

**FIRST REPORT ON CANNIBALISTIC FEEDING BEHAVIOUR
IN POST-FLEXION BLUEFIN LARVAE (*THUNNUS THYNNUS*)
OF THE BALEARIC SEA (NW MEDITERRANEAN)**

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SUMMARY

One of the major sources of mortality during the early life of fish is determined by their feeding ecology. The Atlantic bluefin tuna has been subject to heavy exploitation rates which has propitiated research in tuna larval ecology in recent times. This migratory top predator species spawns mainly in the Gulf of Mexico and in the Mediterranean. This study analyzes the trophic behavior of bluefin tuna larvae ranging from pre- to post-flexion developmental stages (2,7-8,5 mm standard length). Most of the larvae (94%) had at least one prey in its gut. With ontogeny, the diet shifted from copepods and cladocerans in pre-flexion stages to gasteropod larvae and filial bluefin tuna larvae in post-flexion stages (> 6mm). This is the first time that the Mediterranean spawned bluefin larvae are reported to have a cannibalistic feeding behavior.

RÉSUMÉ

L'une des principales sources de mortalité aux premiers stades de la vie des poissons est déterminée par leur écologie trophique. Le thon rouge de l'Atlantique a fait l'objet de taux d'exploitation intensive qui ont justifié la recherche sur l'écologie des larves thonières ces derniers temps. Cette espèce migratoire de prédateur supérieur fraie principalement dans le golfe du Mexique et en Méditerranée. La présente étude analyse le comportement trophique des larves de thon rouge, depuis le stade de pré-flexion jusqu'à la post-flexion (2,7-8,5 mm de longueur standard). La plupart des larves (94%) avaient au moins une proie dans leurs entrailles. Au cours de l'ontogénie, le régime est passé des copépodes et des cladocères pendant la phase de pré-flexion à des larves de gastéropodes et des larves filiales de thon rouge dans les stades de post-flexion (> 6mm). C'est la première fois que les larves de thon rouge frayées en Méditerranée ont un comportement d'alimentation cannibale.

RESUMEN

Una de las principales fuentes de mortalidad durante los primeros años de vida de los peces está determinada por su ecología alimentaria. El atún rojo del Atlántico ha sido objetivo de fuertes tasas de explotación, lo que ha propiciado la investigación sobre ecología de larvas de atún en los últimos tiempos. Esta especie migratoria de depredador superior desova principalmente en el golfo de México y en el Mediterráneo. Este estudio analiza el comportamiento trófico de las larvas de atún rojo desde etapas de desarrollo pre y post flexión (2,7-8,5 mm de talla estándar). La mayoría de las larvas (94%) tenía al menos una presa en su intestino. Con la ontogenia, la dieta cambió de copépodos y cladóceros en etapas pre-flexión a larvas de gasterópodos y larvas de atún rojo filial en etapas post-flexión (> 6 mm). Esta es la primera vez que se detecta que larvas de atún rojo desovadas en el Mediterráneo tienen un comportamiento de alimentación caníbal.

KEYWORDS

*Fish larvae, feeding behaviour, life history, predation, piscivory,
stomach content, zooplankton, genetics, Atlantic bluefin tuna, Thunnus thynnus*

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1. Introduction

The Atlantic bluefin tuna (BFT) is a migrating large predator that has two major spawning regions, the Mediterranean and the Gulf of Mexico. Depending on their origin, the bluefin resource is divided into two main stocks, the Eastern and Western stock. Some common features of their spawning habitat are conspicuous, such as a relatively high degree of oligotrophy (especially acute in the Mediterranean) in conjunction with intense hydrodynamics generating a pattern of frontal interphases and mesoscale eddy structures (Teo *et al.*, 2007; Laiz-Carrión *et al.*, 2015; Reglero *et al.*, 2011; Bakun and Broad, 2013). Thus, the low amount of available food for the initial developmental stages is compensated by these hydrographic structures that allow access to trophic resources for larvae to survive and grow. Bakun and Broad (2013) defined these survival strategies as loopholes from which

Thus, early-life feeding strategies under limited food resources are vital for survival. The TUNIBAL project (Alemany *et al.*, 2010) initiated in 2001 greatly increased previously low tuna larval catches in the Balearic Sea, allowing more representative tuna larvae feeding studies. Catalán *et al.* (2007) reported piscivorous feeding in early post-flexion albacore larvae (*T. alalunga*) while no signs of this feeding behavior appeared in BFT larvae (Catalán *et al.*, 2011). However, Reglero *et al.* (2011) hypothesized, from energetic models' viewpoint, that BFT larvae should become piscivores at post-flexion stages to be able to survive. On the other hand, BFT larvae from Gulf of Mexico showed an intensive piscivorous feeding behavior, since as much as 71% of the 8-10 mm bluefin larval size classes were positive for fish larvae (Llopiz *et al.*, 2015). These observations suggested piscivory also occurs in post-flexion BFT larvae from the Mediterranean, and its confirmation became the aim of the present study.

Larval BFT trophic research has also employed stable isotope analysis (SIA). To understand early life trophodynamics from SIA data of field-captured specimens, Uriarte *et al.* (2016) carried out a larval BFT rearing experiment. This experiment showed that in late-stage BFT larvae (after notochord flexion) the stable isotope of nitrogen (^{15}N) increased exponentially with age/size as a result of being fed reared gilthead seabream larvae (*Sparus aurata*). This pattern of increasing $\delta^{15}\text{N}$ signatures after notochord flexion has not been observed in wild-caught bluefin larvae (García *et al.*, in press), in contrast with the known piscivorous larvae of albacore (*T. alalunga*) (Catalán *et al.*, 2007) and bullet tuna (*A. rochei*) (Morote *et al.*, 2008). The growth of top predator larvae at determined ontogenetic stages demands increasing amounts of protein to meet the metabolic needs of development, and piscivory can fuel the energy expenditure of growth (Llopiz and Hobday, 2015). Despite that several tuna species are reported as piscivorous, none have been reported as cannibalistic.

This feeding habit can have two-sided consequences because, on one hand, predation on tuna siblings can lead to losses of potential recruits (Reglero *et al.*, 2011), but alternatively, cannibalism can achieve the necessary energy requirements for faster growth rates leading to increased survival (Bakun and Broad, 2013). Here we report on the trophic ecology of bluefin larvae in the Mediterranean, including their predation on fish larvae genetically identified as *Thunnus thynnus*, observed during the 2014 bluefin spawning season.

2. Methods

2.1 Field sampling

Larvae were collected during the Bluefin 2014 ichthyoplankton survey, carried out in the Balearic Sea (Western Mediterranean) from June 17 to July 3, coinciding with the BFT spawning season. A total of 98 systematic stations were sampled from the R/V SOCIB. Ichthyoplankton and hydrographic data (CTD) were sampled at each station during daylight hours. Larvae were collected using a square-mouth, 90-cm diameter bongo net carrying out undulating subsurface hauls outfitted with 500, 1000, and 2000 μm mesh nets. Bluefin tuna larvae were sorted on board from the plankton samples and a subset of larvae were preserved in 96% ethanol for carrying out this trophic study. The stations where these larvae were selected for this study are shown in **Figure 1**.

2.2 Laboratory procedures

Preserved individual larvae were identified, photographed and measured for standard length (SL) and mouth size using ImageJ image analysis software, following the method described by Catalán *et al.* (2011). For analyzing gut contents, the entire alimentary canal of each larva was first dissected out, and the gut content was examined with a Leica M205 stereomicroscope, following the method described by Llopiz *et al.* (2010). The consumed prey were identified, measured and enumerated. Fish larvae found in the guts were cleaned of predator residue (**Plate 1**) and stored in ethanol prior to carrying out morphological and genetic identification.

2.3 Genetic identification of preyed fish larvae

Genetic identification of larvae was carried out as described by Puncher *et al.* (2015). Briefly, DNA was extracted from tissue of consumed larvae, and mitochondrial cytochrome C oxidase I (COI) or internal transcribed spacer region 1 (ITS1) were amplified by PCR and sequenced (Stabvida, Caparica, Portugal). Sequence similarity was analyzed according to top percent similarity scores with reference sequences of public databases. COI sequences were submitted to the Barcode of Life Database (BOLD) Identification System (<http://v4.boldsystems.org>) for comparison with all animal species-level barcode records (2,706,083 sequences for 175,327 species and 64,400 interim species at 1 February 2017). The nucleotide Basic Local Alignment Search Tool (BLAST) on the NCBI website (<https://blast.ncbi.nlm.nih.gov/>) was used to analyze sequence similarity of ITS1 sequences. In addition, COI sequences were aligned in MEGA7 (Kumar *et al.*, 2016) using the ClustalW algorithm in order to examine characteristic attributes capable of distinguishing scombrids in the Mediterranean Sea described by (Puncher *et al.*, 2015).

3. Results

3.1 Gut contents

Gut contents were examined from 105 *T. thynnus* larvae collected from 11 stations and ranging from 2.7 to 8.5 mm SL, with a mean of 5.11 mm (SD 1.35) (**Table 1**). A total of 99 larval samples contained at least one prey item in the stomach, which resulted in a feeding incidence of 94%. Although the number of preys ingested by larvae was lower in post-flexion larvae, their prey items were larger. Six BFT larvae from the station with higher density and wider size range of BFT larvae were found to be piscivorous.

Diet composition (by prey numbers) changed with increasing length of larvae (**Figure 2**). Copepods and cladocerans were the most abundant and important prey found in pre-flexion larvae (prior to 6 mm SL). In decreasing importance, the cyclopoid *Farranula* sp. and cladocerans were the most abundant. After 6 mm SL, a diet shift is observed with the introduction of gastropods into the diet, and beyond 7 mm SL, piscivory is observed. An example of an ingested larval fish is shown in Plate 1. The average prey sizes consumed by BFT larvae increased with increasing mouth size and SL (**Figure 3a, b**), exhibiting a significant exponential relationship.

3.2 Genetic identification of consumed larvae

Extracted fish larvae from larval BFT guts showed the morphological attributes of *Thunnus* larvae. However, genetic identification was needed to assure their identification. Community taxonomic information obtained from replica fixed in formaldehyde indicated that the most abundant species at the station where piscivorous BFT larvae were found was in fact *T. thynnus*. The only other scombrid species was *A. rochei*, and therefore the genetic analysis was aimed to discriminate these scombrid species

DNA was extracted from six consumed fish larvae recovered from five BFT larvae, and fragments of mitochondrial COI and nuclear ITS1 were amplified and sequenced. The gut contents recovered from one BFT larvae were not subject of genetic identification as they were too digested and the absence of visible predator tissue residues could not be ensured. Five larvae were genetically identified as *T. thynnus* by BOLD's Identification System (<http://v4.boldsystems.org>) with 100% similarity scores for the top 99 matches, and the sequences obtained from one larva were identified as *Thunnus alalunga* (99.8-100% similarity scores). Characteristic attributes capable of distinguishing scombrids in the Mediterranean Sea described in Puncher *et al.* (2015) were examined confirming BOLD's assignment. Due to the fact that *T. alalunga* mitochondrial DNA has introgressed into *T. thynnus* (Alvarado Bremer *et al.*, 2005), the single larva identified as *T. alalunga* according to mitochondrial COI was further barcoded for nuclear ITS1 and identified as *T. thynnus*, as expected from the observation of the taxonomic study of replica samples. Therefore, all consumed fish larvae recovered from BFT stomach contents were genetically identified as *T. thynnus*.

4. Discussion

These results showed a high incidence of feeding (94%) similar to that described by Catalán *et al.* (2011) in the Mediterranean and Llopiz *et al.* (2015) in the Gulf of Mexico. Nevertheless, observed differences in the diet by size class of BFT larvae were distinguished by the decrease in consumption of copepods and cladocerans with larval size and the corresponding shift in the diet in post-flexion stages where gastropods and fish larvae were the most important prey.

This is first time that piscivory in BFT larvae is attributed to cannibalism in the Mediterranean. Piscivory is rather common in large BFT larvae (>8 mm) in the Gulf of Mexico (Llopiz *et al.* 2015), where cannibalism was also observed. However, this was performed by visually identifying 2 of the 14 extracted scombrid larvae as BFT (other larvae were too digested to confirm their species). Thus, it seems plausible that part of this piscivorous feeding behavior could correspond to sibling cannibalism which is commonplace in BFT larval rearing (Miyashita *et al.* 2001). Although cannibalism may be seemingly detrimental for population growth, there are a number of fish species that resort to this trophic behavior, proving beneficial for survival and development (Payne *et al.*, 2002; Reglero *et al.* 2011). BFT larvae cannot solely survive on zooplankton diets and consequently must prey on fish larvae, which provide the sufficient bioenergetics for survival (Reglero *et al.*, 2011).

Despite piscivory in Mediterranean BFT larvae being evident here, it has not been observed in previous stomach content studies (Catalán *et al.*, 2011) nor in another trophic study based on stable isotope analysis (García *et al.*, in press). Uriarte *et al.* (2016) observed in a BFT larval rearing experiment that $\delta^{15}\text{N}$ signatures increased at post-flexion stages as a result of being fed with yolk sac larvae of gilthead seabream. Additionally, piscivory at early life stages in bullet tuna and albacore larvae suggests that larval predation occurs upon initiating post-flexion (García *et al.*, in press).

The fact that piscivory and cannibalism was observed at only one station out of the 11 sampled, and that this station had the high abundances of fish larvae, indicate that piscivory and cannibalism is likely to be density dependent. Also needed for piscivory is a broad range of size classes—larvae large enough to be capable of piscivory and larvae small enough to be consumed. The station where piscivory was present had the broadest size range of BFT larvae (3.68 – 8.47mm SL) as well as the largest BFT larvae (average SL=6.56 ± 0.96). Since the analyzed specimens of BFT larvae were preserved in 96% ethanol, yielding a large amount of shrinkage, it can be assumed that the specimens showing piscivory were in the advanced post-flexion stages, as hypothesized by García *et al.* (in press).

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References

- Alemany F., Quintanilla L., Velez-Belchí P., *et al.* (2010). Characterization of the spawning habitat of Atlantic bluefin tuna and related species in the Balearic Sea (western Mediterranean). *Prog. Oceanogr.* 86: 21-38.
- Alvarado Bremer, J.R., Viñas, J., Mejuto, J., Ely, B., and Pla, C. (2005). Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Mol. Phylogenet Evol* 36, 169–187.
- Bakun, A., and Broad, K. (2013). Environmental ‘loopholes’ and fish population dynamics: comparative pattern recognition with focus on El Niño effects in the Pacific. *Fish Oceanogr.* 12(4–5):458– 473
- Catalán, I.A., Alemany, F., Morillas, A. and Morales-Nin, B. (2007). Diet of larval albacore *Thunnus alalunga* (Bonnaterre, 1788) off Mallorca Island (NW Mediterranean). *Sci Mar.* 71(2): 347-354
- Catalán, I.A., Tejedor, A., Alemany, F. and Reglero, P. (2011). Trophic ecology of Atlantic bluefin tuna *Thunnus thynnus* larvae. *J Fish Biol.* 78: 1545–1560
- García, A., Laiz-Carrión, R., Uriarte, A., Quintanilla, J.M., Morote, E. Rodríguez J.M. and Alemany, F. (in press) Differentiated stable isotopes signatures between pre- and post-flexion larvae of Atlantic bluefin tuna (*Thunnus thynnus*) and of its associated tuna species of the Balearic Sea (NW Mediterranean). *Deep Sea Res II.*
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 33, 1870–1874.
- Laiz-Carrión R., Gerard. T., Uriarte A., *et al.* (2015). Trophic ecology of Atlantic bluefin tuna (*Thunnus thynnus*) larvae from the Gulf of Mexico and NW Mediterranean spawning grounds: a comparative stable isotope study. *PLoS One* 10: e0138638.
- Llopiz J.K., and Hobday, A.J. (2015) A global comparative analysis of the feeding dynamics and environmental conditions of larval tunas, mackerels, and billfishes. *Deep Sea Res Part II: Topical Studies in Oceanography* 113: 113-124
- Llopiz J.K., Richardson D.E., Shiroza A., Smith S.L. and Cowen, R.K. (2010) Distinctions in the diets and distributions of larval tunas and the important role of appendicularians. *Limnol Oceanogr* 55:983-996
- Llopiz, J.K. Muhling, B.A. and Lamkin, J.T. (2015) Feeding dynamics of Atlantic bluefin tuna (*Thunnus thynnus*) larvae in the Gulf of Mexico. *Collect. Vol. Sci. Pap. ICCAT* 71(4): 1710-1715
- Miyashita, S., Sawada, Y., Okada, T., Murata, O. and Kumai, H. (2001) Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae). *US Fish. Bull.* 99(4): 601-616.
- Morote, E.M., Olivar, P., Pankhurst, P., Villate, F. and Uriarte, I. (2008). Trophic ecology of bullet tuna *Auxis rochei* larvae and ontogeny of feeding-related organs. *Mar Ecol Prog Ser* 353: 243–254
- Payne, A. G. Smith C. and Campbell A. C. (2002) Filial cannibalism improves survival and development of beaugregory damselfish embryos. *Proc. R. Soc. Lond. B*, 269: 2095–2102
- Puncher, G.N., Arrizabalaga, H., Alemany, F., Cariani, A., Oray, I.K., Karakulak, F.S., Basilone, G., Cuttitta, A., Mazzola, S., and Tinti, F. (2015). Molecular Identification of Atlantic Bluefin Tuna (*Thunnus thynnus*, Scombridae) Larvae and Development of a DNA Character-Based Identification Key for Mediterranean Scombrids. *PLOS ONE* 10, e0130407.
- Reglero, P., Urtizberea, A., Torres, A. P., Alemany, F. and Fiksen Ø. (2011) Cannibalism among size classes of larvae may be a substantial mortality component in tuna. *Mar Ecol Prog Ser*, 433: 205–219.

- Teo, S.L.H., Boustany, A. and Block, B.A. (2007). Oceanographic preferences of Atlantic bluefin tuna, *Thunnus thynnus*, on their Gulf of Mexico breeding grounds. *Mar Biol.* 152: 1105–1119
- Uriarte, A., García, A., Ortega, A., De La Gándara, F., Quintanilla, J. and Laiz-Carrión, R. (2016). Isotopic discrimination factors and nitrogen turnover rates in reared Atlantic bluefin tuna larvae (*Thunnus thynnus*): effects of maternal transmission. *Sci Mar.* 80(4): 447-456

Table 1. Number and size range of ethanol-preserved BFT larvae by station examined for gut contents and the number of larvae observed to be piscivorous.

Station	N	SL (mm)	Mean SL (mm) \pm SD	Piscivory
1146	5	3.2 – 5.6	4.29 \pm 1.14	-
1194	5	4.03 – 4.58	4.36 \pm 0.20	-
1234	8	4.77 – 5.69	5.39 \pm 0.30	-
1282	35	3.68 – 8.47	6.56 \pm 0.96	6
1323	5	3.37 – 4.98	3.98 \pm 0.68	-
1499	5	3.82 – 4.89	4.33 \pm 0.44	-
1501	6	3.39 – 3.71	3.57 \pm 0.13	-
1595	8	3.50 – 4.27	3.87 \pm 0.27	-
1795	9	3.41 – 4.21	3.85 \pm 0.29	-
1797	8	2.69 – 6.05	4.16 \pm 1.26	-
1801	10	4.72 – 5.47	5.03 \pm 0.24	-

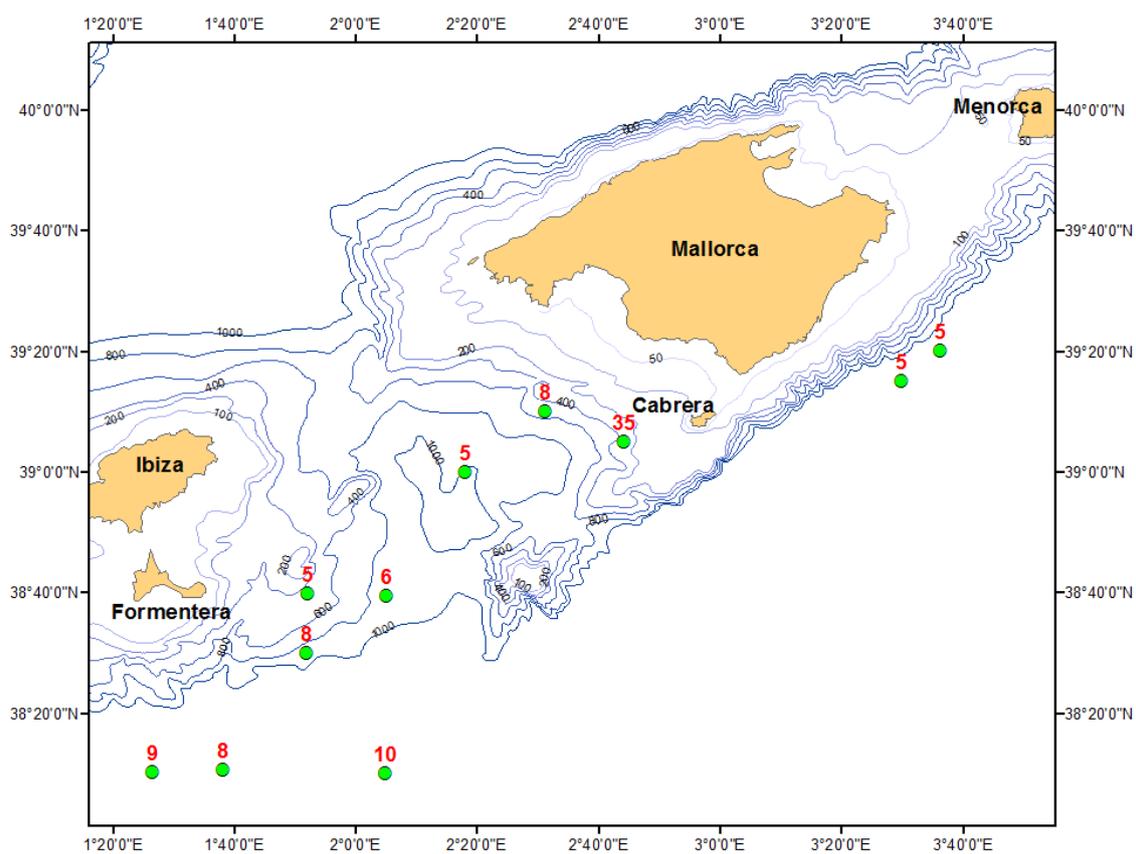


Figure 1. Stations where BFT larvae were selected for this study. The numbers in red correspond to the number of larvae analyzed.

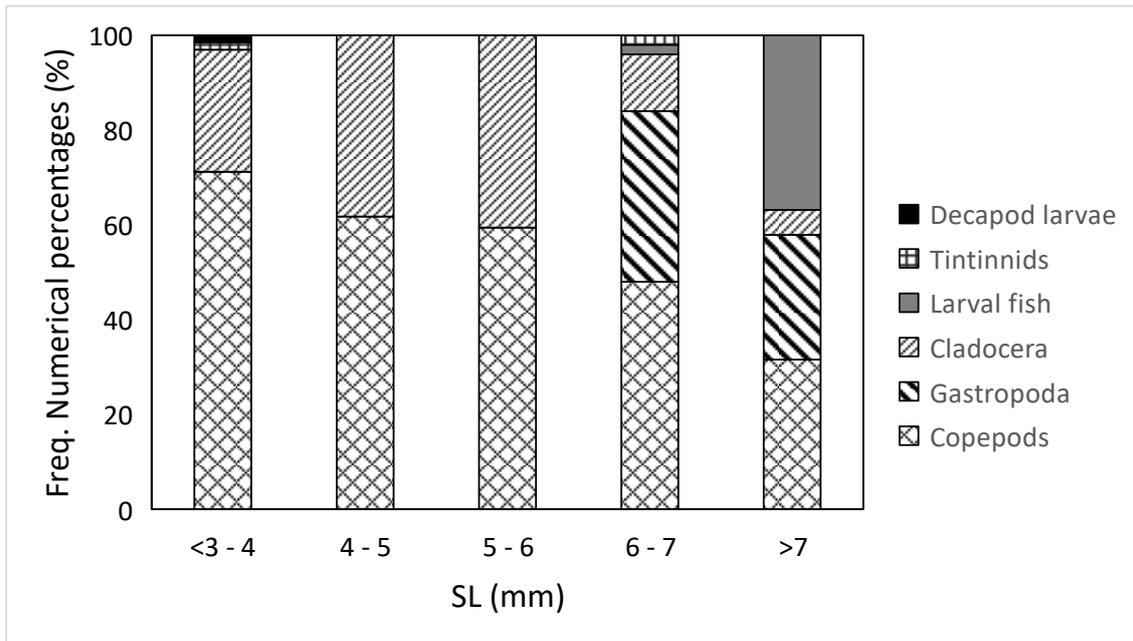


Figure 2. Numerical percentages of prey types by length class of BFT larvae.

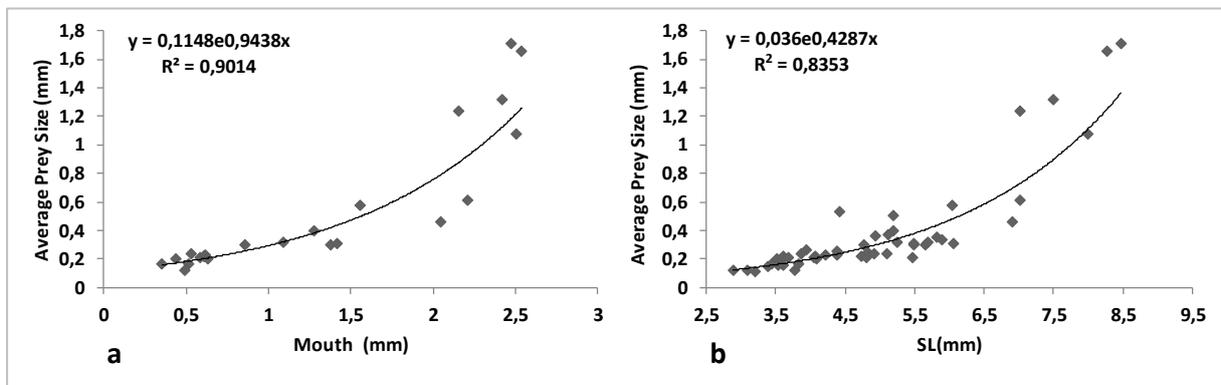


Figure 3. Relationship between average prey size with mouth size (a) and SL (b).

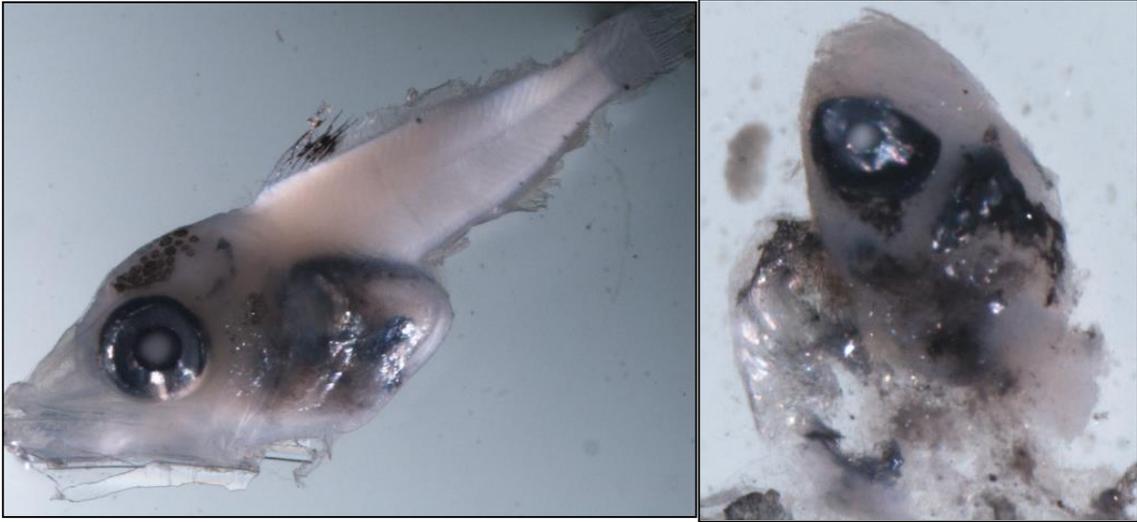


Plate 1. Picture on the left shows a BFT larva containing in its gut a fish larva whose eyes can be observed through the gut wall. Picture on the right is the extracted fish larva.