Information on forage fish ages from otoliths collected in avian stomach contents

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ABSTRACT. Otolith analysis allows studying multiple aspects of fish biology, including age estimation; however, otoliths collected from avian stomach content have not been used to estimate the age of ingested fish. We evaluated the potential ability to estimate the age of Pacific sardine (*Sardinops sagax*) from otoliths collected from blue-footed booby (*Sula nebouxii*) stomach contents sampled in the Galapagos Islands. Blue-footed boobies were selected because they mostly forage close to their colony (<100 m) and were, therefore, more likely to have almost intact otoliths. To estimate age, we employed three previously developed methods for otoliths. We then related these estimates to the fish's standard length and compared these relations statistically using the Akaike Information Criterion (AIC) to determine which method was most parsimonious. We collected 32 otoliths in relatively good condition, of which we aged 31 with the surface-reading method, 26 with the otolith weight method, and 29 with the posterior otolith length method. Average ages obtained with the otolith methods were 5.6 ± 1.7 yr ($\pm$ standard deviation), 5.7 ± 2.0 yr, and 3.9 ± 1.5 yr, respectively. Estimates obtained with the otolith weight method had the highest correlation with standard length and the lowest AIC values of the three suggesting it was the best method for aging. Our results indicate that aging otoliths extracted from bird stomachs are possible; however, their use depends on the availability of previously developed methods and how digested the fish are when the birds return to the colony.

Keywords: *Sardinops sagax*; aging; marine bird; diet; forage fish; Galapagos Islands; eastern Tropical Pacific

INTRODUCTION

Avian diets have been used to investigate and monitor basic biological aspects of fish populations (Roby et al. 2010, Gladics et al. 2014, Depot et al. 2020). However, key biological information about individuals and the population can be estimated in cases when otoliths or fish ear bones are used. The otolith information includes species identification since otolith shape is species-specific (Jobling & Breiby 1986, Takasuka et al. 2003, Gladics et al. 2014). Three features of an otolith also allow age estimation: 1) length and 2) weight of an otolith increases with a fish's length and age, and 3) fish lay growth rings on their otoliths at regular time intervals (Samame 1977, Stevenson & Campana 1992, Fletcher 1995). Although fish larvae have been aged when collected from fish stomachs (Takasuka et al. 2003), to our knowledge, age has not been estimated from otoliths recovered from bird stomach contents. This missed opportunity could be exploited under the right circumstances.

The nature of seabirds' collection of fish from their stomach content means that the digestion process may compromise a sample's quality. For some species or families (e.g. Procellariiformes), all but the most rudimentary otolith techniques, such as identification, will be unusable; only uneroded otoliths should be used for the surface-reading, weight, and length aging methods (Schuiteman 2006). However, for other seabirds, like blue-footed boobies (*Sula nebouxii*) that mostly forage very close to their colony (~100 m) and can return with mostly whole prey in their stomachs,

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otoliths could be obtained in better condition and a larger number of them could therefore be used for estimating age (Anchundia et al. 2014).

Because even uneroded otoliths recovered from stomach contents may be stained by digestive processes, comparing multiple methods for aging will allow us to determine the most accurate. To test if fish can be aged from otoliths from bird regurgitations, we used Pacific sardines (*Sardinops sagax*) from blue-footed booby stomach contents collected in the central Galapagos Islands.

MATERIALS AND METHODS

Study site and fieldwork

Stomach contents were collected by regurgitation from adult blue-footed boobies (*Sula nebouxii*) at breeding colonies in North Seymour, Baltra, and southern Santa Cruz (Playa de Los Perros) Islands in the central region of the Galapagos Islands, Ecuador (Fig. 1). We sampled during four periods: July 2017, September 2017, December 2017, January 2018, and March-April 2018. Regurgitations were obtained after sunset when boobies had returned to the colony. Post-capture, birds were inverted for 30 s to stimulate regurgitation, then released. A total of 64 regurgitations with 371 fish items were collected (Table 1). Many fish were partially digested, and 76 nearly-intact fish that could be measured and thought to have otoliths were saved for analysis. These fish' standard lengths (SL, in cm) were measured in the field with calipers.

Lab analysis

Fish were first assigned to species by their morphological characteristics, following Grove & Lavenberg (1997). Species identifications were then visually confirmed for partial individuals using otolith shape. Visual confirmation was possible due to the significant difference in shapes and sizes among otoliths (Fig. 2) and the relatively good conditions of the fish. We identified *Sardinops sagax*, Galapagos thread herring (*Opisthonema berlangai*), and sharp nose or slender anchovy (*Anchoa ischana*) in bird stomach contents. Because photographs of the otoliths of *O. berlangai* and *A. ischana* have not been published, we used otoliths from intact individuals and were therefore identified positively using morphology (Fig. 2).

Estimating fish age

The estimation of age using otoliths in Osteichthyan fishes is based on the assumptions that older fish are longer than younger fish, their otoliths are longer and
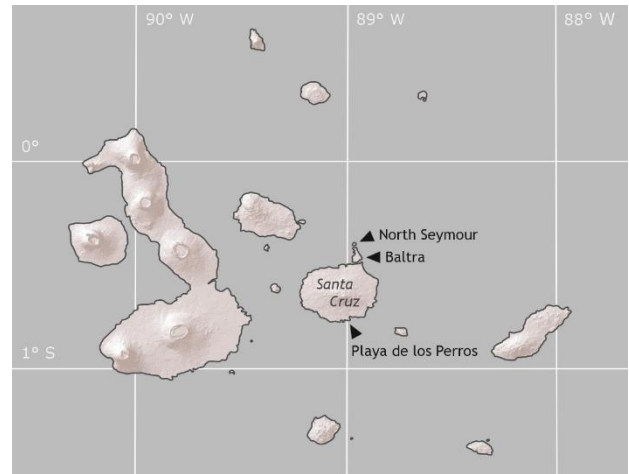

Figure 1. Map of Galapagos Archipelago showing the islands where fish from blue-footed booby (*Sula nebouxii*) stomach content regurgitations were collected. Far Northern Islands of Darwin and Wolf are not included.

heavier, and the fact that rings are laid at a regular time interval throughout life (Stevenson & Campana 1992). These four assumptions have been confirmed by data for *S. sagax* (Samame 1977, Fletcher 1995, Soares et al. 2007), but not in *O. berlangai* and *A. ischana*. We were, therefore, only able to estimate the age of *S. sagax*. However, the length of the otoliths of the individuals from the other two species was measured to determine the relationship with fish SL to validate the assumption that longer fish have longer otoliths than younger fish.

We estimated the age of *S. sagax* with three methods based on otolith characteristics, which have been previously evaluated (Samame 1977, Fletcher 1995, Soares et al. 2007), related them to the fish's SL, and then statistically compared these relationships. We used SL as a way to evaluate the best method because of the strong relationship that has been found to occur between age and length in this species (Samame 1977, Butler et al. 1996). The three otolith methods were surface-reading otolith annual rings, and two other methods that are based on two models that require otolith weight (g, from here on referred to as "otolith weight method") and distance from the core to the posterior side of the otolith (mm, referred to as "posterior otolith length method", Fig. 3) (Samame 1977, Fletcher 1995). The surface-reading method has been used and evaluated throughout the fish's distribution.

This was not another paragraph the otolith weight and posterior length methods were developed with *S. sagax* from south-western Australia and Peru. To surface-read their rings, we submerged both right and left otoliths in glycerin (96%), side by side, and viewed them under transmitted or reflected light, depending on their condition (Fig. 3).

Table 1. Number of samples collected by date and sites in central Galapagos, blue-footed boobies (*Sula nebouxii*) sampled, fish regurgitated, and the subsample of fish collected for otolith analysis. Number of samples by date and site of *Sardinops sagax*, *Opisthonema berlangai,* and *Anchoa ischana* collected during each date, respectively.

| Date	Colony site	Birds regurgitated	Total fish	Subsampled fish	*Sardinops sagax*	*Opisthonema berlangai*	*Anchoa ischana*
July 17, 2017	N. Seymour	5	48	10	5	1	4
July 19, 2017	Baltra	6	38	10	2	8	---
July 20, 2017	Santa Cruz	6	18	4	4	---	---
September 23, 2017	N. Seymour	6	29	3	---	3	---
September 30, 2017	Baltra	7	181	10	2	8	---
December 16, 2017	N. Seymour	8	73	15	---	6	9
January 13, 2018	Baltra	8	13	4	4	---	---
March 23, 2018	N. Seymour	6	26	11	10	1	---
March 30, 2018	Baltra	6	14	4	4	---	---
April 5, 2018	Santa Cruz	6	75	5	1	4	---
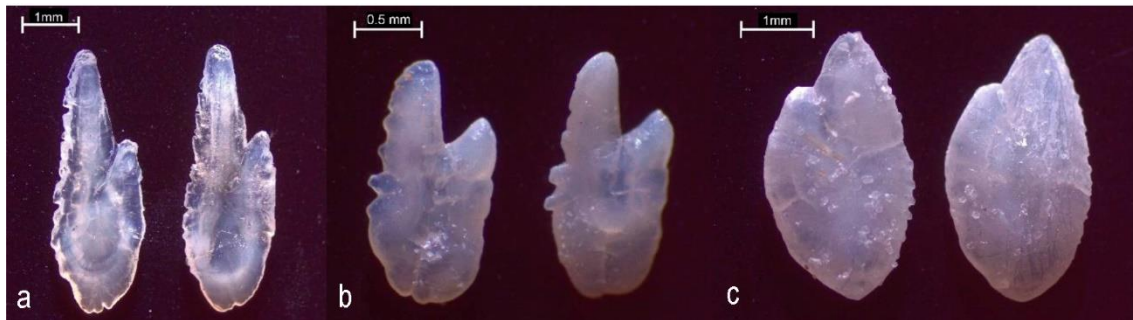
Figure 2. Photos of a) *Sardinops sagax* (23.3 cm total length), b) *Opisthonema berlangai* (6.6 cm standard length, SL), and c) *Anchoa ischana* (9.8 cm SL) otoliths collected from blue-footed booby regurgitations at three islands in central Galapagos during 2017-2018.

Figure 3. Photos of *Sardinops sagax* otoliths collected from blue-footed booby (*Sula nebouxii*) regurgitations at three islands in central Galapagos from 2017 to 2018. Posterior otolith length measurement is described on the otolith on the left and annual rings on the otolith on the right. Methods are further detailed in the text.

Each otolith was read two times by JRMJ (author initials) at least one week apart, without knowledge of the fish's SL. A third reading was conducted when the readings did not match (9 of 32 otoliths). Before their reading, otoliths were weighed on an analytical scale (0.0001 g precision) and their total length was measured from photographs taken with a dissecting scope fitted with a digital camera (Leica EZ4W) LAS EZ software. To estimate variation among the two or three surface readings and three otolith methods, the coefficient of variation (CV) was calculated. To compare the age estimates from the three models and confirm the assumption that older fish are longer even when birds have ingested them, we used simple linear regressions between the three otolith-based estimates of age and SL. We then compared the three regression models using Akaike Information Criterion (AIC) for small sample sizes with the dredge function in R software. The model with the lowest AIC value was selected as the most parsimonious. To further compare these models, delta, the difference between the model with the lowest AIC and the other models, and the weight,

or the relative likelihood of the model, are presented. Assumptions for linear regressions were tested using Q-Q plots and Bartlett tests (Sokal & Rohlf 1981). Statistical analysis was conducted using the R software and MuMin package (R Core Team 2021, Barton & Barton 2015).

RESULTS

From the stomach contents, we identified 32, 31, and 13 individuals of *Sardinops sagax*, *Opisthonema berlangai*, and *Anchoa ischana*, respectively (Table 1). SL of these fish averaged 19.1 ± 3.0 cm (SD) for *S. sagax*, 8.1 ± 3.9 cm for *O. berlangai*, and 8.3 ± 1.7 cm for *A. ischana*, and was significantly positively related to otolith total length ($P < 0.05$, Fig. 4).

We were able to age 31 *S. sagax* individuals with otolith surface readings, 26 with the otolith weight method, and 29 with the posterior otolith length method. None of the otoliths extracted showed signs of digestion, but some were stained (Figs. 2-3). Correlation of *S. sagax* age estimates and SL were highest for the otolith weight ($r = 0.81$, $P < 0.0001$), followed by the surface reading ($r = 0.60$, $P = 0.002$), and posterior otolith length method ($r = 0.57$, $P = 0.002$, Fig. 5). The coefficient of variation among the surface readings and three otolith methods averaged 14.26 ± 12.62 and 27.46 ± 14.85, respectively. Because ages were below 10, the variability among age estimates was considered high (CV > 10), suggesting the precision among estimates was low potentially because of how difficult it was to surface read the otoliths due to staining. Average age values were highest when fish were aged with the otolith weight method (average ± SD: 5.7 ± 2.0 yr, range: 3 to 8 yr) followed by surface reading (5.6 ± 1.7 yr, 2 to 8 yr), and otolith posterior length (3.9 ± 1.5 yr, 1.4 to 6.8 yr) (Fig. 5), respectively. Estimates were, in at least two methods, from all ages between 2 and 8 years, and 3 to 7 years in all methods (Fig. 5). Finally, the model that best fit the observed SL data was based on age estimates from the otolith weight method (Table 2).

DISCUSSION

To our knowledge, this study is the first to use otoliths to age fish in the stomach contents of piscivorous birds. This study is important because sardine populations are doing poorly worldwide (Hill et al. 2016). Moreover, the population of blue-footed boobies in Galapagos has been reduced in size by more than 50% in the last 40 years, a decline attributed to the reduction of *Sardinops sagax* from its diet, which has been tracked since 1986
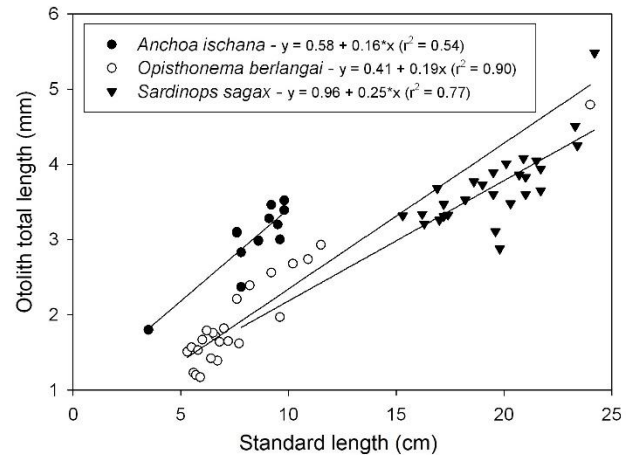

Figure 4. Relationship between standard length (cm) and total otolith length (mm) of three fish species collected from stomach contents regurgitated by blue-footed boobies (*Sula nebouxii*) in three islands in central Galapagos during 2017 and 2018.

(Anderson 1989, Zwolinski & Demer 2012, Anchundia et al. 2014, Tompkins et al. 2017). Our comparison of estimates from three different methods suggests the otolith weight method ages were best related to SL. These results could have occurred because otoliths grow in three dimensions and not in two, making the weight method better related to SL than the posterior otolith length. In the present study, the otoliths did not seem to have been digested, only stained, making surface reading more difficult and potentially less accurate. Despite the fact that the otolith weight method was found to produce the best model, the range of ages observed in all methods was within ages observed in waters off Ecuador (1-7 yr, the late 70s to early 80s), Peru (1-8 yr, early 1990s), Baja California, and southern California (1-6 yr, 1994), Oregon (1-8 yr, early 2010s), and Washington (1-10 yr, suggesting that on average these estimates were within the correct range (Dawson 1986, Butler et al. 1996, Csirke et al. 1996, Krutzikowsky & Smith 2012, Wargo & Hinton 2016). Interestingly, the sardines in this study were larger and appeared to be older than fish consumed in 2014 in Galapagos (21.2 ± 3.2 *vs*. 6.8 ± 3.2 cm fork length; Anchundia et al. 2014) during an ongoing decline in blue-footed booby numbers (Anchundia et al. 2014).

Although our samples were small, this study confirms that otoliths can be used to estimate the age of fish consumed by marine birds, which to our knowledge has never been conducted, and identify their prey, as has been shown in other locations (Jobling & Breiby 1986, Takasuka et al. 2003, Gladics et al. 2014). At the same time, there are caveats to using an otolith length or weight method developed for different populations without considering annual variation in growth
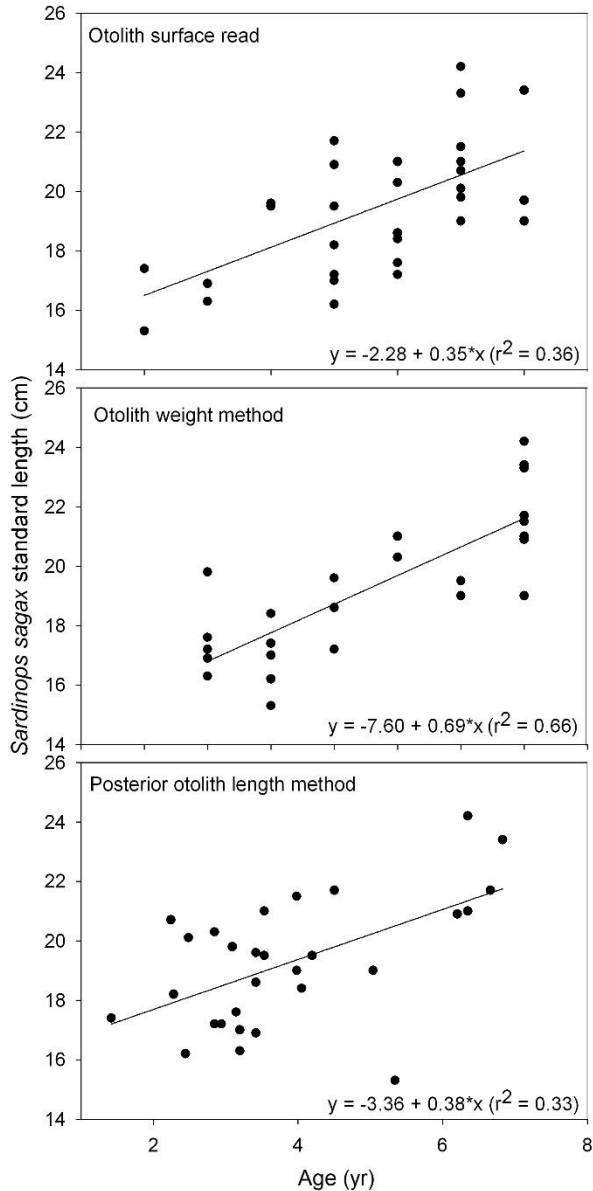
Figure 5. Relation between *Sardinops sagax* standard length (cm) with age (yr). Age was estimated using the otolith ring surface reading, otolith weight method, and posterior otolith length method. Methods are further detailed in the text.

(Samame 1977, Fletcher 1991, 1995). Methods not requiring visible annual rings (such as otolith weight) may be more appropriate in these circumstances because staining can affect rings. The strong relationship we observed between fish SL and age estimates from the otolith weight method suggests our strategy is effective and that using otoliths collected from bird stomach contents is a viable method to estimate fish age. These results also highlight the value of developing other methods to estimate fish age besides reading otolith rings, particularly in parts of the world where the expertise and equipment to read oto-

Table 2. Comparing simple linear regression models of *Sardinops sagax* age estimates with three otolith-based methods and their standard length (cm, Fig. 5). Comparisons were conducted using Akaike Information Criterion values for small sample sizes (AICc and delta) and log-likelihoods (logLik).

Model	logLik	AICc	Delta	Weight
Weight	-43.162	93.5	0	0.46
Posterior otolith length	-49.479	106.1	12.63	0.001
Surface reading	-52.338	111.8	18.35	0

liths are unavailable. Future work should focus on estimating the age of the Pacific sardines collected in the ocean around the Galapagos Islands, developing growth models, analyzing the size and age frequency distribution, and the potential of size-selective predation by these charismatic marine birds.

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