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First description of post-flexion larvae of the West Atlantic trumpetfish, *Aulostomus maculatus*

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Abstract

The West Atlantic trumpetfish *Aulostomus maculatus* is a species of little commercial importance, but it is frequently used as a study organism in behavioural ecology, and it has been traded in the aquarium industry to some extent. The adult life stage is well described, however its early life history is nearly unknown. This paper provides the first description of post-flexion larvae of *A. maculatus*, including detailed illustrations, photographs, morphological data, and collection site data of specimens collected during a multipurpose research survey conducted within the Sargasso Sea Subtropical Convergence Zone. The collection site also implies a geographic range expansion, off the continental shelf, of the pelagic larvae stage. This paper hence advances the scientific knowledge about the early life stages, distribution and ecology of this species.

Introduction

West Atlantic trumpetfish, *Aulostomus maculatus*, typically inhabit coastal waters near reefs or grass beds in the western central Atlantic from Bermuda to the northern coasts of South America (Wheeler, 1955; Richards, 2005; Pollom, 2015; Froese and Pauly, 2024). They can also be found further out at sea (Bowen *et al.*, 2001, and references therein), with a few records of larval specimens far offshore in the Sargasso Sea (data from online database Ocean Biogeographic Information System, OBIS, http://www.iobis.org, mainly derived from FishNet2 Marine Data: Fishnet2 Portal, http://www.fishnet2.net). The species is of little or no commercial importance but has been traded in the aquarium industry to some extent (Monteiro-Neto *et al.*, 2003; Richards, 2005; Pollom, 2015; Froese and Pauly, 2024). Scientifically, it has been extensively used as study organism in behavioural ecology, particularly in studies of predator–prey interactions (Eibl-Eibesfeldt, 1955; Aronson, 1983; Matchette *et al.*, 2022; Matchette *et al.*, 2023). This is mainly due to their intriguing and unusual hunting strategy, called shadowing, whereby the trumpetfish swims closely behind or next to a 'host' species, hiding behind it to facilitate the capture of prey (Longley and Hildebrand, 1941; Eibl-Eibesfeldt, 1955; Wheeler, 1955; Aronson, 1983; Sterrer and Schoepfer-Sterrer, 1986; Matchette *et al.*, 2022; Matchette *et al.*, 2023). Studies on *A. maculatus* have been performed using adults, and hence this life stage is relatively well described. Their reproduction is however unknown, including spawning and description of eggs (Leis and Carson-Ewart, 2000; Bowen *et al.*, 2001; Richards, 2005). On the contrary, the pelagic eggs of the Chinese trumpetfish, *A. chinensis*, have been described (Leis and Carson-Ewart, 2000). It should be noted that the online database FishBase (2024) describes the eggs of *A. maculatus* as pelagic (Froese and Pauly, 2024), citing Patzner (2008). In Table 9.1 of Patzner (2008), this information can indeed be found (page 322), however, the reference for the table is FishBase (2004), meaning that the reference is circular, and that no original source is cited. In communication with Patzner, it was stated that the results of Patzner (2008) could no longer be reproduced (R. Patzner, personal communication, see email conversation on figshare, link below under Data availability statement). We therefore conclude that the information provided in Leis and Carson-Ewart (2000), Bowen *et al.* (2001), and Richards (2005) is valid, i.e., that there is yet no description of eggs.

The early life history pattern is poorly known, but likely includes a pelagic phase with planktonic larvae (Leis and Carson-Ewart, 2000; Bowen *et al.*, 2001; Richards, 2005). The larvae stage referred to here consist of the developmental stages: yolk sac, pre-flexion, flexion, and post-flexion, whereafter the fish is referred to as a juvenile (Ahlstrom and Ball, 1954; Ahlstrom and Moser, 1976; Leis and Carson-Ewart, 2000). One description of a pre-flexion larvae *A. maculatus* (i.e., the developmental stage after the yolk sac stage, ending at the start of upward flexion of the notochord) can be found in Leis and Carson-Ewart (2000) (a 8.2 mm pre-flexion larvae), reprinted in Richards (2005). Descriptions of post-flexion (i.e., the developmental stage after the flexion stage, from formation of the caudal fin to attainment of full external fin rays) larvae and small juveniles are lacking (Leis and Carson-Ewart, 2000; Bowen *et al.*,

2001; Richards, 2005). Bowen *et al.* (2001), contains references to personal communication regarding juvenile specimens having been collected in mid-oceanic plankton surveys, and juveniles settling in Caribbean Panama (the latter measured to 89–104 mm standard length, aged to 80–94 days using otoliths). Although the species is listed as 'Least Concern' according to the 2015 IUCN Red List of Threatened Species, it is stated that more research is needed on life history and ecology (Pollom, 2015). Research is also needed on population size, distribution and trends as well as habitat trends (Pollom, 2015). Here, we detail the first description of post-flexion larvae of *A. maculatus*, including drawn illustrations, photographs, morphological as well as collection site data, thereby providing valuable knowledge on the early life history stage of this species.

Material and Methods

West Atlantic trumpetfish, *Aulostomus maculatus*, larvae were collected during a multipurpose research survey with the German fishery research vessel Walther Herwig III, conducted from March to April 2023 (cruise number WH-465). The main purpose of the triennial survey is to investigate the distribution, abundance, and ecological aspects of early developmental stages of the European eel (*Anguilla anguilla*) and American eel (*A. rostrata*). The sampling design and specific methods of the recurring survey have been described previously in publications focussing on different species (Hellenbrecht *et al.*, 2019; Miller *et al.*, 2019; Sundin *et al.*, 2023), and are therefore described here in short. During the 465th cruise, a total of 45 stations were sampled along two north–south transects, located between 31° to 19° N and 67° to 64° W (Figure 1). Sampling stations varied between

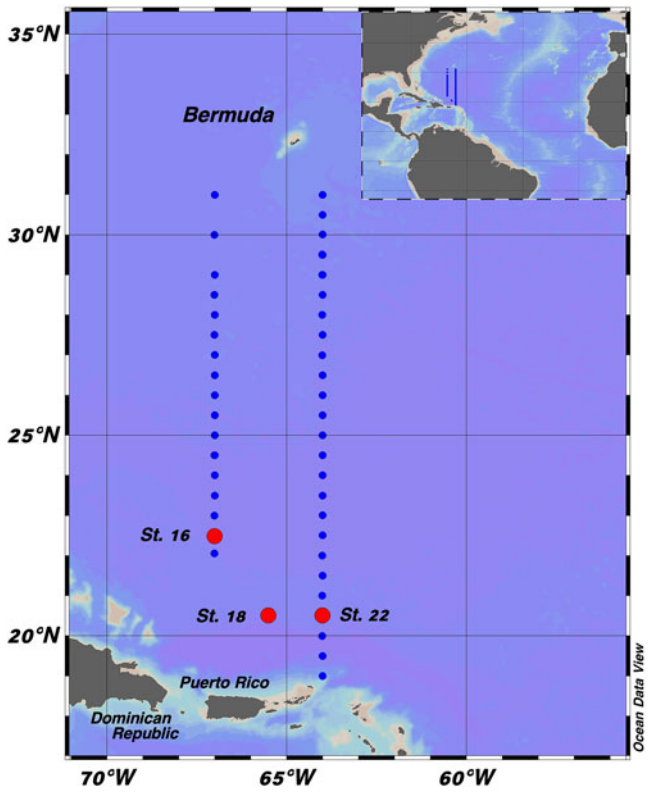

Figure 1. Locations (stations) where post-flexion larvae of the West Atlantic trumpetfish, *Aulostomus maculatus*, were collected in the Sargasso Sea using an Isaacs-Kidd Midwater Trawl during a multipurpose research survey conducted between March and April 2023. All locations (stations) visited during the cruise are indicated on the map, red circles (station 16, 18, 22) show where trumpetfish were collected.

0.5° and 1° along the latitudinal transects. Water depth in the sampled area ranged roughly from 5000 to 7000 m.

Sampling was conducted using an Isaacs-Kidd Midwater Trawl (IKMT) (mesh size 500 µm, mouth opening 6.2 m^2, length 10 m, Hydro-Bios Apparatebau GmbH). The IKMT was deployed using double-oblique tows between the surface and a maximum depth of 300 m during night and day. Plankton samples were manually sorted on-board immediately upon collection. In total, three trumpetfish larvae were collected at three different stations (stations 16, 18, 22, Figure 1). They were identified to the species level on-board according to a region-specific identification guide (Richards, 2005). The first two collected specimens were photographed (using a Canon EOS 5D Mark III camera with a Canon EF 100 mm/2.8 l Macro lens) on a light board equipped with a millimetre scale. All three specimens were fixed in ethanol (99%) at room temperature. Morphometric measurements were then derived from the photographs using the open-source software ImageJ (version 1.53) (Schneider *et al.*, 2012), using the tool for straight or segmented lines at appropriate magnification. A detailed scientific illustration was drawn (Figure 2) based on the photos of the fresh samples (mainly the largest individual) and based on examination and photos of the specimens after fixation in ethanol (Figure 3) (photos of samples stored in ethanol taken using a Leica M165 FC stereo microscope equipped with a Leica DMC6200 Pixel Shift Camera, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany).

Species identification was genetically verified by DNA barcoding and BLAST analysis after the ship's arrival back at the Thünen Institute of Fisheries Ecology, Bremerhaven, Germany. The methods are described in Sundin *et al.* (2023). In short, DNA was extracted using a Chelex-based method (Walsh *et al.*, 1991) and amplified by PCR for DNA barcoding using the mitochondrial marker Cytochrome c oxidase I (COI). The sequencing of the PCR-products was carried out by a service lab (StarSEQ GmbH, Mainz, Germany) and forward and reverse DNA sequences were checked and trimmed before generating consensus COI sequences for each sample. To verify the species identification all sequences were aligned and compared with BioEdit Sequence Alignment Editor (Copyright 1997–2013 Tom Hall) and verified by nucleotide BLAST against NCBI database (Altschul *et al.*, 1990).

Results and Discussion

The three trumpetfish larvae were identified on-board as the West Atlantic trumpetfish, *Aulostomus maculatus*. The morphological species identification was later genetically confirmed with 100% certainty (for COI sequence alignment, see data on figshare, link below under Data availability statement). *Aulostomus maculatus* are characterized by a laterally compressed, elongated and slender body, with a relatively small mouth at the tip of the pipette-like tubular snout (Figure 2) (Wheeler, 1955; Sterrer and Schoepfer-Sterrer, 1986; Richards, 2005). Adult *A. maculatus* are characterized by a distinct barbel on the tip of the lower jaw, and 8–13 short isolated dorsal spines (Wheeler, 1955; Sterrer and Schoepfer-Sterrer, 1986; Richards, 2005; Froese and Pauly, 2024). Those characters were however not found in the larval specimens (Figure 2). The pectoral fins are located close to the head, with 15–16 fin rays (according to Richards (2005), no fin-ray counts could be performed on the collected specimens, for any of the fins). The opposed soft dorsal and anal fins are located at the last quarter of the body, with 21–25 or 24–28 fin rays in the dorsal fin, and 22–25 or 22–25 in the anal fin (according to Wheeler (1955) and Richards (2005), respectively, fin counts vary between sources). According to the online database FishBase, both the dorsal and anal fin have 21–25 fin rays (Froese and Pauly, 2024). The pelvic fin has 6 fin rays (according to: Richards,

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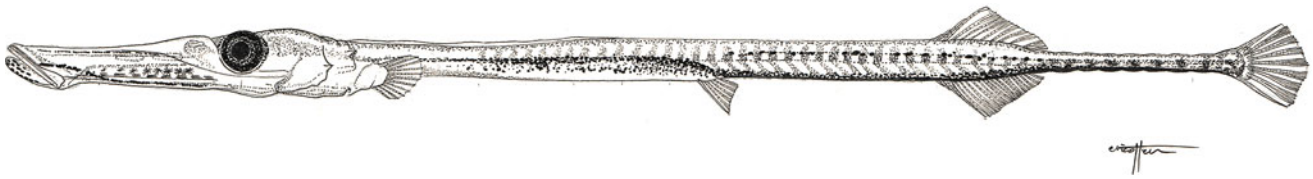

Figure 2. Illustration of a post-flexion West Atlantic trumpetfish, *Aulostomus maculatus*, collected in the Sargasso Sea using an Isaacs-Kidd Midwater Trawl during a multipurpose research survey conducted between March and April 2023. ©Eric Otten.

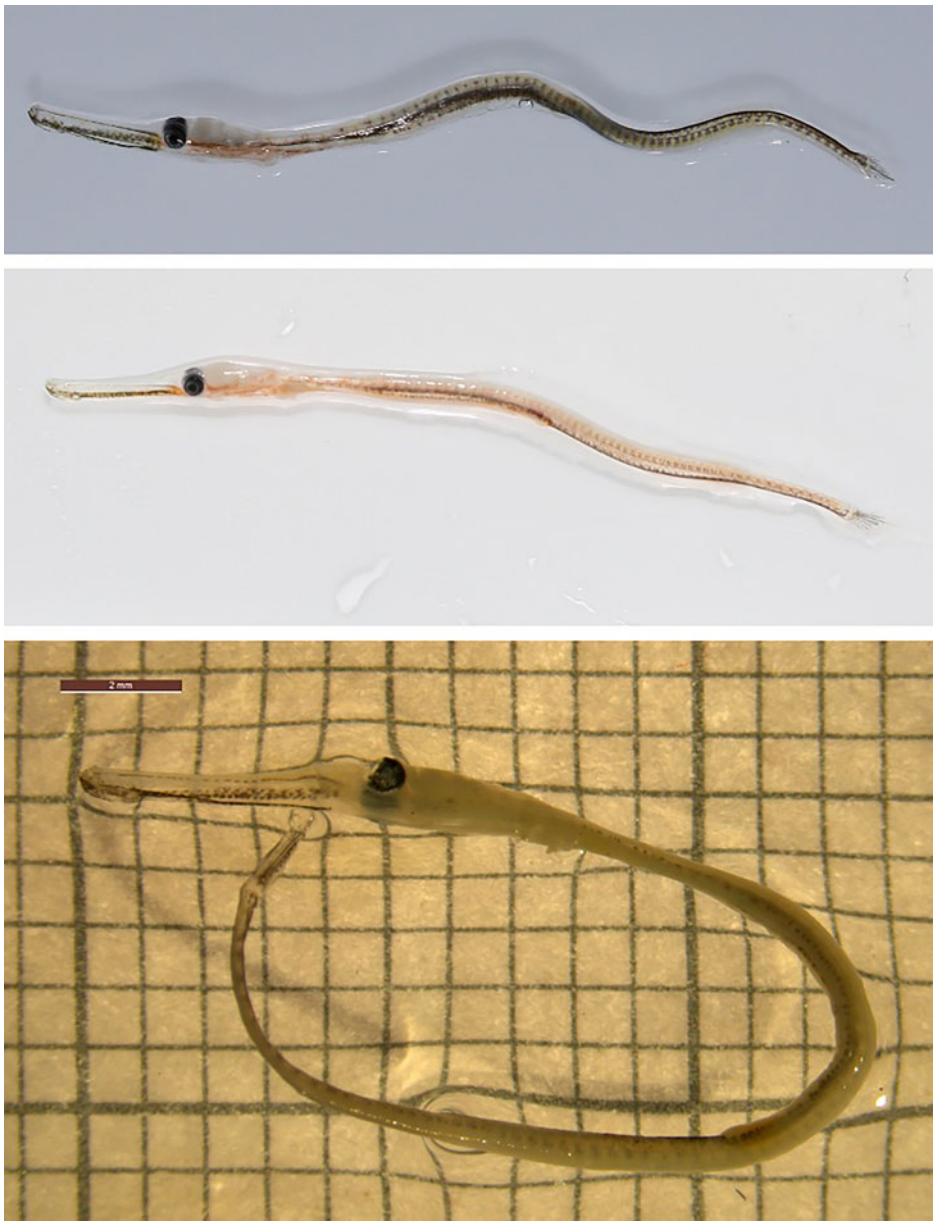

Figure 3. Post-flexion larvae of the West Atlantic trumpetfish, *Aulostomus maculatus*, collected in the Sargasso Sea using an Isaacs-Kidd Midwater Trawl during a multipurpose research survey conducted between March and April 2023. Photos of fresh samples taken on-board the research vessel upon collection, and of an ethanol stored sample.

2005). The caudal fin is rounded and convex (Figure 2). It should be noted that the sampled individuals showed signs of mechanical damage from the trawl (Figure 3). Although this did not cause any issues with species identification, it made it difficult to perform exact fin ray counts, in particular after fixation in ethanol. The total length of the two collected specimens that were photographed was 20.2 and 31.5 mm, snout length was 3.1 and 5.0 mm (Table 1). The average total length of the three collected specimens (after 6–8 days of fixation in 99% ethanol) was 26 mm (Table 1). The maximum length of adult *A. maculatus* varies between sources, from maximum 80 cm (Orr and Pietsch, 1998) to 100 cm (Sterrer and Schoepfer-Sterrer, 1986, Froese and Pauly, 2024). The collected specimens had a distinct line of pigment along the body, from the snout to tail (Figures 2 & 3). This has also been reported for pre-flexion larvae (Richards (2005), illustrated for an 8.2 mm (NL) larvae in Leis and Carson-Ewart (2000). This specific trait can be used to distinguish larval trumpetfish (Aulostomidae) from pipefishes (Syngnathidae), which also have an elongate body and snout (Richards, 2005). Except for the line of pigment, the samples were mostly translucent with a light pink colouration (Figure 3), however the individuals were collected dead from the trawl and hence photographed as such, meaning that colours could have already changed. As a result, colour was not used as a species identification trait. In adults,

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Table 1. Data for larval West Atlantic trumpetfish, *Aulostomus maculatus*, samples: date collected, collection site number (station), latitude and longitude, total length ethanol (measured on board 2023-04-06, i.e., after 6–8 days of fixation in 99% ethanol), total length (TL), standard length (SL), and snout length (from tip of snout to front edge of eye), measured from photos using ImageJ. The last specimen collected was not photographed, hence no measurements were made except for total length after six days fixation in ethanol

Date	Station	Lat (°N)	Long (°W)	TL ethanol (mm)	TL (mm)	SL (mm)	Snout (mm)
2023-03-29	16	22°30	66°59	31	31.5	29.9	5.0
2023-03-30	18	20°29	65°26	20	20.2	19.6	3.1
2023-03-31	22	20°30	63°59	28	NA	NA	NA

colour is variable, and has been studied to some extent in relation to possible colour change and colour matching of their 'host' species during shadowing (Eibl-Eibesfeldt, 1955; Wheeler, 1955; Aronson, 1983; Lochmann, 1989; Matchette *et al.*, 2022).

The distribution of *A. maculatus* ranges from the Bahamas and Bermuda to South America and St. Paul's Rocks off Brazil, including Gulf of Mexico and the Caribbean Sea (Bowen *et al.*, 2001; Pollom, 2015). Our three specimens were collected in the Sargasso Sea Subtropical Convergence Zone, between 20°29–22° 30 latitude and 66°59–63°59 longitude (Figure 1). This, while still in relative proximity to the continental shelf of the Antilles, is further offshore than most other records (Pollom, 2015; Froese and Pauly, 2024). In the Fishnet2 Portal, only two previous records were found that extended into the Sargasso Sea; one adult collected at 31°94 lat., 63°96 long. (location listed as: Sargasso Sea, Ocean Acre Area, Bermuda), and one larva collected at 37°63 lat., 66°73 long. (location listed as: North Sargasso Sea) (data accessed from the FishNet2 Portal, http://www.fishnet2.net, 2024-01-20). The samples collected here hence not only provide the first description of post-flexion *A. maculatus*, but also add valuable data on the distribution of this species and extends the geographic range of the pelagic larvae stage to include areas off the continental shelf.

Data. The data used for descriptive statistics are archived in the figshare repository, together with high-resolution images of the trumpetfish samples, the COI sequence alignment, and personal communication with Patzner regarding published information on *A. maculatus* eggs (https://figshare.com/s/03e82b45a394ce42b45a, DOI: 10.6084/m9.figshare.25198916), following best practices guidelines for public data archiving (Roche *et al.*, 2015).

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Competing interest. The authors have no *conflict* of interest to declare.

Ethical Standards. Animal ethical approval is not applicable for sampling in international waters.

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