



**UNIVERSIDADE FEDERAL FLUMINENSE
INSTITUTO DE GEOCIÊNCIAS
DEPARTAMENTO DE GEOLOGIA/LAGEMAR
PROGRAMA DE PÓS-GRADUAÇÃO EM
DINÂMICA DOS OCEANOS E DA TERRA**



TESE DE DOUTORADO

**Crescimento, ciclo reprodutivo e concentração de mercúrio total de peixes
comerciais explorados na região de Cabo Frio**

JULIANA MONTEIRO DA SILVA VIEIRA

Niterói, RJ

Junho, 2023

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Tese de doutorado apresentada ao Programa de Pós-graduação em Dinâmica dos Oceanos e da Terra da Universidade Federal Fluminense, como parte dos requisitos necessários à obtenção do grau de Doutor. Área de Concentração: Ecologia Marinha.

Orientador: Dr. Paulo A. S. Costa

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–Christopher Paolini

Resumo

Esta tese teve como objetivo descrever a dinâmica populacional (idade, crescimento e reprodução) de duas espécies de pequenos atuns: bonito-pintado (*Euthynnus alletteratus*) e bonito-cachorro (*Auxis thazard*), provenientes de desembarques da pesca artesanal de cerco de praia e da frota de traineiras da região do Cabo Frio (no sudeste do Brasil). Adicionalmente, foram quantificadas as concentrações de mercúrio total (HgT) em três espécies de pequenos atuns exploradas no sudeste do Brasil: o bonito-cachorro (*A. thazard*), o bonito-pintado (*E. alletteratus*) e o bonito-listrado (*Katsuwonus pelamis*). Todos os espécimes foram coletados entre 2017 e 2019 e amostras de espinho da nadadeira dorsal, fígado e gônadas foram retiradas para os estudos de idade, crescimento, reprodução, e amostras de músculo foram utilizados nas análises de contaminação por mercúrio. Os dois primeiros capítulos desta tese são centrados na descrição e avaliação da idade, do crescimento e dos aspectos reprodutivos de *E. alletteratus* e *A. thazard*. No **capítulo 1**, através da análise de 345 exemplares, foi observado que período reprodutivo de *E. alletteratus* ocorreu do fim da primavera e ao longo do verão, aparentemente associado a ressurgência costeira da Água Central do Atlântico Sul (ACAS) na região de Cabo Frio, em temperaturas entre 15–18°C, um aspecto documentado pela primeira vez para a espécie. A idade da primeira maturação sexual foi ao 1 ano, em comprimentos entre 423 e 492 mm. 314 espinhos foram seccionados e registrados, e idades entre 0+ e 5 anos foram observadas a partir da formação de um incremento anual para peixes de 2 e 3 anos. O coeficiente de variação (CV) entre leituras sucessivas foi de 9,2%. As estimativas dos parâmetros de crescimento da equação de von Bertalanffy para todos os espécimes foram: $L_{\infty}=791,9$ mm, $k=0,42$ ano⁻¹ e $t_0=-0,97$ ano⁻¹. No **capítulo 2**, 548 espinhos de *A. thazard* foram seccionados e registrados, e idades entre 0+ e 4 anos foram observadas. O CV foi de 11%. O crescimento diferiu entre os sexos, e os parâmetros de crescimento foram $L_{\infty}=471$ mm; $k=0,47$ ano⁻¹ e $t_0=-1,46$ ano⁻¹ para fêmeas e $L_{\infty}=498$ mm; $k=0,35$ ano⁻¹ e $t_0=-2,01$ ano⁻¹ para machos. O índice gonadossomático atingiu o pico em dezembro, associado a ressurgência da ACAS, em temperaturas de aproximadamente 16 °C. O Comprimento de primeira maturação sexual foi de 345,4 mm para as fêmeas e 329,8 mm para os machos. No **capítulo 3**, um total de 82 amostras de músculo de 3 espécies de pequenos atuns (*Auxis thazard*, *Euthynnus alletteratus* e *Katsuwonus pelamis*) foram utilizadas para a quantificação de mercúrio total (HgT) e análise da influência da ecologia trófica e preferência de habitat no acúmulo de HgT utilizando isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). Adicionalmente, o risco à saúde humana em relação ao consumo foi avaliado através da estimativa de ingestão semanal

(*estimated weekly intake*, EWI) e pela ingestão semanal tolerável provisória (*provisional tolerable weekly intake*, PTWI) para os consumidores em geral, homens adultos e mulheres adultas. Variáveis como tamanho e idade estiveram positivamente correlacionadas ao mercúrio, sugerindo bioacumulação ao longo do tempo. A posição trófica parece influenciar positivamente as concentrações de mercúrio para *A. thazard* e *K. pelamis*, cuja relação entre $\delta^{15}\text{N}$ e HgT foi significativa. No entanto, para *E. alletteratus*, a falta de relação sugere que os valores de $\delta^{15}\text{N}$ não variam para peixes adultos (> 300 mm). Para nenhuma das espécies avaliadas nos três cenários as concentrações de HgT ficaram acima do limite tolerável estabelecido para o mercúrio pelos órgãos internacionais brasileiros, conforme indicado pelo %PTWI. Esse achado sugere que o consumo dessas espécies capturadas no sudeste do Brasil nas quantidades consideradas não apresenta risco para adultos saudáveis.

Palavras-chave: Idade, crescimento, reprodução, peixe marinho, bioacumulação, ecologia trófica, sudeste do Brasil

Abstract

This thesis aimed to describe the population dynamics (age, growth and reproduction) of two small-tunas: the little tunny (*Euthynnus alletteratus*) and frigate tuna (*Auxis thazard*) sampled from artisanal landings off the Cabo Frio region (Southeast Brazil). Additionally, total mercury concentrations (THg) were quantified in five species of economic importance in southeastern Brazil: the frigate tuna *Auxis thazard*, the blue runner *Caranx crysos*, the little tunny *Euthynnus alletteratus*, the skipjack tuna *Katsuwonus pelamis* and the red porgy *Pagrus pagrus*. All specimens were collected between 2017 and 2019 and dorsal fin spines, liver and gonads were sampled for age, growth, reproduction studies, and muscle samples were used in mercury contamination analyses. The first two chapters of this thesis are centered on the description and evaluation of the age, growth and reproductive aspects of *E. alletteratus* and *A. thazard*. In **chapter 1**, through the analysis of 345 specimens, it was observed that the reproductive period of *E. alletteratus* occurred from the end of spring and throughout the summer, most likely associated with coastal upwelling of the South Atlantic Central Water (ACAS) in the Cabo Frio region, at temperatures between 15–18°C, a first-time documented aspect for the species. Age at first maturity was of 1-year-old, at lengths between 423 and 492 mm. 314 spines were sectioned and recorded, and ages between 0+ and 5 years were observed from the formation of an annual increment in 2 and 3-year-old fish. The coefficient of variation (CV) between successive readings was 9.2%. Estimates of the growth parameters from the von Bertalanffy function for all specimens were: $L_{\infty}=791.9$ mm, $k=0.42$ yr⁻¹, and $t_0=-0.97$ yr⁻¹. In **chapter 2**, 548 spines of *A. thazard* were sectioned and recorded, and ages between 0+ and 4 years were observed. The CV was 11%. Growth differed between sexes, and growth parameters were $L_{\infty}=471$ mm; $k=0.47$ yr⁻¹ and $t_0=-1.46$ yr⁻¹ for females and $L_{\infty}=498$ mm; $k=0.35$ year⁻¹ and $t_0=-2.01$ year⁻¹ for males. Gonadosomatic index peaked in December, associated with the upwelling of the ACAS, at temperatures of approximately 16 °C. The Length-at-first-maturity was 345.4 mm for females and 329.8 mm for males. In **chapter 3**, A total of 82 muscle samples from 3 small-tuna species (*Auxis thazard*, *Euthynnus alletteratus* and *Katsuwonus pelamis*) were used for quantification of total mercury (THg) and analysis of the influence of trophic position and habitat preference on Hg accumulation using isotopes from carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Additionally, the risk to human health in relation to consumption was assessed by estimating weekly intake (EWI) and by Provisional Tolerable Weekly Intake (PTWI) for general, adult male consumers. and adult women. Variables such as size and age were correlated to mercury concentrations, indicating a process of bioaccumulation over time. The

trophic position appears to significantly influence mercury concentrations for *A. thazard* and *K. pelamis*, whose relationship between $\delta^{15}\text{N}$ and THg was significant. However, for *E. alletteratus*, the lack of relationship suggests that $\delta^{15}\text{N}$ values do not vary for adult fish (> 300 mm). For none of the evaluated species in the three scenarios THg concentrations were above the established tolerable limit for mercury by Brazilian international organs, as indicated by the %PTWI. This finding suggests that the consumption of those species caught off the southeast Brazil at the considered amounts does not present a risk for healthy adults.

Keywords: Age, growth, reproduction, marine fish, bioaccumulation, trophic ecology, southeastern Brazil

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ESTRUTURA DA TESE

Esta tese de doutorado está estruturada em três capítulos, todos em formato de manuscritos de artigos científicos na língua inglesa. No **Capítulo 1** foram descritos e analisados a idade, o crescimento e a aspectos reprodutivos do bonito-pintado *Euthynnus alletteratus* na costa sudeste do Brasil. No **Capítulo 2** foram investigados a idade, o crescimento e a reprodução do bonito-cachorro *Auxis thazard*. No **Capítulo 3** foram quantificadas e analisadas as concentrações de mercúrio no tecido muscular de *E. alletteratus*, *A. thazard*, e de outras 3 espécies-alvo (pargo-rosa *Pagrus pagrus*; xerelete *Caranx crysos* e bonito-listrado *Katsuwonus pelamis*). Adicionalmente, as concentrações de mercúrio total foram comparadas em relação a características ecológicas e populacionais por meio de análise de isótopos estáveis (SIA). Por fim, foi determinado o risco potencial das concentrações observadas quando comparadas aos limites estabelecidos por órgãos brasileiros e internacionais.

Introdução Geral

Peixes são responsáveis por quase 20% da proteína animal que é ingerida por mais de um terço da população mundial (FAO, 2018). Peixes ósseos, em particular, correspondem a 85% do total de espécies marinhas capturadas em volume e são importantes componentes socioeconômicos em pescarias artesanais e comunidades costeiras em todo o mundo (FAO, 2022; Rousseau et al. 2019; Watson e Tidd, 2018). Dentre esses peixes, os atuns apresentam particular importância, devido ao fato de serem amplamente consumidos. Neste grupo, que pertence à família *Scombridae*, existem pequenos-atuns (ICCAT, 2018). Os mesmos consistem em espécies geralmente menores que 120 cm, observadas em águas tropicais e subtropicais distribuídas pelo mundo, como o *K. pelamis*, três espécies do gênero *Euthynnus* (*Euthynnus affinis*; *E. alletteratus* e *E. lineatus*) e os bonitos do gênero *Auxis* (*Auxis thazard* e *A. rochei*) (Boyce et al., 2008; Collette & Nauen, 1983; Muhling et al., 2017).

Katsuwonus pelamis, uma espécie migratória e cosmopolita, encontrada em águas tropicais e temperadas quentes em todo o mundo, representa quase 40% da captura anual de atuns do mundo e ocupa o terceiro lugar em tonelagem de captura na pesca marítima global (2,8 × 106 t em 2020; FAO, 2022). Já no Atlântico, *K. pelamis* tem grande relevância social e econômica para o mercado pesqueiro, principalmente para a indústria de atum enlatado no Brasil, ao passo que *Euthynnus alletteratus* é capturado principalmente sazonalmente como captura acessória em pescarias de arrasto e redes com retenida na costa africana e capturados principalmente para uso como isca no Golfo do México, além de ser o pequeno atum mais capturado no Oceano Atlântico junto ao *Sarda sarda* e o *Auxis thazard* (Cabrera et al. 2005; Gaykov e Bokhanov, 2008; ICCAT, 2021). No Atlântico Oeste, especificamente, a produção por captura do *K. pelamis* atingiu 22.440 ton/ano entre 2018 e 2019 (ICCAT, 2021). Por outro lado, em águas brasileiras, *E. alletteratus* é capturado como um *by-catch*, ou seja, um alvo secundário da pesca de *K. pelamis* junto a outros atuns maiores (*Thunnus spp.*), do *Coryphaena hippurus* e do *A. thazard* (da Silveira-Menezes et al. 2010). Segundo os dados mais recentes da FIPERJ (2015), entretanto, a espécie apresenta relativa importância comercial nos desembarques da frota de cerco de Macaé, Niterói, São João da Barra e Cabo Frio, com o último reportando cerca de 3 t de bonito-pintado desembarcados em 2015, o que é uma redução considerável quando comparado a média de 422 toneladas reportadas por Rossi-Wongtschowski e Cergole (2007) para o período entre 1996 e 1998. Quando considerado todo o Estado, cerca de 36 t foram capturadas em 2015 (FIPERJ, 2015).

Entretanto, apesar da importância socio-econômica da pesca marinha, diversos estudos apontam para uma sobrexploração dos recursos pesqueiros mundiais (Sumaila et al., 2016; Pauly e Zeller, 2016), o que reforça a necessidade de políticas de manejo mais eficientes. Para tal, informações básicas da história de vida como idade, crescimento e maturidade são fundamentais, pois servem como indicadores da dinâmica populacional de peixes submetidos à exploração comercial e para o manejo sustentável das espécies (King 2008; Lucena–Frédou et al. 2017; Pons et al. 2019).

***Euthynnus alletteratus* (Rafinesque, 1810)**

Euthynnus alletteratus é um pequeno atum pertencente à família *Scombridae* (Figura 1) (Uchida, 1981). Sua idade máxima reportada pela literatura é de 8 anos, com a maior parte dos trabalhos estimando uma idade máxima de 5 anos (Jonhson, 1983; Cayrè et al., 1993; Kahraman e Oray, 2001; Santamaria et al., 2005; Valerias et al., 2008; Macías et al., 2009; Adams e Kerstetter, 2014).



Figura 1. Exemplar de *Euthynnus alletteratus* (Rafinesque, 1810).

A respeito de seus aspectos reprodutivos, assim como outros atuns, *E. alletteratus* é um desovante múltiplo, com desenvolvimento assíncrono de ovócitos, capaz de produzir numerosas postas intermitentes por época reprodutiva (Kahraman et al., 2008; Hajjej et al., 2010; Schaefer, 2001). A maturidade sexual é alcançada aos 440 e 420 mm no Golfo da Guiné para machos e fêmeas, respectivamente, 400 mm no Senegal, e em aproximadamente 600 mm para fêmeas aos 3 anos de idade no Mediterrâneo (Rodríguez-Roda, 1966).

Acerca de sua alimentação, poucos estudos descreveram os hábitos alimentares desta espécie. Na costa brasileira, Menezes e Aragão (1977) avaliaram o conteúdo estomacal de juvenis e adultos (>300 mm) de *E. alletteratus* e descreveram sua alimentação como sendo

altamente psicívora, sendo comporta principalmente de Clupeídeos. No Mediterrâneo, esta espécie é descrita como um predador oportunista, que, embora apresente mudanças ontogenéticas na composição de presas, permanece constantemente alimentando-se de pequenos peixes pelágicos, como *Maurolicus muelleri* e clupeiformes, além de crustáceos, peixes, lulas, heterópodos e tunicados (Falautano et al., 2007).

No Brasil, além de ser considerado uma espécie acompanhante de outras de maior interesse econômico, na região sudeste ele apresenta grande importância em capturas de subsistência, sendo frequentemente capturada em pequenas quantidades por pescarias artesanais (da Silveira-Menezes et al., 2010; Nóbrega et al., 2009). No Rio de Janeiro, especificamente, a espécie é fauna acompanhante da pesca da *Sardinella brasiliensis* (Paiva e Motta, 1999), e das capturas de *K. pelamis* pela frota de vara e isca viva (da Silveira-Menezes et al., 2010). No Estado, a produção capturada do bonito-pintado foi de 9 toneladas em 2018, o que representou 0,8% da produção monitorada de vara e isca viva (Martins et al., 2020).

***Auxis thazard* (Lacepède, 1800)**

Auxis thazard constitui um dos menores membros dentre os tunídeos, que consiste dos verdadeiros atuns (Collette e Nauen, 1983) (Figura 2). Seu tamanho máximo é de 650 mm com, geralmente, representantes da espécie ocorrendo entre 25 e 40 cm, dependendo das artes de pesca e da região (Collette e Nauen, 1983). Como todos os bonitos, os mesmos são pertencentes à família *Scombridae*, sendo uma das duas espécies atualmente a compor o gênero *Auxis*.



Figura 2. Exemplar de *Auxis thazard* (Lacepède, 1800).

São poucos os estudos sobre o crescimento sobre esta espécie. Os métodos empregados incluem o uso de espinhos, vértebras e, mais recentemente, métodos indiretos utilizando frequências de comprimento (Ghosh et al., 2012; Grudtsey e Korolevich, 1986; Tao, 2007). A longevidade máxima reportada para a espécie é de 5 anos (Tao, 2007). No sul do Oceano Índico, a época de desova se estende de agosto a abril e no norte do equador é relatada a ocorrência de janeiro a abril (Collette e Aadland, 1996). Collette e Nauen (1983) relatam que em águas japonesas a maturidade sexual é atingida em torno de 290 mm de comprimento furcal (FL), enquanto no Havaí o mesmo ocorre aos 305 mm de comprimento.

Auxis thazard, assim como *E. alletteratus*, é um predador voraz e de topo, que se alimenta principalmente de peixes, em particular anchovas e outros clupeiformes, crustáceos e cefalópodes (Etchevers, 1976). Entretanto, no geral, informações sobre seus hábitos alimentares são ainda mais escassos que os de *E. alletteratus*.

Devido à sua abundância, *A. thazard* é considerado um elemento importante da cadeia alimentar, particularmente como forragem para outras espécies de interesse comercial (Colette et al. 2011). Existe uma falta geral, no entanto, de informação sobre a mortalidade destas espécies como captura acessória, exacerbada pela confusão em relação à identificação de espécies do gênero *Auxis* (ICCAT, 2009).

No Brasil, a espécie é reportada como apresentando desembarques expressivos em Santa Catarina, quando no ano de 2012 pouco mais de 160 toneladas foram produzidas, ao passo que no Rio de Janeiro os dados monitorados de captura por vara e isca viva de 2018 indicaram uma produção de 36,1 toneladas, levando este bonito a se tornar uma das 10 espécies mais capturadas por esta modalidade (Martins et al., 2020; UNIVALI/CTTMar, 2013). Para os anos

entre 1996 e 1998, Rossi-Wongtschowski e Cergole (2007) apontam cerca de 29 ton de bonito-cachorro desembarcadas em média no porto de Cabo Frio, pela frota de cerco. Segundo dados da FIPERJ (2015), no Rio de Janeiro foram produzidas pouco mais de 36 toneladas da espécie, com Niterói sendo o maior produtor (35,9 t). Para o mesmo ano, porém, não há dados sobre os desembarques de Cabo Frio acerca da espécie. Apesar de sua ampla distribuição e da importância para a pesca regional, a maioria das informações sobre o gênero *Auxis* se refere à espécie *Auxis rochei*, com o conhecimento sobre os aspectos de vida de *A. thazard* ainda escassos mundialmente.

Área de estudo

O sistema de ressurgência de Cabo Frio é a mais intensa das áreas de ressurgência ao longo da costa brasileira. É uma região que apresenta uma rica fauna pelágica, que inclui espécies de grande importância econômica, como a sardinha-verdadeira *Sardinella brasiliensis*, o bonito-listrado *Katsuwonus pelamis* e o pargo-rosa *Pagrus pagrus* (Valentin, 2001; Kikuchi et al., 2021). O processo de ressurgência acontece ao longo da primavera e verão austrais, quando predominam os ventos NE-E (Nordeste-Leste) que, junto a uma topografia favorável, levam a ressurgência de águas frias (6 – 18°C) e salinas (34,5 – 36) da Água Central do Atlântico Sul (ACAS), provenientes de uma profundidade de 300 m (Gonzalez-Rodriguez et al., 1992). A produtividade primária criada por este sistema desencadeia um enriquecimento na concentração de nutrientes e causa uma proliferação local de fitoplâncton perto da costa, fator que permite a sobrevivência e alta abundância de uma rica variedade de espécies de peixes carnívoros de altos níveis tróficos, além de desempenhar um papel fundamental na atividade reprodutiva de diversas espécies de peixes, como a sardinha-verdadeira e do pargo-rosa (Costa et al., 2021; Matsuura, 1998; Valentin, 2001).

Mercúrio (Hg)

Mercúrio (Hg) é um elemento que ocorre naturalmente no meio ambiente que circula pela crosta terrestre, atmosfera, oceanos e formas de vida, e pode ser diretamente mobilizado por atividades antropogênicas (Kidd et al., 2012). Quase todo o Hg no músculo do peixe (> 90%) ocorre como metilmercúrio (MeHg), que entra na cadeia alimentar através de bactérias e fitoplâncton e é transferido de forma eficiente através das cadeias alimentares aquáticas, resultando em concentrações de Hg em predadores de topo até milhões de vezes superiores às dos produtores primários ou consumidores (ou seja, biomagnificação; Bloom, 1992). O Hg também se acumula ao longo da vida dos organismos (bioacumulação), o que é muito

dependente de processos físico-químicos (por exemplo, matéria orgânica dissolvida, salinidade, temperatura) e bióticos (por exemplo, tamanho, sexo, ciclo reprodutivo, hábitos alimentares, ambiente de vida) (Kidd et al., 2012; Wang et al., 2012). A concentração também varia entre espécies, e até mesmo de órgão para órgão dentro do mesmo organismo (Manhães et al. 2019).

Quanto à sua toxicidade, a exposição de humanos ao mercúrio tem sido associada à indução de diversas complicações de saúde, incluindo distúrbios neurológicos, anemia, cardiomiopatia e efeitos nos sistemas digestivo, renal e pulmonar (Choi et al., 2012). Também pode reduzir a fertilidade, além da alta toxicidade aos fetos, apresentada como abortos espontâneos, abortos espontâneos e natimortos, e nas crianças, que podem desenvolver retardo mental, reflexos primitivos, estrabismo, etc. (Harada et al., 1999; Rice et al., 2014).

Análise de isótopos estáveis

A análise convencional de conteúdo estomacal, mesmo que fundamental, apresenta diversas limitações inerentes, de modo que a análise de isótopos estáveis tem se tornado cada vez mais útil para reconstruir hábitos alimentares e melhorar a compreensão das teias tróficas nos ecossistemas marinhos (da Silveira et al., 2020; Médieu et al., 2022; Rooker et al., 2006). A variação em isótopos estáveis de carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$) é comumente utilizada como traçadores intrínsecos da composição da dieta, padrões migratórios, além de investigações ecotoxicológicas ao longo de teias alimentares (Hobson, 1999). Esta técnica baseia-se na premissa de que os valores de carbono e nitrogênio refletem os de seus alimentos.

Os valores de $\delta^{13}\text{C}$ são convencionalmente usados para distinguir locais de forrageamento, avaliar a dieta e a migração de espécies, pois o enriquecimento de isótopos de carbono estáveis de um nível trófico para o próximo ($\sim 1\%$) faz com que os valores de $\delta^{13}\text{C}$ das bases da cadeia determinem $\delta^{13}\text{C}$ de predador de topo (Layman et al., 2012; McCutchan et al., 2003; Rosas-Luis et al., 2003; Peterson e Fry, 1987). Por sua vez, os valores de $\delta^{15}\text{N}$ aumentam até 2-4% de um nível trófico a outro, o que os torna úteis na estimativa da posição trófica dos indivíduos e para a avaliação de processos de biomagnificação de contaminantes ao longo da cadeia (DeNiro e Epstein 1978; Di Benedetto et al., 2012; Layman et al., 2012).

Justificativa

Pequenos atuns, com exceção de *K. pelamis*, são espécies relativamente pouco estudadas, apesar de sua ampla distribuição e importância econômica para pescarias regionais (Juan-Jordá et al., 2011; Juan-Jordá et al., 2013). Em relação ao risco de extinção global, ambos bonitos

estão classificados como “menos preocupante” (*Least concern*) pela IUCN. No entanto, o estoque de *E. alletteratus* presente no sudoeste do Atlântico está dentre os estoques de pequenos atuns mais suscetíveis à sobrepesca, sobretudo devido a quantidade escassa de informações sobre seu ciclo de vida na região (Pons et al., 2019). *E. alletteratus* também é considerada uma espécie prioritária para avaliação e implementação de ações de manejo, ao lado de espécies importantes como a cavala-da-índia *Acanthocybium solandri*, a cavala *Scomberomorus cavalla*, o bonito do Atlântico *Sarda sarda* e o bonito-cachorro *A. thazard*, espécie cujas informações no Mediterrâneo, Noroeste e Sudoeste do Atlântico são inexistentes ou escassas (Lucena-Frédou et al., 2017). No Brasil, as capturas de *E. alletteratus* e *A. thazard* não estão sujeitas a políticas de controle ou manejo da pesca, pois os dados de parâmetros de vida para o estoque dessas espécies no sudeste brasileiro não estão disponíveis (Lucena-Frédou et al. 2017). Portanto, a avaliação sobre idade, crescimento, maturidade e outros traços da história de vida dessas espécies são de caráter fundamental para o futuro manejo do estoque capturado no sudeste da costa brasileira. Projetos como o “MULTIPESCA: Ciência para a Sustentabilidade da Pesca, Pescado e Pescadores do Rio de Janeiro”, deste modo, se tornam essenciais (FUNBIO, 2016). O MULTIPESCA, que decorre de uma medida compensatória estabelecida pelo Termo de Ajustamento de Conduta de responsabilidade da empresa Chevron, visa descrever aspectos como idade, crescimento e dinâmica reprodutiva de diversas espécies de recursos pesqueiros provenientes das frotas do norte do Rio de Janeiro (Macaé, Rio das Ostras e Barra de São João) são estudadas com o objetivo de gerar uma pesca sustentável e a conservação dos recursos explorados (FUNBIO, 2016).

Além de informações acerca da ecologia básica, avaliações referentes da qualidade do pescado, em especial contaminantes, também são instrumentos importantes para a manutenção da saúde dos estoques, dos consumidores e o manejo devido das pescarias. Metais como Mercúrio, são reconhecidamente tóxicos mesmo em baixas concentrações, e a principal rota de absorção decorre do consumo de frutos do mar, em especial de peixes, através do processo de bioacumulação ao longo da cadeia trófica, com o ser-humano como consumidor final (Kidd et al., 2012; Zheng et al. 2019). Devido a estes fatores, quantificações do mercúrio presente no músculo de espécies de pescado socioeconomicamente relevantes e avaliações frente aos limites estabelecidos por órgãos nacionais e internacionais também são fundamentais.

Objetivo geral

Estudar os aspectos básicos do ciclo de vida e da dinâmica populacional (idade, crescimento e reprodução) do bonito–pintado (*Euthynnus alletteratus* Rafinesque, 1810) e bonito–cachorro (*Auxis thazard* Lacepède, 1800) capturados na região do Cabo Frio (RJ, Brasil). Adicionalmente, o estudo visou quantificar o mercúrio total presente em três espécies de pequenos atuns de relevância socioeconômica na região sudeste do Brasil (*Euthynnus alletteratus*, *Auxis thazard*, e *Katsuwonus pelamis*).

Objetivos específicos

1. Determinar as frequências de comprimento, peso e a proporção sexual de exemplares de duas espécies de pequenos atuns (*Euthynnus alletteratus* e *Auxis thazard*) capturadas pelas pescarias artesanais e industriais da região do Cabo Frio e Arraial do Cabo;
2. Estimar os parâmetros de crescimento *E. alletteratus* e *A. thazard* a partir da análise das marcas de crescimento no primeiro espinho da primeira dorsal;
3. Descrever a biologia reprodutiva de *E. alletteratus* e *A. thazard*;
4. Determinar o tamanho de primeira maturação e o período reprodutivo de *E. alletteratus* e *A. thazard*;
5. Quantificar e analisar a concentração de mercúrio total presente no tecido muscular de três espécies de pequenos atuns (*Euthynnus alletteratus*, *Auxis thazard*, e *Katsuwonus pelamis*) exploradas na costa sudeste do Brasil;
6. Quantificar os isótopos estáveis de carbono e nitrogênio presentes no tecido muscular de três espécies de pequenos atuns (*Euthynnus alletteratus*, *Auxis thazard*, e *Katsuwonus pelamis*) exploradas na costa sudeste do Brasil.

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CAPÍTULO 1

Age, growth, and maturity of little tunny, *Euthynnus alletteratus* (Rafinesque, 1810) in southeastern Brazil

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Age, growth, and maturity of little tunny, *Euthynnus alletteratus* (Rafinesque, 1810) in southeastern Brazil

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ABSTRACT. This is the first attempt to assess life parameters of little tunny *Euthynnus alletteratus* in the southwestern Atlantic. Fin spines, liver, and gonad information from 345 fishes (330–780 mm fork length) sampled from artisanal landings between March 2018, and February 2019 were used to analyze age, growth, and sexual maturity. Age was estimated by counting and measuring increments in sectioned spines, and the von Bertalanffy growth function was used to fit length–at–age data. There was no significant difference between male and female size distributions. Gonadosomatic index peaked from November to February in both sexes, associated with the South Atlantic Central Water (SACW) upwelling in temperatures between 15–18°C. Reproduction in cold waters has never been reported before and is possibly related to a richer environment for feeding and growth of larvae. The coefficient of variation among successive readings was 9.2%. The oldest fish was five years, and one annual increment for two and 3–year–old fish was observed to form associated with SACW. Little tunny growing in southeastern Brazil shows a higher growth rate and smaller asymptotic length when compared to most stocks in Mediterranean waters. Growth was not significantly affected by sex, and the von Bertalanffy growth parameters for all fish were $L_{\infty} = 791.9$ mm, $k = 0.42$, and $t_0 = -0.97$ yr⁻¹. Length at first maturity is attained by 1–year–old fish of either sex (423–492 mm), suggesting that a small proportion (8%) of juveniles was recorded from fishery landings.

Keywords: *Euthynnus alletteratus*; little tunny; age; growth; reproduction; size; age structure; southwestern Atlantic

INTRODUCTION

Tuna and tuna–like fish consist of several species of worldwide economic importance, belonging to the Scombridae family, and are responsible for 7.9 million metric tons produced

in 2018 (FAO 2020). There are more than 10 species of small-bodied tunas, but only five of these account for about 88% of the total reported catch by weight (ICCAT 2009). The little tunny *Euthynnus alletteratus* (Rafinesque, 1810) is an epipelagic, neritic, small-bodied tuna and one of three species of the genus *Euthynnus* found in tropical and subtropical waters of the world (Manooch III et al. 1985). It is distributed on both sides of the Atlantic Ocean, including the Mediterranean, Black Sea, Caribbean Sea, and Gulf of Mexico (Collette & Nauen 1983).

E. alletteratus is reported as the most abundant scombrid in the epipelagic zone of the Gulf of Mexico (Pruzinsky et al. 2020) and one of the most abundant small tuna species in the Mediterranean Sea (Macías et al. 2009), where it is commercially exploited off the Spanish coast together with bullet tuna *Auxis rochei* and the Atlantic bonito *Sarda sarda* (Baéz et al. 2019). Like the Atlantic bonito and frigate tuna (*Auxis thazard*), little tunny is also among the most captured small scombrid in the Atlantic Ocean (ICCAT 2018). It is captured seasonally in western Africa and the Mediterranean Sea (Gaykov & Bokhanov 2008), considered a secondary target species, and captured mainly as bait in the Gulf of Mexico (Cabrera et al. 2005). Located in southeast Brazil (20–23°S), Rio de Janeiro State is one of the main locations for the skipjack tuna (*Katsuwonus pelamis*) fishery in Brazil, from where little tunny has been reported to be caught as one of the most frequent secondary targets alongside other tunas (*Thunnus* spp.), the common dolphinfish (*Coryphaena hippurus*) and the frigate tuna (*Auxis thazard*) (Da Silveira Menezes et al. 2010). Little tunny's production in 2018 (9 t) represented 0.8% of the monitored rod and live-bait production (Martins et al. 2020). However, capturing *E. alletteratus* along the Brazilian coast is not subjected to fishing control or management policies due to the lack of valid data (Lucena-Frédou et al. 2017).

Small tuna species are relatively understudied (Juan-Jordá et al. 2013) and are expected to be subjected to increasing fishing pressure as several stocks of valuable large-bodied tunas are overfished (Pruzinsky et al. 2020). Despite its wide distribution and economic importance, *E. alletteratus* is classified as least concern by the IUCN (Juan-Jordá et al. 2013), and most studies regarding its biology have been conducted in Mediterranean waters (Rodríguez-Roda 1979, Kahraman & Oray 2001, Macías et al. 2006, 2009, Falautano et al. 2007, Kahraman et al. 2008, Hajjej et al. 2010, Mohamed et al. 2014, Saber et al. 2018). The southwest Atlantic (SWA) studies are scarce and mostly outdated (Menezes & Aragão 1977, Matsuura & Sato 1981, Chatwin & Matsuura 1998). Life traits such as age, growth, feeding ecology, and reproduction are completely unknown in SWA (Lucena-Frédou et al. 2017, Pons et al. 2019).

Like other scombrids, *E. alletteratus* is a multiple spawner, with an asynchronous development of oocytes resulting in numerous intermittent spawning seasons (Schaefer 2001).

Estimates of length at first maturity (L_{50}) ranged from 350 to 448 mm fork length (FL) in Tunisia and Egypt, where the species is exploited by small-scale or recreative fisheries (Hajjej et al. 2010, Mohamed et al. 2014). The smallest L_{50} value (343 mm) was recorded in the Gulf of Mexico (Cruz–Castán et al. 2019) and the largest (570 mm) in Spanish waters (Rodríguez–Roda 1966). Little tunny is mainly caught in both places as a secondary target in artisanal multispecific fisheries, despite being abundant in Spanish waters (Cabrera et al. 2005, Macías et al. 2006).

Little tunny's maximum age is mostly estimated at five years (Rodríguez–Roda 1979, Valeiras et al. 2008, Adams & Kerstetter 2014) and occasionally eight (Cayré & Diouf 1983) and nine years (Kahraman & Oray 2001). In Mediterranean waters (Rodríguez–Roda 1979), Senegal (Diouf 1980), and Gulf of Mexico (Cruz–Castán et al. 2019), age at first maturity is estimated at around two years. Among calcified structures, dorsal fin spines were the most utilized to investigate age and growth of *E. alletteratus* as it has a good relationship between radius and FL (Diouf 1980, Cayré & Diouf 1983, Johnson 1983, Valeiras et al. 2008). Studies using fin spines and vertebrae have reported a formation of one annual growth increment (Rodríguez–Roda 1979, Kahraman & Oray 2001, Valeiras et al. 2008), while two annual growth increments were observed in otoliths from Florida (Adams & Kerstetter 2014). There have been reports of paired increments in fin spines and vertebrae, referred to as "doublets," but they were considered one annual growth mark (Cayré & Diouf 1983, Johnson 1983).

The Cabo Frio upwelling system is the most intense of the upwelled areas along the Brazilian coast and has a rich pelagic fauna that includes large populations of Brazilian sardine *Sardinella brasiliensis* and skipjack tuna (Kampel et al. 1997, Matsuura & Sato 1981, Valentin 2001). The upwelling process happens throughout austral spring–summer when E–NE wind prevails, and the proximity of the 100 m isobath leads to a topography that promotes an upwelling of the cold (6–18°C) and less saline (34.5–36) South Atlantic Central Water (SACW) from a depth of 300 m (Silva et al. 1984, Gonzalez–Rodríguez et al. 1992). In contrast, when the NE wind velocities are reduced, or polar fronts are present, the direction of the wind changes and the thermocline depth fluctuates until the superficial area up to 300 m depth is occupied by the Coastal Water, characterized by the higher temperature (20–24°C) and salinity (>36) (Emílsson 1961, Silveira et al. 2000).

Surface water temperature is one of the most important and used predictors of abundance and distribution of tunas as it regulates physiology, behavior, reproductive activity, and growth (Fonteneau & Soubrier 1996, Worm et al. 2005). Although there are exceptions, spawning generally occurs at sea surface temperatures of about 24°C or higher (Schaefer 2001).

Scombrid's physical adaptations (e.g. vascular current heat exchangers) made them capable of displaying wide temperature tolerance, i.e. bigeye and the yellowfin tuna can be found in water temperatures ranging between 3 and 31°C (Boyce et al. 2008). Little tunny has been reported in water temperatures between 18 and 30°C (Cayré & Diouf 1980, Cruz–Castán et al. 2019). Like other small-bodied tropical tunas such as *Euthynnus affinis* and *K. pelamis*, it has a higher tolerance for warmer waters when compared to cold-tolerant tuna species (e.g. *Thunnus* sp.) (Boyce et al. 2008).

In this paper, the age, growth, and maturity of *E. alletteratus* are described for the first time in the SWA through investigation on transversal sections of fin spines, monthly gonad development, reproductive cycle, and physiological indexes. Additionally, we examined the influence of sea surface temperature on reproductive activity and the formation of growth increments on the first dorsal fin spine.

MATERIALS AND METHODS

Sampling

Samples of *Euthynnus alletteratus* were collected monthly from March 2018 to February 2019 on the southeastern Brazilian coast. Fishes were sampled from artisanal beach seine fisheries in Arraial do Cabo and purse seine fishing landings in the Port of Cabo Frio to cover as much of the size structure and age structure as possible. Beach seine is selective for smaller sizes, while purse seine favors large sizes.

FL was measured to the nearest mm, and total weight (TW) was recorded to the nearest 0.1 g. After that, gonad weight (GW) and liver weight (LW) were recorded to the nearest 0.001 g, and sex was registered. During the sampling period, the sea surface temperature (SST) was continuously recorded by a data logger (HOBO Tidbit UTBI-001), fixed at a rocky shore at Arraial do Cabo in 1 m water depth (Fig. 1). Sea surface temperature (SST) records were used to assess seasonal changes of physiological indicators and the periodicity of formation of growth marks in *E. alletteratus*' spines.

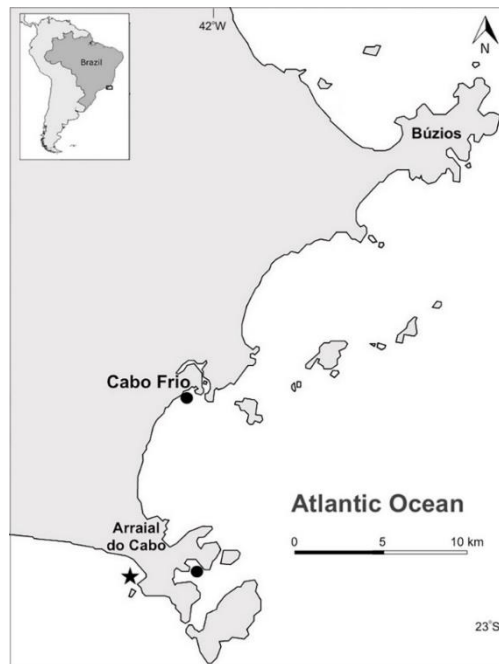


Figure 1. Map of the Cabo Frio region in southeastern Brazil showing the position of sea surface temperature measurements (★) and landing samplings (●).

The length–frequency distribution between sexes by fishing gear comparison was performed by a Kolmogorov–Smirnov test (D). An analysis of covariance (ANCOVA) was used to compare the length–weight relationship parameters of both sexes and the proportion of fishes among fisheries and sex–ratio were analyzed by the chi–squared test (χ^2).

Sexual maturity was visually classified by macroscopic gonad examination following Brown–Peterson et al. (2011) description, which included five phases: immature, developing, spawning capable, regressing and regenerating. Fishes under one of the four latter phases were considered sexually mature (adults). After the macroscopic classification, a sub–sample of gonads was removed, fixed in a solution of alcohol (76.5%), distilled water (8.5%), formaldehyde (10%), and glacial acetic acid (5%) for 24 h and preserved in 70% alcohol. They were subsequently dehydrated in different series of alcohol concentrations and included in paraffin. Transverse sections (5 μ m thick) of tissue were removed with a microtome, mounted on glass microscope slides, stained with hematoxylin–eosin for histological analysis, which was used to validate the macroscopic classification.

The spawning period was evaluated using two physiological indexes, the gonadosomatic index (GSI), calculated as $GSI = (GW / (TW - GW)) \times 100$, and the hepatosomatic index (HI), calculated as $HI = ((TW - LW) / LW) \times 100$. Monthly changes in the condition factor (K) were also recorded to follow the welfare condition of the specimens and calculated as $K = (TW /$

$FL^b) \times 10^4$, where b is the allometric coefficient from the length–weight relationship. Monthly data of the physiological and conditional indexes were tested using Shapiro–Wilk and Levene's tests to verify the normality and homogeneity of the variance of the data before applying an analysis of variance (ANOVA). When restrictions were not met, a Kruskal–Wallis (H) non–parametric test followed by a Mann–Whitney's (U) post hoc test was used (Zar 2010).

Size at first maturity (L_{50}) was estimated as $P = 1 / [1 + \exp^{-r(L-L_{50})}]$, where P is the proportion of mature individuals in the length class, r is the parameter determining the slope of the maturity curve, L is the lower limit of the length class and L_{50} is the fork length at which 50% of the fish are mature. Estimates of the model parameters were performed by non–linear regression, using Solver's quasi–Newton algorithm available in the Microsoft Excel software (Sparre & Venema 1997). Solver optimizes the best combination of parameters, minimizing the differences between mature individuals' predicted and observed proportions.

The first dorsal fin spine obtained from the specimens was extracted and used for age determination. Two cross–sections of 0.8 mm of thickness were taken successively along the length of each spine with a low–speed Buehler–Isomet metallographic saw as close as possible to the condyle. Transversal sections were examined under a stereomicroscope equipped with a micrometer scale and a digital image capture system (Zeiss Stemi 508[®]), applying transmitted light at 40x to 100x magnification. Translucent growth marks were counted, and each section was read twice by one reader, without information on the size or sex of the fish. A third was performed if there was a disagreement between the readings, and specimens whose age estimates still disagreed were removed from further analyses. In several sections was observed the presence of double or triple thinner growth marks with a smaller distance between one another that formed an opaque or translucent band, also reported by Cayré & Diouf (1983), Johnson (1983), and Valeiras et al. (2008). When present, annuli consisting of multiple bands were carefully considered to assess the age. The accuracy of the readings was determined by the average coefficient of variation (CV, Chang 1982) and by the index of average percentage error (APE, Beamish & Fournier 1981).

A translucent band of clear aspect (related to lower growth rates) intercalated with an opaque band of dark aspect (related to higher growth rates) was considered one increment. The spine radius (R_t) and each increment radius (R_i) was measured to the nearest 0.001 mm from the center of the spine (Fig. 2). The radius estimate for the missing initial rings in larger fishes was performed following Hill et al. (1989). A statistical summary of the first two rays of the smaller and younger fish that still had the increments visible was compiled, and a t –test was applied to compare the radii of the fishes with the first two increments visible and compare the

increments' radii between sex. Final corresponding ages were assigned when the radii of at least two successive increments of the first three or four were within the confidence interval limits of the increments' compiled data. Finally, a corrected age estimate was assigned to spines missing up to the second increment by comparing the radii of the first three or four visible increments to the means and the confidence interval limits at 95% of the summarized data.

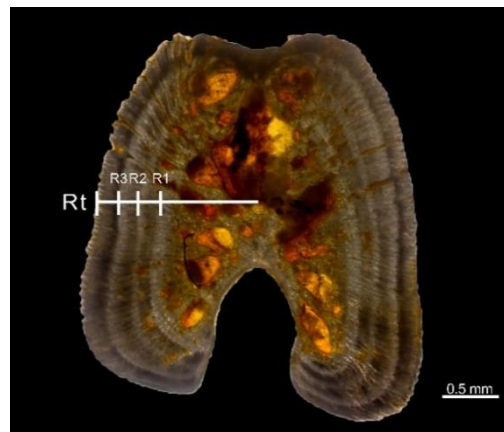


Figure 2. A transversal section of the first spine of a 3+ year female specimen (634 mm FL) showing the spine radius (Rt) measured from the focus to the edge and each increment identified (R1, R2, R3).

The edge aspect of each section (translucent or opaque) was recorded along with the readability of the section, which was classified as 0 (unreadable), 1 (low readability), and 2 (high readability). The precision among readings and the spine radius (Rt) per ring position were analyzed. An ANOVA was performed in order to test mean Rt values. The periodicity in increment formation was assessed by marginal increment analysis (Panfili & Morales–Nin 2002) and by the percentage of edge aspect calculated for fishes of 2 and 3 years, as they were the most frequent among samples. The marginal increment was calculated as $MI = (R_t - R_n) / (R_n - R_{n-1})$, where R_t is the spine radius, and R_n and R_{n-1} are the distance from spine focus to the outermost and the penultimate increment identified, respectively. Differences in marginal increment were tested using Shapiro–Wilk and Levene's tests to verify the normality and homogeneity of the data variance before applying an analysis of variance (ANOVA). When restrictions were not met, a Kruskal–Wallis (H) non–parametric test followed by a Mann–Whitney's (U) *post-hoc* test was used (Zar 2010). Edge aspect proportion (translucent/opaque) during upwelling (SACW) and non–upwelling (CW) was tested using a chi–square test (χ^2).

Von Bertalanffy growth curves were fitted to the observed data applying the standard von Bertalanffy growth function (VGBF): $FL = L_\infty \times [1 - e^{-k(t-t_0)}]$, where FL is the fork length at

age t ; L_{∞} is the asymptotic length; k is the growth coefficient, and t_0 is the theoretical age at zero length. The likelihood ratio test (LRT) was used to estimate the growth parameters (L_{∞} , K , t_0), according to Aubone & Whöler (2000). Curves of females and males were compared using Kimura's likelihood ratio test (Kimura 1980, Haddon 2011).

RESULTS

Size distribution

A total of 345 specimens were sampled, including 174 females (409–780 mm), 169 males (330–780 mm) and two unsexed specimens (361–363 mm). Length classes <400 mm were underrepresented. Size-related distribution did not change between sex according to Kolmogorov–Smirnov test ($D = 0.11$; $df = 1$; $P = 0.22$; Fig. 3a), but it was significantly affected by the fishing gear ($D = 0.79$; $df = 1$; $P < 0.01$; Fig. 3b), although samples were taken from the same population. When size distribution was compared, the chi-squared test pointed towards a significant difference between proportions among fisheries ($\chi^2 > 3.84$; $df = 1$; $P < 0.00$).

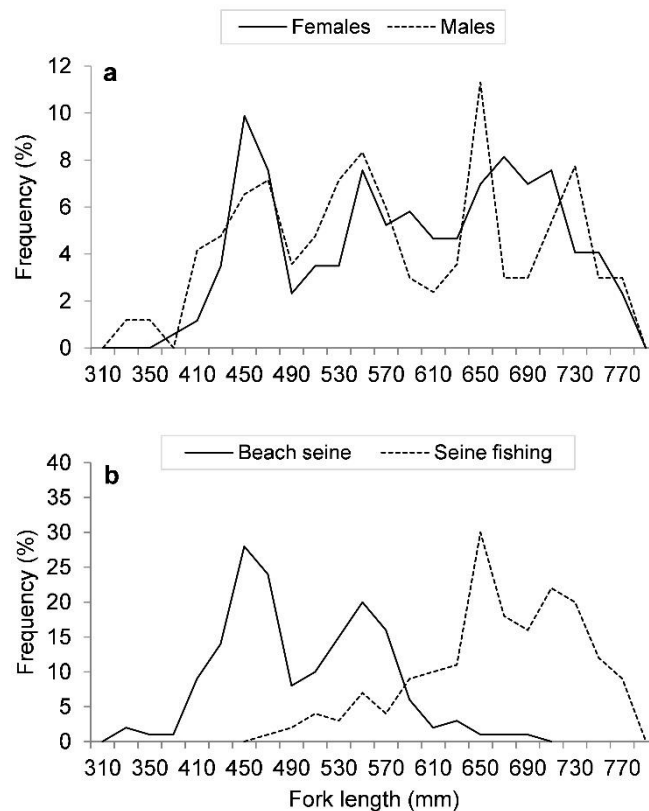


Figure 3. Length–frequency by a) sex and b) gear of *Euthynnus alletteratus* landed in the southeastern Brazilian coast between March 2018 and February 2019.

Parameters of the length–weight relationship did not point to significant difference between sexes (ANCOVA: $F = 0.40$; $df = 1$; $P = 0.52$) or between slopes' angular coefficients

($F = 0.363$; $P = 0.54$). The equations that describe LWR were $TW = 0.0003 \times FL^{2.8711}$ ($R^2 = 0.97$) for females and $TW = 0.0003 \times FL^{2.8737}$ ($R^2 = 0.98$) for males (Fig. 4). For all fishes grouped the equation was $TW = 0.0003 \times FL^{2.8760}$ ($R^2 = 0.98$).

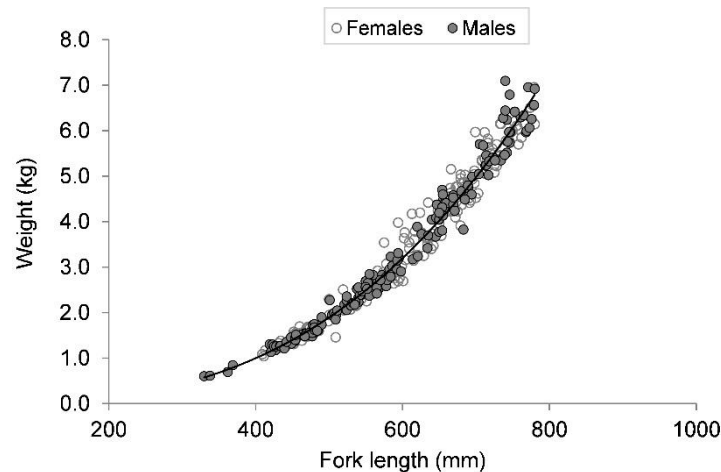


Figure 4. Length–weight relationship for females (○) and males (●) of *Euthynnus alletteratus* landed in the southeastern Brazilian coast between March 2018 and February 2019.

Histological analysis

Females and males of all maturity stages were observed. *Euthynnus alletteratus* ovaries were strongly characterized by oocytes exhibiting asynchronous and discontinuous secondary growth. Primary growth oocytes (PG) were exhibited by immature phase females (Fig. 5a). After reaching sexual maturity, ovaries entered the first phase of the reproductive cycle, the developing phase, with oocytes in the germinative stages of PG and CA (cortical alveolar) present (Fig. 5b). Ovaries in the capable spawning phase had numerous oocytes in the vitellogenic stage (Vit) as well as mature oocytes (MO) with pre-ovulatory hydration distortions, a common aspect after the dehydration process during histological processing (Figs. 5c–d). The "regressing" phase of females, which can be considered as a "partially spent" phase for this species, was characterized by the presence of post-ovulatory follicle complexes (POF) (Fig. 5e). Ovaries in the "regenerating" phase (Fig. 5f) exhibited fibrous nodules (FN) as well as numerous oocytes in PG. Fibrous connective tissue was also observed.

Despite observing males in all maturity stages, testicles' histology micrographs were obtained only for developing and spawning capable phases (Figs. 5g–h). "Developing" testicles exhibited several testicular ducts (TD) with sperm cysts (SCy) in different stages of development along the ducts' walls, and a low number of spermatozoa (Sz) present. In contrast,

Sz cells were observed greatly occupying the testicular lobules and ducts of testicles in the "spawning capable" phase.

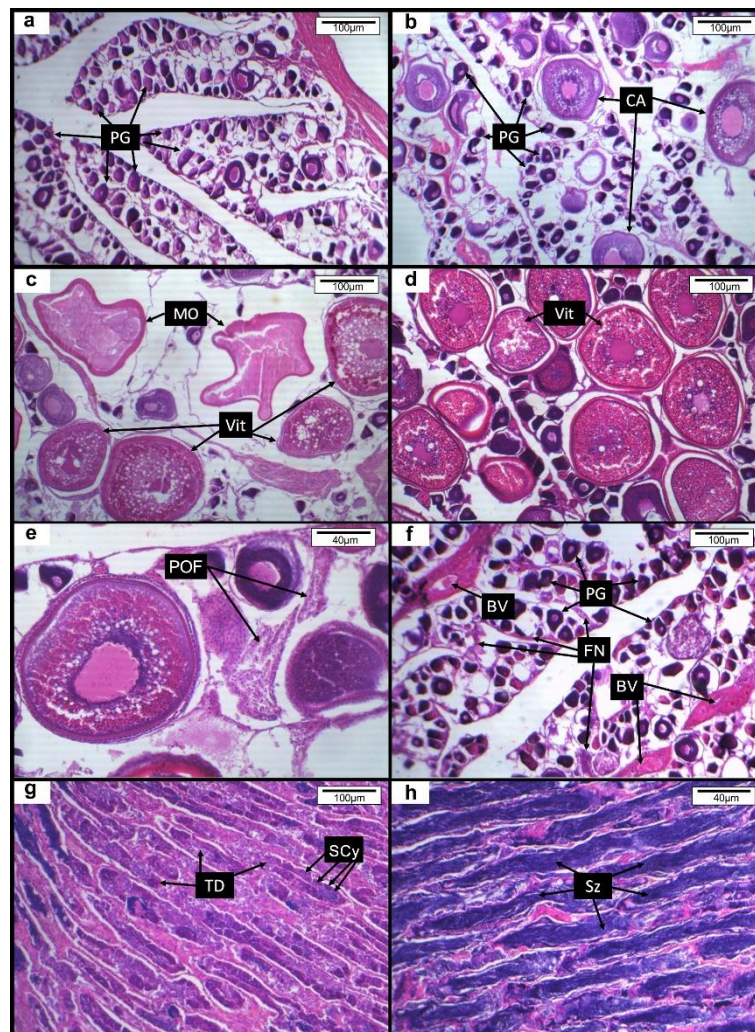


Figure 5. Micrographs from gonad section of females (a–f) and males (g–h) of *Euthynnus alletteratus* stained with hematoxylin–eosin. a) Immature ovary, b) ovary in developing phase, c) ovary in the capable spawning phase, d) ovary in spawning capable phase, e) ovary in the regressing phase, f) ovary in the regenerating phase, g) testicle in the developing phase, h) testicle in the capable spawning phase. PG: primary growth; CA: cortical alveolar; MO: mature oocyte; Vit: oocyte in vitellogenic stage; POF: post-ovulatory follicle complex; BV: blood vessel; TD: testicular duct; SCy: sperm cysts; Sz: spermatozoa.

Spawning period and maturity

The overall female–male proportion didn't change significantly from the equilibrium ($\chi^2 = 0.02$, $df = 1$; $P = 0.88$). When separated among size classes, however, most fishes by class were females ($\chi^2 < 3.84$; $df = 1$; $P < 0.05$), especially in sizes between 600 and 690 mm. Mean GSI values for females and males of *E. alletteratus* indicated maximum gonad weight

corresponding to 1.2 and 1.8% of the body mass, respectively. Monthly mean changes in GSI were significantly different for females ($H = 126.9$; $df = 11$; $P < 0.01$) and males ($H = 125.1$; $df = 11$; $P < 0.01$) (Fig. 6).

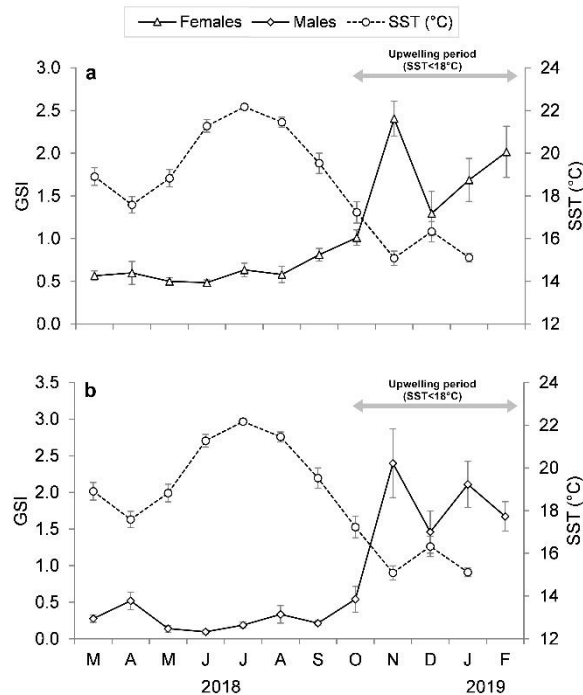


Figure 6. Monthly means of gonadosomatic index (GSI) of a) females and b) males of *Euthynnus alletteratus* in the southeastern Brazilian coast. Open circles (\circ) correspond to monthly sea surface temperature (SST, $^{\circ}\text{C}$). Vertical bars show the 95% confidence interval.

GSI peaked after a long period of temperature decrease, from 22°C in July to 15°C in November and was significantly higher during upwelling periods ($\text{SST} < 18^{\circ}\text{C}$) (Fig. 6), as were HI and K values (Figs. 7a–b). Monthly values of HI (females: $H = 111.2$; $df = 11$; $P < 0.01$; males: $H = 53.5$; $df = 11$; $P < 0.01$) and K (females: $H = 37.17$; $df = 11$; $P < 0.01$; males: $H = 23.69$; $df = 11$; $P < 0.01$) were also significantly different, with HI increasing from December, after spawning period, in a clearer relationship with gonad development when compared to condition factor.

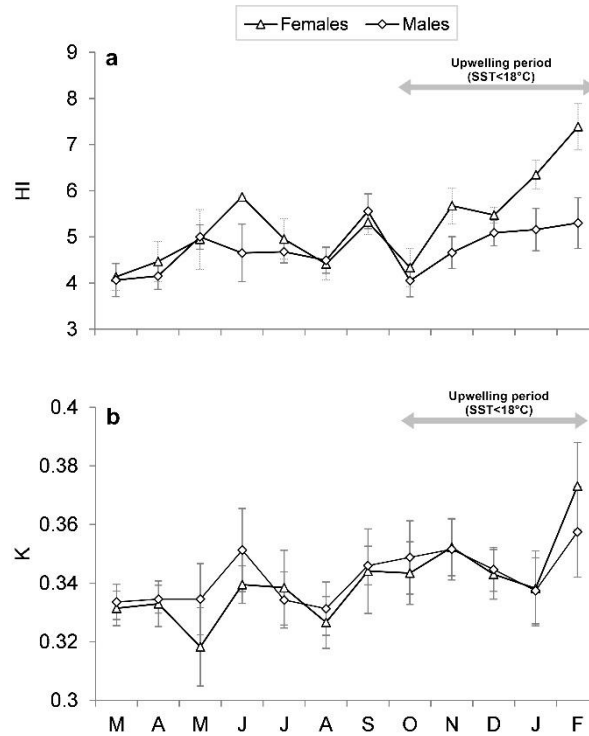


Figure 7. Monthly means of a) hepatosomatic index (HI), and b) condition factor (K) of females and males of *Euthynnus alletteratus* in the southeastern Brazilian coast. Vertical bars show the 95% confidence interval.

The monthly variation on sexual maturity stages (Figs. 8a–b) showed that most developing females were observed from May to October. Testicles in this phase occurred throughout the year. Spawning capable ovaries were most frequent in November and December, matching the period of higher GSI mean values. Partially spent ovaries and testicles increased from winter to the beginning of summer (June–October). Ovaries in the regenerating phase occurred almost year-round, except November when most fish were spawning. Regenerating testicles occurred in May–September and March, associated with warm, coastal waters (SST > 18°C). Immature males (<28%) occurred in low frequencies in June and October, in waters above 17°C.

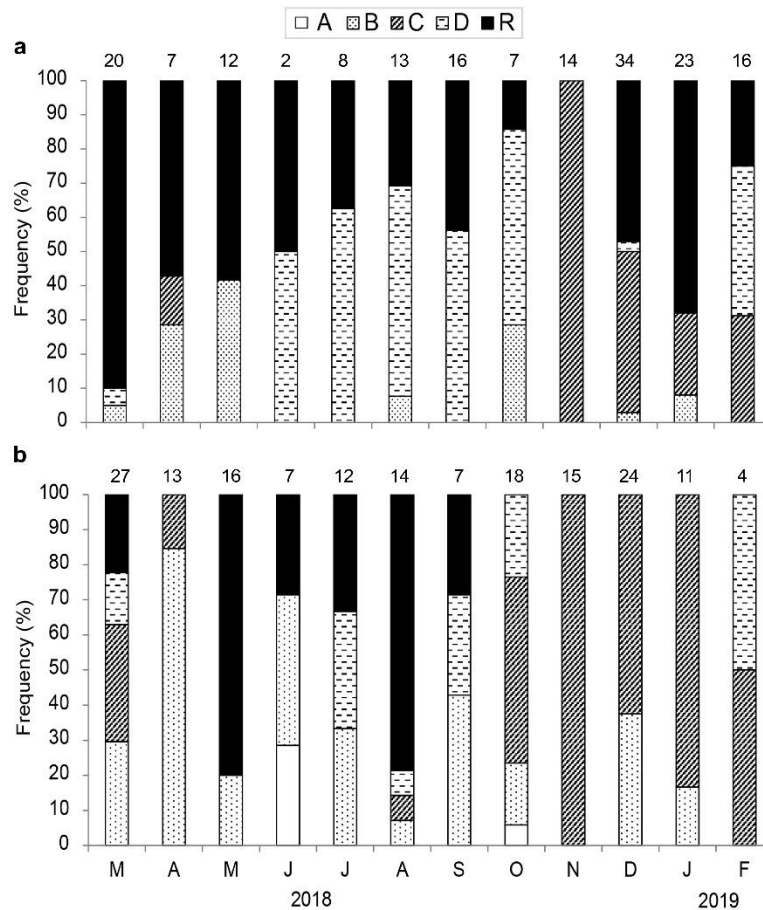


Figure 8. Monthly frequency distribution of maturity phases a) female and b) male of *Euthynnus alletteratus* in the southeastern Brazilian coast. A: immature; B: developing; C: spawning capable; D: regressing/spent; R: regenerating. The numbers above bars correspond to the number of samples per month.

Length at first sexual maturity (L_{50}) was attained by one-year-old fish of either sex and was 423.7 mm FL for females and 492.8 mm FL for males (Fig. 9). Out of 345 fishes, only 3.47% were below the estimated L_{50} . The results suggest that a small proportion (8%) of juveniles came from beach-seine catches.

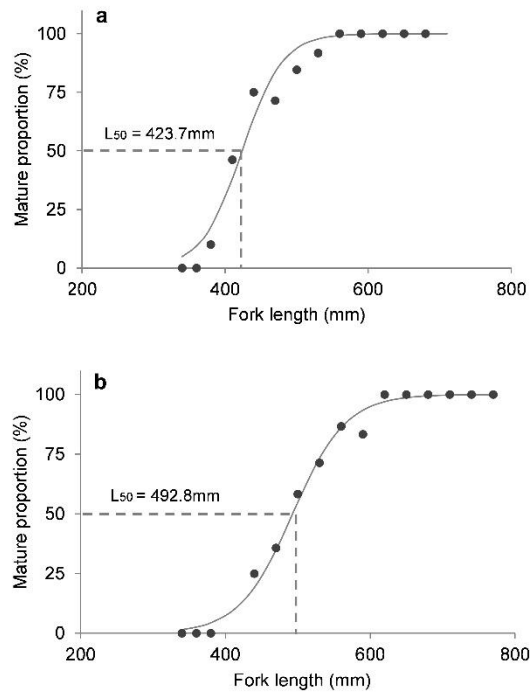


Figure 9. The proportion of mature a) females and b) males *Euthynnus alletteratus* landed in the southeastern Brazilian coast.

Validation

As Rt and FL relationship was similar in females and males (ANCOVA: $F = 0.297$; $df = 1$; $P = 0.58$), data were grouped in marginal increment analysis to reduce variability and increase sample size. A significant correlation between Rt and FL was obtained ($F = 1901.226$; $df = 1$; $P < 0.01$; $R^2 = 0.88$) (Fig. 10).

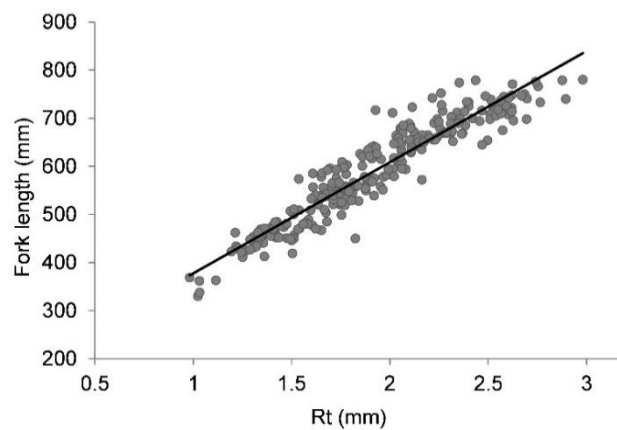


Figure 10. Relationship between spine radius (Rt) and fork length (FL) of *Euthynnus alletteratus* landed in the southeastern Brazilian coast.

Few 0+ fish were sampled, resulting in a high variance. Spine radius was significantly different for all ages (Fig. 11: $H=136,4$; $P < 0.00$), except 0+ and 1. Marginal increment variation was significantly associated to lower temperatures at age 2 (ANOVA: $F = 4.163$; $DF = 1$; $P = 0.04$). At age 3, however, water temperature had no effect in marginal increment (Kruskal–Wallis: $H = 0.001$; $df = 1$; $P = 0.9$).

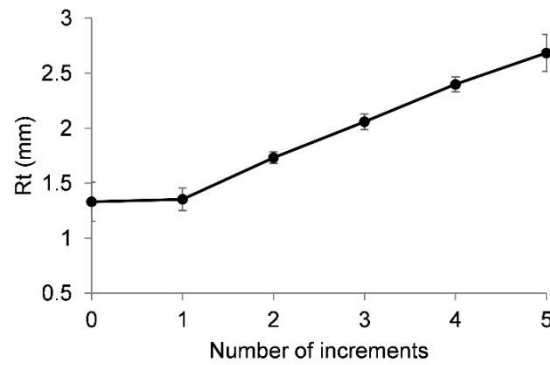


Figure 11. Spine radius (Rt) per number of increments identified.

The edge aspect indicated that, for both ages, one increment is formed during spring–summer (upwelling of SACW), when frequency of opaque edges was significantly higher than translucent ones ($\chi^2 = 5.64$; $df = 1$; $P = 0.018$) (Fig. 12b). During CW, translucent edges were statistically more frequent ($\chi^2 = 4.94$; $df = 1$; $P = 0.026$).

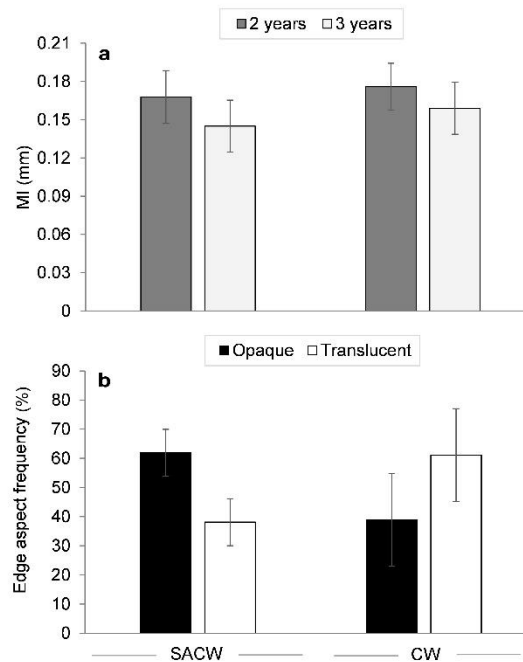


Figure 12. a) Marginal increment (MI) and b) mean frequency of edge aspect in sectioned spines of selected 2- and 3-year old *Euthynnus alletteratus* during upwelling (SACW) and non-upwelling (CW) period.

Readability, age composition, and growth

Readability of the 314 sectioned spines was classified mostly as high (53.1%) and low (44.2%); only 2.7% of spines were unreadable. The APE was 9.3%, and the CV was 9.2%. Assigned ages on spine sections ranged from 0+ to 5 years. Ages 2 (43.3%) and 3 (28.5%) predominated, and males were the most frequent in ages 1, 2, and 5 (60, 52, and 83%, respectively). When analyzed separately, 78% of males and 80% of females were 2–3 years old. Beach seine and purse seine's selectivity overlapped at 575 to 644 mm FL, corresponding to ages 2–3. Individuals between 425–574 mm FL and young one-year-old fish were captured only by beach seine. Larger (645–754 mm FL) and older (4–5 years) fish were restricted to purse seine catches. A wide range of lengths within the same age was observed, especially between ages 2 and 3. Growth parameters were not significantly affected by sex according to Kimura's likelihood test (Table 1). The von Bertalanffy growth coefficients of all fish combined with confidence intervals were: $L_{\infty} = 791.9 \pm 33.4$ mm, $k = 0.42 \pm 0.09$ and $t_0 = -0.97 \pm 0.33$ yr⁻¹ (Fig. 13).

Table 1. Kimura's likelihood test by sex of *Euthynnus alletteratus* from southeastern Brazil. M: male, F: female, df: degrees of freedom.

	Hypothesis	Chi-squared	df	P-value
H_0 vs. H_1	$L_{\infty(F)}$ vs. $L_{\infty(M)}$	1.94	1	0.16
H_0 vs. H_2	$K_{(F)}$ vs. $K_{(M)}$	2.30	1	0.12
H_0 vs. H_3	$t_{0(F)}$ vs. $t_{0(M)}$	0.79	1	0.37
H_0 vs. H_4	$L_{\infty(F)}$; $K_{(F)}$; $t_{0(F)}$ vs. $L_{\infty(M)}$; $K_{(M)}$; $t_{0(M)}$	1.04	3	0.11

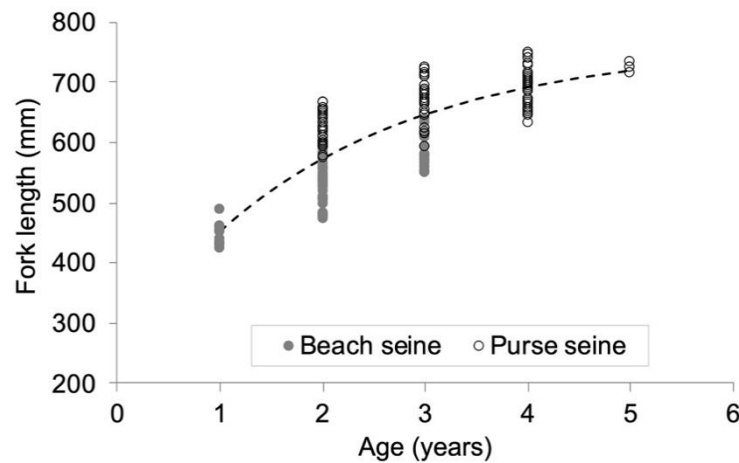


Figure 13. Observed lengths by fishing gear (symbols) and von Bertalanffy growth curve for combined sexes (all data) of *Euthynnus alletteratus* in the southeastern Brazilian coast.

DISCUSSION

The onset of *Euthynnus alletteratus* spawning in the Cabo Frio region was observed in late spring–summer, from November to February, associated with the upwelling of deep colder waters (<18°C) near the coast. The spawning period during summer is in accordance with previous studies in Mediterranean waters (Mohamed et al. 2014, Saber et al. 2018), Gulf of Mexico (Cruz–Castán et al. 2019), and the western coast of Africa (Diouf 1980, Gaykov & Bokhanov 2008). According to Matsuura & Sato (1981), little tunny larvae have been observed in offshore oceanic waters during late spring–summer (December–January), associated with warmer waters (25°C). In a similar condition, larvae of little tunny were recorded in temperatures from 24.1 to 25.4°C in the Gulf of Gabes (Koched et al. 2013). Present results show that water temperature tolerance of little tunny is not only lower than previously reported (Boyce et al. 2008), but also that spawning can happen in cold waters (<18°C), a first–time

documented aspect for the species. Spawning periods related to upwelling events introduce the larvae into a rich habitat (Bakun & Parrish 1990). For some smaller warm–water tunas, adult feeding and spawning grounds greatly overlap (Collette & Nauen 1983, Reglero et al. 2014), and the highest productivity in the Cabo Frio region during the summer favors zooplankton as well as clupeids, an important food group for adults of *E. alletteratus* (Valentin & Coutinho 1990, Menezes & Aragão 1977, Manooch III et al. 1985, Falautano et al. 2007). Spawning period of the skipjack tuna (Martins et al. 2020), sailfish *Istiophorus platypterus* (Mourato et al. 2018), silver porgy *Diplodus argenteus* (David et al. 2005), red porgy *Pagrus pagrus* (Costa et al. 2021), anchovy *Engraulis anchoita* (Bakun & Parrish 1991), the Argentine hake *Merluccius hubbsi* (Costa et al. 2018) and the Brazilian sardine (Matsuura 1971, Matsuura 1998) (indicar nombre científico de las especies en cursiva) are also associated with late spring and summer coastal upwelling in southeastern Brazil. Additionally, *Thunnus thynnus* in Mediterranean waters has also been reported to spawn in temperatures considerably lower than optimal, matching the offspring with ocean productivity and prey availability, in a trade–off between foraging and survival, when higher food abundance increases larval survival (Reglero et al. 2018).

E. alletteratus gonads were also strongly characterized by germinative cells in different developmental stages, resulting from multiple spawner species with an asynchronous oocyte development and indeterminate fecundity (Schaefer 2001, Kahraman et al. 2008). This aspect was observed as all developmental phases occurred simultaneously along the year. Fishes in the capable spawning phase were associated with upwelled waters (<18°C). Spent and regenerating gonads were mostly observed in warm waters, especially during the year's first half, coinciding with low GSI values. Mean GSI values for females and males of *E. alletteratus* were compatible with those recorded for the species in previous studies, where the gonad weight corresponded from 0.8 to 1.2% of the body mass (Kahraman et al. 2008, Hajjej et al. 2010, Mohamed et al. 2014, Cruz–Castán et al. 2019).

Estimates of length at first maturity for *E. alletteratus* in the literature greatly differ and have been given for separated and combined sexes (Table 2). Our findings (females: 423 mm; males: 492 mm) are close to estimates from Senegal (400 mm; Diouf 1980), Tunisia (females: 448 mm; males: 428 mm; Hajjej et al. 2010), Egypt (~430 mm; Mohamed et al. 2014) and Spain (females: 500 mm; males: 434 mm; Saber et al. 2018). Sample classes were similar between these previous studies and ours, although some specimens of 97, 100, and 101 cm were sampled by Hajjej et al. (2010), Mohamed et al. (2014), and Saber et al. (2018), respectively. Fishing gears were similar to those used in our sampling, consisting of gill nets,

drift nets, and beach seine. The lowest mean L_{50} for the species (346–343 mm, F–M; Cruz–Castán et al. 2019) was recently recorded in the Gulf of Mexico due to smaller fishes (360–400 mm FL) caught by gill nets. The authors suggested that little tunny reproduces in smaller lengths in the Gulf of Mexico due to tropical fishes being smaller than those of greater latitudes and places of higher productivity

Table 2. Size–range of fork length (FL, mm), length at maturity (L_{50}), aging method, growth parameters (L_{∞} , k, t_0), maximum age (T_{max}), sea surface temperature (SST, °C) during spawning, and area reported for *Euthynnus alletteratus*. M: male, F: female. V: vertebrae, S: spine, O: otolith. *Sex combined, GOM: Gulf of Mexico.

Reference	FL	L_{50} (M–F)	Aging method	L_{∞}	k	t_0	T_{max}	Area
Rodriguez–Roda (1979)	400–900	570*	V	1.150	0.19	–1.71	5	Spain
Diouf (1980)	200–900	400*	S	995	0.30	–	–	Senegal
Cayré & Diouf (1983)	264–860	–	S	1.120	0.12	–	8	Senegal
Johnson (1983)	315–741	–	S/V					GOM
Kahraman & Oray (2001)	550–850	–	S	1.270	0.10	–4.10	6	Turkey
Kahraman & Oray (2001)	520–970	–	S	1.230	0.12	–3.80	9	Turkey
Valeiras et al. (2008)	480–840	–	S	915	0.39	–0.40	5	Spain
Hajje et al. (2010)	367–978	448–428	–	–	–	–	–	Tunisia
Mohamed et al. (2014)	320–100	420*	–	–	–	–	–	Egypt
Adams & Kerstetter (2014)	250–832	–	O	779	0.60	–0.69	5	USA
Saber et al. (2018)	306–101	434–500	–	–	–	–	–	Spain
Cruz–Castán et al. (2019)	282–807	343–346	–	–	–	–	–	GOM
This study	330–780	492–423	S	791	0.42	–0.97	5	Brazil

This study is the first attempt to interpret increments in sectioned fin spines as a satisfactory method for age assessment of *E. alletteratus* in the southwestern Atlantic. The age composition was the same as the stocks from Mediterranean waters near Spain (Valeiras et al. 2008) and East Atlantic, near the Strait of Gibraltar (Rodriguez–Roda 1979). Ages were also close to those estimated by Kahraman & Oray (2001) in the Aegean Sea (six years), Cayré & Diouf (1983) in the Senegalese coast (seven years), and by Kahraman & Oray (2001) in Mediterranean waters (seven years).

As the little tunny otoliths are very small and difficult to process, we found thin transversal sections of dorsal fin spines to be the easiest technique to apply with satisfactory results, as only 2.7% of the spines were classified as unreadable. The difficulties in reading fin spines included the reabsorption of the first two annuli in older fishes, making it necessary to estimate

the radius of those lacking these growth marks and the existence of multiple thinner bands, which constituted an opaque or translucent band. These problems were reflected in the relatively high CV (9.18%) and APE (9.32%) results, although APE estimates were lower than those by Cayré & Diouf (1983) for the coast of Senegal (10.5%).

Even though it is the most commonly used age validation method, validation by marginal increment analysis is still difficult to interpret and is not an absolute validation method (Campana 2001). According to Cayré & Diouf (1983), the formation of growth marks in tunid spines is probably related to several factors, including migration, spawning, and environmental conditions such as temperature, that work both in combination and separately, affecting physiology, reproduction, and growth. Lower mean marginal increments and highest frequencies of opaque edges in spines for fishes of 2 and 3 years occurred associated with cold upwelled waters (<18°C). Adams & Kerstetter (2014) observed the formation of two translucent increments annually, during summer and winter, in otoliths of little tunny captured in Florida. The authors suggest that one of the increments' formations could be related to changes in fish metabolism for gonad development as little tunny is a tropical and subtropical species and would not face drastic environmental changes. However, in the Cabo Frio system, the water temperature decreases up to 10°C during the upwelling season, affecting fish metabolism, growth, and reproduction. In Brazilian waters, the red porgy (Costa et al. 2021) has been reported to form an *annulus* during summer, from November to February, also associated with SACW upwellings and the reproductive tract activity. The Argentine hake is also known to form a growing mark during spring–summer. However, the formation of increments in juveniles and adults at the same time suggested that the regulating mechanism is not related exclusively to somatic growth or to reproductive activity (Costa et al. 2018).

Previous and present estimates confirm that little tunny is a fish of slower growth when compared to other species of the genus (Juan–Jordá et al. 2013), and capable of attaining larger sizes, with several reports of specimens around 1 m FL and growth coefficient values between 0.1–0.39 yr⁻¹ (Rodríguez–Roda 1979, Diouf 1980, Cayré & Diouf 1983, Kahraman & Oray 2001, Valeiras et al. 2008, El–Hawet et al. 2013). The faster growth (0.6 yr⁻¹) and smaller maximum sizes (779 mm) reported for the species off the coast of Florida appeared to reflect the size composition of the samples, as most fishes were around 700 mm FL (Adams & Kerstetter 2014). The addition of any amount of data from smaller individuals is recommended to reduce the bias in VBGF parameter estimates, and the only situation when combining samples from two or more gears to achieve this goal may not be preferable to single–gear approaches is when both gears miss smaller individuals (Wilson et al. 2015).

In agreement with the hypothesis that fishes in tropical, subtropical, and high productivity areas (e.g. upwelling systems) grow faster and attain smaller sizes when compared to temperate areas counterparts (Pörtner et al. 2005, Watt et al. 2010), our southeastern Brazil results suggest that little tunny has higher growth rates (0.42 yr^{-1}) when compared to stocks in other areas of the Atlantic, as well as the Mediterranean. Furthermore, attain smaller asymptotic lengths (791.9 mm FL) (Table 2), a condition also suggested by Cruz–Castán et al. (2019) for little tunny in the Gulf of Mexico.

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CAPÍTULO 2

Age, growth and maturity of frigate tuna (*Auxis thazard* Lacepède, 1800) in the Southeastern Brazilian coast

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Age, growth and maturity of frigate tuna (*Auxis thazard* Lacepède, 1800) in the Southeastern Brazilian coast

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Abstract

Age, growth and maturity of frigate tuna (*Auxis thazard*) were studied for the first time in Southeast Brazil. A total of 650 fish (265–494 mm Fork Length) were sampled from beach–seine landings between March 2018 and February 2019. Sectioned fin spines from 548 specimens were processed and age was determined by counting and measuring of increments. Growth parameters were fitted to length–at–age data using the von Bertalanffy growth function and were compared to the literature using the growth performance index (Phi). Macro and microscopic characterization of gonads and physiological indexes were used for the analysis of the reproductive cycle. The length distribution did not differ by sex. Assigned ages were between 0+ and 4 years, with annual increments formed during winter. Coefficient of variation between readings was 11%. Growth differed by sex, and the von Bertalanffy growth parameters were $L_{\infty}= 471$ mm; $k= 0.47$ year⁻¹; and $t_0= -1.46$ year⁻¹ for females; $L_{\infty}= 498$ mm; $k= 0.35$ year⁻¹; and $t_0= -2.01$ year⁻¹ for males. Phi was 5.0 for both sexes. Estimated growth rate is among the lowest recorded for the species, possibly related to the method used for age and growth assessment. Gonadosomatic index peaked in December, associated with the upwelling of the South Atlantic Central Waters, at a temperature of approximately 16°C. Spawning activity in colder waters has never been reported for this species and may be influenced by a richer habitat caused by the intrusion of more nutritive rich waters during spring–summer, which allows larval development. Length–at–first–maturity (L_{50}) was 345.4 mm for females and 329.8 mm for males.

Key words: *Auxis thazard* / Age / Growth / Reproduction / Southeast Brazil / Upwelling

1. Introduction

Scombrids sustain some of the most important fisheries in the world due to their high catches, usually high economic value and extensive international trade (Juan–Jordá et al. 2013). They have a wide distribution that includes tropical, subtropical, and temperate oceans (Collette and Nauen 1983). Among the group, there are the small–bodied tunas, which includes species of the genus *Scomberomorus*, as well as the wahoo (*Acanthocybium solandri*), the plain bonito (*Orcynopsis unicolor*), the blackfin tuna (*Thunnus atlanticus*), the skipjack tuna (*Katsuwonus pelamis*), the Atlantic bonito (*Sarda sarda*), the three species of little tunas (*Euthynnus affinis*; *E. alletteratus* and *E. lineatus*) and the frigate and bullet tunas (*Auxis thazard*; *A. rochei*) (Lucena–Frédou et al. 2021). Fish from this group are important socio–economic resources for industrial fleets as well as for artisanal fisheries, despite the lower economic value when compared to larger tunas (Arrizabalaga et al. 2011; Pons et al. 2017).

The frigate tuna (*Auxis thazard*, Lacepède, 1800) is a small, epipelagic, cosmopolitan scombrid found in tropical and subtropical oceans, around islands and occupying continental shelves (Collette and Nauen 1983). It reaches sizes of approximately 500 mm and a maximum age of 4 years in India (Abdussamad et al. 2005; Lelono and Bintoro 2019). In Iran, Indonesia and Taiwan ages of 5 years have been estimated, however, *Auxis thazard* attains lower maximum sizes (Tao et al. 2012; Darvishi et al. 2020). Length–at–first maturity records are variable throughout its distribution (269 to 349 mm) and spawning season is long, with indeterminate fecundity usually associated to warm months (Schaefer 2001; Ghosh et al. 2012; Tao et al. 2012; Jude et al. 2002; Bahou et al. 2016; Tampubolon et al. 2016; Calicdan–Villarao et al. 2017; Herath et al. 2019; Zapadaeva 2021). In the Atlantic Ocean, age and growth assessments are limited to the Northeast and Equatorial areas (Grudtsev and Korolevich 1986; Zapadaeva 2021), whereas there are no reproduction studies. According to Lucena–Frédou et al. (2021), the great morphologic similarities between *A. thazard* and *A. rochei* may create difficulties in obtaining parameter estimates for the species separately.

The frigate tuna in the Atlantic is among the most captured small scombrid alongside the Spanish mackerel *Scomberomorus maculatus*, the Atlantic bonito *Sarda sarda* and the little tunny *Euthynnus alletteratus* (ICCAT 2021). *A. thazard* is often caught as a by–catch (non–targeted species) by industrial purse seines and associated with FADs (fish aggregating devices) (Amandè et al. 2010; Lucena–Frédou et al. 2021). According to the Report of the 2021

Standing Committee on Research and Statistics (ICCAT 2021), frigate tuna corresponded to 13% (12.723 ton) of all reports for small tunas in the Atlantic Ocean and Mediterranean waters for 2020. In Brazil, it is frequently caught by purse seine and pole-and-line together with the skipjack and other tunas (*Thunnus sp.*), although the latter two account for most of the landings (da Silveira-Menezes et al. 2010). In 2018, frigate tuna also represented 3.1% (36.1 ton) of the monitored rod and live-bait production (Martins et al. 2020).

Located in Southwest Atlantic (SWA), the Cabo Frio upwelling system is the most intense of the upwelled areas in the Brazilian coast (Kampel et al. 1997). The upwelling process occurs throughout austral spring–summer, when E–NE wind prevails and the topography promotes an upwelling of the cold (6–18°C), less saline (34.5–36) and nutrient rich South Atlantic Central Water (SACW) from a depth of 300 m (Gonzalez–Rodriguez et al. 1992). As a result, a rich pelagic fauna that includes highly economic important species, such as the Brazilian sardine *Sardinella brasiliensis* and the skipjack tuna, is frequently observed in the area (Matsuura and Sato 1981; Valentin 2001).

Life history information such as age, growth and maturity are fundamental indicators of population dynamics and essential for the sustainable management of species (King 2008). Many approaches have been developed in order to support the correct management of species lacking long-term fisheries, with some based on basic life history information (Lucena–Frédou et al. 2017; Pons et al. 2019). However, except for the skipjack tuna, knowledge on the biology of the small tunas remains incomplete or missing for most species of SWA, even if they are expected to suffer from higher fishing pressure as several valued tuna stocks are overfished (Juan–Jordá et al. 2013; Pons et al. 2017).

Although economically important, catches of frigate tuna off the Southeastern (SE) Brazilian coast are not subjected to fishing control or management policies, as the life parameter data for this species in SWA is not available (Lucena–Frédou et al. 2017). Therefore, our objectives are to investigate age and growth through assessment of increments on transversal sections of fin spines, and describe the maturity using physiological indexes and histological records of gonads. Additionally, we evaluate the relationship between sea surface temperature and the growth and reproductive activity of the species.

2. Material and methods

2.1 Sample processing

Biological samples were collected monthly from the artisanal beach seine fishery in Arraial do Cabo (Rio de Janeiro State, SE Brazil) between March 2018 and February 2019. Fresh fish were kept on ice before fork length (FL, mm), total weight (TW, g), gonad weight (GW, g), and sex were registered. Length–frequency distribution between sexes was compared by Kolmogorov–Smirnov test (D) and an analysis of covariance (ANCOVA) was performed to compare the parameters of the length–weight relationship (LWR) of both sexes. A t test was performed to test the b values estimated against isometry ($b=3$), using the FSA package in R (Ogle, 2017).

Sea surface temperature (SST, C°) from March 2018 to January 2019 was also recorded by a Data logger (HOBO Tidbit UTBI-001) fixed on the shore at 1–m depth, where records were obtained every two/four hours (Fig. 1). This information was used to determine seasonal patterns of spawning activity and the periodicity of increment formation on the spines.

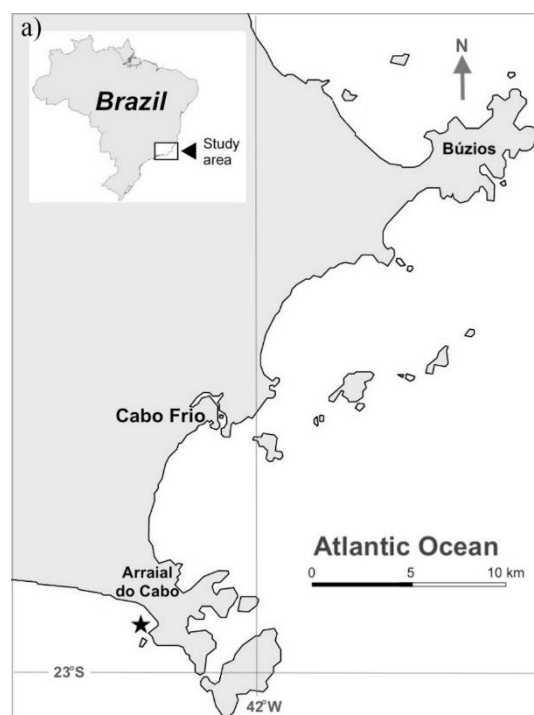


Figure 1. Map of the Cabo Frio region, Southeastern Brazil. (★) corresponds to sea surface temperature measurements.

2.2 Age and growth

The first spine of the first dorsal fin was extracted and used for age assessment. Spines were cleaned and embedded in polyester resin before two thin transversal sections (0.5–0.65 mm) near the condyle were removed using a Buehler–Isomet metallographic saw. The photographic registry of the sections was performed using a stereomicroscope (Zeiss STEMI 508), with transmitted light at 40x to 100x magnification. A translucent band (associated with periods of low growth rates) and an opaque band (associated with periods of high growth rates) were considered to be an increment and the radius of each spine (R_t), as well as the radius of each increment (R_i), was measured to the nearest 0.001 mm (Fig. 2). Opaque bands were counted and each section was read two times, four months apart, by the same reader with no prior knowledge on size, sex, or capture date. When the first two readings differed, a third was performed and in cases of further disagreement the specimen was discarded from subsequent analysis. Reading accuracy was determined by the Coefficient of Variation (CV, Chang 1982) and sections were classified as “Legible”, “Low legibility” and “Illegible”.

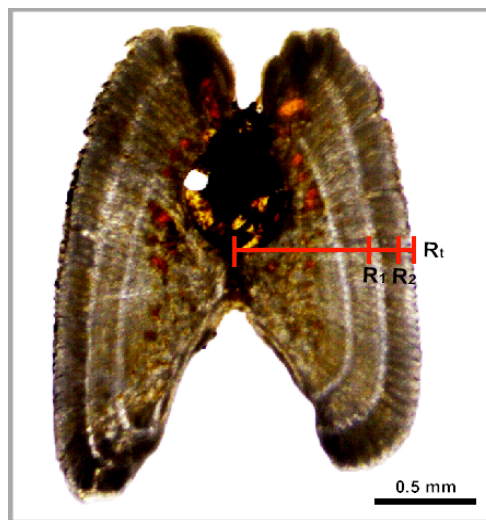


Figure 2. Dorsal fin spine’s transversal cut showing the spine radius (R_t) and identified increment (R_1 ; R_2) measurement position of a 2+ years old female specimen (422 mm FL).

Age frequencies between females and males were compared using a Chi-square test (χ^2) and the periodicity of increment formation was investigated using marginal increment analysis (MI), calculated as $MI = (R_t - R_n) / (R_n - R_{n-1})$ where R_t is the spine radius and R_n and R_{n-1} are the radius of the outermost and penultimate increments, respectively. Monthly MI data for fish of 1 and 2 years, the most frequent, was tested for normality using a Shapiro–Wilk test and for homoscedasticity using a Levene’s test. Thereafter, when restrictions were met, data

was compared using an ANOVA followed by a Tukey's pairwise *post-hoc*. When the restrictions were not met, a non-parametric Kruskal–Wallis analysis (H) was applied before a Mann–Whitney (U) *post hoc* (Zar 2010).

Growth parameters were adjusted for observed lengths on the terms of the von Bertalanffy growth function (VGBF): $FL = L_{\infty} \times [1 - e^{-k(t-t_0)}]$, where L_{∞} is the asymptotical length, k is the growth rate constant i.e., the rate of approaching L_{∞} and t_0 is the age when fish length is theoretically zero (von Bertalanffy 1938). The likelihood ratio test was used to estimate the growth parameters (L_{∞} , K , t_0) for fish from 1 to 4 years (Aubone and Wöhler 2000). Fish of 0+ years were not included in the estimation as the data for added too much noise to the estimates. Growth curve estimates of females and males were compared using Kimura's likelihood test (Kimura 1980; Haddon 2011) and the growth performance index (Phi) was calculated as $\phi' = \log(k) + 2 * \log(L_{\infty})$, and used to compare the obtained growth parameters with those from literature.

2.3 Gonad maturity, spawning period and length-at-first-maturity (L_{50})

Gonad maturation phases were assigned for each fish as described by Brown–Peterson et al. (2011): “Immature”, “Developing”, “Spawning capable”, “Resting”, and “Regenerating”. All fish included in one of the latter four stages were considered as adult. After visual classification, gonad sub-samples were fixed in a solution composed of alcohol (76.5%), distilled water (8.5%), formaldehyde (10%) and glacial acetic acid (5%) for 24h and preserved in 70% alcohol. Afterwards, each sample was dehydrated in different series of alcohol concentration and included in paraffin before transverse sections of tissue (5 μ m–thick) were sectioned with a microtome, mounted on glass microscope slides and stained with hematoxylin–eosin. Microscopic analysis was used to validate the macroscopic assignment of sexes.

The spawning period was assessed using the gonadosomatic index (GSI), calculated as $GSI = (GW / (TW - GW)) \times 100$, and the percentage of maturity stage by month. Monthly data was tested using Shapiro–Wilk and Levene's tests to verify the normality and homogeneity of the variance of the GSI data. Subsequently, an ANOVA followed by a Tukey's pairwise *post hoc* was applied, if restrictions were met. When restrictions were not met, a Kruskal–Wallis (H) non-parametric test followed by a Mann–Whitney's (U) *post hoc* was applied (Zar 2010).

Sex ratio was calculated by month and by size class, and observed results were compared to expected results using a Chi-square test (χ^2).

Length-at-first-maturity (L_{50}) was estimated using the logistic regression in the SizeMat package in R (Torrejon-Magallanes, 2020).

3. Results

3.1 Size distribution and Length-weight relationship (LWR)

A total of 650 frigate tuna were sampled, including 321 females, 300 males, and 29 specimens without assigned sex. Females ranged between 269 mm and 494 mm (Mean: 338.2 mm) and males ranged between 265 mm and 452 mm (Mean: 340.5 mm). Length distribution was not different by sex (Kolmogorov-Smirnov: D: 0.074; P=0.34) (Fig. 3a). Fish between 305 and 355 mm comprised 71% of the samples.

Comparison of LWR by sex did not indicate significant differences between parameters (ANCOVA: F=0.733; DF=1; P=0.39) or regression's slopes (ANCOVA: F=2.459; P=0.11) (Fig. 3b). The equations that described LWR were $TW=0.00007 \times FL^{3.14}$ for females ($R^2=0.96$), $TW=0.00004 \times FL^{3.22}$ for males ($R^2=0.97$) and $TW=0.00005 \times FL^{3.19}$ for all fish ($R^2=0.96$).

When tested against isometry, both sexes exhibited a positive allometric increase in weight by size ($P<0.00$), with b between 3.06 and 3.24 for females and 3.17 and 3.32 for males, with 95% confidence.

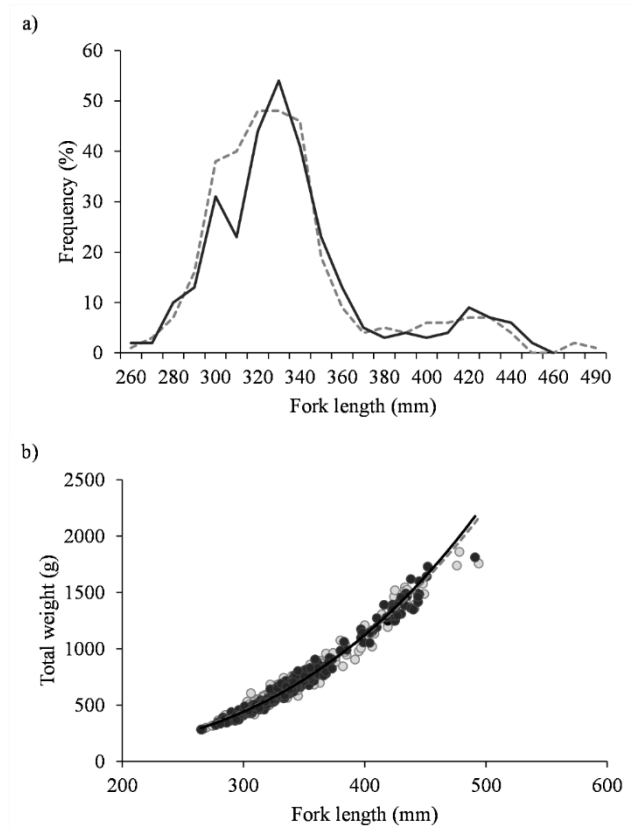


Figure 3. a) Fork length frequency for females (—) and males (---) and b) Length–Weight relationship and tendency lines for females (○; —) and males (●; ---) of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019.

3.2 Age determination and validation

A total of 548 spines were sectioned, with 495 (90.32%) successfully aged. From all (n=548) sections analyzed, 327 (59.65%) were classified as legible, 168 (30.67%) with low legibility, and 53 (9.68%) as illegible. The coefficient of variation between readings was 11%.

Increments were formed during winter, when Coastal Waters (CW) of SST>18°C were registered. MI values were significantly different for 1 y.o. (ANOVA: F= 2.685; DF= 9; P < 0.005) and for 2 y.o. fish (H= 19.7; DF= 11; P= 0.03) (Fig. 4).

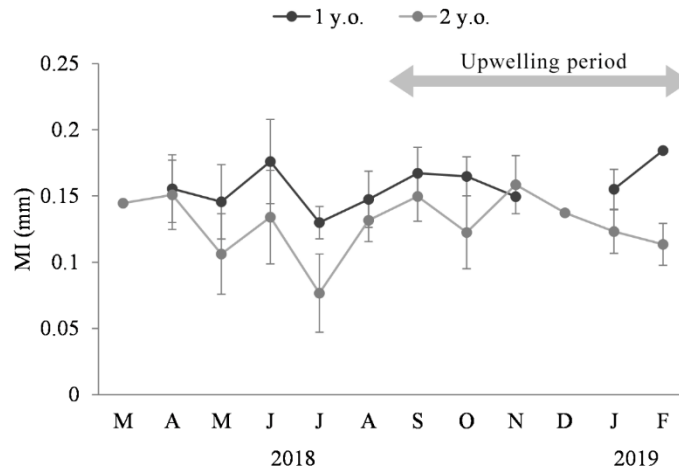


Figure 4. Monthly means of marginal increment (MI) for 1-year (●) and 2 years-old (●) *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. Vertical bars denote 95% confidence interval.

The relationship between Rt and FL did not point towards differences in spine growth between sex (ANCOVA: $F=2.133$; $DF=1$; $P=0.144$) so, as a mean to increase information and reduce variance, female and male data was grouped for the regression, which was significant ($R^2=0.84$; $F=2413.4$; $DF=1$; $P<0.00$) (Fig. 5).

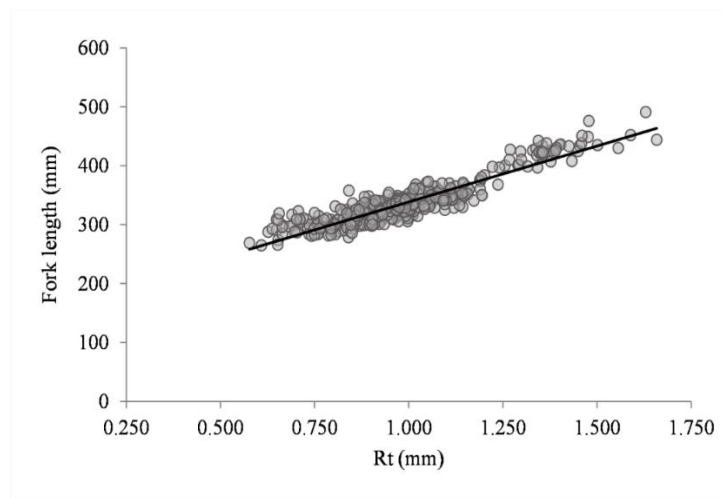


Figure 5. Relationship between fork length and spine radius (Rt) of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019.

For females and males assigned age ranged from 0+ to 4 years (Fig. 6). The most frequent age class was 1 year for both sexes, which constituted of 54% of all aged fish. When analyzed separately, the frequency differed by sex ($\chi^2=0.02$; $DF=1$; $P=0.8$).

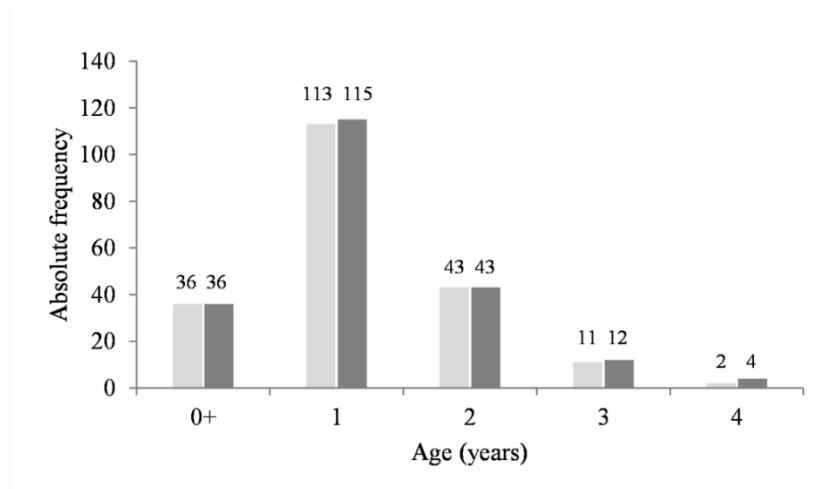


Figure 6. Age composition of females (■) and males (■) of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. Numbers above bars correspond to number of aged fishes.

3.3 Growth curves

Growth parameters estimated using the standard von Bertalanffy growth function (VBGF) were $L_{\infty} = 471$ mm; $k = 0.47$ year⁻¹; and $t_0 = -1.46$ year for females, $L_{\infty} = 498$ mm; $k = 0.35$ year⁻¹; and $t_0 = -2.01$ year for males (Fig. 7).

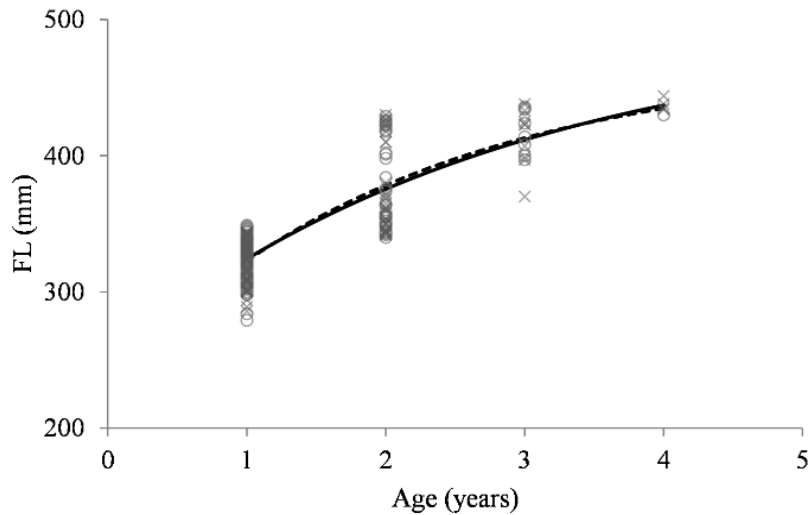


Figure 7. von Bertalanffy growth curves for females (—) and males (---) of *Auxis thazard* landed in Southeastern Brazil between March 2018 and February 2019. Symbols represent observed lengths for females (○) and males (×).

The Kimura likelihood test did not point towards differences between coincident curves ($\chi^2 = 4.63$; DF=3; P = 0.20). However, when parameters were analyzed separately, L_∞ ($\chi^2 = 4.48$; DF=1; P=0.03) and k ($\chi^2=4.25$; DF=1; P = 0.04) were different by sex, whereas t_0 was not ($\chi^2 = 3.75$; DF=1; P = 0.05) (Table 1). Females and males estimated growth performance index (ϕ') was 5.

Table 1. Kimura's likelihood test for *Auxis thazard* landed in Southeastern Brazil between March 2018 and February 2019. M–Male; F–Female.

	Hypothesis	χ^2	df	p-value
H ₀ vs H ₁	$L_{\infty(F)}$ vs $L_{\infty(M)}$	4.48	1	0.03
H ₀ vs H ₂	$K_{(F)}$ vs $K_{(M)}$	4.25	1	0.04
H ₀ vs H ₃	$t_{0(F)}$ vs $t_{0(M)}$	3.75	1	0.05
H ₀ vs H ₄	$L_{\infty(F)}$; $K_{(F)}$; $t_{0(F)}$ vs $L_{\infty(M)}$; $K_{(M)}$; $t_{0(M)}$	4.63	3	0.20

3.3 Gonad characterization

Immature ovaries were strongly characterized by the presence of oocytes in primary development (PG) (Fig. 8a). Ovaries at the beginning of the developing phase showed numerous PG oocytes, but also oocytes in the CA (Cortical–Alveolar) phase (Fig. 8b) and during final development CA oocytes were present in greater numbers, alongside oocytes in the Vitellogenic phase (Vit) (Fig. 8c). “Spawning capable” ovaries had mature oocytes (MO) in bigger sizes and a displacement of the germinal vesicle to the animal pole (Fig. 8d), and when fish had partially spawned, ovaries exhibited postovulatory follicle complexes (POF) (Fig. 8e). Regenerating ovaries had fibrous nodules (NF) among numerous oocytes in PG and few CA oocytes (Fig. 8f).

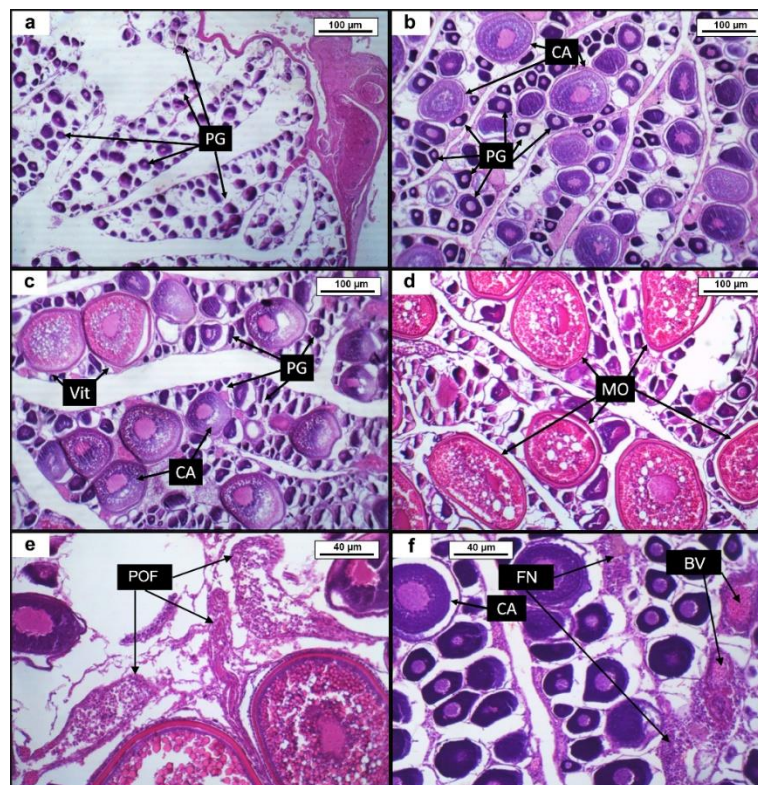


Figure 8. Micrographs of the gonad section stained with hematoxylin–eosin from female *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019 (a–f). a) Immature, b) developing, c) spawning capable, d) spawning capable, e) regressing/spent, f) regenerating. PG: primary growth; CA: cortical alveolar; Vit: oocyte in vitellogenic stage; MO: mature oocyte; POF: post–ovulatory follicle complex; BV: blood vessel; FN: Fibrous nodule

Immature testicles exhibited spermatid cysts (SCy) on the walls of the testicular lobules (Fig. 9a). Developing testicles, however, had numerous testicular ducts (TD) with spermatid

cysts in different development stages alongside its wall (Fig. 9b). Mature testicles showed spermatozoid cells occupying the testicular lobules and testicular ducts (Fig. 9c), and regenerating testicles displayed empty testicular ducts (Fig. 9d). Spermatid cysts were observed to occur only in the periphery of the testicles, restarting the spermatozoid production.

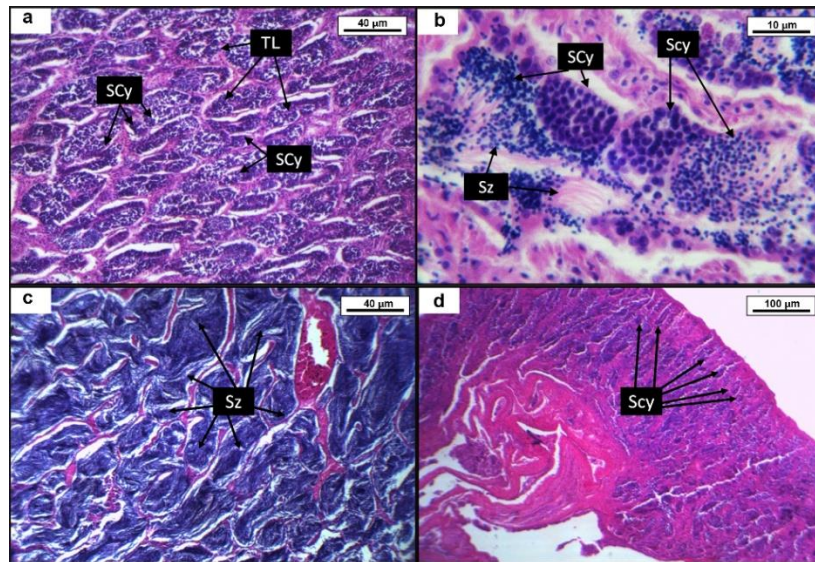


Figure 9. Micrographs of the gonad section stained with hematoxylin–eosin from male *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. a) Immature; b) development phase; c) spawning capable; d) regeneration. TD: testicular duct; SCy: sperm cysts; Sz: spermatozoa.

3.4 Sex ratio, reproductive cycle and length–at–first–maturity

The overall sex ratio (321:300) for *A. thazard* landed in Cabo Frio was 1:1 (Female:Male). The chi–square test did not indicate differences in sex ratio by month ($\chi^2=0.10$; $P=0.75$) (Table 2), although there were a few significantly different months. Sex ratio between size classes also did not differ ($\chi^2=0.11$; $P=0.74$), despite females dominating numerous classes (Table 3).

Table 2. Monthly variations in sex ratio of *Auxis thazard* landed in Southeastern Brazil between March 2018 and February 2019. χ^2 =chi-squared test.

	Number		Frequency (%)		χ^2	p-value	F:M
	F	M	F	M			
Mar	53	49	51.5	48.5	0.09	0.76	1.1:1
Apr	40	44	47.6	52.4	0.23	0.63	0.9:1
May	4	5	44.4	55.6	1.23	0.27	0.8:1
Jun	36	36	50.0	50.0	0.00	1.00	1:1
Jul	20	18	52.6	47.4	0.28	0.60	1.1:1
Ago	10	10	50.0	50.0	0.00	1.00	1:1
Sep	10	18	35.7	64.3	8.16	0.00	0.6:1
Oct	74	49	60.2	39.8	4.13	0.04	1.5:1
Nov	16	17	48.5	51.5	0.09	0.76	0.9:1
Dec	25	25	50.0	50.0	0.00	1.00	1:1
Jan	29	23	55.8	44.2	1.33	0.25	1.3:1
Feb	4	6	40.0	60.0	4.00	0.05	0.7:1
Mean	321	300	51.6	48.4	0.11	0.74	1.1

Table 3. Variations by Fork length class (FL, mm) for sex ratio of *Auxis thazard* landed in Southeastern Brazil between March 2018 and February 2019. χ^2 =chi-squared test.

FL (mm)	Number		Frequency (%)		χ^2	p-value	F:M
	F	M	F	M			
265	2	2	50.0	50.0	0.00	1.00	1:1
275	3	8	27.3	72.7	20.66	0.00	0.4:1
285	12	10	54.5	45.5	0.83	0.36	1.2:1
295	23	18	56.1	43.9	1.49	0.22	1.3:1
305	48	29	62.3	37.7	6.09	0.01	1.7:1
315	38	29	56.7	43.3	1.80	0.18	1.3:1
325	50	60	45.5	54.5	0.83	0.36	0.8:1
335	54	49	52.4	47.6	0.24	0.63	1.1:1
345	28	30	48.3	51.7	0.12	0.73	0.9:1
355	14	16	46.7	53.3	0.44	0.50	0.9:1
365	5	10	33.3	66.7	11.11	0.00	0.5:1
375	6	2	75.0	25.0	25.00	0.00	3.0:1
385	2	1	66.7	33.3	11.11	0.00	2.0:1
395	6	6	50.0	50.0	0.00	1.00	1:1
405	5	3	62.5	37.5	6.25	0.01	1.7:1
415	8	7	53.3	46.7	0.44	0.50	1.1:1
425	8	8	50.0	50.0	0.00	1.00	1:1
435	4	7	36.4	63.6	7.44	0.01	0.6:1
445	2	4	33.3	66.7	11.11	0.00	0.5:1
455	0	0	0.0	0.0	–	–	–
465	0	0	0.0	0.0	–	–	–
475	2	0	100.0	0.0	100	0.00	–
485	1	1	50.0	50.0	0.00	1.00	1:1
Mean	321	300	51.7	48.3	0.11	0.74	1.1

Monthly data on gonadosomatic index showed that reproductive activity is inversely associated to SST progression for the frigate tuna in SE Brazil. Adults' GSI values increased from October onwards and maximum spawning activity was reached during spring–summer, when SST was at approximately 16°C (Females: H=46.99; DF=1; P<0.00; males: H=78.37; DF=1; P<0.00) (Fig. 10a and b).

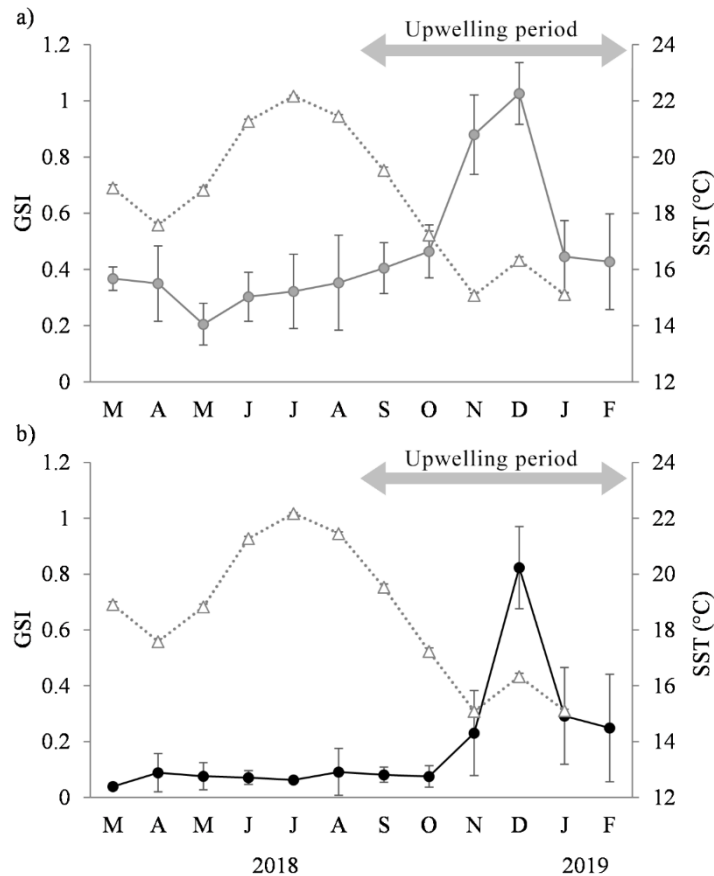


Figure 10. Monthly means of Gonadosomatic index (GSI) for a) females and b) males of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. Triangles (Δ) correspond to Sea surface temperature (SST, $^{\circ}\text{C}$). Vertical bars correspond to the standard deviation.

Variation of gonad development stages showed that most sampled immature fish were associated to coastal waters ($\text{SST} > 17^{\circ}\text{C}$) (Fig. 11). In contrast, developing and spawning fish were observed almost year-round, with no clear association with water temperature, despite spawning capable gonads constituting 84% of all gonads in November, right before maximum GSI values in December. Regressing (or partially spent) ovaries and testicles were the smallest fraction among samples (3.5%) and the highest frequency of gonads in this phase occurred in March, when only fish in spawning, regressing and resting phases were identified. Regenerating gonads were observed very sparsely.

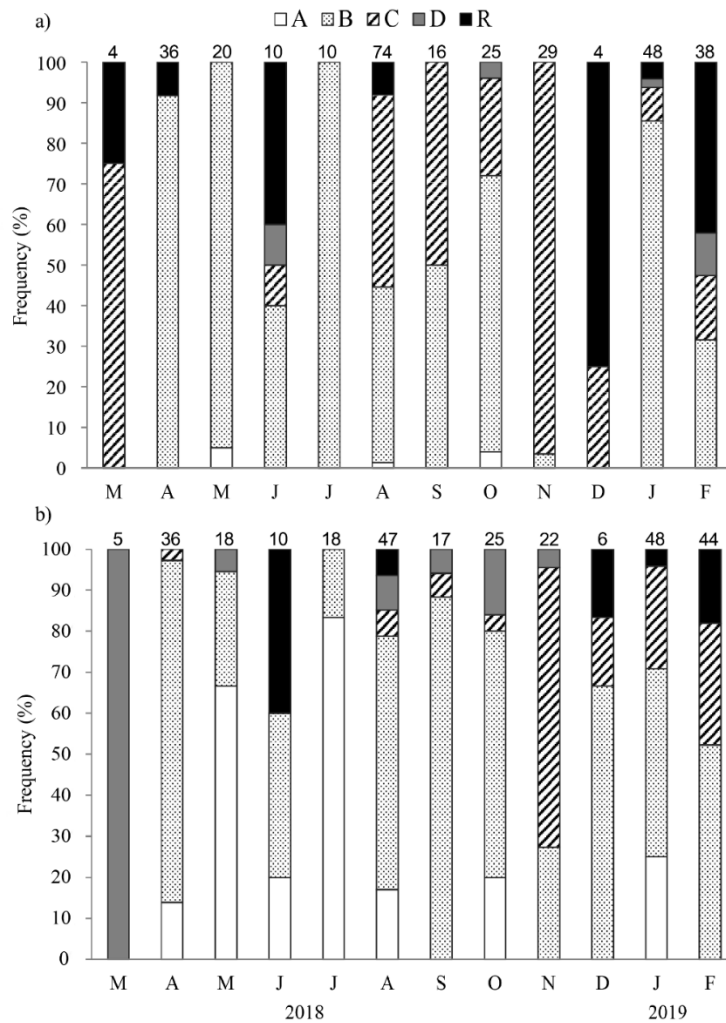


Figure 11. Monthly frequency distribution of maturity phases a) females and b) males of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. A: immature; B: developing; C: spawning capable; D: regressing/spent; R: regenerating. Numbers above bars correspond to the number of samples per month.

Length-at-first-maturity was 345.4 mm for females (95% CI: 334.5–356.6 mm) and 329.8 mm for males (95% CI: 320.6–337 mm) (Fig. 12).

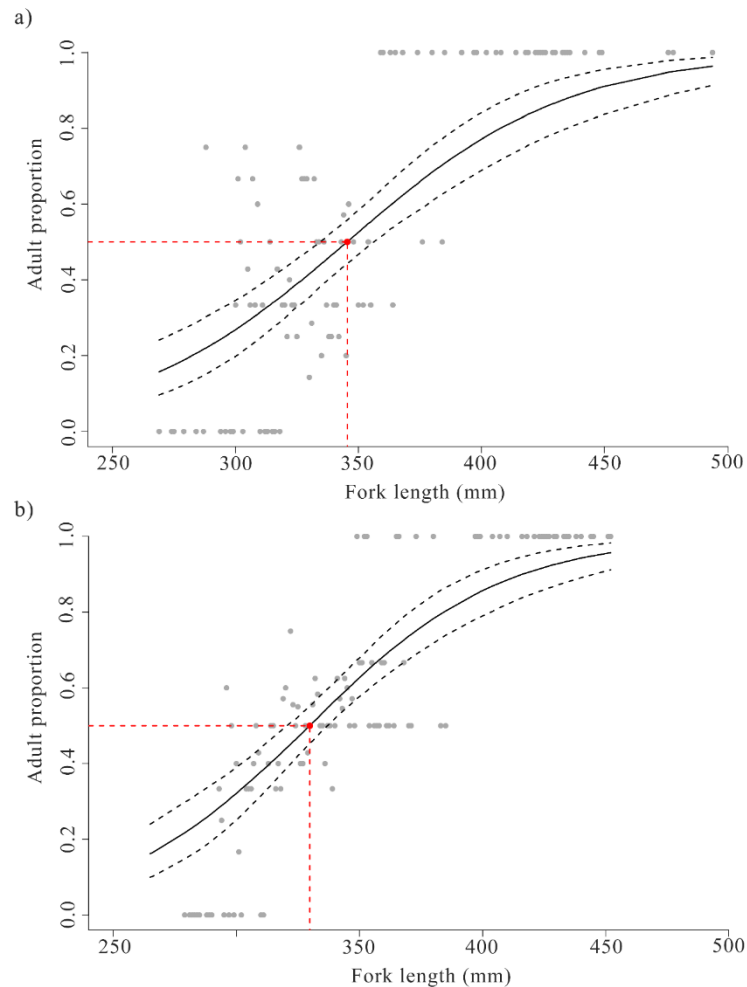


Figure 12. Length-at-first-maturity (L_{50}) for a) females and b) males of *Auxis thazard* landed in the Southeastern Brazil between March 2018 and February 2019. Dotted lines (—) denote 95% confidence interval.

4. Discussion

The small-tunas are an important social and economic resource for coastal communities, having comprised 17% of the total catches of tuna and tuna-like species between 1950 and 2018, according to the ICCAT official catch statistics (Lucena-Frédou et al. 2021). Conversely, there are several gaps in their life history information, which are fundamental determinants of fish's population dynamics as well as essential to understand the impacts of fishing and to promote sustainable fishing practices (King 2008; Juan-Jordá et al. 2013; Lucena-Frédou et al 2021). The frigate tuna stock, in particular, has been identified as a priority to be evaluated by the ICCAT (Lucena-Frédou et al. 2017), and Pons et al (2019) classified the data on life-history parameters of the species in the SWA as insufficient for a proper assessment of the stock.

In Brazilian waters, the scarce knowledge on *A. thazard* also hinders the management for the stock, even though it comprises of a target species for artisanal fisheries in SE Brazil (da Silveira-Menezes et al. 2010; Martins et al. 2020). Additionally, the overexploitation of more valuable fish stocks has resulted in their replacement by new target species, which includes some small-tunas (Fogliarini et al. 2021).

In line with these observations, this study provides new information regarding life-history parameters (age, growth, and maturity) of frigate tuna, which are essential for the assessment of the stock in SWA.

4.1 Length–weight relationship (LWR)

The positive allometric growth ($b > 3$) observed in our results suggests that frigate tuna in SE Brazil increases faster in weight rather than length. Likewise, frigate tuna in the Indian Ocean, Northeast Atlantic and Gulf of Guinea also exhibits positive allometric growth (Bahou et al. 2016; Tampubolon et al. 2016; Abekan et al. 2017; Herath et al., 2019, Darvish et al. 2020). For the North West Indian coast, Mudumala et al. (2018) calculated $b < 3$ for *A. thazard*, indicating a negative allometric growth, which the authors suggested to be caused by the absence of juveniles in the samples. Moreover, *A. thazard* caught on Veraval (India) was observed to have an isometric growth (Ghosh et al. 2010). The variations of the b parameter are possibly related to ecosystem and biological conditions such as temperature, salinity and food availability, and generalizations for the species should only be made after considering all the variables (Froese 2006).

4.2 Increment formation

The completion of opaque bands occurred during winter when translucent increments, which reflect periods of slow somatic growth, started to deposit. Tropical fish growth is most likely to be influenced by temperature, as it directly affects metabolism (Morales–Nin and Panfili, 2005). However, the formation of increments in calcified structures (e.g., spines and otoliths) can also be influenced by spawning activity and/or food availability (Beckman and Wilson, 1995). Experiments on otoliths have shown that translucent material can be deposited in response to slow somatic growth caused by food restriction (Høie et al. 2008) and in the Cabo Frio upwelling system the wind direction changes during cold months (March to August, during autumn and winter), which leads the superficial depths to be occupied by warm, coastal waters that have less nutrients (Valentin, 2001).

Thus, the deposition of translucent material may be related to lower nutrient availability during winter, while more organic material is deposited due to the presence of highly productive waters of the SACW. This association was also corroborated by our samples' stomach content, as food was only present from September to February, during spring–summer (unpublished data). The formation of translucent growth marks during austral winter has also been reported for the little tunny *E. alletteratus* exploited off the Brazilian coast (Vieira et al. 2021).

4.3 Age and Growth parameters

Reports of maximum age for the frigate tuna in the literature ranges from 3 to 5 years (Kasim 2002; Abdussamad et al. 2005; Ghosh et al. 2010; Tao et al. 2012; Darvishi et al. 2020). Maximum age in our study was of 4 years, similar to recent observations in India (Mudumala et al. 2018); however, most studies in India have reported maximum ages of 3 years (Kasim 2002; Abdussamad et al. 2005; Ghosh et al. 2010). Additionally, for Taiwan, Indonesia and Iran, the frigate tuna attains up to 5 years (Tao et al. 2012; Lelono and Bintoro 2019; Darvishi et al. 2020).

Estimates of growth parameters (L_{∞} , k , t_0) from the literature are summarized in table 4. Growth rate was similar and particularly high for maximum age in the Indian Ocean, where data was estimated using length frequency analysis (Kasim 2002; Abdussamad et al. 2005; Ghosh et al. 2010; Mudumala et al. 2018). The exception is the most recent report by Lelono and Bintoro (2019), where the growth rate was 0.58 year^{-1} for frigate tuna caught in two landing sites off the Indonesian coast. Taiwanese estimates were also lower ($k=0.50\text{--}0.52$) and similar to one another, despite using length frequency analysis and vertebrae, respectively (Lu et al. 1991; Tao et al. 2012). Regardless of variations, our estimates are among the lowest reported for growth rate, an aspect possibly related to the different methods and locations, since most assessments for the species have been conducted using length frequency analysis. This hypothesis is further corroborated by Grudtsev and Korolevich (1986), whose estimates for frigate tuna in Eastern Central Atlantic using spines were close to ours ($L_{\infty}=514.5 \text{ mm}$; $k=0.32$). The growth performance index calculated herein ($\phi'=5.0$) reflects the lower growth rates for asymptotic length obtained for *A. thazard* caught off SE Brazil. Our results were also among the lowest calculated values from previous studies' data, which varied from 4.8 to 5.6 (Table 4). The lowest value ($\phi'=4.8$) was associated with the lower L_{∞} (354 mm) estimated for the Indonesian population (Lelono and Bintoro 2019).

Table 4. Size–range (FL, mm), ageing method, growth parameters (L_{∞} , k , t_0), maximum age (Tmax), growth performance index (ϕ') and area reported for *Auxis thazard*. Ageing method: LF–Length frequency, V–Vertebrae, S–Spine; NE–Northeast; EC–Eastern Central

Reference	FL	Method	L_{∞}	k	t_0	Tmax	ϕ'	Area
Kasim 2002 (Female)	–	LF	512.0	1.30	–0.00	3	5.5	India
Kasim 2002 (Male)	–	LF	490.0	1.30	–0.00	3	5.5	India
Abdussamad et al. 2005	180–300	LF	529.0	0.68	–0.28	3	5.3	India
Ghosh et al. 2010	200–470	LF	466.0	0.93	–0.01	3	5.3	India
Mudumala et al. 2018	160–500	LF	473.8	1.40	–0.23	4	5.5	India
Lu et al. 1991	201–469	LF	484.4	0.50	–0.40	–	5.1	Taiwan
Tao et al. 2012	198–456	V	481.8	0.52	–0.33	5	5.1	Taiwan
Calicdan–Villarao et al. 2017	160–550	LF	409.5	0.60	–	–	5.0	Filipinas
Calicdan–Villarao et al. 2017	160–550	LF	462.0	0.70	–	–	5.2	Filipinas
Lelono & Bintoro 2019	–	LF	354.0	0.58	–0.26	4.8	4.8	Indonesia
Darvishi et al. 2020	210–490	LF	532.0	0.80	–1.17	5	5.4	Iran
Grudtsev & Korolevich (1986)	–	S	514.5	0.32	–0.83	–	4.9	EC Atlantic
Zapadaeva (2021)	–	LF	486	0.48	–	–	5.1	NE Atlantic
Present study (Female)	269–494	S	471.0	0.47	–1.46	4	5.0	Brazil
Present study (Male)	265–452	S	498.0	0.35	–2.01	4	5.0	Brazil

4.4 Reproductive cycle

The onset of the frigate tuna’s reproduction associated with cold waters was an unexpected result of our study, as most tunas are known to have a universal requirement for warm waters ($>20^{\circ}\text{C}$) to spawn (Schaefer 2001). The *Auxis* genus has been observed to spawn throughout its range, in coastal to oceanic waters of tropical and subtropical areas (Collette and Nauen 1983). Off the Indian coast, the reproduction occurs during summer, while in Gulf of Mexico it happens during spring, at SSTs above 20°C (Ghosh et al. 2010). In Sri Lanka spawning season is from May to August, during autumn–winter, and in the Philippines reproductive activity peaks twice a year, in January and from March to April, in winter/spring (Calicdan–Villarao et al. 2017; Herath et al. 2019). In Côte d’Ivoire, spawning occurs from

July to October, at temperatures between 22.14 and 25.15°C, although gravid females were observed at temperatures between 27°C and 28°C (Bahou et al. 2016). In Brazilian waters, while previous data on the genus' reproduction is limited, Matsuura and Sato (1981) have described the distribution of *Auxis sp.* larvae along the SE Brazilian coast from samples collected in considerably varied SST (16°C–28°C), but mostly from temperatures between 22°C to 26°C.

Spawning activity of *A. thazard* seems to favor more productive continental shelf and slope environments, and during spring–summer the intrusion of deeper and more nutritious waters of the SACW causes an enrichment of the habitat's productivity and enables the feeding and growth of larvae and adults (Valentin 2001; Pruzinsky et al. 2020). This characteristic was also observed by Bahou et al. (2016) in Côte d'Ivoire, as peak spawning season occurred during main upwelling season. For some smaller tunas, adult feeding and spawning grounds considerably overlap, and sardines and anchovies, which are important food items for frigate tuna, are commonly found associated to upwelled waters in Brazil (Collette and Nauen 1983; Valentin 2001; Reglero et al. 2014; Herath et al. 2019). In SE Brazil, other tunas also seem to synchronize their reproductive activity with upwelling events, such as the little tunny *E. alletteratus* (Vieira et al. 2021) and the skipjack tuna *K. pelamis* (Soares et al. 2019).

The peak of spawning activity for frigate tuna was restricted to spring–summer; however, fish in developing and spawning phases were observed almost year–round, as illustrated by the monthly evaluation of gonad development, and the presence of germinative cells in different developmental stages simultaneously. Like other tunas, *A. thazard* has an asynchronous oocyte development that leads to long and multiple spawning seasons throughout the year, as observations in India and Gulf of Guinea suggest (Rudomiotkina, 1984; Schaefer 2001; Ghosh et al. 2012; Abekan et al. 2017). Collette and Nauen (1983) have also mentioned the occurrence of adult fish throughout the year in the eastern Pacific, although spawning activity increases during some months.

4.5 Length–at–first–maturity (L_{50}) and sex–ratio

Length–at–first–maturity for *A. thazard* has been often estimated for combined sex. For other locations, L_{50} range between 269 to 349 mm, with most estimates under 330 mm (Ghosh et al. 2012; Jude et al. 2002; Tao et al. 2012; Bahou et al. 2016). Although within the range, our estimates ($L_{50} = 329.6$ – 345.6 mm) are among the highest for the species and are comparable to those of Tampubolon et al. (2016) ($L_{50} = 348.9$) and Jude et al. (2002) ($L_{50} = 308$ – 328) from Indonesia and India, respectively. When separated by sex, our results are similar to reports in

India (Jude et al. 2002), where females of frigate tuna mature at greater sizes when compared to males. In contrast, the opposite has also been reported for the Taiwanese population (Tao et al. 2012).

The balanced sexual ratio (1:1, F:M) indicated the absence of sexual segregation in the area. Our findings were also similar to previous observations in other locations, such as Indonesia, Sumatra and the Philippines (Noegroho et al., 2013; Tampubolon et al., 2016; Calicdan–Villarao et al. 2017). When samples were analyzed by month, the number of females and males was not significantly different, despite fluctuations over time, in contrast to observations in the Gulf of Guinea, where males were dominant all year–round (Abekan et al. 2017). Conversely, when samples were divided by size, females were most numerous, although only a few classes significantly diverged from the 1:1 ratio. This suggests that both sexes are equally vulnerable to the fishery and that there is no size–related segregation for this species.

5. Conclusions

This study validated the use of fin spine as an age assessing method for the *A. thazard* caught in SE Brazil. The deposition of growth marks was completed during winter and the longevity of the frigate tuna (4 years) was close to observations in other areas, although the growth rate was among the lowest recorded for the species. Moreover, our observations of spawning gonads from fish in upwelled cold waters during spring–summer contradict the assumption that the frigate tuna only spawns at temperatures above 20°C.

Small tunas such as the frigate tuna are important social and economic resources for coastal communities. However, the available data on this species is still scarce for management aims. Therefore, our study provides the ICCAT and other management institutions essential information on *A. thazard*'s biology in SWA, where no investigations on age, growth and maturity have been conducted. In Brazilian waters, particularly, there are no management policies for the frigate tuna's fishery and catches are underestimated, a problem aggravated by the deficiency of knowledge on the species.

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CAPÍTULO 3

Stable Isotope relations and risk of human exposure of Total mercury in three small-tunas in southeastern Brazil

Artigo a ser submetido ao periódico Environmental Research

Stable Isotope relations and risk of human exposure of Total mercury in three small-tunas in southeastern Brazil

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ABSTRACT

Mercury (Hg) bioaccumulation can be highly variable for marine species. Its concentration is influenced by several physico-chemical and biotic processes, including size, age, diet composition and foraging habitats. Herein, we addressed total mercury (THg) concentrations on three economically important small-tuna species in South Eastern Brazil: the frigate tuna *Auxis thazard*, the little tunny *Euthynnus alletteratus* and the skipjack tuna *Katsuwonus pelamis*. Samples were collected from commercial landings in the SE Brazilian coast from 2017 to 2019. The influence of trophic ecology and habitat preference on Hg accumulation was assessed through stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Additionally, the human health risk regarding the species consumption was assessed by the estimated weekly intake (EWI) and the provisional tolerable weekly intake (PTWI) for total consumers, adult men and adult women. Variables such as size and age were positively correlated to Hg, suggesting bioaccumulation over time. Trophic position appears to positively influence the mercury concentrations for *A. thazard* and *K. pelamis*, whose relationship between $\delta^{15}\text{N}$ and THg was significant. For *E. alletteratus*, the lack of relationship suggests that $\delta^{15}\text{N}$ values do not vary for adult fish (> 300 mm). For none of the evaluated species in the three scenarios THg concentrations were above the established tolerable limit for mercury by Brazilian international organs, as indicated by the %PTWI. This finding suggests that the consumption of those species caught off the southeast Brazil at the considered amounts does not present a risk for healthy adults.

Keywords: Metal bioaccumulation; Feeding habitat; Trophic ecology; Human exposure; Southwest Atlantic; Brazilian coast

1. INTRODUCTION

Mercury (Hg) is a naturally occurring element in the environment that cycles through the earth's crust, atmosphere, oceans, and life forms, and can be directly mobilized by anthropogenic activities (Kidd et al., 2012). This heavy metal, mainly in the form of methylmercury (MeHg), enters the food chain through bacteria and phytoplankton and it is efficiently transferred across aquatic food webs, resulting in Hg concentrations in top predators up to millions of times higher than those in primary producers or consumers (*i.e.*, biomagnification; Bloom, 1992; Kidd et al., 2012). Hg also accumulates throughout the life of organisms (bioaccumulation), which is very dependent on physico-chemical (*e.g.*, dissolved organic matter, salinity, temperature) and biotic processes (*e.g.*, size, sex, reproductive cycle, feeding habits, living environment) (Kidd et al., 2012; Wang et al., 2012).

Although Hg can be absorbed through different sources, the main route of human exposure to Hg is the consumption of fish and fish-derived products (Fitzgerald et al., 2007). Fish meat provides proteins of high biological values and fats (*e.g.*, Omega 3) and it is particularly important for coastal communities worldwide. Small-scale artisanal and subsistence fisheries contribute with over one-quarter of the catches in volume and represents half of the world's fishing effort (Watson and Tidd, 2018; Rousseau et al., 2019; Mishra, 2020). Among these fish species, tunas are of primary importance, as they are widely consumed. The *Katsuwonus pelamis*, a cosmopolitan migratory species found in tropical and warm temperate waters worldwide, comprise of nearly 40% of the annual catch of the world's tunas, and ranks third in catch tonnage in global marine fisheries (2.8×10^6 t in 2020; FAO, 2022). In Atlantic waters, *K. pelamis* has a high social and economic relevance for the fishery market, especially the tuna canning in Brazil, while the *Euthynnus alletteratus* is mostly caught seasonally as a by-catch in trawl and purse-net fisheries off the African coast and captured mainly as bait in the Gulf of Mexico (Cabrera et al. 2005; Gaykov and Bokhanov 2008; Schmidt et al., 2019). In fact, alongside the *Sarda sarda* and the *Auxis thazard*, *E. alletteratus* is the most captured small scombrid in the Atlantic Ocean (ICCAT, 2021). In Southeastern (SE) Brazilian waters specifically, *K. pelamis*' catch production reached 18,000 t/year in the last decade (FAO, 2014). While, *E. alletteratus* is caught as one of the most frequent secondary targets of the *K. pelamis*'s fisheries alongside other tunas (*Thunnus* spp.), the *Coryphaena hippurus* and the *A. thazard* (Da Silveira-Menezes et al. 2010).

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopic variation is frequently used as intrinsic tracers of diet composition and migration, as well for ecotoxicological investigations

through food webs (Le Croizier et al., 2019; Hobson, 1999). The Stable Isotope Analysis (SIA) of consumers is based on the premise that carbon and nitrogen values reflect those of its food. The $\delta^{13}\text{C}$ values are conventionally used for distinguishing foraging locations (*e. g.* coastal/oceanic and pelagic/benthic), as they indicate original sources of dietary carbon (Layman et al., 2012; McCutchan et al., 2003; Rosas-Luis et al., 2021). On the other hand, $\delta^{15}\text{N}$ values increase up to 2-4‰ with increasing trophic level (DeNiro and Epstein 1978; 1981), making nitrogen isotopes conventionally used for trophic position estimation (Layman et al., 2012). As most mercury is absorbed through the food chain, SIA is a valuable tool for the analysis of diet and feeding habit's contributions to the processes of mercury accumulation (Kidd et al., 1995). Conventional stomach analysis has several inherent constraints, making SIA increasingly useful to reconstruct feeding habits and improve the understanding of trophic webs in marine ecosystems (da Silveira et al., 2020; Médiéu et al., 2022; Rooker et al., 2006).

The SE Brazilian coast is highly populated and hosts an industrial complex and important rivers (Ovalle et al., 2013). Oceanographically, it is greatly influenced by the warm Brazil Current flowing southwards and the seasonal upwelling of the cold and nutrient-rich waters of the South Atlantic Central Water (SACW) from a depth of 300m during spring-summer (Gonzalez-Rodriguez et al., 1992). As a result, it is associated with a rich pelagic fauna, which includes highly economic important species, such as the *Katsuwonus pelamis* (Valentin, 2001). Despite the association between upwelling process in this area and inorganic Hg input, as well as enrichment of upper layers (Cossa et al., 2004; Figueiredo et al., 2013; Galvão et al., 2020), few studies have focused on stable isotope measurements coupled with Hg determination in the study area (Bauer et al., 2021; Bisi et al., 2012; Costa et al., 2019; Muto et al., 2014; Di Benedetto et al., 2011, 2012, 2021).

The present study aims to update information on THg concentrations in the white muscle of three small-tuna species caught off the SE Brazilian coast: the little tunny *Euthynnus alletteratus*, the skipjack tuna *Katsuwonus pelamis* and the frigate tuna *Auxis thazard*. We also evaluate the impacts of ecological and populational attributes (length, age, feeding habits and displacement from the coast) using stable isotope analysis. Additionally, the potential risk of the observed concentrations when compared to limits established by Brazilian and international organs was also determined and discussed.

2. MATERIAL AND METHODS

2.1 Sample collection

A total of 82 specimens were analyzed for THg determination and stable isotope measurements. Specimens were sampled from commercial landings in Cabo Frio, Macaé and Arraial do Cabo, in the southeastern Brazilian coast (Fig. 1), between 2017 and 2019. Sex of each fish, as well as fork length (FL, 0.5 cm) were recorded while samples were still fresh. White muscle samples (3–4g) were removed from the region between the head and the first dorsal fin and kept frozen prior to analysis. Age of each fish was assigned by applying growth parameters from literature (Soares et al., 2019; Vieira et al., 2021, 2022) to the inverse function of the von Bertalanffy growth equation: $t = t_0 - \frac{LN(1 - L/L_\infty)}{k}$, where L is the length at age t ; L_∞ is the asymptotic length; k is the growth coefficient, and t_0 is the theoretical age at zero length.

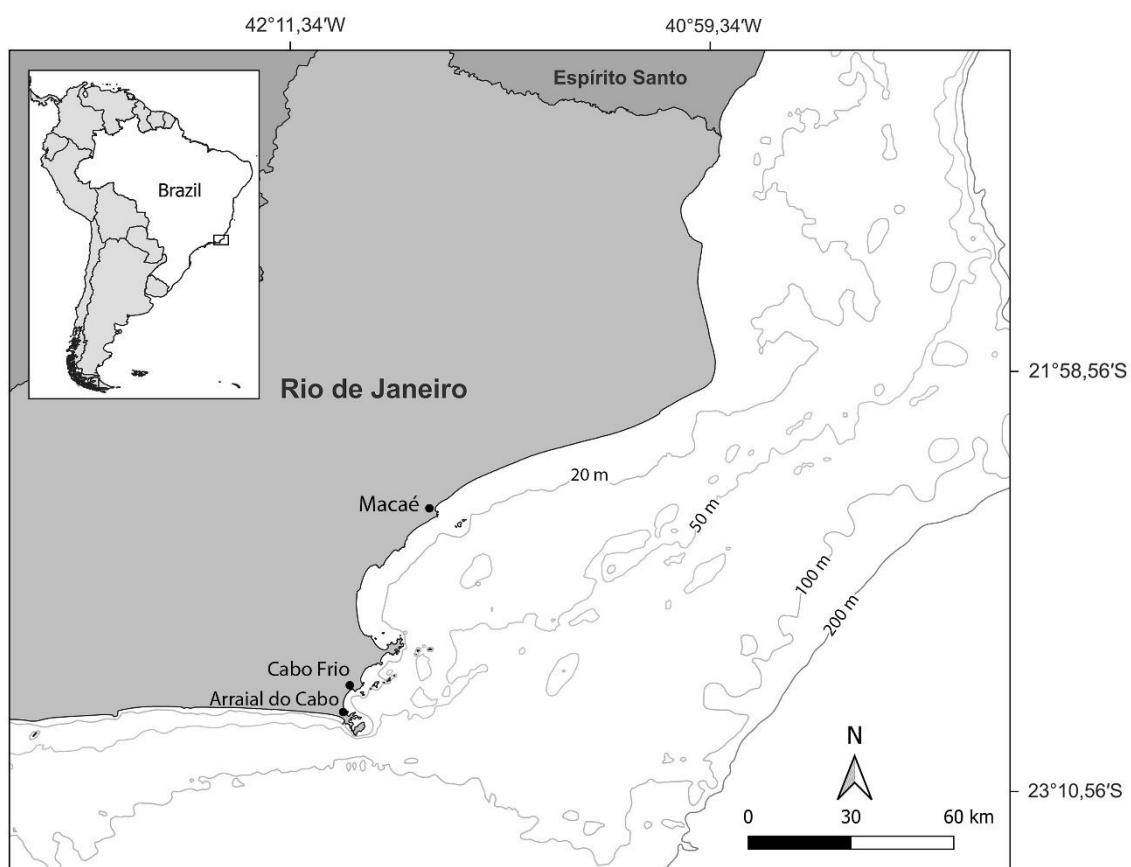


Figure 1. Location of the sampling ports (black dots) in the Rio de Janeiro State, SW Atlantic.

2.2 Analytical analysis

2.2.1 Total mercury concentrations

THg quantification was performed as described by Bastos et. al. (1998). Wet muscle samples were weighted freeze-dried before the register of dry-weight (dw). Aliquots between 0.02 and 0.04g dw were homogenized for total mercury (THg) determination. All samples were duplicated and solubilized using 1mL of hydrogen peroxide and 3mL of concentrated sulfonitric solution (HNO₃:H₂SO₄; 1:1) and kept on a thermal bath for 2h. Thereafter, 5mL of potassium permanganate was added and samples were placed on thermal bath of another 15 min. After 24h, the mixture was reduced using 1mL of hydroxylamine hydrochloride and diluted up to 14mL using high purity deionized water (18.2 MΩ cm) from a Milli-Q system.

Total mercury concentration on muscle samples were obtained by atomic absorption spectrometry with cold vapor generation using the Flow Injection Mercury System (FIMS-400, Perkin-Elmer). Aliquots of the certificated reference materials (CRM) DOLT-5 (fish liver) was used to validate the procedure. Mean THg concentration for DOLT-5 was 463.3 µg/kg (n=7), providing a mean recovery of 107%. Results were only accepted when the coefficient of variation between duplicates did not exceed 15%. The term THg concentration for all analysis refers to total wet-weight (ww) concentrations, considering a water percentage of 70%.

2.2.2 Carbon and nitrogen isotope analysis

In order to evaluate possible differences in feeding habits, a quantification of carbon and nitrogen isotopes was conducted. Wet muscle samples were collected from the dorsal region, freeze-dried, powdered, and weighed (~1mg) in tin capsules for δ¹³C and δ¹⁵N isotope determination at the University of New Mexico Center for Stable Isotopes. Samples were combusted in an Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometer (Europa Hydra 20/20). SIA values were expressed using delta notation (δ) in parts per thousand (‰), as determined by $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} and R_{standard} indicate the molar ratios of C¹³/C¹² and N¹⁵/N¹⁴ of the samples and the reference material (standards), respectively. Internationally accepted standards were used for δ¹⁵N, the atmospheric N₂, and δ¹³C, the Vienna Pee Dee Belemnite (V-PDB). Internal laboratory standards were used to estimate analytical precision: Casein with δ¹⁵N and δ¹³C values of 6.3‰ and -25.5‰, respectively; and Tuna muscle with δ¹⁵N and δ¹³C values of 13.1‰ and -15.5‰. Analytical precision for δ¹³C and δ¹⁵N values was <0.2‰.

2.3 Statistical analysis

Data on total mercury concentrations and C and N values were tested using Shapiro-Wilk and Levene's tests for normality and homogeneity of the data variance before applying an analysis of variance (ANOVA), if restrictions were met. If not, the non-parametric Kruskal-Wallis (K-W, H) test was used followed by an Unequal N HSD post-hoc was used (Zar, 2010). In order to investigate the influence of biological parameters (length and age) on total mercury concentrations, Spearman's correlation test was applied.

2.4 Estimation of potential human health risks

The estimated dietary exposure to THg was expressed in micrograms per kilogram per week and it was compared to the reference values established by the European Food Safety Authority (EFSA) and the Brazilian legislation for MeHg (Brasil, 2013; EFSA, 2012). As MeHg comprises approximately 90% of the consumed Hg, the same limits established for MeHg were used as threshold in the present study (Bloom, 1992; Wang, 2012).

For the estimation of weekly intakes three scenarios were considered, according to the IBGE (Instituto Brasileiro de Geografia e Estatística) on fresh fish Brazilian consumption for years 2017-2018: I) Total consumers: 91.7 g/week; II) Adult men: 104.3 g/week; III) Adult women (pregnant women were not considered herein): 80.5 g/week (IBGE, 2013). For assessments, the considered body weight (bw) was of 60 kg. The estimated weekly intake (EWI $\mu\text{g}/\text{kg}$ bw) of Hg was calculated using the equation: $\text{EWI} = (\text{C} \times \text{WIR}) / \text{BW}$, where C is the THg concentration in fish muscle, WIR is the mean weekly intake rate of fish and BW is the mean individual body weight (60kg) (FAO/WHO, 2010). According to the Brazilian legislation, the tolerable limits of for Hg in Brazilian for top fish predators is of 1000 $\mu\text{g}/\text{kg}$. For the European Food Safety Authority (EFSA), the threshold of provisional tolerable weekly intake of MeHg is 1300 $\mu\text{g}/\text{kg}$ bw (EFSA, 2012). In order to investigate the risk of THg exposure by consuming fish meat, Provisional Total Weekly Intake was calculated as: $\% \text{PTWI} = (\text{EWI} \times 100) / \text{PTWI}$, where EWI is the estimated weekly intake previously and PTWI is 1000 or 1300 $\mu\text{g}/\text{kg}$ bw, as established by the Brazilian regulations for predator fish and EFSA, respectively (Brasil, 2013; EFSA, 2012; Formula adapted from: WHO/FAO, 2004). Percentages of PTWI higher than 100 represent health risks.

3. RESULTS

3.1 THg concentrations and Biologic parameters

Mean THg concentrations in muscle ranged from 68.5 $\mu\text{g}/\text{kg}^{-1}$ ww for *A. thazard* to 416.0 $\mu\text{g}/\text{kg}^{-1}$ ww in *E. alletteratus* (Table 1). When THg concentrations were compared, *A. thazard* was observed to have significantly lower concentrations of all three tunas (Kruskal-Wallis; $H_{2,82} = 27.39$; $p < 0.0001$). As for *E. alletteratus* and *K. pelamis*', although mean concentrations were close, *K. pelamis*' was significantly lower (ANOVA; $F_{1,56} = 4.18$; $p = 0.04$) (Figure 2).

Table 1. Sample size (*n*), fork length (mm), age (years), mean values \pm standard deviation (SD), median, minimum (Min) and maximum (Max) of THg concentrations in $\mu\text{g}/\text{kg}$ wet-weight (ww) of the three small-tuna species in the present study.

Species	<i>n</i>	Fork length	Age	[THg] $\mu\text{g}/\text{kg}$ ww			
				Mean \pm SD	Median	Min	Max
<i>Auxis thazard</i>	24	309-389	1-2	152.3 \pm 48.4	148.2	68.5	260.3
<i>Euthynnus alletteratus</i>	28	452-588	1-4	285.0 \pm 85.6	283.2	152.1	416.0
<i>Katsuwonus pelamis</i>	30	390-738	2-6	240.9 \pm 75.4	445.0	117.1	245.1

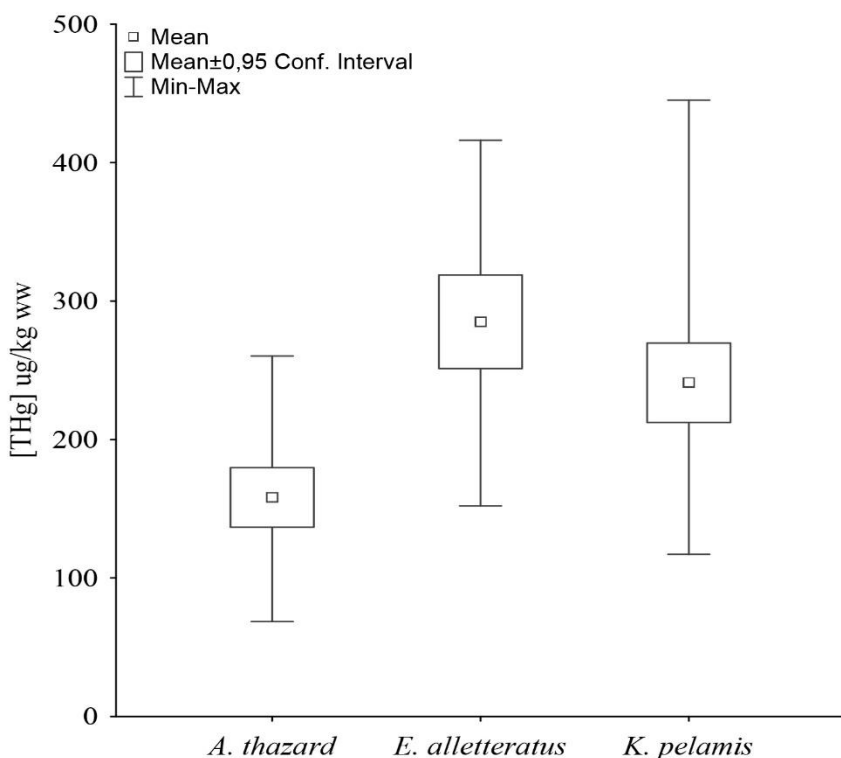


Figure 2. Total mercury concentrations for *A. thazard*, *E. alletteratus* and *K. pelamis* caught off the southeast Brazilian coast. □ = Mean; Outside box = Mean ± Confidence Interval (95%); Bars = Minimum and Maximum values of THg concentrations.

Significant positive relationships between THg concentration and fork length and age were observed for all taxa (Spearman's correlation, $p < 0.05$) (Table 2; Figure 3). In order to eliminate possible bias of THg concentration towards size per age, mercury concentrations for fishes of 2 years, the only age class representative for all species, were compared (*A. thazard*: $n = 16$; Mean FL \pm SD = 373.5 ± 10.7 , *E. alletteratus*: $n = 13$; Mean FL \pm SD = 535 ± 38.4 , *K. pelamis*: $n = 12$; Mean FL \pm SD = 420 ± 30.1). Total mercury was significantly higher in the muscle of *E. alletteratus* (Kruskal-Wallis test; $H_{2,38} = 15.49$; $p = 0.004$).

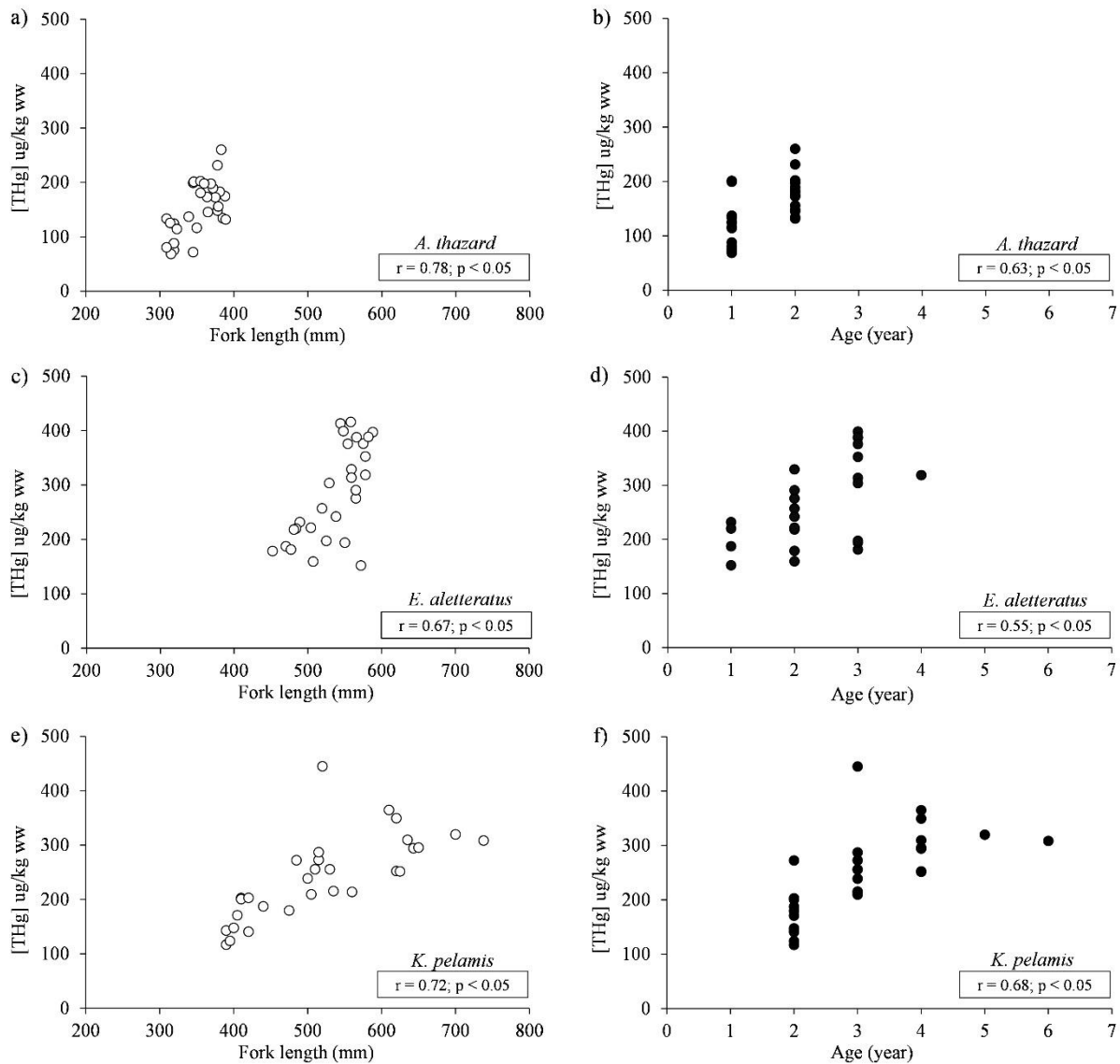


Figure 3. Linear regressions between total mercury concentrations (THg, $\mu\text{g}/\text{kg ww}$) and Fork length (a, c and d), and age (b, d and e) for *A. thazard*, *E. alletteratus* and *K. pelamis* caught off the southeast Brazilian coast.

3.2 Stable Isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in fish

Summaries for carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) values are represented on the table 2 and figure 4. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly different among fish (Kruskal-Wallis; $H_{2,82} = 59.39$, $p < 0.0001$ and ANOVA; $F_{2,79} = 35.79$; $p < 0.0001$, respectively).

Carbon isotope values ($\delta^{13}\text{C}$) values ranged from -21.8‰ to -17.2‰ across species. *Katsuwonus pelamis* exhibited significantly higher $\delta^{13}\text{C}$ values, while *E. alletteratus* the lowest. As for $\delta^{15}\text{N}$, values ranged from 6.8‰ for *K. pelamis* to 14.8‰ in *A. thazard*. While *K.*

pelamis was observed to be significantly depleted in nitrogen when compared to the other tunas, *E. alletteratus* and *A. thazard*'s values did not differ from each other.

Table 2. Total mercury concentrations (THg) and fork length's: Sample size (*n*), intercept (a) and slope (b) of the regression, Spearman's correlation (r) and *p*-value for the relationship between total mercury concentrations (THg) and the fork length. Stable Isotope Analysis: Sample size (*n*), values from the stable isotope analysis (SIA), minimum (Min), maximum (Max) and Spearman's correlation (r) and *p*-value of the linear regression between THg concentrations and stable isotope values (¹³C and ¹⁵N).

Species	[THg] and fork length					Stable Isotope Analysis						
	<i>n</i>	a	b	r	<i>p</i> -value		<i>n</i>	Mean ± SD	Min	Max	r	<i>p</i> -value
<i>Auxis thazard</i>	24	-390.3	1.56	0.8	<0.05	δ ¹³ C	24	-18.9 ± 0.76	-19.9	-17.5	0.11	0.65
						δ ¹⁵ N	24	12.2 ± 1.86	7.8	14.8	0.86	0.001*
<i>Euthynnus alletteratus</i>	28	-521.06	1.50	0.7	<0.05	δ ¹³ C	28	-20.4 ± 0.79	-21.8	-18.3	0.06	0.77
						δ ¹⁵ N	28	13.1 ± 1.22	9.5	14.6	0.36	0.08
<i>Katsuwonus pelamis</i>	30	-45.44	0.55	0.7	<0.05	δ ¹³ C	30	-17.8 ± 0.38	-18.9	-17.2	0.09	0.61
						δ ¹⁵ N	30	9.6 ± 1.51	6.8	12.3	0.47	0.02*

*Statistically significant relationship

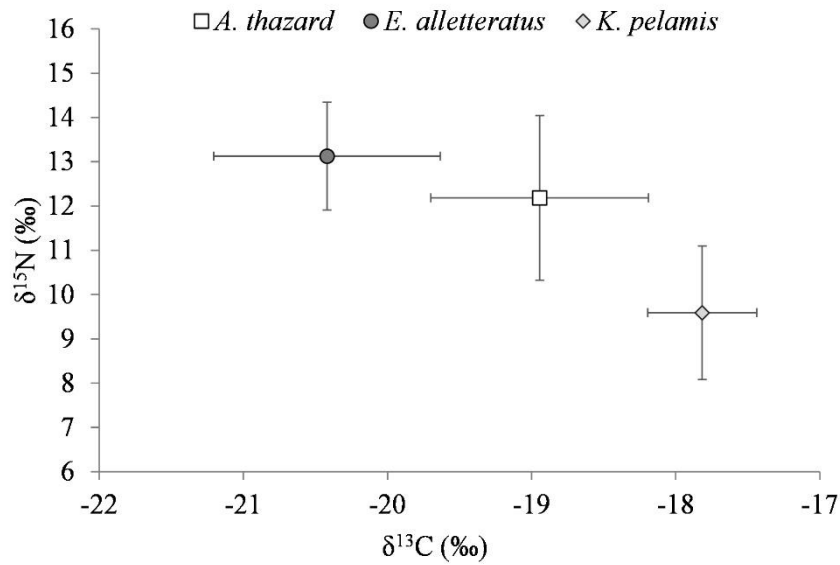


Figure 4. Mean and standard deviation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for *A. thazard*, *E. alletteratus* and *K. pelamis* caught off the southeast Brazilian coast.

3.3 Linear regression between THg concentration and stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in fish muscle

The linear regression between THg concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is represented in figure 5 and table 2. For $\delta^{13}\text{C}$, the relationship was not significant for any species (Spearman's correlation, $p > 0.05$). As for $\delta^{15}\text{N}$, it was positively correlated to THg for *A. thazard* and *K. pelamis* (Spearman's correlation, $p < 0.05$), while not significant for *E. alletteratus*.

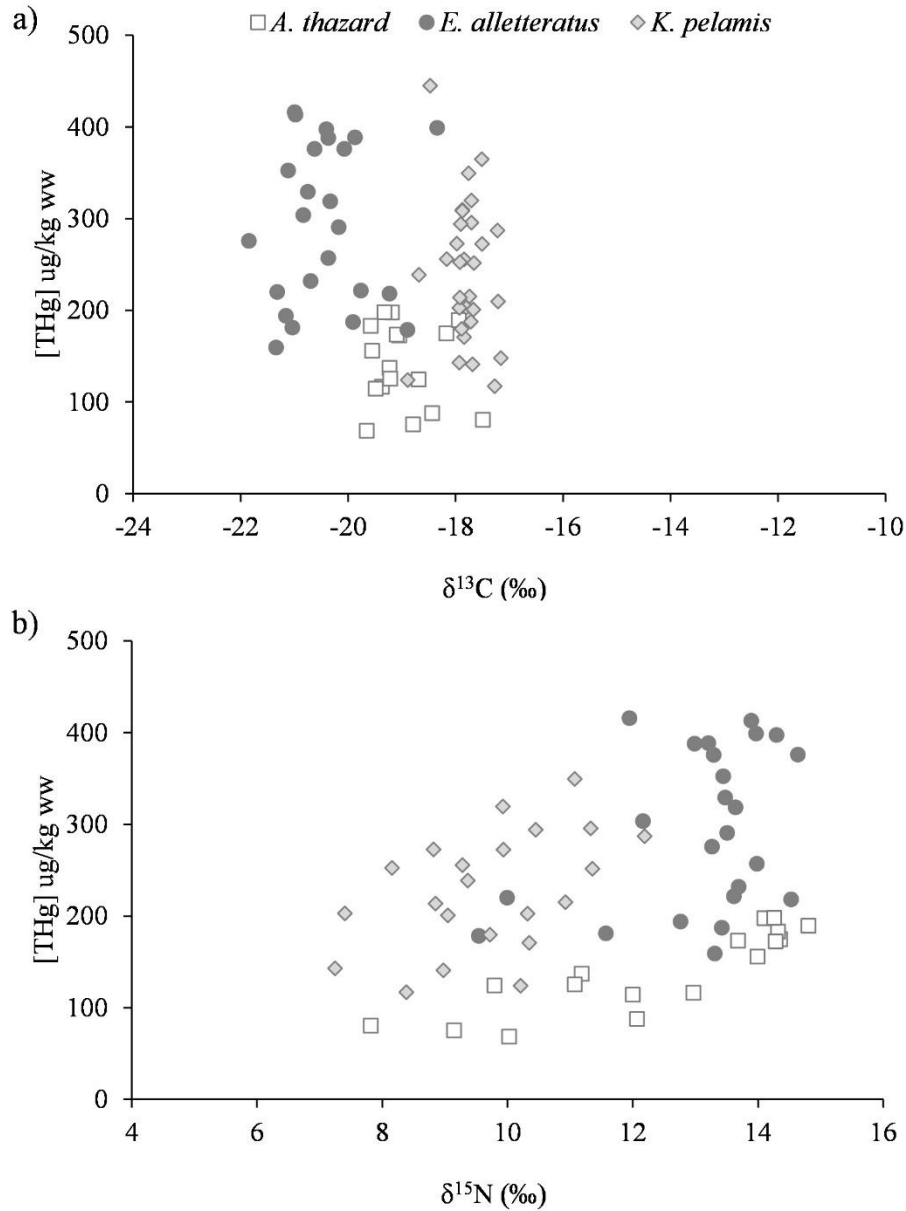


Figure 5. Linear regressions between total mercury concentration and a) $\delta^{13}\text{C}$ and b) $\delta^{15}\text{N}$ for *A. thazard*, *E. alletteratus* and *K. pelamis* caught off the southeast Brazilian coast.

3.4 Public health risks

Estimated weekly intake for analyzed species ranged from 241.7 to 368.3 $\mu\text{g}/\text{kg ww}$ for total consumers, and 275.0 to 495.5 $\mu\text{g}/\text{kg}$ for adult men and 212.2 to 382.4 $\mu\text{g}/\text{kg}$ for adult women (Table 4). According to our estimates, PTWI of THg did not exceed 100% for any species regarding limits established by Brazilian or international organizations.

Table 3. Mean THg concentrations ($\mu\text{g}/\text{kg ww}$) of three small-tuna landed in southeastern Brazil in terms of I) Total consumers; II) Adult men and III) Adult women sample size (n), estimated weekly intake (EWI $\mu\text{g}/\text{kg ww}$), and percentual of provisional tolerable weekly intake (%PTWI) for a limit of 1000 $\mu\text{g}/\text{kg}/\text{week}$ and of 1300 $\mu\text{g}/\text{kg}/\text{week}$.

I) Total consumers (average = 91.7 g/week)			
	EWI (g/week)	%PTWI (1000 $\mu\text{g}/\text{kg}$)	%PTWI (1300 $\mu\text{g}/\text{kg}$)
<i>Auxis thazard</i>	241.78	24.18	18.60
<i>Euthynnus alletteratus</i>	435.64	43.56	33.51
<i>Katsuwonus pelamis</i>	368.31	36.83	28.33
II) Adult men (average = 104.3 g/week)			
	EWI (g/week)	%PTWI (1000 $\mu\text{g}/\text{kg}$)	%PTWI (1300 $\mu\text{g}/\text{kg}$)
<i>Auxis thazard</i>	275.00	27.50	21.15
<i>Euthynnus alletteratus</i>	495.50	49.55	38.12
<i>Katsuwonus pelamis</i>	418.92	41.89	32.22
III) Adult women (average = 80.5 g/week)			
	EWI (g/week)	%PTWI (1000 $\mu\text{g}/\text{kg}$)	%PTWI (1300 $\mu\text{g}/\text{kg}$)
<i>Auxis thazard</i>	212.25	21.22	16.33
<i>Euthynnus alletteratus</i>	382.43	38.24	29.42
<i>Katsuwonus pelamis</i>	323.33	32.33	24.87

4. DISCUSSION

4.1 Stable isotopes in fish muscle

4.1.1 Carbon isotopes ($\delta^{13}\text{C}$)

Carbon isotope analysis is an important tool used to assess diet, habitat use and migration of species, as the enrichment of stable carbon isotopes from one trophic level to the next (~1‰) consequently causing the $\delta^{13}\text{C}$ values of the preys to determine $\delta^{13}\text{C}$ of top predator (Peterson and Fry, 1987; McMahon et al., 2010).

Euthynnus alletteratus have been regarded by the literature as a very coastal species as well as highly migratory, although less than other tunas (Collete and Nauen, 1983; FAO, 1994; ICCAT, 2006; Serdy, 2004; Yoshida, 1979). Previous carbon isotope quantifications in the Gulf of Mexico are in agreement with this classification, as higher $\delta^{13}\text{C}$ values were observed for *E. alletteratus* when compared to the present study (approximately -16.4 ‰), and the lack of regional differences in Hg concentrations are in agreement with the previously reported highly migratory movements for this species, indicating a wide plasticity for this species (Cai et al., 2007; FAO, 1994). However, As opposed to previous reports, in the present study *E. alletteratus* was depleted in carbon when compared to more migratory and oceanic species, such as *K. pelamis* (Ely et. al., 2005; ICCAT, 2006). *Euthynnus alletteratus* is known to be a highly piscivorous species, and our isotope results most likely reflect this species' prey preferences in SE Brazil, as this tuna mostly feed on pelagic preys, possibly farther from the coast, which usually have lower carbon signatures compared to the benthic/coastal fauna (Bahou et al., 2007; Falautano et al., 2007; Micheiner and Schell, 1994; Peterson, 1987). In fact, in SE Brazilian waters *E. alletteratus* is caught not only by beach seine, but also by purse seine fisheries, which suggest that this species may be found in coastal areas as well as farther from the coast, where it may feed (Vieira et al., 2021).

Similarly, *K. pelamis* $\delta^{13}\text{C}$ values are close to carbon signatures for cephalopods and the mesopelagic lanternfish from sampled the stomach content of specimens previously caught off the SE Brazilian coast (Coletto et al., 2020). This evidence the higher importance of the depth in which the preys inhabit in terms of different carbon source for these species' trophic web.

Despite not considered a highly migratory species, fish from the *Auxis* genus are known to be found in both coastal and open waters, much like *E. alletteratus* (Collete and Nauen, 1983; Maguire et al., 2006). *Auxis thazard*, specifically, inhabit environments across shelves, but sometimes can be found offshore and around oceanic islands, an aspect in agreement with our isotope results, as $\delta^{13}\text{C}$ values were intermediary between the more coastal *E. alletteratus*

and the oceanic *K. pelamis*. (Andrade, 2003; Bezerra et al., 2020; Collete and Nauen, 1983; Ely et al., 2005; Yoshida, 1979). Rosas-Luis et al. (2021), who has previously quantified carbon and nitrogen isotopes for fish of the *Auxis* genus, observed $\delta^{13}\text{C}$ values close to our results and attribute this to the fact that *Auxis spp.* may move to low productivity areas and consume prey from more pelagic habitats when compared to other tunas such as *K. pelamis*. Indeed, as observed in the present study, $\delta^{13}\text{C}$ values were closer to *E. alletteratus*, who also feeds on pelagic preys when compared to *K. pelamis*.

4.1.2 Nitrogen isotopes ($\delta^{15}\text{N}$)

As for nitrogen isotopic values, *K. pelamis* was the most nitrogen-depleted across species, with the $\delta^{15}\text{N}$ values in agreement with the findings by Coletto et al. (2021) in SE Brazilian waters. Ontogenetic shifts in diet are frequent featured in fish, therefore, changes in the trophic position with growth are expected (Pizzochero et al., 2018). For *K. pelamis*, shifts in prey composition have been also observed: from lanternfish and krill in juveniles, to a larger proportion of cephalopods and small pelagic fish, in adults (Muto et al., 2014; Coletto et al., 2020; Coletto et al., 2021). In our study, most samples (66%) were represented by adult fish (>470 mm), thus, the nitrogen isotope signatures, in a similar way to carbon values, reflect their diet, as overall $\delta^{15}\text{N}$ values for this species are closer to values for krill, cephalopods and particularly the small pelagic fish (Coletto et al., 2020; Coletto et al., 2021). In SE Brazil, the $\delta^{15}\text{N}$ signatures of krill and lanternfish, the main food composition of adults, are around 3.7‰ and 10.0‰, while clupeids and engraulids are higher, from 11.7‰ to 12.3‰, respectively (Coletto et al., 2021; Di Benedetto et al., 2015).

Euthynnus alletteratus and *A. thazard*, on the other hand, did not present significant differences in $\delta^{15}\text{N}$ values. Among other factors, nitrogen isotope values may vary in consequence of the type and the size of the preys and a similar diet may explain the close values for nitrogen (Hussey et al., 2014; Post, 2002). The food of *E. alletteratus* described from stomach content, as mentioned beforehand, consists mainly of clupeids and engraulids, and, even though detailed information on the diet composition of *A. thazard* is scarce, it has been reported to also feed on small pelagic species such as clupeids and engraulids (anchovies) (Etchevers, 1976; Uchida, 1981). Similar to carbon results, both groups in SE Brazil, according to Di Benedetto et al. (2015), present $\delta^{15}\text{N}$ values close to observed for *E. alletteratus* as well as for *A. thazard* (*S. brasiliensis*: $11.7 \pm 1.4\text{‰}$ and engraulid fish: $12.3 \pm 0.4\text{‰}$, respectively). This theory further corroborated by the composition of schools of *A. thazard*, which are known

to mix with other tuna and tuna-like species, such as *E. alletteratus*, an aspect also observed during our samplings (Uchida, 1981; Yoshida, 1979).

4.2 Influence of biological parameters and foraging sites on Hg accumulation

Hg bioaccumulation and biomagnification in organisms are affected by several factors: some abiotic, such as water chemistry and mercury bioavailability and some biotic, such as body size, growth rate, age, sex, diet and foraging habits (Kidd et al., 2012).

4.2.1 Body size and age

As mercury has a tendency to bioaccumulate over time, body size has been one of the major investigated biological parameters to possibly influence mercury concentrations (Dang and Wang, 2012; Storelli et al., 2007). Our results are in agreement with the premise, as the small-tunas' sizes appeared to be the primary factor influencing Hg concentrations. Moreover, previous observations for the assessed species have also shown the positive relationship between length and mercury concentrations (Adams, 2004; Manhães et al., 2020; Voegborlo et al., 2006). Manhães et al. (2020), in fact, attributed the observations in *E. alletteratus* and *K. pelamis* to larger individuals possibly accessing larger and more contaminated prey, which would lead to higher amounts of mercury for the larger fishes. However, for *A. thazard*'s muscle, Voegborlo et al. (2007) found Hg in quantities close to observations in the present study (44-201 µg/kg ww, mean = 108 µg/kg) for fish caught in Ghana, even though their samples' size ranged from 355 to 532 mm and ours between 309 and 389 mm. This highlights the importance of other factors apart from body size in evaluating the influences of biological parameters on Hg concentrations, such as mercury availability and diet.

When concentrations were overall compared between species, *A. thazard* exhibited the lowest values, most likely a reflection of the smaller samples size and the shorter life span (2 years) when compared to the other tunas. This would, therefore, result in a shorter time to accumulate Hg when compared to *E. alletteratus* and *K. pelamis*. Additionally, when restricting the comparison to 2-year-old fishes, THg concentrations were the highest for *E. alletteratus*, hence emphasizing the importance of body size in the Hg accumulation, as *E. alletteratus* had the biggest sizes for the 2-year-old age class among all three tunas.

4.2.2 Foraging habits

Foraging habits and environmental conditions are also known to influence mercury concentrations in fish. Mercury accumulation is generally positively correlated with trophic level

as determined by $\delta^{15}\text{N}$ (Di Benedetto et al., 2012), an aspect previously observed for several fish species off the Brazilian coast as well as for *A. thazard* and *K. pelamis* in the present study (Bauer et al., 2021; Bisi et al., 2012; Di Benedetto et al., 2011; Dorneles et al., 2020; Kehrig et al., 2013; Muto et al., 2014). Bisi et al. (2012) investigated carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and total mercury (THg) concentrations in the muscle of several species, including crustacean, cephalopod, fish, and dolphin. The study compared 3 different coastal sites influenced by estuaries and observed the highest mean $\delta^{15}\text{N}$ values in fish and benthic invertebrate feeders as well as demersal species. Additionally, THg and $\delta^{15}\text{N}$ were positively correlated for two out of the three studied bays. A positive relationship between Hg levels and trophic position suggests mercury biomagnification across food web for those species (Kidd et al., 1995).

As for *E. alletteratus*, although ontogenetic shifts in food composition have been reported before in Mediterranean waters, this species appears to remain constantly feeding on small pelagic fish, such as *Maurolicus muelleri* and clupeiforms (Falautano et al., 2007). While *M. muelleri* have never been described as prey of *E. alletteratus* in Brazilian waters, teleosts, particularly clupeids, are still the most important prey group for juveniles and adults (> 300 mm) of this species in SE Brazil (Menezes and Aragão, 1977). Therefore, as there are no indications of major shifts in prey composition for this tuna caught off SE Brazil, *E. alletteratus* may occupy essentially the same trophic position or have the similar feeding habits throughout its life, resulting in a small variability in $\delta^{15}\text{N}$ values and the lack of correlation between THg and $\delta^{15}\text{N}$. This aspect is further corroborated by the lowest variability in $\delta^{15}\text{N}$ values among analyzed species, as shown by the standard deviation.

4.2.3 Environmental conditions

Southeast Brazil not only hosts industrial areas and flow of important rivers, but during spring-summer there are upwelling events that serves as a great input from sediment and deep layers to the surface, making upwelling systems hot spots for mercury biomonitoring (Bowman et al., 2016; Cossa et al., 2017; Le Croizier et al., 2019; Ovalle et al., 2013).

Mercury contamination in carnivorous demersal reef fish in SE Brazil have been previously assessed by Galvão et al. (2020). The authors addressed the importance of the upwelling phenomenon in enhancing biomagnification across the food web in the area, especially of demersal species. The MeHg concentrations in those species, according to the authors, may be affected by the high productivity caused by the upwelling (which results in increasement of the organic carbon in the water, where Hg may stay adsorbed), and even by

the extension of trophic webs, that would cause top predators to absorb higher amounts of mercury.

On the other hand, Silva et al (2011) quantified THg and MeHg concentrations in the muscle tissue of pelagic and demersal fish, including the *K. pelamis*. The authors found concentrations to be much lower when compared to the present study, most likely a result of the limited sizes for *K. pelamis*' samples in their study. Moreover, the authors did not observe particularly high concentrations their analyzed species, with the exception of *Cynoscion striatus*, a demersal fish, which presented Hg above the limits for ingestion suggested by health organizations, which suggest a higher influence of upwelling movements and mercury displacement from the seabed, where Hg is more available, in benthic/coastal species than in pelagic ones, a pattern previously observed in other areas as well (Cossa et al., 2017; Le Croizier et al., 2019). This process may explain the low Hg observed for the tunas for our study. Even though they are top predators, these small-tunas do not tolerate low oxygen concentrations and temperatures, therefore, living mostly in shallow waters above the thermocline, where there are lower methylmercury concentrations, as well as feeding on relatively smaller preys with lower Hg concentrations (Choy et al., 2009; Monteiro et al., 1996). However, when it comes to fish of high swimming capacity and migrating species, tracking the influence of local environmental factors is more complex, especially for globally distributes tunas such as *A. thazard* and *K. pelamis* (Colette and Nauen, 1983; Médiu et al., 2022; Nicklisch et al., 2017).

4.3 Public health risks

Quantification of THg concentrations in muscle tissue of commercial fish is an acceptable way to assess the risk of human exposure to MeHg, as most of the mercury content in this tissue (~90%) consists of MeHg (Bloom, 1992). Due to the effects of mercury exposure for humans even at low concentrations, Brazilian legislation has established safe limits for THg in for predatory commercial fish is 1000 µg/kg/bw (ww) (Brasil, 2013, Karagas et al., 2012). As for EFSA, for exposure from fish, the PTWI for MeHg is of 1300 µg/kg/bw (EFSA, 2012).

All the species in the present study are of local and/or worldwide importance, especially because of the socio-economic value to artisanal fisheries, in the case of *E. alletteratus* and *A. thazard*. Thus, an assessment of consumed meat quality is vital in terms of public health risks. Our results, however, should be treated with caution as the analyzed fish are highly migratory (FAO, 1994). In addition, evaluations using local samples are always preferable in order to fully estimate the potential risk for human health considering regional variabilities in fish consumption and environmental conditions (Goyanna et al., 2022).

In all fishes analyzed herein mercury concentrations were in accordance with the 1000 µg/kg/bw limit set by the Brazilian legislation and the 1300 µg/kg/bw recommended by EFSA, suggesting that the potential risk of exposure to Hg is minimal for consumption in amounts close to the considered (Table 4). Particularly for *K. pelamis*, which is of low market price and globally well consumed, %PTWI was very low, reaching a maximum of almost 42% when considering a limit of 1000 µg/kg/week (FAO, 2020). Taking the current limits into consideration, our results suggest that consuming these small-tunas caught off the southeast Brazil at the current amount does not present a risk, in terms of public health, for healthy adults.

5. CONCLUSIONS

The present study provides new and updated information regarding mercury concentrations and stable isotopes in the muscle of three small-tuna species. Our results for *E. alletteratus* and *K. pelamis* indicate constant concentrations when compared to previous observations in the area. Body size, foraging habits and age appear to be the most important parameters to influence mercury concentrations. Length and age results in particular suggest a process of mercury bioaccumulation for all analyzed species. Using SIA, we observed a positive relationship between trophic position and THg concentrations for *A. thazard* and *K. pelamis*, however, for *E. alletteratus*, the lack of variability in $\delta^{15}\text{N}$ values caused a lack of significance between variables. This indicates that this species may occupy the same trophic position or have the similar feeding habits throughout its life. In an unexpected result, SIA results also revealed *E. alletteratus* the most carbon-depleted, a pattern possibly related to the prey composition of this species in SE Brazil, which consists majorly of pelagic preys. As for *A. thazard*, the overlap of $\delta^{15}\text{N}$ values with *E. alletteratus* suggest a possible degree of prey partitioning among both species, while *K. pelamis* exhibited the lowest values, a reflection of a more varied prey composition that includes mesopelagic species. To conclude, according to our %PTWI results, mercury concentrations for all fishes are in accordance within the limits set by the Brazilian legislation and recommended by EFSA and does not pose a risk, if consumed in quantities close to considered, to healthy adults and non-pregnant women.

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Conclusões gerais

- O presente estudo é o primeiro a descrever os parâmetros da história de vida (idade, o crescimento e a biologia reprodutiva) de *Euthynnus alleteratus* e *Auxis thazard* no Sudoeste do Atlântico;
- A contagem dos anéis de crescimento presentes no primeiro espinho da primeira nadadeira dorsal de *E. alleteratus* e *A. thazard* se mostrou um método confiável para a aferição da idade para ambas as espécies;
- Este é o primeiro registro do pico do reprodutivo de *E. alleteratus* e *A. thazard* em temperaturas abaixo de 18°C, durante o período de primavera e verão austrais;
- O comprimento de primeira maturação sexual (L_{50}) é atingido por *E. alleteratus* na idade de 1 ano para ambos os sexos (entre 423 e 492 mm);
- Os valores de L_{50} estimados para *A. thazard* apresentaram diferenças por sexo, sendo 345.6 mm para fêmeas e 329.8 mm para os machos;
- O tamanho corporal, os hábitos alimentares e a idade parecem ser os parâmetros mais importantes a influenciar as concentrações de mercúrio.
- Utilizando análise de isótopos estáveis, foi observada uma relação positiva entre a posição trófica e as concentrações de HgT para *A. thazard* e *K. pelamis*, porém, para *E. alleteratus*, a relação não foi significativa;
- A pouca variabilidade entre os HgT e os valores de $\delta^{15}\text{N}$ de *E. alleteratus* indica que esta espécie pode ocupar a mesma posição trófica ou ter hábitos alimentares;
- As concentrações de mercúrio para todos os peixes estão de acordo com os limites estabelecidos pela legislação brasileira e recomendados pela EFSA e não representam risco, se consumidos em quantidades próximas às consideradas, para adultos saudáveis e mulheres não grávidas.
- O presente estudo fornece informações básicas e indispensáveis para o manejo pesqueiro de espécies exploradas na região de Cabo Frio (Rio de Janeiro). Adicionalmente, também foram produzidas novas informações e atualizações acerca das concentrações de mercúrio e isótopos estáveis no músculo de três espécies de pequenos atuns (*E. alleteratus*, *A. thazard* e *K. pelamis*).