TESTING METHODS TO ESTIMATE THE AGE OF BLACKSPOT PICAREL (*SPICARA MELANURUS)* USING OTOLITHS, FROM THE WATERS OF CAPE VERDE ISLANDS

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ABSTRACT

Blackspot picarel (*Spicara melanurus*) is a fish species from the Sparidae family and is an important species for the artisanal fishery in Cape Verde. Despite being commercially important, stock assessment is not performed for this species due to limited knowledge of life story traits and a lack of methodology to investigate the age of the species. In this study, methodology to estimate age of blackspot picarel from Cape Verde waters, was investigated by counting otolith increments and by measuring otolith size. Otoliths from 134 specimens ranging in fork length from 4.5 cm to 29 cm were analysed. Increments became visible once the otolith was sectioned, polished, image taken, and the image enhanced. An age-length key was produced. The precision of the ageing method was estimated by replicating increment count for 23% of otoliths. For replicas, the mean coefficient of variation was 8.76% which is acceptable. The maximum age estimated was 17 years and the von Bertalanffy growth parameters were $L_\infty = 36.17\, cm,\ k = 0.09\, year^{-1},\ t_0 = 0.89\, year$. This suggests that blackspot picarel is a slow-growing and a long-lived species. Maturity-at-age was 7.1 years. The relationship between specimen length and otolith dimension, and between specimen age and otolith dimensions was strong and explained 89% or more of variance in otolith dimensions. The age-length key, maturity-at-age, and growth rate provide vital information for stock assessment.

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1. INTRODUCTION

Sustainable use of marine resources provides food security and economic prosperity, whereas overfishing can increase poverty and hunger (FAO, 2018). Assessing fish stock size is vital to estimate stock productivity and sustainable catch volume. Stock assessment demands knowledge of various biological life history traits of the target stock, such as length-/weight-at-age, age-at-maturity, age structure of the stock and somatic growth rate (Haddon, 2011). Therefore, accurately, and precisely estimating the age of individuals of a specific species is important for the stock to be sustainably harvested.

In the Cape Verde Islands, approximately 150 commercial fish species are harvested annually (González & Tariche, 2009). However, a method to estimate age has only been developed for one species, a grouper *(Cephalopholis taeniops)*. The results suggest an annual formation of increments in their otoliths (Tariche *et al.*, 2014). In 2005, during the 7th Annual Meeting of the National Institute of Fisheries Development (INDP) Scientific Council, it was recommended to the INDP that methods to estimate age of more commercial fish stocks in Cape Verde islands be developed, including the blackspot picarel (*Spicara melanurus*) (INDP, 2007).

There is no universal method to estimate age of fish. For each species, an ageing method must be developed. The most common methodology is to estimate age from otoliths, a calcified structure collected from the inner ear of the fish (Stevenson & Campana, 1992). In otoliths the deposition rate of minerals and growth in size is positively related to somatic growth of the fish (Campana, 1999). Otoliths vary in shape, size, and visibility of annual growth increments between fish species; hence many methods have been developed to count increments (Campana, 1999). Counting of increments in blackspot picarel otoliths has never been researched and it is not known which method functions best for the purpose.

1.1 Goal and objectives

The goal of the current project is to establish a methodology to estimate age of blackspot picarel, found in Cape Verde Islands, by counting otolith increments and by measuring otolith size. A toolbox of methods currently used to count increments of various fish species will be tested, starting with the most common methodology, until a suitable method is established. Furthermore, various life history parameters related to age and vital for stock assessment will be calculated.

Objectives

- Estimate the age of blackspot picarel by developing a methodology for counting otolith increments.
- Calculate the relationship between the number of annual otolith increments and otolith size, length, and width.
- Estimate life history parameters related to age of which many are vital for stock assessment, such as length-at-age, weight-at-age, age-at-maturity, and growth parameters (e.g. von Bertalanffy).

2. BACKGROUND CAPE VERDE ISLANDS

The Cape Verde archipelago is formed by ten islands and five islets that are in the Atlantic Ocean 375 miles west off the coast of Senegal and Mauritania (Figure 1). The archipelago has a total area of 4033 km^2, shelf area of 5382 km^2 and a coastline of 965 km (FAO, 2018). Fisheries are economically and socially important in Cape Verde. The population is 535 thousand and thereof 4800 individuals are employed in fishing (FAO, 2018). Fish products constitute 7% to 10% of gross domestic product (GDP) (Medina & Gomes, 2015). However, the export value of fish products is much higher than its GDP. In 2016 fisheries export represented 80% of the national monetary value exported (INE, 2017). Service is the largest industry in Cape Verde and that industry has limited export value, hence the relatively high export value of fish products. Fish is also an important source of food for Cape Verdeans and the average per capita consumption is approximately 12 kg per year which provides 10% of annual animal protein consumption (FAO, 2018).

Figure 1. The Cape Verde archipelago (http://legacy.lib.utexas.edu).

2.1 The fisheries

Fishing activity in Cape Verde is traditionally divided into artisanal fishery and industrial fishery based on fleet characteristics, especially boat length and engine power. The artisanal fleet is composed of small vessels with length ranging from 3 to 11 m and width between 1.5 and 2.5 m. They are made of wood and most are equipped with outboard engines of up to 25hp (INDP, 2011). The industrial fleet consists of larger vessels up to 25 m and inboard engine powered up to 500 hp (MAAP, 2004).

Artisanal fisheries operate inside 3 nautical miles from the coast and the industrial fleets from 3 nautical miles to about 12 miles (INCV, 2016).

Annual catches range from approximately 8 thousand to 15 thousand tonnes (figure 2). Landings are similar for both fleets in most years, except in the period from 2012 to 2015 when the industry landings were higher (INDP, 1995-2012); (data for 2013-2015 personal communication from INDP statistical division).

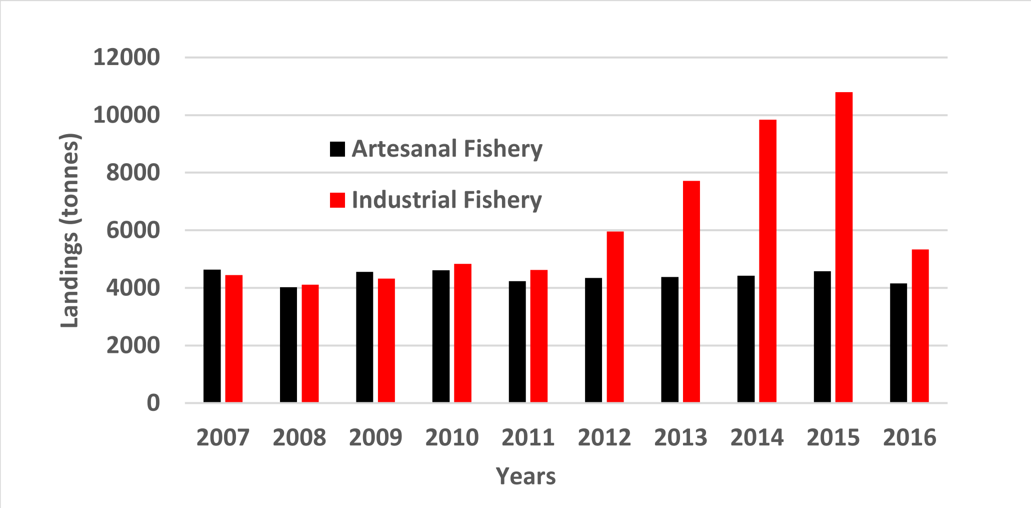

Figure 2. Total fish landings in Cape Verde 2007 to 2016, for both fleets (source: INDP).

The Cape Verde artisanal fishery has neither catch or effort limits. Various fishing gear is used, the main being handlines, purse seine, gillnets and beach seines (MAAP, 2004). Approximately 150 commercially important fish species are caught. The species catch depends on their habitat (table 1) (González & Tariche, 2009). The most valuable species category, in volume of catch, is oceanic pelagic which is composed of migratory species that inhabit the Atlantic Ocean far from the coast of Cape Verde, such as tuna. The second most catch species category is coastal pelagic which is composed mainly of the small pelagic species that live close to the coast. Demersals represent the last category most captured.

Blackspot picarel (*Spicara melanurus*) has been one of the most valuable coastal pelagic species for the last two decades (figure 3) (INDP, 1995-2012); (data from 2013-2015 personal communication of INDP statistical division). The blackspot picarel is mostly captured in the artisanal fishery (94% of total landings) using gillnets (approximately 80%), and to a small extent as bycatch in industrial fisheries. During the last twenty years, black picarel annual catch ranges from approximately 150 tonnes to 1200 tonnes (figure 4). Catches peaked around 2000 and then declined until 2012, with a notable increase in the catch from 2012 to 2015.

Table 1. Main target species in artisanal fisheries taking into consideration all fishing gear.

Group	**Species**
Ocean Pelagics	*Katsuwonus pelamis, Thunnus albacares, Acanthocybium solandri, Thunnus obesus, Euthynnus alletteratus* and *Auxis spp.*
Coastal pelagic	*Spicara melanurus, Decapterus macarellus, Selar crumenophthalmus, Decapterus punctatus, Caranx spp* and *Seriola spp*
Demersal rocky bottoms	*Cephalopholis taeniops,* Serranus, Epinephelus, Mycteroperca, Murenídeos, Lutjanus, *Apsilus fuscus, Lethrinus atlanticus* and *Spondyliosoma cantharus*
Demersal of sandy bottoms	*Lithognathus mormyrus, Diplodus spp, Galeoides decadactylus, Pseudupeneus prayensis, Mullus surmuletus,* Priacantídeos and *Pomadasys incisus*

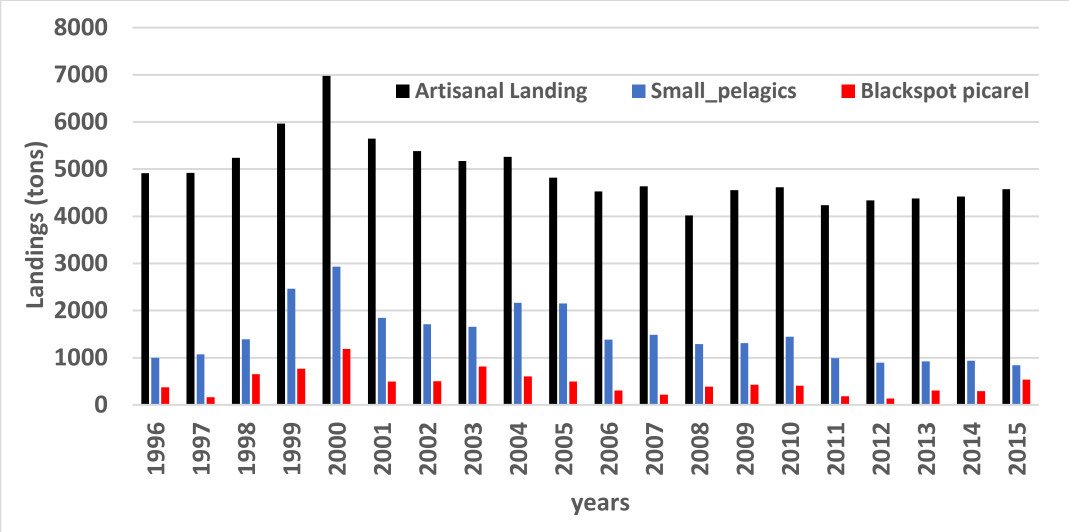

Figure 3. Annual landings for the artisanal fishery, for all species in the small pelagic group and for blackspot picarel in Cape Verde from 1996 to 2015 (source: INDP).

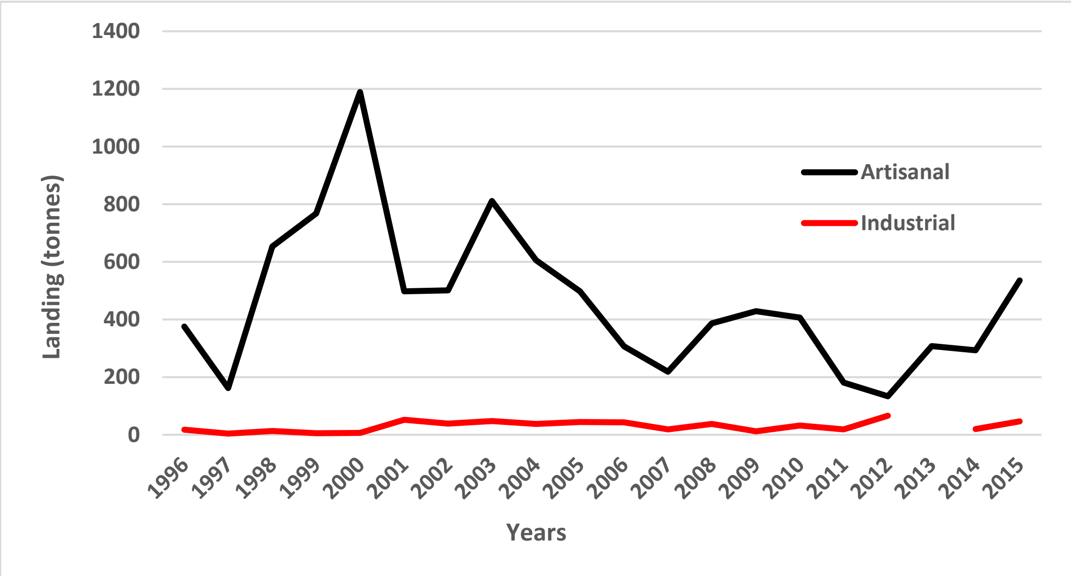

Figure 4. Total landing of blackspot picarel from 1995 to 2015 (source: INDP).

2.1 Biology and ecology of blackspot picarel

Blackspot picarel is a small pelagic fish of the Sparidae family. The geographic distribution of the species is limited to the continental shelf of Cape Verde, from Senegal to Angola, and Madeira (figure 5) (Carpenter et al., 2014). The blackspot picarel, as with all fisheries resources in Cape Verdean waters, is considered to be one stock (Almada & da Veiga, 2016). Blackspot picarel has not been researched much and limited information is available.

Figure 5. Blackspot picarel (left, source: adapted from FishBase, 2018) and the map of the geographic distribution of the species (right), the Cape Verde archipelago is marked by a circle (Carpenter, *et al.*, 2014).

Blackspot picarel is a small pelagic fish species that inhabits the continental shelf, usually where bottom depth ranges from 100 m to 250 m (Reiner, 2005). They aggregate in schools with several individuals (personal communications by fishermen and divers). They mostly feed on small crustaceans (Monteiro, 2008). Blackspot picarel recorded maximum fork length is 30 cm, however, the most common size of mature individuals is 25 cm (Monteiro, 2008). The species is a protogynous hermaphrodite (Costa, 2007). Microscopic analysis of gonads, collected monthly for one year, identified four spawning seasons in January and February, April, July, and November. Afterwards, based on a visual gonad analysis, it was identified that the species spawned throughout the year (Tariche & Martins, 2011).

2.2 Fisheries management of blackspot picarel

Management of the blackspot picarel stock is limited and one of the challenges is the lack of efficient stock assessment. The only attempt to estimate how much fishing the stock can sustain was a qualitative estimate of maximum sustainable yield (MSY) conducted in 2012. Indicated by a range between 200 and 300 tonnes (DeAlteris, 2012). It was recommended that the fishery should be limited to catches below MSY until an analytical stock assessment could be conducted, and information on age structure of the stock was urgently needed as input data for stock assessment (DeAlteris, 2012). However, the catch of 581 tonnes in 2015 was almost double the recommended MSY. Age-based analytical stock assessment has never been performed for the blackspot picarel stock despite its economic importance for the last 20 years. It is important to develop age reading methods for blackspot picarel as that is a key variable lacking for stock assessment which is vital for sustainable harvest of the stock.

Two management measures are applied for the species. A minimum size, for catch and commercialisation, of 17 cm was established in 2016. And the minimum mesh size for gillnet was set at 30 mm (INCV, 2016).

2.3 Environmental conditions

Cape Verde is in the subtropics and has a warm and dry climate with two distinct seasons. The period from December to June is defined as the cold and dry season, with an average sea surface temperature (SST) of 21 – 22 ºC, and the period from July to November as the warm and wet season with SST oscillating between 26 ºC and 27 ºC (Almada, 1993). Mixed layer depth ranges from 25 m to 40 m whereas thermocline depth extends down to 100 m (González & Tariche, 2009). Temperature in these layers changes with the seasons. Below the thermocline, the temperature gradually decreases to approximately 10 ºC and 6 ºC at 500 m and 1000 m depths respectively (González & Tariche, 2009).

3. OTOLITHS

Otoliths are ridged calcified structures localised in the head of all teleost fish that have been used to estimate age for the last century (Jones, 1992). Minerals deposited in the otolith are positively correlated to somatic growth, where each year forms a pair of increments (Campana, 1999). The annual increment pair has a narrow transparent increment formed during the slow somatic growth season of the year and a wide opaque increment formed during the fast growth season (Campana, 1999). Importantly, otoliths are not reabsorbed during stressful conditions, (Mendoza, 2006), hence they provide a continuous record of fish age from hatching and also their dimension is correlated to the somatic size and age of the fish (Granadeiro & Silva, 2000).

Otoliths are in the inner ear and aid with hearing and balance (Campana, 1999). Fish have three pairs of otoliths called lapilli, sagittae, and asterisci which differ in size and location *(Stevenson & Campana, 1992)*. The sagittae pair is most often used to estimate fish age, since they are the largest and easiest to extract among the three pairs (Oliveira, *et al.*, 2014). Otoliths are three dimensional and grow at different rates between dimensions. The size and shape of otoliths varies between species from oblong, to round, and to having prominent peaks (figure 6) (Poper *et al.*, 2005).

Figure 6. Different shapes of the sagittal otolith from different species. From left to right *Merluccius bilinearis*, *Halargyreus johnsoni*, *Lampris guttatus*, *Urophycius tenuis* and *Lopholatilus chamaeleonticeps*. Adapted from Poper, *et. al*, (2005).

Given the diverse shape and size of otoliths between species, it is not surprising that one method cannot be used to estimate age from otoliths of all fish species. A toolbox of methods has been developed to estimate age of an individual from its otoliths. The most common methods are direct observation of the whole otolith under a stereoscope and the cross-section method where a slice is cut from the middle of the otolith and observed in a microscope (Mendoza, 2006). Age is determined by counting pairs of narrow and wide increments, each pair corresponds to one year.

For each fish species, the best method to estimate age by counting otolith increments needs to be developed by testing the different methods available. This has been done for one species from Cape Verde waters, a grouper (*Cephalopholis taeniops*) (Tariche *et al.*, 2014). The grouper otoliths had narrow translucent increments and wide increments. Narrow increment formation occurred when sea surface temperature was around 21.5 °C, and wide increments when temperature was 26-27 °C (Tariche *et al.*, 2014). Increment formation in blackspot picarel has not been investigated. Given the seasonal difference observed in the temperature of their habitat, the formation of narrow and wide increments on annual basis is likely.

4. METHODOLOGY

4.1 Black picarel biological sampling

Biological samples of blackspot picarel, have been collected from the artisanal fishery from 2005 onwards by INDP (table 2). The samples are bought at the fish market of Sao Vicente Island. For each sample, 75 specimens are haphazardly selected from the sale stalls. Sampling is done twice a month throughout the year, when blackspot picarel is available in the fish market. At the INDP lab, fork length total weight, liver weight and gonad weight were measured. The specimen's sex was visually classified as immature, female, male or hermaphrodite. Maturity stage was also visually determined using a 7-stage scale (Almada, 1997). The number of specimens sampled annually ranges from 742 to 1526. The sagittae otoliths were collected from 25 specimens on each sampling date and more than 2000 pairs of otoliths collected. Otoliths were not sampled from 2012 onwards.

In addition, a scientific beach seine survey was done in the Sao Vicente Island in April 2008 by INDP. A total of 35 blackspot picarel were caught ranging in fork length from 4.5 cm to 15 cm. The specimens were measured like artisanal samples and their otoliths were collected.

Table 2. Number and biological information for blackspot picarel sampled 2005- 2017.

Year	L (cm)	W (g)	Sex				MS (g)	GW (g)	LW (g)	N Otolith (estimate)
			F	I	M	M/F				
2005	1526	1526	754	13	754	5	1526	1526	1526	300
2006	978	978	449	1	526	2	977	977	977	250
2007	1197	1197	657	2	535	3	1196	1195	1196	300
2008	1050	1050	582	5	463		1050	1045	1050	300
2009	1500	1500	870		628	2	1499	1500	1500	300
2010	1339	1339	683		655	1	1339	1339	1339	275
2011	1049	1049	533		515	1	1049	1049	1049	225
2012	1040	1040	563		477		1040	1040	1040	275
2013	1447	1447	764		683		1446	1447	1447	0
2014	1109	1109	547		561	1	1109	1109	1109	0
2015	963	963	511		452		963	963	963	0
2016	742	742	398		343	1	742	742	742	0
2017	1114	1114	565		548	1	1113	1114	1114	0

Note: L=Fork Length; W=Total Weight; I=Immature/undifferentiated; F=Females; M=Males; M/F=hermaphrodite; MS – Maturation stage; GW=Gonad Weight and LW= Liver Weight.

4.2 Laboratory methods

4.2.1 Otolith subsampling

For the current study, a sample of blackspot picarel otoliths from INDP was used, in total 785 specimens with fork length ranging from 4.5 to 29 cm (table 3). A database was created, and each specimen was given a unique identification number (ID). To create the ID number information was used relating to the month, the year, and the sequential number of the otolith in the month according to the otoliths database previously created. An example of ID recording is Jan11_09 that means the sample is from January 2011 and the number 9 refers to the month in the database.

Table 3. Number of otoliths sent from Cape Verde to Iceland.

Length (cm)	**2004**	**2005**	**2006**	**2007**	**2008**	**2011**	**2012**	**Total**
4.5					1			**1**
5					2			**2**
5.5					1			**1**
8					1			**1**
9					7			**7**
9.5					1			**1**
10					11			**11**
11					5			**5**
12					3			**3**
13					1			**1**
14		5			1			**6**
15		6			1			**7**
16		5						**5**
17		7						**7**
18		8					4	**12**
19		4		7		4	16	**31**
20		9		19		2	38	**68**
21		13	1	12		7	44	**77**
22	34	11	4	9		24	24	**106**
23	36	19	7	9		29	17	**117**
24		13	3	12		46	36	**110**
25		23	5	5		45	32	**110**
26		9	4	2		34	25	**74**
27		2	1	2		6	6	**17**
28						1	3	**4**
29							1	**1**
Total	**70**	**134**	**25**	**77**	**35**	**198**	**246**	**785**

A subsample of 152 specimens was selected for the aging study (table 4). The selection of the subsample was conditioned by the high number of otoliths brooked due to the bad storage condition of the otoliths. It is important to analyse otoliths from the whole size range to understand how the otolith grows. The aim was to analyse otoliths from ten specimens for each 1 cm length interval. It is also important to understand how the increments grow during the two annual growth seasons, hence otoliths were selected from the warm fast growth season and the cold slow growth season. The aim was to analyse otoliths from ten specimens for each 1 cm length bin and for the different seasons within a year. Not enough otoliths were collected in one single year for the complete length range from 4.5 cm to 29 cm. The highest number of otoliths were available from 2012, total 246 otoliths. Hence, otoliths from 2012 were used for majority of the length range. The only otoliths available from the two seasons within the same year and the same 1cm length interval was 22 cm and 23 cm long specimens in 2004.

Table 4: Number of otoliths sampled per 1 cm length interval for counting of annual increments, all otoliths for 4.5 to 18 cm and 28 to 29 cm length fish were used.

Length (cm)	**4.5 - 18**	**19**	**20**	**21**	**22***	**23***	**24**	**25**	**26**	**27**	**28 - 29**	**Total**
Otoliths (n)	32	14	10	11	21	20	10	13	10	7	4	**152**

Otolith selection by year:

- length range from 4.5 to 18 cm, otoliths collected from years 2005, 2008 and 2012,
- length range from 19 to 21 cm and 24 to 29 cm from the year 2012, except the 27 cm that was completed with otoliths from 2011,
- length 22 and 23 cm were taken from the year 2004, to compare the formation of translucent and opaque rings in the otoliths between the cold and warm season.

4.2.2 Otolith processing

In the laboratory, a picture was taken of the intact otolith using a stereoscope, length and width of the otolith was measured using image analysis software Adobe Photoshop Elements 2019. The maximum length was measured between the rostrum and a posterior region (horizontal arrow) and the maximum width (vertical arrow) as a distance perpendicular to the otolith length (figure 7). Then, using the scale bar of the figure, the real dimensions were calculated in millimetres.

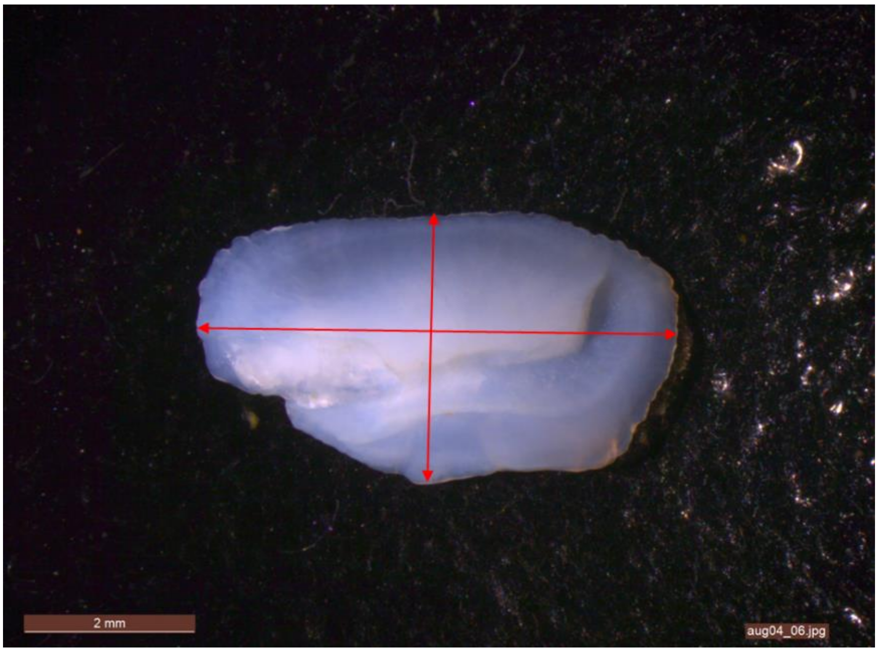

Figure 7. Dimensions measured for each otolith, length (horizontal arrow) and width (vertical arrow).

To estimate the age of the specimens, different methods were tested for counting increments starting with the most common method of direct observation of the whole otolith under a stereoscope Leica M125 C (magnification of 12.5x) (Appendix 1). Using this method, increments were either invisible or blurry (Figure 8). Next method tested was the cross-section method where the otoliths are fixed in black resin and transversely sectioned of 1 mm from the core of the otolith, hence the slice includes the otolith nucleus. Then the otolith slice was stained using neutral red for one hour and observed under the microscope. This method did not reveal any increments. The third method tested was to polish the otolith slice first using a 3 µm polishing film and then a 0.5 µm film. Next a photograph is taken of the slice using a stereoscope Olympus SZX9 (Magnification 6.3x to 12x) connected to a camera Olympus SC50 with a reflected light below the otolith shining through it. The image was enhanced using Adobe Photoshop Elements 2019. This preparation revealed a pattern of narrow and wide increments. The number of narrow increments counted using the enhanced otolith image displayed on a computer screen. The assumption is that number of narrow increments represent age of specimen in years.

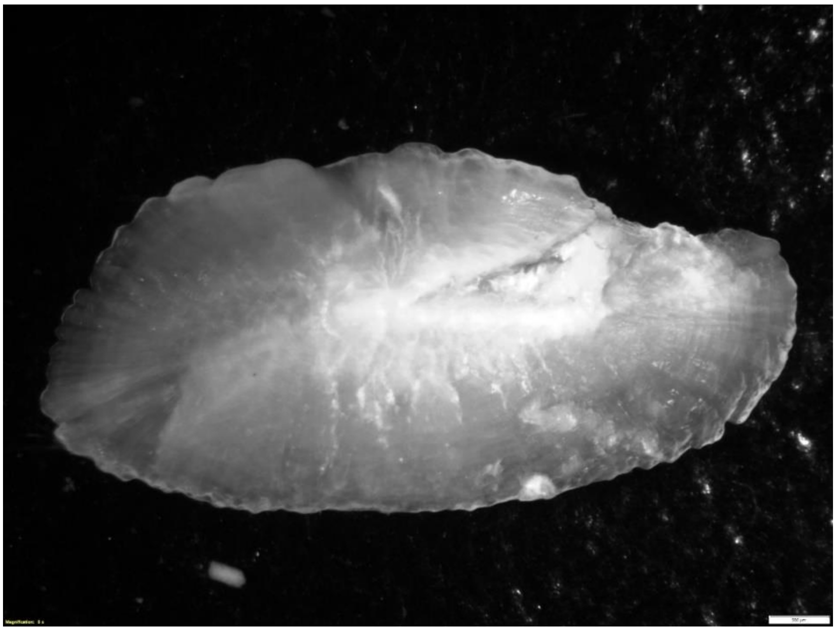

Figure 8. A whole otolith visualised with a stereoscope; some increments visible but not clear enough to be counted. Specimen fork length was 21 cm.

The above description of otolith processing applies to the specimens with fork length > 10 cm. There were 24 specimens with fork length ranging from 4.5 cm to 10 cm and their otoliths were too small for slicing, due to the otolith small size. Therefore, another method was needed to count increments of small specimens. Annual increments were visible when observing the whole otolith under a stereoscope. For ageing them, a photograph was taken of the whole otolith.

All the images taken were digitally manipulated to highlight the annual increments using image software (Adobe Photoshop Elements 2019). During the image editing, the level of grayscale was activated for the otoliths, where the grey level was enhanced and adjusted. Posteriorly by an unsharp mask filter using a radius was manipulated in a range between 10–20 pixels and amounts in a range of 100%–200% (Campana, *et al.*, 2016). To count the increments points were added marking the increments in the image (figure 9).

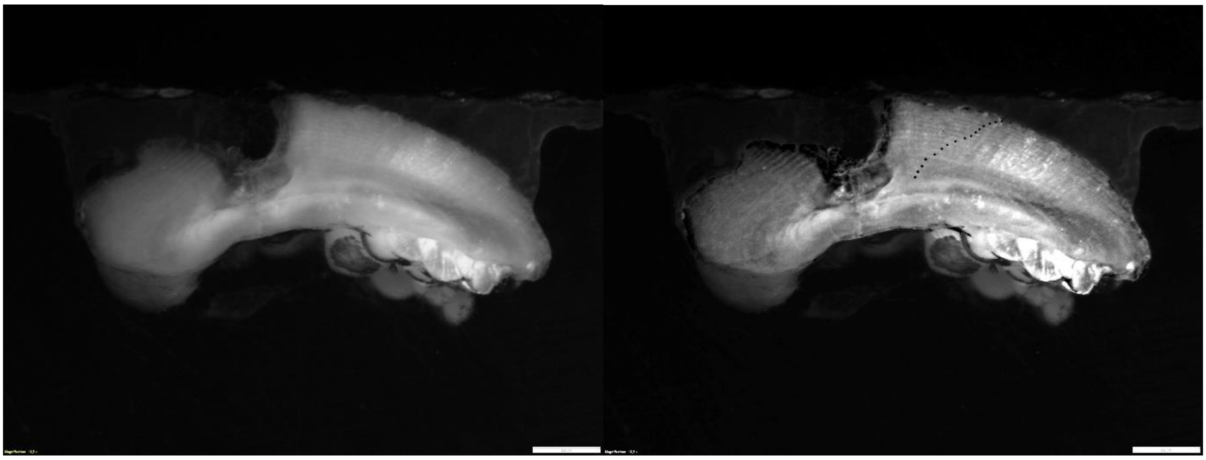

Figure 9. Difference in an otolith image before (left) and after (right) image enhancement. Annual narrow growth increment identified (solid black dots). The specimen had fork length of 28 cm.

Quality checking of increment counting is an important stage when estimating fish age using otoliths (Campana, 2001). To evaluate precision of increment counting 23% of the number of otoliths readings were selected for a blind recounting. In total 31 otoliths were randomly selected from the total length range with otolith recounted, table 5.

Table 5. Distribution of the frequency length of the 23% of the otolith readings to evaluate the precision of the increment counting.

Length	9	14	15	17	18	19	20	21	22	23	24	25	26	27	28	29
N	3	1	1	1	1	1	2	4	2	2	3	4	3	1	1	1

4.3 Statistical analysis

The relationship between fork length and total weight was estimated using linear regression on log-transformed data and was fitted using the equation:

$$W = a * L^b \quad (1)$$

where W is weight (g), L is length (cm), and a and b are parameters whose values are estimated.

The relationship between specimen length and otolith size length was investigated using linear regression (Zar, 1999) that was fitted to the data using the equation:

$$OL = a * L + b \quad (2)$$

where OL is otolith length (mm), L is fork length of the specimen, and a and b are parameters whose values are estimated. Linear regression is also used to investigate the relationship between specimen length and otolith width and was fitted to the data using the equation:

$$OW = a * L + b \quad (3)$$

where OW is the otolith width (mm).

Precision of repeated increment counting was tested using several methods including a paired t-test (Zar, 1999), average per cent error (APE) (Beamish & Fournier, 1981), coefficient of variation (CV) (Chang, 1982), and index of precision (D) (Chang, 1982). For all other analyses presented, results from the first increment count were used. The APE is calculated for each otolith with replicated increment counting using the equations:

$$APE_j = 100\% * \frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - xX_j|}{X_j} \quad (4)$$

where R is the number of replicate readings, X*ij* is the *i*th replicated increment count for the *j*th specimen, and X*j* is the average increment count for the *j*th specimen (Beamish & Fournier, 1981). The CV is calculated for each otolith with replicated increment counting using the equation:

$$CV_j = 100\% * \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij}-X_j\right)^2}{R-1}}}{X_j} \tag{5}$$

Where CV_j is the age precision estimate for the *j*th fish. The D is calculated by the equation:

$$D_j = \frac{CV_j}{\sqrt{R}} \tag{6}$$

To estimate somatic growth rate of blackspot picarel the von Bertalanffy growth equation was fitted to data:

$$L_t = L_\infty \left(1 - e^{-k(t-t_0)}\right) \tag{7}$$

where L_t is the fork length of blackspot picarel (cm) at age *t* (years), L_∞ the asymptotic length, k is a growth coefficient (year^{-1}) and t_0 is the age at zero length (Jennings et al., 2001).

For estimate maturity-at-age or the age at 50% individuals attain the sexual maturity was fitted a logistic curve to find the relationship between the proportion of mature and the age.

$$P = 1/(1 + e^{-r(t-Tmat)}) \tag{8}$$

where P is the proportion of mature at age *t* (years), T_{mat} is the maturate age and r is a constant of the equation (Jennings *et al*., 2001).

The relationship between specimen age and total weight was estimated using linear regression on log-transformed data and was fitted using the equation:

$$W = a * t^b \tag{9}$$

where *W* is weight (g), *t* is age (year), and *a* and *b* are parameters whose values are estimated.

The relationship between specimen age and otolith size length was investigated using logarithmic regression (Zar, 1999) that was fitted to the data using the equation:

$$OL/OW = a * \ln(t) + b \tag{10}$$

where, *OL* is otolith length, *OW* is the otolith width, *t* is age of the specimen, and *a* and *b* are parameters whose values are estimated.

Statistical analyses were performed in Microsoft Excel (2016) or in R 3.5.1.

5. RESULTS

5.1 Biological parameters

5.1.1 Length frequency distribution

Blackspot picarel recorded fork length ranged 4.5 cm to 37 cm (figure 10). However, specimen > 31 cm were excluded from otolith analysis due their weight indicates obvious measurement errors at time of sampling. Annual average length ranged from 21.7 cm to 24.7 cm (table 6) and was not statistically significant between the years (ANOVA: $F (11, 149) = 0.83$, $p = 0.61$). For annual length distribution see appendix 2.

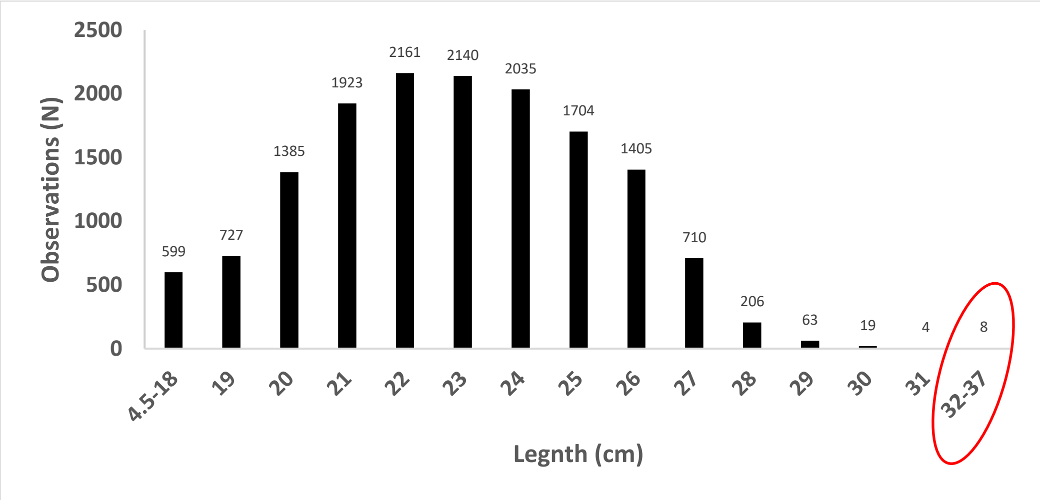

Figure 10. Blackspot picarel fork length frequency from all biological samples collected from 2005 to 2017 (n =15089). Length ranges from 32 cm to 37 cm. Eight specimens were considered outliers and were excluded from otolith analysis (appendix 3).

Table 6. Blackspot picarel annual minimum length (Lmin), maximum length (Lmax), mean length, and standard deviation of the mean (StdDev) from 2005 to 2017. Number of specimens also reported (N).

Year	Lmin (cm)	Lmax (cm)	Mean (cm)	StdDev	N
2005	12	28	22.1	2.2	1526
2006	17	29	22.4	2.1	978
2007	15	30	23.0	2.9	1195
2008	4.5	29	21.7	3.4	1085
2009	17	29	22.7	2.1	1499
2010	17	29	23.6	2.1	1339
2011	18	32	23.9	1.8	1046
2012	17	29	22.4	2.4	1040
2013	16	29	21.8	2.6	1447
2014	16	29	22.7	2.6	1109
2015	17	31	22.8	2.9	963
2016	18	30	23.7	2.1	742
2017	19	29	24.7	1.9	1113

Length distribution of the 785 specimen whose otoliths were made available for the current project (figure 11).

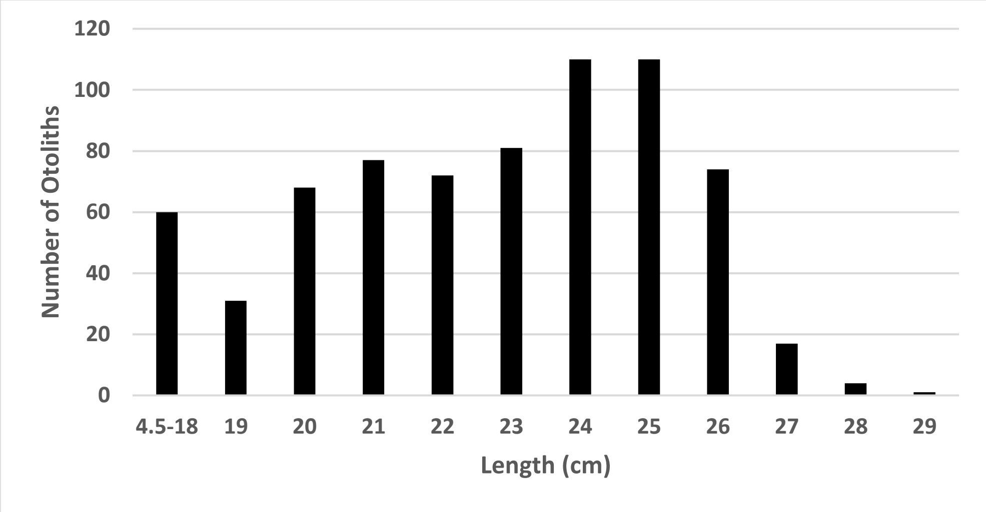

Figure 11. Length distribution of the otolith sample included in the current study.

5.2 Otolith analysis

5.2.1 Relationship between specimen size and otolith dimensions

Blackspot picarel otolith length ranged from 1.54 mm to 8.27 mm (figure 12a) and the width ranged from 0.93 mm to 3.87 mm (figure 12b). Positive linear relationship was between blackspot picarel fork length and otolith dimensions (Figure 12a, b). Specimen length explained 94% and 92% of respective variance in otolith length and otolith width.

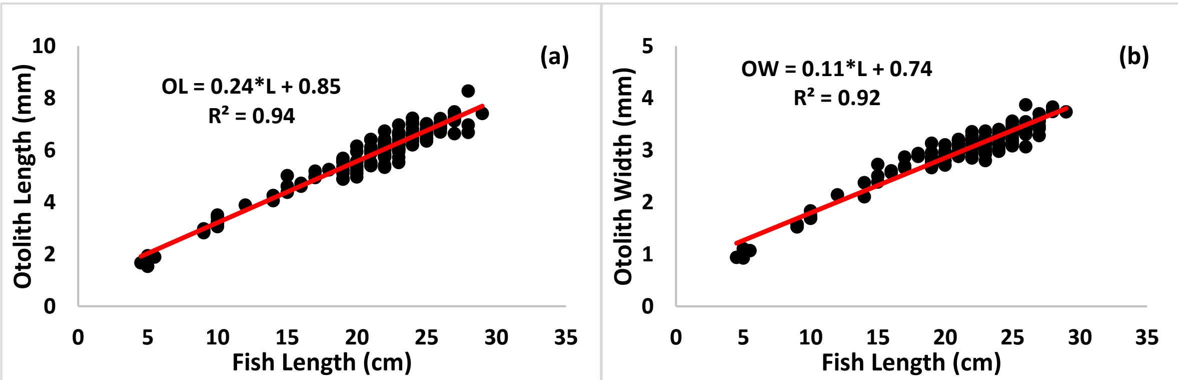

Figure 12. Relationship between specimen fork length and otolith length (a) and with otolith width (b). Regression equation and explained variance (R^2) included (n = 139).

5.2.2 Age estimate

Otolith increments were successfully counted in 134 specimens ranging in fork length from 4.5 cm to 29 cm (Table 7). Their estimated age ranged from two to seventeen years, assuming each increment represents one year. The specimen average length was 20.7 cm and their average age was 10.6 years. Blackspot picarel age-length key is presented in table 8.

Eighteen otoliths were lost during processing, twelve were damaged during preparation and six did not display visible increments. The otoliths were not replaced as no more otoliths were available in their length interval.

Table 7. Fork length (cm) and estimated age of blackspot picarel (n = 134).

Length (cm)	**Age (years)**																**Total**
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
4.5	1																1
5	1	1															2
5.5		1															1
9			4	1													5
10			3	2	1												6
12																	0
14						1											1
15						3											3
16							2										2
17							1	2									3
18							1		2								3
19							3	4	3	3							13
20						1		3	4	1							9
21								2	1	2	3	1					9
22								2	7	4	3	1	1				18
23										5	3	7	1				16
24										1	5	1	2	1			10
25											2	3	4	2			11
26											1	5	4				10
27												3	3			1	7
28													1	1	1		3
29															1		1
Total	**2**	**2**	**7**	**3**	**1**	**5**	**7**	**13**	**17**	**16**	**17**	**21**	**16**	**4**	**2**	**1**	**134**

Table 8. Blackspot picarel age-length key (n = 134).

Length	**Age Proportions**															
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
4.5	1.0															
5	0.5	0.5														
5.5		1.0														
9			0.8	0.2												
10			0.5	0.3	0.2											
12																
14						1.0										
15						1.0										
16							1.0									
17							0.3	0.7								

18							0.3		0.7							
19							0.2	0.3	0.2	0.2						
20						0.1		0.3	0.4	0.1						
21								0.2	0.1	0.2	0.3	0.1				
22								0.1	0.4	0.2	0.2	0.1	0.1			
23										0.3	0.2	0.4	0.1			
24										0.1	0.5	0.1	0.2	0.1		
25											0.2	0.3	0.4	0.2		
26											0.1	0.5	0.4			
27												0.4	0.4			0.1
28													0.3	0.3	0.3	
29															1.0	

5.2.3 Replicate counting of otolith increments

Number of increments were counted for the second time for 23% of otoliths. There was a significant difference in estimated age between readings (paired t-test: $t_{2,30} = 2.04$, $p = 0.039$). The linear regression between readings had a slop of 0.9, hence suggesting lower number of increments in the second reading (figure 13).

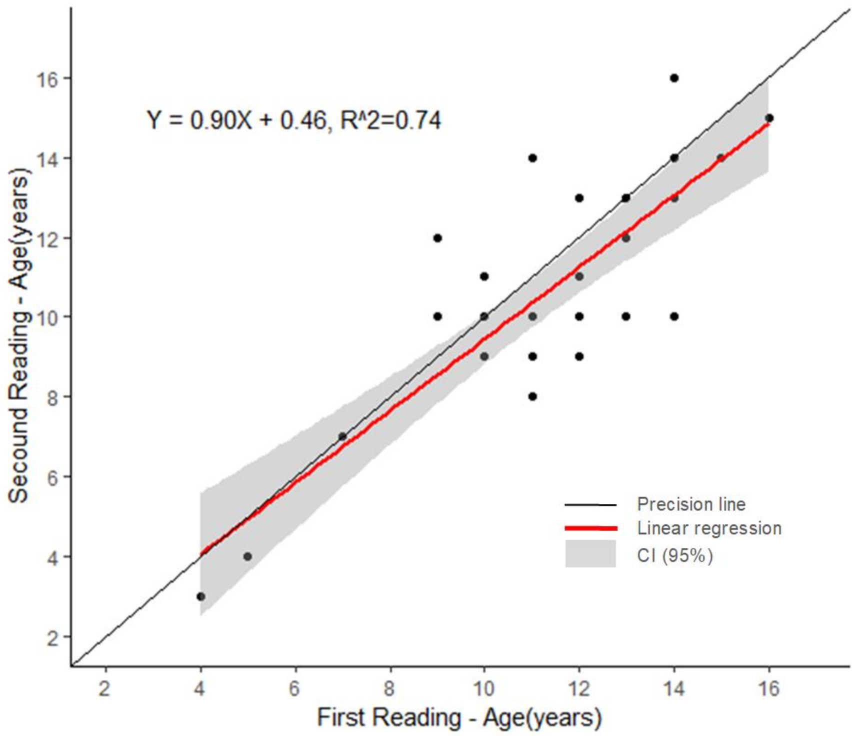

Figure 13. The relationship between the first and second replicas of otoliths increment counting, the black line represent the precision line, the red line the linear regression and the grey area the confidence Interval of 95% (n = 31).

The results of the different ageing precision tested for the study shown an APE average = 7.15%, CV average = 8.76% (figure 14) and D average = 6.19%. Value for all readings are in appendix 4.

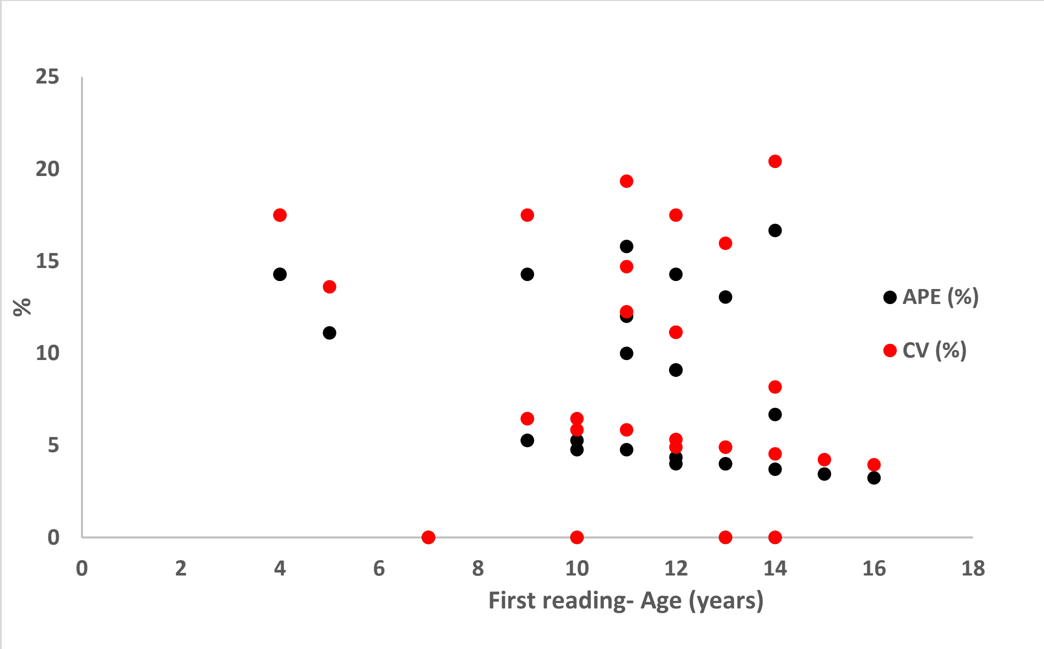

Figure 14. The results of the APE and CV for each otolith compared for the replica 1 for the sample.

5.2.4 Estimating growth parameters

The age-length key was used to estimate the mean fork length per age. Next the von Bertalanffy equation was used to calculate growth parameters for the blackspot picarel (figure 15). The growth parameters were estimated as $L_\infty = 36.17\ cm$, $k = 0.09\ year^{-1}$, $t_0 = 0.89\ year$.

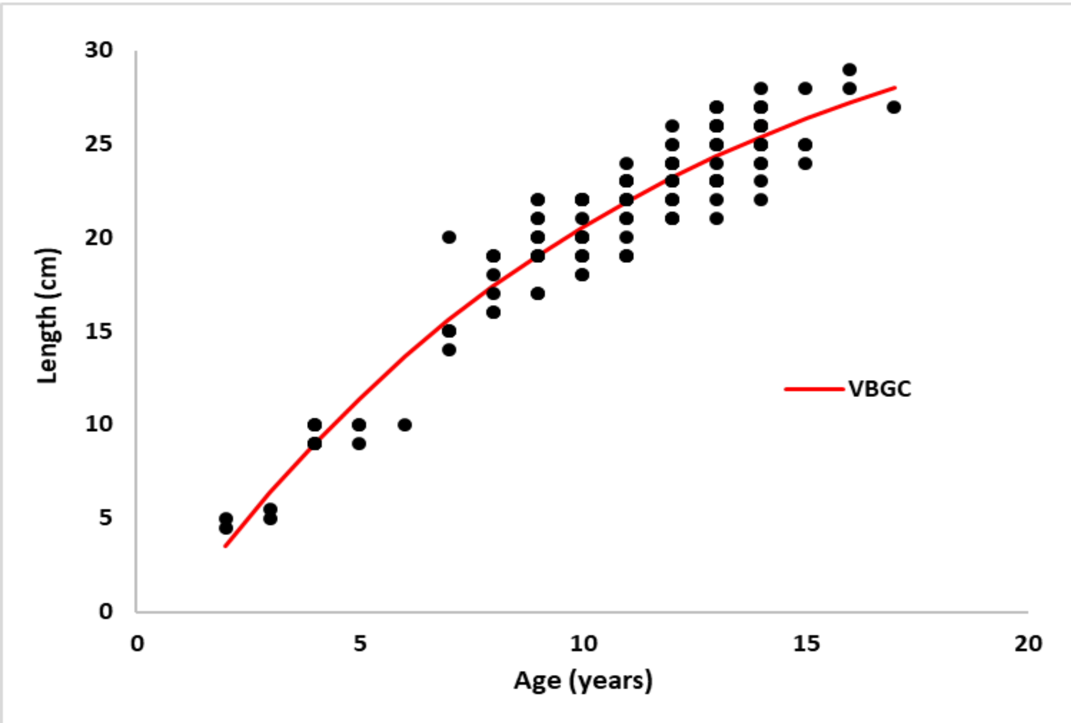

Figure 15. von Bertalanffy growth curve for blackspot picarel (n = 134).

5.2.5 *Maturity-at-Age*

The maturity-at-age was estimated using the maturity information available for all the specimens aged. The logistic curve obtained show that 50% of the blaskspot picarel are mature the age of 7.06 years (figure 16). Individuals aged 2 to 6 years (n=15) were all immature.

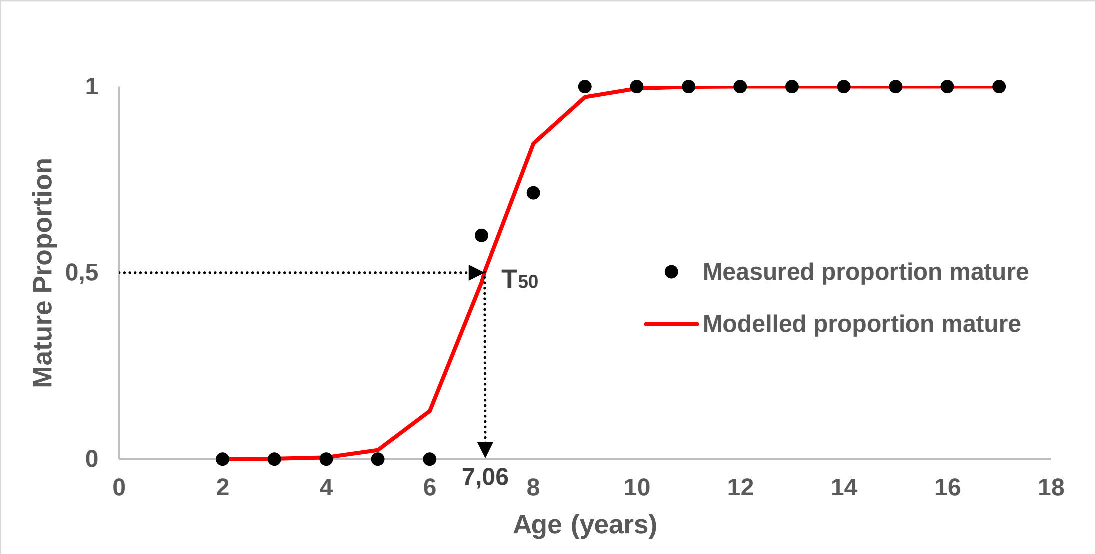

Figure 16. Proportion of mature blackspot picarel for each age and a fitted logistic model. The arrows shown the age that 50% of the individuals are mature.

5.2.6 *Age – weight relationship*

Total weight of specimen used in the otolith analysis ranged from 1.78 g to 423 g. The relationship between weight and age is best described by exponential growth curve (figure 17).

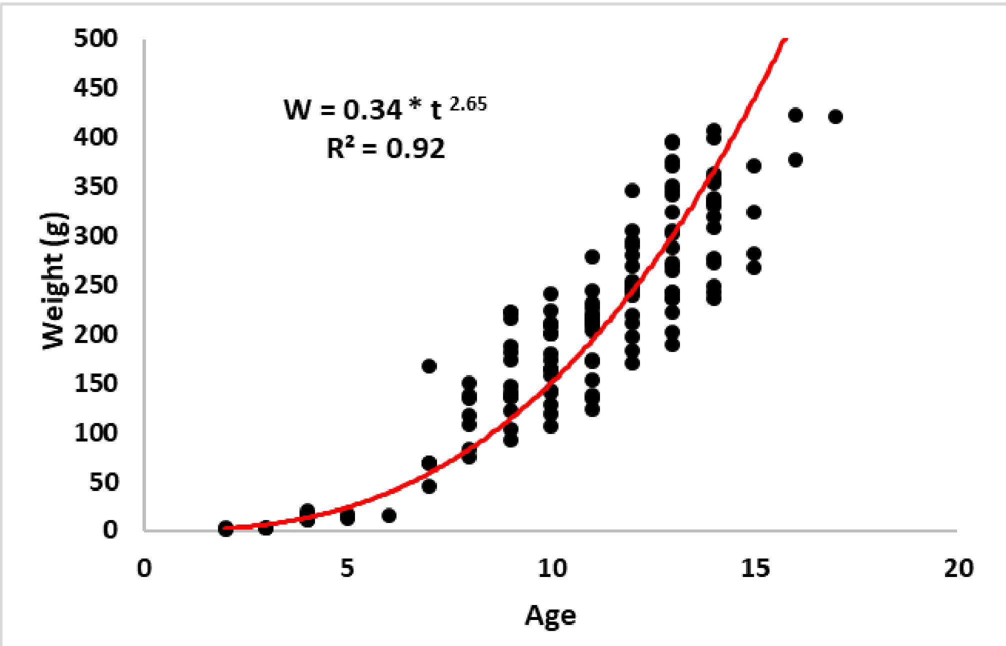

Figure 17. The relationship between blackspot picarel age and total weight with age explaining 92% of variance in weight (n = 134).

5.2.7 Relationship between specimen age and otolith dimensions

A positive relationship was between blackspot picarel age and otolith dimensions (Figure 18a and b). Specimen age explained 89% of the variance in otolith length and otolith width.

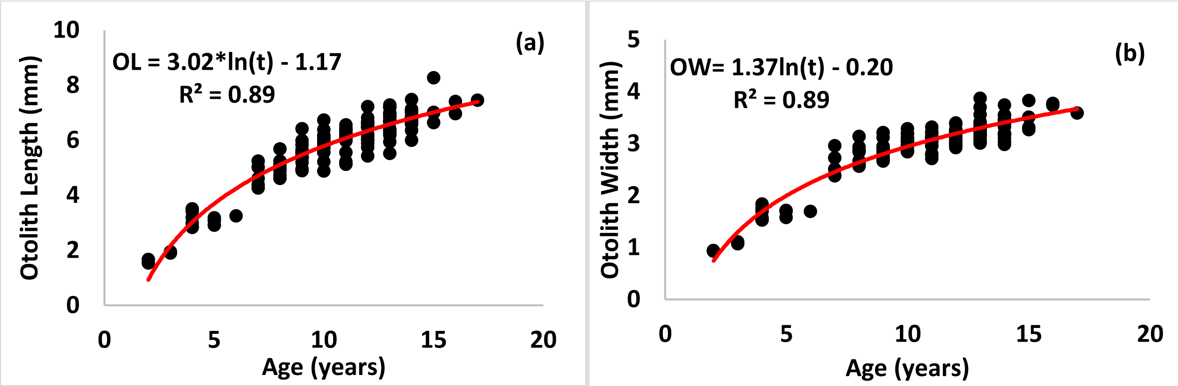

Figure 18. Relationship between the fish age and the otoliths dimensions, otolith length (a) and otolith width (b); n = 134.

6. DISCUSSION

6.1 Otolith analysis

Age determination of fish stocks is important to estimate life history traits, to understand stock dynamics, and is vital for sustainable fisheries management. This study is the first attempt to investigate age of blackspot picarel. Several methods were tested to identify and count otolith increments. We successfully identified otolith increments for small specimen (< 11cm) using the whole otolith, and for larger individuals by sectioning the otolith through the core and counting increments from an enhanced image of the slice.

The otolith analyses suggest that the ageing methods developed can be used to age blackspot picarel. Precision of replicated increment counts, estimated using several metrics, was below average but within the range of values reported for otolith studies (figure 4 in Campana, 2001). There is no specific threshold for acceptable precision for an ageing method. However, Campana (2001) calculated the median CV from 117 publications to be 7.6% (range 0% – 26%) which is lower than our CV (8.8%) *i.e.* precision of our study was lower than the median. Precision decreased with age suggesting that the otoliths structure becomes fuzzier as the otolith grows. Many factors can influence ageing precision, such as the level of difficulty identifying increments for the species, experience of the age reader, and sample size. It is outside the scope of the current study to identify which factors reduce precision.

Our study design included specimens ranging in size from 4.5 cm to 29 cm which include the recorded size range from biological samples collected since 2005. Otoliths in the age-length key were collected from many different years as no single year had otoliths collected for the whole size range presented in biological sampling. Traditionally, an age-length key is constructed from specimens sampled during the same calendar year, and constructed for each year. It is impossible to estimate if and how merging data from several years influences the age-length key presented in the current study. However, until a more detailed study is conducted the age-length key provided in the current study can be used to convert catch length distirbution to age distribution for stock assessement.

Age determination of tropical fish using otoliths is considered challenging (Zekeria *et al*., 2006) and is rare compared to temperate fish species. However, Longhurst & Pauly (1987) suggested that increments would be deposited in otoliths of tropical fish when seasonal variation in ambient temperature was ≥ 4 ° C. Given the two seasons observed in Cape Verde, where the temperature of the sea water at temperature ranges from 21-22 ° C in the cold season compared to 26-27 ° C in the hot season, it is to be expected that increments are deposited in otoliths of blackspot picarel.

Identification of the first increment in the blackspot picarel otolith was difficult for larger specimen. In the otolith slice, the core was not always evident, although the otolith was cut where the core was identified. To discard the possibility that the otoliths are being cut in the wrong location, a group of otoliths was aligned in a block, fixed with a transparent resin, and visually monitored that the centre of the otolith was cut. This experiment showed how some otoliths had an opaque core making it difficult to identify the first increment compared to others. For smaller specimen presence of false increments complicated ageing. Furthermore, otoliths shape varied between specimen with no obvious otolith axis visible. To measure increment width a specific axis in the otolith must be identified and all otoliths measured along this predefined axis (Panfili & Morales-Nin, 2002). One objective of the present study was to measure increment formation throughout the year, between the hot season and the cold season. That goal was not reached since no axis was identified in the otolith.

Ageing method has been developed for another commercial fish speceis in Cape Verde, the speceis *C. taeniops*, species with a slow growth. The k for the species was 0.135 year^{-1} and the CV obtained comparing the two reading methods used was 8.5% and 9.1% for the reader 1 and reader 2 (Tariche *et al.*, 2014). The result of the CV are similar to the results from the present study.

Campana (2001) suggested splitting the development of a fish stock ageing program into four steps with development of an ageing method being the first step and the next step being age validation. Validation is confirming that the number of increments present the age of the specimen in years. This demands extensive experiments where individuals are reared from hatch until old age, then slaughtered and number of otolith increments compared to known age of the specimen. It is recommended that such a study be conducted in the future.

The linear relationship between fish size and otoliths dimensions can be used in ecological studies to convert blackspot picarel otoliths, sampled in stomach samples, to specimen size and to analyse size distribution in archaeological samples (Harvey et al., 2000). There was a strong relationship between number of increments and otolith size, and between otolith size and fish size. The relationship between fish age and otoliths dimensions also show a strong relationship, that means the otoliths dimensions can be used to estimate the age.

6.2 Life history parameters

The growth parameters obtained using the age-length key suggest that blackspot picarel should be considered a slow growing species. Currently, the species is classified as a small pelagic with rapid somatic growth. There is no other study on blackspot picarel growth in the literature to the authors knowledge. Information on growth rate is available for two species in the *Spicara* genus, *S. maena* and *S. smaris* with their k-values ranging from 0.165 to 0.489 year^{-1} and 0.125 to 0.929 year^{-1} respectively (Froese & Pauly, 2018) which is 36% to 1010 % larger than k-value reported in the current paper (Appendix 5). Given the slow growth of blackspot picarel reported in the current study it should be classified as a slow growing species.

Estimated age at maturity was 7.06 years. Analysis of the biological data shows that specimen in the age range from 2 to 6 years were immature, ages 7 and 8 years were a mixture of immature and mature specimens, and that all specimens age 9 years and older were mature. The age at maturity is not currently estimated for the species in Cape Verde waters due the absence of age information for them, but for the *C. taeniops* is was estimated as 4.2 years (Tariche, 2002). This value is lower than the T_{50} obtained for the blackspot picarel in the present study, however our T50 was calculated using a small sample.

7. CONCLUSIONS AND RECOMMENDATIONS

A method to age blackspot picarel using otoliths was developed using biological samples collected from 2004 to 2017 in Cape Verde. An age-length key for specimen ranging in fork length from 4 cm to 27 cm was produced. Ageing precision was slightly below median compared to Campana´s (2001) overview of fish otolith studies. The recommendations for the current study are:

1) to use the present age-length key for stock assessment and fisheries management purposes until an annual age-length key can be produced for blackspot picarel,

2) carry out annual studies of the age-length key including specimens from the whole size spectrum of the species,

3) to conduct studies on accuracy of the ageing method. That is, to test if the number of otolith increments represents the true age of the specimen,

4) trained staff and laboratory infrastructure for otolith analysis needs to be established at INDP to facilitate future otolith work; that is much needed for blackspot picarel and other species,

5) it is advisable that blackspot picarel be reclassified as a slow growing species but not a fast growing small pelagic as it is currently classified.

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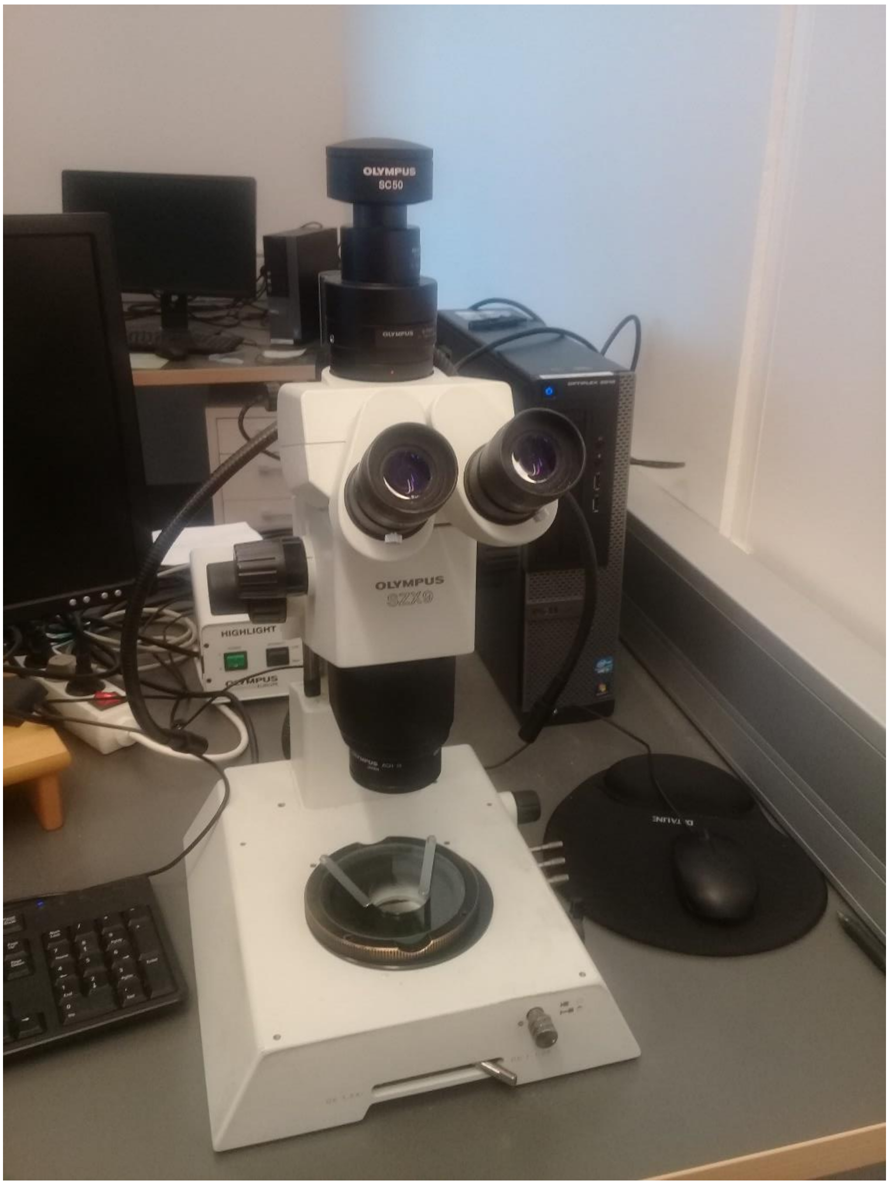
10. APPENDICES

Appendix 1. Equipment used to count increments in otoliths.

Equipment used to take the whole otoliths pictures stereoscope Leica M125 C connected to a screen monitor.

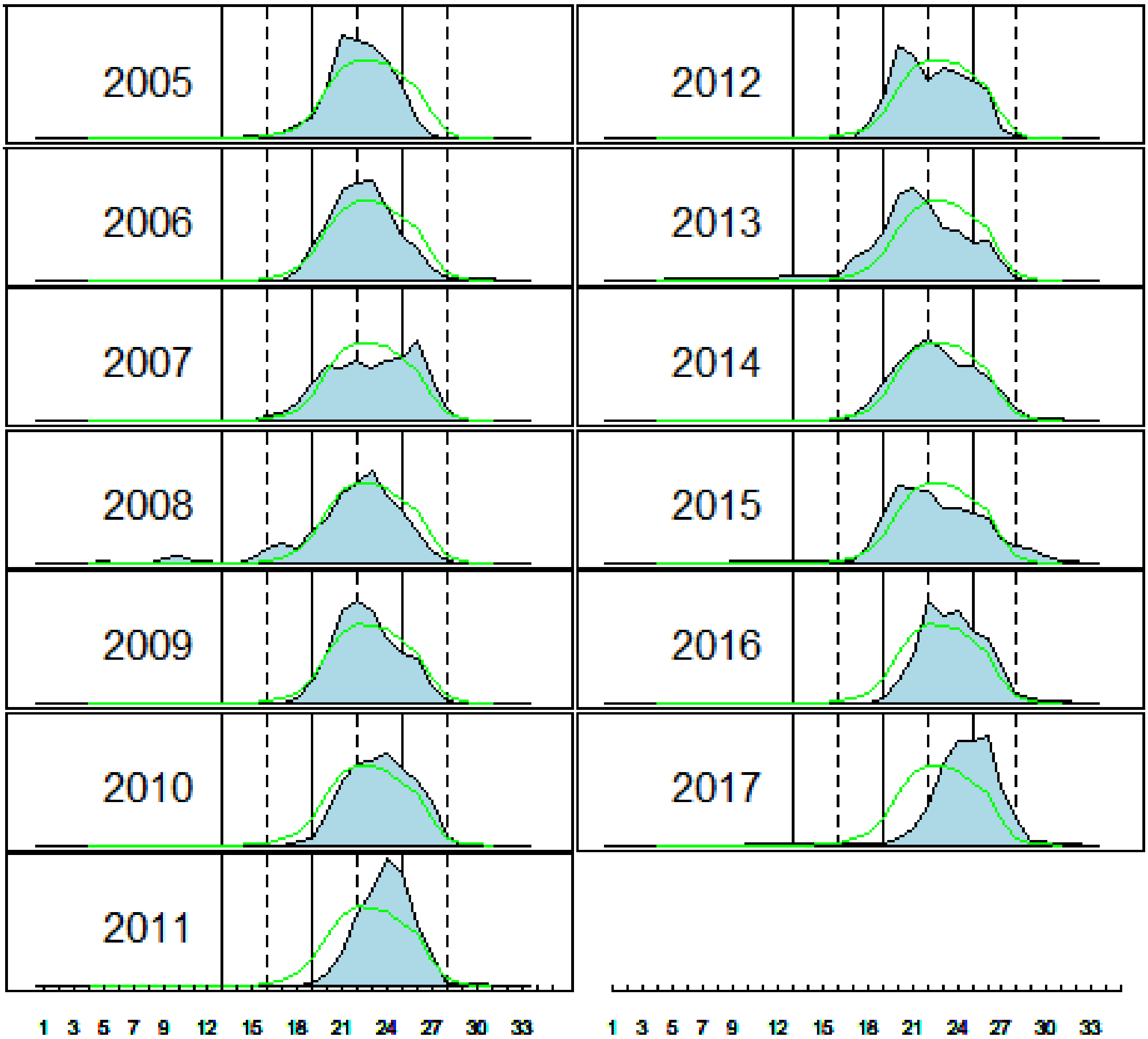
Process of the line step of the otoliths where they were prepared to be cut.

The otolith cutting machine used in the process.



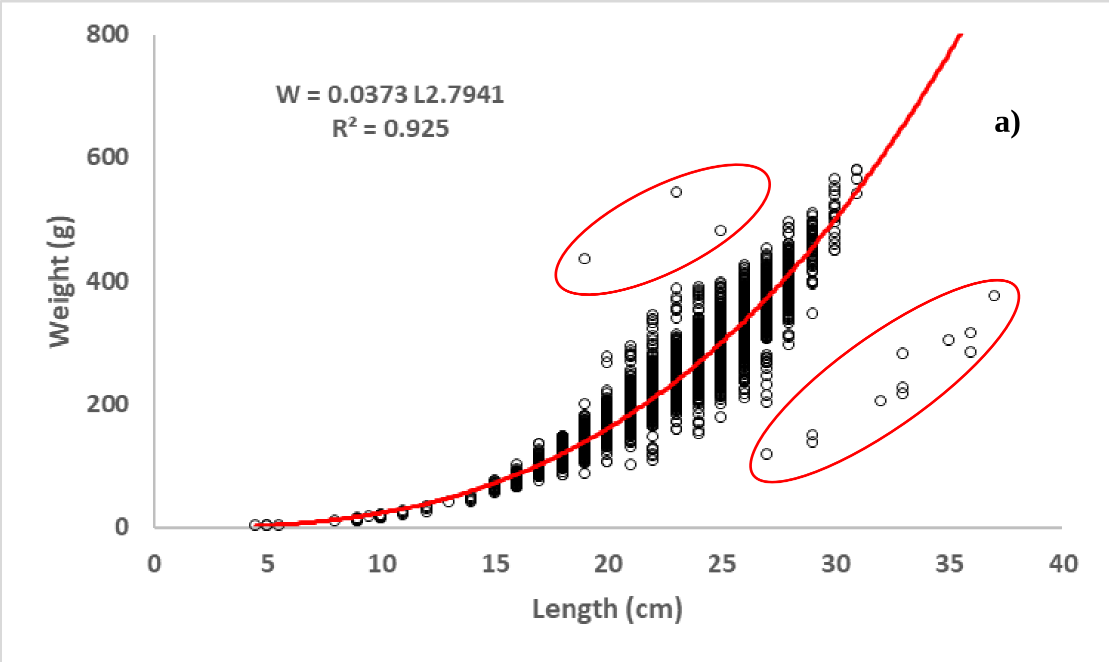
Stereoscope Olympus SZX9 connected to a camera Olympus SC50, they were connected to a screen monitor.

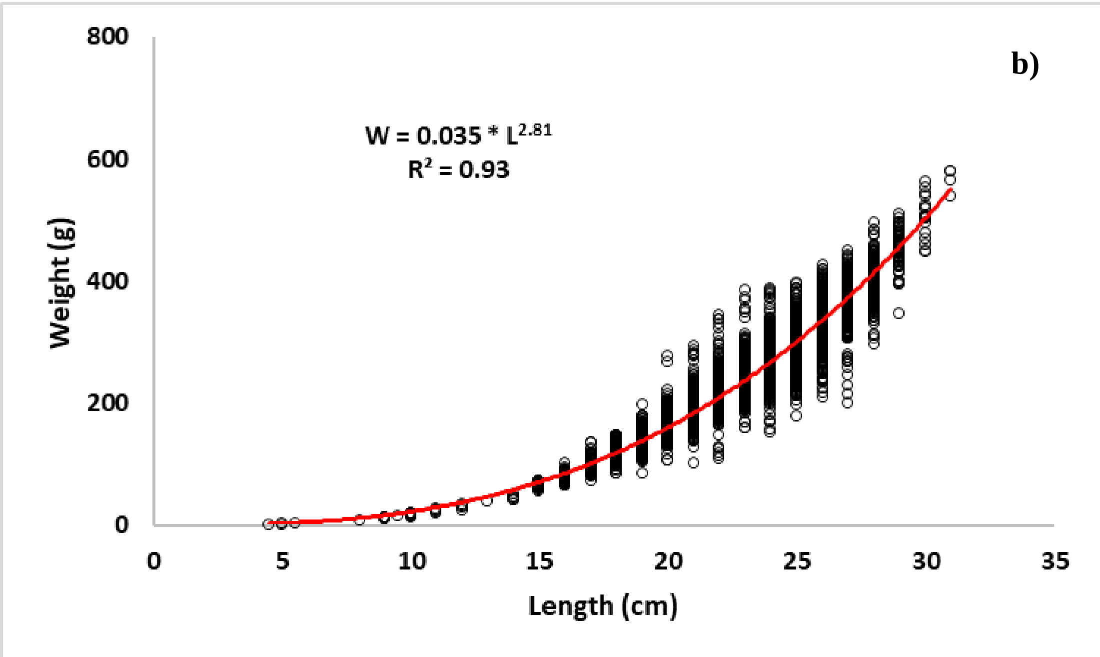
Appendix 2. Plot of the blackspot picarel length distribution during the period 2005 to 2015.



The green line represents the average length for thetimeseries from 2005 to 2012, the blue shaded area represents the length frequency distribution for each year and the vertical lines with an interval of 3 cm are for support in visualising the graphs.

Appendix 3. Length-weight relationship: a) with outlier inside red line and b) after removing the outlier.





Appendix 4. The results from average percent error (APE), coefficient of variance (CV) and index of precision (D) calculations for each otolith with the replicas 1 and 2 and the average.

	Length (cm)	Replica 2	Replica 2	Mean_age	APE (%)	CV (%)	D (%)
	9	5	4	4.5	11.11	13.61	9.62
	9	4	3	3.5	14.29	17.50	12.37
	9	4	3	3.5	14.29	17.50	12.37
	14	7	7	7	0.00	0.00	0.00
	15	7	7	7	0.00	0.00	0.00
	17	9	10	9.5	5.26	6.45	4.56
	18	10	11	10.5	4.76	5.83	4.12
	19	10	9	9.5	5.26	6.45	4.56
	20	11	10	10.5	4.76	5.83	4.12
	20	10	10	10	0.00	0.00	0.00
	21	11	8	9.5	15.79	19.34	13.67
	21	9	12	10.5	14.29	17.50	12.37
	21	12	9	10.5	14.29	17.50	12.37
	21	11	9	10	10.00	12.25	8.66
	22	9	10	9.5	5.26	6.45	4.56
	22	11	14	12.5	12.00	14.70	10.39
	23	14	10	12	16.67	20.41	14.43
	23	13	12	12.5	4.00	4.90	3.46
	24	12	10	11	9.09	11.13	7.87
	24	12	10	11	9.09	11.13	7.87
	24	13	13	13	0.00	0.00	0.00
	25	12	11	11.5	4.35	5.32	3.77
	25	12	10	11	9.09	11.13	7.87
	25	13	12	12.5	4.00	4.90	3.46
	25	14	16	15	6.67	8.16	5.77
	26	12	13	12.5	4.00	4.90	3.46
	26	14	14	14	0.00	0.00	0.00
	26	13	10	11.5	13.04	15.97	11.30
	27	14	13	13.5	3.70	4.54	3.21
	28	15	14	14.5	3.45	4.22	2.99
	29	16	15	15.5	3.23	3.95	2.79
Average	**21.23**	**10.94**	**10.29**	**10.61**	**7.15**	**8.76**	**6.19**

Appendix 5. Comparation of the result of *Linf* and *k* obtained in this study with the fish base data base information (https://marine.rutgers.edu/~cfree/what-combinations-of-von-bertalanffy-growth-parameters-are-possible).