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ASSESSING THE SPAWNING STOCK BIOMASS OF ALBACORE (*THUNNUS ALALUNGA*) IN THE WESTERN MEDITERRANEAN SEA FROM A NON-LINEAR LARVAL INDEX (2001-2019)

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SUMMARY

*Larval abundance indices express retrocalculated abundances of larval densities at hatching time. They provide a proxy for assessing spawning stock biomass and are applied to assess population status of various species in the Gulf of Mexico and in the Balearic Sea. Recently, the methodological approach to calculate the indices was improved to accommodate for non-linear responses of environmental effects on catchability. This improved methodology is routinely applied in the Balearic Sea to assess the bluefin tuna (*Thunnus thynnus*) spawning stock biomass. Here we apply the same methodology to update the larval index of albacore (*Thunnus alalunga*) from surveys conducted from 2001 to 2019 in the Balearic Sea, the most relevant spawning ground of this species in the Western Mediterranean. Albacore larval abundances show a decreasing trend and significant lower abundances from 2013 onwards, despite a slight recovery between 2016 and 2017. This larval index, standardized for gears, sampling coverage, salinity, date and sea surface temperature, provides information on the dynamics of the western Mediterranean stock of albacore, which is considered a data poor stock.*

RÉSUMÉ

*Les indices d'abondance larvaire expriment les abondances rétrocalculées des densités larvaires au moment de l'éclosion. Ils fournissent une approximation pour évaluer la biomasse du stock reproducteur et sont appliqués pour évaluer l'état de la population de diverses espèces dans le Golfe du Mexique et dans la mer des Baléares. Récemment, l'approche méthodologique pour calculer les indices a été améliorée pour tenir compte des réponses non linéaires des effets environnementaux sur la capturabilité. Cette méthodologie améliorée est régulièrement appliquée dans la mer des Baléares pour évaluer la biomasse du stock reproducteur du thon rouge (*Thunnus thynnus*). Dans ce document, la même méthodologie est appliquée pour mettre à jour l'indice larvaire du germon (*Thunnus alalunga*) issu des prospections menées de 2001 à 2019 dans la mer des Baléares, la frayère la plus importante de cette espèce en Méditerranée occidentale. L'abondance larvaire du germon montre une tendance à la baisse et des abondances significativement plus faibles à partir de 2013, malgré une légère reprise entre 2016 et 2017. Cet indice larvaire, standardisé pour les engins, la couverture d'échantillonnage, la salinité, la date et la température de surface de la mer, fournit des informations sur la dynamique du stock de germon de la Méditerranée occidentale, qui est considéré comme un stock pauvre en données.*

RESUMEN

*Los índices de abundancia larval expresan las abundancias retrocalculadas de las densidades larvales en el momento la eclosión. Proporcionan una aproximación para evaluar la biomasa del stock reproductor y se aplican para evaluar el estado de la población de diversas especies en el golfo de México y en el mar Balear. Recientemente, se ha mejorado el enfoque metodológico para calcular los índices con el fin de tener en cuenta las respuestas no lineales de los efectos medioambientales en la capturabilidad. Esta metodología mejorada se aplica de forma rutinaria en el mar Balear para evaluar la biomasa del stock reproductor de atún rojo (*Thunnus thynnus*). en este documento se aplica la misma metodología para actualizar el índice larval del atún blanco (*Thunnus alalunga*) de las prospecciones realizadas desde 2001*

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hasta 2019 en el mar Balear, la zona de desove más importante de esta especie en el Mediterráneo occidental. La abundancia larval del atún blanco muestra una tendencia descendente y abundancias significativamente menores desde 2013 en adelante, a pesar de una ligera recuperación entre 2016 y 2107. Este índice larval, estandarizado para los artes, la cobertura de muestreo, la salinidad, la fecha y la temperatura de la superficie del mar, proporciona información sobre la dinámica del stock de atún blanco del Mediterráneo occidental, que se considera un stock con pocos datos.

KEYWORDS

Abundance, Albacore, Fish larvae, CPUA

1. Introduction

Larval abundance indices are standardized abundances of larval densities retrocalculated to the hatching time. They have been used in ICCAT for assessing trends of the spawning stock biomass for different species. A methodology was developed to assess tuna species in the Gulf of Mexico (Ingram *et al.*, 2010) and, with some improvements, applied to routinely assess the spawning stock biomass of Bluefin tuna in the Balearic Islands since 2014 (Ingram *et al.*, 2015,2017). A larval index for albacore (*Thunnus alalunga*) was developed from surveys conducted in the Balearic Sea between 2001 and 2015, which was applied to assess the spawning stock biomass in the area (Alvarez-Berastegui *et al.*, 2017b). The index was included in the stock assessment performed by the ICCAT Albacore species group that year. It is the only abundance index of spawning stock biomass for the Mediterranean albacore that is independent from the fishery and provides a valuable source of information considering the low number of other abundance indices available for assessing the Mediterranean population (ICCAT, 2017).

Different sources of information, from the spatial distribution of early life stages (Almemany *et al*., 2010, Torres *et al.*, 2011), and from the spatial distribution of mature adults (**Figure 1**), show that the Balearic Sea is the main spawning ground for Albacore in the Western Mediterranean (Alvarez-Berastegui *et al.*, 2018a). Besides, oceanographic analysis has shown that the Balearic Sea acts as a larval retention area at the western Mediterranean scale, potentially aggregating larvae spawned in other adjacent areas (Díaz-Barroso *et al*., 2018). The surveys included in the calculation of the Albacore larval index cover these retention areas and the main albacore spawning grounds in the Balearic Sea, showing a larval index trends that are well correlated with other indices of albacore spawning stock biomass in the Mediterranean (Alvarez-Berastegui *et al.*, 2018a).

The Albacore larval index from 2017 used a former approach that applied generalized linear models to determine the relationships between environmental model and presence-absence and abundance of albacore (Alvarez-Berastegui *et al.*, 2017b). In the present work, we add newly processed data from 2017 and 2019. But because functional responses of larval abundances and environmental variables are often non-linear (Alvarez-Berastegui *et al.*, 2014), we move a step forward and revise the methodological approach to include generalised additive models. The revised methodology is in line with that applied to Bluefin tuna and recently accepted by ICCAT species group (Alvarez-Berastegui *et al.*, 2021). This approach also incorporates the estimation of annual mean indices and associated errors using least-squared errors for two-stage models. Hence, we produce a revised larval index of albacore abundance for the Balearic Islands. The methods applied account for changes in fishing methods over time, using calibration models from experimental fishing. Catches are standardized to the effects of various factors affecting catchability, such as changes in hydrographic conditions, fishing date and time and geographical location of the sampling.

We present here the results of the albacore larval index covering ten years of data from 2001 to 2019, with updated methodologies, providing fishery independent information informing about the trends of the spawning stock biomass in the region.

2. Material and methods

2.1 Ichthyoplankton surveys

Albacore larval samples were collected from ichthyoplankton surveys around the Balearic Sea (**Figure 2**) along twelve surveys in two periods, between 2001 and 2005 and between 2012 and 2019. Sampling design consists of a regular 10x10 nautical miles sampling grid, covering an approximate area of 86,351 Km^2 around the Balearic Islands during the months of June-July (see details in **Table 1** and supplementary data **Figure S1**).

Collection of larvae was performed with bongo nets performing oblique tows covering the whole species depth range, specific gear description and operations are presented in **Table 1**. In all haul-types, flow meters were fitted to the net mouths for determination of the volume of water filtered. Plankton samples were fixed on board with 4% formaldehyde in seawater. In the laboratory, all fish larvae were sorted under a stereoscopic microscope. Albacore larvae were then identified to species level (Alemany 1997) and standard length measured by means of an Image Analysis System. In addition, at each station, a vertical profile of temperature, salinity, oxygen, turbidity, fluorescence and pressure was obtained using a CTD probe SBE911.

The development of a larval abundance index requires selecting the most representative dataset to avoid potential biases on the inter-annual variability of the mean abundances. To do so, sampling dates, filtered volumes and individual larval lengths were visually inspected through box whisker plots (supplementary data, **Figure S2.1** and S2.2), together with the environmental data known to affect the timing and spatial distribution of albacore spawning in the area (Alemany *et al.*, 2010, Reglero 2012). The earliest larva recorded during the year occurred on the 18^{th} of June at a sea water temperature of 20.9ºC (average temperature in the mixed layer depth). Hence, following Alvarez-Berastegui *et al.*, (2017b), we selected field sampling campaigns that coincided with the timing of the gonad maturity of the species and with the presence of larvae in the Balearic Sea, as well as showing similar sea temperature ranges. Hence, the samplings performed in 2002 and 2003 were excluded from the analysis as they were conducted too early and too late in the season respectively (See details on data selection in Alvarez-Berastegui et al. 2017b). The selected sampling campaigns were representative of the spawning time in the area since larvae were observed coincident with mature adults as observed from gonad analyses in June-July (Saber *et al.*, 2015b). Box whisker plots were performed by means of the 'ggplot2' package (Wickman 2016).

2.2 Data processing

The abundance of albacore was standardised to larvae at reference length of 2 mm to avoid the exponential decay due to natural mortality and changes on catchability of older larvae (following a methodology proposed for bluefin tuna, see Alvarez-Berastegui *et al.* 2017a, 2021).

$$N_{2mm} = 9183.5856\, e^{-0.8998\, L_i} \quad \text{(Eq. 1)}$$

where N_{2mm} is the number of larvae at 2 millimetres and L_i total length of larvae, in mm. Second, the catch per unit area ($CPUA_{2mm}$, in N larvae_2mm / m^2) is obtained using the equation:

$$CPUA2mm = \frac{N2mm}{V_{filt}}\, D_{tow} \quad \text{(Eq. 2)}$$

where V_{filt} is the volume of water filtered by the net (in m^3) and D_{tow} is the towing depth (in m), obtaining a CPUA at 2mm.

Standardization for changes in fishing methods.

CPUA was standardised for changes in fishing methods in the periods 2001-2005 (Bongo 60 with 333 μmmesh size) and 2012-2019 (Bongo 90 down to 30 meters and 500 μm mesh size), following an exponential relationship between B60 deep oblique and B90 mixed layer oblique obtained from experimental hauls on bluefin tuna larvae, following Alvarez-Berastegui *et al.* (2018b):

$$CPUA_{B90} = 0.58\, CPUA_{B60}\, e^{0.00115\, CPUA_{B60}}, R^2 = 0.998 \quad \text{(Eq. 3)}$$

The inter annual variability of the CPUA of larvae at 2 mm was modeled using a two-stage generalised additive modelling (GAM) approach using the 'mgcv' package (Wood 2004, 2006), following a similar approach to that applied for bluefin tuna larval index in the study area (Álvarez-Berastegui *et al.*, 2020). This method combines a binomial submodel predicting probabilities of larval presence and a log-normal submodel to predict log-transformed positive abundances. Environmental variables were included to improve the standardiSation of the larval indices. The explanatory variables considered in the modelling were year, day time (night or day), mean salinity in the mixed layer depth (SMEZCLA) accounting for the spatial distribution of water masses (Balbín *et al.*, 2014) that affect the spawning of albacore (Alemany *et al.* 2010, Reglero *et al.*, 2014), the day of the year (jd) accounting for differences on sampling dates in relation to the beginning of the spawning, and the residual temperature (tempres) as the residuals of a linear model where temperature was fitted to the day of the year (R^2=0.48, p-value < $2.2e^{-16}$). The residual temperature allowed including inter annual differences of temperature in the models considering the existing significant correlation between the mean temperature in the mixed layer depth and the day of the year (R^2=0.70, p-value < $2.2e^{-16}$). Binomial and log-normal GAM submodels were fitted following a stepwise forward method, starting from models with only one variable and subsequently adding significant variables (p-value<0.05) by means of restricted maximum likelihood (REML; Wood 2011). REML is more efficient than other available methods like general cross-validation (Marra and Wood, 2011). The degree of smoothness of each particular variable was limited in order to avoid overfitting, i.e. a maximum of 3 knots for single variable relationships and 9 knots for interactions between two variables.

Model performance was assessed by inspection of the Area Under the Curve (AUC) plot and density plot of real positive and negative estimates, for the presence probability (binomial) submodel. For the log-transformed positive abundance submodel, model performance was evaluated through the deviance of the residual versus theoretical quantiles, residuals versus linear predictor, histogram of the residuals and the response versus fitted values.

The index value for each year (I'_y) was calculated as follows:

$$I'_y = c'_y \, p'_y \text{ (Eq. 4)}$$

where c'_y is the back-transformed mean CPUA from the lognormal submodel in the year 'y' and p'_y probability of presence of albacore larvae estimated from the binomial submodel, both compensated accounting for changes in factors among years by means using estimated marginal means with the 'emmeans' package (Searle *et al.*, 1980, Lenth R. 2020). The estimation of the standard error (*se'*) was also computed using the compensation for unbalanced factors with 'emmeans'. The upper and lower 95% confidence intervals (*UCI* and *LCI*) for the index were calculated to measure the precision of the mean, using the approximation for non-normal data as proposed by Ingram *et al.*, (2010), according to the following equations:

$$UCI = I'_y \times C \text{ (Eq. 5)}$$

$$LCI = \frac{I'_y}{C} \text{ (Eq. 6)}$$

where $C = e^{2 x \sqrt{log\,(1-CV'^2)}}$, *CV'* is the coefficient of variation of the index I'_y, computed as the standard error of the index (*se'*) divided by the value of the index (I'_y), both compensated for unbalanced factors. All calculations were computed in R software (R Core Team 2020).

3. Results

Summary statistics of the larval abundance and nominal CPUA based on the 2 mm standardized larvae are presented in **Table 3**. In the binomial submodel, the finally included variables were the smoothed interaction between latitude and longitude (lat, lon), the day of the year (jd) and salinity down to the mixed layer depth (SMEZCLA) and the year as a factor of the form:

Probability of Presence ~ as.factor (year) + s (lon, lat) + s (jd) + s (SMEZCLA)

Model summary, diagnostic plots and model responses are shown in Supplementary materials (**Table S3.1**, **Figure S4.1** and **Figure S5.1**, respectively). Diagnostic plots of the binomial model showed AUC value of 0.79, and the histograms of probabilities at real presences and absences presented a good separation of both categories, with a better performance of the binomial model to assign low values to real absences than to real presences (**Figure S4.1**). Response functions of the environmental data showed a higher probability of presence at lower salinity levels (**Figure S5.**1) indicating a preference for recent Atlantic waters located at the southern area of the Balearic oceanic front.

For the log-normal submodel, the finally selected model explained a 32.6% of the deviance and was:

log (CPUA) ~ as.factor (year) + s (jd) + s (SMEZCLA) + s (tempres)

Model summary, diagnostic plots and response function of the exploratory variables for the log-normal model are presented in supplementary materials (**Table S3.2**, **Figure S4.2** and **Figure S5.2**, respectively). Similar to what was observed in the binomial model, water masses on the proximity of the Balearic front with lower salinity present higher log-transformed abundances of albacore. The day of the year had a positive effect (**Figure S5.2**). Residual temperature showed a positive effect evidencing a preference for the lower temperatures than average (**Figure S5.2**).

The values of the larval index (I'_y) as well as the associated dispersion parameters resulting from the modelling approach shows a decreasing trend along the time series, with a sharp decrease occurring between 2012 and 2013, with stable lower values since then (**Table 2**, **Figure 3**).

4. Discussion

The present work revises the larval abundance index for albacore (*Thunnus alalunga*) presented in 2017 (Alvarez-Berastegui *et al.*, 2017), to include data collected from 2001 to 2019 in the main spawning area of the species in the Western Mediterranean. Larval abundances obtained in the ichthyoplankton hauls were standardised for differences in gears, sampling depth, day of the year and water environmental parameters known to have an influence on the species' spawning. The index was estimated by means of a two-stage GAM model and error estimation was performed by least-squares mean errors (or marginal means) to account for different weighting of the explicative variables. Time series of albacore larval abundance shows a declining trend with stable lower values since 2013.

The inclusion of environmental variables in the standardisation process is key to develop reliable abundance indices and avoid confounding effects that could introduce some bias in the estimates. Salinity was significant in both submodels showing an avoidance of albacore larvae of the areas with higher salinity. These values coincide geographically with the main spawning ground located at the East of the Balearic archipelago. Disentangling the potential dispersion and retention patterns from this area will improve our knowledge on albacore larval distribution and the larval index standardization process. The residual temperature had an effect only in the abundance submodel, showing an increase in abundance with temperatures above the typical temperature in a particular day of the year. The day of the year also showed positive effects in both submodels, showing that larvae presence probabilities and abundances are both affected by the sampling date.

In general, functional responses of larval abundances and environmental variables are non linear (Alvarez-Berastegui *et al.* 2014) so linear models may show restricted capabilities to incorporate that information into the standardization of larval abundances.

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Table 1. Ichthyoplankton survey, fishing and gear characteristics and sampling effort.

Year	Dates	Gear	Haul type	Nº samples	Mean tow depth
2001	16Jun-7Jul	B60	Deep oblique	162	69
2002	8Jun-29Jun	B60	Deep oblique	171	70
2003	3Jul-29 Jul	B60	Deep oblique	197	68
2004	18Jun-10Jul	B60	Deep oblique	166	69
2005	27Jun-23Jul	B60	Deep oblique	186	70
2012	21Jun-9Jul	B90	Mixed layer oblique	153	34
2013	20Jun-9Jul	B90	Mixed layer oblique	124	28
2014	12Jun-29Jun	B90	Mixed layer oblique	92	29
2015	23Jun-9Jul	B90	Mixed layer oblique	94	25
2016	21Jun-12Jul	B90	Mixed layer oblique	95	25
2017	26Jun-12Jul	B90	Mixed layer oblique	92	25
2019	19Jun-29Jun	B90	Mixed layer oblique	108	31

Table 2. Summary of the raw biological data and of the input and output incorporated in the modelling approach: amount of hauls (Nº samples), amount of larvae captured in the hauls (Larval counts, n larvae), number of larvae standardised at 2 mm, gear, depth and filtered volume (Nominal CPUA, n larvae at 2 mm/m^2), larval index estimated by means of a two-stage GAM approach with marginal mean estimation of the error (Index, n larvae at 2 mm/ 10 m^2), precision of the index estimated as the coefficient of variation of the mean (CV, %) and the 95% lower and upper confidence intervals (LCI and UCI).

	Raw data		Input	Output			
Year	Nº samples	Larval counts	Nominal CPUA	Index	CV (%)	LCI	UCI
2001	162	166	1.65	7.92	29.3	14.04	4.46
2004	166	313	1.28	8.79	22.2	13.63	5.66
2005	186	771	2.80	8.84	17.4	12.50	6.26
2012	153	510	1.00	5.72	22.1	8.85	3.70
2013	124	80	0.28	1.72	34.9	3.38	0.87
2014	92	46	0.12	2.10	37.3	4.31	1.02
2015	94	77	0.16	0.67	34.2	1.30	0.34
2016	95	144	0.21	1.37	38.9	2.90	0.65
2017	92	421	1.22	2.65	25.5	4.38	1.61
2019	108	57	0.08	1.47	34.8	2.90	0.75

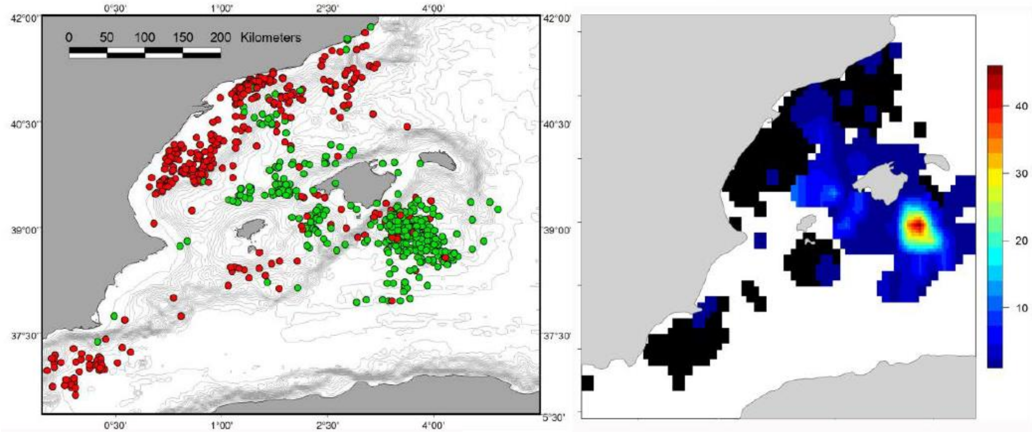

Figure 1. Spatial distribution of adult albacore tuna from longline commercial fisheries catches. Left: location of the original fishing sets, positive (green) and zero (red). Rigth: Raster map of catches (Number of fishing sets with positive catches per cell, black color indicating catches equal to zero).
Source: Alvarez-Berastegui *et al.*, 2018a.

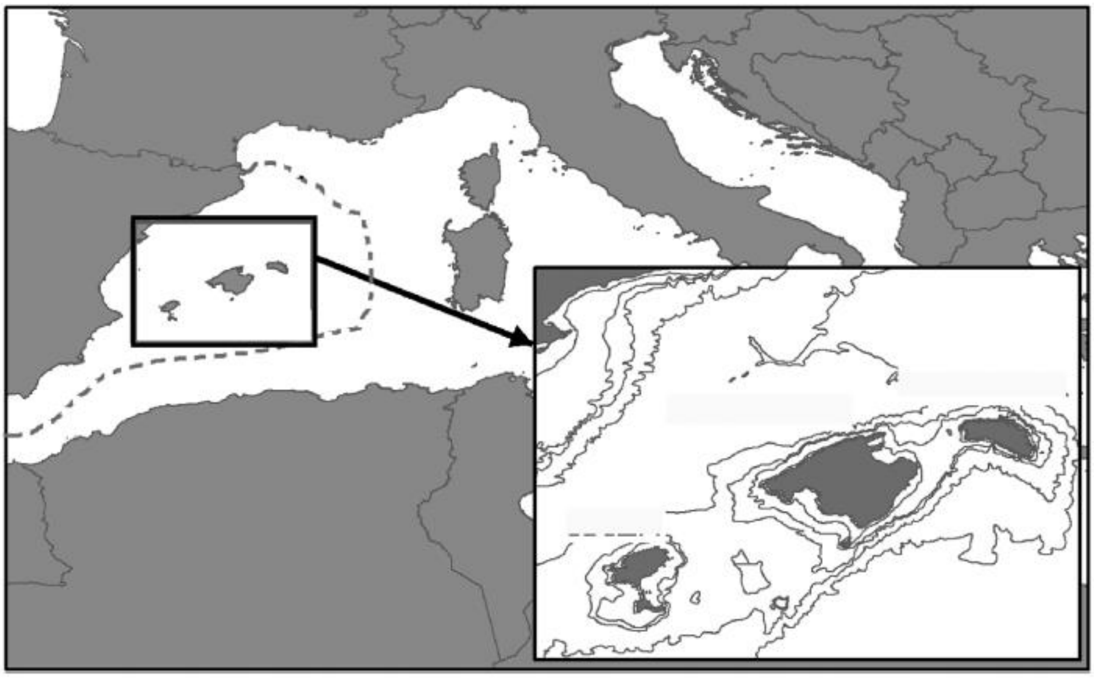

Figure 2. Study area in the Balearic Islands.

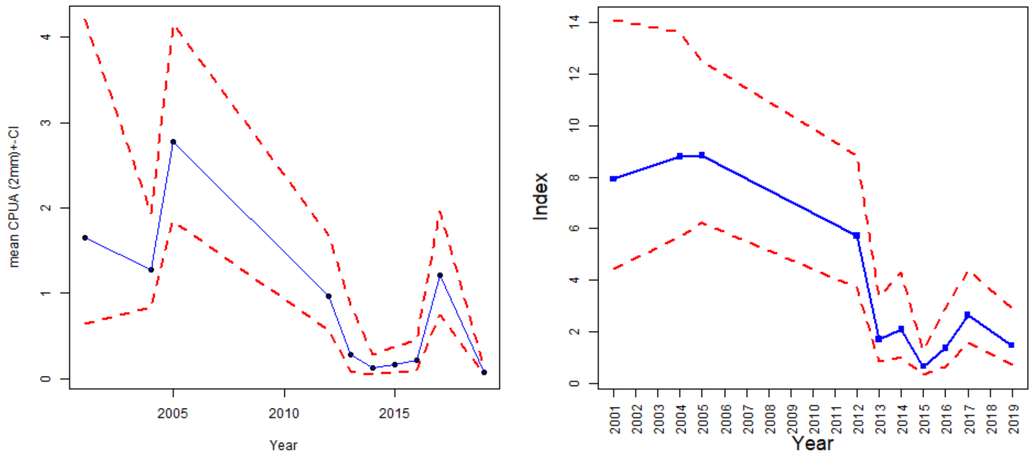

Figure 3. Albacore CPUA (left) and larval index (right) for the period 2001-2019.

Supplementary data

S1. Sampling locations

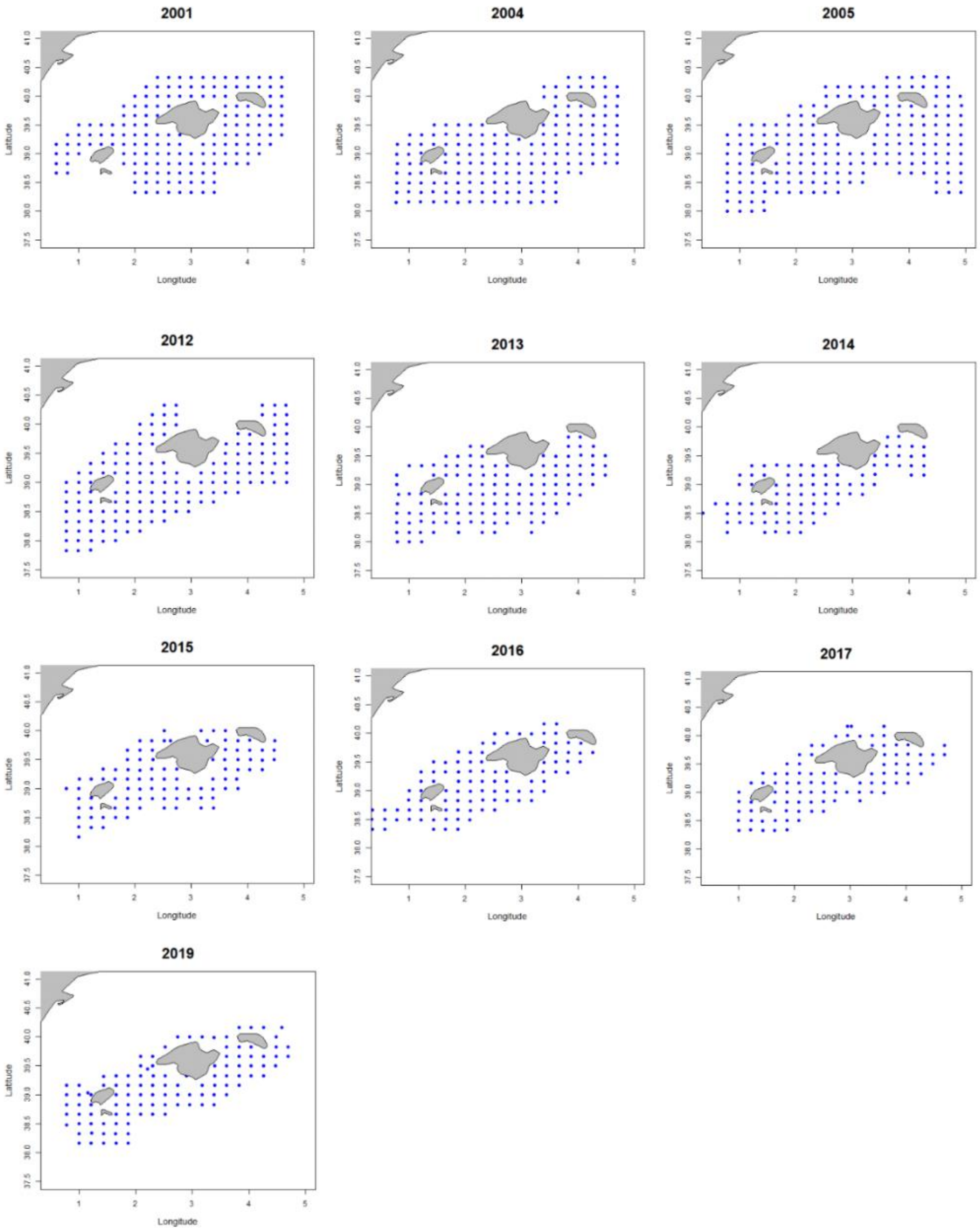

Figure S1. Sampling locations of the ichthyoplankton surveys used in the estimation of the larval index.

S2. Data explorations for volumes of water filtered and environmental variables

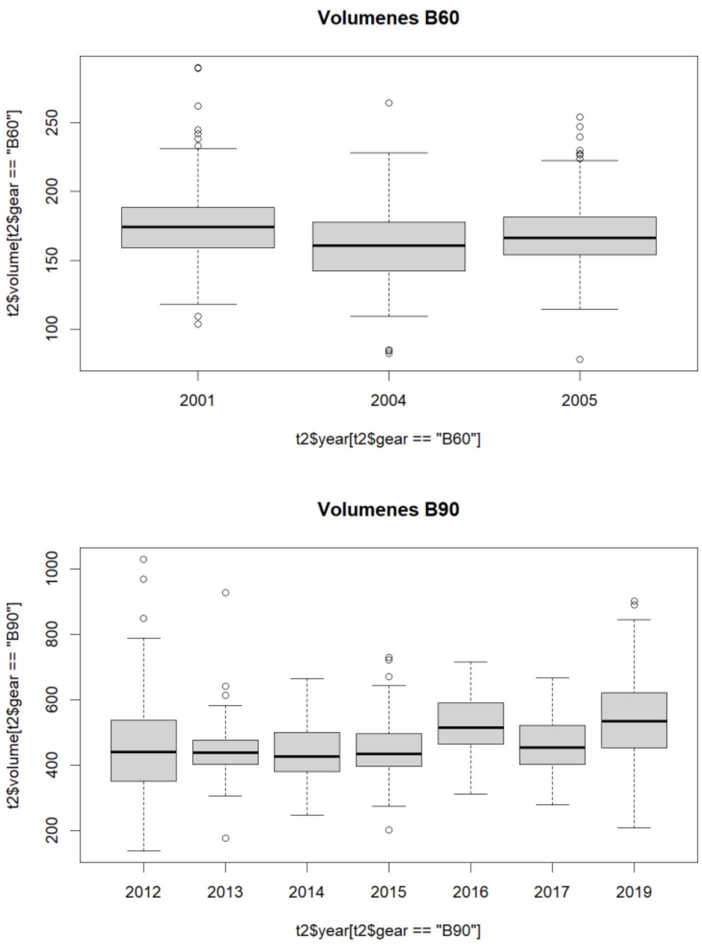

Figure S2.1. Boxplot of the volumes of water filtered per year for the two fishing deployments: bongo-60 with 333 μm and bongo-90 with 500 μm.

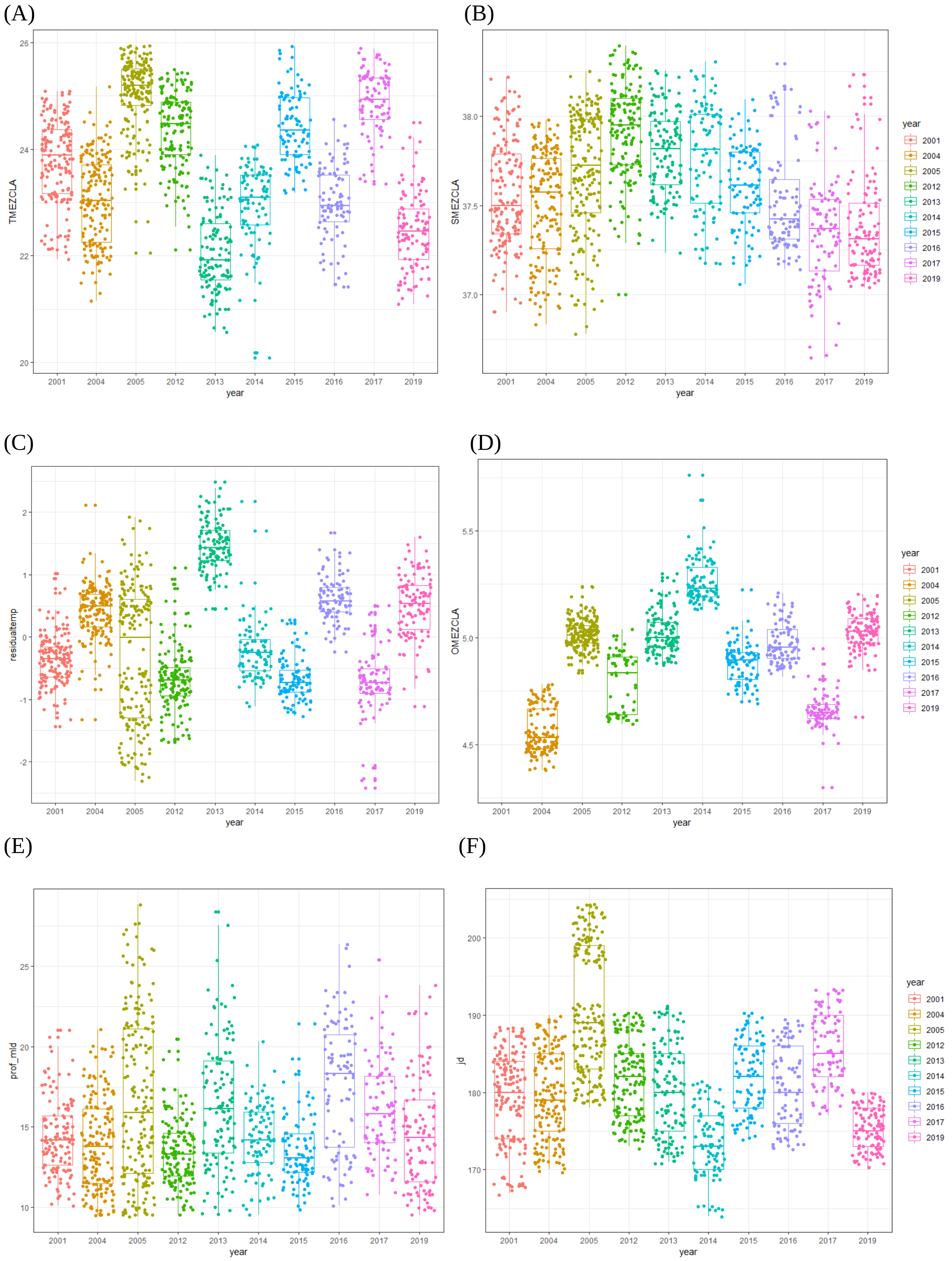

Figure S2.2. Boxplot of the environmental variables: (A) Temperature in the mixed layer depth (TMEZCLA); (B) Salinity in the mixed layer depth (SMEZCLA); (C) Residual temperature in the mixed layer depth (extracted the effect of the day of the year using a linear model); (D) Oxygen in the mixed layer depth (OMEZCLA); (E) Mixed layer depth; (F) Day of the year.

S3. Summary of the models

Table S3.1. Summary of the binomial model

Family: binomial (link function="logit")
Formula: lpres ~ as.factor(year) + s(lat, lon) + s(jd, k = 3) + s(SMEZCLA, k = 3)

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.49252	0.21939	-6.803	1.02e-11	***
as.factor(year)2004	0.76067	0.27040	2.813	0.00491	**
as.factor(year)2005	1.47934	0.33078	4.472	7.74e-06	***
as.factor(year)2012	1.26989	0.31967	3.972	7.11e-05	***
as.factor(year)2013	0.25552	0.33416	0.765	0.44447	
as.factor(year)2014	0.63126	0.37477	1.684	0.09211	.
as.factor(year)2015	-0.06157	0.34123	-0.180	0.85682	
as.factor(year)2016	-0.31116	0.35450	-0.878	0.38008	
as.factor(year)2017	0.78828	0.33977	2.320	0.02034	*
as.factor(year)2019	0.37001	0.32214	1.149	0.25072	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(lat,lon)	9.1890	29	34.91	1.82e-06	***
s(jd)	0.9517	2	18.77	5.12e-06	***
s(SMEZCLA)	1.8483	2	30.64	2.89e-08	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) = 0.217 Deviance explained = 19.7%
-REML = 674.29 Scale est. = 1 n = 1265

Table S3.2. Summary of the abundance model

Family: gaussian (link function="identity")
Formula: log(ALBab_gs) ~ as.factor(year) + s(jd, k = 3) + s(SMEZCLA, k = 3) + s(tempres, k = 3)

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.3582	0.2146	15.649	< 2e-16	***
as.factor(year)2004	-0.3837	0.2692	-1.426	0.154733	
as.factor(year)2005	-0.7094	0.2714	-2.614	0.009266	**
as.factor(year)2012	-1.0613	0.2827	-3.754	0.000199	***
as.factor(year)2013	-1.7072	0.3617	-4.720	3.24e-06	***
as.factor(year)2014	-1.7439	0.3671	-4.750	2.80e-06	***
as.factor(year)2015	-2.4305	0.3419	-7.108	5.19e-12	***
as.factor(year)2016	-1.5198	0.3695	-4.113	4.71e-05	***
as.factor(year)2017	-1.5957	0.2928	-5.449	8.70e-08	***
as.factor(year)2019	-1.9352	0.3402	-5.689	2.42e-08	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(jd)	0.9571	2	11.160	1.2e-06	***
s(SMEZCLA)	0.8479	2	2.774	0.01074	*
s(tempres2)	0.8746	2	3.485	0.00471	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) = 0.307 Deviance explained = 32.6%
-REML = 698.99 Scale est. = 1.5085 n = 427

S4. Partial effects of the environmental variables in the selected models

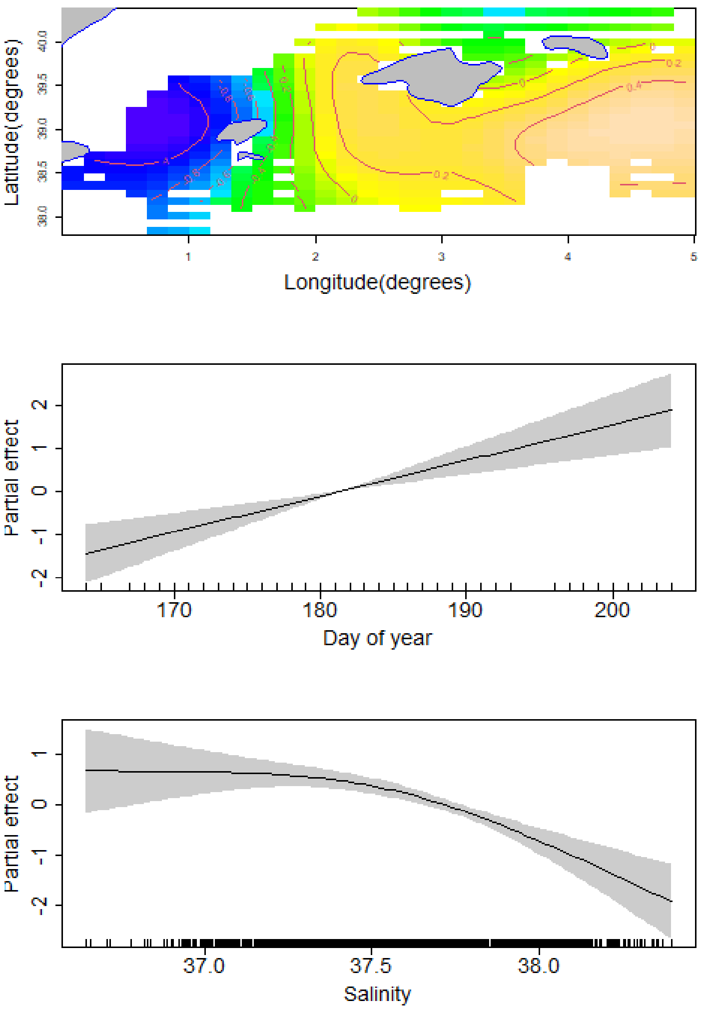

Figure S4.1. Partial effects of the significant variables included in the final selected model for albacore presence-absence.

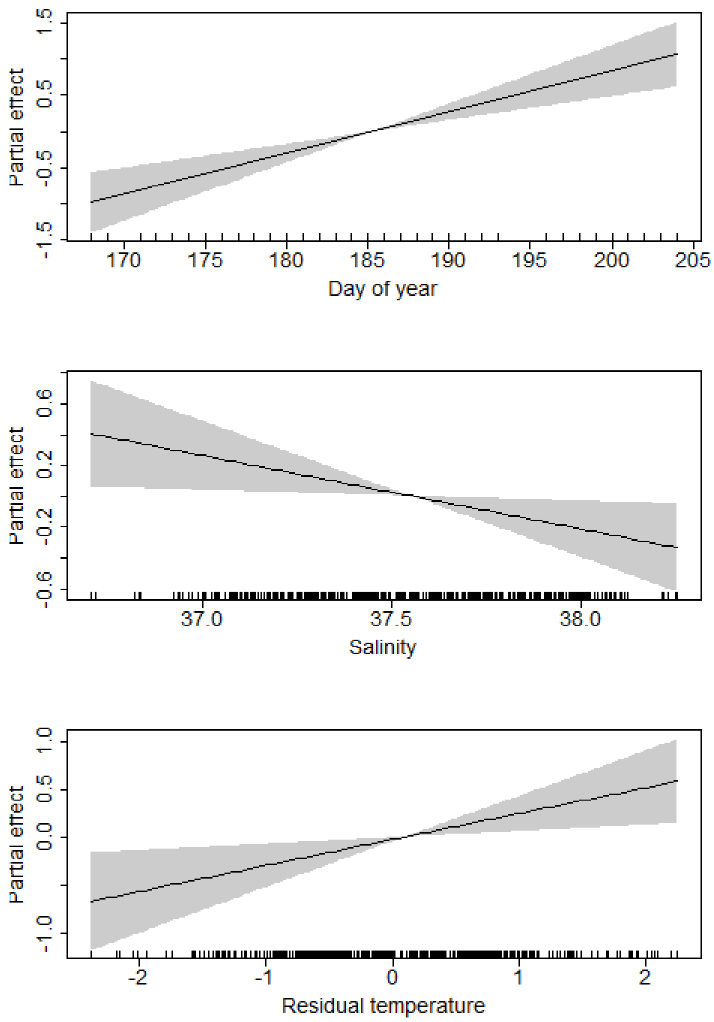

Figure S4.2. Partial effects of the significant variables included in the final selected model for albacore abundance.

S5. Model performance

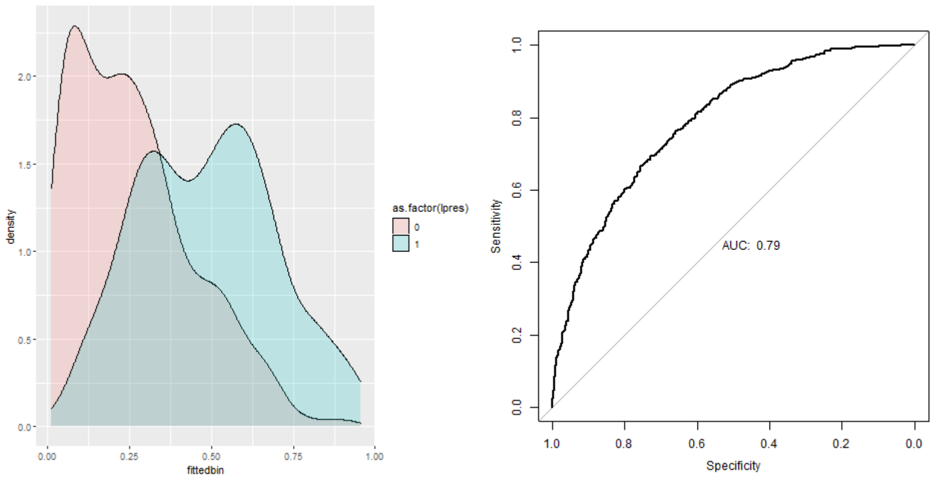

Figure S5.1. Model performance of the binomial model.

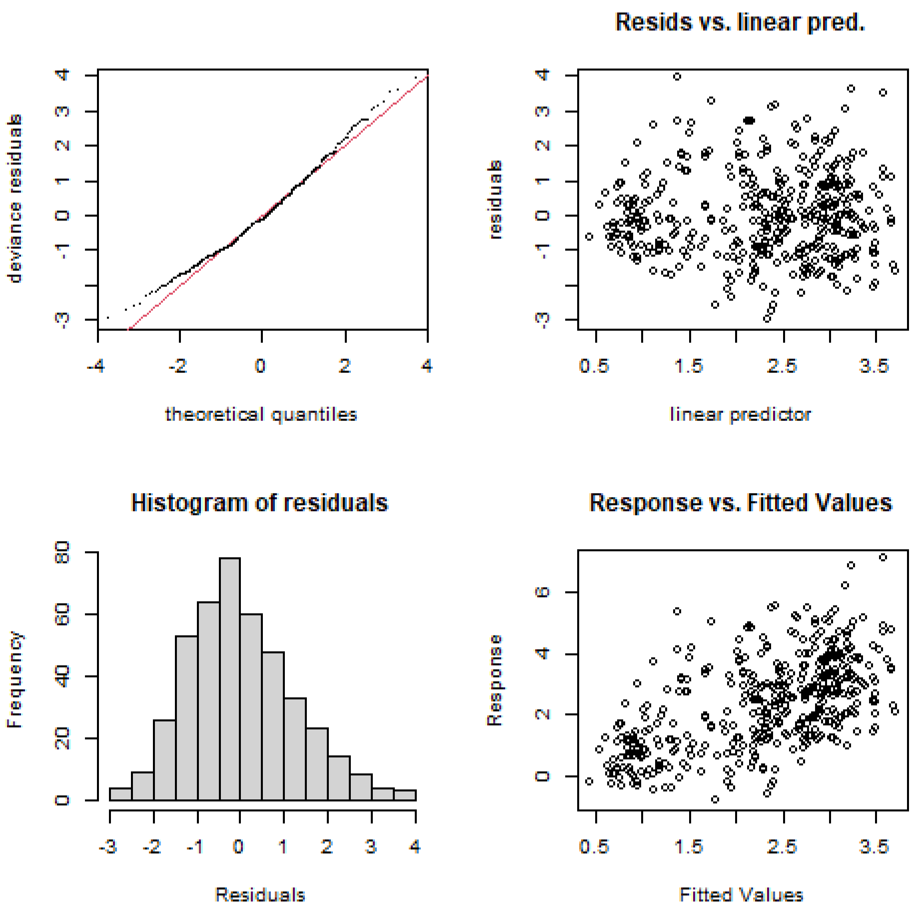

Figure S5.2. Model performance of the abundance submodel.