CHANGES OF BLUEFIN TUNA (*THUNNUS THYNNUS*) LARVAE FISHING METHODS OVER TIME IN THE WESTERN MEDITERRANEAN, CALIBRATION AND LARVAL INDICES UPDATING

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SUMMARY

Bluefin tuna larval abundances in the western Mediterranean Sea provide a fishery independent estimator of the temporal trends of the eastern stock spawning biomass of this species. The index is calculated from time series of ichthyoplankton surveys where fishing techniques (gear type and tow depth, changed over time. Here we revise assumptions on previous gear standardization methods by studying the vertical distribution of bluefin tuna larvae in the Balearic Sea, providing a new gear calibration approach to improve larval index calculations. We apply the new calibration to update larval indices including data from the 2015 ichthyoplankton survey. Results show that fishing techniques covering the full range of the larval vertical distribution can be standardized to calculate larval indices, ensuring that observed temporal variability is not derived from differences in fishing methods. The larval index shows an increase in abundances from the 2001-2005 period to the 2012-2015 period, with a fluctuating trend over the last years.

RÉSUMÉ

Les abondances des larves de thon rouge dans la mer de la Méditerranée occidentale constituent un estimateur indépendant des pêcheries des tendances temporelles de la biomasse reproductrice du stock oriental de cette espèce. L'indice a été calculé à partir de séries temporelles de prospections d'ichthyoplancton dans le cadre desquelles les techniques de pêche (type d'engin et profondeur de remorquage) ont changé au cours du temps. Dans le cadre du présent document, les postulats consacrés aux méthodes antérieures de standardisation des engins ont été révisés en étudiant la distribution verticale des larves de thon rouge dans la mer des Baléares, en fournissant une nouvelle approche de calibration des engins visant à améliorer les calculs de l'indice larvaire. La nouvelle calibration a été appliquée pour actualiser les indices larvaires, y compris les données de la prospection d'ichthyoplanctons de 2015. Les résultats montrent que les techniques de pêche couvrant toute la gamme de la distribution verticale des larves peuvent être standardisées pour calculer les indices larvaires, en veillant à ce que la variabilité temporelle observée ne soit pas calculée à partir des différences de méthodes de pêche. L'indice larvaire présente une augmentation des abondances de la période 2001-2005 à la période 2012-2015, avec une tendance fluctuante au cours des dernières années.

RESUMEN

Las abundancias de larvas de atún rojo en el Mediterráneo occidental proporcionan un estimador independiente de la pesquería de las tendencias temporales de la biomasa reproductora del stock oriental de esta especie. El índice se calculó a partir de series temporales de prospecciones de ictioplancton en las que las técnicas de pesca (tipo de arte y profundidad de remolque) cambiaban con el tiempo. En el documento se revisan los supuestos de métodos anteriores de estandarización del arte mediante el estudio de la distribución

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vertical de las larvas de atún rojo en el mar Balear, ofreciendo un nuevo enfoque de calibración del arte para mejorar los cálculos del índice larvario. Se aplicó la nueva calibración para actualizar los índices larvarios, incluyendo los datos de la prospección de ictioplancton de 2015. Los resultados muestran que las técnicas de pesca que cubren toda la zona de distribución vertical de las larvas pueden estandarizarse para calcular índices larvarios, garantizando que la variabilidad temporal observada no se deriva de las diferencias en los métodos de pesca. El índice larvario muestra un aumento en la abundancia desde el período 2001-2005 hasta el período 2012-2015, con una tendencia fluctuante en los últimos años.

KEYWORDS

Abundance, bluefin tuna, catchability, fish larvae, fishery sciences, mathematical models, pelagic environment, spawning, tuna fisheries

Introduction

In 1993, data from the monitoring of Atlantic bluefin tuna early life stages in the Gulf of Mexico allowed developing a fisheries independent index of abundance for the Western stock spawning biomass (Scott *et al.* 1993). Since then the larval index of the western stock has been applied to identify temporal trends in the population improving assessment models applied in the framework of ICCAT. During the last decade the techniques to estimate the index were improved by the application of the delta log-normal models (Ingram *et al.* 2008). This approach, appropriated for zero-inflated distributed data, implied a relevant advance in the standardization of larval catches regarding changes in fishing gear, effort and timing.

In 2013 similar modeling techniques allowed developing a larval index for the Eastern stock population of bluefin tuna (Ingram *et al.* 2013). The larval index is calculated from data collected during ichthyoplankton surveys in the Balearic Sea during the bluefin tuna reproductive season since year 2001. From then onwards, ichthyoplankton surveys have been conducted using two different types of bongo nets and meshes, bongo 90 (B90) fitted with 500 microns meshes and bongo 60 fitted with 333 microns ones, towed at three different depths. Therefore, three different of data sets of larval abundances are available (see **table 1**): i) the bongo 60 (B60) deep oblique towed down to 70 meters depth ("B60do", available from 2001 to 2005 campaigns); ii) the bongo 90 subsurface, towed down to 5 meters depth ("B90ss", available during the 2004 and 2005 campaigns); and iii) the bongo 90 oblique towed down to the thermocline, throughout the mixed layer depth, what is about 20-30 meters in the study area during the June-July months ("B90ml", in the 2012-2015 campaigns).

During the 2004 and 2005 campaigns, the B60 deep oblique and B90 subsurface tows were conducted in all stations. This replication of the two fishing techniques allowed including the factor "gear type" in the delta log-normal models. Using this standardization method assumed that the catchability ratio between B60do, B90ss is constant, and that the catchability of the B90ss and the B90do is equal. These two assumptions imply accepting a homogeneous structure of the Bluefin tuna larval community in abundances and lengths from surface to the maximum depth of the larval distribution in the water column. A non-homogeneous vertical distribution would cause the B60ss to provide information on larval abundances and lengths non representative for the entire water column, that would bias the calibrations between the B60do and B90ml sampling gears. If so, differences in the larval index values between the 2001-2005 period and the 2012-2015 period could be influenced by changes in fishing techniques. An alternative option for the standardization of the catches from the different fishing methodologies is to develop experimental fishing tows over larval patches of various densities and calculate calibration functions to be applied before the computation of the delta lognormal models.

Here we present the summary of experimental fishing activities directed to investigate the vertical distribution of the Bluefin tuna larvae community and to improve the calibration among fishing methods. We also present the results of the larval index update in the Balearic Islands, computed considering the results from the calibrations among the different methodologies and including data till 2015.

2. Material and methods

2.1 Vertical distribution of larvae

Two research surveys were conducted off the Balearic Islands, Western Mediterranean, in 2011 and 2012, including two different sampling strategies. The first consisted on series of ichthyoplankton oblique hauls from 30 m depth using bongo 90 nets equipped with 500 micrometers meshes over a systematic 10 by 10 miles grid, aiming at detecting areas with high density of tuna larvae. Once one of these dense identified tuna larval patch was considered appropriate for vertical distribution studies, its position was monitored with an Iridium lagrangian buoy and a series of hauls using multiple opening-closing plankton nets were performed. In 2011, an HYDRO-BIOS multi-net was used, sampling five depth strata (0-5 m, 5-10 m, 10-15 m, 15-20 m, 20-30m), whereas in 2012, the sampling device was a Multiple Opening Closing Net and Environmental Sensing System (MOCNESS), which allowed sampling six depth strata (0-10m, 10-20 m, 20-30m, 30-40m, 40-50m, 50-60m). The net mouth openings were 0.25 and 1 m^2, respectively, and the mesh size was 333 μm for both nets. Both devices were repeatedly towed at ~2 knots during day and night. While following the tuna larval patches, 14 stratified vertically stratified sampling operations between the 20-22nd of June 2011 and 5 between the 9-11th of July 2012, were conducted. Once in the laboratory, samples were subsequently sorted for fish larvae, which were identified to species level following Alemany (1997). Mean larvae densities (number of larvae/100 m^3) were computed separately for day (between sunrise and sunset) and night (between sunset and sunrise) fishing operations.

2.2 B90 and B60 gear catchability analyses

In the year 2013 a bluefin tuna larvae sampling campaign was conducted around the Balearic Islands from 20th June to 5th July, using bongo 90 nets fitted with 500 microns meshes towed down to 20 – 30 meters, covering the whole mixed layer depth in this area and season (Torres *et al.* 2014). Fishing operations were conducted at low speeds, around 2 knots, during 8–10 minutes, similarly as the standard procedure used during the period 2012-2015. Right after the bongo 90 fishing operations, the presence of bluefin tuna was evaluated and plankton samples were immediately preserved with 4% formalin buffered with borax. In 13 stations, where larval catches were positive for BFT, the boat returned to the initial location of the first fishing operation and carried out a second tow using bongo 60 nets fitted with 333 microns meshes towed down to 70 meters depth, similarly to the standard procedure used during the period 2001-2005. Once in the laboratory, number of larvae were counted and standardized to capture per unit area (CPUA, equation 1), obtaining the total larvae abundance per square meters. The functional relationship between of B90ml and B60do CPUAs was modelled using an exponential curve.

(1) CPUA= (Number of larvae / volume filtered (m^3)) x Tow depth (m)

2.3 Updating the western Mediterranean Bluefin tuna larval index

The larval indices for the western Mediterranean were computed from the ichthyoplankton surveys around the Balearic sea (See **figure 1**) along 9 years between 2001 and 2015 (see **table 1**). Calculation of the habitat corrected larval index was based on the delta lognormal modeling approach that includes a variable (potential habitat quality indicator, "PHAB") accounting for the probability of that sample to be within appropriate larval habitat (see details in Ingram *et al.* 2015). This update of the larval index presents three major modifications in relation to previous versions presented in ICCAT meetings (Ingram 2013 & 2015): 1) the B90 subsurface samples from the 2004 and 2005 campaigns have been excluded from the computation; 2) the functional equation relating B90do and B90ml obtained in section 2.2 have been applied to standardize abundances among gears; and 3) the numbers of larvae at each station have been adjusted to the number of 2 millimeter larvae using the decay in numbers at size derived from a unique length-based catch curve, computed using an exponential model from samples of both gears. Previous updates used separate curves for each gear.

Definitions of input variables for the delta lognormal model are presented in **table 2**. For the variable selection all possible models were developed while including the year factor in order to investigate the inter-annual variability of the larval index (see Ingram et al, 2015 for details). To estimate the PHAB variable associated to each sampled station for a given year, the dataset (nine years of data), was split into two datasets, the prediction data set and the fitting datasets. The first set contained data from the considered year and the second one data from the other eight years. Using the fitting data set, a quasi-binomial general additive model (GAM, Wood 2006) was designed to fit the larvae presence to the following variables: latitude, longitude, sea surface salinity, day of the year and residual sea surface temperature (defined as the residual of sea surface temperature against

the day of the year, as both variables were strongly correlated). The fitted model was then applied to predict larval presences in the considered year. This inter-annual cross-prediction approach allowed considering non linear effects of environmental variables on the prediction of larval habitat (see details in Alvarez-Berastegui 2016). This process was applied for each sampling campaign, so predictions of PHAB for each year were always based on data from the remaining eight years. All calculations related to the PHAB were processed with the R software (Rdevelopment Core Team, 2008) using the MGCV package (Wood 2006).

We provide descriptive statistics (Box whisker plots) showing the inter annual variability of relevant parameters affecting the standardization of larval catches: filtered volumes, individual larval lengths, and hydrographical parameters (temperature and salinity in the mixed layer depth) affecting the spatial distribution of spawning habitats in the area (Reglero 2012, Alvarez Berastegui 2016)

3. Results

3.1 Vertical distribution of larvae

The mean larval densities from the 5 MOCNESS operations show a vertical distribution limited to the first 20 meters (**figure 1.A**), corresponding with the mixed layer depth (Torres *et al.* 2014). The sampling design using the HYDROBIOSS multinet allowed a more precise analysis of the vertical distribution of the larvae within the 0-20 meters depth range (**figure 1.B**). During the day, maximum densities appear in the first 10 meters while the maximum values during the night were found below 10 meters, with a peak on the 15 meters strata.

3.2 B90 and B60 gear catchability analyses

CPUA (number of larvae/m^2) obtained from the B60 deep oblique and B90 mixed layer oblique follow a exponential relationship (**Figure 2**, equation 2) with a $R^{2=}0.998$.

*(2) CPUA-B90ml = (a*CPUA B60do)*(exp(b*CPUA B60do); a= 0.5823 ; b=0.00115*

This function is then selected for standardization of the abundances from the B60 deep oblique fishing collected during the first five years of the time series, in relation to the abundances of B90 mixed layer currently applied as standard methodology.

3.3 Updating the western Mediterranean Bluefin tuna larval index

The result of the adjustment of the exponential model (equation 3) to fit the decay in the number of larvae at length is presented in **figure 4** (R squared =0.999).

(3) $N_{2mm} = a\ (EXP^{(-b\ (length))})$; N2mm= number of larvae at 2 millimeters; a= 1092944,8; b= 1,52144;

The total number of bluefin tuna larvae per year collected during the ichthyoplankton surveys is presented in **table 3**, together with the number of larvae at 2 mm and nominal abundance parameters (frequency, density and CPUA). All parameters show a general increasing trend over the time series. The mean number of larvae fished in the period 2012-2015 is 91 times higher than the number of larvae fished during the period 2001-2005. The larval indices are provided in **table 4**, together with dispersion parameters and the "scaled index" (larval index scaled to a mean of 1, **figure 5**). The larval indices confirm the increasing trend of abundances among the two periods. For the 2012-2015 period the index fluctuates around a mean value that is over five times higher than the mean of the 2001-2005 period.

Box whisker plots show that the campaigns used for the larval index calculation were developed at variable conditions of salinity and temperature distributions (**Figure 6**). Fishing efforts per tow (volume filtered) were homogeneous for the two gears used (**Figure 7**). Length distribution of larvae presents variability along years with higher mean lengths in year 2014 (**Figure 8**).

4. Discussion

The analyses here presented show that the vertical distribution of Atlantic Bluefin tuna (*Thunnus thynnus*) larvae collected around the Balearic archipelago is restricted to the mixed layer, which is above 20-30 meters depth during the spawning season (Torres *et al*. 2014). Within this bathymetric range the abundance of larvae is not uniformly distributed, and the abundance peak can be found at different depths indifferent years. This is relevant regarding the design of methods to obtain quantitative data for the calculation of larval indices and also for the standardization of larval abundances collected with different fishing techniques.

The results from the experimental fishing with oblique bongo 90 towed through the mixed layer (down to 20-30 meters) and the bongo 60 deep oblique (towed down to 70 meters), show that the capture per unit area (CPUA, number of larvae/m^2) obtained from both methods can be standardized through an exponential relationship with optimal results (R^2>0.99). The ratio of the CPUA between the two gears is close to one (=1.2), demonstrating that the two methods present similar catchability. Then we conclude that time series of larval abundances from the ichthyoplankton surveys using these two methodologies in the study area, can be appropriately standardized to calculate larval indices, ensuring that observed temporal variability is not derived from differences on fishing methods. On the other hand, computing larval indices from ichthyoplankton surveys using fishing tows not covering the full bathymetric range of the larvae may derive in biased results, as captures may not be representative for the total abundance in the water column and could also present selectivity for specific lengths. Changes in length selectivity is a relevant issue for the computation of larval indices since the index rely in larval abundances at 2 millimeters calculated from decay curves obtained from length distributions.

The updated larval index confirms a change in the population status from the period 2001-2005 to the period 2012-2015. The fluctuations of the larval index over the last years may indicate a new equilibrium on the adult population. Following updates will allow verifying whether the abundances follow an increasing trend in the last five years or if they vary around a mean value.

The box and whisker plot of the fishing effort per tow (volume filtered) shows no relevant differences among field work campaigns. In relation to the hydrographical conditions, the inter annual variability of the mean salinity in the mixed layer depth show that during later years, mainly 2012, 2013 and2014, values are higher. Changes on the temperature are more relevant, probably due to the combined effect of the inter annual variability of the environmental conditions and changes on the dates of the campaigns, especially for the 2003. These results reinforce the need of developing larval index models that properly account for the environmental variability over the survey years, allowing to standardize campaigns to the larval habitat sampled which is strongly affected by hydrographic conditions (Alemany *et al.* 2010, Muhling *et al.* 2013). Other way, inter annual variability of the indices could be strongly affected by spatio-temporal changes on the location of those habitats.

It is worth noting the variability on the length distributions of the larvae among years. The correct standardization to individuals at any length to the number of larvae at two millimeters from the decay curves is relevant for the calculation of larval indices. At present that calculation does not consider the temperature effects on larval growth. Further developments on the growth and mortality models as function of the environmental conditions could improve the quality of the larval indices.

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Table 1. Ichthyoplankton surveys, type of fishing and sampling effort.

Year	Dates	Gear/Haul type	Number of samples	Mean tow depth
2001	16Jun-7Jul	B60 deep oblique	162	70
2002	7Jun-30Jun	B60 deep oblique	171	70
2003	3Jul-29Jul	B60 deep oblique	198	68
2004	18Jun-10-Jul	B60 deep oblique	166	69
		B90 subsurface*	166	3
2005	27Jun-23Jul	B60 deep oblique	186	69
		B90 subsurface*	187	3
2012	21Jun-9Jul	B90 mixed layer oblique	153	32
2013	20Jun-10Jul	B90 mixed layer oblique	124	27
2014	12Jun-29Jun	B90 mixed layer oblique	92	28
2015		B90 mixed layer oblique	94	24

***** Samples excluded in the 2015 update of the larval index.

Table 2. Candidate variables for the binomial and log-normal submodels of the delta modeling approach.

Variable name	Acronym	Definition	Variable type
CPUA of larvae at 2mm	CPUA2mm	Capture per 10 m^2 area of larvae back calculated to 2mm using in situ decay function	Response variable
Year	Year	Year of sampling (n=9)	Factor
Day of the year	DY	From 1 to 365	Continuous
Day-night	DN	2 level variable (day or night)	Factor
Potential habitat quality indicator	PHAB	Probability of the sample to be located within larval habitat (0 to 1)	Continuous

Table 3. Number of larvae and nominal abundance parameters.

Year	*Number Larvae*	*Number Larvae at 2mm*	*Nominal Frequency*	*Nominal density*	*Nominal CPUA*
2001	121	6926	0.166	0.23	16.75
2002	135	2592	0.093	0.08	5.63
2003	211	39258	0.121	0.95	67.17
2004	264	66511	0.150	2.21	154.70
2005	182	67760	0.198	1.85	129.95
2012	28764	937181	0.686	12.06	361.97
2013	24749	524777	0.620	9.05	271.74
2014	3456	299090	0.521	7.40	222.10
2015	10106	594017	0.776	13.62	408.79

Table 4. Larval index (n larvae/ 10m^2) and dispersion parameters, scaled larval index**.**

Year	Index	var	se	cv	LCI	UCI	Scaled index*
2001	5,50	1,03	1,015	0,185	3,81	7,93	0,214
2002	2,76	0,52	0,719	0,261	1,65	4,61	0,062
2003	13,40	11,47	3,386	0,253	8,15	22,04	0,653
2004	9,03	3,10	1,760	0,195	6,13	13,28	0,410
2005	3,56	0,37	0,608	0,171	2,54	5,00	0,106
2012	41,05	9,03	3,006	0,073	35,47	47,52	2,189
2013	21,83	3,09	1,758	0,081	18,59	25,64	1,121
2014	25,41	6,92	2,631	0,104	20,67	31,24	1,320
2015	54,29	14,75	3,841	0,071	47,13	62,53	2,924

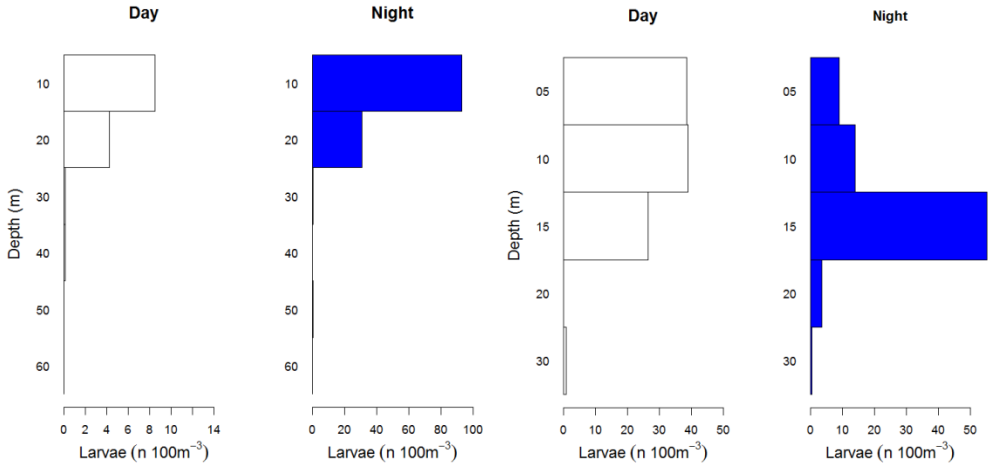

Figure 1. Vertical distribution of bluefin tuna larvae densities. A) data from MOCNESS 2012, B) data from MULTINET 2011.

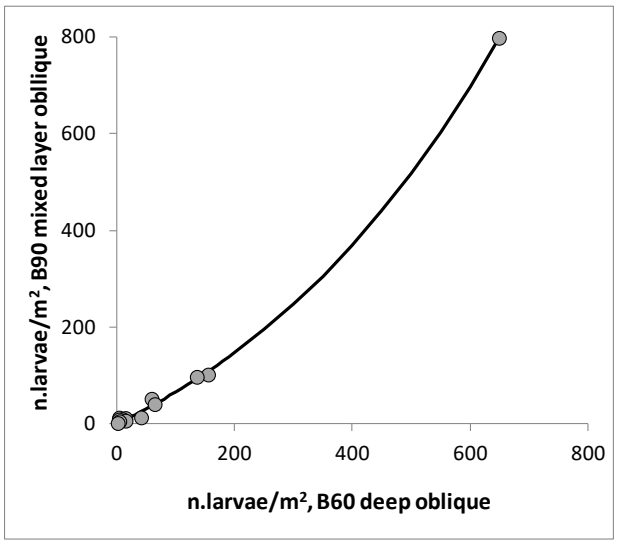

Figure 2. Larvae abundances (number of larvae per square meter) of B90ml and B60do from 2013 replicated stations. Left: all samples included (n=13), right: Maximum value excluded (n=12).

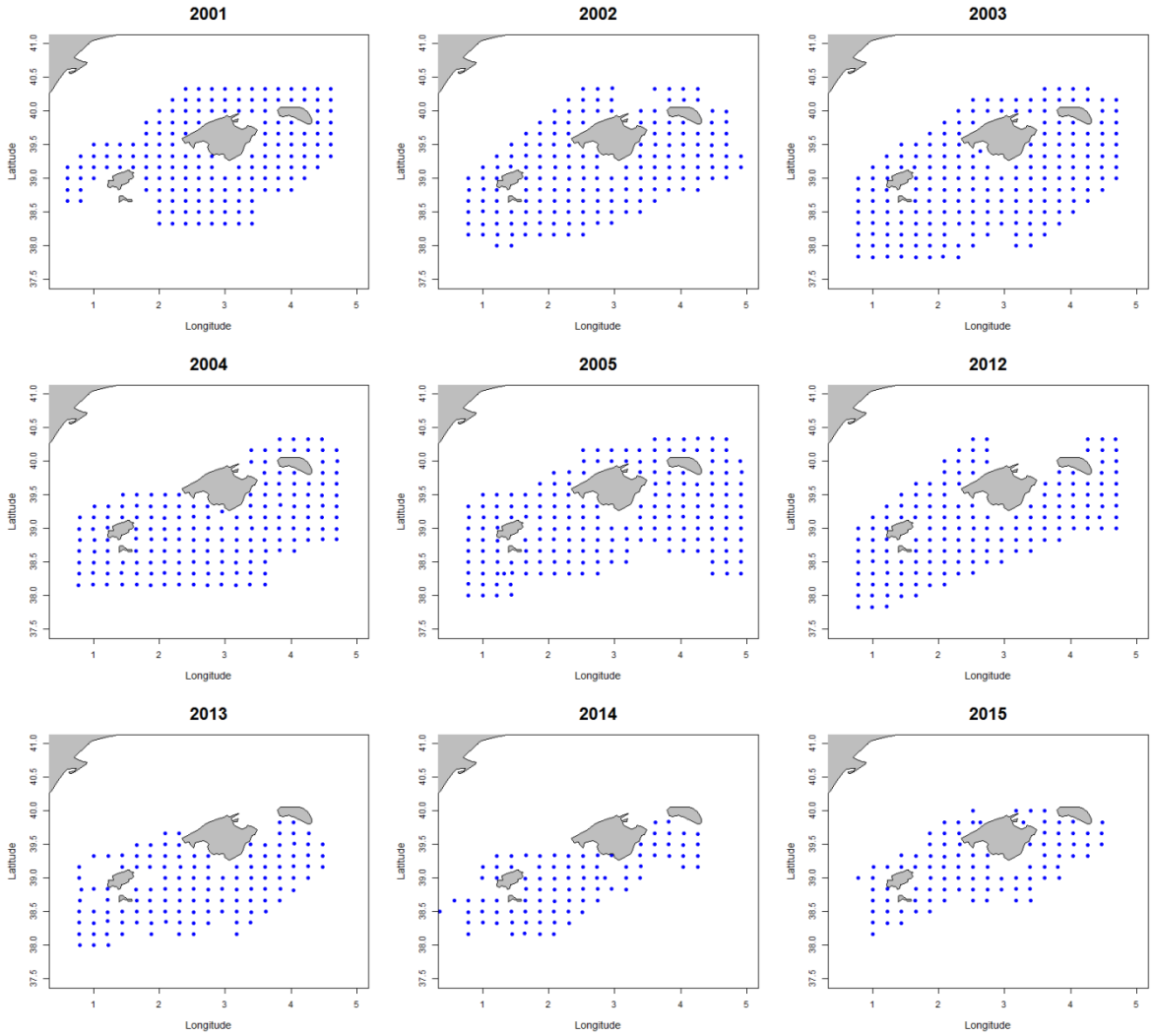

Figure 3. Spatial distribution of the samples per campaign.

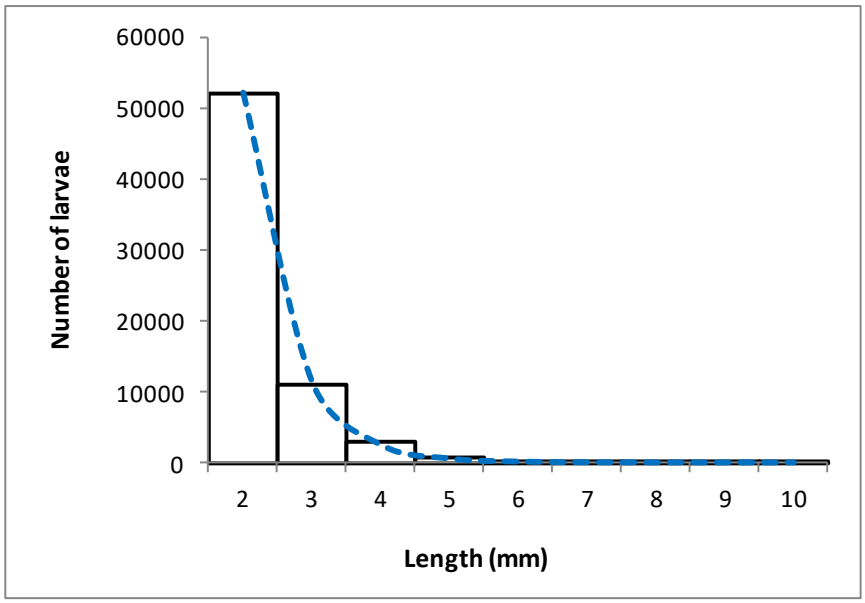

Figure 4. Distribution of number of larvae at length (bars) and adjusted exponential model (dashed line) used for calculating the number of larvae at 2 millimeters.

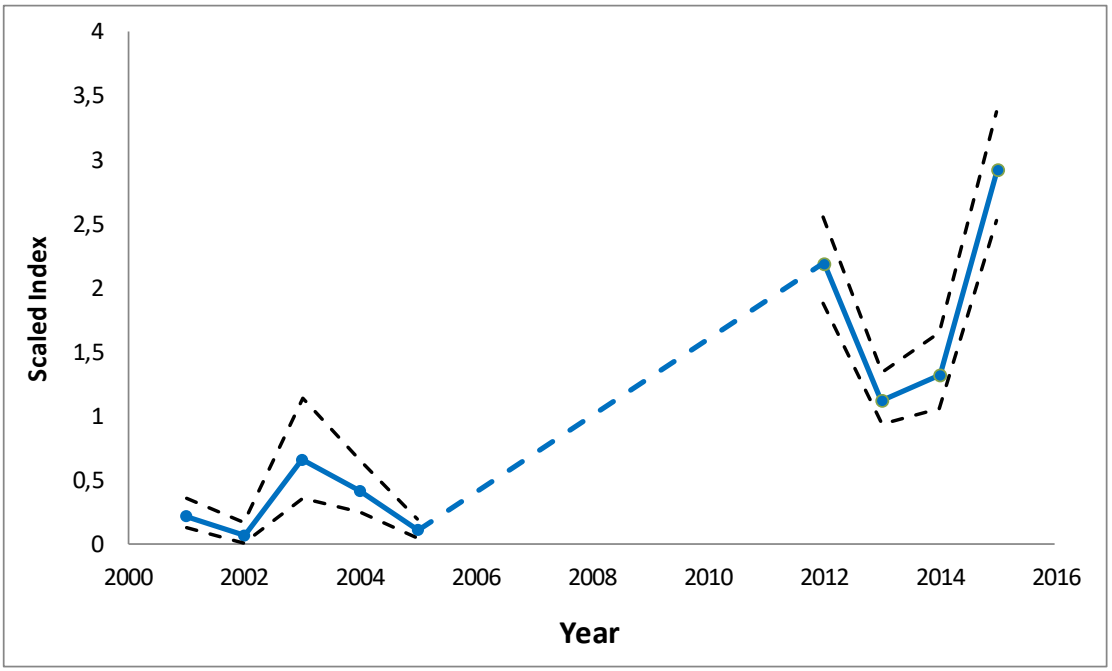

Figure 5. Scaled index for larval Atlantic bluefin tuna collected with both the bongo-60 and bongo-90. Black dashed lines show lower and upper confidence limits of 95%.

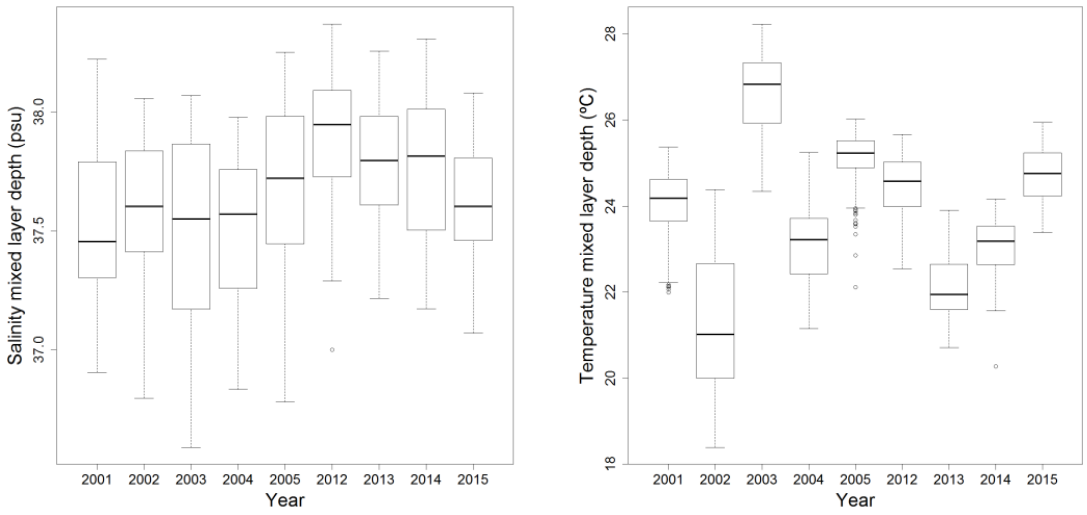

Figure 6. Environmental variability across campaigns: distribution of mean salinity (left) and mean temperature (right) in the mixed layer depth.

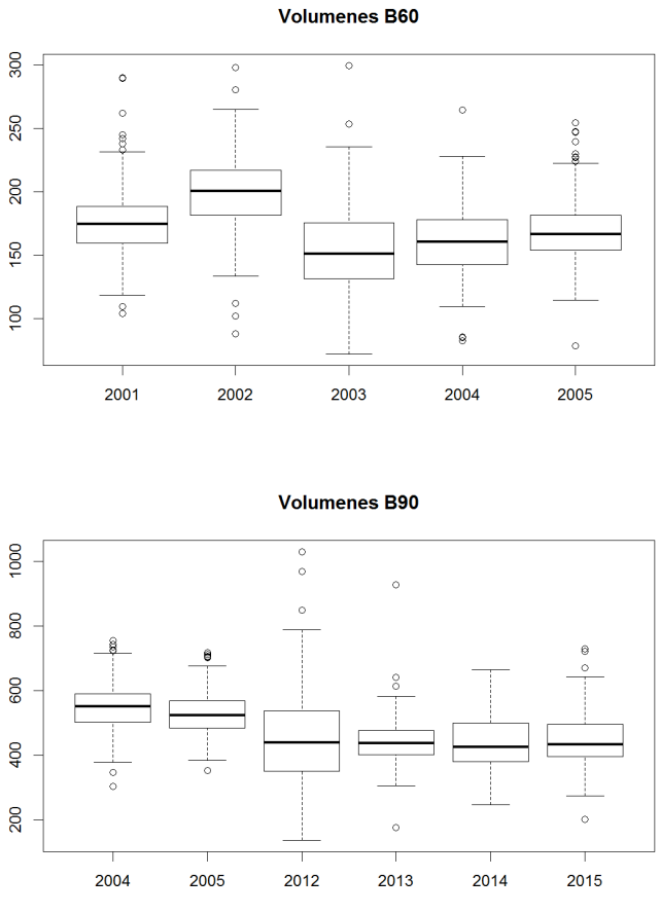

Figure 7. Variability of the volume filtered among years per sample and gear (above: Bongo 60 deep oblique, Below: Bongo 90 mixed layer).

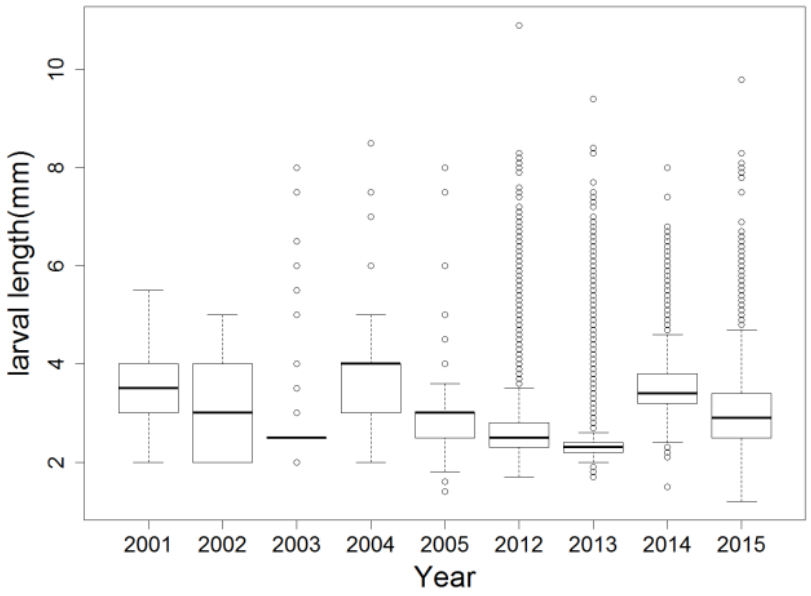

Figure 8. Larval length distribution per year.