry has modified classic concepts about the life history of certain fish species, such as eels (*Anguilla anguilla, A. japonicus, A. rostrata*), not more considered as mandatory catadromous fish species (Tsukamoto *et al.*, 1998; Daverat *et al.*, 2006).

The existent studies concerning the fish migration through a salinity gradient are mainly based in Sr and Ba concentrations in otoliths (Campana, 1999; Mai *et al.*, 2014). High Sr:Ca ratios characterize the sea water environments. The opposite usually occurs with Ba:Ca concentrations in otoliths (Kraus & Secor, 2004; Elsdon *et al.*, 2008). Therefore, the ratios of these elements in addition with the chronological attributes of otoliths provide information about individual movement within a given population (Elsdon & Gillanders, 2006). Moreover, the join study of these structural and chemistry features incorporated in the otoliths it is more advantageous when compared to other techniques used for migration studies. Thus, otoliths are considered natural tracers of fish migration (Campana, 1999; Elsdon & Gillanders, 2006; Secor, 2010).

Centropomus parallelus is considered an amphidromous species, migrating between freshwater, brackish and saltwater environments during part of its life cycle (Silvano *et al.*, 2006 and Dutka-Gianelli, 2010). Moreover a recent study showed that the young juveniles of this species can live in different salinity environments throughout their life cycle (Daros *et al.*, 2016). Once the conservation measures planned for this species in Brazil provide only a minimum catch size, 300 mm of furcal length (Brasil, 2003) and, the most complete measure, of the Paraná State (Paraná, 2009), does not include all precautions necessary for the species conservation at this location, it is necessary to know more about the species. Therefore is needed understand the migratory patterns and habitat use for taking appropriate measures for the sustainable conservation and management of a species, it must be determined if the adults of this species, subject to fisheries exploitation, also share the observed environmental plasticity.

In view of this, the main purpose of this study was to investigate the migration pattern and habitat use of *C. parallelus* adults in Paranaguá Estuarine Complex through different environmental salinity areas using the Sr:Ca and Ba:Ca ratios recorded in otoliths.

Material and Methods

Study Area

The Paranaguá Estuarine Complex (PEC) is composed of a mosaic of ecosystems: mangroves, creeks, canals, beaches and rivers, totaling an area of 612 km^2. In its longitudinal extension it has a gradient of physical-chemical characteristics, namely salinity, that defines different PEC regions: (i) euhaline, (ii) polyhaline and (iii) oligo-mesohaline (Lana *et al.*, 2001). The three sampling points were selected due to its salinity characteristics: oligohaline (0.02 psu), mesohaline (6 - 15 psu) and polyhaline/euhaline (29 - 31 psu) for Cachoeira River, Faisqueira River and Ponta do Poço, respectively (Fig. 1).

The PEC is also classified according to their morphological and hydrological characteristics, grouping a total of five sectors and 12 sub-estuaries. The Cachoeira River (CR), an oligohaline area and Faisqueira River (FR), with mesohalines features, are located in Antonina sector and sub-estuary Cachoeira, the largest, covering 926.7 km^2 of drainage area. Ponta do Poço (PP), which is located in the Mistura sector has the highest salinity between the selected points because suffers greater tidal influence (Noernberg *et al.*, 2006).

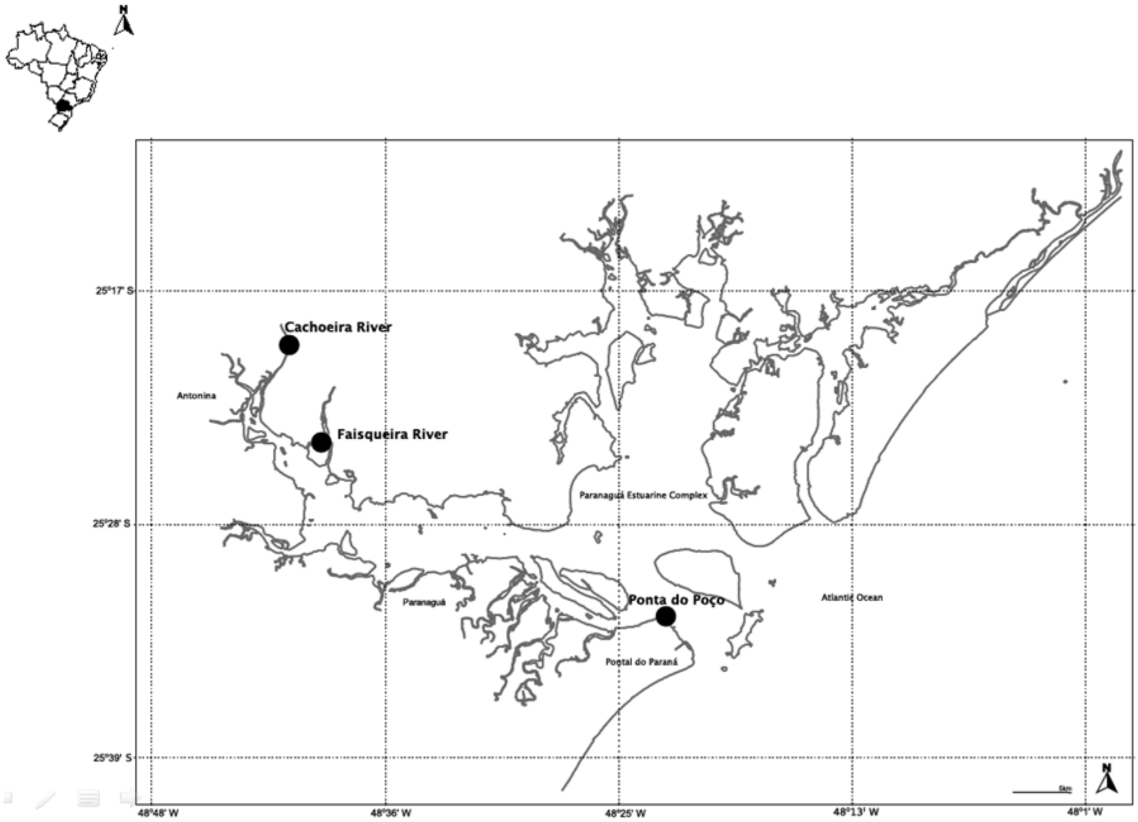


Fig. 1. Sampling areas of the *C. parallelus* adults used in this study and collected in three areas within the Paranaguá Estuarine Complex (Cachoeira River - oligohaline, Faisqueira River - mesohaline and Ponta do Poço - polihaline).

Fish Sampling

Ninety adults (30 per sampling area) of *C. parallelus* were collected with different fishing gears, namely spear fishing and hooks, from October of 2015 to March of 2016. The sampling was target to individuals approximately 170 to 300 mm total length as they are the most caught in sport fishing tournaments (Moro, 2008). In laboratory the fishes were weighted (W) (g), standard length measured (SL) (mm) and sagittal otoliths were removed, cleaned and stored in Eppendorf vials.

Water Sampling

Water samples were collected at different water column depths (surface, middle and bottom), filtered, preserved by addition of nitric acid 1.27% and kept at 4° C until further analysis. Sr, Ba and Ca concentrations were determined using ICP-OES. Salinity and temperature data were recorded *in situ* using a YSI CTD CastAway at the time the fish were collected.

Otolith Preparation for Microchemistry Analysis

The left sagittal otoliths were cleaned using ultrapure water, air dried and mounted in epoxy resin (Struers, Epofix) with the sulcus facing down. The blocks were sectioned (approximately 2 mm thickness) to cut away the excess resin around the otoliths with a low speed diamond saw (Isomet Low Speed Saw, Buehler) at 6000 rpm. Otoliths were ground in the transverse plane to expose the core with 800, 1200 and 2400 silicon carbide papers (Hermes) and further polished with 6, 3, 1 and 0.25 μm diamond pastes (Metadi II, Buehler). Regular optical inspections were made under a metallographic microscope (Meiji, ML7100) to check the polishing plane. Finally otoliths were cleaned in an ultrasonic bath with ethanol and ultrapure water (Milli-Q water).

Otolith Microchemistry Analysis

Otoliths were carbon coated by high vacuum evaporation, and Sr, Ba and Ca concentrations (% dry mass) were measured from the core to the edge (Fig. 2) along the ventral ridge of the sulcus using an X-Ray Electron Probe Micro-Analyser (EPMA, JEOL JXA-8500F). Accelerating voltage was 15 kV and beam current was 20 nA. The electron beam used has a diameter of 10 μm and analyzes were measurements every 50 μm. The acquisition time was 70 s (30 per element, 30 s for the peak counting and 20s each background) per point. The detection limits achieved with this operating conditions were 160, 146 and 115 for Sr, Ba and Ca respectively. For the core and edge analysis a single ablation point was used. Celestite ($SrSO_4$), barite ($BaSO_4$) and apatite [$Ca_5(PO_4)^3$] were used as standards respectively.

The Sr:Ca values used to determine in which environment the fish was, were based on the values found for the juveniles of this species in a previous work. Thus for migration patterns classification of *C. parallelus* habitat it was considered that Sr:Ca ratios in otoliths between 2–5, 5–8 and greater than 8 as freshwater, brackish and salt water proxies, respectively (Daros *et al.*, 2016). For Ba:Ca, the values utilized were 0.005-0.218, 0.00051-0.004 and lower than 0.004 to freshwater, brackish and salt water respectively (Tabouret *et al.*, 2010).

The terminology used to classify the hereby observed migration patterns based on ototith profiles were the same validated for juveniles of the species (Daros *et al.*, 2016). For new patterns found, new terminologies were created.

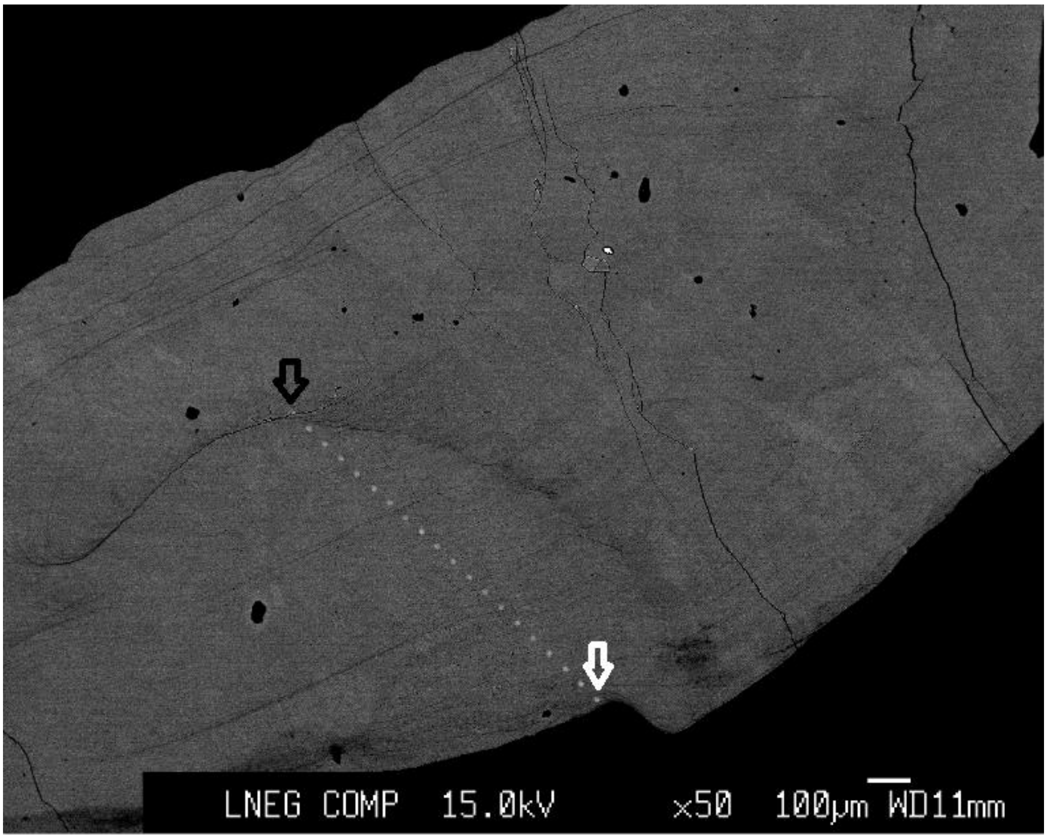


Fig. 2. Transverse section from a sagittal otolith of *Centropomus parallelus* individual (SL = 235 mm; Age Group 2+) collected in Cachoeira River showing the location of the burn depressions made by the electron microprobe from the core (black arrow) until the edge (white arrow).

Age Estimates

The same otoliths were re-polished using alumina solution and immersed in a clearing mixture of ethanol and glycerol (1:1) for 24 hours. The annual growth increments, a translucent and an opaque zone (an annuli, Fig. 3) of the otoliths were counted under a compound stereoscope (Meiji, EMZ-13TR) by reflected light against dark background (Correia *et al.*, 2012). After image software acquisition, growth annuli from otoliths have been blind count by three different readers. The readers fully agreed on ages. The age was calculated by counting the translucent increments, but taking into consideration the date of birth (1st July following the rules in the southern hemisphere) and the date of capture (Panfili *et al.*, 2002).

Fig. 3. The annual growth increments, a translucent (white arrow) and an opaque zone (black arrow), from a sagittal otolith of *Centropomus parallelus* (individual collected in Ponta do Poço with 243 mm of SL and 2 years old).

Statistical Analysis

The statistical analysis were performed using R (3.1.2 version, car package). Prior to data analysis, results were checked for normality and homoscedasticity. Differences between Sr:Ca and Ba:Ca ratios of core and edge in otoliths were evaluated using One-Way ANOVA, Tukey test *a posteriori* and T test (independent samples). One subsample of 59 individuals, the most abundant age group (2+), was used to make additional inferences about the Sr:Ca and Ba:Ca ratios along the otoliths radius. Statistical tests were performed with a level of significance of 0.05. Data are presented as Mean Values ± Standard Error.

Results

The *C. parallelus* adults used in this study ranged from 170 to 290 mm and 96 to 583 g (Table 1). There were significant differences in the SL of the *C. parallelus* adults between sampling regions (One-Way Anova: $F_{2,87} = 17.25$, $p < 0.05$), with the Ponta do Poço showing smaller individuals (Tukey test, $p < 0.05$).

Table 1. Collection sites, geographic co-ordinates (LAT; LONG), sample size (N), standard length (SL) and weight (W) of *Centropomus parallelus* adults used in this study. Values were presented as means ± standard errors.

Site	LAT; LON	N	SL (mm)	W (g)
Cachoeira River	25°20'22"S; 48°41'28"W	30	190-290 (241 ± 5)	130-583 (297 ± 20)
Faisqueira River	25°24'45"S 48°39'46"W	30	198-290 (240 ± 4)	145-460 (271 ± 15)
Ponta do Poço	25°33'3"S 48°21'56"W	30	170-250 (206 ± 4)	96-365 (181 ± 12)

Water physicochemical characteristics varied according to sampling areas. Strontium increased with increasing salinity. Barium, in turn, does not showed any relationship with salinity. The temperature ranged from 23°C to 26°C (Table 2). There was no difference for Sr:Ca ratios of otolith edges of males and females (T test: $t_{77.66}$ = -1.74, p = 0.08).

Table 2. Temperature (T), salinity (S), strontium (Sr) and barium (Ba) of the water samples collected in the sampling points. Values presented as means ± standard errors.

Site	T (°C)	S (psu)	Sr (mg/L)	Ba (mg/L)
Cachoeira River	22.68 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.13 ± 0.01
Faisqueira River	26.03 ± 0.13	9.29 ± 0.53	1.21 ± 0.01	0.20 ± 0.01
Ponta do Poço	23.27 ± 0.00	28.68 ± 0.01	5.34 ± 0.05	0.13 ± 0.00

The estimated annual age for the collected specimens ranged from one to four years old, but most of the individuals (66%) showed to be two years old (Fig. 4). The overall Sr:Ca concentrations vary very little in the different sampling areas. Considering a subsample (2+ individuals), *C. parallelus* mainly remained in salt water during through their life-time, except for individuals collected in oligohaline waters who migrated to brackish water from the middle of second winter (Fig. 5).

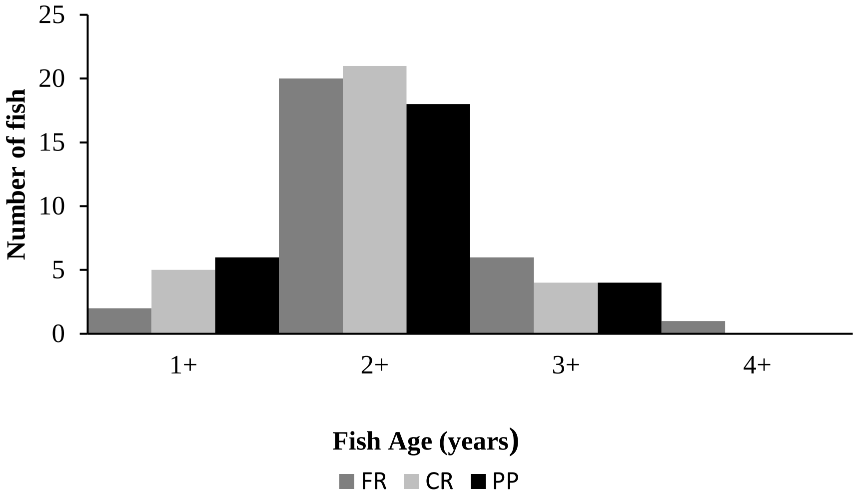


Fig. 4. Frequency of *C. parallelus* estimated age by sampling area. Cachoeira River (CR); Faisqueira River (FR); Ponta do Poço (PP).

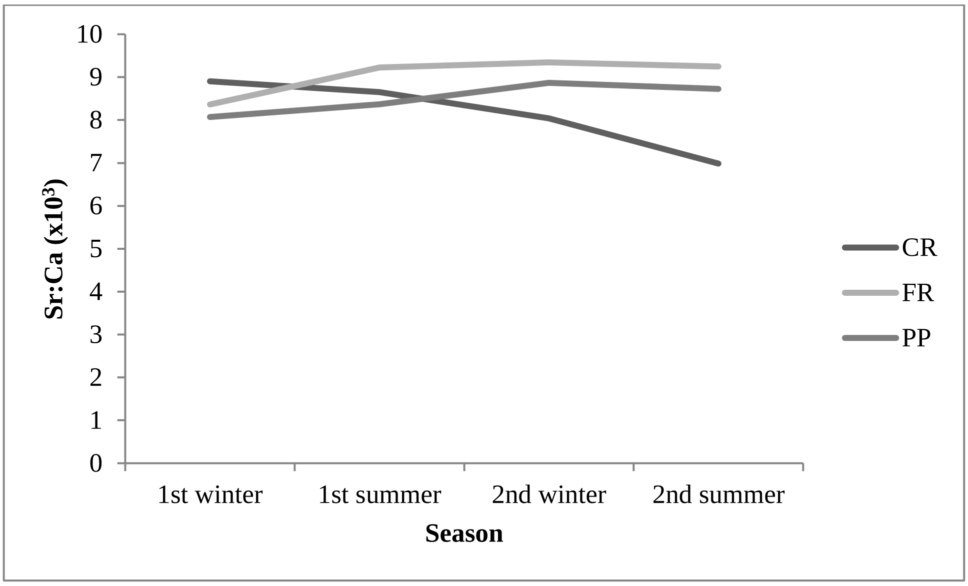


Fig. 5. Average Sr:Ca concentrations for season considering a sub-sample of 59 individuals, age group 2+. First and Second winter; First and Second summer by sampling area. Cachoeira River (CR); Faisqueira River (FR); Ponta do Poço (PP).

The *C. parallelus* showed no significant differences in their otolith cores (One-Way Anova: $F_{2,87} = 0.25$, $p = 0.78$) and edges (One-Way Anova: $F_{2,87} = 1.85$, $p = 0.16$) for Ba:Ca concentrations. For Sr:Ca concentrations there were differences for otolith's core between sampling locations (One-Way Anova: $F_{2,87} = 3.60$, $p = 0.03$), the Cachoeira River add higher values comparatively to the other collection points (Tukey –

test, p = 0.03). Otolith edges also have different values (One-Way Anova: $F_{2,87} = 11.74$, $p < 0.05$) and Cachoeira River showed smaller values than the other sampling areas (Tukey – test, $p < 0.05$) (Table 3).

Table 3. Collection sites, otolith's core and edge concentrations for the Sr:Ca and Ba:Ca [(mass % (x10^3)]. Mean values ± standard errors.

Site	Core Sr:Ca	Edge Sr:Ca	Core Ba:Ca	Edge Ba:Ca
Cachoeira River	8.96 ± 0.31	6.98 ± 0.43	1.51 ± 0.10	1.55 ± 0.22
Faisqueira River	8.21 ± 0.23	9.51 ± 0.38	1.42 ± 0.09	2.17 ± 0.44
Ponta do Poço	7.92 ± 0.31	8.64 ± 0.31	1.48 ± 0.08	1.45 ± 0.09

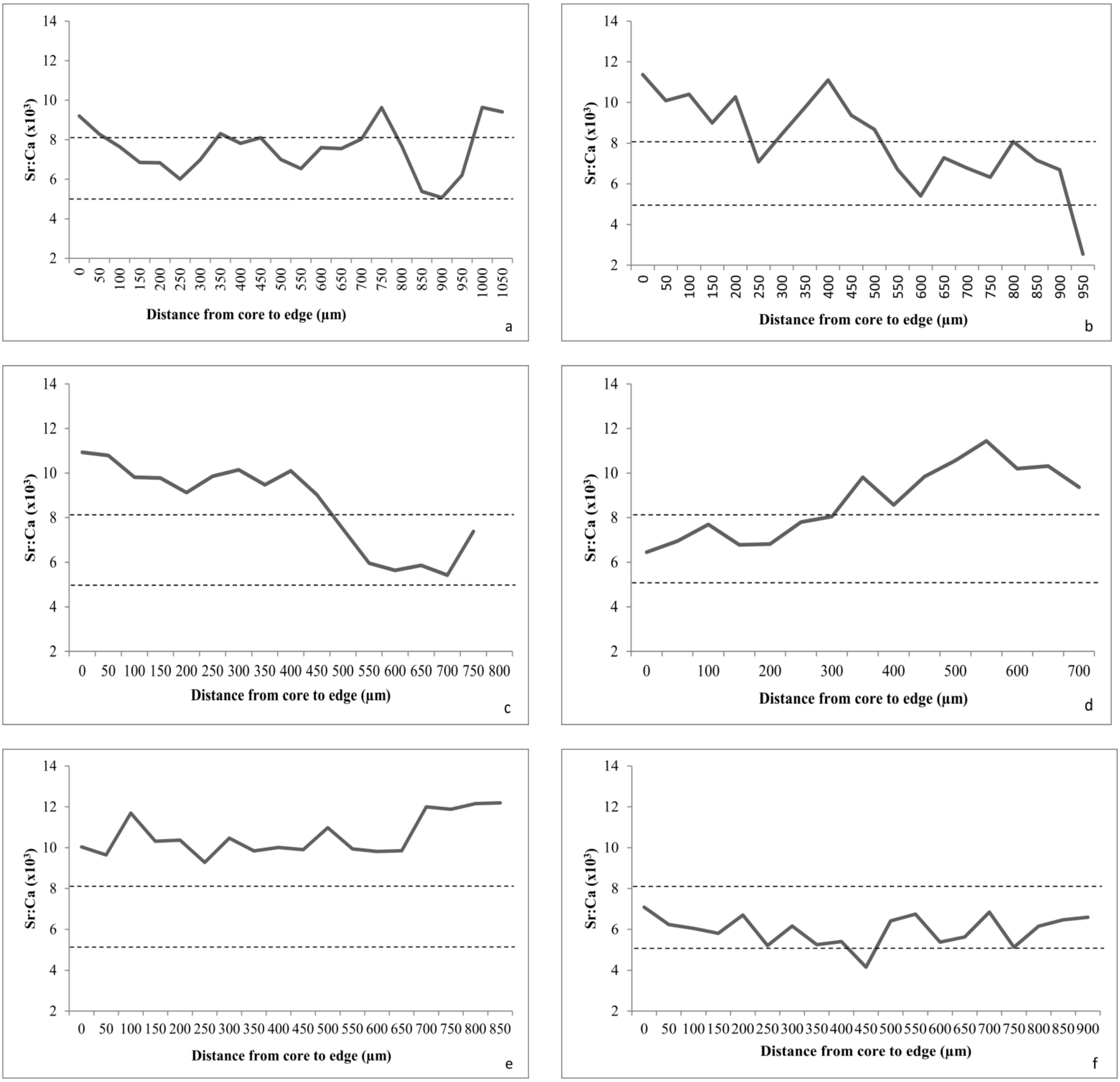
The Sr:Ca ratios recorded for otoliths showed different migration patterns. Eight different patterns were identified for *C. parallelus* adults (Fig. 6, Table 4). Forty seven individuals (52.2%) collected in PEC showed Sr:Ca otolith concentrations ranging from values corresponding to meso and polyhaline waters (marine brackish migrants), that are characterized by spending all life, from hatching to the sampling, migrating between salt and brackish water environments (Fig. 6a).

Eighteen *C. parallelus* (20%) presented varied Sr:Ca ratios, corresponding to the three presented salinities (marine freshwater migrants). They migrate randomly between oligo, meso and polyhaline environments during their life cycle (Fig. 6b).

The remaining 27.8% are divided into six different migration patterns. Those with medium Sr:Ca concentrations in the early stages (brackish water) followed by high Sr:Ca ratios (salt water) until catch date (marine migrants). This type of migration was found in six *C. parallelus* (6.7%) (Fig. 6c). It was also reported a pattern contrary of this, it means that first fishes shows high Sr:Ca concentrations (salt water) and followed by medium values (brackish water). Seven individuals showed this pattern (brackish migrants) this represents 7.7% of the total sample (Fig. 6d).

Another pattern was also found in adults was marine resident. They are those in which was recorded high Sr:Ca concentrations from core to the edge otoliths. This eight individuals (8.8%) were at sea all the time (Fig. 6e). One more type was brackish resident with occasional freshwater entry, as the name suggests the fish lives in brackish water and had a transitory entry in oligohaline water. Only one specimen (1.1%) showed this movement pattern (Fig. 6f).

Another pattern is represent only by one individual, the brackish resident in which the Sr:Ca ratios recorded from core to edge was medium values, corresponding to brackish waters (Fig. 6g). The last one was represented by 2 (2.2%) individuals and was characterized by low Sr:Ca ratios in the early stages of life (freshwater) followed by medium Sr:Ca concentrations referring to brackish water (freshwater migrant) (Fig. 6h).



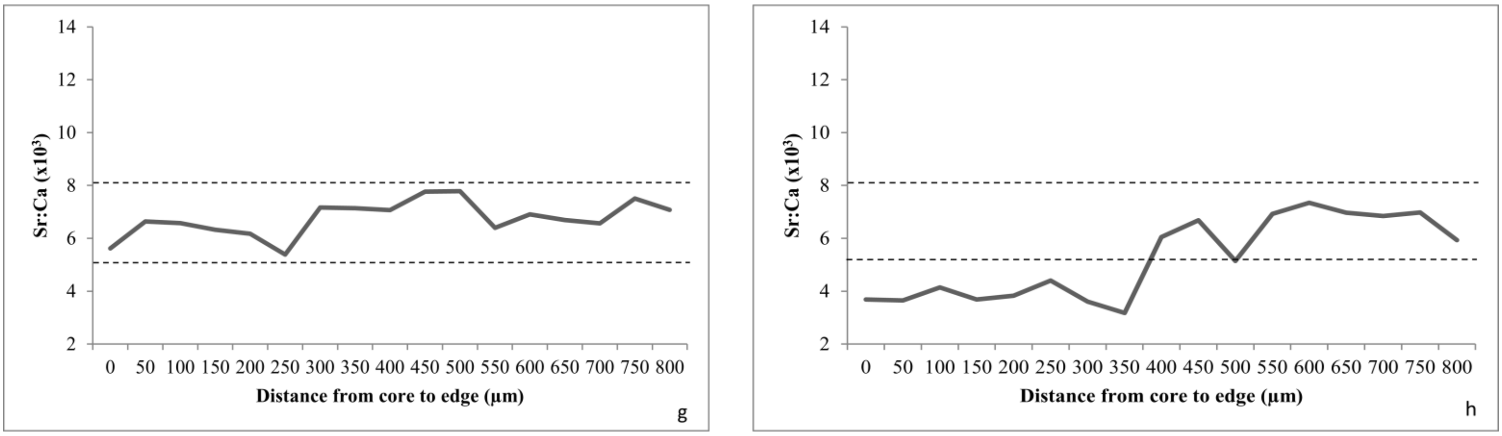
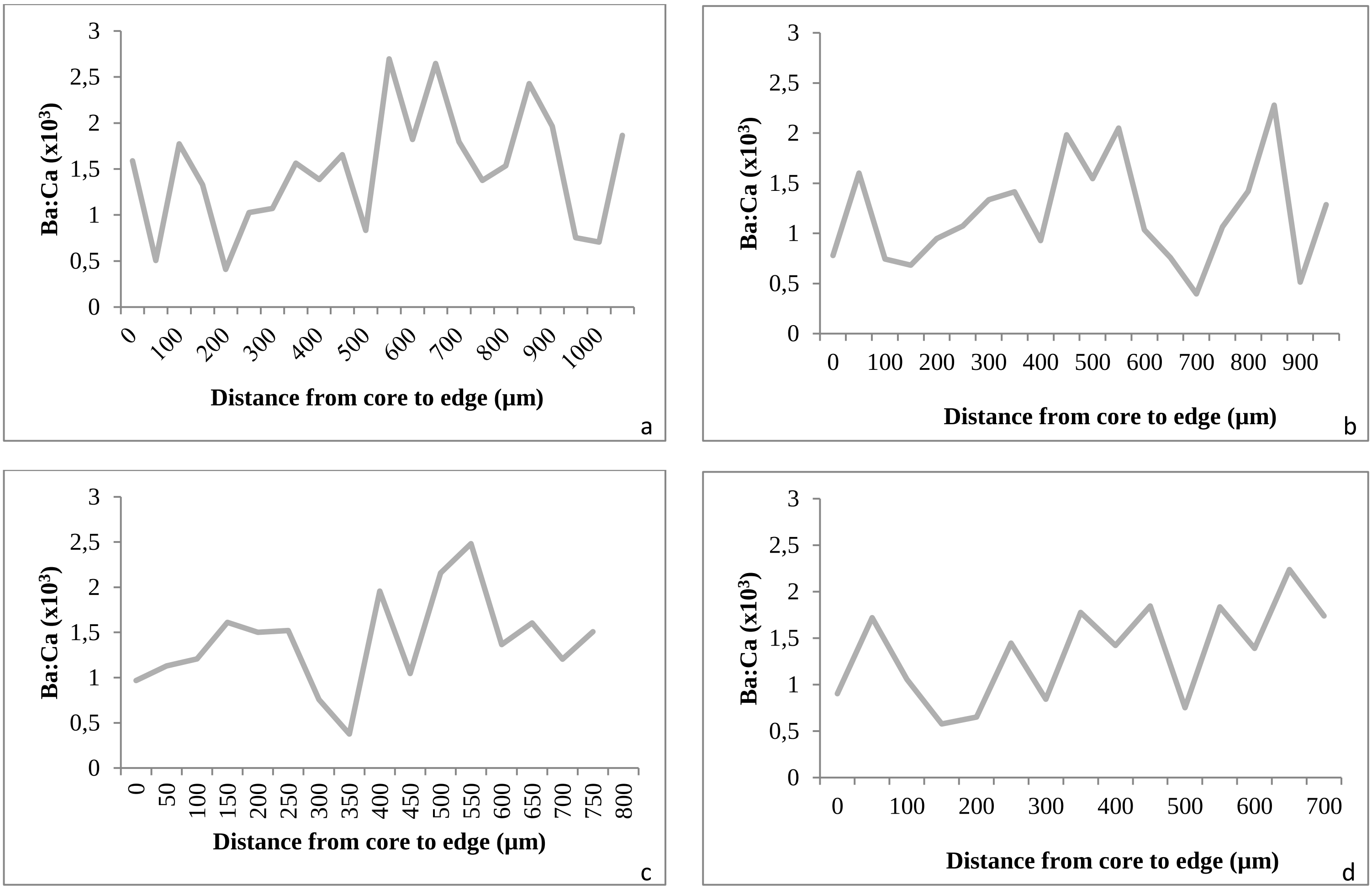


Fig. 6. Eight different patterns founded in *Centropomus parallelus*. a) marine brackish migrant, n = 47; b) marine freshwater migrant, n = 18; c) marine migrant, n = 6; d) brackish migrant, n = 7; e) marine resident, n = 8; f) brackish resident with occasional freshwater entry, n = 1; g) brackish resident, n = 1; h) freshwater migrant based on Sr:Ca otoliths concentrations, n = 2. The dotted lines show the limits of the different salinities (freshwater 2-5; brackish water 5-8; salt water higher than 8).



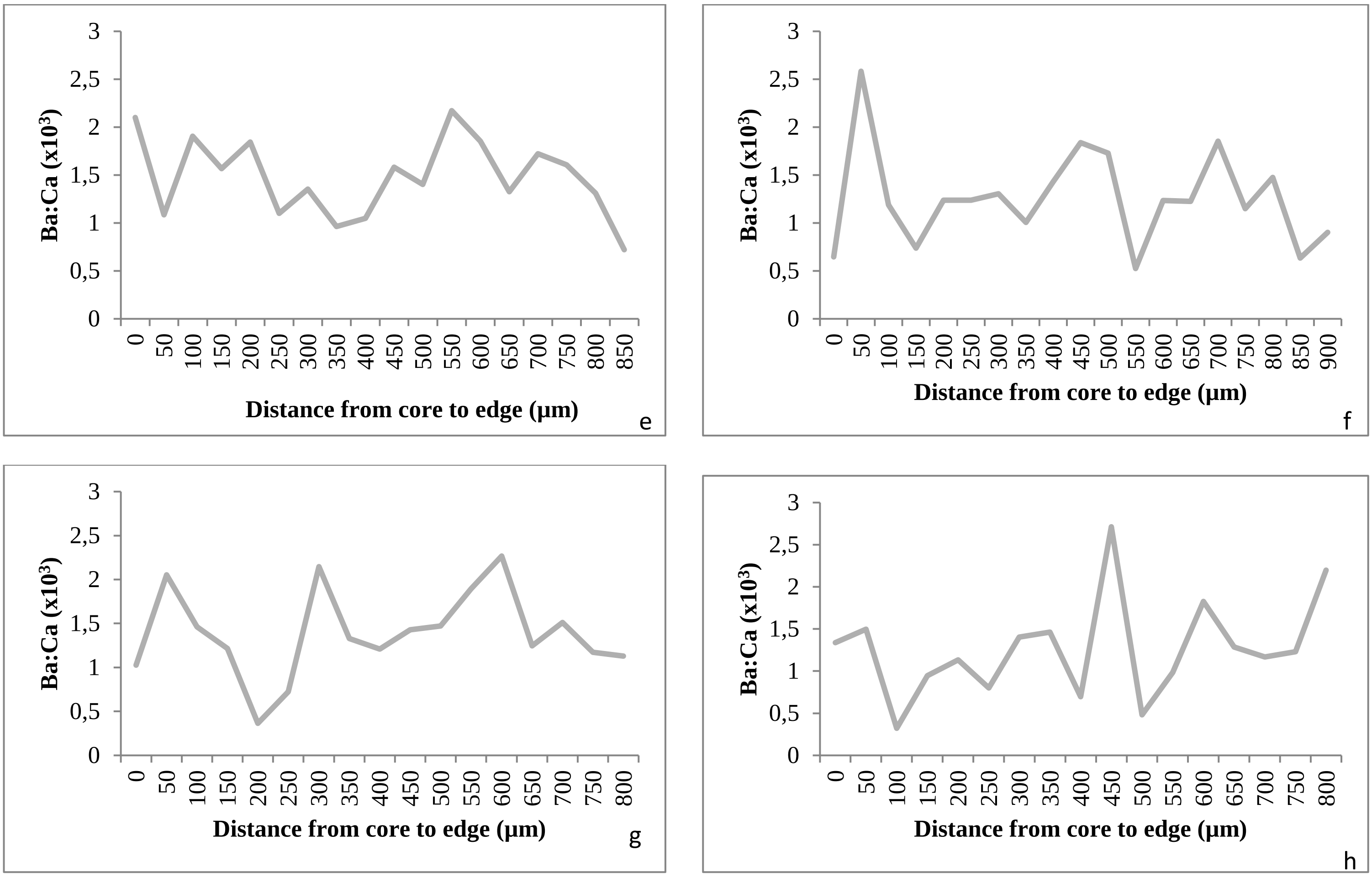


Fig. 7. Ba:Ca otolith concentrations of the same eight different patterns founded in *Centropomus parallelus* that were identified from the Sr:Ca concentrations respectively.

Table 4. Migration types, description and frequency (%) of *C. parallelus* adults.

Migration Type	Description	Frequency (%)
marine brackish migrant	characterized by spending all life, from hatching to the sampling, migrating between salt and brackish water environments	52.2
marine freshwater migrant	migrate randomly between oligo, meso and polyhaline environments during their life cycle	20.0
marine resident	recorded high Sr:Ca concentrations from core to the edge otoliths, were at polyhaline waters all the time	8.8
brackish migrants	a pattern contrary of above, it means that first fishes shows high Sr:Ca concentrations (salt water) and	7.7

	followed by medium values (brackish water)	
marine migrants	medium Sr:Ca concentrations in the early stages (brackish water) followed by high Sr:Ca ratios (salt water) until catch date	6.7
brackish resident	Sr:Ca ratios recorded from core to edge was medium values, corresponding to brackish waters	2.2
freshwater migrant	characterized by low Sr:Ca ratios in the early stages of life (freshwater) followed by medium Sr:Ca concentrations referring to brackish water	2.2
brackish resident with occasional freshwater entry	lives in brackish water and had a transitory entry in oligohaline water	1.1

Discussion

The hereby water samples showed, as expected, that Sr content increases with salinity. However no relationship was found for Ba. The small variation in temperature in sampling areas cannot explain the observed results for Ba, since Sr and Ba concentrations in water vary mainly due to salinity changes and are little affected by temperature (Elsdon and Gillanders, 2003). In addition, other factors can influence the elemental concentrations in water, such as the geological composition of the sampling area (Walther and Limburg, 2012).

Although many authors use the Ba:Ca ratio in migration pattern studies and consider this measure to be reliable (Tabouret *et al.*, 2010; Reis-Santos *et al.*, 2012) other studies have shown that this ratio is not always as expected (Hamer *et al.*, 2015). Jessop *et al.*, 2012 suggested that Ba:Ca should be used with caution and that it could be used only as a complement to the information coming from the Sr:Ca rations because they did not find a critical value that separated freshwater from estuaries, as they find for Sr:Ca. Moreover they claim that the relationship of the environment salinity with the Sr:Ca otolith concentrations are more consistent than the relationships with otolith Ba:Ca, mainly in brackish water.

In addition, Ba unlike Sr, is adsorbed onto suspended particles in the river (Coffey et al., 1997) and then is removed by biological or chemical processes (Coffey et al., 1997; Guay and Falkner, 1998). Thus, the peak location of dissolved Ba depends on the density of hydrological conditions and river particulate matter.

In this work we also found unexpected values for Ba:Ca, since they were higher than those recorded for other migratory species (Tabouret *et al.*, 2010; Reis-Santos *et al.*, 2012, Mai *et al.*, 2014), probably due to unexpected high concentration of Ba in the water. Because of this, the classification of migration patterns on the hereby study were made using Sr:Ca as a proxy.

Centropomus parallelus is classified as amphidromous species (Dutka-Gianelli, 2010; Fortes, *et al.*, 2014), it means a fish who migrates between marine and freshwater with no purpose of breeding (Myers, 1949). It is able to live in a range of abiotic characteristics, as temperature and salinity, as shown in controlled laboratory experiments (Sterzelecki *et al.*, 2013), and in the natural environment (Vitule *et al.*, 2013; Daros *et al.*, 2016). Thus, although we expected that some individuals would remain in the same salinity environment during a long period to avoid physiological stress, the species showed a great variety of movement patterns and high environmental plasticity, probably to search for food and to escape to predators as observed for other fish groups (Capoccioni *et al.*, 2014).

Another reason for this wide variety of environments occupancy can be the need for high salinity for egg hatching (Chaves and Nogueira, 2013) and subsequent need for low salinity for optimal juvenile development (Rocha *et al.*, 2005), followed by no salinity dependence for adults, but a possible demand for milder temperatures, since above 31 degrees the snook growth efficiency decays, as described for *C. undecimalis* (Tucker, 2003).

Thus the mosaic of PEC with different salinity habitats could provide a variety of environmental conditions for this species. This could explain for instance the eight observed different patterns of fish migration. Half of these observed patterns have been already been described for *C. parallelus* juveniles, but with different proportions (Daros *et al.*, 2016). The brackish resident fish pattern with some individuals having freshwater occasional entries (considered the same as brackish resident for juveniles) represents just 2.2% of adults total sampled. For juveniles, instead, it represents 30%. The same happen with other three movement types that adults and juveniles have in common. For marine migrant adults presents 6.6% while juveniles presents 33.3%. For marine resident was recorded in 8.8% adults and 16.6% juveniles. Finally for brackish migrant was found 7.7% adults and 20% juveniles.

The two most abundant patterns (72% together), the marine brackish migrant and marine freshwater migrant, in adults are with the most "mobility" migration strategies. This may be due to the fact that the fish adults of genus *Centropomus* are more resistant to environmental factors than juveniles (Shafland and Foote, 1983 and Tucker, 2003) and they have the swimming system fully developed, for these reasons, they must have a greater ability to adapt to environmental adversities, both biotic and abiotic.

Other migration patterns were recorded for Centropomidae but not the same found in *C. parallelus* (Trotter *et al.*, 2012). In *C. undecimalis*, for example, a pre-spawn aggregation behavior was demonstrated in which the invidious migrate to the higher salinity regions in order to promote egg viability, while for *C. parallelus* no such relationship was found (Rolls, 2014). Therefore for those who have not yet been found, we suggest names based on patterns previously described.

It is usual to combine two or more terms to name migration patterns, at least one regard to the location at which the fish live and another one that refers to the time that it remains at this location, for example freshwater resident (Arai and Chino, 2012). To describe fish movements without seasonality or without any clear temporal regularity of environmental change the term "nomadic" has been proposed (Daverat *et al*., 2006), but it is little used, moreover "migrant" it has been validated for *C. parallelus* (Daros *et al.*, 2016). Thus a combination of location (or locations) where the fish spend more time (either during the early or late life stage) with its migrant characteristic (movement between different habitats) or resident (stays in the same habitat) was determined to each one of the patterns described in this paper.

Migration besides being considered as a response to predation, it is considered a response to variation of food quality or abundance (Gauthreaux, 1982). It was well know for fish populations (Tableau *et al*., 2015) and work as a trade-off, it means, the balance between the energy expenditure of movement and food availability. This balance may work differently for different individuals of the same population, resulting in partial migration.

C. parallelus adults can be classified as partial migratory species because they have, in the same population, both resident and migrant individuals. There are many examples of partial migration within Perciformes (Chapman *et al.*, 2012). Although, types of seasonal partial migration have been usually described (Chapman *et al.*, 2011), we did not find any seasonality for *C. parallelus* migration in the different collection points.

The observed values of Sr:Ca ratios of otoliths edges were higher than expected for the less salt sites comparatively with a previous study. Those that were caught in brackish water showed Sr:Ca concentrations higher than eight (salt water) and individuals collected in freshwater showed values attributed to brackish water (Daros *et al.*, 2016). Since the edge values theoretically represents a short period immediately before the fish collection, it could mean that time required for incorporation of the chemicals in the otoliths is not enough to imprint the salinity signal of the sampling location. Furthermore, little is known about the time required for incorporation of environmental elements in the otolith. Some experiments were done of how the temperature, salinity and the interaction of these factors influence the incorporation but only for some species. Other variables must be considered, such as time of exposure to environmental variables, ontogeny, age and physiology of the species (Elsdon and Gillanders, 2003).

The fish movements can be affected by a range of reasons like natural mortality, diseases and fishing (Fuji *et al.*, 2016). The habitat selection might be opportunistic, not obligatory. However it is important to know fish movement to our understanding of species ecology and population’s dynamics, key features for an effective conservation and management of fisheries and proper habitat protection strategies (Dutka-Gianelli, 2010; Capoccioni *et al.*, 2014).

The high number of migration patterns recorded here, twice as described for the juveniles, suggests that adults have a greater adaptability and plasticity than young fish. Furthermore most migratory patterns includes estuaries as occupation area, showing the importance of these environments for the species, probably due to increased food availability and less predation pressure (Paitach, 2015). Therefore species conservation requires preservation of all connectivity habitats, but particular attention should be given to the conservation of estuaries, especially the mangrove areas in mesohaline regions as they are the main areas occupied by recruits and adults.

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